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Breeding Dairy Cattle for Resource Efficiency and Environmental Sustainability

Final Report of the A⁺⁺COW -project (2019-2023)

Terhi Mehtiö, Päivi Mäntysaari, Enyew Negussie, Riitta Kempe, Marja-Liisa Sevón-Aimonen, Arash Chegini, Sanna Hietala, Joel Kostensalo and Martin Lidauer



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Abstract

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Cows consuming less feed than average of the population for a given level of production and body weight are considered as resource and feed efficient. The main aim of the A⁺⁺Cow project, conducted during 2019 and 2023, was to develop tools to improve the feed efficiency of dairy cows through animal breeding, to increase knowledge on the genetic background of these traits and thereby improve the environmental and economic sustainability of dairy production. The development of reliable genomic breeding values for the Nordic dairy population is essential to achieve this goal. Therefore, the four main objectives of the A⁺⁺Cow project were to 1) advance the development of novel phenotypes of feed efficiency, 2) model feed efficiency traits for Maintenance, Metabolic Efficiency and Metabolic Resilience breeding objectives, 3) develop single-step genomic prediction models for Nordic dairy cattle, and 4) assess the economic and environmental benefits, and disseminate the feed efficiency breeding indices.

During the project data were collected from dairy cows at Luke Jokioinen research farm on i.e., feed intakes, milk production and composition, body weights and blood NEFA and BHB levels. When combining the data with the data collected in previous studies, the total data set included 148 715 feed efficiency records from 828 primiparous Nordic Red Dairy cattle cows.

The first Saved Feed index was published in 2019 and the index was included into the Nordic Total Merit index in 2020. Based on the results of A⁺⁺Cow project, the new single-step genomic prediction model of Maintenance, where carcass weight was included in the model, resulted in higher validation reliability and better predictive ability compared with traditional BLUP approach. Current genetic trend in metabolic body weight (MBW) appeared to be somewhat underestimated in all breeds and the new model corrected the genetic trend of MBW. The reliability of the Metabolic Efficiency genomic predictions has been rather low. This could be improved by switching to a model based on regression on expected feed intake (ReFI) that has been developed during the project and which has a better ability to describe the metabolic efficiency of a cow. In addition, more feed intake records are needed. CFIT 3Dcamera imaging could offer a technological solution for recording feed intake on-farms. The accuracy of measuring feed intake by CFIT 3D-cameras was studied in the project and a correlation of 0.71 was found between the average feed intake of 4 to 7 days assessed by CFIT 3D-camera and the average feed intake measured by scales. This is a reasonable correlation, and the technology and algorithms can be further developed in future.

The project assessed animal breeding as highly relevant to improve the sustainability of dairy production – a 10% improvement in resource efficiency would reduce the carbon footprint by 8% and reduce eutrophication impacts by 10%. In addition, the economic impact is significant, and we found that by including two feed efficiency traits, MBW for Maintenance and ReFI for Metabolic Efficiency, into a selection index with production traits and fertility, improved the total economic gain about 30 %.

In addition, the welfare of the cows in early lactation can now be considered through the prediction of blood NEFA and BHB levels from the milk MIR spectral readings. The coefficients of determination were 0.53 and 0.63 for NEFA and BHB, respectively, indicating that the prediction models perform well for both animal breeding and herd management purposes.

Keywords: breeding, genomic predictions, dairy cow, body weight, feed intake, energy status, milk fatty acids, methane, Nordic Red Cattle

Content

1.	Aim of the project	7
2.	Project partners and collaboration	8
3.	Project results	.11
3.1.	Material and Methods	12
	WP1. Novel phenotypes	12
	WP2. Modelling feed efficiency	18
	WP3. Genomic predictions	27
	WP4. Economic and environmental benefits	36
3.2.	Results	44
	WP1. Novel phenotypes	44
	WP2. Modelling feed efficiency	50
	WP3. Genomic predictions	58
	WP4. Economic and environmental benefits	69
3.3	Evaluation of the project implementation	80
3.4	Publications	82
4.	Evaluation of the results	. 89
4.1	Practical applicability of results	89
4.2	Scientific significance of the results	90

Abbreviations

BCS	Body condition score
BD	Body depth
BHB	Beta-hydroxybutyrate
BW	Body weight
BWG	Body weight gain
BWL	Body weight loss
CARW	Carcass weight
CFIT	Cow feed intake technology (3D-camera imaging)
CW	Chest width
DIM	Days in milk
DMI	Dry matter intake
DRP	De-regressed proof
EB	Energy balance
EBV	Estimated breeding value
ECE	Energy conversion efficiency
ECM	Energy-corrected milk
eDMI	Expected dry matter intake
ER	Energy requirement
ERC	Effective record contribution
ES	Energy status
ESI	Energy status indicator
FE	Feed efficiency
FPR	Fat to protein ratio
FY	Fat yield
GHG	Greenhouse gas emissions
HOL	Holstein
ICF	Interval from calving to first insemination
JER	Jersey
LCA	Life cycle analysis
MBW	Metabolic body weight, BW ^{0.75}
ME	Metabolizable energy
MY	Milk yield
NEFA	Non-esterified fatty acids
NTM	Nordic Total Merit index
PY	Protein yield
pMBW	Predicted metabolic body weight
RDC	Nordic Red Dairy cattle
ReFI	Regression on expected feed intake
REI	Residual energy intake
RFI	Residual feed intake
STA	Stature
TMR	Total mixed ratio
UPG	Unknown parent groups

1. Aim of the project

Maintaining Finnish food security requires sufficient high national agricultural production for which a profitable milk production sector is central under boreal climate conditions. At the same time, Finland's government has set ambitious goals to cut the greenhouse gas (GHG) emissions from agriculture and one key part of the strategy is to reduce methane from dairy cattle. Genetic improvement of feed efficiency (FE) has been identified as one of the most cost-effective means to improve profitability and reduce GHG emissions in dairy operations (Ahvenjärvi et al. 2022). The aim of the A⁺⁺Cow project was, by applying an integrated approach involving diverse scientific fields and industry stakeholders, to establish genomic predictions for FE in Finnish and Nordic dairy cattle.

Our overarching hypothesis was that by applying state-of-the-art genomic prediction methodologies that have been established for Nordic dairy cattle and by utilizing all scientific know-how produced in previous research projects, we are able to integrate genomic breeding values for FE into the Nordic Total Merit (NTM) selection index, and by this initiate a continuous improvement of our dairy cows' resource efficiency and the lowering of GHG emissions and eutrophication. Therefore, research in A⁺⁺Cow focused on developing genomic predictions for the following breeding objectives:

Maintenance Cost, which is determined by how much of a cow's total feed consumption is used to maintain its body functions and how much to produce milk. Present-day Finnish dairy cows use over one third of the total feed consumption for maintaining their body functions. Feed requirement for maintenance is a function of the body weight (BW) and cows that have low maintenance requirement, relative to the amount of milk produced, are desirable.

Metabolic Efficiency, which is the cow's ability to efficiently use feed for all her biological activities. A common measure of Metabolic Efficiency is residual feed intake (RFI). It is defined as the difference between actual feed consumption of a cow and the expected feed consumption of a cow based on the cow's metabolic activities, which are determined by BW, growth, milk production, pregnancy stage, etc. More efficient cows will have a lower feed consumption than expected requirements, which for instance can be due to a better ability to digest feed. Modelling Metabolic Efficiency by RFI should make it uncorrelated with *Maintenance Cost*. Hence, both breeding objectives express different parts of the genetic variation in FE.

Metabolic Resilience, which in here is referred to as the ability of a cow to withstand or avoid a severe negative energy status (ES) at the onset of lactation which can lead to metabolic disorders and poor fertility. Best biomarkers for negative ES are the blood plasma non-esterified fatty acids (NEFA) and beta-hydroxybutyrate (BHB) concentration, which can be predicted from milk mid-infrared (MIR) spectral readings. Reliable predictions for negative ES shall allow genetic improvement of *Metabolic Resilience*, which is important when breeding for FE to also underpin the selection for health.

To achieve the overall aim, research in A⁺⁺Cow focused on four main objectives:

- 1. Advancing the development of novel phenotypes
- 2. Modelling feed efficiency traits for the three breeding objectives
- 3. Developing genomic prediction models for Finnish and Nordic dairy cattle
- 4. Assessing the economic and environmental benefits, and dissemination of feed efficiency breeding indices

2. Project partners and collaboration

Participating researchers and main role in the project

Natural Resources Institute Finland (Luke)

Genomics and breeding

Terhi Mehtiö, Ph.D., Research Scientist, project leader 03/2021 onwards, modelling the genetics of feed efficiency traits, development of prediction equations for NEFA and BHB **Martin Lidauer**, Ph.D., Research professor, project leader until 02/2021, modelling metabolic efficiency, genomic prediction models, milk MIR spectral readings

Enyew Negussie, Ph.D., Senior Scientist, genetic analyses of metabolic efficiency and methane emissions

Riitta Kempe, Ph.D., Research Scientist, development of genomic predictions for maintenance and inclusion of carcass weight into the genomic evaluation

Marja-Liisa Sevón-Aimonen, M.Sc., Research Scientist, predicted genetic and economic response, including feed efficiency into breeding goal

Esa Mäntysaari, Ph.D., Research professor, Research Professor, modelling feed efficiency, genomic prediction models

Timo Pitkänen, M.Sc., Research Scientist, body weight analyses **Minna Koivula**, Ph.D., Senior Scientist, genomic predictions

Animal Nutrition

Päivi Mäntysaari, Ph.D., Senior Scientist, management of feed efficiency data, CFIT trials and data analyses

Applied statistical methods

Joel Kostensalo, Ph.D., Senior Specialist, development of prediction equations for NEFA and BHB

Jarmo Mikkola, Ph.D., Senior Specialist, development of prediction equations for NEFA and BHB

Sustainability science and indicators

Sanna Hietala, M.Sc., Research Scientist, environmental impacts of improving feed efficiency

Personnel responsible for data collection, animal experiments and laboratory analyses Päivi Mäntysaari, Terhi Mehtiö, Paula Lidauer, Tomasz Stefanski, Anne Kemppainen, Minna Aalto, Riikka Ahonen, Tuija Hakala, Heli Harju, Taina Jalava, Sari Kajava, Annu Palmio, Riikka Peltonen, Marianne Saha, Jani Tuomola, Widen Juha, Martin Lidauer

University of Helsinki

Department of Agricultural Sciences

Jarmo Juga, Ph.D., Ass. Prof., predicted genetic and economic response, including feed efficiency into breeding goal

Tuomo Kokkonen, Ph.D., Doc., data collection, blood NEFA and BHB assessments

KU Leuven

Ben Aernouts, Ph.D., Research professor, development of NEFA and BHB prediction equations

Valio Ltd

Aleksi Astaptsev, M.Sc., Development manager, Lypsikki LCA model, environmental impacts of improving feed efficiency

Juha Nousiainen, Ph.D., Senior vice president, environmental impacts of improving feed efficiency

Viking Genetics

Søren Borchersen, Ph.D., Head of research & development, CFIT system **Jan Lassen**, Ph.D., Senior project manager, CFIT system

Faba co-op

Jukka Pösö, Ph.D., Research agronomist, responsible expert for NAV genetic evaluations Minna Toivonen, Ph.D., Development manager

Nordic Cattle Genetic Evaluation (NAV)

Gert Pedersen Aamand, Ph.D., General manager, modelling feed efficiency, genomic prediction models

SEGES

Trine Andersen, Consultant, modelling metabolic efficiency **Rasmus Stephansen,** Consultant, modelling metabolic efficiency and maintenance cost

Mtech Digital Solutions Ltd

Hilppa Hietanen, Team leader, MIR database Paula Pääkkö, Lead developer, MIR database

Danish Technological Institute

Rikke Hjort Hansen, Senior project leader, CFIT system

Funding partners and data acquisition partners

MMM Valio Ltd Faba co-op VikingGenetics Mtech Digital Solutions Ltd Nordic Cattle Genetic Evaluation NAV

Project steering group

Eeva Saarisalo, MMM, chairperson Sirpa Karjalainen, MMM Marjukka Mattio, MTK Minna Toivonen, Faba co-op Tuija Huhtamäki, ProAgria Juha Nousiainen, Valio Ltd Søren Borchersen, VikingGenetics

The steering group met eight times during the project.

3. Project results

All research tasks were organized in four work packages (WP). The interconnection between WPs is visualized in Figure 1. Arrows between work packages indicate information flow and likewise the chronological order of the main research activities during the course of the project.



Figure 1. Description of the different work packages and related tasks of the A⁺⁺ Cow project.

3.1. Material and Methods

WP1. Novel phenotypes

Task 1.1 Feed efficiency phenotypes

Päivi Mäntysaari, Enyew Negussie and Terhi Mehtiö

The aim of this task was to collect comprehensive feed efficiency data to be used in modelling feed efficiency and metabolic resilience and for estimation of the necessary variance components required for model developments in WP2.

Motivation

Comprehensive phenotypic data is needed for reliable and accurate calculations and for development of new traits of cow's feed efficiency. Therefore, in this task significant efforts were made to collect more phenotypes to be added into the database of previous projects. The result is a rare and unique data that contain not only production traits but also feed and energy intake, body measurements and daily methane (CH₄) output of the cows. The collected data were used to define efficiency traits; residual energy intake (REI, ME MJ/d), energy conversion efficiency (ECE, kg ECM/ME MJ) and energy balance (EB, ME MJ/d) and to build up new metabolic efficiency traits. The collected data were also used for the genetic analyses of FE traits, associations between FE and environmental impact traits and for studying metabolic resilience of the cows.

Feed efficiency data collection

The phenotypic data for analyses of FE and CH₄ output of primiparous Nordic Red dairy cattle (RDC) cows were collected at Luke Jokioinen experimental farm during 1.2.2019-31.12.2022. Daily measurements of 145 cows included individual dry matter intake (DMI), BW and milk yield (MY) from lactation days 3 to 305. To correct for the daily variation in BW, the daily weights were smoothened using the model of Mäntysaari and Mäntysaari (2015). The milk samples for milk composition analyses (fat, protein, lactose, somatic cell and MIR spectra) were taken in lactation week 2 and 3 and thereafter on routine milk recoding test days. The daily composition of milk was calculated with an assumption of linear change between measurements. The energy-corrected milk (ECM) was calculated according to Sjaunja et al. (1990). All feeds were sampled and analysed to get metabolizable energy (ME) and nutritional values for the feeds (Luke 2017). Based on intake and feed values the daily energy and nutrient intake of the cows were calculated. The body condition scores (BCS) of the cows were assessed on a scale of 1–5 (1=skinny to 5=very fat) with intervals of 0.25 (Edmonson et al. 1989) monthly. Blood samples for plasma non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHB) analyses were taken 1.2.2019–30.3.2021 during lactation weeks 2 and 3 of all the cows in FE data. Stored blood samples from the Feed Efficiency project (1.3.2013-31.10.2016) were analysed for BHB, to increase BHB reference database.

Alongside intake and production recordings, the daily CH₄ output of cows was monitored continuously 24/7 using a portable F10 multigas analyzer (Gasera Ltd, Turku, Finland) that is based on non-dispersive IR cantilever-enhanced photoacoustic detection system. The multipoint F10 gas analyzer was fitted to two feeding kiosks (two sampling points) for continuous

measurement of CH₄ and CO₂ outputs from individual first lactation cows at the Minkiö dairy farm. In this system, whenever a cow visits the feeding kiosk, her breath is sampled and analyzed for the contents of the different gases. Measurements were made alternatively between the two sampling points and every other minute a gas was sampled and analyzed from each sampling point. Sampling of the gas and its analysis took ~30 s each and a single CH₄ and CO₂ concentration measurement from one of the sampling points takes a minute. Under normal conditions, a cow visits feeding kiosks about 3 times a day and during which time cows have an average of 7 usable repeated measurements of the gases recorded per day. During each individual animal measurement, the cow ID, date, time and the concentration of the gases measured were recorded automatically and stored in the internal memory of the F10 equipment. The daily CH₄ output of cows was then estimated from the daily concentration measurements using the method by Madsen et al. (2010). In this method, measurements of CH₄ and CO₂ concentrations in breath of cows were combined with estimates of total CO₂ production from information on ME intake to quantify daily CH4 as described in Negussie et al. (2017).

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Task 1.2 Feed intake predictions

Martin Lidauer, Päivi Mäntysaari, Terhi Mehtiö, Jan Lassen (VikingGenetics) and, Rikke Hansen (Danish Technological Institute)

The aim of this task was to install VikingGenetics' CFIT system at Luke's research farm in Jokioinen and validate against feed intake measurements.

Motivation

Genomic predictions for Metabolic Efficiency will require feed intake measurements from cows at commercial farms to achieve necessary accuracy. A new approach for measuring intake has been developed by one of the project partners, VikingGenetics. During the last seven years, VikingGenetics has been working on predicting feed intake by using a 3D camera system (CFIT). This system predicts cow-specific feed intake volumes non-invasively with minimum interference with daily barn operations. The obtained cow-specific observations are shown to be highly repeatable and correlate with milk production traits similarly as DMI observations do (Lassen et al. 2018). It will be important that CFIT can accurately predict DMI. This can be achieved by recording feed intake for a sufficiently long period of time to reduce the size of the measurement error. This task examined the length of the recording time period CFIT needs to obtain accurate DMI predictions.

CFIT trials

<u>Trial 1</u>. The first CFIT trial was postponed for one year because of COVID restrictions and was carried out between 13.9.2021 and 29.11.2021. There were 3 x 20 cows participating in the first trial. All cows ate from their own places, and between the cows there were separators on the feeding table. Daily feed intake was measured by the CFIT system and by scales for all 60 cows and each cow was followed for three weeks. Daily samples of grass silage and concentrate were taken and analyzed for dry matter to calculate the dry matter content of the total mixed ration (TMR).

<u>Trial 2</u>. The second CFIT trial was carried out between 12.4.2022 and 30.9.2022. There were 2 x 20 cows participating in the second trial. In the second trial cows ate alternatively from a feed sward (S) or from own (O) places with the separators on the feeding table. The sward feeding mimic on-farm feed intake measuring situation. For each cow each feeding alternative was repeated, which means that a cow was for four weeks under recording, where in each week a cow was under different feeding alternatives, either S-O-S-O or O-S-O-S. The feeding table was adapted to have 10 cows under S feeding and 10 cows under O feeding at the same time. After each week the S and O places were switched. Alternating was done to ensure that observations were made at almost the same time, being in almost the same lactation stage and eating almost the same feed. The collected data should allow to assess how much the accuracy reduce when moving from a measuring situation representing Trial 1 to a measuring situation that is more alike on-farm situation. Daily samples of grass silage and concentrate were taken and analyzed for dry matter to calculate the dry matter content of the TMR.



Figure 2. Adaptation of feeding table for Trial I and Trial II.

Data analyses

Daily measurements of intake by scale and CFIT were produced for all cows in Trial 1 and for the cows on own places in Trial 2. In Trial 2 the cows on sward feeding got individual CFIT intake observations but no scale intake observations. The Pearson correlations between CFIT intake and scale observations were calculated using individual observations or 1-day, 2-days, 3-days etc. averages (Trial 1) or the intake observations of the adjacent weeks (Trial 2). The repeatability of measures within measurement methods were estimated using SAS PROC MIXED with the cow as a random effect. The repeatability was calculated to access the accuracy of CFIT feed intake observations when cows are eating from a sward as it is the case on commercial farms.

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Task 1.3 Predicting energy status from milk MIR spectra

Terhi Mehtiö, Joel Kostensalo, Päivi Mäntysaari, Tuomo Kokkonen (University of Helsinki), Paula Lidauer, Ben Aernouts (KU Leuven, Belgium) and Martin Lidauer

The aim of this task was to enlarge the NEFA reference database that has been established during the Feed Efficiency project, and to develop a second version of prediction equations for blood NEFA using mid-infrared (MIR) spectra of milk samples of the enlarged reference database and to develop a prediction equation for BHB.

Motivation

When breeding cows for resource efficiency, it is important to consider Metabolic Resilience and ensure that there will be no emphasis for selecting cows in a severe and deep negative ES in early lactation. Therefore, reliable data on the ES of cows is needed. Obtaining measurements from a biomarker, which is capable to describe the ES of a cow, is of interest (McParland et al. 2012). Blood plasma NEFA concentration is an established indicator, but it is invasive and too expensive for routine applications. We have earlier demonstrated that it is possible to develop prediction equations for plasma NEFA concentration based on milk MIR spectral data (Aernouts et al. 2020). In those studies, the obtained coefficients of determination of cross validation varied from 0.61 to 0.67 depending on the applied cross validation procedure. The analyses showed that there is room to further improvement of the accuracy of the predictions if the reference data will be enlarged as the models seemed to underestimate the level of high (>1.2 mmol/l) NEFA measurements. In addition, based on the results by Mehtiö et al. (2020), the genetic correlations between NEFA predictions and milk BHB predictions varied from 0.41 to 0.73 during the first three months in milk indicating that these energy status indicators are not explaining the same variation in early lactation and an ideal phenotype of energy status could be a combination of these indicator traits. As there were no blood BHB measurements available for Finnish dairy cows, there was an interest to develop predictions also for blood BHB levels based on milk MIR spectral data. In this task the aim was to enlarge the reference database for blood NEFA and update the prediction equations using this enlarged data set, and to develop similar predictions equations for blood BHB levels.

