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Faculty of Biosciences
Department of Animal and Aquacultural Sciences

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Genetic improvement of feed efficiency in dairy cattle

Genetisk forbedring av fôreffektiviteten hos melkeku

Sini Elina Wallén

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PhD Supervisors

Prof. Theo H. E. Meuwissen
Department of Animal and Aquacultural Sciences
Norwegian University of Life Sciences
P.O. Box 5003
1432 Ås, Norway

Dr. Egil Prestløkken
Department of Animal and Aquacultural Sciences
Norwegian University of Life Sciences
P.O. Box 5003
1432 Ås, Norway

Dr. Trygve R. Solberg
Geno Breeding and AI Association
P.O. Box 5003
1432 Ås, Norway

Prof. Harald Volden
TINE SA
P.O. Box 58
1430 Ås, Norway

PhD Evaluation committee

Prof. Nicolas Gengler
University of Liege
Passage des Déportés 2
Belgium

Dr. Yvette de Haas
Wageningen University and Research Centre
Postbus 338, 6700AH Wageningen
Netherlands

Assoc. Prof. Tormod Ådnøy
Department of Animal and Aquacultural Sciences
Norwegian University of Life Sciences
P.O. Box 5003, 1432 Ås
Norway

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Ås, November 2017
Sini Elina Wallén

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Summary

The main objective of the thesis was to investigate the requirements and possibilities for including feed efficiency (FE) in the breeding goal in dairy cattle and hence enable the genetic improvement of feed efficiency. In addition, possible ways to obtain large scale phenotypic data for the genetic improvement of FE were investigated. The data was provided by Norwegian dairy foods company TINE SA (Ås, Norway), NMBU research farm (Ås, Norway), the Norwegian Dairy Herd Recording System (Ås, Norway) and Animal and Grassland Research and Innovation Centre, Teagasc, Moorepark (Co. Cork, Ireland). The data consisted of records from two research farms, the Norwegian dairy herd recording system and mid-infrared (MIR) spectroscopy of milk. In total, data from 160 lactating Norwegian Red dairy cows and 375 lactating Irish Holstein-Friesian dairy cows were used in the thesis, recorded from 2007 to 2015. Individual feed intake (FI), milk yield (MY), concentration of milk, body weight (BW) and milk spectral recordings were included in the dataset.

In Paper I, alternative genomic selection (GS) and traditional Best Linear Unbiased Prediction (BLUP) breeding schemes were compared for the genetic improvement of feed efficiency in simulated Norwegian Red dairy cattle populations. The change in genetic gain over time and achievable selection accuracy were studied for MY and residual feed intake (RFI). When contracted test herds, with genotyped and FE recorded cows as a reference population were used, a reference population size of 4,000 new heifers per year was needed to achieve considerable genetic improvement of feed efficiency. With such a reference population it was possible to reach similar selection accuracies of 0.75 for males than when using progeny testing. It was concluded that the use of contracted test herds with additional recordings (e.g. FE) is a viable option for the genetic improvement of such difficult to record traits.

In Paper II, MIR spectra of milk was used to predict dry matter intake (DMI) and net energy intake (NEI) in Norwegian Red dairy cows. Leave-one-out cross-validation and external validation were used to develop and validate prediction equations using five different models. Predictions were performed using either partial least squares regression (PLS) or BLUP. When using the PLS method, the greatest accuracy (R) for predicting DMI (0.54) and NEI (0.65) in the external validation dataset was achieved when using both BW and MY as predictors in

combination with the MIR spectra. The Best Linear Unbiased Prediction method gave similar accuracies as PLS but the predictions were biased. This study shows that MIR spectral data can be used to predict NEI as a measure of FI in Norwegian Red dairy cattle, and the prediction accuracy can be increased if BW and/or MY is added to the model.

In Paper III, milk MIR data from two countries was combined and used to predict NEI in Norwegian Red dairy cows and effective energy intake (EEI) in Holstein-Friesian dairy cows. Split-sample cross-validation and external validation methods were used to develop and validate prediction equations using four different models. Predictions were performed using either PLS regression, multiple linear regression (MLR) or BLUP methods. Best Linear Unbiased Predictions were implemented either as a single trait or a multi-trait method. Using across country spectra, the R of predicting EEI increased by 0.02 units in both the cross-validation and the external validation compared to the model with spectral information within country only. For NEI, the use of across country MIR decreased the prediction accuracy in the cross-validation by 0.02 units and had no effect on R in the external validation. When NEI was predicted using only the MIR spectral information, single trait BLUP method yielded greater accuracy than PLS. For both NEI and EEI, the greatest accuracy of prediction was achieved using across country MIR spectra. This study shows that MIR spectral data from two countries can be combined and used to increase accuracies of predictions of energy intake (EI) as a measure of feed intake in dairy cattle.

If sufficient quantity of FE phenotypic data is available, genetic improvement of feed efficiency is possible. MIR spectral data can be used to predict NEI as a measure of feed intake in Norwegian Red dairy cattle. Also, across country MIR spectral data can be used to predict different energy intake traits. Before including any measure of FE in the breeding program, genetic correlations between predicted feed intake, actual feed intake and other performance traits, especially health and fertility traits must be estimated, and taken into consideration.

Sammendrag

Hovedformålet med avhandlingen var å undersøke kravene og mulighetene for å inkludere fôreffektivitet (FE) i avlsmålet til melkekyr og dermed muliggjøre den genetiske forbedringen av fôreffektivitet. I tillegg ble mulige måter å oppnå storskala fenotypiske data for genetisk forbedring av FE undersøkt. Dataene ble levert av det norske meieriet TINE SA (Ås, Norge), NMBU-forsøksgård (Ås, Norge), Kukontrollen (Ås, Norge) og Animal and Grassland Research and Innovation Centre, Teagasc, Moorepark (Co. Cork, Ireland). Dataene besto av målinger fra to forskningsgårder, kukontrollen og mid-infrarød spekteranalyse (MIR) av melk. Totalt ble det brukt data fra 160 lakterende melkekyr av rasen Norsk Rødt Fe og 375 lakterende Irske Holstein-Frieser melkekyr i avhandlingen, registrert fra 2007 til 2015. Datasettet inkluderer individuell opptak av fôr (FI), melkeytelse (MY), konsentrasjon av melk, kroppsvekt (BW) og melkespekter.

I artikkel I ble alternativ genomisk seleksjon (GS) og tradisjonelle Best Linear Unbiased Prediction (BLUP) avlssystemer sammenlignet for den genetiske forbedringen av fôreffektivitet i simulerte Norske Røde melkekyrpopulasjoner. Forandringen i genetisk gevinst over tid og oppnåelig seleksjonsnøyaktighet ble studert for MY og restinntak av fôr (RFI). Når det ble brukt begrensede testbesetninger med kyr som har registrert genotype og FE som referansepopulasjon, var det nødvendig med en referansepopulasjonsstørrelse på 4000 nye kviger per år for å oppnå betydelig genetisk forbedring av fôreffektivitet. Med en slik referansepopulasjon var det mulig å oppnå seleksjonsnøyaktigheter på 0,75 for okser, tilsvarende bruk av avkomstgransking. Det ble konkludert med at bruk av testbesetninger med tilleggsregistreringer (for eksempel FE) er et overkommelig alternativ for den genetiske forbedring av egenskaper som er vanskelige å registrere.

I artikkel II ble MIR av melk brukt til å forutsi inntak av tørrstoff (DMI) og netto energiinntak (NEI) i Norsk Rødt Fe. "Holde-en-utenfor" -kryss-validering og ekstern validering ble brukt til å utvikle og validere prediksjonsligninger ved bruk av fem forskjellige modeller. Prediksjoner ble utført ved bruk av enten Partial Least Squares Regression (PLS) eller BLUP metoder. Ved bruk av PLS-metoden ble den største nøyaktigheten (R) for å forutsi DMI (0,54) og NEI (0,65) i det eksterne valideringsdatasettet oppnådd ved bruk av både BW og MY som prediktorer i kombinasjon med MIR-spektrene. Best Linear Unbiased Prediction -metoden ga lignende

nøyaktigheter som PLS, men prognosene var partisk. Denne studien viser at MIR-spektraldata kan brukes til å forutsi NEI som et mål for FI i Norsk Rødt Fe, og prediksjonsnøyaktigheten kan økes dersom BW og/eller MY er lagt til modellen.

I artikkel III ble MIR av melk fra to land kombinert og brukt til å forutsi NEI i Norsk Rødt Fe og effektivt energiinntak (EEI) i Holstein-Frieser melkekyr. "Split-sample" -kryss-validering og eksterne valideringsmetoder ble brukt til å utvikle og validere prediksjonsligninger ved bruk av fire forskjellige modeller. Forutsigelser ble utført ved bruk av enten PLS-regresjon, multiple lineære regresjon (MLR) eller BLUP-metoder. Best Linear Unbiased Predictions ble implementert enten som enkeltegenskap eller en fler-egenskapsmetode. Ved å bruke spekter på tvers av land, økte R for å forutsi EEI med 0,02 enheter både i kryssvalidering og ekstern validering sammenlignet med modellen med spekterinformasjon bare innen land. For NEI reduserte bruken av MIR på tvers av land prediksjonsnøyaktigheten i kryssvalideringen med 0,02 enheter og hadde ingen effekt i R i den eksterne valideringen. Når NEI var forutsatt bare ved bruk av MIR-spekterinformasjonen, enkeltegenskap BLUP-metode ga større nøyaktighet enn PLS. For både NEI og EEI ble den største nøyaktigheten av prediksjon oppnådd ved bruk av MIR på tvers av land. Denne studien viser at MIR-spektraldata fra to land kan kombineres og brukes til å forutsi energiinntak (EI) som et mål for inntak av fôr i melkekyr.

Hvis tilstrekkelig mengde fenotypiske data om FE er tilgjengelige, er genetisk forbedring av fôreffektivitet mulig. MIR-spektraldata kan brukes til å forutsi NEI som et mål for fôrinntaket i Norsk Rødt Fe. Også kan på tvers av land MIR spekterdata brukes til å forutsi forskjellige energiinntaks karakteristikker. Før det inngår noen måling av FE i avlsprogrammet, må genetiske korrelasjoner mellom predikert fôrinntak, faktisk inntak av fôr og andre ytelsesegenskaper, spesielt helse- og fruktbarhetsegenskaper, estimeres og tas i betraktning.

List of abbreviations

BLUP	Best Linear Unbiased Prediction
BW	Body weight
DMI	Dry matter intake
EEI	Effective energy intake
EI	Energy intake
FCE	Feed conversion efficiency
FCR	Feed conversion ratio
FE	Feed efficiency
FI	Feed intake
GS	Genomic Selection
MIR	Mid-infrared spectrometry of milk
MLR	Multiple linear regression
MY	Milk yield
NEI	Net energy intake
NR	Norwegian Red dairy cattle
PLS	Partial least squares regression
R	Accuracy of prediction
REI	Residual energy intake
RFI	Residual feed intake
TMI	Total merit index

List of papers

I. **S. E. Wallén, M. Lillehammer, and T. H. E. Meuwissen.** 2017. Strategies for implementing genomic selection for feed efficiency in dairy cattle breeding schemes. *Journal of Dairy Science 100:6327-6336.*

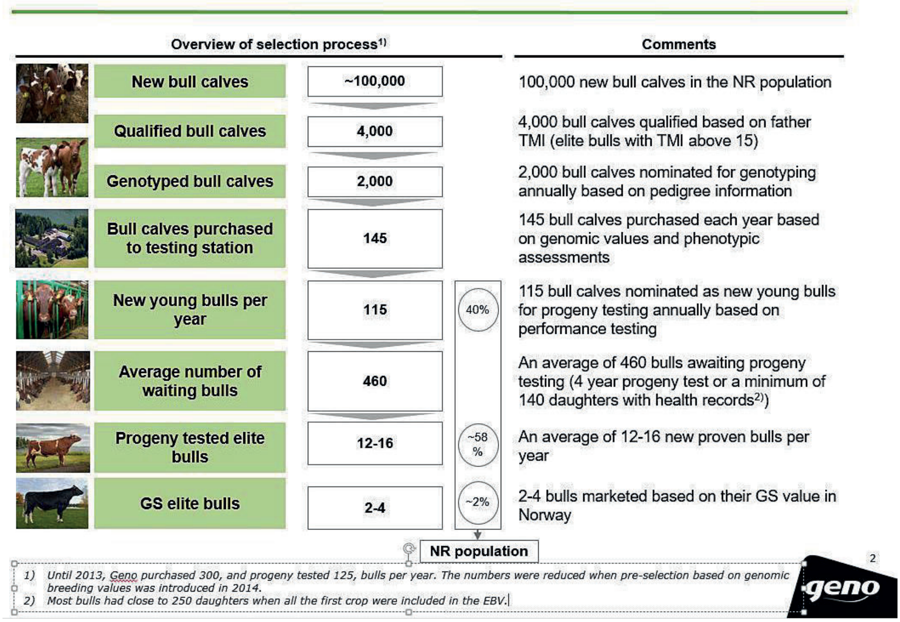
II. **S. E. Wallén, E. Prestløkken, T. H. E. Meuwissen, S. McParland and D. P. Berry.** 2017. Milk mid-infrared spectral data as a tool to predict feed intake in lactating Norwegian Red dairy cows. *Submitted to Journal of Dairy Science.*

III. **S. E. Wallén, S. McParland, D. P. Berry, E. Prestløkken, and T. H. E. Meuwissen.** 2017. Merging of feed intake and milk infrared spectral data across countries increases the accuracy of prediction of feed intake in lactating dairy cows. *Manuscript.*

1. General introduction

1.1. Dairy cattle breeding in Norway

The main dairy cattle breed in Norway is Norwegian Red (NR) with a population size of ~200,000 cows of which 98 % are included in the Norwegian Herd Recording System. The breeding organization for Norwegian Red is Geno SA which is a farmer-owned co-operative and has been active since 1935. Health and fertility traits were included in the breeding program of NR already during 1970's. Before genomic selection was implemented in the breeding program of NR in 2016, the breeding was based on progeny testing including 40 different traits. A weight based on the economic importance of the trait was given for each trait and these weights were used to calculate a total merit index (TMI), which was then used in sire selection (Geno, 2017).



Overview of selection process1)			Comments
New bull calves	~100,000		100,000 new bull calves in the NR population
Qualified bull calves	4,000		4,000 bull calves qualified based on father TMI (elite bulls with TMI above 15)
Genotyped bull calves	2,000		2,000 bull calves nominated for genotyping annually based on pedigree information
Bull calves purchased to testing station	145		145 bull calves purchased each year based on genomic values and phenotypic assessments
New young bulls per year	115	40%	115 bull calves nominated as new young bulls for progeny testing annually based on performance testing
Average number of waiting bulls	460		An average of 460 bulls awaiting progeny testing (4 year progeny test or a minimum of 140 daughters with health records2))
Progeny tested elite bulls	12-16	~58 %	An average of 12-16 new proven bulls per year
GS elite bulls	2-4	~2%	2-4 bulls marketed based on their GS value in Norway

NR population

1) *Until 2013, Geno purchased 300, and progeny tested 125, bulls per year. The numbers were reduced when pre-selection based on genomic breeding values was introduced in 2014.*
2) *Most bulls had close to 250 daughters when all the first crop were included in the EBV.*

2

Figure 1. Earlier progeny testing scheme for Norwegian Red (Geno, 2017).

In the earlier progeny testing scheme, 145 NR bull calves from elite sires and dams were selected for performance testing each year (Figure 1). A total of 115 bull calves were further selected to be test sires and progeny tested based on 140-350 daughters per sire. Based on their TMI and average relationship within the population, 12-16 progeny tested bulls were selected to be elite sires. Overall, 60% of the cows were bred by elite sires and 40% of the cows were bred by test sires. In the current genomic selection scheme, based on their parent average 3,000 bull calves are chosen to be genotyped yearly out of 6,000 bull calves. Based on their genomic values and phenotypic information, 150 bull calves are selected for performance testing and of those 50-60 bulls are selected to be elite sires (Geno, 2017).

1.2. Breeding for improved feed efficiency

1.2.1. Background

The world human population is expanding (FAO, 2009) and there is an increased global demand for animal-derived energy and protein sources. This has led to international interest in sustainable resource use efficiency and increased feed efficiency (FE). Improved FE would reduce nutrient and greenhouse gas emissions per animal (Hurley *et al.*, 2017) hence, many studies have been concentrating on selecting more efficient dairy cattle in order to reduce feed costs and the carbon footprint of dairy production (Connor *et al.*, 2013; Green *et al.*, 2013; Macdonald *et al.*, 2014; Hardie *et al.*, 2015). According to Hurley *et al.* (2017), maintaining the competitiveness of dairy production and meeting consumer demands for animal protein requires more food produced on less feed. Hence, a large-scale global effort has been directed to improve FE since its importance to the dairy industry is well recognized (Berry *et al.*, 2014; de Haas *et al.*, 2015).

