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GENETICS OF NOVEL FEED EFFICIENCY AND RELATED TRAITS IN NORDIC DAIRY CATTLE

DOCTORAL THESIS

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ACADEMIC DISSERTATION

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"It isn't the mountains ahead to climb that wear you out,
it's the pebble in your shoe."
Muhammad Ali

ABSTRACT

Improving the feed efficiency (FE) of dairy cows improves both the economic and environmental sustainability of milk production. However, FE is a complex trait affected by several factors. It is influenced by the genetic ability and physiological state of the cow, along with diet, management and other environmental factors. The overall aim of this thesis was to develop new recording methods for FE traits and explore the genetic background of FE in dairy cows. In particular, the main goals were to develop simple, practical and novel methods for measuring both cow-specific diet digestibility and energy status on farms, develop models for overall metabolisable energy efficiency (MEE) and partial MEE (pMEE), and assess the genetic background in all these traits.

The first objective of the digestibility study was to assess the variability in cow-specific digestibility and investigate the prediction of diet digestibility using near-infrared reflectance spectroscopy (NIRS) scans of faecal samples (I). Feed and faecal samples were collected from 13 Nordic Red Cattle (RDC) and 31 Holstein (HOL) cows at approximately 50, 150 and 250 days in milk (DIM). The results indicated that NIRS can be used to assess cow-specific diet digestibility from faecal samples (I, II). The NIRS-based digestibility traits were dry matter digestibility, which was determined by the indigestible neutral detergent fibre (iNDF) content as an internal marker (DMD_{iNDF}), direct faecal iNDF ($iNDF_f$) and direct faecal organic matter digestibility (OMD). The repeatability estimates for DMD_{iNDF}, and especially for $iNDF_f$ predictions were reasonable, 0.32 and 0.46, respectively. However, the low repeatability and small variability estimates for OMD indicated that OMD predictions by NIRS were not accurate enough to quantify differences between cows. According to the results, collecting representative composite samples from all the cows in a herd every second or third month would yield a suitable data structure for genetic evaluations of diet digestibility.

The second objective was to assess the genetic variation in cow-specific diet digestibility (II). The data for genetic analyses were collected from three research herds in Finland and one in Norway, including a total of 931 records from 283 RDC and 45 HOL cows. Due to differences in faecal sampling protocols, the observations were associated with different accuracies. Therefore, the heritability estimates varied between sampling protocols and ranged from 0.14 ± 0.06 to 0.51 ± 0.24 for DMD_{iNDF} and from 0.13 ± 0.05 to 0.48 ± 0.18 for $iNDF_f$. Estimated genetic standard deviations were 10.5 g/kg and 6.2 g/kg dry matter for DMD_{iNDF} and $iNDF_f$, respectively. The results indicated that recording only the iNDF content of the faeces is sufficient to determine genetic variation in cows' abilities to digest feed if the sampling protocol developed in Study I is applied. The coefficient of genetic variation

for DMD_{iNDF} was rather small (1.7%) but could be utilised if improving diet digestibility pays off the costs of sample collection and processing.

In the third study, two new FE traits were explored: MEE, which is formed by modelling metabolisable energy intake fitting regressions on energy sinks (metabolic body weight, energy-corrected milk, and body weight gain and loss) directly, and pMEE, where the model for MEE is extended with random regressions on energy sinks nested within additive genetic and permanent environmental effects (III). Residual energy intake (REI) was used as a reference trait. The data were collected from experimental herds and included 12 350 weekly ME intake records on 495 primiparous RDC cows from weeks 2 to 40 of lactation. Heritability estimates for REI and MEE were moderate, 0.33 and 0.26, respectively. The MEE allowed more accurate modelling of the data and resulted in better properties for the prediction of breeding values compared with REI. The pMEE models fitted the data even better, but the convergence of the models with random regressions on metabolic body weight presented difficulties. The derived partial heritabilities for energy efficiency in maintenance, milk production and growth were 0.02, 0.06 and 0.04, respectively, indicating that some genetic variation may exist in the efficiency of using ME for various pathways.

In the fourth study, the prediction equations for blood plasma non-esterified fatty acid (NEFA) concentration were developed (IV). Predicted NEFA was based on the mid-infrared reflectance spectroscopy (MIR) of milk samples. A total of 1 585 milk spectral readings and 809 blood samples were collected from 141 RDC cows in three research herds. Blood NEFA concentration was predicted from MIR spectral data using partial least squares regression. The highest coefficient of determination of cross-validation for NEFA was found when using leave-one-out cross-validation with evening milk samples (0.67).

The genetic variation was determined using two NEFA predictions and four other energy status indicator (ESI) traits; the milk fat to protein ratio, milk fatty acid C18:1 *cis*-9, milk β-hydroxybutyrate and milk acetone concentration in the fifth study (V). In addition, the genetic correlations between ESI traits and fertility trait interval from calving to first insemination were studied. The data consisted of 37 424 primiparous RDC cows with milk test-day records between 8 and 91 DIM. Data were analysed using univariate and multivariate linear animal models applied to ESI traits at 8–35, 36–63 and 64–91 DIM. The heritability estimates for all ESI traits were from low to moderate. The genetic correlations between ESI traits and fertility were moderate (0.18 to 0.40) in the first time period, in general slightly lower (0.03 to 0.43) in the second time period and decreased clearly (-0.02 to 0.19) in the third time period.

The results of this thesis provide information needed for genetic improvement in FE of dairy cows and may be utilised on farms in dairy cow nutrition and management.

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CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications:

I Mehtiö, T., Rinne, M., Nyholm, L., Mäntysaari, P., Sairanen, A., Mäntysaari, E.A., Pitkänen, T. & Lidauer, M.H. 2016. Cow-specific diet digestibility predictions based on near-infrared reflectance spectroscopy scans of faecal samples. Journal of Animal Breeding and Genetics 133: 115-125.

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III Mehtiö, T., Negussie, E., Mäntysaari, P., Mäntysaari, E.A. & Lidauer, M.H. 2018. Genetic background in partitioning of metabolizable energy efficiency in dairy cows. Journal of Dairy Science 101: 4268-4278.

IV Mehtiö, T., Mäntysaari, P., Kokkonen, T., Kajava, S., Latomäki, T., Nyholm, L., Grelet, C., Pitkänen, T., Mäntysaari, E.A. & Lidauer, M.H. 2018. Developing an indicator for body fat mobilisation using mid-infrared spectrometry of milk samples in dairy cows. In: Proceedings of the World Congress on Genetics Applied to Livestock Production, Auckland, New Zealand, Feb 7-11 2018. 5p.

V Mehtiö, T., Mäntysaari, P., Negussie, E., Leino, A.-M., Pösö, J., Mäntysaari, E.A. & Lidauer, M.H. 2020. Genetic correlations between energy status indicator traits and cow fertility in primiparous Nordic Red dairy cattle. Animal (accepted 14.2.2020) DOI: 10.1017/S1751731120000439

The publications are referred to in the text by their Roman numerals. Copies of the original articles (I–IV) and the manuscript of an article (V) are included with the kind permission of their respective copyright owners.

The author participated in planning the studies and collecting the data, performed all data editing and statistical analyses, interpreted the results together with the co-authors and was the main writer of all of the articles.

ABBREVIATIONS

AIA	Acid-insoluble ash
BHB	β-hydroxybutyrate
BWG	Body weight gain
BWL	Body weight loss
$BW^{0.75}$	Metabolic body weight
DIM	Days in milk
DM	Dry matter
DMD	Dry matter digestibility
DMI	Dry matter intake
EBV	Estimated breeding value
ECM	Energy-corrected milk
ES	Energy status
ESI	Energy status indicator
FE	Feed efficiency
FPR	Fat to protein ratio
HOL	Holstein
ICF	Interval from calving to first insemination
iNDF	Indigestible neutral detergent fibre
ME	Metabolisable energy
MEE	Metabolisable energy efficiency
MEI	Metabolisable energy intake
MIR	Mid-infrared reflectance spectroscopy
NAV	Nordic Cattle Genetic Evaluation
NEFA	Non-esterified fatty acids
NIRS	Near-infrared reflectance spectroscopy
OMD	Organic matter digestibility
PLS	Partial least squares
pMEE	Partial metabolisable energy efficiency
RDC	Red Dairy Cattle
REI	Residual energy intake
REML	Restricted maximum likelihood
RFI	Residual feed intake

1 INTRODUCTION

Improving the feed efficiency (FE) of dairy cows is of interest for two main reasons: it improves the profitability and reduces the environmental impacts of dairy and meat production. The producer price for milk has dropped by 15% from 2014 to 2018 (Luke, 2018a). In addition, production input prices have increased at an annual rate of 2% to 5%, which has decreased farmer incomes (PTT, 2018). The decrease in producer prices and increase in production input prices lead to poor profitability, which is one of the main factors influencing the change in production structure in Finnish agriculture. In 2000, Finland still had 19 750 dairy farms but 63% of these dairy farms were shut down by 2016, and another 2 800 farms are expected to close by 2025, as predicted by the Natural Resources Institute Finland (Luke, 2018b).

In 2017, the average Finnish meat consumption per capita was 81 kg, 19.4 kg of which was beef. Dairy product (milk, cheese, yogurt, butter) consumption per capita was 173 kg (Luke, 2018c). Thus, meat and dairy products make a significant contribution to Finnish diets. The global demand for animal-based food will additionally increase by nearly 70% from 2010 to 2050 due to population growth but also because of a higher standard of living and economic growth (World Resources Institute, 2018).

There is a high demand from the ecological sustainability perspective to reduce greenhouse gas emissions along with the nutrient discharge from agriculture. The share of total Finnish greenhouse gas emissions from agriculture in 2017 was 12% and 27% if emissions from the land use, land-use change and forestry sector are also accounted for (Tilastokeskus, 2019). The largest share of the agriculture sector's emissions comes from agricultural soils (53%) and the second largest share comes as methane from enteric fermentation (32%). In addition, nutrients (mainly nitrogen and phosphorus) leaching from manure to watercourses contribute to eutrophication. Therefore, it is important to reduce the negative impacts of dairy cattle to the climate and environment.

