GreenDairy

MAKERA PROJECT

Final Report

Developing Genetic and Nutritional Tools to Mitigate the Environmental Impact of Milk Production

(Maidontuotannon ympäristövaikutusten rajoittaminen eläingenetiikan ja ravitsemuksen työkaluja kehittämällä)

MAKERA Project
hankkeen dnro 2667/312/2009

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Yhteenveto

GreenDairy

Maidontuotannon ympäristövaikutusten rajoittaminen eläingenetiikan ja ravitsemuksen työkaluja kehittämällä
Hankkeen dnro 2667/312/2009

Vastuuorganisaatio

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Kesto

2010 – 2013
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Rahoitus

Kokonaiskustannukset	1 186 798.07 euroa
MMM:ltä saatu kokonaisrahoitus	240 000.00 euroa
Tutkimuslaitoksen oma rahoitus	871 798.07 euroa
Muista julkisista lähteistä saatu rahoitus	35 000.00 euroa
Muu ulkopuolinen rahoitus	40 000.00 euroa

Avainsanat: Lypsykarja, metaani, rehuhyötyssuhde, heritabilitet, ympäristövaikutusominaisuudet

Yhteenveto

GreenDairy -projektin tavoitteena oli lisätä ymmärrystä eläingenetiikan ja -ravitsemuksen roolista maidontuotannon ympäristövaikutuksissa, määritellä lehmien ympäristövaikutuksia ja energiankäyttötehokkuutta parhaiten kuvaavat ominaisuudet, sekä kehittää ravitsemuksellisia ja geneettisiä työkaluja maidontuotannon energiatehokkuuden ja ympäristöystävällisyyden parantamiseksi. Maidontuotannon ekologisen jalanjäljen pienentäminen vaatii vahvaa ymmärrystä tuotantoketjun päästöihin liittyvistä geneettisistä ja ruokinnallisista tekijöistä. Tämä puolestaan vaatii tarkkoja mittaustekniikoita, joilla on mahdollista mitata lehmien metaanintuotantoa laajassa mittakaavassa. Tähän asti nopeiden ja luotettavien sekä laajamittaiseen käyttöön soveltuvien metaaninmittaustekniikoiden puute on vaikeuttanut alan tutkimusta. Tässä projektissa sovellettiin ja edelleen kehitettiin kahta erilaista metaaninmittaustekniikkaa. Pienen mittakaavan ravitsemuksellisissa ja fysiologisissa kokeissa käytettiin SF_6 -mitta-ainetekniikkaa. Laajamittaisempaa mittausta varten kehitettiin fotoakustiseen infrapunaspektroskopiaan perustuva yksinkertainen, nopea ja luotettava menetelmä, joka ei vaadi eläimiin kohdistuvia erityisiä toimenpiteitä (F10-kaasuanalysaattori, GASERA Ltd., Turku).

Ravitsemustutkimuksessa arvioitiin erilaisia lypsylehmien metaanintuotantoon mahdollisesti vaikuttavia rehun lisäaineita ja ruokintastrategioita. Fraktioidun ja 22:6n-3 -rikastetun kalaöljyn syöttämisen ei havaittu vaikuttavan mullien pötsissä tapahtuvaan metaanin ja hiilidioksidin tuotantoon kasvavilla annostasoilla 85 g/pv asti. Sen sijaan erilaisia hiivakantoja ja camelinaöljyä sisältävät lisäaineet vähensivät pötsin CH_4- ja CO_2 -tuotantoa ilman että ne juurikaan vaikuttivat pötsin toimintaan, maidontuotantoon tai ravintoaineiden hyödyntämiseen erittäin hyvin sulavaa nurmisäilörehua syövillä lehmillä. Pelkästään camelinaöljyä sisältävät lisäaineet vähensivät myös pötsin CH_4- ja CO_2-tuotantoa, mutta johtivat samalla hieman alentuneeseen rehunsyöntiin, maidontuotantoon ja maidon pitoisuuksiin, vaikka pötsifermentaatio, pötsin mikrobipopulaatiot tai ravintoaineiden sulavuus eivät muuttuneet. Karkearehu:väkirehu -suhteen alentaminen vähensi pötsiperäistä CH_4-tuotantoa ja muutti pötsifermentaatiota propionaattia asetaatin kustannuksella suosivaksi, alensi pötsin pH:ta ja vähensi kuidun sulavuutta.

Tutkimuksessa kerättiin ainutlaatuinen tietokanta lypsylehmien rehun hyväksikäyttö- ja metaanintuotanto-ominaisuuksista. Tietokannassa on 13 958 viikottaista ja 43 735 päivittäistä mittaustulosta pohjoismaista punaista lypsyrotua olevilta lehmiltä, sisältäen eläinkohtaiset tiedot rehunsyönnistä, tuotannosta, elopainosta ja osalta myös metaanintuotannosta. Aineistosta tehdyt analyysit vahvistivat, että energiankäyttötehokkuudesta ja metaanintuotannosta löytyy riittävästi eläinten välisiä eroja mahdollistamaan geneettisen valinnan näiden ominaisuuksien suhteen. Metaanintuotantoa kuvaavien ominaisuuksien (metaanin tuotanto g/pv tai g/kg maitoa) toistuvuus vaihteli 0,2 ja 0,7 välillä lypsykauden eri vaiheissa. Tämä tulos viittaa ominaisuuksissa olevan mahdollisesti myös perinnöllistä vaihtelua, mikä mahdollistaisi jalostusvalinnan käytön yhtenä ympäristövaikutusten vähentämiskeinona. Tutkimusaineiston tarkempi geneettinen analyysi osoitti, että pohjoismaisilla punaisilla lehmillä energiankäyttötehokkuuden periytymisaste vaihteli 0,2 ja 0,4 välillä ja vahvisti että kyseisiä ominaisuuksia olisi mahdollista parantaa jalostusvalinnalla. Energiankäyttötehokkuutta parhaiten kuvaavien ominaisuuksien määrittelyä on kuitenkin vielä tarkennettava ja niiden yhteydet muihin lypsykauden aikana tärkeisiin ominaisuuksiin on selvitettävä. Lypsylehmien energiankäyttötehokkuuden ja ympäristövaikutusten (metaanintuotannon) välillä todettiin olevan vahva yhteys. Jäännösrehunkulutukseltaan alhaiset, eli rehuenergiaa tehokkaasti hyväksikäyttävät lehmät, tuottivat vähemmän metaania kuin vähemmän tehokkaat lehmät samanlaisella maitotuotostasolla. Tämä tulos vahvisti, että valinta rehunkäyttökyvyn suhteen ei pelkästään vähennä rehukustannuksia vaan myös alentaa merkittävästi maidontuotannon hiilijalanjälkeä. Rehun hyväksikäyttöominaisuuksien jalostusvalintaa voisikin käyttää vaihtoehtoisesti maidontuotannon metaanituoton vähentämiseen erityisesti tilanteissa, missä laajamittaiset eläinkohtaiset metaanintuotannon mittaukset ovat vaikeita tai mahdottomia toteuttaa.

Projektissa käyttöönotetut ja edelleen kehitetyt metaaninmittaustekniikat vahvistavat ympäristötieteen tutkimuksiin ja kehitystyöhön tulevaisuudessa tarvittavaa infrastruktuuria. Nämä tekniikat voisivat myös tuottaa maidontuotantotiloilta suoria mittaustuloksia ja realistisia päästötietoja, joita viljelijät voisivat käyttää tuotannonohjaukseen ja päättäjät kansallisen maidontuotantosektorin päästötrendien valvontaan ja kehityksen ennustamiseen. Rehun lisäaineiden ja ruokintastrategioiden tarkastelu ja validointi tuotti tärkeää tietoa käytännön sovellusmahdollisuuksista maitotilojen metaanintuotannon vähentämisessä. Lypsylehmien energiankäyttötehokkuuden ja päästöominaisuuksien fenotyyppiset ja geneettiset analyysit paljastivat ominaisuuksissa olevan eläinten välistä vaihtelua. Tutkimuksessa saatiin myös merkittävää tieteellistä lisätietoa pötsin toiminnasta, ravintoaineiden hyväksikäytöstä, pötsin mikrobipopulaatioista ja metanogeneesistä, joka on julkaistu tieteellisissä julkaisuissa. Tulokset lisäävät ymmärrystä lypsylehmien ravitsemuksesta ja erityisesti

ravitsemuksellisista mahdollisuuksista vähentää maidontuotannon metaanipäästöjä. Lehmien energiankäyttötehokkuuden ja metaanintuotannon geneettiset ja fenotyyppiset tunnusluvut sekä ominaisuuksien väliset yhteydet pohjoismaisilla punaisilla lehmillä ovat ainutlaatuisia genetiikan ja ympäristötieteen alalla. Ne myös muodostavat tärkeän pohjan tuleville ympäristövaikutusten vähentämisstrategioille. Projektin aikana koottu ainutlaatuinen pohjoismaisen punaisen rodun energiatehokkuus- ja päästöominaisuuksien tietokanta on myös tulevaisuudessa korvaamattoman tärkeä tieteellinen resurssi maidontuotannon energiatehokkuutta ja ympäristövaikutuksia käsitteleville tutkimuksille.