However, although FE is economically important, it has still been overlooked in national dairy cattle breeding goals (Hurley *et al.*, 2016), mainly due to a lack of accurate individual feed intake (FI) data on commercial animals (Berry and Crowley, 2013).

1.2.2. Definitions of feed efficiency

Feed efficiency is commonly defined as the relative ability of an animal to turn feed nutrients into a product i.e. units of output per unit of input at the farm level or for an individual animal. For pigs and poultry, genetic selection for FE is common (Emmerson, 1997; Lonergan *et al.*, 2001). Omitting FE from the dairy cow breeding objectives is not only due to lack of available

FI data, but also because of lack of consensus how to define FE and FI in the most appropriate way in dairy cows (Hurley *et al.*, 2017). Having access to individual animal FI recordings and finding appropriate definitions of efficiency are both required in achieving the necessary gains in efficiency (Hurley *et al.*, 2016). Several measures of FE have been proposed and were extensively discussed (Hurley *et al.*, 2017). More than 2 dozen definitions of feed efficiency have been presented in the scientific literature since the 1960s (Archer *et al.*, 1999). Moreover, the definition of FE differs between the species and also within the species FE can be defined and measured differently. The traditional measures of feed efficiency in growing and lactating animals are feed conversion ratio (FCR) and feed conversion efficiency (FCE), respectively (Hurley *et al.*, 2016). Generally, FCR is defined as dry matter intake divided by the average daily gain and more efficient animals have a lower FCR (Berry and Crowley, 2013). Unfortunately, FCR assumes no differences in maintenance efficiency among animals (Berry and Crowley, 2013), which is not necessarily the case (Archer *et al.*, 1999). Feed conversion efficiency for dairy cows is generally defined as kg of energy corrected milk divided by kg of feed dry matter (DM) consumed (Beever and Doyle, 2007). In lactating cows, some currently used feed efficiency definitions (e.g., FCE) do not fully account for body tissue mobilization (Hurley *et al.*, 2016). Several other definitions of feed efficiency exist in lactating animals as well as growing animals and they all have disadvantages and advantages (Berry and Crowley, 2013). Among them, residual feed intake (RFI), traditionally used to measure feed efficiency in growing animals (Berry and Crowley, 2013), has gained popularity also in dairy cattle (Coleman *et al.*, 2010; McParland *et al.*, 2014; Pryce *et al.*, 2014**.** Residual feed intake is defined as the difference between the observed FI and the predicted FI of an individual (Potts *et al.*, 2015), where predicted intake is what an individual is expected to consume based on its production when a regression of milk energy, maintenance energy, metabolic body weight, and change in body weight (BW) has been accounted for (Hardie *et al.*, 2015). Hence, an animal with a negative RFI is defined to be more efficient since it consumes less feed than predicted (Potts *et al.*, 2015). Generally, RFI has been measured during a period of minimal BW and condition change (Tempelman *et al.*, 2015). According to Rathbun et al. (2017), RFI has not been measured during the transition period which is 3 weeks before and 3 weeks after calving. During this period dairy cows mobilize rapidly adipose tissue as fatty acids, and often have elevated circulating ketone body concentrations hence, leading to negative energy balance (Grummer, 1993; Drackley, 1999; Duffield, 2000). Excessive production of ketone metabolites can lead to hyperketonemia and hence, have negative effects on animal production, profitability and health (Baird *et al.*, 1980; Herdt, 2000; McArt *et al.*, 2015). Understanding the effect of negative RFI on animal health and longevity is needed in order to successfully use RFI as a selection tool (Rathbun *et al.*, 2017). International interest in using energy intake (EI) and residual energy intake (REI) as measures of feed efficiency has intensified (Hurley *et al.*, 2016). However, EI is a phenotype which itself encompasses recording errors (McParland *et al.*, 2011) and for example diurnal variation may exist in EI (McParland *et al.,* 2014). Residual energy intake in turn, is defined differently in different dairy cattle studies (Coleman *et al.*, 2010; McParland *et al.*, 2014; Pryce *et al.*, 2014). Consequently, benefits and applications of these FE definitions differ (Hurley *et al.*, 2016), making comparisons between studies challenging.

1.2.3. Genomic Selection

Despite the limited amount of phenotypes, the use of indicator traits (Fogh *et al.*, 2013) and genomic selection (Pryce and Berry, 2014) are the two possible methods which could provide sufficient selection accuracy for feed efficiency. Genomic Selection (GS) uses dense markers covering the whole genome and it addresses most of the genetic differences between the animals (Meuwissen *et al.*, 2001). Since GS can be based on fewer phenotypes than traditional selection, GS would be a useful tool to improve feed efficiency (Pryce *et al.,* 2012; Yao *et al.*, 2017). In GS, reference animals or “ a training” population is used to calibrate the genomic prediction equations (Veerkamp, 2013). As an example, cows from research herds with detailed recording of unique phenotypes can be used as such a training population (Banos *et al.*, 2012). Hence, international co-operatives, the global Dry Matter Initiative (de Haas *et al.*, 2015), the co-operation between United States and Netherlands and the co-operation between Canada, United States, United Kingdom, Australia and Switzerland (Chesnais *et al.*, 2016), for example, have been implemented in order to collect large amount of data from different countries to be used in genomic selection for improving feed efficiency. For expensive or difficult to record traits, such as FE, a new database usually needs to be constructed which can be expensive (Chesnais *et al.*, 2016). Especially, if the heritability of the trait is low and the cost of phenotyping is high, which is the case for many feed efficiency traits, the reference population requires a considerable number of animals in order to achieve adequate prediction accuracy hence, increasing the cost of the reference scheme (Chesnais *et al.*, 2016). Under the circumstances, genotyping all animals with phenotypes is more efficient than using, for example, already available genotypes of those animals' sires (Chesnais *et al.*, 2016). Therefore, the use of a cow reference populations is the most cost effective way to generate genomic evaluations for such traits (Van Grevenhof *et al.*, 2012; Calus *et al.*, 2013). Holstein Association USA has already added a FE component in its selection index. However, this component does not account for variation between individuals in their efficiency of converting feed into product (Chesnais *et al.*, 2016).

1.2.4. MIR

Mid-infrared (MIR) spectroscopy is based on the study of the interaction between matter and electromagnetic waves (De Marchi *et al.*, 2014) in the 900 to 5,000 cm^{-1} region and is routinely used globally to determine fat, protein, and lactose concentration in milk (Soyeurt *et al.*, 2011; De Marchi *et al.*, 2014). McParland *et al.* (2014) documented that FI could be predicted using MIR spectrometry of milk in lactating dairy cows. According to Chesnais et al. (2016), there is an ongoing project in Canada in order to collect MIR records from large amount of cows to develop predictions for FE, methane emissions and milk composition; and produce genetic and genomic evaluations for some of these MIR predictions. Since individual animal milk samples are routinely taken as part of day-to-day dairy herd management, using these samples to also predict feed intake would be a cost-effective strategy for generating data for management purposes as well as for inclusion in a breeding program.

2. Aim and outline of the thesis

The main objective of the thesis was to investigate the requirements and possibilities for including feed efficiency in the breeding goal of dairy cattle. The study also investigates possible ways to obtain large scale phenotypic data for genetic improvement of feed efficiency.

The thesis had three sub goals:

1. To compare alternative breeding strategies for the genetic improvement of feed efficiency

2. To investigate the use of mid-infrared spectral data to predict feed intake in Norwegian Red dairy cattle

3. To investigate if combining mid-infrared spectral data from two different countries would increase the accuracy of predicting feed intake

3. PAPER I:

Strategies for implementing genomic selection for feed efficiency in dairy cattle breeding schemes

S. E. Wallén, M. Lillehammer, and T. H. E. Meuwissen

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Strategies for implementing genomic selection for feed efficiency in dairy cattle breeding schemes

S. E. Wallén,*[1] M. Lillehammer,† and T. H. E. Meuwissen*
*Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, PO Box 5003, 1432 Ås, Norway
†Nofima, PO Box 5010, 1432 Ås, Norway

ABSTRACT

Alternative genomic selection and traditional BLUP breeding schemes were compared for the genetic improvement of feed efficiency in simulated Norwegian Red dairy cattle populations. The change in genetic gain over time and achievable selection accuracy were studied for milk yield and residual feed intake, as a measure of feed efficiency. When including feed efficiency in genomic BLUP schemes, it was possible to achieve high selection accuracies for genomic selection, and all genomic BLUP schemes gave better genetic gain for feed efficiency than BLUP using a pedigree relationship matrix. However, introducing a second trait in the breeding goal caused a reduction in the genetic gain for milk yield. When using contracted test herds with genotyped and feed efficiency recorded cows as a reference population, adding an additional 4,000 new heifers per year to the reference population gave accuracies that were comparable to a male reference population that used progeny testing with 250 daughters per sire. When the test herd consisted of 500 or 1,000 cows, lower genetic gain was found than using progeny test records to update the reference population. It was concluded that to improve difficult to record traits, the use of contracted test herds that had additional recording (e.g., measurements required to calculate feed efficiency) is a viable option, possibly through international collaborations.

Key words: genomic selection, feed efficiency, breeding scheme

INTRODUCTION

Improving feed efficiency is economically important because feed costs constitute the majority of the variable cost in the dairy industry. Hence, some countries have already included feed efficiency (**FE**) in their breeding goals (Pryce et al., 2014). Having access to accurate and low-cost FE measurements is difficult; hence, a lot of research efforts are devoted to this problem (de Haas et al., 2012; Veerkamp et al., 2013). The main problem in including FE in the breeding objective is accessing phenotypic data from a large population of daughters of progeny tested bulls. Because genomic selection can be based on fewer phenotypes than traditional selection, genomic selection would be a useful tool to improve FE, as shown by Pryce et al. (2012) and Yao et al. (2017).

Genomic selection uses dense markers covering the whole genome and addresses most of the genetic differences between the animals (Meuwissen et al., 2001). The total genetic value of selection candidates is predicted based on the estimation of SNP effects, which are estimated using reference individuals that have been genotyped and phenotyped. If the training set is large enough and relevant to the selected population, genomic selection can result in an increase in the accuracy compared with traditional selection (VanRaden et al., 2009). The number of individuals in the training set and the marker density have the greatest effect on accuracy (Hayes and Goddard, 2008; Goddard, 2009). Other factors are heritability (Daetwyler et al., 2008; Goddard, 2009), effective population size (N_e), effective number of segments (Goddard, 2009), relationship between the evaluated animals and training data set (Habier et al., 2010; Wolc et al., 2011; Pszczola et al., 2012), and variance of relationships within the reference population (Habier et al., 2010). For the traits that have low heritabilities, a very large number of records will be required in the training data set to achieve high accuracies of genomic EBV in unphenotyped animals (Hayes et al., 2009). One possibility to overcome the limited size of the training set is to combine data across countries as in the global Dry Matter Initiative (de Haas et al., 2012).

In this study, stochastic simulation was used to investigate how different breeding schemes affect genetic gain without treating accuracy as a fixed value, but rather as an outcome of the simulation. By using stochastic simulation, it is also possible to study complex and

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[1] Corresponding author: sini.wallen@nmbu.no

overlapping generations and the changes in accuracy over time under different schemes (Lillehammer et al., 2011). We used residual feed intake (**RFI**) as a measure of FE. Residual feed intake is defined as the difference between actual and predicted feed (or energy) intake based on the requirements of the animal (Koch et al., 1963; Williams et al., 2011; Berry and Crowley, 2013). The benefits of GS are greatest when selection is for difficult to measure traits, whose recording is either too expensive or phenotypes are not easily accessible (Goddard, 2009). Both these arguments justify the use of GS for improving FE, because FE recording is too expensive to be carried out on large numbers of cows and the FE of milk production cannot be recorded on bulls. In this study, genomic selection strategies were developed for improving FE in Norwegian Red dairy cattle. The objectives of this research were to compare strategies for improving selection accuracy and genetic gain for FE by estimating SNP effects in experimental herds with FE recordings or in large-scale field recordings of FE. Thus, we investigate whether it is possible to use contracted test herds with additional recording for improving traits that are difficult to measure such as FE.

MATERIALS AND METHODS

Historical populations were simulated to create realistic associations between markers and genes and to create founder populations for the breeding schemes. To create these associations and a mutation-drift balance, the simulations consisted of 2,000 generations of random mating following the Fisher-Wright population model (Fisher, 1930; Wright, 1931). The founder population had an effective population size of 200 (100 males and 100 females; Hillestad et al., 2014). The simulated genome consisted of 30 pairs of chromosomes; each was 100 cM in length. The expected number of mutations per meiosis per diploid chromosome was 2. Polymorphisms and recombinations were simulated following Sonesson and Meuwissen (2009). From the created SNP, 3,000 were randomly selected as QTL, and QTL effects were sampled from a normal distribution. Per chromosome, 500 SNP were randomly sampled to be used as genetic markers in the breeding scheme (i.e., a total of 15,000 markers).

Seven different breeding schemes were investigated: basic, milk yield and feed efficiency included in the breeding goal (**MY+FE**), population wide, and 5 test herd simulations. In the basic breeding scheme, only milk yield (**MY**) was included in the breeding goal, whereas in MY+FE and test herd simulations, MY and RFI as a FE trait were included in the breeding goal and they were assumed to be uncorrelated (because RFI as a measure of FE is not correlated with MY) and have equal economic weights (in all the other breeding schemes except test herd 4,000 eco25 and test herd 4,000 eco50 schemes). In the **eco25** scheme, FE had 1/4 of the economic weight of MY, whereas in the **eco50** scheme FE had 1/2 of the economic weight of MY. In test herd simulations, FE test herds were set up (contracted), where RFI and MY were recorded. These test herds varied in total size (500, 1,000, and 4,000) between the schemes. Basic and MY+FE schemes were investigated with both genomic selection (Meuwissen et al., 2001) and with traditional BLUP selection (**ABLUP**; Henderson, 1975). Test herd simulations were investigated only with genomic selection.

In the basic schemes, all cows got records only for MY at 3 yr of age (Table 1 and Figure 1). However, in the MY+FE schemes all cows had records for both MY and RFI at 3 yr of age. In test herd schemes, the test herd females had records for both RFI and MY at 3 yr of age, whereas other cows had records for MY only. No repeated records were assumed for any of the traits, which is conservative with respect to the amount of information that comes from recording a cow. Females were available for selection at 2, 3, 4, 5, and 6 yr of age. All ages refer to the average generation interval that results from their mating (i.e., the actual mating occurs 9 mo earlier). Males were selected to be parents at 3 yr of age in genomic BLUP (**GBLUP**) and at 6 yr of age in ABLUP schemes. Males were progeny tested for both MY and RFI in MY+FE schemes; progeny test results were available at 6 yr of age (Table 1 and Figure 1). However, in the basic and test herd schemes, males were progeny tested only for MY. The progeny test information was hence available when selecting sires in the ABLUP schemes, but not in the GBLUP schemes, due to the shorter generation interval. In GBLUP schemes, progeny information was used to update the reference population. One-third of the females were culled randomly every year starting when they were 3 yr old. Females in the test herds and bull calves born from elite matings were assumed genotyped in GBLUP schemes.

A base generation (generation 0) was created using the animals from the last generation of the founder population and mating them randomly. All 4,000 animals in generation 0 were assumed to be genotyped and have their own records to contribute to progeny testing in all the schemes that involved genomic selection, and those animals were used to estimate SNP effects for MY and RFI. The younger bulls were added to the simulated reference population when their daughters had records for production traits. The simulated breed-

Table 1. Ages (yr) at which recording and selection take place[1]

Item	Age of dam	Age of sire	Milk record dam	RFI record dam	Progeny test sire
Basic ABLUP	2–6	6	3	—	6[2]
Basic GBLUP	2–6	3	3	—	6[2]
MY+FE ABLUP	2–6	6	3	3	6[3]
MY+FE GBLUP	2–6	3	3	3	6[3]
Test herd GBLUP	2–6	3	3	3[2]	6[3]

[1] Ages refer to the generation interval resulting from the mating of the parents (selected for the indicated record). RFI = residual feed intake; ABLUP = best linear unbiased prediction using pedigree relationship matrix; GBLUP = genomic best linear unbiased predictor; MY+FE = milk yield and feed efficiency included in the breeding goal.

[2] Breeding goal includes only milk yield.

[3] Breeding goal includes both RFI and milk yield.

ing schemes closely resembled those of Lillehammer et al. (2011) where earlier progeny-tested bulls were genotyped and used to estimate SNP effects.

True breeding values (TBV) were calculated for all individuals as the sum of the QTL effects:

$$\mathrm{TBV}_i = \sum_{j=1}^{\text{Number of QTL}} x_{ij1}g_{j1} + x_{ij2}g_{j2},$$

where x_{ijk} is the number of copies that individual i has at the jth QTL position and kth QTL allele, and g_{jk} is the effect of the kth QTL at the jth position, which were sampled from the normal distribution. The simulated traits, MY and RFI, were assumed to have heritabilities of 0.3 and 0.15, respectively. Those heritabilities reflect the average heritability of milk production (Hoekstra et al., 1994; Berry et al., 2003) and the average heritability of FE traits (Berry and Crowley, 2013; Varga and Dechow, 2013).