Genetic improvement of FE in dairy cattle contributes to tackling both economic and ecological sustainability challenges. As feed is a large input cost in dairy production, any reduction in feed intake at the same production level will reduce the production costs of dairy products. Concurrently, a reduction in feed intake will decrease methane emissions and nutrient loadings, and save land used for feed production per kg of milk or meat.

1.1 CHALLENGES IN IMPROVING FEED EFFICIENCY

Feed efficiency is influenced by several biological features of a cow: its body weight and maintenance requirements, activity, milk production potential and the magnitude of the intake, along with the efficiency at which nutrients are digested, absorbed and metabolised to milk production, maintenance and growth. This complex system is steered by genetics, nutrition, management and other environmental factors at various stages of lactation.

Easy, cheap and accurate techniques for on-farm measuring of feed intake, body weight and energy status of cows are lacking, which makes evaluating the FE of dairy cows difficult. Therefore, several traits have been developed to describe either overall FE or various specific efficiencies of a cow.

1.1.1 DEFINING THE OVERALL FEED EFFICIENCY

The overall FE of a cow is usually described using various ratio or residual traits. Ratio traits are defined as the ratio of output over input or its inverse. Based on the literature, gross energetic efficiency (Veerkamp & Emmans, 1995; Vallimont et al., 2011), gross feed efficiency (Korver, 1988; Van Arendonk et al., 1991; Spurlock et al., 2012), energy conversion efficiency (Mäntysaari et al., 2012) and feed conversion efficiency (Coleman et al., 2010) are the most studied ratio traits in dairy cattle. The maintenance requirement ratio is one of the most recent FE traits of interest. It describes the expected energy needed for the maintenance per kg of energy-corrected milk (ECM) produced (Lidauer et al., 2018). Ratio traits are easy to define and understand but component traits may be correlated, and thus the expected responses to selection are difficult to predict (Gunsett, 1984).

Residual traits, such as residual feed intake (RFI), can be calculated as the residual from a linear regression for either energy or feed intake on various energy sinks such as milk production, metabolic body weight ($BW^{0.75}$) (for maintenance requirements) and body weight change. Residual energy intake (REI) may be used instead of RFI if the energy intake of the cow is known. Alternatively, these residual traits can be calculated as the difference between actual metabolisable energy intake (MEI) or dry matter intake (DMI) and expected MEI or DMI predicted from animal performance. More efficient cows will consume less feed than expected, which may be because e.g. of their better ability to digest feed.

Commonly, REI or RFI calculations for genetic evaluations are performed by a two-step procedure, where the first step is to model DMI or MEI by a multiple linear regression including ECM production, $BW^{0.75}$ and body weight change to account for body tissue mobilisation and growth (Berry & Crowley, 2013; Liinamo et al., 2015). Certain definitions also include body

condition score changes. Residuals from the models are used as cows' REI or RFI records, which in the second step are modelled against relevant fixed and random effects. However, instead of the two-step process, the fixed and random effects may be directly added into the model for DMI or MEI, which should result in more accurately estimated breeding values (Tempelman et al., 2015).

However, certain concerns in using RFI or REI have been addressed. The observations of these traits accumulate measurement errors that are associated with the component traits. For example, the method of recording feed intake may affect the results. Therefore, recording strategies for DMI need to be reasonably accurate to ensure reliable estimates of cows' genetic merits (Negussie et al., 2019). Moreover, unknown genetic correlations may exist between residual traits and the regressors that are used to predict them (Pryce et al., 2014; Manzanilla-Pech et al., 2016).

1.1.2 DETERMINING COW-SPECIFIC DIET DIGESTIBILITY

Diet digestibility describes the proportion of feed intake that is digested and not excreted in the faeces. It is one important factor affecting FE in dairy cows because a cow with a higher ability to digest feed will have more energy available for production and body functions. Cows with low RFIs are likely to digest and metabolise nutrients more efficiently, and thus diet digestibility may at least partially be an underlying factor in RFI variation (Herd et al., 2004; VandeHaar et al., 2016). Fischer et al. (2018) found a negative phenotypic correlation ($r = -0.26$) between REI and digestibility, indicating that more efficient animals have greater diet digestibility. On the other hand, Fischer et al. (2018) found apparent confounding with other biological traits, such as behaviour, activity, rumen temperature, and suggested that these other traits may either contribute to or be the consequence of digestibility and thus conclusions on biological traits explaining FE differences among cows could not be made. Potts et al. (2017) reported that the relationship between FE and digestibility is diet dependent. In addition to diet composition, the rate of digesta passage through the digestive tract has been established to affect digestive efficiency and thus increasing feed intake reduces diet digestibility (Tyrrell & Moe, 1975). Therefore, an increase in milk production, and a related increase in DMI, may be expected to reduce diet digestibility.

Diet digestibility has traditionally been determined by total faecal collection or from feed and faeces using markers, for example acid-insoluble ash (AIA) (Van Keulen & Young, 1977). However, these methods are too expensive and time-consuming to be used routinely on farms. Near-infrared reflectance spectroscopy (NIRS) has been shown to be a potential method applied to faeces for assessing diet digestibility in cattle and sheep (Fanchone et al., 2009; Nyholm et al., 2009; Decruyenaere et al., 2015). However,

appropriate calibration equations are required for predicting digestibility traits using NIRS. The difficulty in developing such equations is to collect a reference database that is as representative as possible for a given field data diversity.

Even if the effects of feeding level and diet composition on digestibility are well identified (i.e. Huhtanen et al., 2009; Nousiainen et al., 2009), studies on the genetic variation in dairy cattle digestibility have been scarce. This is mostly because of the challenges in determining digestibility and collecting a reasonable-size data set for genetic analyses.

1.1.3 MODELLING PARTIAL EFFICIENCIES

Instead of describing the overall FE of a cow, measuring efficiency with respect to certain metabolic functions, i.e. for milk production, maintenance and growth may be of more interest. This would give more comprehensive information as to why some cows are more efficient than others. Partial efficiencies, such as the efficiency to utilise ME for lactation (k_l), may be calculated by relating milk energy output to ME available for production (Agnew and Yan, 2000) and have traditionally been estimated using calorimetric chambers. Earlier studies have not found clear evidence to assume genetic differences in the partial efficiencies, but high genetic merit cows have been shown to partition the available energy differently from low genetic merit cows (Veerkamp and Emmans, 1995; Agnew and Yan, 2000). From the breeding viewpoint, placing more selection weight on efficiency with respect to certain metabolic functions would be desirable. Therefore, models that are capable of partitioning a cow's efficiency with respect to different pathways would be of interest and have not been studied so far.

1.1.4 ENSURING ENERGY STATUS

Dairy cows in early lactation have a very high energy requirement for milk production that can seldom be fulfilled by feed intake and forces the cows to compensate for energy deficits through their body reserves (Mäntysaari & Mäntysaari, 2010; Mäntysaari et al., 2012). This state of a cow, called a negative energy status (ES) may predispose cows to various health and fertility problems, especially if severe and long-lasting (Roche et al., 2009; Berry & Crowley, 2013). Considering energy status in dairy cattle breeding programmes is important, especially if FE traits are included in the breeding goal. Selecting feed-efficient yet metabolically imbalanced cows is not desirable. Earlier studies have shown that residual traits, such as REI and RFI, and ratio traits, such as gross feed efficiency, are unfavourably correlated with energy balance (Vallimont et al., 2011; Berry & Crowley, 2013; Liinamo et al., 2015; Hurley et al., 2018).

As also with FE traits, measuring ES is challenging. Predicting ES using a calculated energy balance based on milk production and composition, DMI and the energy density of the diet, and body weight, is conceptually problematic. This is because a negative energy balance does not necessarily mean that a cow is in a negative ES. The accuracy of a calculated energy balance may additionally be low due to accumulated measurement errors. Strong association between REI and calculated energy balance can be expected given their mathematical similarity. Thus, failing to account for the mobilisation of body reserves using body weight change may result in selection for negative energy status. Hurley et al. (2018) reported a correlation of 0.96 between REI and energy balance in mid-lactation (90–180 DIM) when the average body weight change was close to zero. Because body weight change is zero, it does not contribute to the REI model, and thus REI is mathematically equivalent to the calculated energy balance. Changes in body condition score and body weight can be used as indicators for ES because the mobilisation of body reserves causes a decrease in body condition score and body weight, but this would require frequent and accurate measuring.

The challenges in estimating energy balance and recording body weight change has led to a growing interest in using various energy status indicator (ESI) traits, which could serve as more precise indicators of cows' ES. These traits are based on body tissue metabolism. Adipose tissue metabolism is highly reactive and finely regulated, and numerous interactions occur between the immune, endocrine and metabolic systems in dairy cows during early lactation (Chilliard et al., 2000; Esposito et al., 2014). However, mobilising adipose tissue principally increases the concentration of non-esterified fatty acids (NEFA) in blood plasma. As the supply of NEFA is overloaded, the production of ketone bodies (acetoacetic acid, acetone and β-hydroxybutyrate) increases in the liver (Chilliard et al., 2000; Veerkamp et al., 2003; Esposito et al., 2014). In addition, the high uptake of long-chain fatty acids (FAs) inhibits de novo synthesis of short-chain FAs by mammary gland tissue and thus causes changes in the milk FA profile (Palmquist et al., 1993). The proportion of FAs originating from adipose tissue (especially C16:0, C18:0 and C18:1 *cis*-9) in milk therefore increases (Stoop et al., 2009; Bastin et al., 2011).

Mid-infrared reflectance spectroscopy (MIR) of milk samples is one of the most promising methods for evaluating blood metabolites, fatty acids and energy status (De Marchi et al., 2014; Bastin et al., 2016). MIR is already used routinely in milk recording to predict major milk components but can also be utilised to predict milk FAs and FA groups with high accuracy (Soyeurt et al., 2011). Fat to protein ratio (FPR) is one of the studied ESI traits, as the postpartum lipolysis results in changes in milk component ratios (Buttchereit et al., 2010; Negussie et al., 2013; Koeck et al., 2014; Pryce et al., 2016). However, the proportion of certain FAs originating from adipose tissue may be used as more accurate indicators (Stoop et al., 2009;

Bastin et al., 2011). In addition, ES-related blood metabolites and hormones, e.g. NEFA, β-hydroxybutyrate (BHB), glucose and IGF-1 (de Roos et al., 2007; Belay et al., 2017; Grelet et al., 2019), can be predicted using milk MIR spectra. The use of milk MIR-predicted blood NEFA as an indicator for ES is a novel approach, and further studies on exploring the genetic relationships for example between NEFA and fertility are needed to ensure that the consequences of selection for lower NEFA concentration in blood would be as expected.