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Vertaisarvioidut tieteelliset artikkelit

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Summary

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Developing genetic and nutritional tools to mitigate the environmental impact of milk production

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Project duration

2010 – 2013
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Financing

Total cost	1 186 798.07 euroa
MMM contribution	240 000.00 euroa
MTT research contribution	871 798.07 euroa
Other public contribution	35 000.00 euroa
External financing contribution	40 000.00 euroa

Key words: Dairy cattle, methane, feed efficiency, heritability, environmental impact traits

Summary

The objectives of GreenDairy project were to understand the role of animal genetics and nutrition in dairy system emissions; to identify environmental impact and efficiency traits and to develop nutritional and genetic tools to improve productive efficiency simultaneously addressing the environmental impact of dairy farming. Any attempt to reduce the ecological foot print of milk production requires a sound understanding of the genetic and nutritional basis of dairy emissions. This in turn requires accurate techniques for the measurement of methane on a large scale. Thus far, however, the lack of fast and reliable techniques for large scale measurement of methane output from individual cows has been a hindrance to this. In this project, two different methane output measurement techniques were adapted and developed. The first was the SF_6 tracer technique which is suitable for small nutritional and physiological experiments. While the second method involved a non-invasive technique that is based on Photoacoustic Infrared Spectroscopy principles as applied in F10 equipment (GASERA Ltd. Turku). With this technique, a simple, fast and reliable means of quantifying the methane output of cows on a large scale was developed.

Feed additives and feeding strategies that have impact on lowering methane output in dairy cows were identified, tested and evaluated. Of the additives, feeding incremental amounts of fractionated fish oil enriched in 22:6n-3 up to 85 g/d showed no effect on ruminal methane and carbon dioxide productions in growing cattle. On the other hand, feed additives containing yeast strains and camelina oil resulted in numerical decreases in ruminal CH_4 and CO_2 production, with relatively minor effects on rumen function, milk production or nutrient utilization in cows fed diets based on highly digestible grass silage. Supplements of only camelina oil also decreased ruminal CH_4 and CO_2 emissions, changes that were accompanied by slightly lowered intake, yields of milk and milk constituents in the absence of changes in ruminal fermentation, rumen microbial populations or total tract nutrient digestibility. A feeding strategy with decreases in the dietary forage:concentrate ratio was found to lower ruminal CH_4 production that was associated with alterations in rumen fermentation towards propionate at the expense of acetate, lower ruminal pH and lower ruminal fiber digestibility.

A rare and unique database containing dairy feed efficiency and methane output traits was compiled. The database has 13 958 weekly and 43 735 daily measurements from Nordic Red cows on feed intake, production, weight and part individual cows methane output measurements. The analysis of these data for energy efficiency as well as methane output traits confirmed that there is enough between-animal variation amenable to genetic selection. The repeatability for methane phenotypes (methane output *gm* per day or per *kg* milk yield) ranged from 0.2 to 0.7 during lactation. This indicated a potential genetic variation suggesting that genetic selection for lower methane output can be one mitigation strategy. Detailed genetic analysis of same data for dairy energy efficiency traits showed that heritability in Nordic Red cows ranged from 02-0.4 confirming that improvement via selection is possible. However, this needs further assessment of optimum definition of energy efficiency traits in dairy cows and its association with other traits during lactation. Strong association was found between dairy cows energy efficiency and environmental impact traits (methane output). Feed efficient cows selected phenotypically based on their residual energy intake were found to produce less methane than their less feed efficient counterparts at more or less similar level of production. This confirmed that selection for feed efficiency will not only reduce feed cost but it will also have a marked effect in reducing the carbon foot print of milk production systems. Therefore selection for feed efficiency traits could be an alternative to mitigate methane output from milk production systems particularly when large scale measurements of methane phenotypes are difficult or impossible.

The methane measurement techniques adapted and developed by the project strengthened the infrastructures needed for future research and development in environmental science. The techniques could also provide direct measures and realistic emission figures from the dairy systems which can be used by farmers for management purposes as well as by policy makers for monitoring and prediction of national dairy system emission trends. The testing and validation of identified feed additives and feeding strategies provided very important information about the applicability of techniques which have the potential to lower methane output from the dairy systems. The phenotypic and genetic analyses of dairy energy efficiency and emission traits revealed the existing between-animal variations. A wealth of scientific knowledge has also been generated in the areas of rumen function, nutrient utilization, rumen microbial populations and methanogenesis that are shared with our scientific publications. This contributes important knowledge to the science of dairy nutrition and particularly to the nutritional options in the mitigation of dairy system emissions. In the field of genetics, the estimated genetic and phenotypic parameters for dairy energy efficiency, emission traits and associations between the various traits for Nordic Red cows are unique contributions to genetics and environmental science. They also form important basis in the design of mitigation strategies. Besides, the rare and unique database built for dairy energy efficiency, emission and etc. particularly for the Nordic Red Cattle serves as an indispensable scientific resource for supporting future scientific research and development work in the areas of dairy systems energy efficiency and environmental impact.

Publications

Refereed scientific journal publications

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GreenDairy

MAKERA PROJECT

Final Report

Developing Genetic and Nutritional Tools to Mitigate the Environmental Impact of Milk Production

(Maidontuotannon ympäristövaikutusten rajoittaminen eläingenetiikan ja ravitsemuksen työkaluja kehittämällä)

MAKERA Project
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1. BACKGROUND AND OBJECTIVES

Methane is one of the most important greenhouse gases that is produced as a by-product of the normal digestive process in ruminants. Methane released to the atmosphere by domestic ruminants represents the largest global source of methane (29%) (Global Methane Initiative, 2010). Methane production and eructation is an energetically wasteful process to the ruminant animal and on a purely energy basis, it is considered as a feed conversion inefficiency. This is because feed energy converted to methane and exhaled by dairy cows cannot be used by the animal for maintenance, growth or production. As a result, dairy cows lose on average about 2 to 12 % valuable feed energy as eructed methane. Therefore, mitigation of methane output in dairy cows will improve milk productivity through improved feed utilization efficiency.

Methane emission by dairy cows is not only a significant loss of feed energy for milk production or feed utilization efficiency. It also signifies a major concern for the environment. Methane from enteric fermentation by dairy cows and other domestic ruminant livestock species represents a potent greenhouse gas that contributes to the global warming. Methane has about 21 times the Global Warming Potential (GWP) of carbon dioxide (CO_2) and it is one of the most potent greenhouse gases coming from livestock agriculture. Therefore mitigation methane output from dairy production system not only improves the feed utilization efficiency of dairy cows but it also reduces the carbon foot print of milk production which is important to ensure a sustainable dairy production system.

A sustainable dairy production system must meet the food needs of the population while minimizing the social, economic and environmental impact. In view the ever increasing demand for more natural resources and the increasing effects of global warming, the sustainability of the dairy production will continue to be a significant issue. As a result, continued efforts should be made to mitigate the environmental impact of dairy production systems. It is, therefore, essential that dairy producers and policy makers identify opportunities to adapt or adopt management practices that promote environmental stewardship and resource conservation. In this regard, reducing the carbon footprint of the dairy sector is a key element of sustainable milk production. It is with this background that the GreenDairy project titled **"Developing genetic and nutritional tools to mitigate the environmental impact of milk production"** was started in 2010 with the following main objectives: 1) to understand the role of animal genetics and nutrition in dairy system emissions; 2) to identify environmental impact and efficiency traits and 3) to develop practical tools that help farmers improve productive efficiency simultaneously addressing the environmental impact of dairy farming. The project's specific objectives were the following:

1) Dietary ingredients (vegetable oils, specific fatty acids and probiotic products) that have effect in reducing methane production without any adverse effect on milk production and composition identified and developed.
2) Feeding strategies that combine the inclusion of lipids and levels of forages in dairy cow diets that increase dairy cow efficiency and reduce ruminal methane emissions tested and developed for use by farmers.
3) Between-animal variations and heritabilities of methane emission, production, functional and dairy cow efficiency traits estimated.
4) The genetic and phenotypic associations between methane production and other production, functional and dairy efficiency traits estimated and least-cost indicators of feed efficiency and environmental impact traits developed.
5) New traits related to environmental impact identified. The environmental impact of current breeding goals quantified and new breeding goals that include traits related to environmental impact developed.

The mitigation of methane emissions from dairy systems could be approached through both genetic and/or nutritional strategies. For this, a clear understanding of the genetic and nutritional basis of dairy system emissions is an essential first step for developing tools for its mitigation. Thus far, however, very little has been done to understand the mechanisms and develop tools for its mitigation. The GreenDairy project was initiated to fill some of these missing gaps and below is a brief report of the results and achievements of the project. For more detailed information, project results and achievements are given by work packages and tasks in Appendix1.

2. PROJECT PARTNERS AND COLLABORATION

GreenDairy is a multidisciplinary project. It has drawn together the disciplines of nutrition, biological modeling, statistics, quantitative and molecular genetics and environmental science. Experts from different departments of MTT and with several years of experience were involved in the different project tasks and activities. Specifically, members of the Animal Production Research department of MTT Agrifood Research were involved in the nutritional and physiological studies whilst those from the Biotechnology and Food Research, Biometrical Genetics

department were involved in the genetics area of the studies. Both groups participated in the developments of different methane measurement methods in addition to the collaborative work done with the experts from Plant Production Research department of MTT and the Agrobiotechnology department of Helsinki University. There was also fruitful collaboration with the industry nationally and internationally. For instance, in identifying and developing feed additives targeted to lower methane outputs we have worked with Suomen Rehu of Finland and the Lallemand Animal Nutrition of France. In developing non-invasive methane measurement technique we have worked in collaboration with Aarhus University, Folum Agricultural Research Center of Denmark.

3. PROJECT RESULTS
3.1. Methods and data

3.1.1. Methane measurement techniques for Ruminants

In this project two different methane measurement techniques were adapted and developed. The first one was a **tracer technique** which is more appropriate for intensive nutritional and physiological studies whilst the second one was a **non-invasive technique** that is based on Photoacoustic Infrared Spectroscopy (PAS) technique and is more suitable for the measurements of methane output on a large scale.