The accuracy of the genomic breeding values was calculated, according to Sonesson and Meuwissen (2009), as the correlation between the estimated genomic breeding values and the true breeding values. Genomic breeding values were estimated by summing the marker effects:

$$\mathrm{GEBV}_i = \sum_{j=1}^{n} x_{ij}a_j,$$

where x_{ij} is the jth SNP effect of individual i, a_j is the BLUP estimate of the jth SNP effect, and n is the number of SNP (15,000). To ensure that direct com-

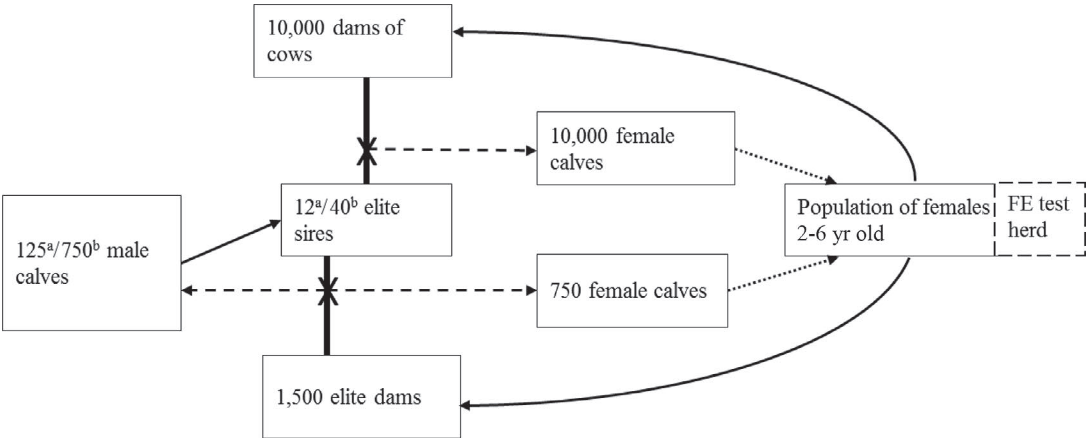

Figure 1. Overview of the breeding schemes. Solid lines with a cross represent matings, and dashed arrows represent progeny produced by the matings. Dotted arrows represent that animals move from one category to another due to aging. Solid arrows represent selection of animals. [a] In BLUP using pedigree relationship matrix (ABLUP) schemes, 125 male calves were progeny tested and 12 elite sires were selected. [b] In genomic BLUP schemes, 750 male calves were progeny tested and 40 elite sires were selected. FE = feed efficiency.

parison between traditional and genomic EBV was possible, all EBV were scaled so that $b = 1$, where $b = \mathrm{Cov}\left[\mathrm{TBV}_i;(\mathrm{G})\mathrm{EBV}_i\right] / \mathrm{Var}\left[(\mathrm{G})\mathrm{EBV}_i\right]$. This is important for the selection of females, which is across genomic EBV and traditional EBV for some of the schemes.

Phenotypes were simulated by adding a normally distributed random error term to the true breeding value:

$$P_i = \mathrm{TBV}_i + \varepsilon_i,$$

where ε_i is an error term for animal i, which was normally distributed $\left(0,\sigma_e^2\right)$. To express the results in genetic standard deviations and create phenotypic records with the desired heritability, the genetic variance $\left(\sigma_g^2\right)$ was scaled to 1 for both of the traits and the residual variance $\left(\sigma_e^2\right)$ was adjusted following Sonesson and Meuwissen (2009).

The value of 1 genetic standard deviation of MY was arbitrarily set to 100 monetary units. When the economic value of RFI equaled that of MY, a genetic standard deviation of RFI also represented 100 monetary units. In schemes with reduced economic values for RFI, eco50 and eco25 schemes, 1 genetic standard deviation of RFI represented 50 and 25 monetary units, respectively.

The BLUP method (Meuwissen et al., 2001) was used for the estimation of marker effects. The statistical model used to estimate individual marker effects was

$$y_i = \mu + \sum_{j=1}^{n} X_{ij} a_j + e_i,$$

where y_i is the record of individual i; μ is the overall mean; X_{ij} is the marker genotype; a_j is the random effect of the jth marker, with variance equal to the total genetic variance divided by the number of markers; and e_i is a random residual.

Simulated population sizes were smaller than those of the real Norwegian Red dairy cattle population to make stochastic simulation computationally possible. Population sizes were rescaled as described by Lillehammer et al. (2011), so that selection steps for conformation traits of bulls and bull dams were not considered in the simulation and selection intensities for the included traits were maintained at realistic levels when population size was reduced. The ABLUP schemes were designed to mimic the breeding structure of Norwegian Red before implementation of genomic selection, whereas the GBLUP schemes mimic the current breeding structure of Norwegian Red after genomic selection was implemented (Figure 1; Lillehammer et al., 2011).

For each scheme, 50 replicates were run and simulations were performed over a 20-yr period. Genetic gain and selection accuracy for males and females were reported as an average over yr 10 to 20 of the simulations. In all the schemes, total genetic gain was calculated by summing up the genetic gain (in monetary units) for MY and RFI. When results of the simulation study are reported, omitting the first years of the simulation avoids the problem of the non-steady-state population structure at the start of the scheme, where all animals are of the same age and all base generation animals are assumed to be genotyped and progeny tested, which affects early simulation results.

RESULTS

Figure 2 shows the total genetic gain (in monetary units) for MY and RFI when RFI was included in the breeding scheme. The highest total genetic gain was found when using the MY+FE GBLUP scheme, where bulls were progeny tested for both traits. The ABLUP schemes gave lower total genetic gain when compared with a similar GBLUP scheme. Increasing the number of cows in the test herds caused an increase in genetic gain. Genotyping 500 or 1,000 cows in test herds resulted lower genetic gain than using progeny test records to update the reference population. However, a test herd size of 4,000 cows gave slightly lower genetic gain than the MY+FE GBLUP scheme. Using smaller economic values for FE in test herd 4,000 GBLUP eco schemes decreased the total genetic gain.

As expected, the basic scheme gave the highest genetic gain for MY of the ABLUP schemes (Table 2 and Figure 3), and GBLUP schemes gave higher genetic gain for MY than similar ABLUP schemes. As expected, introducing a second trait in the breeding goal reduced genetic gain for MY. This is due to the fact that if selection pressure is devoted to more traits, the progress for each of the original traits decreases.

The highest genetic gain for RFI was reached using the MY+FE GBLUP scheme (Table 2 and Figure 4), where all cows had RFI records. Obtaining RFI records from test herds of limited size gave less gain for RFI, but increasing the number of genotyped cows in the test herd schemes increased the genetic gain for RFI. At a test herd size of 4,000 genotyped cows, the genetic gain for RFI was very similar to obtaining records from all cows in the population. As expected, test herd 4,000 GBLUP eco schemes gave lower genetic gain for RFI

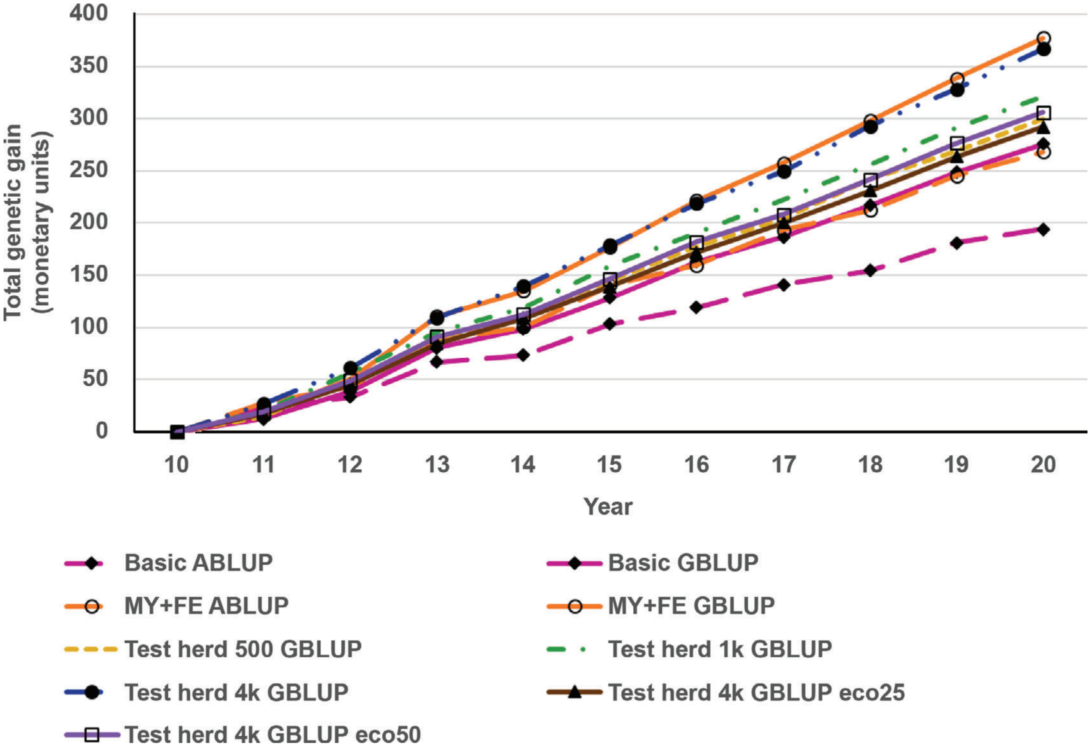

Figure 2. Total genetic gain measured as monetary units relative to yr 10 onward. In the basic schemes, genetic gain is only for milk yield, whereas in the schemes with milk yield and feed efficiency included in the breeding goal (MY+FE) and test herd schemes, genetic gain is the total genetic gain when summing up the genetic gain for both residual feed intake (RFI) and milk yield. In eco25 and eco50 schemes, 1 genetic SD of RFI represented 25 and 50 monetary units, respectively. ABLUP = BLUP using pedigree relationship matrix; GBLUP = genomic BLUP. Color version available online.

Table 2. Average genetic gain (ΔG) as monetary units with SE when $h^2 = 0.3$ for milk yield and $h^2 = 0.15$ for residual feed intake[1]

	Milk yield		Residual feed intake	
Breeding scheme[2]	ABLUP, ΔG	GBLUP, ΔG	ABLUP, ΔG	GBLUP, ΔG
Basic	19.64 (0.2)	28.52 (0.2)	—[3]	—[3]
MY+FE	14.76 (0.2)	21.74 (0.3)	12.45 (0.2)	17.28 (0.3)
Test herd 500	—	18.37 (0.3)	—	12.49 (0.2)
Test herd 1,000	—	18.99 (0.3)	—	13.88 (0.3)
Test herd 4,000	—	20.06 (0.3)	—	17.18 (0.2)
Test herd 4,000 eco25	—	28.74 (0.06)	—	1.23 (0.06)
Test herd 4,000 eco50	—	26.08 (0.15)	—	5.21 (0.13)

[1] Average of genetic gain measured as genetic SD of yr 10 to 20. The value of 1 genetic SD of milk yield was arbitrarily set to 100 monetary units. In eco25 and eco50 schemes, 1 genetic SD of residual feed intake represented 25 and 50 monetary units, respectively.

[2] Milk yield and feed efficiency included in the breeding goal (MY+FE) and test herd schemes include both milk yield and residual feed intake in the breeding goal; the basic scheme includes only milk yield.

[3] Residual feed intake is not included in the basic scheme.

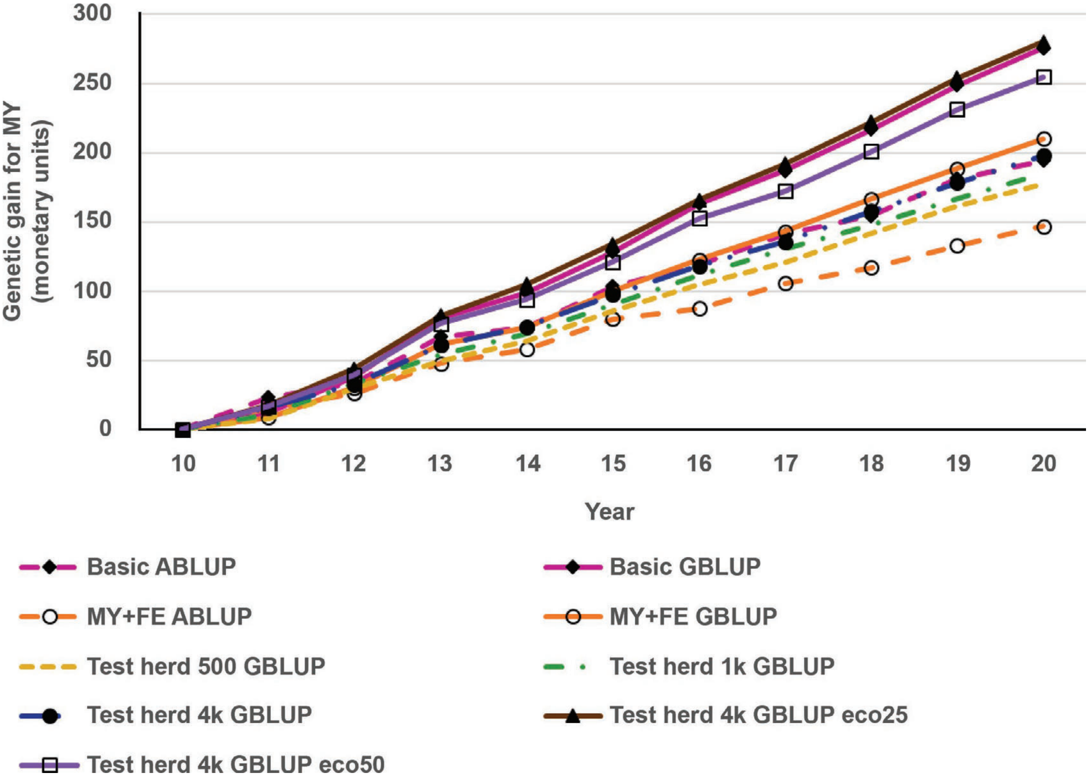

Figure 3. Genetic gain for milk yield (MY) measured as monetary units relative to yr 10 onward. In the basic schemes, genetic gain is only for MY, whereas in the schemes with MY and feed efficiency included in the breeding goal (MY+FE) and test herd schemes, genetic gain is the total genetic gain when summing up the genetic gain for both residual feed intake (RFI) and MY. In eco25 and eco50 schemes, 1 genetic SD of RFI represented 25 and 50 monetary units, respectively. ABLUP = BLUP using pedigree relationship matrix; GBLUP = genomic BLUP. Color version available online.

than other GBLUP schemes where RFI was included, which is due to the smaller economic value for RFI in eco schemes.

Selection accuracies for males ranged from 0.65 to 0.79 in GBLUP schemes and 0.94 to 0.96 in ABLUP schemes (Figure 5 and Table 3). Using lower economic values for RFI in the test herd, 4,000 GBLUP eco schemes slightly increased the selection accuracy for males (Figure 5). However, the selection accuracy for females was approximately 0.6 in all the other schemes except the test herd schemes (Table 3). The test herd scenarios caused a decrease in the selection accuracy for females because only a fraction of the females obtained RFI records. However, increasing the test herd size resulted in an increase in the female selection accuracy. The highest selection accuracy for females was reached using basic schemes, where the breeding goal included only MY.