1.2 HERITABILITY ESTIMATES OF FEED EFFICIENCY AND RELATED TRAITS

Genetic variation in overall FE has been studied intensively in dairy cattle in recent years. Heritability estimates for ratio traits have been reported to range across lactation from 0.14 to 0.32 for the feed conversion ratio (Vallimont et al., 2011; Spurlock et al., 2012), and from 0.16 to 0.47 for the energy conversion efficiency (Liinamo et al., 2015; Lidauer et al., 2018). Lidauer et al. (2018) studied genetic variation in the maintenance requirement ratio and reported a heritability estimate of 0.50. The heritability estimates reported for DMI have varied between 0.10 and 0.34 (Berry et al., 2014; Liinamo et al., 2015; Li et al., 2016). The heritability estimates found for RFI and REI range from 0.06 to 0.29 and the estimates are reported to change during lactation (Liinamo et al., 2015; Tempelman et al., 2015, Li et al., 2017; Hurley et al., 2018).

However, the literature on genetics behind diet digestibility in dairy cattle is scarce. Earlier studies presented that only little variation exists among cows in their ability to digest feed, particularly when intake levels are standardised (Korver, 1988; Veerkamp & Emmans, 1995). More recent studies have indicated genetic differences in digestibility among cows (Berry et al., 2007) and between breeds (Beecher et al., 2014). Berry et al. (2007) reported heritability estimates ranging from 0.08 to 0.45 in diet digestibility when random regression models were fitted on a data set from 238 HOL (Holstein) cows.

High phenotypic correlation has been reported between REI (or RFI) and the calculated energy balance, suggesting that negative REI animals (i.e. more efficient ones) are also in a more negative energy balance (Liinamo et al., 2015; Hurley et al., 2018). Hurley et al. (2018) concluded that REI could be used as a breeding objective, but health and fertility traits need to concurrently be considered in the breeding programme. Heritability estimates for calculated energy balance have been reported to vary from 0.02 to 0.49 across lactation, being highest during early lactation (Berry et al., 2007; Hüttmann et al., 2009; Spurlock et al., 2012; Liinamo et al., 2015).

Many studies have explored the genetic variation in potential milk MIR-predicted indicators for metabolic status and their correlations with health and fertility (Bastin et al., 2016; Pryce et al., 2016; König & May, 2019). Bastin et al. (2012) reported a heritability estimate of 0.13 for C18:1 *cis*-9 at 5 DIM and estimates increased as lactation progressed. Heritability estimates for milk BHB and acetone concentrations have varied between 0.10 and 0.30 in early lactation (van der Drift et al., 2012; Koeck et al., 2014; Lee et al., 2016). Negussie et al. (2013) and Koeck et al. (2014) have reported heritability estimates between 0.12 and 0.23 for FPR.

Oikonomou et al. (2008a) studied a data set with weekly measured blood metabolites and found heritability estimates ranging across lactation from 0.08 to 0.40 and 0.08 to 0.35 for BHB and NEFA, respectively. The genetic variance found for both traits was particularly high during the first weeks of lactation (Oikonomou et al., 2008a).

1.3 FEED EFFICIENCY TRAITS IN BREEDING PROGRAMMES

In the past, improvements in dairy cattle feed efficiency were mainly achieved through a dilution of the maintenance requirement. With increased milk production per cow, more energy per cow is consumed, but a greater portion of the energy is partitioned toward milk production instead of maintenance and growth. However, the effect of dilution has diminished for modern high-producing dairy cows (VandeHaar et al., 2016). Thus, there is a growing emphasis placed on selecting for digestive and metabolic efficiency, but to date only a few countries have included FE traits as breeding objectives. One reason is that feed intake, diet digestibility and ES are all difficult and expensive to accurately measure from a large number of individual cows. Therefore relatively small data sets mainly from research herds can currently be used for genetic analyses and evaluations. Here genomic selection has its advantages.

In genomic selection, the cumulative effects of markers positioned densely across the whole genome on the trait of interest are estimated using genotypes and phenotypes obtained from a reference population (Meuwissen et al., 2001). Such prediction equations allow predicting breeding values for the population using only the genotypes of the animals. Genomic prediction equations may therefore be applied to animals with genotypes but no phenotypes. However, the size of the reference population has to be reasonable large and relevant to the selected population to obtain reliable genomic predictions (VanRaden et al., 2009).

Australia, the Netherlands and the Nordic countries have made the first attempts to include FE traits into breeding programmes, but predictions still have low reliability due to small reference populations. For example, FE is

included into the national breeding objective in Australia and "Feed Saved" breeding value estimates have been released since 2015 (Pryce et al., 2018). Breeding value includes a genomic component for RFI, combined with an estimated breeding value (EBV) for body weight predicted from type traits. The mean reliability of the Feed Saved EBV was 37% in 4 416 genotyped HOL sires without phenotypes (Pryce et al., 2018).

In the Netherlands and Flanders, the breeding values for DMI were estimated using observations of 3 200 cows, 1 300 of which were genotyped (de Jong et al., 2016). In addition, the majority of sires were genotyped. When correlated traits (milk yield, fat yield, protein yield and body weight) were also included in the evaluation model, the reliability of DMI breeding values for bulls was reported to average 59% (de Jong et al., 2016). Breeding values for DMI for Dutch bulls and cows have been published since 2014. Since December 2017, the EBV's for Saved Feed for maintenance, which is based on the difference between feed intake and feed used for production, have also been published for all the bulls (CRV, 2018).

Nordic Cattle Genetic Evaluation NAV (Denmark, Finland, Sweden) is currently developing an index for Saved Feed. This index comprises two components: maintenance and metabolic efficiency (Lidauer et al., 2019). In the launching phase in 2019, the index contained only the maintenance part. Maintenance is based on breeding values for $BW^{0.75}$, for which the breed-specific genomic predictions for HOL, Nordic Red dairy cattle (RDC) and Jersey dairy breeds were developed (Lidauer et al, 2019). The development of genomic evaluation for metabolic efficiency based on RFI is still under progress.

As soon as the challenges in developing reliable FE indices are solved, considering ES in the breeding programmes will also be necessary to avoid selection for negative ES in early lactation. Then the ES indicators, i.e. blood metabolites and milk fatty acids, can be predicted from milk samples using MIR spectrometry and utilised in the selection (De Marchi et al., 2014; Bastin et al., 2016; Pryce et al., 2016; König and May, 2019).

2 OBJECTIVES OF THE STUDY

The overall aim of this thesis was to develop new recording methods for FE traits and to explore the genetic background of FE in dairy cows. In particular, the aims were to develop novel, simple and practical methods for measuring cow-specific diet digestibility and ES on farms, model for metabolisable energy efficiency (MEE) and partial MEE (pMEE) and assess the genetic backgrounds in all these traits.

The objectives of this research were (article numbers in parentheses):

1. to determine the variability in cow-specific diet digestibility within lactation and across dairy cows (I);
2. to develop an optimal sampling protocol for faecal samples and study the feasibility of NIRS predictions (I, II);
3. to determine the genetic variation in cow-specific digestibility (II);
4. to assess the genetic differences in metabolisable energy efficiency and efficiency in partitioning metabolisable energy in various pathways (III);
5. to develop an indicator for body fat mobilisation using mid-infrared spectrometry of milk samples (IV); and
6. to determine genetic variation in ESI traits and their correlations with fertility (V).

The original articles of the thesis were designed to support each other. Thus, the faecal sampling protocol developed in Study I was used for data collection in Study II. The prediction equations for energy status indicators were developed in Study IV and were utilised in Study V. Study III was carried out as a separate study.

3 MATERIALS AND METHODS

3.1 RESEARCH DATA SETS

3.1.1 DIET DIGESTIBILITY DATA

The data used first for developing the sampling protocol for faecal samples and later for assessing the genetics of cow-specific diet digestibility were collected from research farms between 2012 and 2016 (I, II). In the first trial, composite faecal samples were compiled from ten individual samples collected twice daily (in the morning and evening) during each sampling week at approximately 50, 150 and 250 days in milk (DIM) from the Luke Maaninka research herd between 2012 and 2013. The composite samples from 13 RDC and 31 HOL cows were obtained by aggregating 100 g of faeces from each sampling time over the whole 5-day sampling period. These ten samples formed one composite sample (one from each lactation stage), resulting in three observations for each cow (C10 protocol). In addition, an individual sample set from a subset of 20 cows with daily individual morning and evening faecal samples were retained. These samples were used to estimate the variation within individual samples and the repeatability of digestibility predictions across lactation, which were used to assess an optimal faeces sampling protocol.

In the second trial, during April 2013 and December 2014, the first prototype of the sampling protocol (based on the preliminary results from the first trial) was used on the three Finnish research farms. The protocol involved collecting faecal samples for three consecutive days every morning at approximately 50, 150 and 250 DIM. The collected three samples were combined into one composite sample (protocol C3).

The third trial was carried out during January 2015 and April 2016. Here, the sampling protocol developed in Study I was used. Thus, samples from all cows lactating between 29 and 294 DIM were collected bi-monthly for three consecutive days every morning and combined into one composite sample (protocol B3). During this collection, an individual sample from day one (protocol B1) and a composite sample from both days one and two (protocol B2) were also retained for further analyses. Protocol B3 was followed in Norway, as faecal samples were collected during February and March 2015.

All composite faecal samples collected from the different trials were used for the genetic analyses (II). In Finland, these samples were collected from 153 RDC cows from Luke Jokioinen research farm, 49 RDC and 45 HOL cows from Luke Maaninka research farm, 34 RDC cows from the University of Helsinki research farm in Viikki and from 47 RDC cows from the Norwegian University of Life Sciences research farm in Norway. This data set (FULL)

included a total of 931 records from 328 cows. In addition, a subset of 441 faeces samples from 144 cows in the FULL data set that were collected according to sampling protocol B3 along with samples based on protocols B2 and B1 were analysed separately (BI-MONTHLY data set).