In the tracer technique, the tracer gas sulfur hexafluoride (**SF_6**) was used. The main concept in this technique was that CH_4 emissions can be measured provided the release rate of a tracer gas from the rumen is known. The technique relies on the use of SF_6 filled permeation tubes placed in the rumen and collection of gases from the rumen headspace. Thin wafer permeation tubes containing SF_6 with SF_6 release rate of approximately 1.0 mg/d were placed into the rumen of four steers at the beginning of the experiment. The actual release rate of SF_6 was determined gravimetrically over the course of the experiment and was used in the calculations. Sampling was carried out between four to six days and the concentration of SF_6 and CH_4 in the collected gases were determined by gas chromatography.

The non-invasive technique was based on ***Photoacoustic Infrared spectroscopy*** *(PAS)* principle which provides an efficient technology for gasseous measurement and monitoring purposes as implemented in the F10 multigas analyzer equipment procured from GASERA Ltd. Turku, Finland. At MTT, the F10 multi-gas analyzer was adapted and developed to a two-point sampling technique. The technique was then used to measure individual cow CH_4, CO_2 and acetone outputs from the breath sample of cows via sampling tubes fitted to two separate individual concentrate feeding kiosks. The feeding kiosks are visited by cows several times during the day. During each visit, the breath of a cow was sampled several times and analyzed for the contents of the different gases and the ID, date, time and its measurements were recorded automatically. Repeated daily F10 measurements of the gases were used to calculate the daily mean CH_4:CO_2 ratio for each cow. The CH_4:CO_2 ratios were then used to estimate the daily methane output of cows using the method by Madsen *et al.* (2010).

In this method, the estimation of CH_4 output is based on measurements of CH_4 and CO_2 concentrations in air near the animals combined with an estimation of the total CO_2 production from information on intake of metabolizable energy (ME) or heat producing units. The mean daily methane output was then calculated by multiplying total CO_2 production with the CH_4:CO_2 ratio. Based on these calculations four different methane output phenotypes were identified and described. The mean daily methane output of the cows was described as total output in gram/day (CH_4g) or per unit of product or intake as: CH_4g/kg milk (CH_4mk), CH_4g/kg DM intake (CH_4dm) or feed energy lost as methane as percentage of gross energy intake (CH_4GE). These were the four different methane output phenotypes which are stable and repeatable measures of dairy emission that were identified and used in subsequent data analyses.

3.1.2. Nutritional strategies and additives

The nutritional strategies to mitigate methane emissions in ruminants was aimed at understanding the basic biological and physiological mechanisms underlying rumen fermentation, as well as evaluating the effects of feeding strategies and additives in dairy system emission. For this, two different areas were considered. The first one evaluated the effects of various feed additives and probiotics whilst the second one looked at the effects different feeding strategies (levels of forages and concentrates) on rumen fermentation characteristics and methane emission.

Feed additives. The first study evaluated the effect of incremental amounts of fractionated fish oil enriched in 22:6n-3 on ruminal CH_4 production, rumen fermentation and apparent nutrient digestibility in growing steers fed grass silage based diets. Four Aberdeen Angus steers with live weight of 621.8 ± 39.8 kg fitted with rumen cannula

were used. Steers were offered total mixed rations based on restrictively fermented grass silage and cereal based concentrates (forage: concentrate ratio 60:40 on a dry matter, DM, basis) fed at a rate of 85 g DM/kg metabolic live-weight/d equivalent to 95% of ad libitum intake. Experimental treatments were comprised of 0.0, 21.4, 42.9 and 85.7 g/d of a fractionated fish oil (Aker BioMarine, Oslo, Norway) containing 70 g of 22:6n-3/100 g total fatty acids which were intended to supply 0, 15, 30 and 60 g/d of 22:6n-3, respectively. The oil supplements were offered as two equal amounts by mixing with 0.5 kg of concentrate components immediately before feeding the total mixed ration. A total faecal collection was performed on d 18-22 of each experimental period. Samples of the gas produced in the rumen were collected during d 23 to 25 of each period and daily gas production was determined by the SF_6 tracer gas technique.

The other study on feed additives evaluated the potential of probiotics namely, two strains of live yeasts or camelina oil enriched in polyunsaturated fatty acids (**PUFA**) to lower ruminal CH_4 and CO_2 emissions, and the associated effects on rumen function, rumen microbial populations, nutrient utilization and milk production of lactating cows fed grass silage-based diets. Four multiparous dairy cows in mid lactation fitted with rumen fistula were used to examine the effects of yeast strains and camelina oil. Treatments consisted of a total mixed ration (forage to concentrate ratio 50:50) based on grass silage (control), the same basal ration with 10^{10} cfu/d of one of two live yeast (*Saccharomyces cerevisiae*) strains A or B, supplied as 0.5 g/d of a highly concentrated dried product (Lallemand Animal Nutrition, Blagnac, France) or 60 g of camelina oil /kg dry matter (**CO**). The oil replaced concentrate ingredients in CO treatment. Feed intake was measured during d 24 to d 28 of each period. Whole tract apparent digestibility coefficients were determined by total faecal collection during d 24 to 28. Total urine was collected along with the faecal collection. Ruminal CH_4 and CO_2 output was also measured from d 24 to 28 of each period. Cows were milked twice daily and mean yields of milk and milk constituents were measured on d 24 to 26 of each experimental period.

Feeding strategies. The study on feeding strategies evaluated the effects of dietary Forage:Concentrate (F:C) ratio and supplements of sunflower oil (**SFO**) on ruminal fermentation, milk yield and composition, ruminal gas emissions and nutrient digestibility in lactating cows offered grass silage based diets. Here also four multiparous dairy cows in mid lactation fitted with rumen fistula were used to examine the effects of forage level and sunflower oil supplements during 35 d experimental period. The experimental treatments consisted of isonitrogenous diets (CP 150 g/kg) containing high (**H**) or low (**L**) proportions of forage (F:C ratio 65:35 and 35:65 on a dry matter basis, respectively) and either 0 (**O**) or 50 g SFO/kg diet dry matter (**S**) formulated to induce variable effects on milk fat synthesis. The forage component of the diet was comprised of restrictively fermented grass silage. The dietary concentrates were comprised of rolled barley, ground wheat, rapeseed expeller meal, urea and vitamin and mineral premix providing g/kg 405 or 280 NDF, and 127 or 304 starch for H and L diets, respectively. Feed intake and milk yield were recorded during days 22-25 of each period and samples of milk were collected at each milking and submitted for milk compositional analysis. Ruminal CH_4 and CO_2 output were measured during d 16 to d 21 of each period using the SF_6 tracer gas technique.

3.1.3. Genetics of environmental impact traits

Improving the efficiency of dairy animals is one of the best ways of reducing feed costs. This first requires a clear understanding of the genetic and phenotypic parameters of energy efficiency traits and the magnitude of its association with other traits. Thus far the magnitude of associations between dairy energy efficiency and other environmental impact traits are largely not known. One of the main problems for this is the lack of a well organized data on feed intake and emission traits. To address this the project compiled a long-term data and collected new ones to establish a database of dairy energy efficiency and, energy balance, feed intake, production and emission traits.The data base contained all measurements taken during lactation weeks 2 – 40.

From the database two dairy energy efficiency traits and the above mentioned methane output phenotypes were extracted for estimation of genetic and phenotypic parameters. The two energy efficiency traits calculated were residual energy intake (REI) and energy conversion efficiency (ECE). Residual energy intake (ME MJ/d) was defined as the difference between total energy intake of each animal, and the energy required for milk, maintenance and body weight change whilst energy conversion efficiency was defined as the ratio of ECM yield to ME intake in MJ (ECM kg/MEI MJ/d). These traits were analysed fitting different statistical models to estimate the genetic and phenotypic parameters for dairy cows energy efficiency traits and to quantify between-animal variations in daily methane output of dairy cows. Furthermore, the relationship between dairy energy efficiency and methane phenotypes was assessed by dividing cows into divergent FE (high *vs.* low) groups based on REI in order to

ascertain whether cows selected for divergent FE phenotypes also exhibit divergent methane phenotypes. The divergent FE phenotypes were selected by ranking cows into high REI (REI > SD above the mean, low feed efficiency), medium (REI±SD from the mean) and low (REI < SD below the mean, high feed efficiency) groups and their relationships with methane output phenotypes (CH_4 per kg DM intake, CH_4dm) were assessed.

3.2. Results

3.2.1. Two different methane output measurement techniques developed

The SF_6 tracer technique was successfully developed for the measurement of methane output in ruminants. It was then used to measure ruminal CH_4 production and CO_2 productions in our three different nutritional and physiological experiments. In all these experiments, the tracer technique provided reliable estimates of ruminal CH_4 and CO_2 productions as adjudged from comparisons with reports in the literature. Average CH_4 production (mean ± SD) of 400 ± 73 g/d determined in these experiments were similar to 406 ± 46, 411 ± 50 and 347 ± 21 reported in literature. The SF6 technique can be used as a reliable alternative to indirect respiration chambers, but total CH4 production will be underestimated (ca. 5%) because CH4 from hindgut fermentation is not accounted for. While it is useful for research purposes, the costs of the technique would prevent wide scale application to large groups of animals or implementation on-farm. On the other hand, the non-invasive technique developed at MTT provided a fast, simple and reliable method for estimation of methane output from a large number of individuals. This is a prerequisite for any genetic studies on dairy emission traits. With this method four methane output phenotypes (traits) were identified. So far over 5000 observations on daily methane output of cows were collected from about 87 cows. The analyses of this data showed that the mean daily methane output in first-lactation cows fed on a ration composed of silage and concentrate was about 330g per day and during lactation ranged from 220 - 458 g per day. When expressed on per kg of milk yield bases or per kg of feed intake, the mean estimates were 13 g/kg milk yield and 17 g/kg dry matter intake. The average amount of feed gross energy (GE) lost as exhaled methane as percentage of GE intake was about 5.7%. The technique is currently undergoing validations against other techniques. Thus far, the estimates we had in general are close and consistent with the estimates from most accurate techniques for the same class of stock fed on more or less similar kind of diets. Apart from providing a fast and reliable method to quantify the methane output of dairy cows, the technique has the advantage of using very small sample volume and it is stable, portable, suitable for the measurement of difficult gases e.g., such as those with high humidity and is ideal for use in dairy barns.