DISCUSSION

This study compared different designs of implementing genomic selection and traditional BLUP selection for the genetic improvement of FE, and investigated how genetic gain accumulates over time and the selection accuracies that are achievable through the addition of genotyped females in the reference population. We used RFI as a FE trait because it is by definition the component of feed intake that is uncorrelated with MY. Practical breeding schemes may select directly for MY and against feed intake, but also here only the component that is uncorrelated with MY will be

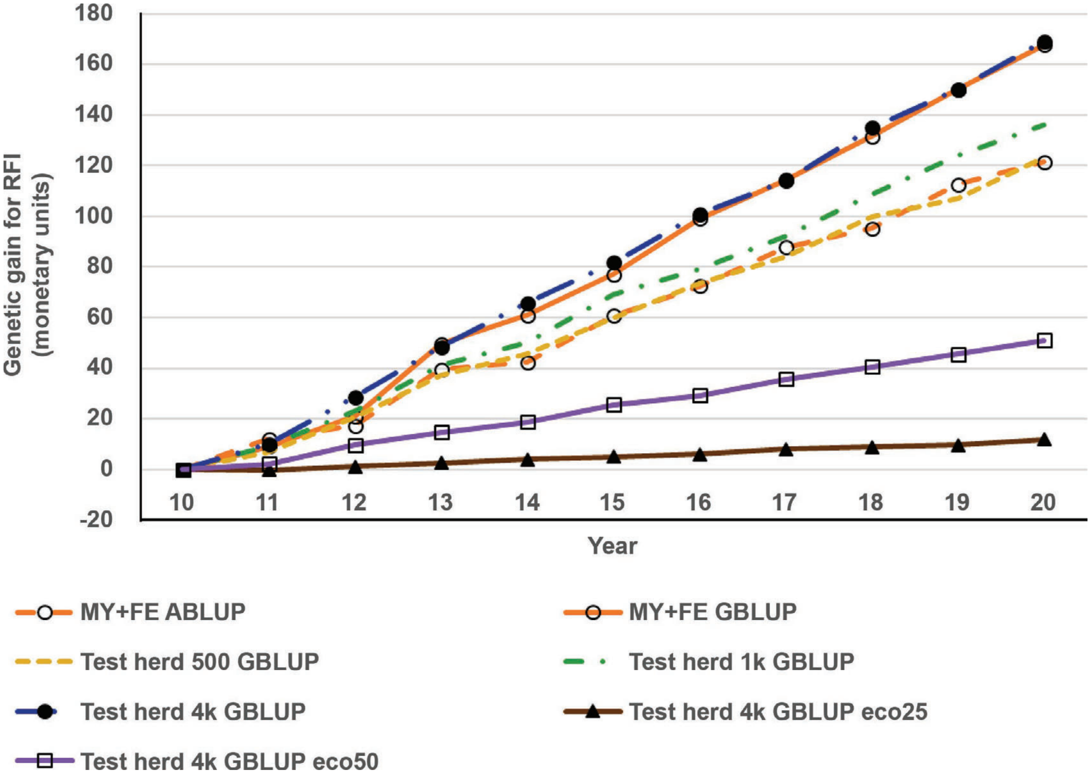

Figure 4. Genetic gain for residual feed intake (RFI) measured as monetary units relative to yr 10 onward. In the schemes with milk yield and feed efficiency included in the breeding goal (MY+FE) and test herd schemes, genetic gain is the total genetic gain when summing up the genetic gain for both RFI and milk yield. In eco25 and eco50 schemes, 1 genetic SD of RFI represented 25 and 50 monetary units, respectively. ABLUP = BLUP using pedigree relationship matrix; GBLUP = genomic BLUP. Color version available online.

reduced, whereas the component of feed intake that is associated with MY will increase together with the general increase in MY.

Table 3 showed that it is possible to achieve high selection accuracies for males when including FE in GBLUP schemes. This can be done either by obtaining phenotypes from all cows in the population and hence get progeny information for genotyped bulls that can be used to update a reference population, or by updating the reference population through genotyping of cows with records. The latter will be preferable if genotyping is cheap compared with phenotyping. These results are in line with Chesnais et al. (2016) who found, in real data, that the accuracies of genomic selection for FE varied between 0.45 and 0.58. When using genomic selection to improve low heritability traits, the number of records in the reference population has to be sufficiently large to achieve high selection accuracies (Hayes et al., 2009). Our study showed that 4,000 cows had to be phenotyped and genotyped every year to achieve a similar selection accuracy of genomic selection as if all cows were phenotyped, but when only bulls are genotyped.

Females were always selected on ABLUP, except in test herd schemes, where the genotyped test-herd females obtained genomic breeding values. The female selection accuracy were first of all affected by whether the females had records for the trait under selection or not, giving higher female selection accuracy for schemes where phenotypes for all traits under selection were available for the entire cow population (Table 3). When test herds were used, the females belonging to these herds will have more accurate breeding values than the cows outside the test herds, due to their phe-

Table 3. Average selection accuracy of yr 10 to 20 for males (M) and females (F) in the total breeding goal with SE when $h^2 = 0.3$ for milk yield and $h^2 = 0.15$ for residual feed intake (RFI)

Breeding scheme[1]	ABLUP, Accuracy M	GBLUP, Accuracy M	ABLUP, Accuracy F	GBLUP, Accuracy F
Basic	0.96 (0.0005)	0.75 (0.002)	0.61 (0.002)	0.62 (0.001)
MY+FE	0.94 (0.0007)	0.72 (0.002)	0.58 (0.001)	0.59 (0.001)
Test herd 500	—	0.65 (0.002)	—	0.21 (0.003)
Test herd 1,000	—	0.67 (0.002)	—	0.24 (0.003)
Test herd 4,000	—	0.75 (0.001)	—	0.42 (0.003)
Test herd 4,000 eco25	—	0.79 (0.001)	—	0.60 (0.002)
Test herd 4,000 eco50	—	0.78 (0.002)	—	0.53 (0.004)

[1] Milk yield and feed efficiency included in the breeding goal (MY+FE) and test herd schemes include both milk yield and residual feed intake; the basic scheme includes only milk yield. In eco25 and eco50 schemes, 1 genetic SD of RFI represented 25 and 50 monetary units, respectively.

notypes and genotypes. The female selection accuracy will hence depend on the fraction of the cows that are included in the test herds.

Genetic gain will depend on both male and female selection accuracy, although the male selection accuracy has the greatest effect because of the higher intensity of selection. Genetic gain was therefore similar in test herd 4,000 GBLUP as in MY+FE GBLUP, reflecting the similar accuracy of the genomic breeding values in the 2 schemes. The small advantage of MY+FE GBLUP,

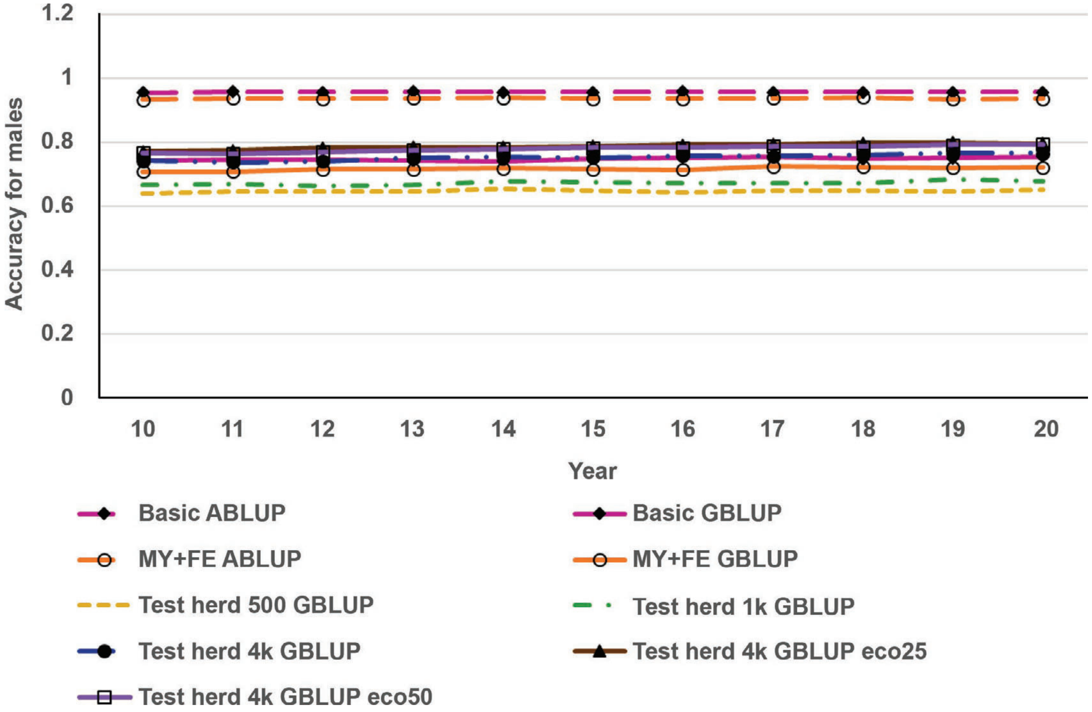

Figure 5. Selection accuracy for males relative to yr 10 onward. In the basic schemes, genetic gain is only for milk yield, whereas in the schemes with milk yield and feed efficiency included in the breeding goal (MY+FE) and test herd schemes, genetic gain is the total genetic gain when summing up the genetic gain for both residual feed intake (RFI) and milk yield. In eco25 and eco50 schemes, 1 genetic SD of RFI represented 25 and 50 monetary units, respectively. ABLUP = BLUP using pedigree relationship matrix; GBLUP = genomic BLUP. Color version available online.

compared with test herd 4,000 GBLUP, may increase if a more intense selection of females is used. However, if selection of females were also based on genomic selection, this difference could disappear, as the fraction of the female population with RFI phenotypes becomes less important. The general level of the genetic gains agrees with those found by Lillehammer et al. (2011).

We also investigated how the reduced economic values for RFI affects the genetic gain and the accuracy of selection by comparing the test herd 4,000 GBLUP at a half and a quarter of its original economic value of RFI. As expected, test herd 4,000 GBLUP eco schemes gave higher genetic gain for MY and lower genetic gain for RFI compared with other schemes. Lower economic values for RFI increased the selection accuracy of males and especially females because many more phenotypes were available for MY than for RFI in the test herd scheme. Total genetic gain was reduced for the schemes with lower economic values for RFI. To build up test herds to facilitate genomic selection for traits with low economic value might hence not be economically defendable, as the expected gain is sensitive to the weight put on these traits.

In these simulations, we assumed a large reference population at the start of the breeding scheme, which might be optimistic. However, Figure 5 shows that genomic selection accuracies during yr 10 to 20 remain stable, implying that the gain of accuracy due to the genotyping of new relevant reference animals is compensated for old reference animals becoming less relevant (i.e., the start reference population is becoming less and less relevant during yr 10 to 20). The results of Table 3 and Figure 5 show that if progeny testing for FE is not feasible, genotyping females in test herds that enter a reference population may compensate for the lack of progeny testing. However, this requires the genotyping and phenotyping of 4,000 test females annually because smaller test herd sizes resulted in markedly reduced genetic gains. Obtaining large amounts of animals with multiple recordings is possible using for example collaboration between countries (de Haas et al., 2012; Veerkamp et al., 2013) or milk mid-infrared predicted FE records. McParland et al. (2014) showed that mid-infrared spectrometry of milk could be used to predict RFI as a measure of FE in lactating dairy cows. Because individual animal milk samples are routinely taken as part of the dairy herd management, using these samples to also predict feed intake and efficiency would be cost effective and a relatively undemanding approach to obtain large numbers of FE phenotypes.

In this study, we used RFI as a measure of FE. However, earlier studies showed that weak unfavorable genetic correlations exist between RFI and fertility (Vallimont et al., 2013). This is probably due to the mathematical similarity in the calculations of RFI and energy balance and a failure to account correctly for body tissue mobilization, which might lead to selection for a trait that is similar to selecting for a negative energy balance (Pryce et al., 2014). Therefore, genetic correlations with other traits (especially fertility traits) must be accounted for when including RFI into the breeding scheme (Pryce et al., 2014). That is, a multitrait selection index where genetic correlations with other traits are properly accounted for is required if RFI is to be included in the selection objective.

Feed efficiency is a trait that is difficult to measure and as such is difficult to include in the routine progeny test evaluations. Our results show that for these kind of traits, the use of rather large contracted test herds with additional recording is a viable option. This strategy would give close to similar accuracy of genomic selection as recording this trait in the whole female population. This implies that the male selection, which is the most intense selection, would be as effective with contracted test herds of genotyped females as when a routine progeny test would be performed for this trait, as long as a sufficient number of cows (4,000) is included in the test herds.

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4. PAPER II:

Milk mid-infrared spectral data as a tool to predict feed intake in lactating Norwegian Red dairy cows

S. E. Wallén, E. Prestløkken, T. H. E. Meuwissen, S. McParland and D. P. Berry

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Milk mid-infrared spectral data as a tool to predict feed intake in lactating Norwegian Red dairy cows

S. E. Wallén,*[1] E. Prestløkken,* T. H. E. Meuwissen,* S. McParland,† and D. P. Berry†

*Norwegian University of Life Sciences, Dept. of Animal and Aquacultural Sciences, Ås, Norway

†Teagasc, Moorepark, Co. Cork, Ireland

[1]Corresponding author: sini.wallen@gmail.com

ABSTRACT

Mid-infrared (MIR) spectroscopy of milk was used to predict dry matter intake (DMI) and net energy intake (NEI) in 160 lactating Norwegian Red dairy cows. A total of 857 observations were used in leave-one-out cross-validation and external validation to develop and validate prediction equations using five different models. Predictions were performed using either partial least squares (PLS) regression or Best Linear Unbiased Prediction (BLUP) methods. Both methods were implemented either using just the MIR spectral information or using MIR together with milk yield (MY), body weight (BW) or NEI from concentrate (NEIconc). In the BLUP methods, the MIR spectra were always treated as random effects whereas, MY, BW and NEIconc were considered as fixed effects. Accuracy of prediction was defined as the correlation between the predicted and observed feed intake test-day records. When using the PLS method, the greatest accuracy for predicting DMI (0.54) and NEI (0.65) in the external validation dataset was achieved when using both BW and MY as predictors in combination with the MIR spectra. When using the BLUP method, the greatest accuracy of predicting DMI (0.54) in the external validation was when using MY together with the MIR spectra. The greatest accuracy of predicting NEI (0.65) in the external validation using BLUP was achieved when the model included both BW and MY in combination with the MIR spectra or when the model included both NEIconc and MY in combination with MIR spectra. However, while the linear regression coefficients of actual on predicted values for DMI and NEI were not different from unity for the PLS method, they were less than unity when using the BLUP method. This study shows that MIR spectral data can be used to predict NEI as a measure of feed intake in Norwegian Red dairy cattle and the accuracy is augmented if additional, often available data, are also included in the prediction model.

Key words: mid-infrared spectroscopy, dry matter intake, net energy intake, prediction.

INTRODUCTION

Dairy cattle breeding goals have advanced from being traditionally narrow-focused, to now being more holistic and including functional traits (Miglior et al., 2005). In the Nordic countries, health and fertility traits have been included in the breeding goal since the 1970's (Philipsson and Lindhé, 2003). Traits not currently explicitly included in most dairy cow breeding goals include feed intake, product quality, and the environmental footprint (Berry, 2015). Breeding goal traits must be economically important, must exhibit genetic variation, and should ideally be (easily) measured at a low cost in a large population of animals or, at least, should be genetically correlated with heritable traits that can be routinely measured. Improving feed efficiency is economically important because feed is a major single variable cost in dairy production (Shalloo et al., 2004). Genetic variability in feed intake (and efficiency) in dairy cows is known to exist (Svendsen et al., 1993; Berry and Crowley, 2013; Hurley et al., 2017). Thus, the main factor inhibiting the inclusion of feed intake directly in a breeding objective is routine access to phenotypic data of feed intake from a large population of animals, ideally at a low cost, to achieve a high accuracy of selection.

McParland et al. (2014) documented that feed intake in lactating dairy cows could be predicted using mid-infrared (MIR) spectrometry of milk. MIR spectroscopy is based on the study of the interaction between matter and electromagnetic waves (De Marchi et al., 2014) in the 900 to 5,000 cm^{-1} region and is routinely used globally to determine fat, protein, and lactose concentration in milk (Soyeurt et al., 2011; De Marchi et al., 2014). Since individual animal milk samples are routinely taken as part of day-to-day dairy herd management, using these samples to also predict feed intake would be a cost-effective strategy for generating data for management purposes as well as for inclusion in a breeding program. McParland et al. (2014), however, only evaluated the prediction of feed intake in lactating Holstein-Friesian dairy cows. Traditionally, the partial least squares (PLS) regression method has mainly been used for developing the prediction equations in MIR studies (McParland et al., 2011, 2012, 2014; De Marchi et al., 2014) but recently Ferragina et al. (2015) suggested the use of genomic prediction approaches for MIR-based predictions. In the present study, we wanted to validate whether MIR spectral data are suitable for predicting feed intake in lactating Norwegian Red dairy cows. Our objective was therefore to assess whether (1) MIR spectra can be used as selection criteria to achieve genetic gain in feed efficiency in Norwegian Red cattle breeding programs, and (2) if the method is accurate enough to be adopted as a monitoring tool for day-to-day herd management. We also wanted to investigate the use of the Best Linear Unbiased Prediction (BLUP) methodology, which is commonly used in genetic and genomic prediction, for the prediction of dry matter intake (DMI) and net energy intake (NEI) using MIR data.

MATERIALS AND METHODS

Data

Data from six different feeding experiments were collected from the dairy research farm at the Norwegian University of Life Sciences (NMBU, Ås, Norway) between the years

2007 and 2015, with the exception of the years 2010 and 2012 from which DMI data could not be recovered. Traits periodically available included individual cow DMI, milk yield (MY), fat, protein and lactose concentration in milk, as well as individual cow body weight (BW) (Table 1). Data were available from 204 lactations from 160 cows and the total amount of observations for each trait was 857. Estimates for each performance trait were obtained from interpolation of the actual observations for DMI, NEI, MY, fat, protein and lactose concentration in milk and BW; cubic splines with 6 knot points at 20, 70, 120, 170, 220 and 270 DIM were fitted through individual test-day records of the traits to facilitate the interpolation. After interpolation, several restrictions were implemented to improve the integrity of the data. Milk yield and milk composition records were discarded if MY was < 10 kg. Records were also excluded if fat concentration in milk was > 6.5%, total DMI was > 30 kg, or if BW was < 400 kg. Net energy intake values <55 MJ or >400 MJ were also excluded from the dataset. A total of 15 data points (i.e., 1.7% of the data) were discarded during this data editing step.