The pedigrees used for the genetic analyses were traced back six generations including 4 034 informative animals for the FULL data set and 2 389 animals for the BI-MONTHLY data set (II).

The digestibility traits were dry matter digestibility (DMD), organic matter digestibility (OMD) and indigestible neutral detergent fibre (iNDF) concentration in faeces. Faecal samples were analysed using NIRS to obtain direct predictions of cow-specific OMD (OMD_f) and $iNDF_f$. In Study I, the samples from the first trial were also assessed by the AIA method (Van Keulen & Young, 1977) to obtain reference measurements for DMD (DMD_{AIA}) and OMD (OMD_{AIA}). The iNDF concentration in the diet ($iNDF_d$) was also predicted by NIRS for silage and diet fed as total mixed ration. The iNDF content in separately fed concentrates was determined based on a 12-d *in situ* incubation in rumen, as described in Ahvenjärvi et al. (2006), or calculated based on an earlier determined average iNDF content of the concentrate ingredients. Thus, it was possible to use iNDF as an internal marker for calculating the DMD (DMD_{iNDF}) using formula:

$$DMD_{iNDF} = 1 - (iNDF_d/iNDF_f).$$

Moreover, $iNDF_f$ was used as an indicator trait for DMD.

After freezing, drying and milling all feed and faecal samples were analysed by NIRS at Valio Ltd. laboratory by scanning duplicate cyvettes for each sample between 400 and 2500 nm in 2-nm increments using a FOSS NIRSystems 6500 spectrometer (Foss Electric A/S, Hillerød, Denmark). Applied NIRS prediction equations for OMD_f and $iNDF_f$ were developed from a reference data including 221 and 236 samples collected in earlier trials, respectively (I). Digestibility was determined from these samples either by total faecal collection or the AIA method.

In Study II, the reference data for developing NIRS calibration equations for $iNDF_f$ were enlarged by including 476 samples collected from earlier trials. The iNDF concentration for these samples was determined using ruminal incubation for 12 days in small pore size nylon bags using samples ground through a 1-mm screen (Krizsan et al., 2015). The statistics of the best NIRS calibration models are shown in Table 1.

Table 1 Statistics of the best NIRS calibration models for organic matter digestibility (OMD), and forage and faecal indigestible neutral detergent fibre (iNDF; g/kg).

	N obs	SEC	SECV	R^2	R^2_{cv}	RPD_{cv}
OMD_{faeces}	221	17.0	17.7	0.69	0.66	1.7
$iNDF_{forage}$	448	13.8	14.9	0.87	0.85	2.6
$iNDF_{faeces}$	236	15.3	16.8	0.85	0.82	2.3
$iNDF_{faeces}$	476	15.3	16.6	0.86	0.83	2.4

SEC = standard error of calibration (g/kg); SECV = standard error of cross-validation (g/kg); R^2 = coefficient of determination; R^2_{cv} = cross-validated coefficient of determination; RPD_{cv} = ratio of performance to deviation of cross-validation

3.1.2 FEED EFFICIENCY DATA

The data used in assessing the genetic differences in metabolisable energy efficiency and efficiency in partitioning metabolisable energy in various pathways were collected from Luke's research farms Rehtijärvi (tiestall) and Minkiö (loose housing) in Jokioinen between 1998 and 2014 (III). Data were from 495 primiparous RDC cows, 291 of which were from different feeding trials and 204 had been measured after starting routine measuring of feed intake since 2009. The analysed data included 12 350 weekly observations from weeks 2 to 40 of lactation. The pedigree used for genetic analyses was traced back four generations from the cows with records and included 2 409 informative animals.

The final data included MEI (MJ/d), ECM production (kg/d, calculated according to Sjaunja (1990), metabolic body weight ($kg^{0.75}$), body weight loss (BWL, kg/d), body weight gain (BWG, kg/d) and REI (MJ of ME/d). Metabolisable energy intake was based on the DMI of the feeds and their energy values. The energy values (MJ of ME/kg of dry matter) of the feeds were calculated according to Luke (2015). The daily MEI was corrected by the total DMI and the concentration of ME and protein in the diet according to the correction equation given by Luke (2015). Residual energy intake was estimated by modelling MEI with multiple linear first-order regression including ECM, $BW^{0.75}$ and piecewise regressions on BWL and BWG. Weekly cow-wise means of residuals from the model were used as cows' REI measures.

3.1.3 ENERGY STATUS INDICATORS AND FERTILITY DATA

The reference data used for developing blood NEFA concentration predictions by milk MIR spectra were from 141 RDC cows in Luke Jokioinen, Luke Maaninka and the University of Helsinki Viikki research farms between

2013 and 2016 (IV). The data included in total 1 585 milk spectral readings and 809 NEFA concentration records measured from blood samples.

MIR spectral readings of the milk samples, based on two MilkoScan FT6000 spectrometers (Foss, Hillerød, Denmark) calibrated and standardised regularly, were provided by Valio Ltd milk laboratory. The MIR spectral data consisted of 1 060 data points, which represent infrared light absorbance through the milk sample in a wavelength area of 925 to 5 011 cm^{-1}. The first derivative of spectral data observations was used and 212 informative spectral points were utilised in the analyses.

For the genetic analyses, the data consisted of 37 424 primiparous RDC cows with test-day records between 8 and 91 DIM (V). Early lactation was divided into three time periods: 8–35, 36–63 and 64–91 DIM, and thus the cows may have one, two or three ESI records in the data set. MIR spectral data have been automatically stored for test-day milk samples from Valio Ltd. herds after May 2015. In June 2018, milk FAs and $NEFA_{MIR}$ were predicted for all milk MIR spectral records collected using the previously developed prediction equations by Soyeurt et al. (2011) and in Study IV, respectively. Blood $NEFA_{FA}$ was predicted according to Mäntysaari et al. (2019) with a model including DIM, milk FPR and milk FAs C10:0, C14:0, C18:1 *cis*-9 and C14*C18:1 *cis*-9. Milk BHB and acetone concentrations were recorded during November 2015 to October 2017 and were predicted with calibration equations from FOSS (based on de Roos et al., 2007). Milk BHB and acetone were $\log_e$-transformed to normalise their distribution for genetic analyses. Interval from calving to first insemination (ICF) was used as the fertility trait because of its susceptibility to negative energy status in early lactation. The pedigree for genetic analyses was traced back four generations from the cows with records and contained 121 542 informative animals.

3.2 METHODS

3.2.1 MODELLING COW-SPECIFIC DIET DIGESTIBILITY

3.2.1.1 Assessment of NIRS prediction reliability

The reliability of NIRS predictions was assessed by comparing the means and standard deviations and by correlations between digestibility traits measured using the standard AIA method and NIRS (I). To correct for environmental effects, the residuals from fitting a linear model were used. The applied model removed environmental variation due to lactation stage and diet. To account for the diet, an interaction between concentrate proportion in the diet (either 22% or 49%) and collection week was modelled. Correlations between repeated observations from various lactation stages were modelled using an unstructured covariance structure for error term.

3.2.1.2 Development of the sampling protocol

Correlations between sample averages and composite samples for DMD_{iNDF} and $iNDF_f$ were explored to assess how many samples are required to gain a representative composite sample (I). The results varied considerably depending on which day or days of the week were used in the analyses. Hence, the analyses were repeated with independent sets of observations and obtained correlations were averaged.

The covariances between individual cow samples were utilised to study the effect of using sample averages, i.e. composite samples, for the repeatability of DMD_{iNDF} and $iNDF_f$ across lactation stages. The applied linear mixed model included the fixed effects of sampling day within the lactation stage, lactation stage (50, 150 or 250 DIM) and an interaction between concentrate proportion in the diet and week of collection. Random effects were animal and animal × lactation stage interaction, with variances σ^2_a and σ^2_{as}, respectively, and the error term with variance σ^2_ε. Each constructed matrix had the form:

$$\begin{bmatrix} (\sigma_a^2 + \sigma_{as}^2)\mathbf{J_5} & \sigma_a^2\mathbf{J_5} & \sigma_a^2\mathbf{J_5} \\ \sigma_a^2\mathbf{J_5} & (\sigma_a^2 + \sigma_{as}^2)\mathbf{J_5} & \sigma_a^2\mathbf{J_5} \\ \sigma_a^2\mathbf{J_5} & \sigma_a^2\mathbf{J_5} & (\sigma_a^2 + \sigma_{as}^2)\mathbf{J_5} \end{bmatrix} + \sigma_\epsilon^2\mathbf{I_{15}},$$

where $\mathbf{J_5}$ is a 5 × 5 matrix of ones and $\mathbf{I_{15}}$ is an 15 × 15 identity matrix. The matrices were used to assess repeatabilities of observations that are based either on a single-day sample or on 2-day, 3-day, 4-day or 5-day averages.

3.2.1.3 Genetic analyses of cow-specific diet digestibility

In Study II, the genetic parameters based on the FULL data set with 328 cows were obtained by fitting a univariate repeatability animal model described as:

$$y_{ijklmno} = B_i + P_j + LS_k + FL_l + HYM_m + b\times iNDF_{d_n} + a_o + pe_o + \epsilon_{ijklmno},$$

where $y_{ijklmno}$ is DMD_{iNDF} or $iNDF_f$ record of cow o, B_i is the fixed effect of breed i (RDC or HOL), P_j is the fixed effect of parity j (primiparous or multiparous), LS_k is the fixed effect of lactation stage k (<100 DIM, 100–199 DIM and >199 DIM), FL_l is the fixed effect of feeding level l (three levels: the basic and two divergent levels), HYM_m is the fixed effect of herd-year-month m (54 classes), b is a regression coefficient, $iNDF_{d_n}$ is the diet-specific iNDF content of diet n, a_o is the random additive genetic effect for animal o [$a \sim N(0, \mathbf{A}\sigma^2_a)$, where $\mathbf{A}$ is the additive genetic relationship matrix among animals and σ^2_a is the additive genetic variance], pe_o is the random permanent environmental effect for animal o [$pe \sim N(0, \mathbf{I}\sigma^2_{pe})$, where $\mathbf{I}$ is an

identity matrix and σ^2_{pe} is the permanent environmental variance] and $\epsilon_{ijklmno}$ is the random error term. The HYM classes were designed to describe the contemporary groups of animals fed the same diet. However, some variation occurred between iNDF content in diet within the same HYM class of observations collected under C10 and C3 protocols and thus iNDF was included in the model as a covariate. Four heterogeneous residual variance classes were fitted in the model due to different sampling protocols.