3.2.2. Feed additives and feeding strategies that have effect on methane output identified and evaluated

Feed additives: The first study evaluated the effects of fractionated fish oil enriched in 22:6n-3 on ruminal CH_4 production, rumen fermentation and apparent nutrient digestibility in growing steers fed grass silage based diets. Results from this study showed that incremental amounts of fractionated fish oil enriched in 22:6n-3 up to 85 g/d had no effect on ruminal methane and carbon dioxide productions in growing cattle fed restricted amount of grass silage based diets. On the other hand, the study directed at evaluating the effects of yeast strains and camelina oil concluded that ruminal administration of yeast strains resulted in numerical decreases in ruminal CH_4 and CO_2 production, with relatively minor effects on rumen function, milk production or nutrient utilization in cows fed diets based on highly digestible grass silage. Supplements of camelina oil decreased ruminal CH_4 and CO_2 emissions, changes that were accompanied by slightly lowered intake, yields of milk and milk constituents in the absence of changes in ruminal fermentation, rumen microbial populations or total tract nutrient digestibility. Decreases in methanogenesis to camelina oil can be explained, in the most part, by the lower intake, with some evidence to suggest that other mechanisms including changes in rumen VFA profile may have also been involved. However, it can be expected that modifications in the function of specific microorganisms especially fibre degrading communities may contribute to the positive effects of camelina oil or probiotic yeasts on reducing ruminal CH_4 and CO_2 emissions.

Feeding strategies: The study on feeding strategies evaluated effects of dietary Forage:Concentrate (F:C) ratio and supplements of sunflower oil (**SFO**) on ruminal fermentation, milk yield and composition, ruminal gas emissions and nutrient digestibility in lactating cows offered grass silage based diets. Results from this study concluded that decreases in the dietary forage:concentrate ratio lowered ruminal CH_4 production that was associated with alterations in rumen fermentation towards propionate at the expense of acetate, lower ruminal pH and lower ruminal fibre digestibility. Sunflower oil supplements also lowered ruminal CH_4 production which can be explained, at least in part, by less extensive digestion of organic matter in the rumen. There was no evidence that the effects of concentrate feeding level and oil supplementation on methanogenesis were additive.

3.2.3. Genetics of environmental impact traits

Database for dairy efficiency and emission traits compiled and built

The project has compiled, collected and built a rare and unique database for dairy energy efficiency and emission traits particularly for Nordic Red cows. The dataset included individual production, functional and efficiency traits of the primiparous cows at MTT Minkiö herd collected from November 2009 till June 2013 (Minkiö data) as well as individual records of primiparous cows collected in Rehtijärvi herd (Rehtijärvi data) with measurements from 1998 till 2009. At this moment the database has records on 436 cows with 13 958 weekly and 43 735 daily measurements. Traits recorded on animals were cows' feed intake (kg DM/d), milk production (kg/d), milk composition, body condition score and body weight (kg). The latest data particularly from Minkiö herd contained daily methane output measurements for all first lactation cows.

Genetic & phenotypic variations in energy efficiency among Nordic Red cows estimated

The phenotypic analysis two energy efficiency traits in Nordic Red cows showed that although the average efficiency measurements were close to what would be expected, marked between-animal variation was observed in energy efficiency traits among cows. The proportion of total variance due to animals was 0.46 for REI and 0.48 for ECE. In the latest data, the proportion of total variance due to animals for REI (i.e., 0.46) corresponds to 12 MJ/d that is about 6 % of the average daily energy intake of the cows. Our findings clearly indicate that there is true phenotypic variation between-animals in the energy efficiency among Nordic Red Dairy cows and which could be utilized via genetic selection.

The scope of any improvement in energy efficiency traits in dairy cows ultimately depends on the magnitude of the genetic variation and on the proportion of this variation that is heritable. To address this, we did a genetic analysis of the energy efficiency traits fitting several different statistical models. The result obtained showed that across lactation heritability estimates were moderate in the beginning of lactation (0.20 - 0.4), dropped close to zero after lactation week 10, and started to rise again towards mid- to late lactation (up to 0.2 - 0.3). Genetic correlations of residual energy intake and energy conversion efficiency were strong and positive with energy corrected milk estimated with fixed regression model**.** Residual energy intake was also positively correlated with dry matter intake, while energy conversion efficiency had strong negative correlations with body weight and energy balance. In general, the heritability estimates and genetic correlations obtained in this study suggest that feed efficiency traits are moderately heritable. This means that improvement of energy efficiency in dairy cattle via genetic selection is possible. However it should be noted that these traits have partially different physiological backgrounds and differing genetic mechanisms at different stages of lactation. Besides due to their uneven relationship with health, fertility traits applying either feed efficiency trait in practical breeding selection may not be straightforward. Therefore, assessing the relationship between energy efficiency and functional traits at different stages of lactation and looking for an optimum definition of the feed efficiency or for biologically relevant direct indicator traits for describing feed utilization efficiency in dairy cows is required.

Methane emission phenotypes identified and between-animal variations in methane output estimated

Using the non-invasive measurement technique four different methane output phenotypes were identified and their repeatabilities were estimated. Repeatability, as a ratio of between-animal to total variation indicates the potentially available animal variation which will predict the scope of lowering methane emission via selection. Our estimates of repeatability for the three different methane phenotypes CH_4g, CH_4mk, CH_4GE were relatively higher and ranged from 0.2 to 0.7 during lactation. The estimates were higher in early lactation, moderate in mid lactation and started to rise again towards late lactation. Such moderate to high estimates of repeatability for methane phenotypes (0.2 to 0.7 during lactation) indicated that there is potential genetic variation in these traits suggesting that genetic selection for lower methane output should be considered as one mitigation strategy. The dairy emission data so far collected is very small and was only from about 87 cows. As a result no detailed genetic analysis was made on methane output traits.

Relationships between dairy feed efficiency and methane emission ascertained

The study on the relationships between feed efficiency (FE) and methane output traits involved dividing cows into divergent feed efficiency groups based on REI. The result showed that feed efficient cows (i.e., those with lower REI) had also lower methane output. At more or less similar levels of production, the less feed efficient cows consumed 18.6% more feed and produced 2g/kg DM intake more methane than their high feed efficient counter parts. In other words the high FE group had relatively lower feed intake and hence lower daily methane output than the low feed efficient group at relatively similar level of production. The difference in the output of methane as a

fraction of DM intake between the high and low feed efficient groups indicated that there is innate difference in methane producing abilities of the divergent FE lines selected. This result confirms that selection for feed efficiency traits will not only result in lowering feed costs to farmers, it could also be an alternative to reduce the carbon foot print of milk production systems particularly when large scale measurements of methane phenotypes are difficult or impossible. However the superiority of the high FE group should be validated at different stages of lactation and the consequences of selection on energy efficiency traits on other production and functional traits needs to be validated.

3.3. Evaluation of the project implementation phase

During the implementation of the project some schedule changes and also some delays were encountered. In the nutritional and physiological experiments there were some changes in the schedule due mainly to the availability fistulated cows in the right numbers and conditions. The other was the inability of collecting feed intake data during the summer months when the cows are out on pasture. At the beginning we have also encountered a serious delay in the procurement of the F10 multigas analyzer that is used in the development of the non-invasive methane measurement technique. This has caused a delay in the methane measurement output data collection. Soon after F10 procurement factors related to technical issues (electricity, networking etc. problems) of the equipment in operating under the dairy barn environment had caused a serious stops and disruptions in the continuous measurement and data collection process. Although this all issues have now been resolved it has slightly affected the collection of enough dairy methane mission data. At this moment, in the database we have daily methane output estimates for a total of 87 cows. This size of data is too small for any reasonable genetic analysis and as a result part 2 of the WP 3 which was aimed at developing new breeding goals was not completed. However, this part of the work will be completed when more data is collected in the newly started project that looks into dairy feed efficiency. The other limitation was that we can only get 40 first lactation cows per year for the Minkiö farm. This in part has contributed to the smaller number of animals with simultaneous measurements on energy efficiency and methane output phenotypes.

3.4. Publications

Results and achievements of the GreenDairy project have been disseminated through several means. Some of the results are already published in refereed scientific articles, in several national and international proceedings of conferences and seminars as well as in news papers and farmer's professional articles and magazines. In general, the project has published 8 papers on refereed scientific journals, 12 papers on national and international conferences and seminars, 3 papers on farmers' professional magazines and also one news paper article and 2 invited talks were given. Detailed list of publications is given below:

Refereed scientific journal publications

Bayat, A. R., P. Kairenius, T. Stefański, H. Leskinen, S. Comtet-Marre, E. Forano, F. Chaucheyras-Durand, and K. J. Shingfield. Effect of camelina oil or live yeasts on enteric methane production, rumen microbial populations, milk production, and milk fatty acid composition in lactating cows fed grass silage diets. J. Dairy Sci. (Submitted).

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4. EVALUATION OF PROJECT RESULTS

4.1. Practical implications of the results

The project has generated results that are of great practical significance. For instance the developments of methane measurement techniques (both the tracer and non-invasive techniques) have improved MTT's capacity and its scientific infrastructures for undertaking more research in environmental aspects. Secondly, these techniques have also great potential to provide first hand direct measures and realistic emission figures from the dairy systems which can be used by farmers for management purposes as well as by policy makers for monitoring and prediction of dairy system emission trends for Finland. The testing and evaluation of the different feed additives and feeding strategies have also important practical implications. The addition of yeast strains to the diet has shown reduction in CH_4 and CO_2 with just minor effects on rumen function, milk production and nutrient utilization. On the other hand, supplements of camelina oil decreased CH_4 and CO_2 emissions. However, these effects were associated with slightly lowered yields of milk and milk constituents. Therefore great care should be taken when feeding camelina oil supplements to avoid unfavourable correlated responses.