In all experiments, cows were fed timothy-grass-based silage combined with grain-based-concentrate (Appendix A). The concentrate contained mainly barley and oats supplemented with rapeseed cake and soybean meal as main protein feed. The protein concentration of the feed varied from 12 and 20% of dry matter (DM) (Appendix A). Concentrates were fed according to individual cow MY (4 of the 6 feed treatments) or in fixed amounts (2 of the 6 feeding treatments) using automatic feed stations. In all instances, silage was fed either *ad libitum* or restricted, using feed bins fitted with vertical feed gates and weighing cells underneath.

The DM of the silage was calculated based on the feed analyses. For concentrate, DM was calculated based on feed analyses, or tabulated information in the feed tables from the Nordic feed evaluation system (NorFor) (Volden et al., 2011). Individual feed intake and DM of feed were used to calculate DMI of silage and concentrate separately. Daily energy intake, expressed as NEI, was calculated based on the NorFor evaluation system and is described in detail in Appendix B. Net energy intake was calculated from the DMI of silage and concentrate separately. Net energy intake for silage (MJ/kg DM) was calculated based on the chemical composition of the feed using standard feed values in NorFor. Net energy intake for concentrate was calculated using a default DM of 86% and an energy content of 7.3 MJ/kg DM. Both DMI and NEI for silage and concentrate were summed up to get total DMI and NEI per cow.

MIR Data. Cows were milked twice daily at 0615 to 0900 h and at 1500 to 1715 h. Milk samples were collected with varying frequency for each of the different experiments (Table 2). All milk samples were conserved with Bronopol (2-bromo-2-nitropropane-1,3-diol) and then stored at 4°C. All milk samples were analyzed using the same MIR spectrometer (MilkoScan FT6000; Foss Electric A/S, Hillerød, Denmark) and the resulting spectra were stored. The absorption of infrared light through the milk sample at wavelengths in the 900 to 5,000 cm^{-1} region is represented by 1,060 data points in the Foss MIR spectrum. Mid-infrared wavelength regions known to be related to water absorbance were not considered in the analysis (Zimmermann and Kohler; 2013). Preliminary tests of alternative wavelength regions yielded the most suitable wavelength regions for each of the two traits. For NEI, the wavelength regions of 926-1601, 1701-1805, and 2693-3069 cm^{-1} were used which reflect the protein and fat regions. For DMI, the regions used were between 926-1593, between 1745-3061, and between 3781-5149 cm^{-1}.

In the experiments carried out between the years 2007 and 2014, spectral data were only available from a composite morning and evening milk sample. In the experiment undertaken in the year 2015, spectral data were taken separately from the morning and evening milkings. A weighted average of fat, protein and lactose concentration as well as each spectrum wavelength was therefore calculated for data collected in the year 2015 so the data from all years was comparable. Each wavelength value of the spectrum was scaled such that the mean and standard deviation of each of the wavelength were 0 and 1, respectively. As a separate treatment, Savitzky-Golay smoothing was applied to the untreated MIR spectral data in order to smooth a signal by fitting a polynomial to a sliding window of MIR data. Different degrees of polynomial and window sizes were tested using the Unscrambler X program (Version 10.3, Camo Software AS, Oslo, Norway).

Development and Validation of Prediction Equations

Prediction models were developed using leave-one-out cross-validation. In this approach, a single observation was held out, one-by-one, as a single-element test set while all the other observations were included in the calibration set. This was iterated until every sample had been predicted once. To obtain a more appropriate representation of the accuracy and robustness of the prediction equations, external validation was also performed. This was achieved by randomly stratifying animals into five external validation datasets ensuring that all the records of a given animal appeared only in one validation set, so that the data of an animal was never present in both the calibration and validation dataset at the same time; observations in each external validation dataset were then predicted using the model developed from only the observations in the respective calibration dataset.

Prediction equations were developed and cross-validated using five different model constructed: (1) using observations of MY, fat, protein and lactose concentration in milk (with no MIR data), (2) using only the MIR spectral information, (3) using the observation of both MY and the associated MIR spectral information, (4) using the observation of both MY and the associated MIR spectral information as well as the BW of the cow, and (5) using the observation of both MY and the associated MIR spectral information as well as the energy intake from concentrate (NEIconc). Models 1 to 4 were used to predict both DMI and NEI while model 5 was used to predict only NEI. The same dataset was used for all model construction and validation.

Model 1 was implemented as a multiple linear regression model. For models 2-5, two alternative statistical approaches were used to predict either NEI or DMI: partial least squares (PLS) regression (PROC PLS; SAS Institute Inc., Cary, NC) and BLUP. BLUP was implemented using the same approach as in genomic prediction except that marker loci were replaced by the wavelengths of the spectra (Meuwissen et al., 2001). In BLUP, the MIR spectra wavelengths were always treated as random effects; when fitting models 3 to 5, MY, BW and NEIconc were always considered as fixed effects. Matlab (Matlab (R), The MathWorks, Inc. R2016a) scripts were used to perform all BLUP analyses. The model for the BLUP analysis was:

$$y = Xb + Zu + e$$

where y is a vector of NEI or DMI records which were scaled such that the mean and standard deviation of each trait were 0 and 1, respectively; b is a vector of fixed effects containing an overall mean, and where the appropriate additional effects of MY, BW and NEIconc were scaled such that the mean and standard deviation of each trait were 0 and 1, respectively; X is the design matrix containing columns of covariates for the effects in b; Z is a matrix of scaled MIR spectra; and u are the random effects of the spectral wavelengths with $\mathrm{Var}(u)=I\sigma_u^2$; and e is a vector of residuals with $\mathrm{Var}(e)=I\,\sigma_e^2$. The variance components σ_u^2 and σ_e^2 are unknown, but solving the mixed model equations in order to obtain solutions for b and u only requires knowing the ratio of the variances $\lambda=\sigma_e^2/\sigma_u^2$. Different values of λ were tested on the dataset and the λ value which resulted in the greatest accuracy of prediction in the external validation were chosen separately for each trait and model. The impact of different values of λ on leave-one-out cross-validation was also considered.

The square root of the coefficient of determination from the regression model of true on predicted values (i.e. the correlation between true and predicted values) was used as a measure of the accuracy (R) of prediction. In the PLS regression analysis, a variable number of explanatory factors is used to explain the maximum amount of variation of the correlated wavelength values (as well as their correlation with the dependent variable). Increasing the maximum number of explanatory factors permitted in the prediction models can improve the accuracy of cross-validation but may reduce the accuracy of prediction in external validation. Determining the maximum number of explanatory factors in the model was achieved by visually inspecting the changes in R for leave-one-out cross-validation and external validation. When undertaking the external validation of the PLS analysis, model performance was also assessed by the mean bias of prediction as well as both the root mean square error (RMSE) of prediction and the linear regression coefficient (b) of true values on their respective predicted values using simple least squares regression.

RESULTS

The mean phenotypic values for the different performance traits of the 160 cows, on days where MIR data were also available, are summarized in Table 1. Average daily DMI and NEI were 19.8 kg and 125.6 MJ, respectively. The average NEI lactation profile on days with MIR spectral data is in Figure 1. When including just MY, fat, protein and lactose concentration in milk in the model, the prediction accuracy was lower in the external validation were R was 0.57 for NEI and 0.49 for DMI compared to R of cross-validation (0.59 and 0.52, respectively). For this model, the average linear regression coefficient of actual on predicted NEI and DMI in the external validation was not different from 1. Also, the mean bias for NEI and DMI in the external validation for this model was not different from 0 indicating unbiased prediction. Using smoothed MIR wavelengths did not improve the prediction accuracies; reported results therefore only relate to the analyses using the untreated spectra.

Partial Least Squares Regression (PLS)

The accuracy of predicting DMI and NEI from PLS is presented in Table 3. The accuracy of prediction for the traits was dependent on the maximum number of explanatory factors permitted in the prediction equations (Figure 2.) The greatest accuracy of predicting NEI when using only MIR spectral information in the leave-one-out cross-validation was achieved when the maximum number of permitted factors in the prediction equations was between 10 and 20 and in the external validation when the maximum number of permitted factors was between 12 and 20. The number of prediction factors in the PLS models resulting in the greatest accuracy of prediction were chosen. The number of prediction factors used in the PLS models ranged between 6 and 8 depending on the trait and model (Table 3). The R of predicting DMI and NEI was generally lower for external validation compared to cross-validation.

The R of prediction using the external validation ranged from 0.38 to 0.54 for DMI and from 0.49 to 0.65 for NEI. Compared to the model with spectral information only (i.e., Model 2), including MY in the prediction model increased the R of predicting DMI by 0.14 units in both the cross-validation and external validation and the R of NEI by between 0.13 (cross-validation) and 0.14 (external validation) units (Table 3). The greatest accuracy of predicting DMI (0.54) and NEI (0.65) in the external validation was achieved when both BW and MY were included in the model as predictors, together with the MIR spectra (i.e., Model 4).

The average linear regression coefficients of actual on predicted NEI and DMI in the external validation were not different from 1, which indicates that predicted differences in NEI and DMI were close to the actual values (Table 3). The mean bias for NEI and DMI in the external validation was not different from 0 indicating unbiased prediction.

Best Linear Unbiased Prediction (BLUP)

The accuracy of the BLUP predictions of DMI and NEI is presented in Table 4. The R of predicting DMI and NEI was lower in the external validation dataset compared to the cross-validation dataset. The R of prediction using the external validation ranged from 0.30 to 0.54 for DMI and from 0.50 to 0.65 for NEI. Compared to the model with spectral information only (i.e., Model 2), including MY in the model (i.e., Model 3) increased the R of predicting DMI by between 0.07 (cross-validation) and 0.14 (external validation) units and the R of predicting NEI by between 0.07 (cross-validation) and 0.13 (external validation) units (Table 4). The greatest accuracy for predicting DMI in the external validation (0.54) was achieved when predicted from the MIR spectra and MY combined (i.e., Model 3). Treating BW as a fixed effect (i.e., Model 4) when predicting DMI resulted in considerable less accuracy especially in the external validation than using models 2 and 3. The greatest accuracy for predicting NEI in the external validation (0.65) was achieved when using a model including BW, MY and MIR spectra (i.e., Model 4) or using a model that included NEIconc, MY and MIR spectra (i.e., Model 5).

The linear regression coefficients of actual on predicted NEI and DMI in the external validation were all less ($P<0.01$) than 1, irrespective of the model and the mean bias of prediction were also sometimes different ($P<0.05$) from 0 indicating biased prediction in some models (Table 4). Based on the regression coefficient and the bias, the best model for predicting NEI using BLUP included BW, MY and the MIR spectra and for DMI the best model included MY together with MIR spectra.

DISCUSSION

The objective of the present study was to predict NEI and DMI as measures of feed intake in lactating Norwegian Red cows using milk MIR spectral data, sometimes accompanied in the prediction model by other often available data. Accurate and easily assessable information about individual cow feed intake and efficiency could help manage feed costs and might also enable genetic improvement of feed efficiency by providing large scale data for breeding value evaluation. Wallén et al. (2017) showed that in order to genetically improve feed efficiency using genomic selection, 4,000 additional genotyped and phenotyped heifers are needed to be added to the reference population annually. Generating such large quantities of low-cost feed efficiency measurements is challenging. Hence considerable research has focused on solving this conundrum, one of which is to pool data from different populations (de Haas et al., 2012; Veerkamp et al., 2013). Furthermore, McParland et al. (2011) proposed that MIR spectral data may be useful to predict energy intake since fat-to-protein ratio and milk fatty acid composition, which are reported to be associated with energy balance (Reist et al., 2002; Friggens et al., 2007), are already predicted from MIR spectra. McParland et al. (2011) successfully related milk MIR spectral data to both body energy status and energy intake in lactating Holstein dairy cows. The hypothesis therefore for the present study was that MIR spectral data could be used as a predictor of NEI and DMI; of particular interest was the marginal improvement in predictive ability by considering data from potentially informative traits often available at the time of milk sampling but also the usefulness of BLUP approaches in improving the predictive ability over the commonly used PLS approaches. Moreover, studies to-date have been confined to

Holstein-Friesian cows but Norwegian Red cows predominate in Norway and the ability of milk MIR to predict intake in this breed is currently unknown.

The Prediction Equations

The number of explanatory factors permitted in the prediction model affected the resulting prediction accuracy in the present study. This is in line with the results from both McParland et al. (2011) who predicted body energy status in Holstein cows using PLS on milk MIR and Martínez et al. (2017) who studied a model selection criterion for PLS regression using simulations. In the present study, the impact was greater in the external validation dataset than in the cross-validation datasets. Too few explanatory factors in the model contributed to a reduction in R most likely due to an over-simplified prediction model; on the contrary, too many permitted explanatory factors also resulted in a reduction in R in external validation, probably due to an over-parameterized model. The optimum number of permissible factors in the present study appears to be 10-12 when predicting NEI (Figure 2). Whereas, the optimum number of permissible factors in the study of McParland et al. (2011) was 20 for body energy status when predictions were across lactation. For this reason, the maximum number of explanatory factors in the prediction models was limited to 12 in the present study which was lower than the maximum number of explanatory factors permitted of 20 in the study of McParland et al. (2011), and is also greater than the 16 factors permitted by Soyeurt et al. (2011) who also used PLS but in the prediction of milk fatty acids from milk MIR in dairy cows.

The results in the present study regarding smoothing agree with those of McParland et al. (2011) who used MIR of milk to predict animal-level phenotypes but contradict with those of Soyeurt et al. (2011) who predicted milk fatty acid composition. The apparent discrepancy between studies could simply be due to whether or not the MIR data originated from one or multiple spectrometers; Soyeurt et al. (2011) exploited data from multiple spectrometers while only data from one spectrometer was used by both McParland et al. (2011) and in the present study.

Comparison between PLS and BLUP methods

Partial least squares methods have traditionally been used to develop the prediction equations in dairy cows from milk MIR (McParland et al., 2011, 2012, 2014; De Marchi et al., 2014). Best Linear Unbiased Predictions approaches, however, are the norm in genetic and genomic evaluations. Because one of the objectives of the present study was to develop prediction models for possible use in breeding programs, consideration was given to whether BLUP approaches could also be used to predict NEI and DMI thus simplifying the pipelines used. Furthermore, since BLUP provides the opportunity to treat some of the predictors as fixed effects (i.e., MY and BW), and there is strong prior knowledge to indicate these are informative in predicting feed intake (Berry and Crowley, 2013; McParland et al., 2014), we expected that BLUP could also result in improved prediction accuracy relative to PLS. Although R in cross-validation was best for BLUP, the R of both BLUP and PLS was similar in the external validation (Tables 3 and 4). Worryingly, however, was the evidence of mean bias and a linear regression coefficient of the actual values on BLUP-predicted values differing from unity; such biases were not evident for PLS. McParland et al. (2011) reported a linear regression coefficient of the actual values of energy balance on PLS-predicted values not differing from unity. However, the linear regression coefficient of actual on predicted energy content was different from unity (0.77-0.83 ± 0.06). McParland et al. (2011) also reported a biased prediction for energy content. Mean biases however are not necessarily a big issue for genetic evaluations since genetic evaluations are all undertaken within contemporary group and in doing so remove the mean contemporary group effect from the individual records. One possible reason why BLUP resulted in biased predictions is that λ was chosen based on external-validation R values and was not estimated using the variance components. Thus, based on our results PLS seems to be a better method for predicting feed intake using milk MIR than BLUP.

Improving Feed Efficiency

In the present study, the most accurate model for predicting both DMI and NEI included BW, MY and MIR spectra when using PLS. Even though, BW itself is not that easily available on commercial dairy farms, it could be estimated using for example chest width since a high genetic correlation between body weight and chest width has been reported (0.75-0.86; Veerkamp and Brotherstone., 1997). It is clear that using only MIR spectra in the model does not result in high prediction accuracy for NEI or DMI; in fact the accuracy of prediction using just the MIR data was always inferior to a model that used just MY, fat, protein and lactose concentration. The use of MY and BW together with MIR spectra in the prediction model increased the prediction accuracy considerably and resulted R of 0.65 for NEI in the external validation which is however, lower compared to the study of McParland et al. (2014) where PLS was used to predict effective energy intake using just MY and milk MIR resulting R of 0.70 in the external validation. The differences in the results between McParland et al. (2014) and the present study could be due the fact that within each experiment, the cows did not receive exactly the same diet and the DM content of feed differed between different experiments, which could have an impact to the relationship between MIR and DMI/NEI in the present study.