Collected reference data

Before the A⁺⁺Cow project started we had collected 806 NEFA observations and corresponding morning and evening milk samples with MIR spectra data. We continued collecting blood samples once a week after morning milkings from all cows in lactation weeks 2 and 3 at Luke Jokioinen research barn as described in Task 1.1. The last blood samples were collected in spring 2021 and NEFA and BHB assessments for all blood samples were performed during autumn 2021. In total the reference data set had increased to 1642 blood NEFA records, 1488 blood BHB records and 3190 milk MIR spectral readings (Table 1).

Table 1. Number of validated observations, means, interquartile range (IQR), minimum and
maximum values of the reference data set of blood plasma NEFA and BHB concentrations
(mmol/l).

		Ν	Mean	IQR	Min	Мах
NEFA	All	1,642	0.49	0.34	0.04	1.95
	Week 2	598	0.58	0.35	0.09	1.95
	Week 3	890	0.48	0.31	0.05	1.73
	Week 20	154	0.13	0.06	0.04	0.42
BHB	All	1,488	0.95	0.44	0.29	4.66
	Week 2	598	0.91	0.38	0.29	3.98
	Week 3	890	0.98	0.49	0.30	4.66
	Week 20	-	-	-	-	-

MIR data pre-processing and development of prediction equations

The MIR data pre-processing routine was developed together with Luke and Mtech and all MIR data, as well as predictions for NEFA and BHB levels and milk fatty acid concentrations, are now available routinely in Mtech database.

The prediction models for NEFA and BHB are based on the same partial least regression (PLS) method applied and explained by Aernouts et al. (2020). The cross-validation procedure of the R-package *pls* was applied. In this procedure 10 splits are used, i.e., the data is fitted 10 times with 90% training and 10% testing data. In addition, the models were cross validated by fitting the model using two herds and testing on third herd, which provided a test for a completely independent data set.

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WP2. Modelling feed efficiency

Task 2.1 Modelling maintenance cost

Terhi Mehtiö, Anna-Maria Leino, Timo Pitkänen, Esa Mäntysaari, Riitta Kempe, Enyew Negussie and Martin Lidauer

The aim of this task was to develop a model that will utilize all useful information for the prediction of metabolic body weight breeding values for the Finnish and Nordic dairy cattle breeds.

Motivation

Larger cows need more energy for maintenance than smaller cows, and literature has shown that body weight (BW) has an unfavourable correlation with efficiency traits, indicating that heavier cows are less feed efficient (Vallimont et al. 2011). Therefore, there is an interest to include a trait describing the maintenance cost into the selection index.

Basically, the energy requirement for maintenance is related to the BW, and more precisely to the metabolic body weight (BW^{0.75}, MBW), of the cow. During the last decades, dairy cows' BW has been recorded in Finland by heart girth measurements for feeding management purposes and this has generated over 800,000 BW observations since 1990. At the same time, recording of BW by scale has been increasing slowly in the Nordic countries, mainly in Denmark. Thus, the sizeable amount of BW records available in Finland and Denmark allowed the development of the MBW evaluations. However, lately recording of BW by heart girth has been decreasing whilst recording by scale is increasing only slowly, which may weaken the maintenance evaluation in future. Therefore, the benefit of including also correlated traits, like carcass weight or conformation traits, to the maintenance evaluation is of interest.

To study this, we estimated the genetic variation and genetic correlations for eight traits describing the energy requirement for maintenance including: first, second and third parity MBW based on heart girth measurements, carcass weight (CARW) and predicted MBW (pMBW) based on predicted slaughter weight, and first parity conformation traits stature (STA), chest width (CW) and body depth (BD) (Mehtiö et al. 2021).

Data

The data used in this study consisted of 21,329 records from RDC and 9,780 records from Holstein (HOL) breed cows in Finland (Table 2).

Table 2. Number of records, means and standard deviations for MBW in three parities (MBW1, MBW2, MBW3), first parity conformation traits (STA, CW, BD) and traits based on slaughter information (CARW, pMBW) for RDC and HOL breeds separately.

		RDC			HOL	
Trait	Records	Mean	SD	Records no.	Mean	SD
	no.					
MBW1 (kg ^{0.75})	14,832	112.9	9.6	6,620	119.7	10.6
MBW2 (kg ^{0.75})	9,876	119.0	9.7	4,347	127.2	10.6
MBW3 (kg ^{0.75})	6,066	123.4	10.3	2,557	131.9	10.8
STA (cm)	12,612	138.7	4.6	6,277	146.1	4.4
CW (score)	12,612	5.0	1.1	6,277	5.0	1.2
BD (score)	12,612	5.1	1.0	6,277	5.3	1.0
CARW1 (kg)	858	253.1	40.2	363	262.3	41.4
CARW2 (kg)	1,826	272.8	46.1	856	278.9	45.6
CARW3 (kg)	1,734	279.7	46.1	821	285.1	46.2
pMBW1 (kg ^{0.75})	858	117.81	6.9	363	125.2	6.7
pMBW2 (kg ^{0.75})	1,826	124.4	6.4	856	131.4	5.8
pMBW3 (kg ^{0.75})	1,734	128.1	6.9	821	134.8	6.4

Predicted metabolic body weight

Because the relationship between CARW and MBW traits is not linear, we were interested to predict BW at the time of slaughtering, to see if pMBW would have a stronger genetic correlation with MBW than CARW. In addition, the fat class of carcass was used in predicting MBW, which could bring more information and improve the breeding value estimates. The pMBW observations were developed as explained by Mehtiö et al. (2021).





Genetic analyses

Two multiple trait linear mixed animal models with seven traits were applied for genetic analyses where both models had the same six traits (MBW [1,2,3], STA, CW, and BD) and the seventh trait was either CARW or pMBW. The models are explained in detail by Mehtiö et al. (2021) paper.

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Task 2.2 Modelling metabolic efficiency

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The aim of this task was to develop a model for predicting metabolic efficiency in dairy cows.

Motivation

The biological efficiency of a cow to utilize the feed, for which the term "metabolic efficiency" is used by the Nordic dairy cattle breeding community, is the most important efficiency trait as it comprises the efficiencies of all biological processes in dairy cows, i.e., the efficiency to digest the feed, the ability to have low energy loss by methane exhalation, and the efficiency of using ME for the different body functions and production.

The most used metrics for modelling the genetic variation of metabolic efficiency is RFI, which is used in the breeding of other species (Gilbert et al. 2017). It is defined as the difference between actual feed intake and predicted feed intake. Predicting feed intake for dairy cows is difficult because the energy requirement is a sum of different physiological processes, which are changing significantly during lactation. Therefore, continuous recording is needed, and random regression models have been suggested for modelling such RFI data in dairy cows (e.g., Mehtiö et al. 2018). However, these models require comprehensive and complete data collection which is often difficult under on-farm conditions. Furthermore, numerous studies on applying RFI for dairy cattle give indications that the classical RFI metric may be not suitable for dairy cattle.

New approaches to model metabolic efficiency

In this research task we focused on two approaches to model metabolic efficiency. One novel approach is to model the component traits of **RFI by a multiple-trait RFI approach**, where applied variance components are transformed so that estimates for intake will resemble RFI. This multiple-trait model is robust to accommodate incomplete records. The second approach, **regression on expected feed intake (ReFI)**, is a new approach that was developed in this project and aims to overcome problems associated with the classical RFI metric.

A) Residual feed intake via multiple-trait approach

In the classical RFI model, where partial regression coefficients and breeding values are estimated in a single step (RFI_{ST}), DMI is regressed on energy sink traits ECM, MBW, BW loss (BWL), and BW gain (BWG). Whereas in the new multi-trait RFI (MT_{PF}) approach it involves

Cholesky decomposition of the multi-trait variance covariance matrix of component traits to express DMI in a sequential relationship that is conditional on each of the energy sinks. In so doing, the MT_{RFI} approach avoids some of the shortcomings of the classical RFI model and allows the effective use all available information on a cow. In addition, the heterogeneity of residual variations across lactation and modelling of body weight changes during lactation as body weight loss (BW_{LOSS}) and body weight gain (BW_{GAIN}) traits were tested.

In this study data from 731 Nordic Red cows from four Finnish research farms that included 20,533 weekly records were used. The effects of heterogeneity of residual variances and

modelling of body weight change during lactation on the estimates of MT_{RFI} genetic parameters were investigated. The whole lactation period was divided into five residual classes: <5, 5-8, 9-12, 13-32 and >33 lactation weeks and a MT repeatability animal model was used for the estimation of MT variance covariance (VCV) components. Then, a square-root free and modified Cholesky decomposition was applied on MT-VCV matrix to derive the MT_{RFI} genetic

parameters. Using these parameters, a comparison between the classical RFI_{ST} and MT_{RFI} was made in terms of animal evaluations. Correlations between RFI_{ST} and $MT_{_{RFI}}$ breeding values for

selected groups of animals and standard deviation of estimated breeding values (EBV) and genomic enhanced breeding values (GEBV) were calculated. Using leave-one-out method, the cross-validation reliability of BLUP and ssGBLUP models were also calculated.

B) Regression on expected feed intake (ReFI)

During the course of this project, together with the Nordic research group, we have developed genomic predictions for metabolic efficiency in Nordic dairy cattle (Task 3.2). The genomic prediction is based on a RFI model. However, when thoroughly validating the genomic breeding values, we observed that some breeding values are illogical. We were able to identify the problem, which is that the RFI model had problems in modelling correct expectations for the feed intake observations. Especially for high yielding cows the feed intake expectations where too low, which resulted for those cows incorrect genomic breeding values. Alternative models for RFI did not improve the modelling of expected feed intake. Similar problems have been observed in other studies (Tempelman et al. 2015). Therefore, we developed an approach, regression on expected feed intake (ReFI), where the expectations for feed intake are based on dairy cow nutrition studies, rather than estimating the expectations directly from the feed efficiency data. Moreover, the approach is based on a random regression model that allows to account for the multiplicativity of feed efficiency (Lidauer et al. 2022), which simplifies modelling the large variation in the daily amount of feed a cow consumes, and the effect of the diet on efficiency. We have tested different ReFI models and a basic ReFI model can be described as following:

 $DMI_{ijk} = \beta_i \times eDMI_{jk} + \psi_k \times eDMI_{jk} + \alpha_k \times eDMI_{jk} + \varepsilon_{ijk},$

where DMI_{ijk} is a feed intake observation, β_i fixed regression coefficient for herd × year × season contemporary group i; ψ_k is a random regression coefficient for the nonhereditary animal effect of cow k; α_k is a random regression coefficient for the additive genetic animal effect of cow k; ε_{ijk} is the random regression coefficient for the additive genetic animal effect of cow k; ε_{ijk} is the random regression coefficient for the additive genetic animal effect of cow k; ε_{ijk} is the random regression coefficient for the additive genetic animal effect of cow k; ε_{ijk} is the random regression coefficient for the additive genetic animal effect of cow k; ε_{ijk} is the random regression coefficient for the additive genetic animal effect of cow k; ε_{ijk} is the random regression coefficient for the additive genetic animal effect of cow k; ε_{ijk} is the random regression coefficient for the additive genetic animal effect of cow k; ε_{ijk} is the random regression coefficient for the additive genetic animal effect of cow k; ε_{ijk} is the random regression coefficient for the additive genetic animal effect of cow k; ε_{ijk} is the random regression coefficient for the additive genetic animal effect of cow k; ε_{ijk} is the random regression the expected feed intake, first we calculated the expected energy requirement (ER) applying the following formulation:

 $ER_{jk} = 4.81 \times ECM_{jk} + 0.603 \times MBW_{jk} - 27.6 \times BWL_{jk} + 34.8 \times BWG_{jk}$

where the ER coefficients for ECM, MBW, BWL and BWG were based on the study by Agnew et al. (2003). Dividing the ER values by the energy density of the feed gives expected dry matter intake (eDMI) covariables.

Data

We investigated the ReFI model with weekly feed efficiency records from 645 research farm cows recorded in Jokioinen (n=575), Maaninka (n=36) and Viikki (n=34) research farms (Table 3).

Trait	N obs	Mean	SD
DMI	18,581	19.7	2.84
ECM	18,542	29.3	4.43
MBW	18,573	120.0	9.19
BWL	3,602	0.374	0.383
BWG	14,443	0.353	0.222
MEI	18,574	215.0	28.3
RFI	18,030	0.085	1.98
REI	18,027	-4.60	20.2
ECE	18,505	0.137	0.020

Table 3. Descriptive statistics of daily value of measured and derived traits for primiparous Nordic Red dairy cattle, recorded at Finnish research farms between 2006 and 2021.

Abbreviations: DMI = dry matter intake [kg]; ECM = energy corrected milk [kg]; MBW = metabolic body weight [kg^{0.75}]; BWL = body weight loss [kg]; BWG = body weight gain [kg]; MEI = metabolizable energy intake; RFI = residual feed intake [kg] calculated as DMI minus expected DMI; REI = residual energy intake [MJ] calculated as MEI minus expected energy requirement; ECE = energy conversion efficiency [kg/MJ] that is ECM/MEI.

Comparison with a residual feed intake model and a genetic residual feed intake model

We compared the above explained new ReFI metric with a classical RFI model, which had the following form:

 $\mathrm{DMI}_{ijk} = \mathrm{HYS}_i + \mathrm{LW}_j + \gamma_E \times \mathrm{ECM}_{jk} + \gamma_M \times \mathrm{MBW}_{jk} + \gamma_L \times \mathrm{BWL}_{jk} + \gamma_G \times \mathrm{BWG}_{jk} + p_k + a_k + e_{ijk},$

where a DMI_{*ijk*} observation is same as for the ReFI model; HYS_{*i*} is the fixed effect of herd × year × season contemporary group *i*; LW_{*j*} is the fixed effect of lactation week *j*; γ_E , γ_M , γ_L and γ_G are partial regression coefficients on a cow *k*'s observations in her lactation week *j* for ECM, MBW, BWL and BWG, respectively, where inhere BWL and BWG have either zero or a positive value; p_k is the random nonhereditary animal effect of cow *k*; a_k is the random additive genetic animal effect of cow *k*; and e_{ijk} is the random residual effect.

For comparison with the genetic residual feed intake (gRFI) metric (Kennedy et al. 1993), we first estimated variance and covariance components by a multivariate analysis that included the traits DMI, ECM, MBW, BWL and BWG applying for each trait a model with the following effects:

$$\mathbf{Y}_{t:ijk} = \mathbf{HYS}_{t:i} + \mathbf{LW}_{t:j} + p_{t:k} + a_{t:k} + e_{t:ijk},$$

where $Y_{t:ijk}$ is an observation for trait t, $HYS_{t:i}$ is a herd x year x season effect, $LW_{t:j}$ is a lactation week effect, $p_{t:k}$ is the random permanent environmental effect, $a_{t:k}$ is the additive genetic effect, and $e_{t:ijk}$ is the random residual effect. Breeding values for gRFI have been calculated following (Kennedy et al. 1993).

For the genetic analyses we modelled the relationship between the cows by a relationship matrix that included 4329 animals. We estimated variance components and breeding values based on the three metrics and compared the phenotypic observations of the 10% superior cows when selection was based either on the ReFI, on the RFI or on the gRFI model.

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Task 2.3 Metabolic Resilience

Terhi Mehtiö, Martin Lidauer, Enyew Negussie, Päivi Mäntysaari and Esa Mäntysaari

The main aim of this task was to develop a model that describes the genetic variation in energy status at the onset of lactation by utilizing milk MIR readings collected routinely from dairy cows. The developed model shall allow estimating breeding values that can be used to enhance metabolic resilience in cows.

Motivation

Selecting for feed efficiency may favor cows with a disposition for negative ES unless metabolic resilience is accounted properly in the selection index. The prediction equations developed for NEFA and BHB in Task 1.3 allow predicting ES at the onset of lactation from routine milk recording samples. In this task we were interested to study the genetic associations between traits that could serve as energy status indicators (ESI), the predicted NEFA, BHB, acetone, certain milk fatty acids and fat to protein ratio (FPR), and fertility.

Based on the results we wanted to develop a model, utilizing all collected milk MIR spectral data since 2015, to describe best the genetic variation in the cows' ES at the onset of lactation, and the breeding values from this model shall enable selection for Metabolic Resilience.

Data

To estimate the genetic variation in ESI traits and their genetic correlation with female fertility in early lactation a data with 37 424 primiparous RDC cows with milk test-day records between 8 and 91 days in milk (DIM) was used. Routine test-day milk samples were analysed by MIR for blood plasma NEFA, milk fatty acids, milk BHB and milk acetone concentrations. Six ES indicators were considered and included in the analyses: plasma NEFA concentration (mmol/l) either predicted by multiple linear regression including DIM, milk fat to protein ratio (FPR), and fatty acids C10:0, C14:0, C18:1 *cis*-9, C14:0*C18:1 *cis*-9 (NEFA_{FA}) or predicted directly from milk MIR spectra (NEFA_{MIR}), C18:1 *cis*-9 (g/100 ml milk), FPR, BHB (mmol/l milk), and acetone (mmol/l milk). The interval from calving to first insemination (ICF) was considered as the fertility trait. The data is described in detail in Mehtiö et al. (2021) article.

Genetic analyses

Multivariate linear mixed animal models were applied to ES indicator traits at 8 to 35, 36 to 63, and 64 to 91 DIM. In addition, univariate analyses were made for each lactation period separately. In matrix notation, the model can be written as:

$y = X\beta + Za + e_{,}$

where **y** is a vector of observations, $\boldsymbol{\beta}$ is a vector of fixed effects of herd, year-month of the test-day for energy status indicator traits and year-month of calving for ICF, age at calving, and regression on DIM for energy status indicator traits; **a** is a vector of random animal additive effects; **e** is a vector of random residuals; and **X**, and **Z** are the corresponding design matrices. Random effects were assumed to be normally distributed with means equal to zero and the covariance matrix for **a**, var(**a**) = **G**₀ \otimes **A**, where **G**₀ was the covariance matrix for the random additive genetic effects and **A** was the additive genetic relationship matrix, and the

covariance matrix for **e**, $var(e) = \mathbf{R}_0 \otimes \mathbf{I}$, where \mathbf{R}_0 was the covariance matrix for the random residuals and \mathbf{I} was an identity matrix.

Genetic analyses were made first within each ESI trait separately, applying a multi-trait model for all three periods to assess the genetic correlations between the periods as well as applying single-trait analyses within each period. Secondly, the correlations between the six different ESI traits and ICF were assessed applying multi-trait models within each period. Variance components were estimated using REML applying Average Information (AI-REML) method in DMU package (Madsen & Jensen 2013). Standard errors for heritability estimates and genetic correlations were approximated using Taylor series expansions.

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WP3. Genomic predictions

Task 3.1 Single-step genomic predictions

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The aim of this task is to develop optimal single-step models for the models developed in WP2 for Maintenance, Metabolic Efficiency, and Resilience.

Motivation

Genomic prediction has been officially introduced into Finnish and Nordic dairy cattle evaluations in 2012. In the current practice, the animals are first analysed with conventional pedigree based genetic evaluations, and then those genetic evaluations are converted to "pseudo" phenotypes that are used as input for genomic evaluations. Breeding animals, usually AI bulls or top cows are first genotyped with dense whole genome SNP panels, and next their pseudo phenotypes are used to solve associations between SNP variants and the traits. After the associations are estimated, the "SNP-key" can be used to predict the breeding values of young animals with own genotypes but without phenotypes. This makes genomic evaluations well suited to genetic evaluations of novel traits and traits that are too expensive to measure from all animals. Since then, genomic methodologies have continuously evolved and an approach (Aguilar et al. 2010, Christensen & Lund 2010), which allows modelling simultaneously information from genotyped and non-genotyped animals, named "single-step model", has been introduced. This cures the problem of having two evaluations: genomic evaluations for nucleus animals, and genetic evaluations for the most. Single-step genomic prediction models are the models of choice for developing genomic predictions for feed efficiency traits.

Development of genomic models

A) Genomic prediction model for Maintenance Cost

Phenotypic data and pedigree. The data contained all available BW and conformation traits observations of Danish, Finnish and Swedish RDC cows born in the years 1990 to 2017. This included BW observations from Finnish cows, which were measured by tape (heart girth), BW observations from Danish cows, which were measured by scale, and first parity conformation observations from cows of all countries. The raw BW observations were pre-processed as explained by Lidauer et al. (2019) to obtain for each lactation, that had BW records, one MBW observation with an associated weighting coefficient that accounted for the type and number of the raw BW observations. The first parity conformation observations were taken from the NAV routine conformation evaluation (NAV, 2019) with the following trait definition: ST (linear, cm), CW (linear score from 1 to 9) and BD (linear score from 1 to 9). After editing, the data consisted of 1.28 million MBW observations and 2.94 million conformation observations. The pedigree of the cows with observations included 2.99 million animals and the base population was described with 182 unknown parent groups (UPG).