The study on feeding strategies on the other hand clearly showed that decreases in the dietary forage: concentrate (35:65) ratio lowered enteric methane output associated with alterations in rumen fermentation towards propionate at the expense of acetate. Similarly, the addition of a limited amount (50g/kg diet dry matter) of sunflower oil also lowered ruminal CH_4 production which in part explained by less extensive digestion of organic matter in the rumen. In general, the indications are that feeding strategies with decreases in levels of forage:concentrate ratio and use of sun flower oil could be suitable mitigation strategies provided that proper balance is maintained to avoid any adverse effect on feed intake and production.

Thus far, one of the problems to understand the genetic basis of dairy system efficiency and to estimate the magnitudes of its association with environmental impact traits was a lack of an organized database with records on feed intake and emission traits of individual animals. The database compiled and built by this project for these and other traits will have a significant practical impact and form an outstanding basis of reliable source of information for future research and understanding in the area of dairy energy efficiency and dairy system emissions.

The genetic and phenotypic analysis of the dataset set has also provided several results of practical significance. The phenotypic analyses of the data concluded that there is a true between-animal variation in energy efficiency among Nordic Red cows. And the genetic analyses of energy efficiency traits provided estimates of genetic variation in dairy energy efficiency traits, its heritability and genetic associations with other production and functional traits. These all are of great significance, first in providing parameters estimates for scientific literature and secondly such parameter estimates are the necessary inputs during development of new breeding goals or estimate breeding values in order to identify bulls or cows families which are feed efficient and productive animals.

Furthermore the study on dairy emission traits identified methane output phenotypes that are stable and repeatable. It also provided a new insight about the existence of between individual variation in dairy cows methane output traits. This indicated that genetic selection can be one of the methane mitigation strategies from the dairy production systems. Most importantly, the analysis of associations between dairy cows energy efficiency and methane output traits clearly indicated that selection for feed efficiency could also reduce the carbon foot print of milk production systems. Therefore, the most sustainable and long term solution to increase dairy production and simultaneously reduce the environmental impact lies on increasing overall efficiency of dairy cows. With the increase in feed efficiency, health and reproductive and productive efficiency we can reduce the amount inputs and at the same time reduce the environmental impact.

4.2. Scientific impact or significance of the project

This project has tested, evaluated and identified feed additives and feeding strategies that have effect on rumen fermentation characteristics and methane emission. During this process, a wealth of knowledge has been generated in the areas of rumen function, nutrient utilization, rumen microbial populations and methanogenesis. This

knowledge has been shared and will be shared to the scientific community through our several publications. This adds an important knowledge to the science of dairy nutrition and particularly to the nutritional options in the mitigation of dairy system emissions.

In the field of livestock genetics, there was little information on the clear understanding of the genetic and phenotypic basis of dairy systems energy efficiency and emission traits. Particularly, information on the Nordic Red cattle was largely missing. The results from this study especially information on the genetic and phenotypic parameters estimates along with estimates of the magnitude of associations between the various traits are therefore unique and significant contributions to genetics and environmental science.

In addition, the rare and unique database built for dairy energy efficiency, emission and other traits particularly for the Nordic red Cattle will be an indispensable scientific resource for future scientific research and development work in the areas of dairy systems energy efficiency and environmental impact. Besides, most of our publication on refereed scientific journals contributed a wealth of information to the scientific literature and highlighted most important future research directions in the areas feed efficiency and environmental science. Most importantly, the partnership and collaboration built by the project with our both national and international scientific and industry collaborators in the area of dairy system efficiency and environmental impact has laid a cornerstone for our future scientific global collaboration in addressing this global problem.

Summary of project budget

Table 1. Financial summary of the GreenDairy project (**MAKERA**) by year, cost items and sources of financing.

	2010	**2011**	**2012**	**2013**	**TOTAL**	**Remark**
COST ITEMS:						
Realized Work man months	13.0	45.9	25.1	24.0	**108.00**	
Direct personal cost	69 653.99	236 676.40	152 106.42	136 217.96	**594 654.77**	
Overhead cost	60 245.51	245 068.11	123 421.47	114 370.88	**543 105.97**	
Analytical cost and consumables	63.38	13 501.59	3 343.23	13 538.89	**30 477.09**	
Travel	0	6 233.72	5 636.81	6 719.71	**18 590.24**	
TOTAL	**129 962.88**	**501 479.82**	**284 507.93**	**270 847.44**	**1 186798.07**	
FINANCING SOURCES:						
MAKERA	44 104.51	96 042.44	86 622.89	13 230.16	**240 000.00**	15% after final repo
MTT Strategic	0	0	0	0	0	
MTT	50 858.37	385 437.27	177 885.04	257 617.28	**871 798.07**	
Lallemand Animal Nutrition	0	20 000.00	20 000.00	0	**40 000.00**	
Suomen Rehu	35 000.00	0	0	0	**35 000.00**	
TOTAL	**129 962.88**	**501 479.71**	**284 507.93**	**270 847.44**	**1 186798.07**	

Annex 1. Summary of the final report

Appendix 1. Project results by work package and tasks

Appendix 1

GreenDairy

Maidontuotannon ympäristövaikutusten rajoittaminen eläingenetiikan ja ravitsemuksen työkaluja kehittämällä

(Developing genetic and nutritional tools to mitigate the environmental impact of milk production**)**

MAKERA Final Report

April 2014

WP 1. Nutritional strategies to mitigate methane emissions in ruminants

Task1. Establish the use of SF_6 tracer technique to measure methane emissions in individual animals

One of the major challenges to lowering GHG from the dairy systems is the need for accurate and reliable techniques for the measurement of methane (CH_4). Task 1 evaluated a tracer gas technique for the measurement of CH_4 output for individual cows.

The concept of the sulfur hexafluoride (**SF_6**) technique is that CH_4 emissions can be measured provided the release rate of a tracer gas from the rumen is known. For this purpose a non-toxic, physiologically inert and stabile gas is required that behaves similarly to CH_4 in the rumen. The SF_6 tracer was chosen because it meets the necessary criteria, is inexpensive, with an extremely low detection limit that is straightforward to analyze. The technique developed at MTT in task 1 relies on the use of SF_6 filled permeation tubes being placed in the rumen and the collection of gases in the rumen headspace. Thin wafer permeation tubes containing SF_6 (i.d. 16.4 × 46.5 mm; Fine Permeation Tubes, Spadafora, Italy) with SF_6 release rate of approximately 1.0 mg/d were placed into the rumen of the steers in the beginning of the experiment. The actual release rate of SF_6 was determined gravimetrically over the course of experiment and used in the calculations. Sampling is typically carried out between four to six days. The concentration of SF_6 and CH_4 in the collected gases is determined by gas chromatography.

The gas sampling lines and equipments were designed to constantly collect gas samples directly from the rumen cannula using plastic tubes (i.d. 2 mm) connected to the sampling canisters. The beginning of the line was 5 to 10 cm inside the rumen, so that the tube passed through the cannula into the rumen headspace. A 20 ml plastic bottle, equipped with T shape valves, was attached to the line to serve as a "dead trap" for rumen liquid. The sampling tube between the cannula and the dead trap was inserted into a spiral tube (i.d. 8 mm) allow the free movement of sampled animals. After the dead trap, a syringe filter (type 0.2 µm and 25 mm diameter) was used to prevent flow of rumen liquid and small particles to the capillary tube. After the filter, 110 cm of capillary tubing (PEEK 1.6 mm × 0.13 mm i.d., VICI® Valcro Instruments Co, Houston, TX, USA) was used to control gas flow ca. 2.0 mL/min. The sampling line was connected to an evacuated (-0.85 bar) canister (5.5 L) to collect the gas sample. The canister was built from PVC pipe with 110 cm diameter and equipped with a valve and a fast connector. Each canister was replaced after 24 hour, over pressurized with nitrogen gas to 1.1 bar and left 2 hours to mix. The gas was released slowly from the canister, sub-sampled in triplicate, and transferred into evacuated 10 mL glass tubes equipped with a rubber stopper, and analyzed for CH_4, CO_2, and SF_6 concentrations using a gas chromatograph (HP 6890 Series, GC System, Hewlett Packard, USA) equipped with flame ionization (**FID**) and electron capture (**ECD**) detectors, autosampler, and a nickel catalyst for converting CO_2 to CH_4. Chromatography was achieved using a 1.8 m pre-column and 3.0 m analytical column packed with 80/100 mesh Hayesep Q (Supelco Inc., Bellefonte, PA, USA), nitrogen as a carrier gas and a mixture of argon and CH_4 (5%) as a make-up gas (1.4 mL/min) to increase the sensitivity of the ECD. The GC oven, FID, and ECD were operated at 70°C, 300°C, and 350°C, respectively (Regina and Alakukku, 2010). Peaks were identified by retention time comparisons with authentic standards (AGA Ltd., Espoo, Finland). Concentration of gases was determined based on calibration curves constructed using authentic standards (range 2.5-25% for CO_2 and CH_4, and 0.00625-0.125 ppm for SF_6) and the following equation was used to calculate daily CH_4 and CO_2 emissions:

$$CH_4\ (L/d) = SF_6\ (L/d) \times [CH_4]/[SF_6]$$
$$CO_2\ (L/d) = SF_6\ (L/d) \times [CO_2]/[SF_6]$$

where SF_6 is the predetermined release rate from the permeation tube and $[CH_4]$, $[CO_2]$ and $[SF_6]$ are the concentrations of CH_4, CO_2 and SF_6 in samples.