The prediction accuracy of 0.65 (external validation) for NEI seems promising, especially if we consider that energy intake is a phenotype which itself encompasses recording errors (McParland et al., 2011). For instance, diurnal variation may also exist in energy intake

(McParland et al., 2011), which makes the maximum achievable prediction accuracy less than 100% (possibly 75% for body energy status according to McParland et al., 2011 and 70% for energy intake according to McParland et., 2014). McParland and Berry (2016) reported heritable genetic variability for energy intake, energy balance and residual energy intake predicted using milk MIR and reported that phenotypic differences in energy intake existed among animals which were stratified based on their EBV for energy intake predicted from MIR. Hence, genetic improvement of feed efficiency could be possible. Feed intake itself is the actual gold standard trait and can be used when actual feed intake data are available. Hence, as more actual feed intake data accumulates for an animal or a sire, the importance of the MIR predicted feed intake diminishes. Nonetheless, the accuracy of prediction of feed intake from daughter phenotypes can never be greater than the genetic correlation between the predicted phenotypes and the actual feed intake.

Results from the present study suggest that MIR spectral data can be used to predict NEI as a measure of feed intake in Norwegian Red dairy cattle. When using PLS with 8 factors, the most accurate model of predicting NEI included in BW, MY and MIR spectra as predictors. The BLUP predictions yielded similar accuracies but were biased. Nonetheless, no matter which feed efficiency measure would be chosen, before including the trait in the breeding program, genetic correlations between predicted feed intake, actual feed intake and other performance traits, especially health and fertility traits need to be estimated to derive the selection index weights. The use of MIR spectra data to predict energy intake could also be adopted as a monitoring tool for day-to-day herd management since the milk samples are taken routinely in dairy production.

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Table 1. Phenotypic records[1] at the days with milk mid-infrared spectral information for 160 cows and 204 lactations.

Trait	Mean	s.d.
DMI (kg/d)	19.83	3.52
MY (kg/d)	24.79	5.93
Fat %	4.37	0.55
Protein %	3.42	0.28
Lactose %	4.65	0.18
BW (kg)	557.38	54.91
NEI (MJ/d)	125.58	22.00

[1] DMI=dry matter intake, MY=milk yield, Fat=fat concentration in milk, Protein=protein concentration in milk, Lactose=lactose concentration in milk, BW=body weight, NEI=net energy intake, Mean=the mean value of each trait, s.d.=standard deviation for each trait.

Table 2. Summary[1] of feeding experiments.

Feeding experiment	Length of test period (d)	No. of cows	No. of collected rec.	Frequency
A	127	33	33	1.00
B	109	43	119	2.77
C	84	14	14	1.00
D	109	22	61	2.77
E	371	44	128	2.91
F	54	48	502	10.46

[1] Length of test period=length of the test period in each feed experiment, No. of cows=total number of cows within each feed experiment, No. of collected rec.=total number of collected records for dry matter intake, milk yield, fat, protein and lactose concentration in milk, body weight and net energy intake within each feeding experiment, Frequency=how frequently records were collected per cow and within each feeding experiment.

Table 3. Average number of factors (Fac), root mean square error (RMSE), correlation coefficient (R), mean bias (SE in parentheses),[a] and slope (b; SE in parentheses)[b] obtained from predicting dry matter intake (DMI) and net energy intake (NEI) using centered and scaled mid-infrared (MIR) spectra, tested using leave-one-out cross-validation and external validation methods in partial least squares regression analysis

Trait		Leave-one-out cross-validation			External validation				
		Fac	RMSE	R	Fac	Bias (SE)[a]	b (SE)[b]	RMSE	R
DMI (kg/d)	Model 2	6	3.15	0.45	6	0.01 (0.11)	0.88 (0.16)	3.27	0.38
	Model 3	8	2.84	0.59	8	-0.01 (0.10)	0.88 (0.11)	3.01	0.52
	Model 4	7	2.84	0.59	7	-0.02 (0.10)	0.90 (0.11)	2.98	0.54
NEI (MJ/d)	Model 2	6	18.49	0.54	6	0.16 (0.65)	0.95 (0.12)	19.17	0.49
	Model 3	8	16.35	0.67	8	-0.16 (0.59)	0.95 (0.09)	17.15	0.63
	Model 4	8	16.02	0.69	8	-0.17 (0.57)	0.94 (0.08)	16.83	0.65
	Model 5	8	16.13	0.68	8	0.04 (0.58)	0.94 (0.09)	17.03	0.63

Model 2: Using only MIR spectral information as predictors.

Model 3: Using observations of milk yield together with MIR spectral information as predictors.

Model 4: Using observations of body weight and milk yield together with MIR spectral information as predictors.

Model 5: Using observations of energy intake from concentrate and milk yield together with MIR spectral information as predictors.

[a] Average difference between predicted values and true values in external validation dataset.

[b] Linear regression coefficient of true value on predicted value.

Table 4. Lambda (λ), root mean square error (RMSE), correlation coefficient (R), mean bias (SE in parentheses),[a] and slope (b; SE in parentheses)[b] obtained from predicting dry matter intake (DMI) and net energy intake (NEI) using centered and scaled mid-infrared (MIR) spectra, tested using leave-one-out cross-validation and external validation methods in BLUP analysis[c]

Trait			Cross-validation		External validation			
		λ	RMSE	R	Bias (SE)[a]	b (SE)[b]	RMSE	R
DMI (kg/d)	Model 2	4	2.93	0.56	0.18 (0.11)	0.61 (0.02)	3.25	0.40
	Model 3	20	2.74	0.63	0.24 (0.10)	0.81 (0.01)	2.96	0.54
	Model 4	20	2.96	0.54	0.49 (0.12)	0.49 (0.02)	3.40	0.30
NEI (MJ/d)	Model 2	4	17.10	0.63	0.95 (0.65)	0.66 (0.02)	19.14	0.50
	Model 3	10	15.77	0.70	1.08 (0.59)	0.89 (0.01)	17.18	0.63
	Model 4	10	15.49	0.71	0.99 (0.57)	0.93 (0.01)	16.78	0.65
	Model 5	4	15.43	0.71	1.02 (0.57)	0.90 (0.01)	16.78	0.65

[c] λ=Error variance/variance of the spectra, Cross-validation=Leave-one-out cross-validation, BLUP=Best Linear Unbiased Prediction, In models 3-5, milk yield, body weight and net energy intake from concentrate are treated as fixed effects.

Model 2: Using only MIR spectral information as predictors.

Model 3: Using observations of milk yield together with MIR spectral information as predictors.

Model 4: Using observations of body weight and milk yield together with MIR spectral information as predictors.

Model 5: Using observations of energy intake from concentrate and milk yield together with MIR spectral information as predictors.

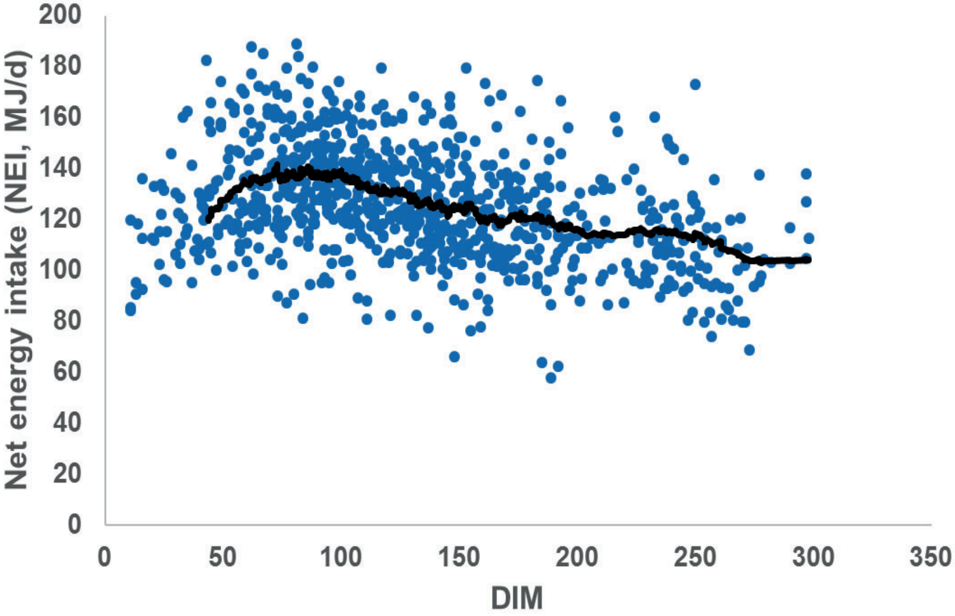

Figure 1. Lactation profile of the net energy intake (NEI) at days with milk mid-infrared spectral recordings with an average trend line; DIM = days in milk.

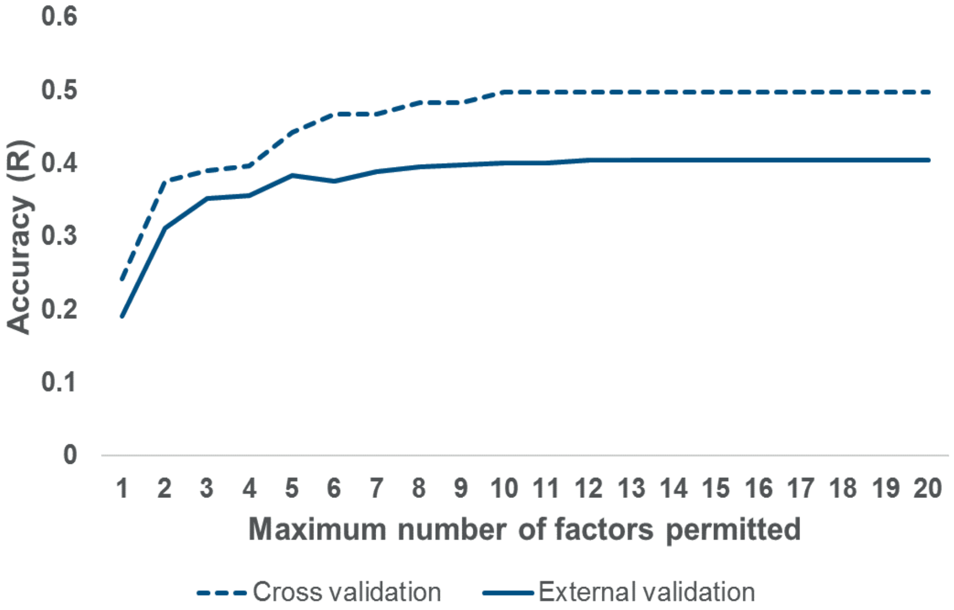

Figure 2. Influence of maximum number of predictive factors permitted in the calibration equation on the accuracy (R) of the leave-one-out cross-validation and the external validation when predicting net energy intake using only milk mid-infrared spectral information.

APPENDIX A

Table A1. Description of the diets[1] in the feed experiments.

Diet[a]	N	Type of feed	DM (g/kg)	Ash (g/kg DM)	OMD (%)	CP (g/kg DM)	Cfat (g/kg DM)	NDF (g/kg DM)	iNDF (g/kg NDF)	Starch (g/kg DM)	NE (MJ/kg DM)
A (2007)	33	Grass silage	297.3	69.7	75.4	141.3	30.9	537.0	143.7	.	6.18
		Concentrate	924.5	77.5	80.1	202.5	60.9	198.5	322.0	328.0	7.46
B (2008)	43	Grass silage	260.0	65.1	79.2	147.0	34.5	479.0	105.5	.	6.68
		Concentrate	880.0	56.6	85.0	159.1	44.5	181.8	322.5	435.5	7.11
C (2009)	14	Grass silage	289.0	79.0	75.0	161.5	53.5	466.0	125.0	12.0	6.15
		Concentrate	907.0	43.0	76.0	122.0	26.0	178.0	250.0	534.0	7.80
D (2011)	22	Grass silage	283.0	78.0	74.3	123.0	52.0	487.0	104.0	.	6.20
		Concentrate	875.0	42.0	82.0	163.0	81.0	287.0	350.0	330.0	7.70
		Addit. Feed	879.5	214.4	67.5	87.0	28.8	158.8	275.0	400.0	.
E (2013-2014)	44	Grass silage	336.5	64.3	76.5	152.0	39.5	495.5	137.5	.	6.52
		Concentrate	883.5	103.0	83.5	200.5	57.5	187.5	180.0	333.5	7.20
F (2015)	48	Grass silage	367.3	65.0	72.9	111.3	20.1	551.9	151.6	36.1	6.29
		Concentrate	864.0	57.3	77.4	151.0	43.0	219.1	180.0	391.0	6.82

[1] N=number of cows, Addit. feed=additional feed, DM=dry matter in feedstuff, OMD=apparent total digestibility of organic matter, CP=crude protein in feedstuff, Cfat=crude fat in feedstuff, NDF=neutral detergent fibre in feedstuff, iNDF=indigestible NDF in feedstuff, NE=net energy content.

[a] Name of the diet and the year(s) when feed experiment was carried out.

APPENDIX B

Formulas used to calculate energy intake based on NorFor (Volden et al., 2011).

Gross energy intake (GEI):

$$GEI = \frac{24.1 * \sum_i (DMI_i * CPcorr_i) + 36.6 * \sum_i (DMI_i * CFat_i) + 18.5 * \sum_i (DMI_i * \left(OM_i - CPcorr_i - CFat_i - \frac{CP_i}{6.25} * \frac{NH3N_i}{1000}\right))}{1000}$$

where GEI is expressed as MJ/d, and for feedstuff i, DMI_i is the dry matter intake (kg/d), $CPcorr_i$ is the content of ammonia- or urea-corrected crude protein, $CFat_i$ is the crude fat content (g/kg DM), OM_i is the organic matter content (g/kg DM), CP_i is the crude protein content (g/kg DM) and $NH3N_i$ is the ammonia or urea N content (g/kg CP), (i=1,...,n).

Metabolizable energy intake (MEI):

$$MEI = \frac{18.0 * tdCPcorr + 37.7 * tdCFat + 14.5 * (tdCHO - \sum_i DMI_i * SU_i) + 13.9 * \sum_i DMI_i * SU_i}{1000}$$

where MEI is expressed as MJ/d, DMI_i is the dry matter intake of the i=1...n'th feedstuff (kg/d), SU_i is the sugar content in the i=1...n'th feedstuff (g/kg DM), tdCPcorr is the total tract digestion of ammonia- or urea-corrected crude protein, tdCFat is the total tract digestion of crude fat, and tdCHO is the total tract digestion of carbohydrates.

Net energy intake (NEI):

In NorFor, NEI is based on the equations of Van Es (1975; 1978). NEI per individual was calculated based on metabolizable and gross energy of feed:

$$NEI = 0.6 * \left(1 + 0.004 * (q - 57)\right) * MEI$$

where NEI is expressed as MJ/d, q is the ratio (%) between metabolizable energy and gross energy.

5. PAPER III:

Merging of feed intake and milk infrared spectral data across countries increases the accuracy of prediction of feed intake in lactating dairy cows

S. E. Wallén, S. McParland, D. P. Berry, E. Prestløkken, and T. H. E. Meuwissen

Manuscript

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Merging of feed intake and milk infrared spectral data across countries increases the accuracy of prediction of feed intake in lactating dairy cows

S. E. Wallén,[*] S. McParland,† D. P. Berry,† E. Prestløkken,[*] and T. H. E. Meuwissen[*1]

[*]Norwegian University of Life Sciences, Dept. of Animal and Aquacultural Sciences, Ås, Norway

†Animal and Grassland Research and Innovation Centre, Teagasc, Moorepark, Co. Cork, Ireland

[1]Corresponding author: theo.meuwissen@nmbu.no

ABSTRACT

Mid-infrared (MIR) spectroscopy of milk was used to predict net energy intake (NEI) in 160 lactating Norwegian Red dairy cows and effective energy intake (EEI) in 375 lactating Holstein-Friesian dairy cows. A total of 857 observations were used in NEI predictions and 1341 observations in EEI predictions when split-sample cross-validation and external validation methods were used to develop and validate prediction equations using four different models. Predictions were performed using either partial least squares (PLS) regression, multiple linear regression (MLR) or Best Linear Unbiased Prediction (BLUP) methods. The methods were implemented using the MIR spectral information within country or across two countries. Best Linear Unbiased Predictions were implemented either as a single trait or a multi-trait method. Accuracy of prediction was defined as the correlation between the predicted and observed energy intake test-day records. Combining the spectra from two countries, increased the R of predicting EEI by 0.02 units in both the cross-validation and the external validation compared to the model with spectral information from one country only. Whereas for NEI, combining the spectra from two countries decreased the prediction accuracy in the cross-validation by 0.02 units and had no effect in R in the external validation. When predicting NEI using only the MIR spectral information, it was more beneficial to use single trait BLUP method compared to PLS. Both single trait and multi-trait BLUP methods resulted greater R for EEI compared to PLS models including MIR spectra from one country only. This study shows that MIR spectral data from two countries can be combined and used to predict energy intake as a measure of feed intake in dairy cattle.

Key words: mid-infrared spectroscopy, pooled data, effective energy intake, net energy intake, prediction.