Best linear unbiased prediction model. The BLUP model developed for the NAV routine MBW evaluation was the basis for building the single-step genomic predication model. The BLUP model is a multiple-trait animal model with six traits, which are MBW in 1st, 2nd, and 3rd parity, and first parity ST, CW, and BD. The applied heritabilities for these traits were 0.46, 0.51, 0.56, 0.60, 0.18, and 0.26, respectively. A detailed description of the model and the applied variance components is given in Lidauer et al. (2019). The correlated information from the conformation traits is important because there is no BW information available from Swedish cows. Moreover, recording of BW by tape is reducing in Finland and measuring BW by scale is slowly becoming more common. Genetic correlations between the three MBW traits and the conformation traits were on average 0.67, 0.55, and 0.49 with ST, CW, and BD, respectively. Furthermore, to improve predictions of Finnish herds the observations from HOL cows were also included to increase contemporary group sizes. The estimated breeding values (EBV) for MBW were used to form for each animal one combined MBW EBV by weighting 1st, 2nd, and 3rd parity EBVs with the weights 0.30, 0.25, and 0.45, respectively, and changing the sign of the index values so that a higher index value refers to a lower MBW.

Marker data. Genotype information from RDC animals only was included for developing the single-step model. Bulls were genotyped using the Illumina Bovine SNP50 Bead Chip (Illumina, San Diego, CA) and the cows using the lower-density EuroG MD chip (<u>http://www.-eurogenomics.com/</u>). The unobserved genotypes with the lower-density chip were imputed to the 50K density. After editing there were 46,914 single nucleotide polymorphisms (SNP) markers available for 51,417 animals.

Multi-step genomic prediction model. For building the multi-step genomic prediction model, which was based on SNP-BLUP (msSNPBLUP), all genotyped bulls which had at least 20 daughters and a MBW EBV reliability of at least 0.5 and all genotyped cows that had observations were included. This resulted in 5,554 bulls and 43,276 cows and in total 48,830 genotyped animals. De-regressed proofs (DRP) based on the combined EBV for MBW of 1st, 2nd, and 3rd parity were used as observations for the SNP-BLUP evaluation and each animal's observation was weighted by the animal's effective record contribution (ERC). In addition to marker effects, the SNP-BLUP model included a polygenic effect explaining 10% of the genetic variation.

Single-step genomic prediction model. Relationships between animals in the single-step genomic prediction model (ssGBLUP) were described by the H matrix (Aguilar et al. 2010, Christensen & Lund 2010) which was implemented as described by Koivula et al. (2021). The Van-Raden method I (VanRaden 2008) was used for building the genomic relationship matrix by blending the G matrix with the residual polygenic effect that accounts for 10% of the genetic variation. The QP transformation was applied to account for the UPG. A combined genomic enhanced breeding value (GEBV) for MBW was formed in the same way as the combined EBV for MBW.

Model validation. The ssGBLUP model was validated by forward prediction cross-validation, which was done for all the three models. For the cross-validation, we selected 1721 cows and 354 bulls that had no own information in the reduced data set. For the evaluation with the reduced data, observations from most recent four years of bulls and their progenies were excluded. The same pedigree and genomic information were used as for the full data set evaluation to obtain breeding values (either EBV or GEBV) for candidates (BV_c). Thus, in the SNP-

BLUP evaluation for the reduced data all candidate animals were included but had no own data. The cross-validation reliability (r_{cv}^2) was calculated as:

 $r^{2}_{cv} = corr (DRP, BV_{c})^{2} / r^{2}_{DRP},$

where DRP were calculated separately for both the bull validation candidate, and the cow validation candidate group, to ensure proper calculation of ERC weights used for the de-regression. The second statistic we applied was the regression of full data breeding values on reduced data breeding values (Legarra & Reverter, 2018), which has an expectation of b_1 =1.0

B) Genomic prediction model for regression on expected feed intake

This research is a continuation of Task 2.2. The objectives of this study were: 1) to use different energy requirement formulations (Agnew et al. 2003, Finnish feeding norms Luke 2022, and NRC 2021) to calculate eDMI and apply this different expected dry matter intake values in predicting breeding values for metabolic efficiency by a regression on expected feed intake (ReFI) model, 2) to solve single-step genomic prediction models for each of above-mentioned ReFI models with both full- and reduced-dataset in order to compare predictive ability of these models.

Data. Three data files including weekly phenotypic records (22,071 records pertained to 791 individuals), pedigree (5,604 individuals) and SNP markers (for 1489 animals; 762 males and 727 females) were available. 10 genetic groups were defined for animals with unknown parents according to their birth year and origin.

Statistical analysis. Three different equations were used in this study to calculate estimated dry matter intake. Feed requirement formulations were extracted from Agnew et al. (2003), from the Finnish feeding norms (Luke 2017), and Nord American nutrient requirements for dairy cattle (NRC 2021). Based on model selection, it was found that Herd-Production-Year (HPY) and Herd-Trial-Month (HTM) are the best factors to be included in the models. The ReFI repeatability model for the single-step genomic prediction was as follows:

 $DMI_{ijk} = \beta_i \times eDMI_{ijk} + \kappa_j \times eDMI_{ijk} \psi_k \times eDMI_{ijk} + \alpha_k \times eDMI_{ijk} + e_{ijk},$

where β , κ , ψ and α are the regression coefficients of observed dry matter intake (DMI) on expected dry mater intake (eDMI) nested within HPY, HTM, permanent environmental (PE) and additive animal (A) effects, respectively; e is the residual effect. The regression coefficients for the HTM effect were considered random since the number of records in each class of HTM was small. Three different formulations for calculating eDMI were tested.

Additionally, single-step genomic predictions were carried with a residual feed intake model where partial regression coefficients have been replaced by a regression on eDMI (RFI*). The RFI* repeatability model was as follows:

 $\mathsf{DMI}_{ijk} = \mu + \gamma \times \mathsf{eDMI}_{ijk} + \mathsf{PE}_i + \mathsf{A}_j + \mathsf{e}_k,$

where μ is the mean; γ is the regression coefficients of DMI on eDMI with eDMI calculated based on Agnew et al. (2003); PE is a random permanent environmental effect; A is the random additive genetic effect; and e is the random residual effect.

In the first step, variance components were estimated using MiX99 program which were needed for forward validations process. Data was split into two parts: a training set and a validation set. For the validation set 88 youngest cows with birth year in 2017 to 2019 were included. For the forward validation, we used two validation metrics. The first validation metric was regression of feed deviation (FD; phenotypes adjusted for fixed effects) on pedigree-based or genomic-based estimated breeding values (EBV and GEBV, respectively). The second metric was linear-regression validation introduced by Legarra and Reverter (2018) which was regression of EBV_f and GEBV_f using full data on EBV_r and GEBV_r using reduced data. Furthermore, Pearson correlations between GEBV from the different models for animals with record were calculated.

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Task 3.2 Genomic predictions for Nordic countries

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The aim of this task is to develop genomic prediction models for feed efficiency traits applicable for genomic predictions in Finnish, Danish and Swedish dairy cattle. First, prediction models based on the currently used "multiple-step" approach will be developed following by the development of "single-step" genomic prediction models.

Motivation

Feed efficiency research carried out in the Nordic countries for more than ten years aimed to develop the necessary scientific knowhow, phenotypes, methods, and models that allows to come up with research solutions applicable in daily Nordic dairy cattle breeding. Results from the previous work packages (WP1 & WP2), combined with research carried out in Denmark, allowed Nordic Cattle Genetic Evaluation (NAV) to implement first genomic prediction models for Maintenance Costs and Metabolic Efficiency in the years 2019 and 2020, respectively.

For Maintenance Cost, a multi-step genomic prediction is implemented. In a first step, breeding values, which are estimated based on the models for genetic evaluation of MBW that we have developed in WP2, are de-regressed to obtain for all genotyped animals de-regressed proofs (DRP). In a second step these DRP's are used to estimate genomic breeding values for genotyped animals based on a SNP-BLUP model. However, a genomic prediction model that combines all steps and estimates genomic breeding values for all animals (single-step model) is preferable and therefore, one aim for this task was to develop single-step genomic prediction models for the Nordic dairy breeds.

For Metabolic Efficiency, single-step genomic prediction models have been implemented by NAV for the Nordic dairy breeds based on our work in (see Task 3.1) and the work of the Danish research group. The models are based on a classical RFI model. However, in this project we found that a new metric, ReFI, would have advantages over using the RFI metric. Therefore, for the extended period of the project, one aim was to investigate whether a ReFI model would give similar results for all three Nordic breeds as we have found for the Finnish RDC breed.

A) Single-step genomic prediction models for metabolic body weight in Nordic Holstein, Red dairy cattle and Jersey

Materials & Methods

Phenotypic data and pedigree. The data contained all available BW observations (kg), the first parity stature (STA) observation (cm), which was taken from the NAV routine conformation evaluation (NAV, 2019) and carcass weight (kg) in five parities. Observations were from the Danish, Finnish and Swedish RDC, HOL, Jersey (JER) cows born in the years 1990 to 2020. This included BW observations from Finnish cows, which were measured by tape (heart girth), BW observations from Danish cows, which were measured by scale, and first parity STA observations from cows of all countries (Table 1). The raw BW observations were pre-

processed as explained by Lidauer et al. (2019) to obtain for each lactation with BW records one MBW observation (BW^{0.75}, kg) with an associated weighting coefficient that accounted for the type and number of the raw BW observations. After editing, the RDC and HOL data consisted of 1.30 and 1.46 million MBW observations (Table 4). JER data had only 11448 MBW observations. The number of STA and CARW records are presented in Tables 4 and 5. The pedigree of the RDC, HOL and JER cows with observations included 4.7, 9.3 and 0.9 million animals and the base population was described with 182, 202 and 70 unknow parent groups (UPG), respectively.

Table 4. Number of records per country per trait in RDC, HOL and JER breeds. Traits are metabolic BW in three parities (MBW1, MBW2, MBW3), first parity stature (STA) and carcass weight (CARW).

	RDC			HOL			JER		
	FIN*	DNK	SWE	FIN*	DNK	SWE	FIN	DNK	SWE
MBW1	514,809	5,915	0	200,603	90,898	4	0	6,064	0
MBW2	315,346	3,133	1	122,792	49,580	6	0	3,458	0
MBW3	91,905	1,383	2	45,447	26,457	1	0	1,926	0
STA	188,850	177,746	187,577	107,534	1,004,200	195,892	0	305,861	1,548
CARW	386,134	397,229	743,703	243,038	3,078,986	840,851	0	467,329	7,538

*HOL records not counted in Finnish RDC data and RDC records not counted in Finnish HOL data in this table.

Table 5. Number of records, means, and SD for metabolic BW in three parities (MBW1, MBW2, MBW3), first parity stature (STA) and carcass weight (CARW) in RDC, HOL and JER dairy cows.

	RDC				HOL			JER		
	Ν	Mean	SD	Ν	Mean	SD	Ν	Mean	SD	
MBW1	520,724	107.89	9.63	291,505	113.26	10.38	6064	86.16	5.43	
MBW2	318,480	115.34	10.09	172,378	123.04	10.44	3458	97.18	5.72	
MBW3	93,290	120.13	11.05	71,905	128.50	10.88	1926	101.49	6.28	
STA	554,173	139.50	4.40	1,307,626	146.18	4.06	307,409	127.50	3.20	
CARW	1,527,066	286.06	50.78	4,162,875	290.10	53.06	474,867	179.18	37.52	

*HOL records not counted in Finnish RDC data and RDC records not counted in Finnish HOL data in this table.

Best linear unbiased prediction (BLUP) model. The BLUP model developed for the NAV routine MBW evaluation (Lidauer et al. 2019) was the basis for building the single-step genomic predication model. The NAV routine BLUP model was updated by dropping the traits CW and BD and including CARW. This resulted a multiple-trait BLUP model with five traits, which are MBW in 1st, 2nd, and 3rd parity, first parity ST, and CARW. The heritabilities and variance components for these traits are presented in Table 6. The correlated information from STA and CARW is important because there is no BW information available from Swedish cows. Moreover, recording of BW by tape is reducing in Finland and measuring BW by scale is slowly becoming more common. Furthermore, to increase contemporary group size in Finnish herds, all observations from all breeds were included in each breed-specific model. The EBV for MBW were used to form for each animal one combined MBW EBV by weighting 1st, 2nd, and 3rd parity EBV with the weights 0.30, 0.25, and 0.45, respectively, and changing the sign of the index values so that a higher index value refers to a lower MBW.

Multiple trait linear mixed animal models for the first (MBW1), second (MBW2) and third (MBW3) parity MBW were:

$$y_{ijklmn} = lt_i\phi(d) + cage_i + ym_k + h5y_l + hy_m + a_n + e_{ijklmn},$$

where y_{ijlkmn} is either a MBW1, MBW2 or MBW3 observation; $lt_i\phi(d)$ is the fixed regression function on dim d nested within country × breed × time period, where $\phi(d)$ is a vector containing the covariates of linear and quadratic term of Legendre polynomial for dim d plus the exponential $e^{-0.1d}$ (based on Mäntysaari & Mäntysaari, 2015); cage_j is a fixed effect of calving age j nested within breed, ym_k is a fixed effect of year × month k; h5y₁ is a fixed effect of herd × 5-years period l; hy_m is a random effect of herd × year; a_n is the random additive genetic effect of animal n; and e_{ijklmn} is the random residual effect.

The linear model for the first parity ST was:

 $y_{ijlkm} = cage_i + cmy_j + lstg_k + h5y_l + hy_m + a_n + e_{ijklmn}$

where y_{ijlkm} is a ST observation; cage_i is a fixed effect of calving age i × year × country, cmy_j is a fixed effect of calving year j × month × country, $lstg_k$ is a fixed effect of lactation stage k × year × month × country, $h5y_1$ is a fixed effect of herd × 5-years period l, hy_m is a random effect of herd × year m, a_n is a random additive genetic effect of animal n, and e_{ijklmn} is the random residual.

The linear models for CARW was:

$$y_{ijklm} = lt_i \varphi(d) + parity_i + sage_j + sym_k + hy_l + a_m + e_{ijklm}$$

where y_{ijklm} is a CARW observation, $It_i \phi(d)$ is the fixed regression function on dim nested within breed × parity × country -interaction i , where $\phi(d)$ is a vector containing the covariates of linear and quadratic term of Legendre polynomial for DIM d plus the exponential $e^{-0.1d}$ (based on Mäntysaari & Mäntysaari, 2015); sage_j is a fixed effect of age in months at slaughtering j, sym_k is a fixed effect of slaughter year × month k, hy_l is a fixed effect of herd × year l, a_m is a random additive genetic effect of animal m, and e_{ijklm} is the random residual.

	MBW1	MBW2	MBW3	Stature	CARW
MBW 1	0.46	0.97	0.95	0.65	0.77
MBW 2	-	0.51	0.98	0.70	0.84
MBW 3	-	-	0.56	0.68	0.85
Stature	-	-	-	0.59 (RDC & HOL)	0.59
				0.41 (JER)	
CARW	-	-	-		0.52 (RDC & HOL)
					0.36 (JER)

Table 6. Heritabilities (on diagonal) and genetic correlations for RDC, HOL and Jersey breeds.

Marker data. Genotype information from RDC, HOL and JER animals were included for developing the single-step model. Bulls were genotyped using the Illumina Bovine SNP50 Bead Chip (Illumina, San Diego, CA) and the cows using the lower-density EuroG MD chip (<u>http://www.eurogenomics.com/</u>). The unobserved genotypes with the lower-density chip were imputed to the 50K density.

	RDC	HOL	JER
Pedigree records	4,669,968	9,270,696	882,137
Unknown parent groups (UPA)	182	202	70
Animals in data	2,987,705	6,229,638	608,356
Accepted data records	2,987,294	6,229,093	606,569
Genomic data			
All genotyped animals of NAV	207,219	384,029	107,836
Genotyped animals in MBW-CARW data	92,448	129,269	45,070
Ref.pop. tot*	89,815	123,873	43,736
Ref.cows	83,419	115,527	42,082
Reference cows with CARW obs	37,957 (45%)	41,703 (36%)	17,535 (42%)
Reference bulls	6,396	8,346	1,654
SNP markers available	46,914	46,342	41,897

 Table 7. Phenotypic and genomic data.

*Criteria for reference population according to NAV official instructions. All genotyped bulls which had at least 20 daughters and the MBW EBV reliability of at least 0.5, and all genotyped cows that had observations were included.

Single-step genomic prediction model. Single-step models were run with ssGTaBLUP (Mäntysaari et al. 2017) where the key computations involving the G^{-1} matrix are replaced by a dense T matrix of size m by n where n is the number of genotyped animals and m is the number of SNP markers. The VanRaden method I (VanRaden, 2008) was used for building the genomic relationship matrix by blending the G matrix with the residual polygenic effect that accounts for 30% of the genetic variation. The genetic groups were accounted for in the single-step models using the so-called partial QP transformation that omitted G^{-1} in QP (Koivula et al. 2022). The QP transformation was applied to account for the UPG. A combined genomic enhanced breeding value for MBW was formed in the same way as the combined EBV for MBW. Detailed information on genomic data is in Table 7.

Model validation. The ssGTaBLUP model was validated by forward prediction cross-validation, which was done for two models. For the cross-validation, we selected 45388 RDC cows and 250 RDC bulls, 78022 HOL cows and 323 HOL bulls and 21518 JER cows and 133 JER bulls that had no own information in the reduced data set. For the evaluation with the reduced data, observations from most recent four years of bulls and their progenies were excluded. The same pedigree and genomic information were used as for the full data set evaluation to obtain breeding values (either EBV or GEBV) for candidates (BV_c). Thus, in the ssGTaBLUP evaluation for the reduced data all candidate animals were included but had no own data. The cross-validation reliability (r^2_{cv}) was calculated as:

 r_{cv}^2 = corr (DRP, BV_c)² / $r_{DRP.}^2$

The second statistic we applied was the linear regression of full data breeding values on reduced data breeding values (Legarra & Reverter 2018), which has an expectation of b_1 =1.0.

B) Genomic prediction models for Metabolic Efficiency in Nordic Holstein, Red dairy cattle and Jersey

The implemented single-step genomic prediction model is based on a classical RFI model and is explained in detail by Stephansen et al. (2021). In this task we compared the currently implemented RFI model with a regression on expected feed intake (ReFI) model. The outcome of this study should clarify whether it would be beneficial to upgrade the current single-step evaluation to a single-step evaluation based on ReFI.

Data sets. We used all available feed efficiency data from CFIT herds and research farm herds for the three breeds Holstein, RDC and Jersey, which comprised of 149288, 70292, 62760 records, respectively.

Models. We applied four different approaches to model Metabolic Efficiency. Approach A: currently applied routine RFI model, which is a two-step modelling approach, where first RFI observations are calculated by a least squares model, and then these observations are used for the single-step genomic prediction. Approach B: was same as Approach A, but in the least squares model the partial regression coefficients were replaced by a regression on expected DMI using feed requirement formulations as used in Task 2.2. Approach C: the RFI observations were defined as requirement RFI, which was the difference between feed intake and expected feed intake. Approach D: ReFI, applying a similar model as in Task 4.1. The approaches and models are described in detail by Lidauer et al. (2022).

We compared the different approaches based on estimated variance components, and the phenotypic means of the genetically 10 % best cows. All analyses were carried out by Trine Andersen from SEGES Innovation, Denmark.

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WP4. Economic and environmental benefits

Task 4.1. Quantifying economic benefits

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The aim of this task is to study the genetic and economic impacts of different breeding program scenarios.

Motivation

As soon as feed efficiency breeding values are used for the selection, economic and environmental benefits will be realized. Breeding values for 90 different breeding traits of dairy cattle are combined into five trait groups which are production, fertility, health, conformation and functionality. The trait groups are combined into the NTM index by weighting each trait based on its economic value. The NTM comprises the breeding goal for the Nordic dairy cattle and is used for genomic selection of breeding candidates. Over the last years, NAV has been active in revising the economic weights of the NTM index, and the Saved Feed index was included in NTM in 2020.

In this task we wanted to estimate the additional economic response of including the developed feed efficiency traits into the NTM. We estimated the expected genetic response for the feed efficiency traits by considering the economic values developed by NAV and utilizing the required variance components. In addition, the genetic correlations between feed efficiency, production and fertility were needed to design a selection index and to assess the economic impact of including feed efficiency into dairy cattle breeding programme.

Data

The data used for variance component estimation was the FE data collected from research herds in Finland during 2006-2021. The data included in total 18 581 weekly records from 645 primiparous RDC cows. The traits of interest were seven different FE, production and fertility traits. The FE traits were ReFI (developed in Task 2.2), RFI and MBW. The production traits in this study were milk yield (MY), protein yield (PY), fat yield (FY), and the female fertility trait was ICF. For ICF yield deviations records from the official genetic evaluation of fertility for the cows in the pedigree were used (n=3037). For variance component estimation records from the data that were deviating more than 4 SD from the mean were removed. The pedigree included in total 5650 RDC animals.

	N obs	Mean	SD	Min	Max
DMI (kg/d)	18,581	19.7	2.8	8.6	31.0
eDMI (kg/d)	18,030	19.8	2.1	8.4	29.2
MY (kg/d)	18,559	27.4	4.6	8.7	44.5
PY (kg/d)	18,536	1.00	0.15	0.40	1.54
FY (kg/d)	18,556	1.21	0.21	0.37	2.05
MBW (kg)	15,899	120.2	9.4	83.4	157.6
MBW (kg)*	18,573	120.0	9.2	85.4	156.7
ICF**	3,037	1.1	24.0	-77.3	107.6

Table 8. Number of records, means, standard deviations (SD), minimum and maximum values for DMI, eDMI, MY, PY, FY, MBW, and ICF records.