The SF_6 technique adapted and developed at MTT was used to measure ruminal CH_4 production in three experiments: the first experiment in growing cattle fed diets supplemented with fish oil, the second experiment with lactating cows receiving probiotic yeasts and camelina oil and the third experiment with lactating cows receiving diets containing different proportions of concentrate and/or sunflower oil. The tracer technique provided reliable estimates of ruminal CH_4 and carbon dioxide (**CO_2**) productions as adjudged from comparisons with reports in the literature. Average CH_4 production (mean ± SD) of 400 ± 73 g/d determined in Task 2.2. and Task 3.1. are similar to those reported previously of 406 ± 46, 411 ± 50 and 347 ± 21 from (Corré 2002), IPCC (1997) and Kirchgessner et al. (1995), respectively.

Conclusions and implications: The SF_6 technique can be used as a reliable alternative to indirect respiration chambers, but total CH_4 production will be underestimated (ca. 5%) because CH_4 from hindgut fermentation is not accounted for. Considerable between-day variation in enteric CH_4 production requires the collection of gases for at least 4 days. No attempt was made to modify the technique for use in intact animals, but it is probable that the sampling period would need to be extended in these situations. While useful for research purposes the costs of the technique would prevent wide scale application to large groups of animals or implementation on-farm.

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Task2.1. Effect of incremental levels of docosahexaenoic acid enriched oil on ruminal lipid metabolism and ruminal methane production in steers

Rationale

Ruminant livestock systems are a significant source of CH_4 and nitrous oxide (**N_2O**) into the environment that contribute to global warming. Numerous countries have agreed in principle to decrease greenhouse gas (**GHG**) emissions, targets that have to be met within the context of increased global demand for food. The main GHG from livestock production are CH_4, N_2O and CO_2 Ruminal CH_4 production accounts for between 3.5 and 7.5% of gross energy intake (O'Hara et al., 2003), and is considered to be the most important GHG released into the environment from ruminant livestock (Ogino et al., 2007). Several approaches for decreasing enteric CH_4 production have been examined including vaccination, defaunation, use of probiotic treatments and alterations in diet composition (Martin et al., 2010). Inclusion of oils and oilseeds in the diet typically depress enteric methanogenesis (Beauchemin et al., 2008; Martin et al., 2010), effects thought to be related to the anti-protozoal properties of 12:0 and 14:0 and inhibitory effects of unsaturated ≤ 18 carbon fatty acids on the growth of rumen methanogens. Dietary supplements of plant oils rich in polyunsaturated fatty acids have been shown to lower CH_4 emissions from cattle (Martin et al., 2008; Beauchemin and McGinn, 2006). Studies in vitro have also provided evidence that 20:5n-3 and 22:6n-3 contained in fish oil and marine algae inhibit methanogenesis (Dong et al., 1997; Fievez et al, 2003; 2007). However, data on the efficacy of the very long chain n-3 fatty acids on ruminal CH_4 production in vivo are limited. In the current experiment, the effect of incremental amounts of fractionated fish oil enriched in 22:6n-3 on ruminal CH_4 production, rumen fermentation and apparent nutrient digestibility was examined in growing steers fed grass silage based diets.

Methodology

Four Aberdeen Angus steers with live weight of 621.8 ± 39.8 kg fitted with rumen cannula and a rigid T-piece cannula located within 50 mm of the pylorus were used in a 4 × 4 Latin square design with 28 d experimental periods comprising 14 d adaptation, 11 d sampling and 3 days washout periods. Steers were housed in a dedicated metabolism unit and offered daily rations as equal meals at 06.00 and 18.00 h. Steers were offered total mixed rations based on restrictively fermented grass silage and cereal based concentrates (forage: concentrate ratio 60:40 on a dry matter, DM, basis) fed at a rate of 85 g DM/kg metabolic live-weight/d equivalent to 95% of ad libitum intake measured immediately before the start of the experiment. Experimental diets comprised (g/kg DM) grass silage (650), barley(87.5), oats (87.5), molassed sugar beet pulp (56.9), solvent extracted rapeseed meal (105) and a vitamin and mineral premix (13.1). Treatments comprised 0.0, 21.4, 42.9 and 85.7 g/d of a fractionated fish oil supplement (Aker BioMarine, Oslo, Norway) to supply 0 (**FO0**), 15 (**FO15**), 30 (**FO30**) and 60 g/d (**FO60**) of 22:6n-3, respectively. Fish oil contained (g /kg), *cis*-11 22:1 (24.2), 20:5n-3 (91.3), *cis*-15 24:1 (10.8), 21:5n-3 (13.8), 22:5n-6 (16.7), 22:5n-3 (41.9), 22:6n-3 (713.9) and total fatty acids (959). Oil supplements were offered as two equal amounts by mixing with 0.5 kg of concentrate components immediately before feeding the total mixed ration. A total faecal and collection was performed on d 18-22 of each experimental period. Samples of the gas produced in the rumen were collected during d 23 to 25 of each period and daily gas production was determined by the SF_6 tracer gas technique.

Results and Discussion

Cattle were fed restricted amounts of silage and concentrates to minimize the influence of variation in feed intake on the measured parameters. Under these circumstances, fish oil enriched in 22:6n-3 had no effect ($P > 0.05$) on intake. Fish oil is known to lower intake in a dose dependent manner in growing steers, irrespective of dietary forage to concentrate ratio (60:40) or forage species (Keady and Mayne, 1999; Lee et al., 2008; Shingfield et al., 2010). Incremental supplementation of fish oil progressively increased ($P < 0.01$) fatty acid intakes (264, 285, 305 and 346 g/d for treatments FO0, FO15, FO30 and FO60, respectively). Apparent total tract OM or CP digestibility was unaffected ($P > 0.05$) by dietary treatments, whereas fish oil increased linearly ($P < 0.05$) neutral detergent fibre (**NDF**), potentially digestible NDF (**pdNDF**) and acid detergent fibre (**ADF**) digestion (Table 1). However, there was no clear effect of treatment ($P > 0.05$) on ruminal fibre digestibility (Table 1). An increase in total tract fibre digestibility coefficients to fish oil is in contrast to previous studies in cattle fed grass silage or red clover silage (Lee et al., 2008) or total mixed rations based on maize silage (Shingfield et al., 2010) in which fish oil had no effect. In lactating cows, fish oil has been shown to increase fibre digestion (Doreau and Chilliard, 1997; Keady et al., 2000; Shingfield et al., 2003). A higher total tract apparent digestibility of NDF with increasing the level of fish oil in the diet has been attributed to the lower DM intake in some studies (Keady et al., 2000). In this experiment, fish oil

increased total tract apparent fibre digestibility due to numerical increases in ruminal digestion and more extensive digestion in the hindgut. Keady et al. (2000) suggested that one or more of the fatty acids in fish oil may affect the metabolism of cell wall degrading microorganisms, especially those involved in the digestion of hemicelluloses with the support that NDF but not ADF digestibility was increased by fish oil.

Oil supplements had no effect ($P > 0.05$) on ruminal CO_2 production, pH, ammonia nitrogen or total VFA concentrations (Table 1). However, fish oil decreased linearly molar proportions of acetate ($P < 0.01$), valerate ($P < 0.05$) and caproate ($P < 0.05$) and decreased linearly ($P < 0.05$) molar proportions of propionate and isovalerate. Fish oil had no effect on rumen pH ($P > 0.05$), that at least in part, reflects the similar intake across treatments. Consistent with previous studies (Shingfield et al., 2012; Fietze et al., 2003), fish oil caused a shift in rumen fermentation towards propionate at the expense of acetate. Daily enteric CH_4 production tended ($P = 0.08$) to increase in a quadratic manner, being greatest on FO30. However, the effect of treatment was not ($P > 0.05$) evident when CH_4 production was expressed as a function of OM or NDF digested in the rumen (Table 1). Moate et al. (2013) feeding up to 75 g/d 22:6n-3 from algal meal reported no effect on CH_4 production in dairy cows. However, incubations of fish oil or marine algae containing 20:5n-3 and 22:6n-3 with mixed rumen bacteria (Dong et al., 1997; Fievez et al., 2003; 2007) has been reported to decrease CH_4 production in vitro up to a maximal inhibition of 80%. The results of this experiment provided no support that supplements of 22:6n-3 substantially alters CH_4 production in growing cattle fed grass silage based diets. In this case, fish oil stimulated numerical improvements in ruminal fibre digestion such that any increase in CH_4 production arising from higher retention in the rumen were compensated for a shifts in ruminal fermentation towards propionate at the expense of acetate. It appears that other fatty acids in fish oil and marine algae may be responsible for the effects observed in vitro. Fish oil and marine algae are relatively abundant in 14:0. Studies in lactating cows have shown that supplementing the diet with 14:0 decreases CH_4 production (Dohme et al., 2004; Odongo et al., 2007) suggesting that the possible effects of marine oils on methanogenesis in ruminants are related to the concentrations of medium chain saturated fatty acids in these lipid supplements.

Table 1. Effect of dietary fish oil supplements enriched in docosahexaenoic acid on ruminal gas production, and apparent ruminal and whole tract nutrient digestibility in growing cattle

	22:6n-3 (g/d)					*P*-value[3]	
Parameter[1]	0	15	30	60	SEM[2]	L	Q
Enteric CH_4							
g/d	224	232	236	226	9.1	0.93	0.08
g/kg OM digested in rumen	55.3	54.3	54.8	52.6	3.55	0.18	0.73
Enteric CO_2							
g/d	1559	1692	1679	1661	72.1	0.38	0.21
g/kg OM digested in rumen	387	397	391	386	32.9	0.83	0.69
Apparent total tract digestibility (g/kg)							
OM	731	726	730	748	10.6	0.20	0.38
CP	696	685	703	697	8.7	0.59	0.97
NDF	625	629	631	657	9.6	< 0.05	0.45
pdNDF	767	767	773	811	9.2	0.01	0.17
ADF	635	645	645	669	7.8	< 0.05	0.67
Apparent ruminal digestibility (g/kg)							
OM	451	475	478	475	12.1	0.14	0.12
NDF	566	560	585	586	15.8	0.16	0.85
pdNDF	698	690	720	721	19.3	0.16	0.86
ADF	578	573	589	598	17.4	0.22	0.86

[1]OM, organic matter; CP, crude protein; NDF, neutral detergent fiber; pdNDF, potentially digestible NDF; ADF, acid detergent fiber

[2]SEM for n = 16 measurements; error degree of freedom = 6.