The model used for the BI-MONTHLY data set with 144 cows was otherwise the same as for the FULL data set, but the fixed effects of feeding level and various residual variances were omitted. In addition to the univariate models, a bivariate model was fitted for DMD_{iNDF} and $iNDF_f$ on the B3 data set to estimate the genetic correlation between the traits.

3.2.2 MODELLING METABOLISABLE ENERGY EFFICIENCY

Several models were investigated to assess the genetic differences in metabolisable energy efficiency and efficiency in partitioning metabolisable energy in various pathways (III). However, the reference trait of REI was first explored, because it is currently the most commonly used FE trait in the literature.

The repeatability animal model for REI was:

$$REI_{ijl}=rym_i+lw_j+a_l+pe_l+\epsilon_{ijl},$$

where REI_{ijl} = residual energy intake (ME MJ/d), rym_i = fixed effect of recording year-month i, lw_j = fixed effect of lactation week j, a_l = random additive genetic effect for animal l [$a \sim N(0, \mathbf{A}\sigma^2_a)$, where $\mathbf{A}$ is the additive genetic relationship matrix among animals and σ^2_a is the additive genetic variance], pe_l = random permanent environmental effect for animal l [$pe \sim N(0, \mathbf{I}\sigma^2_{pe})$, where $\mathbf{I}$ is an identity matrix and σ^2_{pe} is the permanent environmental variance], and ϵ_{ijl} = a random error term with variance σ^2_ε.

In the studied data, lactation class averages for REI deviated from the expectation of zero, reflecting that on average the calculated energy requirements and actual MEI differed among various stages of lactation. Likewise, regressions on energy sinks were hypothesised to differ across lactation, and if so, these regressions should be nested within lactation stages to avoid systematic bias in estimates for additive genetic effects of animals with lactation in progress. Therefore, a least square model was fitted for MEI to assess whether regression coefficients vary markedly during lactation.

The least square model for MEI was:

$$MEI_{ijls}=rym_i+lw_j+b_{1s}BW_{jl}^{0.75}+b_{2s}ECM_{jl}+b_{3s}BWG_{jl}+b_{4s}BWL_{jl}+\epsilon_{ijls},$$

where MEI_{ijls} = metabolisable energy intake (MJ/d), rym_i = fixed effect of recording year×month i, lw_j = fixed effect of lactation week j, b_{cs} = fixed regression coefficient b_c nested within lactation class s, where c = 1, 2, 3, 4 are for maintenance, milk production, BWG and BWL, respectively, and where lactation classes s are: 2–5, 6–10, 11–15, 16–20, 21–25, 26–30, 31–35 and 36–40 weeks of lactation, $BW_{jl}^{0.75}$ = metabolic body weight (kg), ECM_{jl} = energy corrected milk (kg/d) and BWG_{jl} = body weight gain (kg/d) of cow l in lactation week j, BWL_{jl} = body weight loss due to mobilisation of tissue energy for milk production (kg/d) for cow l in lactation week j, ϵ_{ijls} = a random error term with variance σ^2_ε.

The model for MEE included regressions on energy sinks as fixed effects. Otherwise the applied repeatability model included the same effect as the model for REI.

Repeatability animal model for MEE was as follows:

$$MEI_{ijls}=rym_i+lw_j+b_{1s}BW_{jl}^{0.75}+b_{2s}ECM_{jl}+b_{3s}BWG_{jl}+b_{4s}BWL_{jl}+a_l+pe_l+\epsilon_{ijls},$$

where MEI_{ijls} = metabolisable energy intake (MJ/d), rym_i = fixed effect of recording year×month i, lw_j = fixed effect of lactation week j, b_{cs} = fixed regression coefficient b_c nested within lactation class s, where c = 1, 2, 3, 4 is a regression coefficient for maintenance, milk production, BWG and BWL, respectively, and with eight lactation classes s (eight classes: 2–5, 6–10, 11–15, 16–20, 21–25, 26–30, 31–35, 36–40 weeks of lactation), $BW_{jl}^{0.75}$= metabolic body weight (kg), ECM_{jl} = energy corrected milk (kg/d), BWG_{jl} = body weight gain (kg/d) of cow l in lactation week j, BWL_{jl} = body weight loss due to mobilisation of tissue energy for milk production (kg/d) for cow l in lactation week j, a_l = random additive genetic effect for animal l [$a \sim N(0, \mathbf{A}\sigma^2_a)$, where **A** is the additive genetic relationship matrix among animals and σ^2_a is the additive genetic variance], pe_l = random permanent environmental effect for animal l [$pe \sim N(0, \mathbf{I}\sigma^2_{pe})$, where **I** is an identity matrix and σ^2_{pe} is the permanent environmental variance], and ϵ_{ijls} = a random error term with variance σ^2_ε.

Applying random regression models would allow the estimation of cow-specific energy sink efficiencies. Therefore, the model for MEE was extended with regressions on energy sinks nested within additive and permanent environmental effects.

The random regression model for pMEE was:

$$MEI_{ijls}=rym_i+lw_j+b_{1s}BW_{jl}^{0.75}+b_{2s}ECM_{jl}+b_{3s}BWG_{jl}+b_{4s}BWL_{jl}+a_{ol}+a_{1l}B\dot{W}_{jl}^{0.75}+a_{2l}E\dot{C}M_{jl}+a_{3l}B\dot{W}G_{jl}+a_{4l}BWL_{jl}+pe_{ol}+pe_{1l}B\dot{W}_{jl}^{0.75}+pe_{2l}E\dot{C}M_{jl}+pe_{3l}B\dot{W}G_{jl}+pe_{4l}BWL_{jl}+\epsilon_{ijls},$$

where MEI_{ijls} = metabolisable energy intake (MJ/d), rym_i = fixed effect of recording year×month i, lw_j = fixed effect of lactation week j, b_{cs} = fixed regression coefficient b_c nested within lactation class s, where c = 1, 2, 3, 4 is a regression coefficient for maintenance, milk production, BWG and BWL, respectively, and with eight lactation classes s (eight classes: 2–5, 6–10, 11–15, 16–20, 21–25, 26–30, 31–35, 36–40 weeks of lactation), a_{cl} = random additive genetic regression coefficient c = 0 (intercept), 1 (maintenance), 2 (milk production), 3 (body weight gain), 4 (body weight loss) for animal l, pe_{cl} = random permanent environmental regression coefficient c = 0 (intercept), 1 (maintenance), 2 (milk production), 3 (body weight gain), 4 (body weight loss) for animal l, $BW_{jl}^{0.75}$ = metabolic body weight (kg), ECM_{jl} = energy corrected milk (kg/d), BWG_{jl} = body weight gain (kg/d) of cow l in lactation week j, BWL_{jl} = body weight loss due to mobilisation of tissue energy for milk production (kg/d) for cow l in lactation week j, $B\dot{W}_{jl}^{0.75}$, $E\dot{C}M_{jl}$ and $B\dot{W}G_{jl}$ are the covariables standardised as $\dot{x}=\frac{x-\mu_x}{\sigma_x}$, where x is the original covariable, and μ_x and σ_x are the mean and standard deviation of covariable x, respectively, and ϵ_{ijls} = a random error term with variance σ^2_ε. The covariance matrices were var(**a**)=$\mathbf{G}_{5x5}\otimes\mathbf{A}$ and var(**pe**)=$\mathbf{P}_{5x5}\otimes\mathbf{I}$, where **G** was the covariance matrix for the random additive genetic effects, **A** was the additive genetic relationship matrix, **P** was the covariance matrix for the random permanent environmental effects and **I** was an identity matrix.

As the model is complex, simpler random regression models were also explored. These two sub-models differed in the number of random regression effects included in the permanent environment and additive genetic animal effects. The alternative models included the following random regressions: intercept and ECM (pMEE1); intercept, $BW^{0.75}$, ECM and BWG (pMEE2); and intercept, $BW^{0.75}$, ECM, BWG and BWL (pMEE3). Hence, model pMEE3 was equal to the model described above.

3.2.3 MODELLING ENERGY STATUS

3.2.3.1 Development of a prediction model for NEFA

Partial least squares regression was used to predict blood plasma NEFA concentration and energy balance from milk MIR spectral data (IV). Two models were tested, either using only MIR spectra or using MIR spectra and milk yield as predictor variables. The models were validated with leave-one-out cross-validation and by randomly leaving out 20% of the cows for cross-validation (five random replicates).

Mid-infrared reflectance spectroscopy spectra from morning and evening milks were studied separately to examine whether blood plasma NEFA concentration observed in the morning could be more accurately predicted using milk samples collected the same day at morning or evening milking.

The robustness of the developed prediction equation for blood plasma NEFA concentration was studied by calibrating the prediction equations with records (evening MIR spectral readings) from cows in two herds and then predicting NEFA for cows in the third herd.