* Records modelled by Mäntysaari and Mäntysaari (2015)

** Yield deviations from the genetic evaluations of fertility

Genetic analyses

Single-trait genetic analyses were performed to estimate the variance components of the traits. For ReFI the energy requirements by Agnew et al. (2003) were used and the model was:

 $DMI_{ijkl} = \beta_i \times eDMI_{ijkl} + \kappa_j \times eDMI_{ijkl} \psi_l \times eDMI_{ijkl} + \alpha_l \times eDMI_{ijkl} + e_{ijkl},$

where β_i is the fixed regression coefficient for the herd-feed year i, κ_j is the random regression coefficient of herd-test-month j, ψ_i is the random regression coefficient for the permanent environmental effect of cow I, α_i is the random regression coefficient for the additive genetic effect of cow I, and e_{ijkl} is the random residual.

For comparison we used the classical RFI, which was modelled as:

 $DMI_{ijkl} = HTM_i + \beta_1 ECM_j + \beta_2 MBW_j + \beta_3 BWG_j + \beta_4 BWL_j + LACTCURVE_k + pe_l + a_l + e_{ijkl},$

where HTM_i is the fixed effects of herd-test-month i, β_1 , β_2 , β_3 , β_4 are the fixed regression coefficients of the energy sinks nested within herd-feeding year j,LACTCURVE_k is the fixed lactation curve modelled by the 4th order Legendre polynomial and Wilmink function in days in milk k, pe_l is the random permanent environmental effect of cow l, a_l is the random additive genetic effect of cow l, and e_{ijkl} is the random residual. The MBW, BWG and BWL records were modelled as by Mäntysaari and Mäntysaari (2015).

Metabolic body weight and production traits MY, PY and FY were modelled as:

 $Y_{ijkl} = HTM_i + LACTCURVE_{jk} + CAGE_l + pe_m + a_m + e_{ijklm},$

where HTM_i is the fixed effect of herd-test-month i, LACTCURVE_{jk} is the fixed lactation curve modelled by the 4th order Legendre polynomial and Wilmink function in days in milk j nested within herd-5year-period k, CAGE_l is the fixed calving age modelled by 2nd order polynomial in calving age, month l, pe_m is the random permanent environmental effect of cow m, a_m is the random additive genetic effect of cow m, and e_{ijklm} is the random residual.

To estimate the genetic correlations between all seven traits bi-variate analyses of yield deviations from single trait analyses for all the traits were used. Genetic analyses were performed using MiX99 software.

Simulation studies on MoBPS

The genetic response and economic impact of including either ReFI or RFI into dairy cattle breeding program was studied. Genetic parameters obtained in this task were applied. However, for MBW we applied the variances and heritability used in the Nordic evaluation because a higher heritability is expected for MBW from research farm data where MBW is measured daily. Economic values based to those used in NTM (NAV, 2022) were applied. For MBW the applied economic value was larger than that one used by NAV to account also for the savings in feed cost for replacement by considering that a replacement cow is during the whole growing period 98.8% smaller in size, which is proportional to 1 kg^{0.75} MBW reduction for an average primiparous cow, i.e., 630 kg instead of 638 kg. All economic values are presented as per cow and year and assuming that an average cow produces 10,000 kg milk, 340 kg protein and 420 kg fat.

Various breeding program scenarios were be compiled and compared to assess the genetic and economic impact using interface version of R package MoBPS (Pook et al. 2020). In all simulation scenarios, the same breeding program was assumed. The simulation is simplified and was based only on bull selection. The number of bulls was reduced to about a third of that of what is currently used in the RDC breeding program, but the selection intensity was kept the same by selecting the 2.5% bulls with the highest index. 5 repetitions and 10 breeding cycles were run for each scenario. Generation interval was assumed to be three years. All animals were assumed to be genotyped and all the cows were phenotyped. The simulation included 7 traits (ReFI, RFI, MBW, MY, PY, FY, and ICF).

Four different selection indices were compared:

Scenario	Included traits in the selection index
1. Yield:	MY, PY, FY, ICF
2. +MBW	MY, PY, FY, ICF, MBW
3. +MBW+RFI:	MY, PY, FY, ICF, MBW, RFI
4. +MBW+ReFI:	MY, PY, FY, ICF, MBW, ReFl

Breeding values were estimated by single trait GBLUP and correlations between traits were considered when calculating the index weights. The selection was made based on the indices according to scenarios. When calculating the index weights for a specific scenario, only the genetic parameters and economic values of the traits that were included in the index were considered. In the simulation, however, all 7 traits with genetic parameters were included to get correlated response for all traits. For all scenarios, the economic response was calculated

the same way by multiplying the changes in the genetic breeding values by the economic values for the traits MY, PY, FY, ICF, MBW and ReFI. The ReFI metrics was chosen for the metabolic efficiency trait because it is calculated on feed requirement formulations.

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Task 4.2 Genetic response – environmental impact

Enyew Negussie, Jarmo Juga (University of Helsinki) and Martin Lidauer

The aim of this task is to quantify the impact of a unit change in a feed efficiency trait on CH4 emissions.

Motivation

Feed efficient animals require relatively less feed and on average they are expected to produce less CH_4 and N_2O per unit product than the population average at a similar level of production. Therefore, improving the feed utilization efficiency of individual animals could be a sustainable alternative for reducing enteric methane emissions from dairy systems (Negussie et al. 2014). Hegarty (2009) showed decreased methane production in animals selected for reduced RFI where reduced RFI is akin to selection for high feed efficiency. As broader breeding goals are the norm in many livestock species, inclusion of FE in dairy cattle selection indices will have environmental impact. However, genetic parameters and particularly genetic associations between RFI, environmental impact, and other production and functional traits are generally lacking. In this task, these important genetic parameters were estimated and correlated response, due to selection for RFI, on CH_4 output and the other production traits included in the breeding goals were calculated. The RFI trait was defined as actual DMI minus expected DMI, where expected DMI was calculated based on Finnish energy requirement formulations.

Data

In this task, production, feed efficiency and methane output traits recorded at the Jokioinen research dairy farm were used. Data was from 310 Nordic Red cows including 13,573 weekly records. Production traits were residual feed intake (RFI), milk yield (MY), fat yield (FY), protein yield (PY) and metabolic body weight (MBW). The CH₄ phenotype included in the analyses was CH₄ production (MeP=CH₄ g/day). Mean MeP, RFI, MY, FY, PY, MBW were 413.1±32.7 g/d, 0.0±19.8 kg/d, 28.0±4.5 kg/d, 1.27±0.19 kg/d, 1.03±0.13 kg/d, and 119.6 kg^{0.75}, respectively.

Genetic analyses

A multi-trait repeatability animal model was fitted for the estimation of variance components and to calculate the associated genetic parameters. The models included fixed effects of age, kiosk number, lactation week, measurement year-month and random permanent environment and animal effect.

Analyses of genetic response

From an economic viewpoint it is important to assess how correlated characters change when animals are selected for a certain characteristic. In this case, our aim was to see how CH₄ emission and other production traits change when selection is on RFI to improve feed efficiency. Particularly, the focus was to see the correlated response in CH₄ emission when selection is on RFI. For this, a multi-trait selection index theory as described in Kennedy et al. (1993) was used and the correlated response due to selection for RFI was calculated as Δg =b'Gi/ σ_i , where response in component traits equals b= index weight, G= genetic variance covariance matrix, i= intensity of selection and σ_i = standard deviation of the index.

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Task 4.3 Quantifying environmental benefits

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The aim of this task was to quantify the environmental impact of improving feed efficiency on GHG mitigation and land use. Also eutrophication potential was assessed.

Motivation

The objective of the conducted research has been in improving the feed efficiency (FE) and developing tools for breeding towards FE and lowered CH₄ emissions from rumination. Even if these breeding goals can be considered as such as improving the environmental performance, the potential trade-offs and magnitude of the improvement remain unclear. Here in task 4.3 guantification and validation of the achieved impacts of breeding at product level were conducted. The environmental impact, and especially the change achieved with breeding, was quantified at product level using Life Cycle Assessment (LCA) method. LCA is well adapted method for assessing environmental impact of a product and already quite standardized especially regarding global warming potential. For the LCA assessment of dairy products, typical system boundaries which are included in the assessment, include feed crop production (including farms own feed crop production and purchased feeds, with their input use), dairy production (including emissions from animals and manure, i.e., rumination and manure management) and related input use, such as electricity use on animal housing, energy used in cereal drying and diesel fuels used in field work and transportation. From the previous LCA assessments of dairy products, it is clear that enteric fermentation and feed crop production are responsible for a large share of the total carbon footprint of milk. As the A⁺⁺Cow project aims in improving dairy production sustainability by developing methods for selection towards more efficient feed utilization and reduced methane generation from rumination, exactly those largest contributors to CF of milk, the project findings regarding these aspects were integrated into LCA assessment model of dairy production. Thus, besides the impact to the single emission sources, LCA analysis gives a detailed overall result for the selected environmental impact category and environmental benefit (or change in impact) can measured in relation to total burden of the production.

Data collection from Finnish dairy farms

The A⁺⁺Cow project was conducted in close collaboration with major Finnish dairy producer Valio. The baseline assessment for the current average Finnish dairy production ("business-as-usual", BAU) was conducted utilizing real farm data collected from 700 Finnish dairy farms. The collected data included herd composition, feed composition and origin, details for own feed crop production (crops, yields, fertilizer inputs, crop protection), manure management system and interval for emptying manure storage, and the yields of raw milk and meat from cull cows.

For assessment of the BAU milk production, data collection from 700 farms was conducted with Valio's Carbo tool which is integrated to Valio's LCA assessment model Lypsikki-LCA. The average farm was formed based on this data and the farm characteristics (which were not affected by breeding) were kept constant for the assessment of the project findings regarding

breeding response. These constant characteristics included herd composition and size, feed composition and manure management systems. Average farm characteristics are presented in Table 9. Project findings regarding breeding response were integrated into the assessment and included DMI, methane generation from rumination, raw milk yield and quality (fat, protein) and BW.

Dairy cows, n	65
Age at first calving, m	25
Dairy cow, live weight, kg	647
Dairy cows, pasture period	74
Feed use, kg dry matter/day/cow	22
Milk production, kg/year/cow	10139
Manure system	slurry, tight cover
Feed forage, %	57 %
Feed concentrate, %	43 %

Table 9. Characteristic of average dairy farm, based on data collected from 700 Finnish dairy farms.

Lypsikki-LCA assessment model

Lypsikki-LCA model has been developed by Valio for dynamic assessment of environmental impacts of dairy production at farm level. The model has been developed to react to the changes in a dairy farm's activities and to optimize diet, balance nutrient flows, simulate cultivation and manure management, predict milk production and herd size changes, and dynamically predict GHG and nutrient emissions.

Lypsikki-LCA model for climate impact of raw milk and beef from culled cows has been conducted by partly following product environmental footprint category rules (PEFCR) of dairy products (European Commission 2018) and IPCC (2006) guidelines. These frameworks have been complemented by national emission prediction models, which present the Finnish production environment. The model is constructed on a nutrient management model described in Nousiainen et al. (2011). Enteric fermentation from rumination is calculated with prediction model by Ramin & Huhtanen (2013) and manure storage emissions are evaluated for each day separately, based on daily amount of manure in the storage and average temperature of that day.

In characterisation of greenhouse gases to carbon dioxide equivalents (CO₂eq) the IPCC (2013) characterization factors with inclusion of climate carbon feedback were used. Thus, the biogenic methane (CH₄) characterization factor was 34 kg CO₂eq kgCH₄⁻¹ and dinitrogen oxide (N₂O) characterization factor was 298 kg CO₂eq kgN₂O⁻¹ (Myhre et al. 2013).

Lypsikki-LCA has system boundary from cradle to farm gate and the analysis was conducted with inclusion of only those feeds which were utilised in dairy production. The life cycle stages within the system boundary included feed crop cultivation (N₂O based on soil type, N₂O from crop residues, N₂O leaching and volatilization, CO₂ from liming), pasture (N₂O from pasture), emissions from animals (methane from rumination), manure management (CH₄ and N₂O) and input production (fertilizers, lime, seeds, purchased crops, bedding materials, electricity, fuels).

In LCA, allocation approach between milk and beef is continuously under debate. Currently, the suggested approach in well adapted PEFCR and IDF guidance's is biophysical allocation, although other allocation methods are also applied. Lypsikki-LCA model includes different allocation options for milk and beef, including also the here-utilized biophysical allocation method for which has been the recommended method in Dairy PEFCR (IDF 2015, European Commission 2018).

The functional unit was set as 1 kg Fat and Protein Corrected Milk (FPCM) as according to FAO (2010), thus the impacts were reported per 1 kg of FPCM and allocated utilizing bio-physical allocation method by IDF between milk and beef (IDF 2015).

Besides the climate change impact assessment, the LCA included estimation of eutrophication potential and land occupation.

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3.2. Results

WP1. Novel phenotypes

Task 1.1 Feed efficiency phenotypes

Feed efficiency data

The final Finnish feed efficiency data (FE-data) of primiparous Nordic Red cows was composed from the measurements collected in current A⁺⁺Cow project and the data of previous projects (Seosrehu, ASMO, GREENDAIRY, Feed Efficiency and A⁺⁺Lehmä pilot). Data from all the projects were reworked and merged to be used in the statistical analyses. In total the merged FE-data will include measurements from 828 cows with 148 715 records (Table 10).

Herd	Years	Cows	Daily observations
Rehtijärvi	1998–2001	146	5,507
Rehtijärvi	2006–2009	145	4,231
Minkiö	2009 →	467	122,923
Maaninka	2013–2016	36	8,944
Viikki	2013–2016	34	7,110
Total		828	148,715

Table 10. Currently available Finnish feed efficiency data.

Data description

The average ECM yield for the cows in FE-data is 28.5 kg/d, the total intake of DM and ME are 19.3 kg/d and 211 MJ/d, respectively (Table 11). The mean body weight of the cows in the FE-data was 589 kg. The FE-data include measurements of the primiparous cow calved be-tween 1998–2022. The development of ECM yield, DMI, ECE and BW during data collection period based on the calving year of the cows is presented in Figure 4. A steady increase in ECM yield can be seen. Cows that started lactation in 1998 had a lower BW than cows that calved in the 2020s, but there is no clear trend in the average weight of cows in the FE data during the last decade.

Table 11. Mean, standard deviation and range (min, max) of cow-wise average milk produc-
tion, feed intake and body weight and condition during lactation days 3-305 (N=828; N in
cow average varied from 10 to 303).

	Mean	SD	Min	Мах
Milk yield, kg/d	26.9	3.54	14.0	37.2
ECM yield, kg/d	28.5	3.55	15.7	39.5
Milk composition, %				
Fat	4.43	0.465	3.23	6.65
Protein	3.64	0.252	3.00	4.60
Lactose	4.58	0.116	3.93	5.00
Intake				
Forage, kg DM/d	10.1	1.29	5.7	15.7
Concentrate, kg DM/d	9.2	1.06	4.9	12.3
Total intake, kg DM/d	19.3	2.19	10.5	26.6
Energy, ME MJ/d	210.9	21.6	123.1	284.7
Crude protein, kg/d	3.29	0.417	1.73	4.46
Body weight, kg	589	54.9	386	809
Body condition score	3.15	0.287	2.26	4.37
REI, ME MJ/d	-5.1	16.8	-70.7	70.0
ECE, kg ECM/ME MJ	0.139	0.017	0.080	0.232



Figure 4. The development of ECM yield, DMI, ECE and BW of the cows in data based on the calving year.

Task 1.2 Feed intake predictions

Validation of CFIT

Data of daily intakes (FI) measured by scale has been processed from both trials. The data include weighted daily FIs and calculated daily dry matter intakes based on weighted scale FIs and analyzed dry matter content of the TMR. Also, the data of CFIT measurements is processed and available from both trials. However, one third of the dataset from Trial 1 will be processed once more to ensure correct values. Therefore, all results are still preliminary results. In Trial 1 the cows ate TMR from their own place with separators on the feeding table and both the scale and CFIT system FI were measured. Also, in Trial 2 cows had own-place FI measurements with separators on the feeding table every second week, totally two weeks. All this own-place data from Trials 1 and 2 were combined to produce a data of 83 cows and 1518 daily observations. The Figure 5 shows the relationship of the scale and the CFIT system FI measures of the cows in the combined data. The average daily scale FI of the cows was 67.3 (SD 8.11) kg/d (= 23.9 kg DM/d) and the average daily CFIT system FI was 59.4 kg/d (SD 8.49) (=21.1 kg DM/d). Thus, the CFIT measured FIs were an average about 12% lower than the scale FIs. Daily intake observations (1-day) were used to build new variables, i.e., 2-days, 3-days etc. averages from both the scale and CFIT FI measurements. The correlation between the scale and CFIT FI measures increased with increased number of days in averages (Figure 5). The correlation between the scale FI and CFIT FI was 0.59 with 1-day observations and with 4-days or more averages 0.71–0.72.



Figure 5. Relationship of the scale and the CFIT feed intake measures (kg/d) (A). The correlation between the scale and the CFIT feed intakes based on 1 to 7 days average intakes (B).

In Trial 2, cows ate every second week from a feed sward (S) and every second week from own places (O) with the separators on the feeding table. The sward-feeding was supposed to mimic on-farm feed intake measuring. As in Trial 1, also in Trial 2 the scale FI measures were higher than the CFIT FI measures on both O- and S-feeding (Table 12). The standard deviation (SD) was clearly higher for the CFIT S-feeding FI than for the scale FI measures. The SD of FI measures was also higher for cows on the CFIT S-feeding than the CFIT O-feeding. The repeatability of 7-days average FIs measured every second week were 0.87, 0.77 and 0.80 when measured by scale, CFIT on O-feeding or CFIT on S-feeding, respectively. The correlations calculated between the scale and the CFIT O-feeding FI measures, between the CFIT S-feeding FI measures and the scale FI averages from the adjacent weeks, and between the CFIT S-feeding FI measures and the CFIT O-feeding FI averages from the adjacent weeks are presented in the Figure 6. All correlations are based on 7-days average FI measures. Naturally, the correlation between the CFIT O-feeding and the scale FI measured on the same week was higher than the correlation between the CFIT S-feeding FI and the scale FI measures which were taken from the adjacent weeks. The correlation between the CFIT S-feeding FI and the scale FI measures which were taken from the adjacent weeks. The correlation between the CFIT S-feeding FI and the CFIT S-feeding FI and the CFIT-O-feeding FI measured on the adjacent weeks was at the same size than the correlation between the CFIT-S-feeding FI and the scale FI from the adjacent weeks. This indicated that there was no marked difference in the accuracy of FI measurements between the CFIT O-feeding and S-feeding. Also, the repeatability of the CFIT O-feeding ing (0.77) and the CFIT S-feeding (0.80) 7-days average FIs of the cows were about the same.

Table 12. Mean, standard deviation (SD) and coefficient of variation (CV) of the daily feed intake of the cows in Trial 2 when measured by scale or by CFIT on own-place (O) feeding or sward-feeding (S).

				Feed intake, kg	ı/d
Intake measured	Cows	Ν	Mean	SD	CV
Scale	40	560	65.5	7.51	0.11
CFIT own-place (O)	40	560	57.6	8.98	0.16
CFIT sward (S)	40	554	58.5	11.01	0.19



Figure 6. The correlations between the scale and the CFIT O-feeding feed intakes measured on the same week, between the CFIT S-feeding feed intakes and the average scale feed intakes of the adjacent weeks and between the CFIT S-feeding feed intakes and the CFIT O-feeding average feed intakes of the adjacent weeks.

Task 1.3 Predicting energy status from milk MIR spectra

Prediction equations for NEFA and BHB

The NEFA model fit with the entire data set is illustrated in Figure 7 with the corresponding R² and RMSE values for the models fitted with and without the transformation of the response variable. In the transformed scale, no heteroscedasticity was observed and there is no obvious systematic error. With the model utilizing the transformation a coefficient of determination of R²=0.53 (RMSE = 0.206 mmol/l, RMSECV = 0.217 mmol/l) was obtained for the 10-fold cross-validation. Furthermore, in independent herd validation RMSEP values 0.186 mmol/l and 0.221 mmol/l were obtained. When the prediction model is built without transforming the data the fit values were very similar, or even slightly better with R²=0.54 (RMSE = 0.204 mmol/l, RMSECV=0.215 mmol/l). However, there are several systematic problems such as some predicted values being negative, systematic error where the predictions for concentrations above 1.2 mmol/l are systematically too small, while predictions for concentrations below this tend to be slightly too large.



Figure 7. The blood test based NEFA values (observed) and the predicted values from a partial least squares model based on mid-infrared milk measurements in a fourth root transformed scale (left panel) and the original scale (right panel).

The BHB model fit with the entire data set is given in Figure 8 with the corresponding R² and RMSE values for the models fitted using the original and the root-transformed scales. While there is no obvious heteroscedasticity in the root-transformed scale, there is some systematic error where the largest values are underpredicted, resulting in fit metrics R²=0.62 (RMSE = 0.328 mmol/l, RMSECV=0.349 mmol/l). Furthermore, independent validation values RMSEP=0.366 mmol/l and 0.431 mmol/l were obtained. A systematic error seems to be present with a small overestimation for BHB<1.0 mmol/l while higher BHB concentrations are underestimated. The use of different transformations was explored as an option, but the results were not satisfactory. In order to address this issue, an ad hoc correction of the form x^{λ} with $\lambda > 1$ was proposed. A value of $\lambda = 1.4$ was obtained via a fitting procedure on the

training data. The corrected values are graphically shown in the leftmost panel of Figure 2. In the original scale without the correction, the RMSE value is 0.328 mmol/l, with uncertainties being smaller for smaller than average concentrations and larger for large concentrations. With the correction the RMSE value is slightly smaller at 0.326 mmol/l (RMSECV = 0.353 mmol/l), but more importantly, the systematic underprediction of larger values is significantly mitigated. The validations in using an independent herd with the ad hoc correction had RMSEP values 0.411 mmol/l and 0.422 mmol/l.