[3]L, linear effect; Q, quadratic effect; the cubic responses to docosahexaenoic acid enriched fish oil were non-significant ($P > 0.05$).

Conclusions

Incremental amounts of fractionated fish oil enriched in 22:6n-3 of up to 85 g/d had no effect on ruminal methane or carbon dioxide production in growing cattle fed restricted amounts of grass silage based diets.

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Task 2.2. Effect of probiotic yeasts or camelina oil in the diet on ruminal gas production, milk yield and milk composition in lactating cows

Rationale

Agriculture accounts for 10-12% of global anthropogenic GHG that includes about 58 and 47% of total anthropogenic N_2O and CH_4 emissions, respectively, with a much smaller contribution to global CO_2 output. Emissions of CH_4 and N_2O due to agricultural activities have increased by 17% from 1990 to 2005 (US-EPA, 2006), while the global output of CH_4 and N_2O from livestock production systems is anticipated to increase from current levels by 60 and 35-60%, respectively by 2030 (FAO, 2003). Nutritional strategies to mitigate CH_4 emissions have sought to identify and investigate various feed additives and dietary ingredients to promote alternative pathways to CH_4 formation for the dissipation of metabolic hydrogen in the rumen (Boadi et al., 2004). Dietary supplements of medium-chain saturated fatty acids (Jordan et al., 2006; Machmüller, 2006) and plant oils are known to lower methanogenesis in lactating or growing cattle(McGinn et al., 2004; Beauchemin and McGinn, 2006; Martin et al., 2008). However, the decreases in ruminal CH_4 formation in response to lipid supplements fed at a relatively high level of inclusion (≥ 50 g/kg DM) are often accompanied by decreases in DMI, nutrient digestion and animal performance (Jordan et al., 2006; Beauchemin and McGinn, 2006; Martin et al., 2008).

Yeast products have been used as feed additives for ruminants to improve feed efficiency, prevent digestive disorders through the scavenging of oxygen within the rumen, provision of microbial growth factors or compete directly with autochthonous species in the rumen (Newbold et al., 1996; Fonty and Chaucheyras-Durand, 2006). However, the effects of dietary yeasts supplements in vivo are inconsistent and vary substantially due to the yeast strain and interactions between the host ruminant and diet composition (Desnoyers et al., 2009). Selection of specific yeast strains for lowering ruminal CH_4 production has been postulated (McGinn et al., 2004; Chaucheyras-Durand et al., 2008; Chung et al., 2011). The present experiment examined the potential of two strains of live yeasts or camelina oil enriched in polyunsaturated fatty acids (**PUFA**) to lower ruminal CH_4 and CO_2 emissions, and the associated effects on rumen function, rumen microbial populations, nutrient utilization and milk production of lactating cows fed grass silage-based diets.

Methodology

Four multiparous dairy cows in mid lactation fitted with rumen fistula were used to examine the effects of two strains of yeast and camelina oilon enteric CH_4 production in a 4 × 4 Latin Square with 42 d experimental periods. Treatments consisted of a total mixed ration (forage to concentrate ratio 50:50) based on grass silage (control), the same basal ration with 10^{10} cfu/d of one of two live yeast (*Saccharomyces cerevisiae*) strains A and B, supplied as 0.5 g/d of a highly concentrated dried product (Lallemand Animal Nutrition, Blagnac, France) or 60 g of camelina oil /kg dry matter (**CO**). The concentrate contained (g/kg dry matter basis) rolled barley 450, molassed sugar beet pulp 240, solvent extracted rapeseed meal 280 and vitamin and mineral premix 30 (3, Onni, Melica Finland Ltd., Vaasa, Finland). Oil replaced concentrate ingredients in the CO treatment. Feed intake was measured during d 24 to d 28 of each period. Whole tract apparent digestibility coefficients were determined by total faecal collection during d 24 to 28. Total urine output was measured at the same time as faecas. Ruminal CH_4 and CO_2 output was measured from d 24 to 28 of each period as outlined in Task 1. Cows were milked twice daily and mean yields of milk and milk constituents were measured on d 24 to 26 of each experimental period.

For microbial assessment, samples of ruminal digesta were collected from four sites within the reticulo-rumen immediately after the collection of rumen fluid at 06.00 h on d 28 of each experimental period. Samples of ruminal digesta were mixed thoroughly, and a sub sample (50 g) was mixed with 100 mL of RNAlater solution (Fisher Scientific, Illkirch, France), split into further aliquots, and stored at -80°C prior to quantitative PCR (**qPCR**) analysis.

Experimental data were analyzed by ANOVA for a 4 × 4 Latin square using the Mixed procedure of SAS (version 9.2, SAS institute, Cary, NC) with a model that included the fixed effects of period and treatment and random effect of cow. Least squares means ± SEM are reported and treatment effects declared significant at $P \leq 0.05$. When the overall effect of treatment was significant, the difference among means was explored further using the Fisher's least significant difference (LSD) test.

Results and Discussion

Compared with the control and yeast treatment B, CO lowered ($P = 0.05$) dry matter intake (19.0, 18.4, 19.2 and 16.7 kg/d for control, yeast treatments A and B, and CO, respectively). By design CO increased ($P < 0.05$) the intake

of fatty acids (1213 vs 403 g/d) relative to the control. When fed in relatively high amounts (> 50 g/kg DM), plant oils typically lower DMI. Adverse effects have often been attributed to the inhibitory effects of unsaturated fatty acids on the growth of ruminal microbial communities, lowered OM and NDF digestion in the rumen, a tendency to shift the site of nutrient digestion from the rumen to the intestines, and elevated plasma gut peptide concentrations (Allen, 2000; Lock and Shingfield, 2004). The decrease in intake to camelina oil was, to some extent associated with lower but non-significant (-4.1%) whole tract OM digestion. Ruminal administration of live yeasts having no effect on DMI is consistent with previous reports in dairy cows (De Ondarza et al., 2010). The influence of live yeasts on intake is thought to be dependent upon plane of nutrition, with positive responses observed at higher DM intakes (Erasmus et al., 2005).

Supplements of live yeasts had no effect ($P > 0.05$) on ruminal CH_4 and CO_2 emissions, whereas CO decreased ($P < 0.\ 05$) ruminal CH_4 and CO_2 emissions (Table 3). The non-significant differences due to live yeasts strains on ruminal CH_4 production (-10.3 and -8.6% for yeasts A and B, respectively) in the present experiment are consistent and marginally higher than -3 and +6% reported by McGinn et al., (2004). However, ruminal CH_4 production per unit total apparent organic matter digestion or kg milk did not differ ($P > 0.05$) between treatments, whereas ruminal CO_2 output per unit milk yield tended ($P = 0.09$) to be lower for CO compared with the control. It is well established that 18:2n-6 and 18:3n-6 exert toxic effects on ciliate protozoa (Girard and Hawke 1978; Ivan et al., 2001) and also on cellulolytic bacteria involved in fiber digestion and hydrogen production that may result in the direct inhibition of ruminal methanogens (Martin et al., 2010). In the present study, the abundance of protozoa in the rumen did not differ between CO and the control. Furthermore, CO did not affect ($P > 0.05$) the abundance of fibrolytic bacteria, despite a tendency to increase the *R. flavefaciens* population. Treatments had no effect ($P > 0.05$) on energy loss as CH_4 that averaged 5.68 % of gross energy intake. Dietary supplements of camelina oil resulted in a 4.9% decrease in CH_4 production per percentage increase in diet DM. The magnitude of this response is similar to a value of 4.8% for cows fed diets containing 58 g of linseed oil/kg diet DM (Martin et al., 2008). Decreases in ruminal CH_4 and CO_2 emissions on the CO treatment of 29.5 and 34.3%, respectively can be explained, at least to some extent, by the associated changes in DMI (-12.0%) and total tract OM digestibility (-4.1%).

Compared with yeast treatment A and the control, CO decreased ($P < 0.05$) the yields of milk and milk protein (Table 3). CO decreased ($P < 0.05$) yields of energy corrected milk (**ECM**) and milk lactose compared with control and yeast treatments, whereas yield of milk fat was lower ($P < 0.05$) for CO compared with control and yeast treatment B which due to lower milk production. Yeast supplements had no influence on milk yield, composition, or energy secretion in milk consistent with no changes in intake or digestibility compared with the control. A recent meta-analysis based on data from 14 experiments including measurements of more than 1,600 cows fed a range of diets indicated that live yeasts can be expected to improve fat corrected milk yield and stimulate an increase in milk protein and fat secretion (De Ondarza et al., 2010).

Relative to the control, CO tended ($P = 0.06$) to decrease rumen pH (6.40 vs. 6.65), whereas treatments had no effect ($P > 0.05$) on ruminal ammonia-nitrogen, total VFA concentration or molar VFA proportions. Live yeast strains having no substantial influence ($P > 0.05$) on ruminal fermentation is in agreement with earlier reports (McGinn et al., 2004). A comprehensive meta-analysis of data from 77 experiments concluded that yeast supplements increase total VFA concentration on average by 5.4% (Desnoyers et al., 2009). Apparent total tract digestibility of nutrients, other than starch and GE were unaffected ($P > 0.05$) by treatment (data not presented). Digestion of starch ($P = 0.05$) and that of GE ($P = 0.08$) tended to be lower for CO compared with the control (0.977 vs. 0.982 and 0.675 vs. 0.700, respectively).