INTRODUCTION

Generating accurate genetic (and genomic) evaluations for any trait is predicated on routine access to phenotypic data on the trait itself or predictors of the trait. Estimated breeding values for feed intake in ruminants in particular suffer from lower than desired accuracy prompting interest in (low-cost) tools to phenotypically predict feed intake (McParland et al., 2014; Wallén et al., submitted). Using a population of 160 lactating Norwegian Red dairy cows, Wallén et al. (submitted) reported an accuracy of predicting energy intake of 0.65 based on milk mid-infrared (MIR) spectroscopy data; similarly, McParland et al. (2014) reported an accuracy of prediction of energy intake in 378 lactating Holstein-Friesian of 0.70 from milk MIR. The main advantage of exploiting milk MIR data is that an infrared spectrum could theoretically be generated on an individual cow basis at each milking (usually twice daily) thus providing a rich data source not only for use in genetic evaluations but also for day-to-day cow and herd management.

The accuracy and robustness of prediction models, however, is a function of how the variation in the data used to generate the model parameters relates to the sample population in which it will be used (Habier et al., 2010; Pszczola et al., 2012). Therefore, combining data from different feed systems when generating prediction models could potentially improve the applicability of the prediction model to a greater range of end-users. Moreover, some populations may not actually have access to gold standard feed intake data to generate the equations and in such situations, the transferability of prediction equations developed elsewhere in a different population is of interest. The objective therefore of this study was to quantify the transferability of MIR-based prediction equations for feed intake between two populations differing in breed and feeding system but also the benefit, if any, of combining data from two populations in the pursuit of more accurate predictions of feed intake. We also wanted to investigate the use of the Best Linear Unbiased Prediction (BLUP) method, which is commonly used in genetic and genomic prediction, for the prediction of energy intake traits using MIR data.

MATERIALS AND METHODS

Norwegian Data

Data from six different feeding experiments were collected from the dairy research farm at the Norwegian University of Life Sciences (NMBU, Ås, Norway) between the years 2007 and 2015, with the exception of the years 2010 and 2012, inclusive from which dry matter intake (DMI) data could not be recovered. Traits periodically available included individual cow DMI (kg/d) and milk yield (MY, kg/d). Estimates for each performance trait were obtained from interpolation (using cubic splines, detailed information in Wallén et al., submitted) of the actual observations for DMI, net energy intake (NEI), and MY. After interpolation, several restrictions were implemented to improve the integrity of the data. Milk yield records were discarded if MY was < 10 kg and DMI records were excluded if total DMI was > 30 kg. Net energy intake values < 55 MJ or >400 MJ were also excluded from the dataset. Detailed information about the data structure can be found in Wallén et al. (submitted). Data (n=857 records) were available from 204 lactations from 160 cows.

In all experiments, cows were fed timothy-grass-based silage combined with grain-based-concentrate (Appendix A in Wallén et al., submitted). Concentrates were fed according to individual cow milk yield (4 of the 6 feed treatments) or in fixed amounts (2 of the 6 feeding treatments) using automatic feed stations. In all instances, silage was fed either *ad libitum* or restricted, using feed bins fitted with vertical feed gates and weighing cells underneath.

Silage DM was calculated based on feed analyses. Concentrate DM was calculated based on feed analyses, or tabulated information from the Nordic feed evaluation system feed tables (NorFor; Volden et al., 2011). Dry matter intake of silage and concentrate were calculated separately and summed. Individual energy intake, expressed as NEI (MJ/kg DM), was calculated separately for concentrate and silage based on the NorFor evaluation system as described in detail in Appendix B in Wallén et al. (submitted); the NEI of concentrate and silage was then summed.

Irish Data

Data from a range of grazing studies (Ganche et al., 2013a,b; McCarthy et al., 2014) were collected from an Irish dairy research herd, located at the Teagasc Animal and Grassland Research and Innovation Center (Moorepark, Fermoy, Co. Cork, Ireland) between the years 2008 and 2013, inclusive. Milk production was routinely recorded weekly on all animals. Detailed information about the data structure can be found in McParland et al. (2014). Data (n=1,341 records) were available from 532 lactations of 375 cows.

The basal diet of the majority of cows was grazed grass. Individual cow DMI was periodically recorded at grass using the *n*-alkane technique and fecal crab samples (Dillon and Stakelum, 1989; Kennedy et al., 2008). In addition, a subset of cows (n = 25) were fed a TMR diet of maize silage, grass silage, soybean meal, and dairy concentrate (Moore et al., 2014). Individual DMI of these 25 cows was recorded daily. The ME content of the grass was assumed to be 0.0157 × digestible OM of grass (AFRC, 1993). Grass ME intake was summed with the ME content of the concentrate fed. This information was used to compute effective energy intake (EEI, MJ/d) according to the formulae of Coffey et al. (2001).

MIR data in both countries

In both countries, cows were milked twice daily between 0615 and 0900 h and between 1500 and 1715 h. Once weekly (Irish samples) or 1-10 times per experiment (Norwegian samples, detailed information in Wallén et al., submitted), milk samples were collected and analyzed using a MIR spectrometer (MilkoScan FT6000; Foss Electric A/S, Hillerød, Denmark). The FOSS FTIR spectrum contains 1,060 data points representing the absorption of infrared light through the milk sample at wavelengths in the 900 to 5,000 cm^{-1} region. The Norwegian data set comprised spectral data from composite morning and evening milk between the years 2007 and 2014. However, separate morning and evening milk spectral data were available in 2015. Therefore, the average of each spectral data point weighted by its corresponding milk yield was calculated for those samples. The Irish data set comprised spectral data from separate morning and evening milkings hence, a plain average of each spectrum wavelength was calculated.

The Savitzky-Golay 1st derivative method was applied to MIR spectra of both countries to bring all spectra to a common baseline and to sharpen absorbance bands. Different degrees of polynomial and window sizes were tested using the Unscrambler X program (Version 10.5, Camo Software AS, Oslo, Norway).

Development and Validation of Prediction Equations

MIR wavelength regions known to be related to water absorbance were not considered in the analysis (Zimmermann and Kohler; 2013). The wavelength regions of 926-1641, 1701-2201 and 2637-3117 cm^{-1} were used to predict NEI and EEI. These regions represent a combination of the wavelengths used in the prediction equations of NEI (Wallén et al., submitted) and EEI (McParland et al., 2014).

Prediction models were developed using split-sample cross-validation. In this approach, every 20th observation were held out as a test set and predicted using a model developed from the data remaining in the calibration dataset. This was iterated until all the observation in each test set had been predicted once. To obtain a more appropriate representation of the accuracy and robustness of the prediction equations, external validation was also performed. This was achieved by randomly stratifying animals into five external validation datasets ensuring that all repeated records of a given animal appeared only in one validation set. Remaining samples were used as calibration data and used to develop the equations to predict samples in each external validation data set.

Prediction equations for NEI and EEI were developed and validated either using MIR spectra within country (Models 1-2) or across countries (Models 3-6).

Within Country Models. Unprocessed within country MIR spectral information was used as predictors in the Model 1 and the first derivative of the within country MIR spectra were used in the Model 2. For models 1-2, partial least squares (PLS) regression (PROC PLS; SAS Institute Inc., Cary, NC) method was used.

Across Country Models. For the Model 3, PLS scores (i.e. latent factors that account for most of the variation in the spectra) were calculated based on the across country MIR spectra. Model 3 was implemented as a multiple linear regression (MLR) model. In the Model 4, PLS method was used for across country MIR spectral information.

Across Country BLUP Models. Across country MIR spectra were used in the Models 5 and 6. A single trait BLUP (sBLUP) method was used for Model 5 and a multi-trait BLUP (mBLUP) for Model 6. For BLUP predictions, the same approach as in genomic prediction, except that marker loci were replaced by the wavelengths of the spectra, was used (Meuwissen et al., 2001). In the multi-trait analysis, the correlation between NEI and EEI was estimated using ASReml (ASReml 4.0, Gilmour et al., 2009). The MIR spectra wavelengths were always treated as random effects in BLUP analysis.

Julia (Julia 0.4.5) scripts were used to construct the G matrix (i.e. variance-covariance matrix of animals based on the spectra data and ASReml to perform all BLUP analyses. The G matrix was calculated as G = XX’, where X is a (number of animals * number of wavelengths) matrix of MIR spectra data. The model for the sBLUP analysis was:

$$y = \mu + u + e$$

where y is a vector of NEI or EEI records which were scaled such that the mean and standard deviation of each trait were 0 and 1, respectively; μ is the mean of y, and u are the random effects of the spectral wavelengths with $Var(u)=GI\sigma_u^2$; and e is a vector of residuals with $Var(e)=I\ \sigma_e^2$. ASReml was used to estimate the variance components. The traits NEI and EEI were also analyzed as two separate traits in a bi-variate analysis using the model (subscripts 1 and 2 denote NEI and EEI, respectively):

$$\begin{bmatrix} y_1 \\ y_2 \end{bmatrix} = \begin{bmatrix} \mu_1 \\ \mu_2 \end{bmatrix} + \begin{bmatrix} u_1 \\ u_2 \end{bmatrix} + \begin{bmatrix} e_1 \\ e_2 \end{bmatrix}$$

and $Var\left(\begin{bmatrix} u_1 \\ u_2 \end{bmatrix}\right) = C \otimes G$, where C is a (2x2) matrix of (co)variances of the two traits that are explained by the spectra and ⊗ denotes the Kronecker matrix product; $Var\left(\begin{bmatrix} e_1 \\ e_2 \end{bmatrix}\right) = R \otimes I_n$, where R is a (2x2) diagonal matrix of residual variances of the two traits and I_n is a (nxn) identity matrix. The residual covariance between the traits is assumed 0, because this covariance cannot be estimated from the current data (none of the cows had recordings on both traits NEI and EEI).

Accuracy of Predictions

The square root of the coefficient of determination from the regression model of true on predicted values (i.e. the correlation between true and predicted values) was used as a measure of the accuracy (R) of prediction. In the PLS regression analysis, a variable number of explanatory factors was used to explain the maximum amount of variation of the correlated wavelength values (as well as their correlation with the dependent variable). Increasing the maximum number of explanatory factors permitted in the prediction models can improve the accuracy of cross-validation but may reduce the accuracy of prediction in external validation. When undertaking external validation, model performance was also assessed by the mean bias of prediction as well as both the root mean square error (RMSE) of prediction and the linear regression coefficient (b) of true values on their respective predicted values using least squares regression.

RESULTS

Partial Least Squares Regression (PLS) and Multiple Linear Regression (MLR)

The accuracy of predicting NEI and EEI from PLS and MLR is presented in Table 1. The R of predicting NEI and EEI was generally lower for external validation compared to cross-validation. Using unprocessed Irish spectra (i.e., Model 1) or taking the first derivative of the Irish spectra (i.e., Model 2) did not affect the accuracy of EEI in the split-sample cross-validation or in the external validation. Whereas, when the first derivative of the Norwegian spectra was used, the prediction accuracy of NEI improved, both in split-sample cross-validation and also in external validation (Table 1). The number of prediction factors in the PLS models and the number of PLS scores in the MLR models were chosen based on the prediction accuracy of the model. The number of prediction factors used in the PLS models ranged between 9 and 18 depending on the trait and model. A higher number of factors (17-18) were used to explain spectral variation related to EEI relative to NEI (9-13).

The R of prediction in external validation ranged from 0.50 to 0.51 for NEI and from 0.66 to 0.68 for EEI. Compared to the model with within country spectral information (i.e., Model 2), combining the spectra from two countries (i.e., Model 3, Figure 1) increased the R of predicting EEI by 0.02 units in both the cross-validation and the external validation. Whereas for NEI, combining the spectra from two countries decreased the prediction accuracy in the cross-validation by 0.02 units and had no effect in R in the external validation.

The linear regression coefficients and mean bias for PLS and MLR models are presented in Table 2. On average, the linear regression coefficient of actual on predicted NEI in the external validation for model 1 was not different from 1, which indicates that predicted NEI were unbiased estimations of actual NEI. All the other models for NEI and EEI resulted in linear regression coefficients that were somewhat less than 1. However irrespective of the model, the mean bias for NEI and EEI in the external validation was not different from 0 indicating unbiased prediction.

Best Linear Unbiased Prediction (BLUP)

The accuracy of the BLUP predictions of NEI and EEI is presented in Table 1. The BLUP predictions were only implemented using the MIR spectral information from both countries (i.e., Models 5 and 6) in the external validation dataset. The R of prediction was 0.67 for EEI and ranged from 0.50 to 0.53 for NEI. Compared to multi-trait BLUP (i.e., Model 6), single trait BLUP (i.e., Model 5) increased the R of predicting NEI by 0.03 units. Whereas for EEI, single trait and multi-trait BLUP resulted in similar accuracy of prediction.

The linear regression coefficients and mean bias for BLUP models are presented in Table 2.The average linear regression coefficient of actual on predicted NEI for mBLUP model was not different from 1; for EEI, both sBLUP and mBLUP models gave similar linear regression coefficients. The single trait BLUP model for NEI resulted linear regression coefficient that was somewhat less than 1.

DISCUSSION

The objective of the present study was to predict NEI and EEI as measures of feed intake in dairy cows using within country or across country milk MIR spectral data. The main objective of this study was to quantify if combining MIR spectra data from two countries would result more accurate predictions of feed intake. Having access to easily assessable and accurate information about individual cow feed intake and efficiency could help in managing feed costs and might also enable genetic improvement of feed efficiency by providing large scale data for breeding value evaluation. Wallén et al. (2017) showed that genetic improvement of feed efficiency using genomic selection requires a large quantity (i.e. 4,000) of additional genotyped and phenotyped heifers to be added to the reference population annually. However, it is challenging to have access to feed efficiency measurements in such a large quantity. To solve this problem, de Haas et al. (2012) and Veerkamp et al. (2013) suggested to pool data from different populations. Milk MIR spectral data has recently successfully been related to predict intake and related traits in Irish, UK and Norwegian dairy cows (McParland et al., 2011; McParland et al., 2014; Wallén et al., submitted). A restriction to previous studies was the size and variability of the data sets used in those studies. McParland et al. (2012) showed that equations developed in one production system were not useful to predict intake in a different system. The hypothesis therefore for the present study was that MIR spectral data from two countries could be used as a predictor of NEI and EEI; of particular interest was the usefulness of BLUP approaches in improving the predictive ability over the commonly used PLS approaches, as the multi-trait BLUP model could account for the fact that NEI and EEI are correlated traits, and not exactly the same traits.

Comparison between PLS and BLUP methods

Many earlier studies (McParland et al., 2011, 2012, 2014; De Marchi et al., 2014) focussed on partial least squares methods to develop equations to predict milk and cow from milk MIR. However, in genetic and genomic evaluations, BLUP approaches are regularly used.

Because one of the objectives of the present study was to develop prediction models for possible use in breeding programs, consideration was given to whether BLUP approaches could also be used to predict NEI and EEI when combining the MIR spectra from two countries. When predicting NEI using only the MIR spectral information, it was more beneficial to use the single trait BLUP method compared to PLS (Tables 1 and 2). In a multi-trait BLUP, there are more parameters to be estimated than in a single trait BLUP method. Estimating those parameters did not yielded greater accuracies of prediction in the present study. However, both single trait and multi-trait BLUP methods yielded greater accuracy of prediction of EEI when compared to PLS models. All the PLS and BLUP predictions were unbiased. However, some of the models had a linear regression coefficient of the actual values on PLS- and BLUP-predicted values different from unity. Our results agree with those from McParland et al. (2011) who reported a linear regression coefficient of the actual values of energy balance on PLS-predicted values not differing from unity. However, the linear regression coefficient of actual on predicted energy content was different from unity (0.77-0.83 ± 0.06). Thus, based on our results single trait BLUP seems to be a better method for predicting NEI, when only milk MIR is available, than PLS. When predicting EEI using milk MIR, calculating PLS scores and using MLR was more beneficial than either one of the BLUP methods.

Improving Feed Efficiency

In the present study, the single trait BLUP method yielded greatest accuracy of prediction for NEI when across country MIR spectral information was used as predictors. For EEI, calculating PLS scores and using MLR method yielded greatest accuracy of prediction when across country MIR spectral information was used. When EEI was predicted using MIR spectra within country, the accuracy of prediction in the external validation (0.66) in the present study was greater than in the study of McParland et al. (2014) where the R for EEI was 0.55-0.64. The differences in the results between McParland et al. (2014) and the present study could be due the fact that different MIR wavelengths were used in different studies and also the datasets used were somewhat different. The greatest accuracy of prediction for both NEI and EEI was achieved using across country MIR spectra.

Results from the present study suggest that MIR spectral data from two countries i.e. from two populations differing in breed and feeding system can be combined and used to predict energy intake as a measure of feed intake in dairy cattle. Our results also indicate that single trait BLUP could have a benefit over the PLS as a method to predict energy intake using the MIR spectra. Nonetheless, before any measure of feed intake can be included in the breeding program, genetic correlations between other performance traits, especially health and fertility traits, predicted feed intake and actual feed intake need to be estimated to derive the selection index weights. Since the milk samples are easily available in dairy production, the use of MIR spectra data to predict energy intake could be adopted as a monitoring tool for day-to-day herd management.