Figure 8. The blood test based BHB values (observed) and the predicted values from a partial least squares model based on mid-infrared milk measurements in a fourth root transformed scale (left panel), the original scale (middle panel), and the original scale with an ad hoc power correction (right panel).

The developed prediction equations for NEFA and BHB were delivered to Mtech in summer 2022 and those are applied and available in the Mtech database since autumn 2022. A manuscript with all results has been submitted to the Animal journal.

Kostensalo, J., Lidauer, M., Aernouts, B., Mäntysaari, PB., Kokkonen, T., Lidauer, P. & Mehtiö, T. 2023. Predicting blood plasma NEFA and BHB concentrations from milk MIR spectra. Animal, submitted.

WP2. Modelling feed efficiency

Task 2.1 Modelling maintenance cost

Main results

Heritability estimates and genetic correlations are presented in Table 13. Estimated genetic correlations among MBW traits were strong (>0.95). Genetic correlations between slaughter traits (CARW and pMBW) and MBW traits were higher (from 0.77 to 0.90) than between conformation and MBW traits (from 0.47 to 0.70). Our results suggest that including information on carcass weight and body conformation as correlated traits into the Maintenance index is beneficial when direct BW measurements are not available or are difficult or expensive to obtain.

Table 13. Heritability estimates on diagonal, genetic correlations in upper triangle and phenotypic correlations in below triangle for MBW in three parities (MBW1, MBW2, MBW3), first parity conformation traits (STA, CW, BD), and traits based on carcass weight (CARW, pMBW) in Nordic dairy cattle. Standard errors for the estimates in parentheses.

	MBW1	MBW2	MBW3	STA	CW	BD	CARW	pMBW
MBW1	0.44	0.97	0.95	0.65	0.58	0.52	0.77	0.84
	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.04)	(0.04)
MBW2	0.65	0.53	0.98	0.70	0.54	0.47	0.84	0.89
		(0.02)	(0.01)	(0.01)	(0.01)	(0.01)	(0.04)	(0.03)
MBW3	0.61	0.72	0.56	0.68	0.52	0.53	0.85	0.90
			(0.02)	(0.01)	(0.01)	(0.01)	(0.04)	(0.03)
ST	0.44	0.48	0.49	0.60	0.18	0.21	0.59	0.67
				(0.02)	(0.01)	(0.02)	(0.07)	(0.05)
CW	0.25	0.23	0.20	0.10	0.17	0.56	0.59	0.57
					(0.02)	(0.04)	(0.29)	(0.21)
BD	0.26	0.23	0.23	0.11	0.31	0.26	0.40	0.42
						(0.02)	(0.29)	(0.21)
CARW	0.47	0.56	0.62	0.41	0.22	0.15	0.52	
							(0.02)	
pMBW	0.51	0.60	0.65	0.47	0.24	0.17		0.54
								(0.05)

Based on these Mehtiö et al. (2021) results it was decided to collect carcass weight records also from Sweden and Denmark and to include carcass weight as correlated trait into the maintenance index. In addition, it was decided to drop CW and BD information from the index due to the low genetic correlations and heritability estimates. This work is described in Task 3.1. Single-step genomic predictions and Task 3.2. Genomic predictions for Nordic countries.

The equations developed in Mehtiö et al. (2021) for predicting MBW at the time of slaughtering can be utilized in estimating the energy requirement for maintenance in national GHG emission inventories to achieve more precise BW estimates for the Finnish dairy cattle population.

Task 2.2 Modelling metabolic efficiency

Main results

A) Residual feed intake via multiple-trait approach

Estimates of heritability from RFI_{ST} and MT_{REI}

Assuming heterogeneity of residual variances, the estimates of heritability for residual classes of 1, 2, 3, 4-8 and 9-11 months for the RFI_{ST} model were 0.16, 0.21, 0.22, 0.22 and 0.21, respectively. Whereas the heritability estimates for the MT_{RFI} model across residual classes were 0.09, 0.13, 0.14, 0.13, 0.10, respectively. Although the heritability estimates from both models were lower at the beginning and end of lactation, in all the cases, estimates from the RFI_{ST} model were consistently higher than those from MT_{RFI} . In general, accounting for heterogeneity of residuals and modelling body weight change gave a more reasonable description of RFI across lactation stages.

	Heritability by residual classes (in months)							
Traits	1	2	3	4-8	9-11			
ECM	0.13	0.19	0.22	0.23	0.21			
MBW	0.75	0.83	0.88	0.90	0.85			
BW _{LOSS}	0.02	0.05	0.05	0.02	0.05			
BW_{GAIN}	0.13	0.26	0.36	0.42	0.39			
DMI	0.30	0.37	0.39	0.37	0.32			
MT _{RFI}	0.09	0.13	0.14	0.13	0.10			
RFI _{ST}	0.16	0.21	0.22	0.22	0.21			

Table 14. Estimates of heritability for classical single-stage (RFI_{ST}), multi-trait RFI (MT_{RFI}) and its component traits at different stages of lactation considering heterogeneity of residual variances

Correlation between RFI_{ST} and MT_{REI} and SD of EBVs/GEBVs

The SD of EBVs from conventional BLUP for cows with at least 20 observations was 0.486 for RFI_{ST} whilst 0.449 for MT_{RFI} model. Whereas the SD from ssGBLUP runs for RFI_{ST} and MT_{RFI} were 0.494 and 0.463, respectively. For cows, the correlation between RFI_{ST} and MT_{RFI} EBVs was 0.89 whereas for ssGBLUP the correlation between the two models was 0.90. Similarly, for bulls with at least 10 daughters the SD of EBVs were 0.536 and 0.512 for RFI_{ST} and MT_{RFI}' respectively. From ssGBLUP runs, the SD of GEBVs were 0.541 and 0.524 for RFI_{ST} and MT_{RFI}' respectively. In general, for bulls the correlation between RFI_{ST} and MT_{RFI} models were 0.92 in both BLUP and ssGBLUP runs indicating a possible re-ranking in top bulls and cows.

Cross validation reliability

The cross-validation reliability (CV r²) calculated using the leave-one-out method showed that for BLUP run the CV r² were 0.003 and 0.142 for RFI_{ST} and MT_{RFI} models with the corresponding inflation factor (b1) of 0.2 and 1.2, respectively. Whereas in the ssGBLUP runs, the CV r² were 0.23 and 0.40 for RFI_{ST} and MT_{RFI} models with the corresponding b1-values of 1.44 and 1.57, respectively. In general, the indications are that the MT_{RFI} model, by being able to use much more data information, gives a more accurate evaluation.

B) Regression on expected feed intake (ReFI)

The estimated variance components for ReFI and RFI are given in Table 15. The estimated additive genetic standard deviation based on ReFI and RFI was 4.7 % and 0.46 kg, respectively, indicating that based on ReFI a twice as large genetic variation in metabolic efficiency was estimated compared to the estimate based on RFI. Same, also the heritability (0.23) and repeatability (0.41) were significantly larger for ReFI compared to RFI. The multivariate analysis, for deriving gRFI, resulted heritability estimates of 0.26, 0.30, 0.90, 0.13 and 0.16 for DMI, ECM, MBW, BWL and BWG, respectively. The genetic standard deviation and heritability for gRFI were lower than those for RFI, and were 0.40 kg and 0.06, respectively.

	ReFI model		RF	l model
Model effect	Parameter Estimate (±SE)		Parameter	Estimate (±SE)
Permanent	$\widehat{\sigma}_{\psi}^2$	0.00169 ±	$\widehat{\sigma}_{p}^{2}$	0.736 ± 0.10
environment		0.00041		
Additive genetic	$\hat{\sigma}^2_{lpha}$	0.00221 ±	$\hat{\sigma}_a^2$	0.208 ± 0.11
		0.00058		
Residual	$\widehat{\sigma}^2_{arepsilon}$	2.24 ± 0.024	$\hat{\sigma}_e^2$	1.70 ± 0.015
Heritability	h^2	0.23 ± 0.06	h^2	0.08 ± 0.04
Repeatability	r	0.41 ± 0.02	r	0.36 ± 0.02

Table 15. Estimated variance components of metabolic efficiency for primiparous Nordic Red dairy cattle based on a regression on feed intake (ReFI) and a residual feed intake (RFI) model.

The estimated partial regression coefficients by the RFI model were significantly lower for ECM, BWL and BWG compared to those applied for the ReFI model (Table 16). This means that by applying a RFI model to describe the metabolic efficiency of a lactating dairy cow, a lower expectation for the required feed intake to produce milk will be modelled, compared to applying a ReFI model. For this data, the estimated partial regression coefficient for ECM of 0.251 equals a ME requirement of 2.74 MJ/kg ECM. This regression coefficient would mean that the k_l -value, the efficiency to use ME for lactation, would have been 1.15, which is impossible and indicates that the estimated partial regression coefficient for ECM is improper. In the literature reported k_l -values are on average 0.64 (Agnew and Yan, 2000). The partial phenotypic regression coefficients derived from the multivariate variance components were close to those estimated by the RFI model (Table 14), which indicated that expected DMI modelled by the RFI model. Thus, also gRFI resulted into a very low partial regression coefficient estimate for ECM and by

this an unrealistic high k_l -value of 1.08. The partial genetic regression coefficients, which are the selection index weights for gRFI, were different from the partial phenotypic regression coefficients and closer to those applied for ReFI.

Table 16. Applied and estimated partial dry matter intake (DMI) requirement coefficients, when use of feed intake is modelled by the four energy sink traits energy corrected milk (ECM), metabolic body weight (MBW), body weight loss (BWL) and body weight gain (BWG).

Model for biological efficiency	ECM	MBW	BWL	BWG
Applied coefficients in ReFI	0.430	0.0539	-2.47	3.11
Estimated coefficients by RFI	0.251	0.0504	-0.85	1.70
Partial phenotypic regression coefficients	0.267	0.0683	-0.75	1.49
Selection index weights for gRFI	0.359	0.0618	-1.86	4.53

Abbreviations: ReFI = regression on expected feed intake; RFI = residual feed intake; gRFI = genetic residual feed intake.

The difference in the regression coefficients between the three alternative metrics (Table 17) has significant effect on the EBV, and by this on the ranking of the animals. When selecting the best 10% cows based on either ReFI, RFI or gRFI, then it can be seen that rather different cow groups will be selected. When selection was based on ReFI EBV, cows had highest ECM which was 2.1 kg above the mean of all cows. In contrast, when selection was based on RFI EBV, then ECM of the superior cows was 1.4 kg below the mean of all cows. The same we found for MBW. When selection was based on ReFI EBV or RFI EBV, MBW of superior cow was 1.4 kg higher or 3.7 kg lower compared to the mean of all cows, respectively. When selection was based on gRFI EBV, then the trait means of the selected cow group were between those of the groups selected by ReFI EBV or RFI EBV. When selection was based on ReFI EBV, then the 10% most efficient cows produced 13% more ECM per unit metabolizable energy intake, whereas the corresponding values were only 4% or 8% when using RFI EBV or gRFI EBV, respectively.

	10% genetica	lly superior cows		
Trait	All cows	ReFI	RFI	gRFI
DMI	19.7	18.7	17.7	18.2
ECM	29.3	31.4	27.9	29.4
MBW	120.0	121.4	116.3	119.2
BWL	0.37	0.55	0.41	0.46
BWG	0.35	0.39	0.35	0.38
MEI	215.5	203.8	195.2	200.2
RFI	0.09	-1.64	-1.17	-1.35
REI	-4.60	-24.2	-15.8	-18.7
ECE	0.137	0.155	0.143	0.147

Table 17. Phenotypic means of all cows and 10% genetically superior cows based on the three alternative breeding values for metabolic efficiency.

Abbreviations: DMI = dry matter intake [kg]; ECM = energy corrected milk [kg]; MBW = metabolic body weight [kg^{0.75}]; BWL = body weight loss [kg]; BWG = body weight gain [kg]; MEI = metabolizable energy intake; RFI = residual feed intake [kg] calculated as DMI minus expected DMI; REI = residual energy intake [MJ] calculated as MEI minus expected MEI; ECE = energy conversion efficiency [kg/MJ] that is ECM/MEI.

The "Regression on expected feed intake (ReFI)" metric was clearly superior in selecting most efficient cows. Selected cows had higher milk production and higher energy conversion efficiency (= gross efficiency of producing milk), i.e., fewer cows and less feed is needed to produce the same amount of milk. The ReFI approach allows fitting of proper expectations for each cow comparison group (Figure 9), which takes into account the energy density and the digestibility of the feed. This is important when feed intake data are collected with different techniques, e.g., by scales or by intake predictions based on feed intake volume that is obtained from analyses of 3D images (CFIT system, see Task 1.2).

A manuscript with all results has been submitted to the Animal journal.

Lidauer, M.H., Negussie, E., Mäntysaari, E.A., Mäntysaari, P., Kajava, S., Kokkonen, T., Chegini, A. & Mehtiö, T. 2023. Estimating breeding values for feed efficiency in dairy cattle by regression on expected feed intake. Animal, submitted.



Figure 9. Regression of dry matter intake on expected dry matter intake for 92 cows of the contemporary groups summer 2008 of herd 1 (green, $\hat{\beta}$ =0.973), and winter 2015 of herd 2 (blue, $\hat{\beta}$ =1.109).

Task 2.3 Metabolic resilience

Main results

Heritability estimates varied during the first three lactation months from 0.13 to 0.19, 0.10 to 0.17, 0.09 to 0.14, 0.07 to 0.10, 0.13 to 0.17, and 0.13 to 0.18 for NEFA_{MIR}, NEFA_{FA}, C18:1 cis-9, FPR, milk BHB and acetone, respectively (Tables 18, 19, 20 and 21). Genetic correlations between all ESI traits and ICF were from 0.18 to 0.40 in the first lactation period (8 to 35 DIM), in general somewhat lower (0.03 to 0.43) in the second period (36 to 63 DIM), and decreased clearly (-0.02 to 0.19) in the third period (64 to 91 DIM). Our results indicate that genetic variation in energy status of cows in early lactation can be determined using MIR-based indicators. In addition, the markedly lower genetic correlation between ESI traits and fertility in the third lactation month indicated that energy status should be determined from the first test-day milk samples during the first two months of lactation.

Natural resources and bioeconomy studies 54/2023

Table 18. Summary statistics, variance components (genetic variance σ_a^2 and residual variance σ_e^2) and heritability estimates (h²) of the data in (1) 8 to 35 days in milk (DIM), (2) 36 to 63 DIM and (3) 64 to 91 DIM for plasma non-esterified fatty acid (NEFA) concentration predicted from milk mid-infrared spectra (NEFA_{MIR}, mmol/l), plasma NEFA concentration predicted from milk fatty acids (NEFA_{FA}, mmol/l), milk fatty acid C18:1 cis-9 (g/100 ml milk), milk fat to protein ratio (FPR), log-transformed milk beta-hydroxybutyrate (BHB, mmol/l milk), log-transformed acetone (mmol/l milk) from multivariate analyses of variables in three time windows and interval from calving to first insemination (ICF) from univariate analysis in primiparous Nordic Red Dairy cows.

	Records, no	Mean	SD	σ²a	σ_{e}^{2}	h²
NEFA _{MIR1}	19,220	0.410	0.206	0.0058	0.0249	0.19
NEFA _{MIR2}	19,329	0.286	0.159	0.0026	0.0163	0.14
NEFA _{MIR3}	19,856	0.213	0.130	0.0021	0.0107	0.16
NEFA _{FA1}	19,214	0.470	0.183	0.0039	0.0190	0.17
NEFA _{FA2}	19,327	0.316	0.141	0.0014	0.0130	0.10
NEFA _{FA3}	19,857	0.237	0.109	0.0010	0.0079	0.11
C18:1 <i>cis</i> -9 ₁	19,193	1.083	0.355	0.0122	0.0741	0.14
C18:1 <i>cis</i> -9 ₂	19,313	0.947	0.266	0.0047	0.0500	0.09
C18:1 <i>cis</i> -9 ₃	19,850	0.867	0.214	0.0036	0.0328	0.10
FPR ₁	19,222	1.342	0.254	0.0043	0.0498	0.08
FPR ₂	19,261	1.316	0.239	0.0036	0.0445	0.07
FPR ₃	19,801	1.273	0.218	0.0035	0.0364	0.09
BHB ₁	12,396	0.055	0.061	0.0005	0.0026	0.16
BHB ₂	12,444	0.054	0.056	0.0004	0.0022	0.15
BHB ₃	12,851	0.050	0.050	0.0003	0.0017	0.15
Acetone ₁	12,310	0.037	0.093	0.0013	0.0060	0.18
Acetone ₂	12,438	0.036	0.079	0.0008	0.0045	0.15
Acetone ₃	12,848	0.028	0.069	0.0006	0.0034	0.15
ICF	32,479	83.35	28.86	17.38	636.51	0.03

Natural resources and bioeconomy studies 54/2023

Table 19. Heritability estimates (on the diagonal) and genetic correlations (above the diagonal) with standard errors in parentheses, and phenotypic correlations (below the diagonal; SE not available) for plasma NEFA concentration predicted from milk mid-infrared spectra (NEFA_{MIR}, mmol/l), plasma NEFA concentration predicted from milk fatty acids (NEFA_{FA}, mmol/l), milk fatty acid C18:1 cis-9 (g/100 ml milk), milk fat to protein ratio (FPR), log-transformed milk beta-hydroxybutyrate (BHB, mmol/l milk) and log-transformed acetone (mmol/l milk) and interval from calving to first insemination (ICF) based on data from 8 to 35 days in milk.

Trait	NEFA _{MIR}	NEFA FA	C18:1 <i>cis</i> -9	FPR	BHB	Acetone	ICF
NEFA _{MIR}	0.17 (0.02)	0.91 (0.02)	0.84 (0.03)	0.44 (0.08)	0.73 (0.05)	0.70 (0.05)	0.39 (0.11)
NEFA _{FA}	0.88	0.17 (0.02)	0.95 (0.01)	0.59 (0.06)	0.71 (0.05)	0.70 (0.05)	0.40 (0.11)
C18:1 <i>cis</i> -9	0.83	0.96	0.14 (0.02)	0.64 (0.06)	0.58 (0.07)	0.56 (0.07)	0.36 (0.12)
FPR	0.50	0.71	0.78	0.08 (0.01)	0.38 (0.10)	0.30 (0.10)	0.18 (0.14)
внв	0.62	0.59	0.54	0.41	0.17 (0.03)	0.95 (0.01)	0.38 (0.12)
Acetone	0.66	0.61	0.53	0.31	0.89	0.18 (0.03)	0.33 (0.12)
ICF	0.04	0.04	0.04	0.03	0.02	0.02	0.03 (0.01)

Trait	NEFA_{MIR}	NEFA_{FA}	C18:1 <i>cis</i> -9	FPR	BHB	Acetone	ICF
NEFA _{MIR}	0.13 (0.02)	0.87 (0.03)	0.66 (0.06)	0.23 (0.11)	0.57 (0.07)	0.55 (0.07)	0.43 (0.11)
NEFA _{FA}	0.84	0.10 (0.01)	0.83 (0.03)	0.32 (0.10)	0.69 (0.07)	0.68 (0.07)	0.28 (0.13)
C18:1 <i>cis</i> -9	0.77	0.94	0.09 (0.01)	0.40 (0.10)	0.53 (0.08)	0.52 (0.09)	0.17 (0.13)
FPR	0.45	0.69	0.76	0.07 (0.01)	0.44 (0.10)	0.28 (0.11)	0.03 (0.14)
BHB	0.55	0.51	0.47	0.36	0.13 (0.02)	0.92 (0.02)	0.29 (0.12)
Acetone	0.57	0.50	0.43	0.24	0.85	0.13 (0.02)	0.16 (0.13)
ICF	0.03	0.04	0.03	0.02	0.03	0.03	0.03 (0.01)

Table 20. Heritability estimates (on the diagonal) and genetic correlations (above the diagonal) from 36 to 63 days in milk.

Table 21. Heritability estimates (on the diagonal) and genetic correlations (above the diagonal) from 64 to 91 days in milk.