3.2.3.2 Genetic analyses of energy status indicator traits and fertility

Multivariate linear animal models were applied to estimate the genetic variation in ESI traits and their correlation with fertility in early lactation. In addition, univariate analyses were performed for each of three lactation periods. Observations from the three lactation periods (defined as: 8 to 35, 36 to 63, and 64 to 91 DIM) were considered to be observations from three different traits. In the matrix notation, the model can be written as:

$$\mathbf{y} = \mathbf{X\beta} + \mathbf{Za} + \mathbf{e},$$

where $\mathbf{y}$ is a vector of observations, $\boldsymbol{\beta}$ is a vector of the fixed effects of a herd, year×month of the test-day for energy status indicator traits and year×month of calving for ICF, age at calving and regression on DIM for energy status indicator traits; $\mathbf{a}$ is a vector of random animal additive effects; $\mathbf{e}$ is a vector of random residuals; and $\mathbf{X}$ and $\mathbf{Z}$ are the corresponding design matrices. There were in total 962 herds, 38 year×month classes for test-days (from May 2015 to June 2018), 40 year×month classes for calvings, and nine age at calving classes, in which < 22 and > 30 were the first and last classes, respectively, and other classes were single months. Random effects were assumed to be normally distributed with means equal to zero and the covariance matrix for $\mathbf{a}$, var($\mathbf{a}$)=$\mathbf{G_o}\otimes\mathbf{A}$, where $\mathbf{G_o}$ was the covariance matrix for the random additive genetic effects and $\mathbf{A}$ was the additive genetic relationship matrix, and the covariance matrix for $\mathbf{e}$, var($\mathbf{e}$)=$\mathbf{R_o}\otimes\mathbf{I}$, where

$\mathbf{R_o}$ was the covariance matrix for the random residuals and **I** was an identity matrix.

Genetic analyses were performed first within each ESI separately by applying multi-trait models for all three time periods to study the correlations between these periods. Secondly, the correlations between different ESIs and ICF were studied by applying multi-trait models within each time period. Heritabilities were estimated also by applying single-trait models.

3.2.4 SOFTWARES

The statistical analyses in Study I were performed using the MIXED - procedure in SAS software version 9.3 (SAS Institute Inc., Cary, NC, USA). In Study II, the variance components were estimated by restricted maximum likelihood (REML) applying the expectation maximisation (EM-REML) method, as implemented in MiX99 software (Vuori et al., 2006).

In Study III, variance components were estimated by REML applying either average information (AI-REML) or EM-REML methods for the most complex model, as implemented in the DMU software package (Madsen & Jensen, 2013). Various sets of heritabilities can be derived for the random regression models and in this case the overall heritability was calculated by summing the (co)variances of $\hat{\mathbf{G}}$ and $\hat{\mathbf{P}}$ with respect to the energy sinks (explained in detail in Study III).

Prediction equations in Study IV were developed using the PROC PLS - procedure in SAS software version 9.3 (SAS Institute Inc., Cary, NC). In Study V, the variance components were estimated by applying the AI-REML method, as implemented in the DMU package (Madsen & Jensen, 2013). Standard errors for heritability estimates in Studies II and V were approximated using Taylor series expansions. The pedigree consistency checking and removal of non-informative animals was performed using the RelaX2 programme (Strandén & Vuori, 2006) in Studies II, III and V.

4 MAIN RESULTS

4.1 COW-SPECIFIC DIET DIGESTIBILITY

The estimated standard deviation for cow-specific dry matter digestibility analysed by AIA was 12.4 g/kg, which is small considering that the average was 723 g/kg (I). Digestibility was lower at the early stage of lactation and increased towards the end of lactation (from 710 to 729 g/kg in RDC cows). Correlations between mid and late lactation were higher than correlations between early and later lactation stages.

4.1.1 DIGESTIBILITY PREDICTIONS BASED ON NIRS

The raw data correlation found between DMD_{AIA} and DMD_{iNDF} was not higher than 0.70, and the correlation was only moderate (0.45) after correcting for environmental effects (I). This may be explained by the relatively large standard errors of the measurements compared to the magnitude of the phenotypic standard deviation. The raw data correlation between DMD_{iNDF} and $iNDF_f$ was quite low (0.25), but the correlation became very high (0.97) after correcting for environmental effects.

When studying differences between digestibility traits measured either by the standard AIA method or by NIRS, only a moderate correlation was found between OMD_{AIA} and OMD_f (0.51), indicating that the prediction of organic matter digestibility directly from faecal samples was not accurate (I). The OMD_f trait was dropped out of the further analyses due to the low R^2cv and only a moderate correlation between OMD_{AIA} and OMD_f.

4.1.2 DEVELOPMENT OF SAMPLING PROTOCOL

The differences in digestibility recordings between morning and evening samples were practically negligible, indicating that the timing of sampling is not very important and may be chosen based on the most convenient practical arrangements (I). Correlations between individual samples and composite samples became stronger when more individual samples were used for estimating the weekly means. However, when more than three samples were compiled to the weekly means, the correlation to the composite sample increased only slightly. Based on the repeatability calculations, similar results were achieved and an additional increase in repeatability was only moderate when using more than three individual samples for building a composite sample.

Based on these findings, a protocol was proposed with composite samples compiled from three individual samples collected either from the mid-lactation period or even more preferably concurrently from all the cows. For genetic analyses, the most suitable sampling design would be to sample cows several times during each lactation and also so that all lactating contemporaries would be sampled concurrently. Thus, the contemporary groups of cows consuming the same feed could be modelled. This protocol was applied in the research farms from 2015 onwards.

4.1.3 GENETIC PARAMETERS FOR DIET DIGESTIBILITY

A relatively high repeatability estimates found for $iNFD_f$ (0.46) indicated that $iNDF_f$ may have potential to be used as an indicator trait for the cow's ability to efficiently digest feed (I). Moreover, the CV of $iNDF_f$ (2.78 %) was larger compared to all other digestibility traits, indicating that future studies on genetic variability in digestibility should concentrate on $iNDF_f$ predictions by NIRS (I).

The estimated genetic standard deviations were 10.5 g/kg and 6.2 g/kg dry matter (DM) for DMD_{iNDF} and $iNDF_f$, respectively, and the coefficient of genetic variation for DMD_{iNDF} was 1.7% when analysing the FULL data set (II). These results were consistent with the results from the BI-MONTHLY data set. Estimated variance components showed heterogeneity for DMD depending on the applied sampling protocol in the FULL data set. Therefore, the heritability estimates varied between sampling protocols and ranged from 0.14 ± 0.06 to 0.51 ± 0.24 for DMD_{iNDF} and from 0.13 ± 0.05 to 0.48 ± 0.18 for $iNDF_f$ (Table 2).

The heritability estimates were generally higher using the B3 protocol compared to the C3 (II). This emphasises the importance of sizeable contemporary groups, as all the cows in the herd were sampled simultaneously every second month when the B3 protocol was applied. Based on the repeatability and heritability estimates, the prediction of diet digestibility was more accurate when using three faecal samples combined into one composite sample, compared to a composite of two samples or just one individual sample (I, II).

Table 2 Variance components and heritability estimates (h^2) with standard errors (se) for dry matter digestibility (DMD_{iNDF}) and iNDF concentration in faeces ($iNDF_f$) using the FULL and BI-MONTHLY data sets with different sampling protocols

	σ^2_{pe} ± se	σ^2_a ± se	σ^2_e ± se	h^2 ± se
DMD_{iNDF}				
$C10_{FULL}$	3.15 ± 50.76	110.67 ± 58.97	102.55 ± 22.98	0.51 ± 0.24*
NOR_{FULL}	3.15 ± 50.76	110.67 ± 58.97	609.24 ± 102.05	0.15 ± 0.07*
$C3_{FULL}$	3.15 ± 50.76	110.67 ± 58.97	687.05 ± 73.36	0.14 ± 0.06*
$B3_{FULL}$	3.15 ± 50.76	110.67 ± 58.97	281.44 ± 22.36	0.28 ± 0.13*
$B3_{BI\text{-}MONTHLY}$	69.0 ± 83.1	76.9 ± 93.3	267.7 ± 23.6	0.19 ± 0.22^{NS}
$B2_{BI\text{-}MONTHLY}$	86.9 ± 68.6	20.2 ± 55.1	335.6 ± 29.0	0.05 ± 0.12^{NS}
$B1_{BI\text{-}MONTHLY}$	91.9 ± 79.4	18.6 ± 81.6	534.2 ± 41.1	0.03 ± 0.13^{NS}
$iNDF_f$				
$C10_{FULL}$	1.11 ± 14.20	38.90 ± 16.89	40.35 ± 7.32	0.48 ± 0.18**
NOR_{FULL}	1.11 ± 14.20	38.90 ± 16.89	253.66 ± 45.61	0.13 ± 0.05**
$C3_{FULL}$	1.11 ± 14.20	38.90 ± 16.89	242.16 ± 24.64	0.14 ± 0.05**
$B3_{FULL}$	1.11 ± 14.20	38.90 ± 16.89	75.05 ± 5.32	0.34 ± 0.12**
$B3_{BI\text{-}MONTHLY}$	13.9 ± 39.9	34.3 ± 42.5	71.8 ± 5.7	0.29 ± 0.35^{NS}
$B2_{BI\text{-}MONTHLY}$	35.4 ± 24.5	5.9 ± 24.5	128.5 ± 9.7	0.03 ± 0.14^{NS}
$B1_{BI\text{-}MONTHLY}$	27.4 ± 29.2	5.8 ± 26.8	145.4 ± 14.4	0.03 ± 0.15^{NS}

σ^2_{pe} = permanent environmental variance, σ^2_a = additive genetic variance, σ^2_e = residual variance

** $p < 0.01$, * $p < 0.05$, NS = non-significant

4.2 GENETIC BACKGROUND IN PARTITIONING OF METABOLISABLE ENERGY EFFICIENCY

4.2.1 METABOLISABLE ENERGY EFFICIENCY

Modelling MEE required including regression coefficients for energy sinks into the model (III). The results showed that estimates for these regression coefficients varied across lactation stages. The overall estimate for ME used for maintenance was 0.81 MJ x $BW^{0.75}$ (kg), and the estimates decreased from 1.09 to 0.48 MJ as lactation progressed. The overall estimate for milk production was 2.67 MJ x ECM (kg/d) and estimates increased from 1.27 to 3.70 MJ as lactation progressed. When fitting a repeatability animal model, heritability estimates of 0.26 and 0.33 were found for MEE and REI, respectively (Table 3).