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Table 1. Average number of factors/scores (Fac/Score), root mean square error (RMSE), and correlation coefficient (R) obtained from predicting net energy intake (NEI, incl. 857 observations) and effective energy intake (EEI, incl. 1341 observations) of mid-infrared (MIR) spectra, tested using split-sample cross-validation and external validation methods in partial least squares (PLS), multiple linear regression (MLR) and Best Linear Unbiased Prediction (BLUP) analysis.

		Split-sample cross-validation			External validation		
Trait		Fac/ Score	RMSE	R	Fac/ Score	RMSE	R
NEI (MJ/d)	Model 1: MIR	9	17.91	0.58	9	19.16	0.50
	Model 2: dMIR	13	17.07	0.63	13	19.05	0.51
	Model 3: MLR	33	17.84	0.61	33	19.10	0.51
	Model 4: PLS				13	19.06	0.51
	Model 5: sBLUP					18.73	0.53
	Model 6: mBLUP					18.56	0.50
EEI (MJ/d)	Model 1: MIR	18	25.89	0.71	18	27.47	0.66
	Model 2: dMIR	18	25.76	0.71	17	27.60	0.66
	Model 3: MLR	75	25.80	0.73	75	26.95	0.68
	Model 4: PLS				17	27.60	0.66
	Model 5: sBLUP					27.32	0.67
	Model 6: mBLUP					26.96	0.67

Model 1: Using unprocessed MIR spectra within country as predictors.
Model 2: Using the first derivative of the MIR spectra within country as predictors.
Model 3: Using the PLS scores calculated based on across country MIR spectra as predictors.
Model 4: Using across country MIR spectra as predictors in PLS analysis.
Model 5: Using across country MIR spectra as predictors in a single trait BLUP analysis
Model 6: Using across country MIR spectra as predictors in a multi-trait BLUP analysis.

Table 2. Mean bias (SE in parentheses),[a] and slope (b; SE in parentheses)[b] obtained from predicting net energy intake (NEI, incl. 857 observations) and effective energy intake (EEI, incl. 1341 observations) of mid-infrared (MIR) spectra, tested using external validation methods in partial least squares (PLS), multiple linear regression (MLR), and Best Linear Unbiased Prediction (BLUP) analysis.

Trait		External validation	
		Bias (SE)[a]	b (SE)[b]
NEI (MJ/d)	Model 1: MIR	-0.22 (0.65)	0.89 (0.11)
	Model 2: dMIR	0.14 (0.65)	0.86 (0.10)
	Model 3: MLR	-0.07 (0.65)	0.87 (0.11)
	Model 4: PLS	0.15 (0.65)	0.86 (0.10)
	Model 5: sBLUP	-2.50E-05 (0.64)	0.91 (0.05
	Model 6: mBLUP	0.29 (0.63)	0.89 (0.12)
EEI (MJ/d)	Model 1: MIR	0.27 (0.75)	0.92 (0.06)
	Model 2: dMIR	-0.03 (0.75)	0.93 (0.07)
	Model 3: MLR	0.09 (0.74)	0.92 (0.06)
	Model 4: PLS	-0.03 (0.75)	0.93 (0.07)
	Model 5: sBLUP	1.22E-05 (0.75)	1.14 (0.03)
	Model 6: mBLUP	0.03 (0.74)	0.98 (0.07)

Model 1: Using unprocessed MIR spectra within country as predictors.
Model 2: Using the first derivative of the MIR spectra within country as predictors.
Model 3: Using the PLS scores calculated based on across country MIR spectra as predictors.
Model 4: Using across country MIR spectra as predictors in PLS analysis.
Model 5: Using across country MIR spectra as predictors in a single trait BLUP analysis
Model 6: Using across country MIR spectra as predictors in a multi-trait BLUP analysis.

[a] Average difference between predicted values and true values in external validation dataset.

[b] Linear regression coefficient of true value on predicted value.

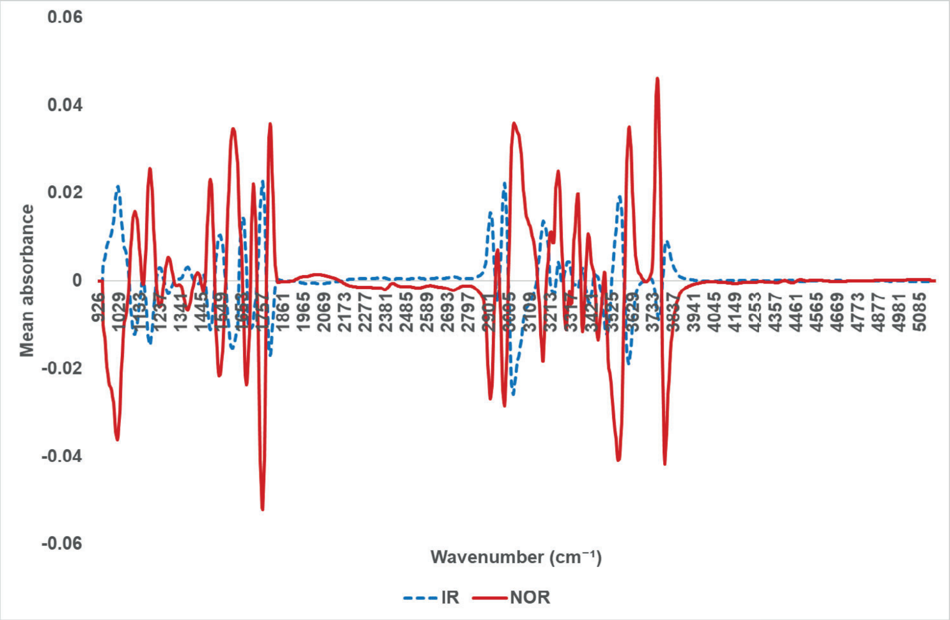

Figure 1. Mean absorbance of Irish and Norwegian mid-infrared spectra of milk.

6. General discussion

The overall aim of this thesis was to investigate the best way to include feed efficiency in dairy cattle breeding objective and what are the requirements for the inclusion. Possible ways to obtain phenotypic data for genetic improvement of FE were also investigated. Accurate and easily assessable information about individual cow feed intake and efficiency is needed to obtain large scale data for breeding value evaluation, enabling genetic improvement of feed efficiency. Having access to such a large quantity of low-cost feed efficiency measurements is however challenging.

6.1. Inclusion of feed efficiency in the breeding scheme

Including a trait in a breeding goal requires that (1) the trait is important, (2) it exhibits genetic variation, and (3) large numbers of accurate phenotypic recordings are available in order to achieve sufficient accuracy of selection. The importance of improving feed efficiency is well established and according to Hurley et al. (2017), exploitable genetic variation exists among several FE traits, and this variation is sufficiently large to justify consideration of including FE in dairy cattle breeding goals. In Paper I, different strategies of including feed efficiency in the breeding scheme of dairy cattle were tested using simulations. The change in genetic gain over time and achievable selection accuracy were studied for milk yield and residual feed intake, as a measure of feed efficiency. The general level of the genetic gains in Paper I agree with those found by Lillehammer *et al.* (2011). Genetic gain of the trait will depend on both male and female selection accuracy, although since selection intensity is higher for males, their accuracy has the highest impact on genetic gain. When progeny testing is not very feasible regarding the properties of the trait of interest (i.e. phenotypes are expensive or difficult to measure), genotyping females in test herds that enter the reference population may compensate the lack of progeny testing. Due to their phenotypes and genotypes, females belonging to these test herds will have more accurate breeding values than the cows outside of the test herds. The selection accuracy for females will hence depend on the fraction of the cows included in the test herds. Based on the results of Paper I, it is possible to achieve high selection accuracies (0.75) for males when FE is included in the genomic selection breeding schemes and that there are two possible ways of updating a reference population to achieve this: obtaining phenotypes from all cows in the population and hence get progeny information for genotyped bulls, or by genotyping cows with records i.e. creating a female test herd. If genotyping is cheap compared to phenotyping, the latter method will be preferable. However when GS is used to improve low heritability traits, such as FE, the number of records in the reference population has to be sufficiently large in order to achieve high selection accuracies (Hayes *et al.*, 2009). Results from Paper I showed that if GS is used, 4,000 additional genotyped and phenotyped heifers are needed to be added to the reference population annually to genetically improve feed efficiency. The use of smaller test herds reduced genetic gain markedly.

In order to have access to such a large quantity of feed efficiency phenotypes, different strategies have been proposed. McParland *et al.* (2014) showed that MIR spectrometry of milk could be used to predict residual feed intake as a measure of feed efficiency in lactating dairy cows. Since, individual animal milk samples are routinely taken in dairy production, using these samples in the prediction FE and feed intake would be cost-effective and a relatively undemanding means for obtaining large numbers of feed efficiency phenotypes. Hence in Paper II, the use of MIR spectral data to predict feed intake in Norwegian Red dairy cattle was investigated using partial least squares regression and Best Linear Unbiased Predictions methods. MIR data of milk was used to predict dry matter intake and net energy intake in lactating Norwegian Red dairy cows. When the PLS method was used, the most accurate model for predicting both DMI and NEI included body weight, milk yield and MIR spectra. The models which included only MIR spectral information resulted lower prediction accuracy for NEI or DMI compared to the models using additional predictors with MIR spectra. However, the accuracy of prediction (R) using just the MIR data was always inferior to a model that used just MY, fat, protein and lactose concentration. The most accurate model for DMI resulted in an R-value of 0.54. The use of MY and BW together with MIR spectra in the prediction model increased the prediction accuracy considerably and resulted in R = 0.65 for NEI in the external validation. However, using only MY and MIR spectra in the model resulted in R = 0.63 for NEI in the Paper II. The latter model could be more practical since BW is not readily available on commercial dairy farms. However, body weight could be estimated using for example chest width since a high genetic correlation between body weight and chest width has been reported (0.75-0.86; Veerkamp and Brotherstone., 1997). Compared to the study of McParland *et al.* (2014) where PLS was used to predict effective energy intake using MY together with milk MIR resulting in R = 0.70 in the external validation, the R for NEI in the most accurate model in the Paper II was somewhat lower. The differences in the results between Paper II and McParland et al. (2014) could be due the fact that within each experiment, the cows did not receive exactly the same diet and the dry matter content of feed differed between different experiments. This could have an impact on the relationship between MIR and DMI/NEI in the Paper II. Also using different wavelengths of the MIR spectra in the studies could have an impact on the prediction accuracies, and the fact that the dataset of McParland et al. (2014) was larger may have improved accuracies.

In Paper II, the BLUP method was also used to predict NEI and DMI. The BLUP predictions yielded similar accuracies as PLS but were biased. Also linear regression coefficients of the actual values on BLUP-predicted values differed from unity, which indicates biases. As a comparison, McParland *et al.* (2011) reported a linear regression coefficient of the actual values of energy balance on PLS-predicted values of about unity. However, their linear regression coefficient of actual on predicted energy content was different from unity. McParland *et al.* (2011) also reported a biased prediction for energy content. Fortunately, mean biases are not necessarily a big issue for genetic evaluations since genetic evaluations are undertaken within contemporary groups and in doing so remove the mean contemporary group effect from the individual records. A possible reason for biased prediction in Paper II is that λ was not estimated using REML (Residual Maximum Likelihood) but instead chosen based on external-validation R values. Thus, based on the results in Paper II, PLS seems to be a better method for predicting feed intake using milk MIR than BLUP.

Another solution to have access to a large quantity of feed efficiency phenotypes, is to pool data from different populations as suggested by de Haas *et al.* (2012) and Veerkamp *et al.* (2013). The impact of combining mid-infrared spectral data from two different countries on the accuracy of predicting feed intake was investigated in Paper III. The use of MIR spectra was tested when predicting NEI and effective energy intake using PLS, multiple linear regression and BLUP methods. Best Linear Unbiased Prediction was implemented either as a single trait analysis or as a multi-trait analysis, where the correlation between NEI and EEI was estimated. When EEI was predicted using MIR spectra within country, the accuracy of prediction in the external validation (0.66) in Paper III was greater than in the study of McParland *et al.* (2014), where the R for EEI was 0.55-0.64. The differences in the results between McParland *et al.* (2014) and Paper III could be due the fact that not exactly the same data was used in the studies and that different MIR wavelengths were used. For NEI, when using the across country MIR spectral information, the single trait BLUP method yielded greater prediction accuracy than PLS. For EEI, both single trait and multi-trait BLUP methods yielded greater accuracy of prediction than the PLS models. All PLS and BLUP predictions in Paper III were unbiased. However, in some of the models, the linear regression coefficient of the actual values on both PLS- and BLUP-predicted values were differing from unity. Based on the results from Paper III, compared to PLS, single trait BLUP seems to be a better method for predicting NEI when only milk MIR is available. When across milk MIR was included in the model for predicting EEI, calculating PLS scores and using MLR yielded greater R than either one of the BLUP methods.

One might argue that using MIR spectra for the prediction of feed efficiency would only detect cows which are in negative energy balance. However, the genetic correlation between MIR predicted energy intake and measured energy balance is quite low (McParland *et al.*, 2015) hence, indicating that use of MIR predicted energy intake would not lead to selection of negative energy balance.

6.2. RFI as a measure of feed efficiency

In Paper I, we used RFI as a measure of feed efficiency. However, earlier studies have shown that weak unfavorable genetic correlations exist between RFI and fertility (Vallimont *et al.*, 2013). According to Pryce *et al.* (2014), this is probably due to the mathematical similarity in the calculations of energy balance and RFI and a failure to correctly account for body tissue mobilization. This could lead to selection for a trait that is similar to selecting for a negative energy balance (Pryce *et al.*, 2014). Therefore, genetic correlations, especially with fertility trait but also with other traits, must be accounted for before including RFI in the breeding scheme (Pryce *et al.*, 2014). I.e. in order to include RFI in the breeding scheme, a multi-trait selection index where genetic correlations with other traits are properly accounted for, is required.

6.3. Data quality

Obtaining reliable NEI data for use in Paper II and III was challenging. The data were selected from several experiments conducted between 2007 and 2015. The main challenge was that frequency of phenotypic recording in several of the experiments was limited and there were several missing records in the dataset. In order to expand the dataset for analysis, missing records were estimated using cubic splines. If those spline estimates were inaccurate that would have had an impact to the NEI results in Paper II and III.

6.4. Challenges in improving feed efficiency

Most of the studies of the FI complex have been derived from dairy cattle in confined production systems, and assumed feed efficiency was genetically the same trait throughout lactation (Pech *et al.*, 2014; Manafiazar *et al.*, 2016)**.** However, based on the results of Hurley *et al.* (2017), it seems possible to genetically improve dairy cow efficiency differently at different stages of lactation. According to Hurley *et al.* (2017), it is also possible to alter the trajectories of the FE traits in order to suit them in a particular breeding objective. However, this demands very precise across-parity estimates of genetic parameters, including genetic correlations with health and fertility traits (as well as other traits) (Hurley *et al.*, 2017). One challenge in improving feed efficiency is the different energy systems used in different countries and comparing those energy values with each other. Different functions involved in energy usage and supply over the entire lactation, for example, body mass changes in lipids and protein, should be accounted for when estimating FE in dairy cattle (Berry *et al.*, 2006). According to Hardie *et al.* (2015) and Rathbun et al. (2017), even though selection towards more feed efficient animals could result in positive progress in reducing environmental impact and feed cost, it is largely unknown how this selection would affect to other phenotypic traits, such as metabolic health. Hence, further research is needed to calculate the correlation and impact of co-selection of these traits (Hardie *et al.*, 2015).

6.5. Recommendations

Feed efficiency is a difficult trait to measure and as such FE is difficult to include in routine progeny test evaluations. A viable option to genetically improve these kind of traits, is to use rather large contracted test herds with additional recording. Using this strategy would give close to similar accuracy of genomic selection as recording this trait in the whole female population. As long as a sufficient number of cows (4,000) is included in the test herds, the male selection would be as effective with contracted test herds of genotyped females as when a routine progeny test would be performed for this trait. Obtaining large amounts of animals with multiple FE recordings is possible by using milk MIR predicted feed efficiency records or using pooled data as a result of collaboration between countries. Based on this thesis, MIR spectral data from two countries i.e. from two populations differing in breed and feeding system can be combined and used to predict energy intake as a measure of feed intake in dairy cattle. The results also indicate that especially single trait BLUP method could have a benefit over the PLS method when EI is predicted using across country MIR spectra. However, before including any kind of feed efficiency trait in the breeding program, genetic correlations between predicted feed intake, actual feed intake and other performance traits, especially health and fertility traits need to be estimated in order to derive the selection index weights for a balanced breeding goal.

7. Conclusions

- Genetic improvement of feed efficiency is possible if sufficient numbers of FE phenotypic data are available (4,000 or more).
- MIR spectral data can be used to predict NEI as a measure of feed intake in Norwegian Red dairy cattle with accuracy of 0.63 to 0.65.
- Across country MIR spectral data can be used to further improve the accuracy of prediction of energy intake traits.

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Norwegian University
of Life Sciences

Postboks 5003
NO-1432 Ås, Norway
+47 67 23 00 00
www.nmbu.no