Trait		NEFA _{FA}	C18:1 <i>cis</i> -9	FPR	BHB	Acetone	ICF
NEFA_{MIR}	0.16 (0.02)	0.79 (0.04)	0.48 (0.07)	-0.02 (0.10)	0.41 (0.09)	0.39 (0.08)	0.19 (0.12)
NEFA _{FA}	0.76	0.12 (0.02)	0.71 (0.05)	0.19 (0.10)	0.47 (0.09)	0.54 (0.08)	0.12 (0.13)
C18:1 <i>cis</i> -9	0.66	0.90	0.10 (0.01)	0.42 (0.09)	0.23 (0.11)	0.26 (0.10)	-0.02 (0.14)
FPR	0.32	0.62	0.74	0.10 (0.02)	0.34 (0.10)	0.16 (0.11)	0.01 (0.14)
внв	0.44	0.40	0.34	0.34	0.14 (0.02)	0.85 (0.03)	0.18 (0.13)
Acetone	0.44	0.39	0.28	0.16	0.83	0.15 (0.02)	0.13 (0.13)
ICF	0.02	0.03	0.02	0.02	0.02	0.01	0.03 (0.01)

All results are published in:

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WP3. Genomic predictions

Task 3.1 Single-step genomic predictions

Main results

A) Genomic prediction model for Maintenance Cost

Correlations between ssGBLUP and msSNPBLUP breeding values of genotyped Nordic Red Dairy cattle animals were 0.98-0.99, indicating that the models gave almost the same genomic breeding values (Table 22). The correlation between BLUP and ssGBLUP or msSNPBLUP breeding values was 0.97 in bulls and 0.84 to 0.86 in cows, respectively. Thus, BLUP breeding values were slightly different in bulls and clearly different in cows compared with genomic breeding values. The ssGBLUP GEBV had 5 to 12% higher standard deviation compared with msSNPBLUP or BLUP breeding values in cows and 0.2 to 5.5% higher in bulls.

The correlations between candidates' BV_c and their future DRP are presented in Table 23. Correlations were the highest when BV_c were estimated with ssGBLUP for both bulls and cows. This resulted also the highest r^2_{cv} with ssGBLUP being as high as 0.91 for the bull candidates. The r^2_{cv} of BV_c obtained with the msSNPBLUP model was higher than that when using BLUP in candidate bulls, but not in cows. For all three models, r^2_{cv} was higher for bulls compared with cows. Mean DRP reliability was for bulls 0.71 and for cows 0.61.

The b_1 estimates were closer to the expectation for ssGBLUP in candidate bulls than those for the BLUP model and msSNPBLUP model (Table 21). In cows, the b_1 estimate for BLUP was slightly closer to expectation than for the ssGBLUP or msSNPBLUP models. The b_1 estimates for msSNPBLUP were larger than 1.0 indicating that BV_c underpredicted the future breeding values, whereas the b_1 estimates for BLUP were below 1.0 indicating that BV_c overpredicted future breeding values. **Table 22.** Standard deviation (Std) and correlation between metabolic body weight breeding values and those with de-regressed proofs (DRP) for reference population animals of the Nordic Red Dairy cattle.

	Prediction			Correlations	
	model ¹	Std	msSNPBLUP	ssGBLUP	DRP
Cows	BLUP	4.11	0.86	0.84	0.88
n=35,075	msSNPBLUP	4.36		0.98	0.75
	ssGBLUP	4.59			0.74
Bulls	BLUP	4.56	0.97	0.97	0.95
n=5,539	msSNPBLUP	4.33		0.99	0.90
	ssGBLUP	4.57			0.91

¹BLUP=best linear unbiased prediction; msSNPBLUP=multi-step genomic BLUP, ssGBLUP=single-step genomic BLUP

Table 23. Cross-validation and Legarra-Reverter estimates; correlation $(r_{(DRP,BVc)})$, validation reliability (r^2_{cv}) , regression coefficient (b_1) and coefficient of determination from the validation regression (R^2) for the Nordic Red Dairy cattle bull and cow candidate groups with different models.

		Cross-val	idation	Legarra	-Reverter
	-	ľ (DRP,BVc)	r ² cv	b 1	R ²
Cows	BLUP ¹	0.67	0.74	0.96	0.51
n=1,721	msSNPBLUP ²	0.65	0.69	1.09	0.70
	ssGBLUP ³	0.73	0.86	1.05	0.84
Bulls	BLUP	0.74	0.77	0.96	0.56
n=354	msSNPBLUP	0.75	0.80	1.10	0.74
	ssGBLUP	0.81	0.91	1.01	0.85

¹ BLUP=best linear unbiased prediction; ²msSNPBLUP=multi-step genomic BLUP, ³ssGBLUP=single-step genomic BLUP; DRP=de-regressed proofs; BVc=breeding value for candidates

Discussion

Breeding values from the single-step model were in good agreement with those from the multi-step model and reasonably different from those of the BLUP model. Correlations between traditional BLUP and genomic models were clearly lower than correlations among genomic models. Also, country-wise (Denmark, Finland, Sweden) investigation of the breeding values confirmed that the single-step model is modelling the data appropriately. The obtained cross-validation reliabilities were higher than those for RDC yield traits ssGBLUP GEBVs (Koivula et al. 2018). This can be explained by the high heritability of the MBW and STA traits, and the high genetic correlation between MBW and STA. The reason why we obtained a lower r^2_{cv} value for the msSNPBLUP evaluation compared with the BLUP evaluation for the cow candidate group was unclear and requires further assessments.

The regression of candidates' full data GEBV on candidates reduced data BV_c were close to the expectation when applying ssGBLUP, and therefore ssGBLUP is preferable over msSNPBLUP for obtaining GEBV for candidates. The results of this study support the

implementation of ssGBLUP for the genomic evaluation of MBW in Nordic RDC and to develop ssGBLUP also for the Nordic HOL and Jersey MBW evaluation.

B) Genomic prediction model for regression on expected feed intake

Heritability of ReFI models were from 0.219 to 0.232 and were close to that of RFI* (which was 0.239) (Table 24). However, ReFI had higher repeatability compared to RFI* (0.481 to 0.502 compared to 0.351).

Table 24. Estimates of genetic, residual, permanent environmental (Var_G, Var_R and Var_{PE}, respectively) variances and heritability (h^2) and repeatability (r) for ReFI repeatability animal model when different energy requirement formulations are used of calculating expected dry matter intake. Agnew= Agnew et al. 2003; FINN= Luke 2022; and NRC2021= NRC 2021.

Criteria\Trait	Var _{нтм}	Var _{PE}	Var _G	Var _R	h²	r
ReFI _{Agnew}	0.874	1.355	1.115	1.759	0.219	0.484
ReFI _{FINN}	0.909	1.511	1.213	1.798	0.223	0.502
ReFI _{NRC2021}	0.864	1.245	1.163	1.738	0.232	0.481
RFI*		0.810	1.738	4.712	0.239	0.351

Correlations between FD (adjusted for fixed effects) and ssGEBV_r for ReFl_{Agnew} and ReFl_{NRC2021} were slightly higher than the correlation between FD and EBV_r (0.164 vs. 0.159 and 0.094 vs. 0.071, respectively) (Table 25). However, correlations between FD and ssGEBV_r were lower than that of FD and EBV_r for ReFl_{FINN} and RFI* (0.170 vs. 0.233 and 0.194 vs. 0.207, respectively). Overall, highest correlations were obtained when applying the Finnish energy requirement formulation and lowest when applying the NRC 2021 energy requirement formulation. Low correlations and inconsistent results when calculating correlation between FD and ssGEBVs could be due to the small data.

Correlation between ssGEBV_f and ssGEBV_r for ReFI criteria were from 0.487 to 0.533 which were higher than that of EBV_f and EBV_r (from 0.325 to 0.458). Regarding to the high correlation between ssGEBVf and EBVf (higher than 0.9 for all models; not shown), it indicates that GEBVs obtained by single-step procedure are able to predict breeding values of individuals with higher reliabilities.

	Reg. of FD on [G]EBVs						Reg. o	of [G]EB [G]EBV _r	V _f on
Models		b0	b1	corr ^δ	corr _{adj} ¥		b0	b1	corr
$ReFI_{Agnew}$	b _{FD.EBVr}	-0.548	0.319	0.079	0.159	b _{PEBVf.EBVr}	-0.005	0.726	0.410
(1/w=2.014)	$b_{\text{FD.ssGEBVr}}$	-0.600	0.204	0.081	0.164	$b_{\text{GEBVf}.\text{GEBVr}}$	0.0008	0.645	0.533
ReFI _{FINN}	$b_{\text{FD.EBVr}}$	-0.617	0.376	0.100	0.233	b _{PEBVf.EBVr}	-0.0055	0.770	0.458
(1/w=2.331)	$b_{\text{FD.ssGEBVr}}$	-0.680	0.182	0.073	0.170	$b_{\text{GEBVf},\text{GEBVr}}$	0.003	0.630	0.528
ReFI _{NRC2021}	b _{FD.EBVr}	-0.438	0.125	0.033	0.071	b _{PEBVf.EBVr}	-0.001	0.564	0.325
(1/w=2.147)	$b_{\text{FD.ssGEBVr}}$	-0.455	0.113	0.044	0.094	$b_{\text{GEBVf}.\text{GEBVr}}$	0.003	0.596	0.487
RFI*	b _{FD.EBVr}	0.130	-0.508	0.128	0.207	b _{PEBVf.EBVr}	0.257	-0.020	0.008
(1/w=1.614)	b _{FD.ssGEBVr}	0.083	0.285	0.120	0.194	b _{GEBVf.GEBVr}	-0.117	0.549	0.341

Table 25. Results of validation process (regression of average of feed intake deviation (FD) and (G)EBVs using full dataset on (G)EBVs predicted using reduced dataset, respectively).

^{*}b_{FD.EBVf}: Regression of average yield deviation on pedigree-based BLUP using full dataset; b_{FD.EBVr}: Regression of average yield deviation on pedigree-based BLUP using reduced dataset; b_{FD.ssGEBVf}: Regression of average yield deviation on single-step GBLUP using full dataset; b_{FD.ssGEBVr}: Regression of average yield deviation on single-step GBLUP using reduced dataset.

 $^{\delta}$ Correlation between [G]EBVs (i. e. full or reduced) with FD.

^{*} In order to adjust correlations, they should be multiplied by their corresponding adjusting coefficient (1/w).

The regression coefficients (b1) were lower than 1.0 in all cases. It shows an overprediction in GEBVs by single-step method when using reduced data. Generally, regression coefficients obtained by linear regression validation procedure were closer to 1.0 and their intercept were closer to zero. Similar results were found by Kudinov et al (2022). Highest and lowest correlations between genomic breeding values were found between ReFI_{Agnew} and ReFI_{FINN} (0.989) and ReFI_{NRC2021} and ReFI_{FINN} (0.817). Also, correlation between GEBV of animals for RFI* and ReFI_{NRC2021} was slightly higher than with other ReFI criteria (0.573) (Table 26).

Table 26. Correlations between ReFI and RFI* GEBVs when applying different energy requirement formulations for ReFI.

Criteria\Trait	ReFI Agnew	ReFI _{FINN}	ReFI _{NRC2021}	RFI*
ReFI _{Agnew}	-	0.989	0.858	0.553
ReFI _{FINN}			0.817	0.475
ReFI _{NRC2021}	-	-	-	0.573

Reference

Kudinov, A.A., Koivula, M., Strandén, I., Aamand, G.P. & Mäntysaari, E.A. 2022. Single-step genomic prediction in small breeds: Finncattle case. WCGALP.

Task 3.2 Genomic predictions for Nordic countries

A) Genomic prediction models for metabolic body weight in Nordic Holstein, Red dairy cattle and Jersey

Body weight recording based on heart girth measurements is decreasing in Finland and Denmark (Figure 10). There are no MBW data available from Sweden. In RDC the MBW data comes mainly from Finland and some from Denmark. In HOL data comes mainly from Denmark and Finland and in the case of Jersey data comes mainly from Danish cows. Therefore, the indicator traits carcass weight (CARW) and stature (STA), that have high genetic correlation with MBW, are of interest. Each cow will get CARW at the end of its life and the number of STA records in each breed is large. The average number of the records (MBW, STA, CW or BD) used in the current evaluation was 0.40, 0.37, 0.33 per cow in RDC, HOL and JER, respectively. In the new evaluation, where CARW was included, the mean of records (MBW, STA or CARW) was 0.71, 0.74, 0.77 in RDC, HOL and JER, respectively. This indicates that STA and CARW strengthen the maintenance evaluation in the Saved feed index.



Figure 10. Number of MBW1, MBW2, MBW3, STA and CARW records by birth year in RDC, HOL and JER cows.

Results showed that the genetic trend of combined MBW (MBW123) is increasing in each breed (Figure 11). The current BLUP model gives lower genetic trend for MBW123 than the new BLUP or ssGTaBLUP models. This indicates that the current trend of MBW123 is underestimated. The new single-step model gives a slightly higher trend compared to new BLUP-model, which was expected result as well as the result were genotyped animals were shown to have slightly higher genetic trend compared to non-genotyped animals. Genetic trend of the future (G)EBVs, which were estimated from the reduced data, follows quite nicely the genetic trend estimated from the full data in RDC and Jersey breeds.

Genetic trend of genotyped and nongenotyped RDC-DFS cows

Genetic trend of MBW123 in DFS-RDC cows





Genetic trend of genotyped and nongenotyped HOL-DFS cows

Genetic trend of MBW123 in DFS-HOL cows





Figure 11. On the left side: genetic trends of MBW123 (G)EBVs by birth year using different models for genotyped (solid line) and non-genotyped (dashed line) RDC, HOL and JER cows. On the right side: trends of MBW123 (G)EBVs by birth year from the full model (solid line) and reduced models (dashed line). (G)EBVs are expressed as standardized breeding values for cows born between the years 2005-2007.

Model	BLUP _{new}	ssGTaBLUP	Std	BLUP _{new}	ssGTaBLUP	Std	
	RDC o	cows (n=73,50	7)	RDC bulls (n=6,155)			
BLUP _{current} ¹	0.93	0.83	4.16	0.93	0.92	4.51	
BLUP _{new}		0.88	4.23		0.98	4.67	
ssGTaBLUP ²			4.77			4.73	
	HOL c	ows (n=103,30)1)	HOL bulls (n=6,322)			
BLUP _{current}	0.91	0.80	3.81	0.90	0.88	4.19	
BLUP _{new}		0.84	3.78		0.97	4.20	
ssGTaBLUP			4.17			4.35	
	JER c	ows (n=36,278	3)	JER	bulls (n=1,592))	
BLUP _{current}	0.86	0.82	2.43	0.82	0.81	3.02	
BLUP _{new}		0.93	2.42		0.98	2.81	
ssGTaBLUP			2.49			2.78	

Table 27. Correlation between MBW123 (G)EBVs using different models and standard deviations (Std) of (G)EBVs in genotyped reference animals.

¹BLUP=best linear unbiased prediction; ²ssGTaBLUP=single-step genomic BLUP

Correlations between new BLUP and ssGTaBLUP breeding values of genotyped reference animals were a bit lower (0.84-0.93) in cows compared to corresponding correlations in bulls (0.97-0.98) (Table 27). This indicates that the new BLUP and ssGTaBLUP models gave almost the same breeding values in genotyped bulls. The correlation between current and new BLUP breeding values were 0.90-0.93 in RDC and HOL animals. In JER, the correlations were lower (0.82-0.86). Thus, BLUP breeding values were slightly different in bulls and cows compared with genomic breeding values. In general, the single-step GEBVs had higher standard deviation than current or new BLUP EBVS, except in genotyped reference JER bulls (Table 25). The ssGTaBLUP GEBV had 10 to 15% higher standard deviation compared with BLUP breeding values in RDC and HOL cows and 1 to 5% higher in RDC and HOL bulls. In JER cows the ssGTaBLUP GEBV had about 3 % higher standard deviation compared to BLUP EBVs. In JER bulls the results were not consistent because current and new BLUP EBVs had 1 to 8% higher standard deviation than ssGTaBLUP GEBVs. The overall trend was that genotyped bulls had a bit higher SD of (G)EBVs than genotyped cows.

The correlations between candidates' BV_c and their future DRP are presented in Table 28. Correlations were the highest when BV_c were estimated with ssGTaBLUP for both bulls and cows in all breeds. Use of ssGTaBLUP-model resulted also the higher validation reliability (r_{cv}^2) compared to BLUP-model without genomic information. Validation reliabilities of ssGTaBLUP-model ranged from 0.59 to 0.72 in the bull candidates and from 0.23 to 0.75 in cow candidates. The validation reliabilities obtained with BLUP-model were from 0.09 to 0.19 in RDC, HOL and JER cows and from 0.25 to 0.42 in bulls. BLUP model without genomic information gave, on average, 33- and 32-percentage-units lower reliability for combined MBW in bulls and cows, respectively.

Mean DRP reliabilities for RDC, HOL and JER bulls were 0.89, 0.92 and 0.87, and for RDC, HOL and JER cows 0.32, 0.33 and 0.23, respectively.

		Cros	s-validatio	on	Legarra-Reverter		
		r _(DRP, BVc)	b 1	r ² _{cv}	b 1	R ²	
RDC cows	BLUP ¹	0.25	1.03	0.19	1.04	0.42	
N=45388	ssGTaBLUP ²	0.49	1.33	0.75	1.09	0.82	
RDC bulls	BLUP	0.62	1.05	0.43	1.05	0.38	
N=233	ssGTaBLUP	0.82	1.07	0.76	1.14	0.74	
HOL cows	BLUP	0.19	0.84	0.11	0.88	0.32	
N=78022	ssGTaBLUP	0.36	1.07	0.39	0.99	0.72	
HOL bulls	BLUP	0.55	0.80	0.32	0.79	0.30	
N=308	ssGTaBLUP	0.75	0.85	0.60	0.94	0.65	
JER cows	BLUP	0.14	0.94	0.09	0.94	0.28	
N=21518	ssGTaBLUP	0.23	1.20	0.23	1.03	0.59	
JER bulls	BLUP	0.47	0.98	0.25	0.98	0.21	
N=130	ssGTaBLUP	0.72	1.02	0.59	1.08	0.59	

Table 28. Cross-validation and Legarra-Reverter estimates; correlation ($r_{(DRP, BVc)}$), regression coefficient (b_1), validation reliability (r_{cv}^2), and coefficient of determination from the validation regression (R^2) for the RDC, HOL and JER bull and cow candidate groups by different models.

¹ BLUP=best linear unbiased prediction; ²ssGBLUP=single-step genomic BLUP; BVc=breeding value for candidates

The b_1 estimates for BLUP were closer to the expectation in candidate RDC bulls and cows than those for the ssGTaBLUP model (Table 28). The b_1 estimates for BLUP and ssGTaBLUP were larger than 1.0 in RDC bulls and cows indicating that BVc underpredicted the future breeding values in RDC. In JER candidate cows and bulls, it seemed that the BLUP-model overpredicted and ssGTaBLUP-model underpredicted the future breeding values. In HOL, the b_1 estimates for ssGTaBLUP were closer to the expectation in candidate bulls and cows than b_1 estimates for BLUP model. The b_1 estimates for ssGTaBLUP were below 1.0 in HOL bulls indicating that BVc overpredicted future breeding values in HOL. However, the reason for the found inflation of HOL bulls' BVc is still under investigation while finalizing this report. We found some inconsistency in the genetic trend of CARW and it needs to be clarified whether this could have affected the trend in MBW. Overall, regression coefficients obtained from the Legarra and Reverter validation procedure were in most cases quite close to the expectation of 1.0, except the b_1 estimates for BLUP in HOL cows and bulls.

The coefficient of determination from the validation regression (R^2) was always higher with the ssGTaBLUP model than by BLUP (Table 28). This indicates a better predictive ability of the model with genomic data.

Discussion

Genomic information is used in genetic evaluation to improve prediction accuracy in dairy cows. In this study, the impact of implementing single-step genomic BLUP was compared with pedigree BLUP for combined MBW in RDC, HOL and JER cattle after adding CARW data into the genetic evaluation as a correlated trait. CARW data significantly increased the amount of phenotypic information used for the genetic evaluation in all studied breeds. This contributes positively to the reliability of the MBW EBVs. Further, the new model, where CARW was included, corrected the genetic trend of MBW to be increasing compared with the current genetic trend which was rather flat from 2010 onwards. Increasing genetic trend of MBW obtained with the new model is more in line with the increasing phenotypic trends of MBW, STA and CARW. Current genetic trend in MBW appeared to be somewhat underestimated in all breeds, perhaps due to a decrease in the amount of MBW data in recent years.

Breeding values from the new single-step model were in good agreement with those from the new BLUP model in genotyped reference bulls but differed somewhat more in genotyped reference cows. The correlations between EBVs obtained from the current BLUP and new BLUP models were high and at the same level in RDC and HOL cows and bulls. In JER the corresponding correlation was somewhat lower perhaps due to the limited number of genotyped JER reference animals along with smaller data set. We found that GEBVs obtained with SSGTaBLUP-model had greater standard deviation than BLUP EBVs indicating more accurate breeding values. Also, genotyped bulls were found to have higher SD than cows and thus more accurate EBVs. Thus, cows will get more accurate breeding value than before if the new ssGTaBLUP-model is used in genetic evaluation.

Our results showed that differences in validation reliabilities among different methods were large and it appears that we achieved better validation reliabilities by using single-step genomic model than traditional BLUP model. Genomic models yielded higher validation reliabilities (r^2_{cv}), and validation regression coefficients (b_1) that were closer to the expectation than conventional BLUP models. Thus, based on the regression coefficients b_1 , the GEBV from the genomic models seemed to be less inflated than the EBV from BLUP models. The ssGTaBLUP provides a good alternative to the evaluation of MBW and the use of CARW as a correlated trait in the model is feasible.

B) Genomic prediction models for metabolic efficiency in Nordic Holstein, Red dairy cattle and Jersey

The estimated heritabilities and genetic variances are given in Table 29 and Table 30, respectively. For all breeds and data sets highest heritabilities were obtained for ReFI (approach D) and lowest for the current RFI (approach A) model. Similarly, also genetic variances were significantly higher based on approach D compared to approach A.