4.2.2 PARTITIONING METABOLISABLE ENERGY EFFICIENCY

Several alternative random regression models were tested when partitioning MEE. All models, except the model with intercept and random regression on ECM (pMEE1), converged poorly in the iterative REML variance component estimation (III). Table 3 shows that the derived overall heritability estimates for random regression models were in line with the estimate for MEE. Most of the variance was explained by the intercept, for which heritability estimates ranged from 0.13 to 0.23 depending on the applied model. The heritability estimates for pMEE for milk production varied between 0.04 and 0.06. The heritability estimates for pMEE with respect to maintenance and growth were 0.02 and 0.04, respectively. Based on pMEE1, the genetic standard deviation estimate for the intercept was 10.8 MJ of MEI/d and the genetic standard deviation estimate for the regression coefficient for milk production was 0.75 MJ of MEI/kg of ECM. The genetic standard deviation estimates for regressions on maintenance and growth were 0.47 MJ of MEI/kg of $BW^{0.75}$ and 18.0 MJ of MEI/kg, respectively, but associated standard errors were large.

Table 3 Heritability estimates (h^2) and partial heritability estimates (η^2) for random regression effects of energy sink specific efficiencies

Model	Additive genetic animal effect	η^2_{int}	η^2_{mBW}	η^2_{ECM}	η^2_{BWG}	η^2_{BWL}	h^2
REI	Intercept						0.33
MEE	Intercept						0.26
pMEE1	Intercept, ECM	0.23		0.04			0.23
pMEE2	Intercept, mBW, ECM, BWG	0.14	0.02	0.06	0.04		0.22
pMEE3	Intercept, mBW, ECM, BWG, BWL	0.13	0.02	0.05	0.04	0.00	0.21

REI = residual energy intake; MEE = metabolisable energy efficiency; pMEE = partial metabolisable energy efficiency; ECM = energy-corrected milk; mBW = metabolic body weight; BWG = body weight gain; BWL = body weight loss; int = intercept

The genetic correlations were 0.44, 0.54 and -0.01 between energy efficiency in milk production and maintenance, energy efficiency in milk production and growth, and energy efficiency in maintenance and growth, respectively. However, the standard errors for genetic correlations were high, indicating uncertainty associated with the genetic covariance estimates.

Phenotypes of the high and low genetic merit cows were compared to assess the response of selection if using EBV's for partial energy efficiencies. For example, comparing cows based on the EBV for the intercept showed that the best 25% of the cows had 11% lower MEI intake, produced 3.7% less milk, had 1.8% lower $BW^{0.75}$ and 17.4 MJ lower REI compared with the average cows.

4.3 ENERGY STATUS

4.3.1 NEFA PREDICTIONS BASED ON MILK MIR SPECTRA

The highest accuracy (R^2cv=0.64, RMSE=0.18 mmol/l) was achieved using milk MIR spectral points as predictors with leave-one-out cross-validation for NEFA (IV, Table 4). Including milk yield as a predictor in the model in addition to milk spectra failed to improve the accuracy when predicting NEFA but improved the accuracy when predicting the calculated energy balance. However, the correlation between observed NEFA and energy balance predicted with MIR spectra was -0.65, while the correlation between observed NEFA and energy balance predicted with MIR spectra and milk yield was only -0.56. Cross-validation results from randomly leaving out 20% of the cows were slightly lower (e.g. NEFA R^2cv = 0.58) than from leave-one-out cross-validation.

Table 4 Statistics[1] from partial least squares regression for NEFA and energy balance using milk MIR spectra (a.m. and p.m.) or MIR spectra and milk yield (my) as prediction variables in the model, validated by leave-one-out or leave 20% of cows out cross-validation

		NEFA			Energy balance		
		N fac	R^2cv	RMSE (mmol/l)	N fac	R^2cv	RMSE (MJ/d)
Leave-one-out	Spectra	20	0.64	0.18	18	0.46	25.82
	Spectra+my	20	0.59	0.18	16	0.64	21.24
Leave 20% of cows out	Spectra	9	0.58	0.19	11	0.45	25.94
	Spectra+my	10	0.56	0.19	12	0.62	21.22

[1] N fac = No of factors used (restricted to 20), R^2cv = coefficient of determination of cross-validation, RMSE = root mean square error

Predictions based on evening milk samples yielded higher coefficient of determination (R^2cv=0.67) than predictions based on morning samples (R^2cv=0.59). When calibrating the equations with leave-one-out cross-validation with evening MIR spectral readings and NEFA records from two herds and then predicting NEFA for cows in the third herd, a R^2 of 0.58 and a RMSE of 0.19 mmol/l were found.

4.3.2 GENETIC VARIATION IN ENERGY STATUS INDICATORS AND CORRELATION WITH FERTILITY

For all ESI traits both genetic and residual variances decreased during lactation (V). Overall, the heritability estimates were highest in the first time period and lowest in the second period for all ESI traits except for FPR, but the differences across time periods were small (Table 5). The estimated

genetic correlations were highest between time periods 36–63 and 64–91 DIM in all ESI traits and varied from 0.89 to 0.99 (V).

Table 5 Heritability estimates (standard errors in parentheses) for energy status indicator (ESI) traits in three time periods of early lactation from single-trait analyses

ESI trait	8–35 DIM	36–63 DIM	64–91 DIM
$NEFA_{MIR}$	0.17 (0.02)	0.13 (0.02)	0.16 (0.02)
$NEFA_{FA}$	0.17 (0.02)	0.10 (0.01)	0.12 (0.02)
C18:1 *cis*-9	0.14 (0.02)	0.09 (0.01)	0.10 (0.01)
Fat:protein	0.08 (0.01)	0.07 (0.01)	0.10 (0.02)
BHB	0.17 (0.03)	0.13 (0.02)	0.14 (0.02)
Acetone	0.18 (0.03)	0.10 (0.01)	0.15 (0.02)

The heritability estimate for ICF was 0.03 (0.01). Genetic correlations between ESI traits and ICF were moderate (0.18 to 0.40) with all ESI traits in the first time period (8–35 DIM), generally lower (0.03 to 0.43) in the second period (36–63 DIM) for all ESI traits except $NEFA_{MIR}$ and decreased clearly (-0.02 to 0.19) in the third period (64–91 DIM) (Table 6). Genetic correlations between ESI traits were also highest in the earliest studied lactation stage and decreased as lactation progressed.

Table 6 Genetic correlations (standard errors in parentheses) between energy status indicator (ESI) traits in three time periods of early lactation and fertility trait interval from calving to first insemination

ESI trait	8–35 DIM	36–63 DIM	64–91 DIM
$NEFA_{MIR}$	0.39 (0.11)	0.43 (0.11)	0.19 (0.12)
$NEFA_{FA}$	0.40 (0.11)	0.28 (0.13)	0.12 (0.13)
C18:1 *cis*-9	0.36 (0.12)	0.17 (0.13)	-0.02 (0.14)
FPR	0.18 (0.14)	0.03 (0.14)	0.01 (0.14)
BHB	0.38 (0.12)	0.29 (0.12)	0.18 (0.13)
Acetone	0.33 (0.12)	0.16 (0.13)	0.13 (0.13)

5 DISCUSSION

5.1 RESEARCH DATA AND METHODS

The designs of experimental studies for diet digestibility (I, II) and for developing prediction equations for milk MIR spectra-based blood NEFA concentration (IV) were unique and the data were especially collected for those studies. The faecal sampling protocol improved on the way, as more results were available from the preliminary studies and thus three different sampling protocols were applied. Collecting faecal samples is expensive and laborious, and therefore most of the limited studies so far have used relatively small data sets for the genetic analyses (Berry et al., 2007, II). The data used for genetic analyses were collected from three research herds in Finland and one research herd in Norway to generate a reasonable data size. Thus, different sampling protocols necessitated the fitting of heterogeneous residual variances. Furthermore, to increase the size of contemporary cow groups consuming the same diet and thereby the statistical power, data were collected from all contemporaries in a herd. Consequently, observations came from both RDC and HOL cows, which was accounted for by including the fixed effect of breed in to the statistical model.

Feed intake, body weight and milk production data have been systematically collected at Luke's experimental farms in Jokioinen since 1998. This is a unique data set that was utilisable for modelling MEE and pMEE (III). The data included 495 cows, which is still a quite limited data size for very complex genetic analyses. Partial energy efficiency breeding values for milk production and growth were reasonable when fitting the random regression models, but when modelling partial energy efficiency for maintenance, the estimate for genetic variance was unexpectedly high with a high standard error. One possible reason could be the size and structure of the data used. Cows in the data were high genetic merit cows of the RDC breeding nucleus, and they have above average FE levels compared to the whole RDC population. Therefore, the variability in the efficiency component traits and MEI between cows may be small.

Blood and milk samples were collected from 141 cows at three research farms in Finland for the development of prediction equations (IV). The data collection for this study was carefully designed to account for variability at various stages of early lactation and for the diurnal variation in NEFA concentration in the blood as well as in the FA profiles in the milk samples. Partial least squares (PLS) regression is particularly useful and a powerful statistical tool to be applied when dependent variables are predicted from a large set of predictors such as when using spectral data. Prediction of PLS regression is based on extracting a set of orthogonal factors called latent variables, which have the best predictive power, from the predictors (Abdi,

2010). In Study IV, the maximum number of latent variables was restricted to 20 to avoid overfitting of the data. This maximum was determined by visually inspecting the changes in R^2 of the cross-validation and external validation associated with increasing the number of latent variables, because the R^2 of cross-validation tends to improve at the expense of the R^2 of external validation (McParland et al., 2012). Cross-validation results from leave-one-out were slightly higher than from randomly leaving out 20% of the cows, probably because the observations used for leave-one-out validation were not independent. However, when first calibrating the equations with leave-one-out cross-validation in two herds and then predicting the observations in the third herd, a R^2 of 0.58 was still observed in the external data set.

The largest data used in this thesis comprised the data used for genetic analyses of ESI traits and fertility (V). Four data sources were used to build the data set for the variance component estimation: MIR spectral data, milk recording test-day data, BHB and acetone data and fertility data. The data were carefully edited and contemporary groups were considered for the genetic analyses of the first three lactation months.

For the genetic analyses, the pedigrees were traced back either six generations (II) or four generations (III, V). In the digestibility study, the data were collected from four herds and two breeds, and therefore increasing the average relationships between cows in different herds involved including more generations to the pedigree (II). In the genetic analyses of MEE and pMEE, all the cows in the data were from the RDC breeding nucleus herd and well-related on both the paternal and maternal sides of their pedigrees. Four generations from the animals were therefore traced back for the pedigree (III). In the genetic analyses of ESI traits and fertility, four generations of animals traced back from the cows with records formed one population and were included in the pedigree (V).