Table 29. Estimated heritability for metabolic efficiency based on four approaches given for different breeds and data sets. RFI_A is same as currently used model, ReFI_D is the proposed regression on expected feed intake model, RFI_B and RFI_C are two intermediate models.

Data set	RFI_A	RFI_B	RFI_C	ReFI_D
Jersey, CFIT data	0.12	0.19	0.16	0.22
Nordic red cattle, CFIT data	0.11	0.21	0.09	0.28
Holstein, CFIT data	0.13	0.19	0.08	0.32
Nordic red cattle, Finnish research				
farms	0.10	0.13	0.09	0.26

Table 30. Estimated genetic variance for metabolic efficiency based on four approaches given for different breeds and data sets. RFI_A is same as currently used model, ReFI_D is the proposed regression on expected feed intake model, RFI_B and RFI_C are two intermediate models.

Data set	RFI_A	RFI_B	RFI_C	ReFI_D
Jersey, CFIT data	0.66	1.40	1.57	2.69
Nordic red cattle, CFIT data	0.62	1.58	0.34	4.73
Holstein, CFIT data	0.87	1.65	0.92	5.81
Nordic red cattle, Finnish research				
farms	0.26	0.33	0.28	1.37

When comparing the phenotypes of the selected 10% genetically best cows (Table 31), we found that rather different cow groups are selected depending which approach is applied. When applying the current approach, then selected cows had lower production and MBW compared to average cows. When selecting based on ReFI (approach D), cows had same or higher production and same or lower MBW compared to all cows. For all breeds and data sets, selected cows had highest feed conversion efficiency when selection was based on ReFI. This was in line with the findings presented under Task 2.2.

Table 31. Phenotypic means of first parity dry matter intake (DMI), metabolic body weight (MBW), energy corrected milk (ECM), milk yield (MY), fat yield (FY), protein yield (PY) and feed conversion efficiency (FCE=ECM/DMI) for all cows with at least 5 observations and for 10% best cows with at least 5 observations selected based on EBV estimated by four approaches. Approach A (RFI_A), B (RFI_B), C (RFI_C) and D (ReFI).

	DMI	MBW	MBW ECM		FY	ΡΥ	FCE		
	(kg)	(kg ^{0.75})	(kg)	(kg)	(kg)	(kg)	(kg/kg)		
Jersey, CFIT data									
All cows	20.3	94.6	28.2	21.6	1.29	0.93	1.39		
top10% RFI_A	17.4	93.0	26.3	20.0	1.21	0.85	1.51		
top10% RFI_B	16.4	90.2	25.5	19.7	1.16	0.83	1.55		
top10% RFI_C	17.0	90.6	28.3	22.4	1.28	0.91	1.66		
_top10% ReFI_D	16.4	90.5	27.5	21.7	1.25	0.89	1.68		
Nordic red cattle, CFIT data									
All cows	25.1	121.3	31.1	29.3	1.27	1.08	1.24		
top10% RFI_A	21.6	117.5	29.5	27.1	1.23	1.01	1.36		
top10% RFI_B	21.6	114.3	29.6	27.4	1.22	1.01	1.37		
top10% RFI_C	22.3	114.7	31.8	29.7	1.31	1.08	1.42		
top10% ReFI_D	22.0	113.2	31.5	29.6	1.31	1.07	1.44		
Holstein, CFIT da	ta								
All cows	24.1	125.0	34.1	33.5	1.37	1.18	1.42		
top10% RFI_A	20.8	123.2	32.9	30.7	1.36	1.12	1.58		
top10% RFI_B	20.3	120.2	32.1	30.2	1.32	1.11	1.59		
top10% RFI_C	21.5	120.5	34.8	33.0	1.43	1.19	1.62		
top10% ReFI_D	20.9	118.5	34.4	32.7	1.42	1.16	1.65		
Nordic red cattle, Finnish research farms									
All cows	19.4	119.4	29.1	27.6	1.20	0.98	1.50		
top10% RFI_A	17.5	118.8	28.2	27.1	1.15	0.96	1.61		
top10% RFI_B	17.2	116.0	28.1	26.7	1.16	0.95	1.63		
top10% RFI_C	18.3	120.2	30.0	28.4	1.24	1.00	1.64		
top10% ReFI_D	17.9	119.0	29.7	27.7	1.25	0.99	1.67		

Results from comparison the four different approaches give strong evidence that upgrading the current genomic prediction model for metabolic efficiency to a genomic prediction model that is based on regression on residual feed intake would be beneficial to enhance genetic progress in feed efficiency of Nordic dairy cows.

WP4. Economic and environmental benefits

Task 4.1. Quantifying economic benefits

Variance components and heritabilities for the traits are shown in Table 32. Heritability for ReFI is presented for the average eDMI (19.8 kg), and it was 0.31. Heritability estimate for RFI was lower than for ReFI, and heritabilities for MBW, production traits and ICF were on the expected level.

Table 32. Variance components and heritabilities for ReFI, RFI, MBW, MY, PY, FY and ICF from the single-trait analyses.

	σ^{2}_{kappa}	σ^{2}_{pe}	σ^{2}_{a}	σ ² e	h ²
ReFI	0.0027	0.0017	0.0030	1.9258	0.31*
RFI		0.77	0.26	1.35	0.11
MBW -		8.93	64.40	4.07	0.83
MY		6.87	5.08	3.65	0.33
PY -		0.008	0.003	0.005	0.21
FY		0.014	0.009	0.011	0.26
ICF -			13.23	565.15	0.02

* Heritability for ReFI calculated for the average eDMI

Genetic and phenotypic correlations between the traits are presented in Table 33. The results showed that the genetic correlations between ReFI and production traits were favourable and from low to moderate. However, the classical RFI was unfavourably genetically correlated with production traits. This indicates that when selecting cows for ReFI, the more efficient cows would be also more yielding cows, but when selecting for RFI the more efficient cows would be less yielding cows. A high genetic correlation between RFI and MBW also indicates that more efficient cows would be smaller in size. These results confirm the findings from Task 2.2. B. There were no genetic correlation found between either feed efficiency traits or fertility. Due to the small data size the standard errors for genetic correlations were high. However, the estimates are reasonable and showing the direction of change in other traits if selecting for RFI or RFI.

	ReFI	RFI	MBW	MY	PY	FY	ICF
ReFI		0.31	0.06	-0.12	-0.11	-0.24	0.09
RFI	0.67		0.56	0.20	0.36	0.13	0.02
MBW	-0.01	0.17		0.27	0.35	0.07	0.23
MY	-0.20	0.09	0.20		0.80	0.74	0.40
ΡΥ	-0.18	0.14	0.21	0.89		0.66	0.23
FY	-0.29	0.00	0.21	0.76	0.76		0.35
ICF	0.08	0.08	-0.06	0.01	-0.01	0.00	

Table 33. Genetic correlations (upper triangle) and phenotypic correlations for ReFI, RFI, MBW, MY, PY, FY and ICF of yield deviation records from the multi-variate analyses.

Yearly genetic gain for each trait (in trait units) and economic values (in €) as total economic gain per year are shown for different scenarios in Table 34. In selection the traits included into scenario are used in total merit index and the gain in other traits based on correlated response. In the total economic gain only the ReFI metric (and not RFI) was included into total economic gain to account for the contribution of metabolic efficiency. The scenario Yield+ICF was assumed as the basic scenario of the breeding program and contained the yield traits and fertility. Adding MBW to the basic program had a favorable effect of increasing economic response by 15%. It strongly reduced MBW and slightly reduced PY and FY. When, in addition to MBW, RFI was added to the breeding program to account for metabolic efficiency, then it weakened ReFI, PY and FY, and thus decreased the overall economic progress by 7%. Adding ReFI instead of RFI to the selection index, then it strongly improved ReFI, had less impact on MBW and yield traits, and had a favorable effect on economic gain by 34%, where most of the increase came from the saved feed.

In this study, the simulated breeding scheme was simpler compared to that one used in the Nordic dairy cattle breeding. Moreover, some of the estimated variance components were associated with large standard errors and also size of the simulated population was small. This all adds to uncertainty in the obtained results. Nevertheless, results give indications that inclusion of both metabolic cost and metabolic efficiency into the total merit index will generate additional economic response. Given the higher economic value we have applied here, including maintenance costs makes almost as large contribution as the inclusion of metabolic efficiency. For the metabolic efficiency, an additional economic benefit was only achieved by applying the ReFI metric.

Table 34. Used heritabilities (h²), yearly based economic values and annual cow based genetic gain in different traits and total gain in € and % compared to basic scenario Yield+ICF. Total merit index traits in different scenarios are Yield+ICF: MY, FY, PY, ICF (basic); +MBW: basic+MBW; +MBW+RFI: basic+RFI+MBW; +MBW+ReFI: basic+MBW+ReFI.

	ReFi	RFI*	MBW	MY	FY	ΡΥ	ICF	Total (€)	Total (%)
Parameters									
h²	0.28	0.11	0.46 **	0.28	0.20	0.22	0.04		
σ _p	1.88	1.54	7.80**	3.91	0.126	0.182	24.1		
Yearly based									
economic values	-63.6	-63.6	-6.4	-16.38	1689.4	559.7	-0.64		
Annual genetic									
gain, €									
1. Yield+ICF	1.27	-1.59	-1.56	-4.58	14.46	6.53	-0.144	15.87	100
2. +MBW	2.56	0.54	3.32	-2.63	8.77	6.24	0.002	18.28	115
3. +MBW+RFI	2.25	2.31	4.63	-2.53	4.85	5.55	-0.060	14.70	93
4. +MBW+ReFI	8.28	-1.06	2.39	-2.73	7.88	5.40	-0.001	21.23	134

*RFI is not included into total economic genetic gain

**h2 for MBW and σ_{p} is from NAV, other from multi-trait model of this task

In Figure 12 the genetic gains for the seven traits are presented for the different scenarios. The simulations were sensitive to genetic parameters and the SE of estimates of correlations were quite high, which needs to be considered when interpreting the results.






Figure 12. Genetic gain for ReFI, RFI, MBW, MY, PY, FY, and ICF by different scenarios Yield+ICF (Scen_Milk), +MBW (Scen_MBW), +MBW+RFI (Scen_ RFI), and +MBW+ReFI (Scen ReFI). Solid line is average genetic gain of five repeat and colored area show 95% confidence interval.

Task 4.2 Genetic response – environmental impact

Estimated heritabilities for methane production (MeP), RFI (calculated as DMI minus expected DMI), MY, MF, MP and MBW were 0.04, 0.06, 0.39, 0.38, 0.22 and 0.75, respectively. Genetic correlations (r_g) between MeP and the rest of the traits namely: RFI, MY, MF, MP and MBW were 0.31, -0.10, 0.13, 0.26 and 0.59, respectively. On the other hand, phenotypic correlations between MeP and the above-mentioned traits were 0.46, -0.05, -0.05, 0.02, 0.16, respectively.

Table 35. Multitrait estimates of genetic parameters. Heritabilities (on the diagonal) and genetic correlations (below diagonal) and phenotypic correlations (above diagonal) in first lactation Nordic Red cattle.

Traits	MeP	RFI	MY	MF	MP	MBW
MeP	0.04	0.46	-0.05	-0.05	0.02	0.16
RFI	0.31	0.06	0.02	-0.05	0.12	-0.03
MY	-0.10	-0.20	0.39	0.78	0.79	-0.18
MF	0.13	0.27	0.78	0.38	0.71	-0.10
MP	0.26	0.19	0.67	0.64	0.22	0.10
MBW	0.59	-0.28	-0.30	-0.31	0.05	0.75

The calculation of correlated response due to selection on RFI showed that when selection is for feed efficiency through lowering RFI, using a selection intensity of 2.1 or improving RFI by about two genetic standard deviation (-2.4 kg), the corresponding correlated response would be that CH₄ reduces by 1.89g/day, MY increases by 0.33kg/day, FY decreases by 0.02 kg/day, PY decreased by 0.01 kg/day and MBW increased by 1.4 kg^{0.75}. In other words, when this expressed in proportion for instance, improving RFI by 5% would result in reduction of feed intake by 1.1 kg/day, CH₄ output by 0.89 gm/day, increase in MY by 0.15 kg/day and no effect on FY (-0.009kg/day) and PY (-0.008 kg/day) but increased BW by 4.2 kg. It should be noted that the available genetic variation in the RDC population for CH₄ is slighter lower than for the Holsteins and the genetic correlation between RFI and CH₄ emission is moderate. The results in general suggest that improving dairy cattle feed utilization efficiency could be one sustainable option for mitigation of CH₄ emission from dairy production systems.

Task 4.3 Quantifying environmental benefits

LCA assessment was conducted with Lypsikki-LCA for the baseline and with integrating the project findings regarding the breeding responses to the assessment. The project findings regarding breeding response are summarized in Table 33. For the assessment, these findings were adjusted to yield a 10% improvement in feed efficiency of cows (-10% from typical DMI 22 kg/d equal -2.2 kg/d), and the remaining parameters were adjusted accordingly (Table 36). The estimates for genetic response are further described in Task 4.1.

	Breeding goal				Response in							
Information on	b1	b2	b3	b4	b5	B6	CH4 (g/d)	RFI (kg)	Milk (kg)	Fat (kg)	Prot (kg)	MBW (kg)
CH4+RFI+MY+FY +PY+mBW	0	-1	0	0	0	0	-1.89	-2.39	0.33	-0.02	-0.01	1.40
Adjusted for 10% response, CH4+RFI+MY+FY +PY+mBW		-0.92					-1.74	-2.2	0.30	-0.02	-0.01	1.29

Table 36. Summary of the project findings utilized in the LCA analysis. RFI = residual feed intake, MY = milk yield, FY = fat yield, PY = protein yield and MBW = metabolic body weight.

The climate change impact (carbon footprint, CF), eutrophication potential and land occupation were assessed for business-as-usual (BAU) and for the A++ breeding response, adjusted with 10% improvement in feed efficiency. For the baseline, the contributions of the different life cycle stages are presented in Figure 13 for climate change impact and Figure 14 for eutrophication potential. Land occupation in BAU system was 1.55 m² kgFPCM⁻¹. In overall, the baseline resulted climate change impact for FPCM of 0.977 kg CO₂eq kgFPCM⁻¹. This is a very typical level for CF of liquid dairy produced in a developed country. Eutrophication potential was 2.86 g PO₄ eq kgFPCM⁻¹. With the integration of the findings from the A⁺⁺Cow project, the 10% improvement in feed intake resulted in CF of 0.899 kg CO2eq kgFPCM⁻¹.



Baseline BAU system, total CF 0.977 kg CO2eq per kg FPCM contribution of stages to CF, %

Figure 13. Contribution of the life cycle stages to the climate change impact (CF) of the baseline systems milk production (1 kg FPCM). CF = carbon footprint, FPCM = fat and protein corrected milk.



Figure 14. Contribution of the life cycle stages to the eutrophication potential (EP) of the baseline systems milk production (1 kg FPCM). EP = eutrophication potential, FPCM = fat and protein corrected milk.



A++ with 10% response to DMI, total CF 0.899 kg CO2 per kg FPCM contribution of stages to CF, %

Figure 14. Contribution of the life cycle stages to the climate change impact (CF) of the dairy production with 10% breeding response to feed efficiency. CF = carbon footprint, FPCM = fat and protein corrected milk.



Figure 15. Contribution of the life cycle stages to the eutrophication potential (EP) of the dairy production with 10% breeding response to feed efficiency. EP = eutrophication potential, FPCM = fat and protein corrected milk.

Similarly for the A++ findings, the contributions of the life cycle stages are presented in Figure 15 for climate change impact (CF) and in Figure 16 for eutrophication potential (EP). Land occupation in the improved system was 1.47 m² kgFPCM⁻¹, which was 5% less than BAU. In overall, the climate change impact for FPCM was 0.899 kg CO₂eq kgFPCM⁻¹. Eutrophication potential was 2.57 g PO₄ eq kgFPCM⁻¹.

Comparison of the global warming potential of raw milk according to the baseline and with A++ findings was conducted per life cycle stage. Results are presented in Table 37.

	Baseline	A++	Improvement, %		
		kg CO₂eq FPCM⁻¹			
Feed crop production and pasture (N ₂ O)	0.209	0.194	-7%		
Methane from rumination (CH ₄)	0.551	0.509	-8%		
Manure management (N2O)	0.042	0.037	-12%		
Manure management (CH ₄)	0.008	0.007	-11%		
Input production (CO2eq)	0.082	0.076	-7%		
Purchased feeds (CO ₂ eq)	0.085	0.075	-11%		
Total, kg CO₂eq FPCM⁻¹	0.977	0.899	-8%		

Table 37. Comparison of the global warming potential of raw milk, produced according to baseline and with A++ findings.

Based on the results, it was seen that the contributions of the different life cycle stages are affected only slightly. The methane generated in the rumination is the major contributor to

the CF of dairy in both cases. Overall, the improvement achieved was 8% in CF of FPCM. Largest percentage of improvements were reflected to manure management and feeds. Manure management emissions are lowered as the DMI reduces and the efficiency of feed utilization increases, less nitrogen is excreted to manure. Due to improved efficiency in feed utilization, feed crops are also needed less, and this is reflected to a lowered contribution of purchased feed crops. As the feed crop production emissions are reduced more efficiently than methane from rumination, this leads to increased contribution of methane from rumination to the total GWP of dairy, even if the absolute amount of methane reduction is larger. As absolute value, the reduction of emissions was clearly largest from enteric fermentation methane (-0.042 kg CO₂eq FPCM⁻¹) and feed production was second largest (own feed crop production and purchased feeds; -0.025 kg CO₂eq FPCM⁻¹), and the remaining smaller effects (manure management and inputs) were in total (-0.012 kg CO₂eq FPCM⁻¹).

Comparison of the eutrophication potential of raw milk according to the baseline and with A++ findings was similarly conducted per life cycle stage. Results are presented in Table 38.

Table 38. Comparison of the eutrophication potential of raw milk, produced with BAU system and with A++ findings.

	Baseline	A ++	Improvement, %	
	g PO₄eq kg FPCM⁻¹			
P leaching, erosion	0.15	0.14	-7%	
N leaching	0.20	0.19	-8%	
NH ₃ fertilisers and manure application	0.80	0.69	-13%	
NH₃ pasture and housing	0.47	0.44	-6%	
NH ₃ manure management	0.62	0.55	-11%	
PO ₄ Purchased feeds	0.60	0.54	-10%	
NOX energy and fuels	0.04	0.03	-6%	
Total, g PO₄eq kg FPCM ⁻¹	2.86	2.57	-10%	

For the eutrophication potential the total reduction of emissions was -10%. Largest contributions were observed in nitrogen input use in cultivation (fertilizer and manure application) and manure management. Also, emissions from purchased feeds were lowered by 10%. All of these reductions are well explained by reduced feed requirement, which is reflected to lowered need of feed crop production. As absolute values, the reduction was largest from the same stages: N input use (-0.11 g PO₄eq kg FPCM⁻¹), manure management (-0.07 g PO₄eq kg FPCM⁻¹) and purchased feeds (-0.06 g PO₄eq kg FPCM⁻¹), while the remaining stages resulted in total -0.06 g PO₄eq kg FPCM⁻¹ reduction.

In overall, the 10% genetic response in feed efficiency would yield a 8% reduction in carbon footprint, 10% reduction of eutrophication potential and 5% reduction in land occupation of FPCM. Here, the 10% genetic response in feed efficiency was only studied and integrated for adult dairy cows impact assessment. However, breeding potentially affects also the feed utilization of young stock and replacement heifers. It should be further investigated how the genetic improvement affects the emissions of youngstock, e.g., heifers and calves.

3.3 Evaluation of the project implementation

General set up of the project and surrounding circumstances

The A⁺⁺Cow project was a large and multidisciplinary project utilising necessary research facilities and laboratories, collecting sizable data needed for genetic analyses, and carrying out complex analyses and developing novel deliverables by the research group. There was significant funding by the industry, larger than the funding by MMM, which allowed conducting a project of this size.

Some unforeseen circumstances were affecting this project. There were delays and restrictions associated with COVID19 pandemic which impacted the smooth progress of some tasks. In Task 1.2 the trials were delayed for one year as the installation of CFIT cameras was not possible during the pandemics and the experts from VikingGenetics, Denmark were not allowed to travel to Finland or visit the Jokioinen research farm. In addition, there was a delay in the blood NEFA and BHB assessment in Task 1.3 due to equipment break down at University of Helsinki and delays in NEFA-kit deliveries due to COVID19 pandemic. Including the development of a reference database for BHB and the developing the prediction equations was not originally planned, but it was found important based on the results by Mehtiö et al. (2020) and also it allows Finland to have BHB phenotypes in future. Terhi Mehtiö was planning a visit to KU Leuven for 2020 for the research in task 1.3., but the visit was cancelled due to COVID19.

Also, some research was allocated differently that was planned when the project started. More research was allocated for research into modelling of metabolic efficiency because we found it important for developing a model that is applicable for prediction of genomic breeding values. In addition, the outcome of our research regarding the use of slaughter weight was very positive, and therefore we started to develop an updated MBW evaluation model including carcass weight, which was not fully budgeted in the original research plan. Moreover, preparing of the necessary data from all three countries took longer than anticipated.