5.2 GENETIC BACKGROUND OF NOVEL FEED EFFICIENCY AND ENERGY STATUS TRAITS

The FE of a cow is complex and affected by many factors. Cows use energy in a cascade of physiological processes for digestion, metabolism, growth and for milk synthesise. All these phenomena experience large variability over time. Selecting for FE in dairy cattle has to account for all this complexity.

This thesis addressed several topics related to FE. One of the novel FE traits studied was cow-specific diet digestibility. Diet digestibility contributes to the overall FE of a cow, and defines how much of the energy intake the cow is able to use for metabolic functions. Results from Studies I and II indicated that NIRS can be used to predict cow-specific digestibility from faecal samples. Indigestible neutral detergent fibre content in the faeces alone was also demonstrated to be utilisable as an indicator for digestibility if the cows

in the same contemporary group consume the same diet. Thus, improving diet digestibility would not require collecting and analysing feed samples. The heritability estimates found were in line with the only estimates reported for dairy cows in the literature (Berry et al., 2007, II). The coefficients of genetic variation were 1.7% and 2.9% for DMD and $iNDF_f$, respectively (II). These are lower than coefficients for production traits in dairy cows (6%, Berry et al., 2003), but showed that selection could be beneficial. Every unit improvement in diet digestibility corresponds to the same amount of savings in feed requirements and thus may become of significant interest in the future. However, analysis of benefits over costs has to be positive, and thus the sampling and processing pipeline for faecal samples should be optimized to provide digestibility records at acceptable costs.

Cow-specific digestibility defines how much energy a cow can have at its disposal, but cows also differ in how efficiently they use ME on a whole or for specific energy pathways. Residual energy intake is one of the most studied overall FE traits. In Study III, a slightly higher heritability estimate of 0.33 for REI was found compared to earlier studies on dairy cows (0.06 to 0.29; Liinamo et al., 2015; Tempelman et al., 2015; Hurley et al., 2018). Modelling MEE requires the inclusion of regression coefficients for energy sinks into a model, and the results showed that the regression coefficient estimates varied across lactation. This together with a slightly higher repeatability for MEE suggests that fitting regressions for energy sinks simultaneously with all other model effects (model MEE) gives better prediction power compared with the model for REI, as even the estimated heritability was lower (0.26) for MEE. These findings are in line with Tempelman et al. (2015) and Li et al. (2017), who also observed heterogeneity in partial regression coefficients when modelling RFI in dairy cows. However, the estimates for the regression coefficients for milk production and maintenance were lower and higher, respectively, than the values from the official Finnish feeding recommendations (III). This is probably due to co-linearity between covariables in the data and therefore the estimation of true biological efficiency coefficients may be better accomplished in designed experiments and with respiration chambers. Moreover, modelling energy use in early lactation is challenging because for example a drop in body weight due to the negative status may not necessarily cause an equivalent drop in energy requirements for maintenance. The model for MEE can be expanded to include random regressions on various energy pathways to model genetic variation in efficiency with respect to specific energy pathways. Including partial regression on ECM (model pMEE1) or partial regressions on $BW^{0.75}$, ECM and BWG (pMEE2) was feasible and the derived partial heritabilities indicated that some genetic variation may exist in the efficiency of using ME for various pathways.

Modern high-yielding dairy cows often undergo a negative energy status during the early postpartum period due to a rapid increase in milk production and the high energy demand that cannot be fulfilled by energy

intake. As earlier studies have shown that FE traits were unfavourably correlated with negative energy balance (Berry & Crowley, 2013; Hurley et al., 2018; Liinamo et al., 2015; Vallimont et al., 2011), considering early lactation ES in the breeding programmes is important.

Several metabolites can be used as biomarkers for negative ES. These biomarkers are related to the consequent changes in hormone and blood metabolite concentrations due to the mobilisation of body fat. Energy status-related blood metabolites and hormones, e.g. NEFA, BHB, acetone, glucose and IGF-1, are predictable from milk MIR spectra (Belay et al., 2017; Grelet et al., 2019). In Study IV, calibration equations were developed to predict blood NEFA concentration from milk MIR spectra. Results indicated that blood NEFA concentration may be more accurately predicted from milk MIR spectra than the calculated energy balance. In addition, the prediction equations for blood NEFA concentration may be further developed as the calibration data set will be increased.

Results from Study V showed that all studied ESI traits were heritable during early lactation. Estimates for blood plasma NEFA concentration heritability have ranged from 0.08 to 0.35, and Oikonomou et al. (2008a) have reported particularly high genetic variance during the first weeks of lactation. These results are in line with the genetic variability found in Study V. The heritability estimates for C18:1 *cis*-9, BHB and acetone were also in line with the results from earlier studies (Bastin et al., 2011; Koeck et al., 2014; Lee et al., 2016; van der Drift et al., 2012). Heritability estimates found for FPR were slightly lower than in the literature (Negussie et al., 2013; Koeck et al., 2014).

The hypothesis was that ICF is prolonged by negative energy status, and thus this fertility trait was used as a reference trait to validate ESI traits. In general, genetic correlations between ESI traits and fertility described by days from calving to first insemination were moderate in the first time period (8–35 DIM) and decreased as lactation progressed (V). These correlations are in line with the literature. For example, Oikonomou et al. (2008b) reported unfavourable genetic correlations between blood NEFA and several fertility traits ranging from -0.17 (between blood NEFA and first-lactation first-service conception rate) to 0.42 (between blood NEFA and presence of metritis).

All studied indicators proved to be promising candidates for evaluating the energy status of a cow for breeding purposes. The newly developed $NEFA_{MIR}$ and $NEFA_{FA}$ were on the same level with C18:1 *cis*-9, BHB and acetone on heritability and genetic correlation with ICF, especially during the first month of lactation.

5.3 IMPLICATIONS AND FUTURE DEVELOPMENTS

Feed efficiency was included in the dairy cattle breeding programmes in Nordic countries in 2019. At first implementation stage in 2019, the Saved Feed index included only maintenance, which is based on the metabolic body weight of dairy cows. Metabolic efficiency (based on RFI) will be added to the Saved Feed index in the next step. Measuring feed intake on a larger scale may become possible in the future, as soon as new measurement technologies (e.g. 3D cameras or feed intake sensors) will become available. In addition, the utilisation of genomic selection is essential when feed intake measurements are sparse. Efforts are currently made to develop genomic predictions for RFI. However, the models developed for MEE and pMEE may be the future models once enough feed intake and body weight records are available (III). These models allow defining the differences between cows in how efficiently they use ME on a whole or for the specific energy pathways.

Cow-specific digestibility describes how much energy will be available and therefore contributes to the FE of a cow. Near-infrared reflectance spectroscopy is a promising tool to be used for predicting cow-specific digestibility from faecal samples (I, II). The coefficients of genetic variation were low, but indicated that selection may be beneficial (II). Nonetheless, every unit improvement in diet digestibility corresponds to proportionally the same amount of savings in feed requirements and thus may become of significant interest in the future.

Considering ES in the breeding programmes will become important once the selection for FE is implemented. The developed ESI traits can be utilised for this purpose (IV, V). Thus, it will be possible to select against severe negative ES, while increasing efficiency and enhancing health and fertility.

The sustainability of food production along with ensuring the food supply for growing global demand will be even more important in the future, and more emphasis will be directed to intensive and efficient food production. Ruminants are able to provide humans with milk and meat from nonhuman-edible plant materials, but with an environmental cost via the ruminal microbiome, i.e. by producing methane. The potential for reducing enteric methane production per kg of energy-corrected milk has been estimated at 15% to 30% if combining possible genetic (including breeding for FE), nutrition and management approaches (Knapp et al., 2014). Direct genetic selection for methane emissions has so far been prevented by the lack of accurate recording techniques utilisable at a large scale (Negussie et al., 2017).

Feed efficiency, enteric methane production and the rumen microbiome are all related to each other, and therefore the development of new tools based on genomics, nutrigenomics and microbiomics will be of interest. Recently, a 1000-cow study showed that a core microbiome was significantly correlated with host genetics and phenotypes such as methane emissions, rumen and blood metabolites, and milk production efficiency (Wallace et al.,

2019). The results indicated that the phenotypes could be predicted from the microbiome and utilised in animal breeding programmes. There is a substantial amount of research, novel openings and ideas occurring in the field of feed efficiency and genetics. In general, the knowledge, and feed efficiency and energy status traits developed in this thesis may be utilised not only in animal breeding but also in precision livestock farming and on the farm level to support the management and feeding of cows. This may therefore contribute to the development of more sustainable dairy production.

6 CONCLUSIONS

Novel phenotypes for predicting cow-specific diet digestibility and energy status, along with a novel approach for partitioning metabolisable energy efficiency were developed in this thesis, and genetic analyses were performed for these traits.

The results showed that NIRS may be used to assess cow-specific digestibility from faecal samples, and that iNDF content in faeces alone may be used as an indicator for digestibility if the cows in the same contemporary group consume the same diet (I, II). Genetic variation found for diet digestibility was low, but may be utilised if it results in greater benefits than costs (II).

Two new feed efficiency traits were proposed: metabolisable energy efficiency (MEE), which is formed by modelling MEI-fitting regressions on energy sinks ($BW^{0.75}$, ECM, BWG and BWL) directly, and partial MEE (pMEE), where the model for MEE is extended with regressions on energy sinks nested within additive genetic and permanent environmental effects (III). MEE allows more accurate modelling of the data and results in better properties for predicting breeding values compared with REI. Results indicated that there may be genetic variation among cows in how efficiently they utilise ME for specific energy pathways.

Results also indicated that blood NEFA, a biomarker for the energy status of a cow, may be predicted from milk spectra using MIR technology (IV). The genetic variation in predicted NEFAs and other energy status indicator traits (FPR, C18:1 *cis*-9, BHB, acetone) were studied and all traits were found to be moderately heritable during the first three months of lactation (V). Moderate genetic correlations were found between energy status indicators and the fertility trait, the interval from calving to first insemination, in the first month of lactation (8–35 DIM), indicating an unfavourable relationship between energy status indicators and fertility (V).

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