Because of these delays a no-cost extension of the project time for another year until March 2023 was applied and approved by the steering group. To get the planned research finalized, the industry partners (VikingGenetics, NAV and Faba co-op) agreed to cover the costs of 8 more working months (60143 €).

Project management

The principal investigator changed during the project; Martin Lidauer led project from the start until January 2021 and Terhi Mehtiö started as the project leader in February 2021.

Project management was challenged by changes in research and technical personnel beyond normal expectations. This was partially due to the reasons mentioned above. The changes were compensated by reallocating data collection, laboratory and research tasks to technical and research personnel who was not involved in the project originally, by a significant increase in workload for researchers central for the project, and by postponing some tasks until a suitable person was found to continue the work. The complexity of the project required a string organization. Research was organized in four work packages and 11 tasks, of which many tasks were rather large. Assigning for each task a responsible researcher was crucial to ensure progress and coordination among tasks. Progress of research was followed up in regular research group meetings, monthly meetings with NAV (50 meetings), Faba, Seges and Växa Sverige and a countless number of spontaneous meetings, and phone and e-mail conversations.

Timetable

The required research farm data collection was carried out over the whole project period as was planned originally for the majority of all data collection activities. Data collection was carried out in a professional manner as well as all analyses in the laboratories. This ensured to make high quality data available for later statistical analyse.

The delays due to unforeseen circumstances, mainly COVID19 pandemic, were already mentioned as well as the changes in allocating the research. In addition, some of the tasks were re-arranged and personnel changed which caused some slowdown in the progress of research. Data analyses were often more demanding than originally anticipated. In addition, there were significant delays in receiving the data sets for analyses in tasks 1.2., 1.3., and 2.3. However, most of the planned research tasks were carried out as planned and agreed with the funding partners, and even taken beyond what was originally planned.

3.4 Publications

Scientific articles:

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 I. 2022. Long-term effects of early-life rumen modulation on dairy cow production performance and methane emissions. Frontiers in Microbiology. <u>https://doi.org/10.3389/-</u> <u>fmicb.2022.983823</u>
- Mehtiö, T., Pitkänen, T., Leino, A.-M., Mäntysaari, E.A., Kempe R., Negussie, E. & Lidauer, M.H. 2021. Genetic analyses of metabolic body weight, carcass weight and body conformation traits in Nordic dairy cattle. Animal 15:(12). <u>https://doi.org/10.1016/j.animal.-</u> 2021.100398
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- Mehtiö, T., Mäntysaari, P., Kokkonen T., Kajava, S., Prestløkken, E., Kidane, A., Wallén, S., Nyholm, L., Negussie, E., Mäntysaari, E.A. & Lidauer, M.H. 2019. Genetic parameters for cow-specific digestibility predicted by near infrared reflectance spectroscopy. Livestock Science 226: 1–6. <u>https://doi.org/10.1016/j.livsci.2019.05.017</u>
- Mäntysaari, P., Juga, J., Lidauer, M.H., Häggman, J., Mehtiö, T., Christensen, J.M. & Mäntysaari, E.A. 2022. The relationships between early lactation energy status indicators and endocrine fertility traits in dairy cows. 2022. Journal of Dairy Science. 105: 6833–6844. <u>https://doi.org/10.3168/jds.2021-21077</u>
- Mäntysaari, P., Mäntysaari, E.A., Kokkonen, T., Mehtiö, T., Kajava, S., Grelet, C., Lidauer, P. & Lidauer, M.H. 2019. Body and milk traits as indicators of dairy cow energy status in early lactation. Journal of Dairy Science 102: 7904–7916. <u>https://doi.org/10.3168/jds.2018-15792</u>
- Negussie, E., Mehtiö, T., Mäntysaari, P., Løvendahl, P., Mäntysaari, E.A. & Lidauer, M.H. 2019. Reliability of breeding values for feed intake and feed efficiency traits in dairy cattle: When dry matter intake recordings are sparse under different scenarios. Journal of Dairy Science 102: 7248–7262. https://doi.org/10.3168/jds.2018-16020
- Strandén, I., Kantanen, J., Lidauer, M.H., Mehtiö, T. & Negussie, E. 2022. Animal board invited review: Genomic-based improvement of cattle in response to climate change. Animal 16(12). <u>https://doi.org/10.1016/j.animal.2022.100673</u>

Doctoral thesis:

Mehtiö, Terhi. 2020. Genetics of novel feed efficiency and related traits in Nordic dairy cattle. Dissertationes Schola Doctoralis Scientiae Circumiectalis, Alimentariae, Biologicae. http://urn.fi/URN:ISBN:978-951-51-5915-1

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- Mehtiö, T., Luostarinen, S., Leskinen, H., Rinne, M., Virkajärvi, P., Huhtanen, P., Perttilä, S., Keto, L., Setälä, J., Vielma, J., Niemi, J., Mäntysaari, E., Kause, A., Leino, A-M., Pastell, M., Tapio, M., Mononen, J., Sévon-Aimonen, M-L., Saastamoinen, M., Schulman, N., Raussi, S., Kuha, R., Vehviläinen, H., Karikallio, H-M., Kaukovirta, A., Vilkki, J., Huuskonen, A., Järvenranta, K. 2023. Luken visio suomalaisesta eläintuotannosta ja sen tutkimuksesta vuoteen 2040. Luonnonvara- ja biotalouden tutkimus 8/2023. Luonnonvarakeskus. Helsinki. 52 s. http://urn.fi/URN:ISBN:978-952-380-606-1
- Mehtiö, T., Leino, M., Virkajärvi, P., Luostarinen, S., and Rinne, M. 2022. Nautakarjatalous osana kotimaisen ruokajärjestelmän kestävyyden tavoittelua. Julkaisussa: Vehmasto, E., Salo, M. & Soini, K. (toim.) 2022. Kestävyyden kehykset Luonnonvaratutkimus kestävyysmurroksessa. Luonnonvara- ja biotalouden tutkimus 74/2022. Luonnonvarakeskus. Helsinki. s. 33–42. <u>http://urn.fi/URN:ISBN:978-952-380-502-6</u>

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Juga, J., Kokkonen, T., Mäntysaari, E.A., Lidauer, M.H. 2019. Tuottavalla eläinaineksella kannattava ja ilmastoviisas kotieläintuotanto. 2019. In. Peltonen, S. (Ed.), Tieto Tuottamaan 145: Ilmastoviisas maatilayritys. ProAgria Keskustenliitto, Vantaa. ISBN-13: 9789518082791, ISBN-10: 9518082790, pp. 59 –61.

Scientific articles under preparation:

- Kostensalo, J., et al. 2023. Predicting blood plasma NEFA and BHB concentrations from milk MIR spectra. (submitted to Animal)
- Lidauer, M.H., et al. 2023. Modelling biological efficiency in dairy cows by regression on expected feed intake. (submitted to Animal)

Oral presentation at congresses:

- BLOCH, V., LIDAUER, M., MÄNTYSAARI, P., MEHTIÖ, T., KEMPPAINEN, A., PASTELL, M. 2020. Long-term BCS monitoring by 3D camera assistant system. The 71st Annual Meeting of the European Federation of Animal Science, Virtual Meeting, December 1–4, 2020, EAAP Book of Abstracts No. 26 (2020), p. 510.
- GREDLER-GRANDL, B., RAYMOND, B., SCHOPEN, G.C.B., CHITNEEDI, P.K., CAI, Z., MANZANILLA-PECH, C.I.V., ISO-TOURU, T., FISCHER, D., BOLORMAA, S., CHUD, T.S., SCHENKEL, F.S., WANG, Y., LI, C., HAILEMARIAM, D., VILLANUEVA, B., FERNANDEZ. A., KUEHN, C., SA-HANA, G., LIDAUER, M.H., PRYCE, J.E., GONZÁLEZ-RECIO, O., PLASTOW, G., BAES, C.F., CHARFEDDINE, N., DE HAAS, Y., VEERKAMP, R.F., BOUWMAN, A.C. 2022. Accuracy of genomic prediction of dry matter intake in Dutch Holsteins using sequence variants from meta-analyses. In: Proceedings, 12th World Congress of Genetics Applied to Livestock Production, Rotterdam, The Netherlands, July 3–8, 2022.
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- KEMPE, R.M., MEHTIÖ, T., LEINO, A.-M., KOIVULA, M., PITKÄNEN, T., STEPHANSEN, R.B., MÄNTYSAARI, E.A., NEGUSSIE, E., PÖSÖ, J., NIELSEN, U.S., FIKSE, W.F., AAMAND, G.P., LIDAUER, M.H. 2022. Single-step genomic prediction for metabolic body weight of Nordic Red dairy cattle. In: Proceedings, 12th World Congress of Genetics Applied to Livestock Production, Rotterdam, The Netherlands, July 3–8, 2022.
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- LIDAUER, M.H., NEGUSSIE, E., MEHTIÖ, T., MÄNTYSAARI, P., KOKKONEN, T., MÄNTYSAARI, E.A., 2022. Modelling multiplicativity in feed efficiency by regression on expected feed intake. In: Proceedings, 12th World Congress of Genetics Applied to Livestock Production, Rotterdam, The Netherlands, July 3–8, 2022.
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Davos, Switzerland, August 30 - September 3, 2019, EAAP Book of Abstracts No. 27 (2021), p. 113.

- LIDAUER, M.H., LEINO, A.-M., STEPHANSEN, R.S., PÖSÖ, J., NIELSEN, U.S., FIKSE, W.F., AAMAND, G.P. 2019. Genetic evaluation for maintenance – Towards genomic breeding values for saved feed in Nordic dairy cattle. In: Proceedings of the 2019 Interbull meeting, Cincinnati, Ohio, USA June 22–24, 2019. Interbull Bulletin 55:21–25.
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- MEHTIÖ, T., NEGUSSIE, E., MÄNTYSAARI, E.A., AAMAND, G.P., AND LIDAUER, M.H. 2022. Genetic correlations between feed efficiency, production and fertility in Nordic Red Dairy cattle. The 73rd EAAP annual meeting 4th - 9th September 2022, Porto, Portugal, Book of Abstracts No. 28, p. 488.
- MEHTIÖ, T., PITKÄNEN, T., LEINO, A.-M., MÄNTYSAARI, E.A., KEMPE R., NEGUSSIE, E., AND LI-DAUER, M.H. 2022. Metabolisen elopainon, teuraspainon ja rakenneominaisuuksien perinnölliset tunnusluvut lypsykarjalla. Maataloustieteen Päivät 2022, 14th – 15th June 2022, Helsinki, Finland, Suomen Maataloustieteellisen seuran tiedote No. 39, p. 35.
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- MEHTIÖ, T., MÄNTYSAARI, P., KOKKONEN, T., KAJAVA, S., LATOMÄKI, T., NYHOLM, L., GRELET, C., MÄNTYSAARI, E.A., LIDAUER, M.H. 2018. Developing an indicator for body fat mobilisation using mid-infrared spectrometry of milk samples in dairy. In: Proceedings, 11th World Congress of Genetics Applied to Livestock Production, Auckland, New Zealand, February 11–16, 2018, 11.225.
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Other presentations:

- LIDAUER, M.H. 2022. Developing genomic predictions for feed utilization in dairy cattle. Invited speaker at the Jahrestagung der Deutsche Gesellschaft für Tierzucht, Genetisch-Statistische Methoden, October 4–6, 2022, Rotenburg a.d. Fulda, Germany.
- LIDAUER, M.H., AND PÖSÖ, J. 2020. Genetiikka avuksi eläinjalostuksella ympäristöystävällisempiä lehmiä. Naudat, ilmasto ja ympäristö -seminar 21.1.2020 Helsinki, Finland.
- LIDAUER, M., KEMPE, R., NEGUSSIE, E., BLOCH, V., MÄNTYSAARI, P., MÄNTYSAARI, E., LEINO, M., PASTELL, M., AND MEHTIÖ, T. 2021. Feed efficiency in the A++ project. Nordic Workshop in Dairy Cattle Genomics 3.5.2021 (online).
- MEHTIÖ, T. 2022. Cattle and Climate. NÖK seminar 25.7.2022 Iceland (online).
- MEHTIÖ, T. 2022. Jalostuksella tehokkaampia ja ympäristöystävällisempiä lehmiä. ProAgria webinar "Jalostuksen vaikutukset maatalouden hiilineutraaliuuteen" 21.1.2022 (online).
- MEHTIÖ, T., LIDAUER, M., NEGUSSIE, E., KEMPE, R., AND MÄNTYSAARI, E. 2021. Aiming for sustainable dairy cattle. Luke, Faba & VikingGenetics, R&D workshop 2.11.2021 (online).
- MEHTIÖ, T. 2022. Tehokkaampia ja ympäristöystävällisempiä lehmiä. Faba webinar 11.2.2021 (online).
- MEHTIÖ, T., LIDAUER, M., AND LEINO, A.-M. 2020. Carcass weight as correlated information for the prediction of metabolic body weight. Nordic Workshop in Dairy Cattle Genomics 27.4.2020 (online).
- MEHTIÖ, T. 2019 Lypsylehmien rehunkäyttökyvyn parantaminen jalostuksen avulla. ProAgria webinar 12.3.2019 (online).

Other publications:

- MEHTIÖ, T. 2023. Tavoitteena ilmasto- ja ympäristöystävällisempi lehmä. Luke's blog: <u>https://www.luke.fi/fi/blogit/tavoitteena-ilmasto-ja-ymparistoystavallisempi-lehma</u> (accessed 28.2.2023)
- MEHTIÖ, T. 2020. Väitös: Uudet ominaisuudet auttavat rehua säästävien lehmien jalostuksessa. Luke's news: <u>https://www.luke.fi/fi/uutiset/vaitos-uudet-ominaisuudet-auttavat-rehua-saastavien-lehmien-jalostuksessa</u> (accessed 10.3.2023)
- MEHTIÖ, T. 2020. Ruokaturvaa, ilmastoa, ympäristöä ja luonnon monimuotoisuutta koskeva kriisi ratkaistaan tutkimuksen ja yhteistyön avulla. Luke's blog: <u>https://www.luke.-</u> <u>fi/fi/blogit/ruokaturvaa-ilmastoa-ymparistoa-ja-luonnon-monimuotoisuutta-koskeva-</u> <u>kriisi-ratkaistaan-tutkimuksen-ja-yhteistyon-avulla</u> (accessed 28.2.2023)

MEHTIÖ, T. 2019. Eläinjalostuksella kohti kestävämpää ruoantuotantoa. Luke's blog: <u>https://-www.luke.fi/fi/blogit/elainjalostuksella-kohti-kestavampaa-ruoantuotantoa</u> (accessed 28.2.2023)

Media:

- HARJU, E. 2020. Maidontuotannon päästöt kuriin uusin keinoin: Suomessa kehitetään vähäpäästöisempiä lehmiä, jotka syövät ja ulostavat nykyistä vähemmän. Iltalehti, 1.2.2020. https://www.iltalehti.fi/kotimaa/a/326113de-cbc0-4a73-abf0-6b665c897eb7
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- LIESPUU, S. 2019. Pienemmällä rehumäärällä parempi maitotuotos. Maatilan Pelllervo, Eläinliite, Syyskuu 2019: 6.
- MEHTIÖ, T. 2022. Tavoitteena ilmasto- ja ympäristöystävällisempi lehmä. Forssan Lehti, 30.12.2022: 2.
- PÖNTINEN, P. 2019. Uusi lehmä. Jokioisilla kehitetään lehmää, joka lypsää vähäpäästöistä maitoa. Suomen Kuvalehti 24 (14.6.2019): 24-31.

Other activities:

Participating and presenting A++COW project poster in Valio's Carbo Science seminar in Helsinki 25th October 2022

Arranging and participating in Naudat, ilmasto ja ympäristö (Cattle, climate and environment) seminar in Helsinki 21st January 2020 (Terhi Mehtiö, Martin Lidauer, Anna-Maria Leino)

- Recording of the seminar: <u>https://www.youtube.com/watch?v=HxwlOzU1j0Q</u>
- Slides of the presentations: <u>Naudat, ilmasto ja ympäristö -seminaari (21.1.2020) by</u> <u>Natural Resources Institute Finland (Luke) / Luonnonvarakeskus (Luke) (slideshare.net)</u> (accessed 3.11.2022)



Figure 16. Summary of the Naudat, ilmasto ja ympäristö seminar in picture made by Tussitaikurit

4. Evaluation of the results

4.1 Practical applicability of results

The overall research aim of this project was to produce new scientific knowledge needed for developing genomic evaluations for feed efficiency in dairy cattle. The project continued research on feed efficiency initiated by the previous "Feed efficiency" and "Lehmien rehunkäyt-tökyky" projects and addressed important research questions identified by that latter project and in the literature. Interdisciplinary research in this project has produced significant amount of new knowledge.

The first Saved Feed breeding values were available in 2019 in Finland, Denmark and Sweden. At first the NAV's Saved Feed index was based only for Maintenance (MBW and conformation records of cows), but then also the Metabolic Efficiency (RFI) was included. The Saved Feed index was included in Nordic Total Merit index in 2020. In A^{++} Cow project we have further developed the genomic predictions of Maintenance by including the carcass information as correlated trait into the models and by developing single-step genomic predictions. This was very important work as the decrease in the recording of body weights of the cows would have weakened the index in the future. CARW data significantly increased the amount of phenotypic information used for the genomic evaluation in all Nordic breeds. This along with the single-step genomic predictions of Maintenance. Thus, animals will get more accurate breeding values than before. NAV is planning to upgrade the current official genomic prediction for Maintenance to the single-step genomic prediction for Maintenance developed within the A++Cow project.

In addition, the model for Metabolic Efficiency has been revised and improved. We simulated alternative breeding programs to study which breeding programme would be the best from an economic and environmental point of view in practical animal breeding. Based on the results, applying the ReFI metric developed in this project would yield in better genetic progress in reducing feed consumption and increasing economic gain when compared to the currently used RFI metric. A more accurate Metabolic Efficiency evaluation developed in this study allows more accurate selection of top bulls and cows with better feed efficiency than the average of the population. This implies that cows with better feed efficiency consume less feed than average of the population for a given level of production and body weight. This in turn practically means reduced feed cost to the farmer and reduced emission of enteric methane which will contributes to the environmental sustainability.

The positive correlations we found between methane production and feed efficiency in dairy cows indicate that selection for feed efficiency is one sustainable strategy to lower methane emissions from dairy production systems. Environmental impact assessment was conducted for average typical Finnish dairy production, according to data collected from 700 Finnish dairy farms, and for improved production, when projects findings were applied to dairy cow processes. It was found that in all three investigated impact categories (climate change impact, eutrophication and land occupation) the project findings of supported mitigation of environmental impacts. It was seen that eutrophication potential was affected most, with 10% decrease in comparison to business as usual. Climate change impact is globally most important of the studied impact categories, in this investigation it was found to decrease by 8%

with breeding towards improved feed efficiency. Globally, but also locally arable land demand is becoming all the time more important. With growing populations adequate nutrition is needed to be produced with more efficient land utilization. In this investigation, it was found that land occupation, the demand for arable land was reduced by 5% when project findings were applied. By observing the impact across different impact categories, it was seen that effect was toward reduction in all cases and no trade-offs between impact categories were found. Even if the investigated findings (improved feed efficiency, lower feed intake, lower methane generation, higher milk yield) can all be considered as reducing environmental impacts of raw milk, new knowledge was produced to understand the magnitude of the effects. In overall, it could be seen that breeding can be considered as powerful tool in mitigating environmental impacts of dairy production.

Research on developing a predictor trait for negative energy status based on MIR spectral data of milk samples has resulted in new opportunities applicable for the practice by developing prediction equations for NEFA and BHB. Cooperation with the OptiMIR partners since the Feed Efficiency project has also allowed Valio Ltd and Luke to use prediction equations developed in the OptiMIR consortium to predict fatty acid profiles for milk samples from Finnish routine milk recording. A routine collection of MIR spectra and transfer to Mtech Digital Solutions Ltd has been established and milk fatty acid, NEFA and BHB predictions are available for all milk samples in the milk recording scheme analysed in Valio laboratory. This allows future utilization of MIR spectral information for animal breeding, animal nutrition and milk quality purposes. We have shown that NEFA and BHB predictions have the potential to be implemented into genetic evaluations and are good indicators of early lactation energy status. In addition, the development of BHB prediction equations allows now also Finland to have BHB phenotypes for the general health selection index.

4.2 Scientific significance of the results

One aim of the project was to publish all results with scientific significance according to the publication activities explained in the report. Results with significant interest for the scientific community are: novel models of Maintenance and Metabolic Efficiency, which strengthen the genetic improvement of feed efficiency; prediction equations developed for NEFA and BHB; and the economic and environmental effect of improving feed efficiency in dairy cows. The scientific significance of the results of the project is notable; the A⁺⁺Cow project was the first one in the world to solve the challenge of using RFI metric in dairy cattle breeding by developing the ReFI model as well as including of carcass weight information of the Maintenance index. In addition, the FE data collected from RDC breed are very unique and made it possible to develop the genomic predictions for the breed. Also, the prediction equations developed for NEFA are unique.

The scientific novelties were published through 9 peer-reviewed scientific articles, 1 PhD-thesis, 19 scientific congress presentations, and numerous of other publications and presentations. Furthermore, 2 scientific manuscripts were submitted before the end of the project in 2023. All publications are listed in 3.4. Publications.



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