Table 3. Effect of dietary supplements of two strains of live yeasts or camelina oil on ruminal gas production, milk yield and milk composition and rumen microbial populations in lactating cows

	Treatment[1]					
	Control	A	B	CO	SEM	*P-value*
Ruminal methane						
g/d	407[a]	365[a]	372[a]	287[b]	22.3	< 0.05
g/kg OMD[2]	32.7	30.4	29.6	27.9	2.12	0.51
g/kg NDFD[2]	57.8	53.3	52.5	49.7	3.60	0.51
g/kg milk	15.6	13.8	15.0	13.1	1.33	0.27
% of GEI	6.31	5.82	5.73	4.86	0.368	0.14
Ruminal carbon dioxide						
g/d	3,493[a]	3,006[a]	3,172[a]	2,295[b]	226.4	< 0.05

g/kg OMD	281	251	252	220	16.8	0.18
g/kg NDFD	497	440	447	392	28.5	0.18
g/kg milk	133	114	127	104	10.4	0.09
Yield						
Milk (kg/d)	27.0[a]	26.5[a]	25.6[ab]	22.4[b]	2.30	< 0.05
ECM (kg/d)[3]	26.4[a]	25.5[a]	25.7[a]	22.1[b]	2. 18	< 0.05
Fat (g/d)	1,070[a]	1,020[ab]	1,062[a]	920[b]	96.4	< 0.05
Protein (g/d)	859[a]	827[a]	824[ab]	696[b]	57.5	< 0.05
Lactose (g/d)	1,240[a]	1,228[a]	1,183[a]	1,017[b]	115.7	< 0.05
Milk composition (g/kg)						
Fat	39.9	38.3	41.6	40.8	1.31	0.24
Protein	32.4	31.2	32.4	31.3	1.08	0.08
Lactose	45.8	46.3	46.2	44.6	1.11	0.45
Microbial numbers (log gene copies/µg of DNA)						
Total bacteria	10.08	10.13	10.16	10.13	0.032	0.44
Methanogens	7.27	7.35	7.30	7.30	0.034	0.26
Protozoa	7.63	7.59	7.60	7.42	0.083	0.23
Fungi	6.10	6.07	5.98	6.02	0.056	0.43
Fibrobacter succinogenes	7.64	7.58	7.77	7.61	0.071	0.31
Ruminococcus flavefaciens	6.50	6.34	6.47	6.75	0.100	0.12

[1]Refers to grass silage based diets containing no additional supplement (Control diet) or supplemented with 0.5 g/d of probiotic yeast strains A or B or 60 g/kg DM of camelina oil (CO).
[2] OMD, Organic matter digested in total digestive tract; NDFD, NDF digested in total digestive tract
[3]ECM, Energy corrected milk= Milk production (kg) × (383 × fat (g/kg) + 242 × protein (g/kg) + 165.4 × lactose (g/kg) + 20.7) / 314

Conclusions

Ruminal administration of yeast strains resulted in numerical decreases in ruminal CH_4 and CO_2 production, with relatively minor effects on rumen function, milk production or nutrient utilization in cows fed diets based on highly digestible grass silage. Supplements of camelina oil decreased ruminal CH_4 and CO_2 emissions, changes that were accompanied by lowered intake, the yields of milk and milk constituents in the absence of changes in ruminal fermentation, rumen microbial populations or total tract nutrient digestibility. Decreases in methanogenesis to camelina oil were explained, in the most part, by the lower intake, with some evidence to suggest that other mechanisms including changes in rumen VFA profile may also be involved. However, it can be expected that modifications in the function of specific microorganisms, particularly y fibre degrading communities, may contribute to the positive effects of camelina oil or probiotic yeasts as supplement for lowering ruminal CH_4 and CO_2 emissions.

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Task 3. Evaluation of different feeding strategies that combine lipids and level of forages in the diet on methane emission in ruminants

3.1. Assess the impact of plant oils and proportion of forage in the diet on methane emission in lactating cows

Rationale

Methane (**CH_4**), N_2O and CO_2 are the main GHG produced by livestock. Ruminal CH_4 emissions, corresponding to loss of 3.5-7.5% of energy intake (O'Hara et al. 2003), is considered to be the most important GHG produced by ruminant animals. Dietary plant oil supplements and increases in the proportion of concentrates in the diet are known to lower ruminal methanogenesis (Martin et al., 2010). However, it has been recently concluded that dietary lipid supplementation results in the most consistent decrease in CH_4 emissions relative to changes in the forage:concentrate (**F:C**) ratio of the diet or other feed additives (Grainger and Beauchemin, 2011). Inclusion of oils and oilseeds in the diet typically depress ruminal methanogenesis (Beauchemin et al., 2008; Martin et al., 2010) via either the anti-protozoal properties of 12:0 and 14:0 or inhibitory effects of unsaturated ≥ 18 carbon fatty acids on the growth of rumen methanogens. Several mechanisms may account for the changes in CH_4 production following the substitution of concentrate ingredients for forages, including alterations in rumen fermentation, ruminal digestion and microbial ecology.

In this study the effects of dietary F:C ratio and supplements of sunflower oil (**SFO**) on ruminal fermentation, milk yield and composition, ruminal gas emissions and nutrient digestibility in lactating cows offered grass silage based diets were examined.

Methodology

Four multiparous dairy cows in mid lactation fitted with rumen fistula were used to examine the effects of forage level and sunflower oil supplements on CH_4 production according to a 4 × 4 Latin Square with a 2 × 2 factorial arrangement of treatments and 35 d experimental periods. Experimental treatments consisted of isonitrogenous diets (CP 150 g/kg) containing high (**H**) or low (**L**) proportions of forage (F:C ratio 65:35 and 35:65 on a dry matter basis, respectively) and either 0 (**O**) or 50 g SFO/kg diet dry matter (**S**). The forage component of the diet comprised restrictively fermented grass silage prepared from mixed timothy-meadow fescue swards treated with a formic acid based ensiling additive. Dietary concentrates contained rolled barley, ground wheat, rapeseed expeller meal, urea and a vitamin and mineral premix. The H and L diets contained (g/kg) 405 or 280 neutral detergent fibre (**NDF**), and 127 or 304 starch, respectively. Feed intake and milk yield were recorded during days 22-25 of each period and samples of milk were collected at each milking, preserved with Bronopol tablets and submitted for fat, crude protein and lactose analysis. Ruminal CH_4 and CO_2 output was measured during d 16 to d 21 of each period as outlined in Task 1.

Results and Discussions

Intake on L diets was higher ($P < 0.01$) compared with H diets, whereas SFO tended ($P = 0.09$) to lower DM intake (19.0, 18.6, 23.3 and 20.7 kg/d for HO, HS, LO and LS treatments, respectively). A higher feed intake with L diets is consistent with greater ruminal OM digestion (0.429 vs. 0.385) despite the decrease in the ruminal digestibility of NDF (0.440 vs. 0.506). The tendency for SFO to decrease DM intake (19.7 vs. 21.2 kg/d) was associated with the lower ($P < 0.05$) ruminal NDF digestibility (0.395 vs. 0.450).

Rumen pH was lower ($P < 0.01$) for L than H, whereas SFO supplements increased ($P < 0.05$ for interaction) rumen pH on L but not on H diets (6.61, 6.51, 6.03 and 6.26 for HO, HS, LO and LS, respectively). Rumen ammonia nitrogen was lower ($P < 0.01$) on L than H diets (5.60 vs. 2.75 mM). Total rumen VFA was highest ($P < 0.05$) on L diet containing no SFO (123 vs. 108 mmol/L) compared with other treatments. Molar proportions of acetate and isobutyrate ($P < 0.05$) were lower, while propionate and valerate ($P < 0.05$) were greater on L than H diets (data not presented). As a consequence, the acetate to propionate ratio was significantly lower ($P < 0.001$) on L than H diets (2.70 vs. 3.60). Lower rumen ammonia nitrogen concentration and acetate to propionate molar ratio on high concentrate diets are consistent with other studies (Stokes and Bull, 1986; Hristove et al., 2001; Ueda et al., 2003).

Decreases in the F:C ratio and SFO supplements lowered ($P < 0.05$) ruminal CH_4 emissions by 13.5 and 22.2%, respectively (Table 3). Corresponding decreases in CH_4 emissions per kg OM apparently digested in the rumen of 34.3 ($P < 0.01$) and 14.8% ($P = 0.06$) and per kg milk were 18.8 ($P < 0.01$) and 17.2% ($P < 0.05$), respectively. The changes in ruminal CH_4 emissions due to low F:C in the diet was associated with alterations in rumen fermentation towards propionate at the expense of acetate (i.e. 25% decrease in acetate:propionate molar

proportion) and lower ruminal pH. These changes are consistent with less extensive digestion of fibre fractions despite greater OM digestion in the rumen. Energy losses as CH_4 was lowered by lower F:C, and decreased by SFO, with responses being greater on the H diet ($P < 0.05$ for the interaction). Ruminal daily CO_2 emissions were decreased ($P < 0.05$) by SFO supplements. Emissions of CO_2 per kg OM apparently digested in the rumen were only decreased when SFO was included in the high forage diet ($P < 0.05$ for interaction). The results of the current experiment show that the inhibitory effects of decreases in dietary F:C ratio and SFO supplements on ruminal methanogenesis are not additive.

Table 3. Effect of dietary forage to concentrate ratio and sunflower oil on ruminal methane (CH_4) and carbon dioxide (CO_2) productions in lactating dairy cows

	Treatment[1]					*P*-value[2]		
	HO	HS	LO	LS	SEM	F	S	F×S
Ruminal CH_4								
g/d	492	362	404	335	20.4	< 0.05	< 0.01	0.16
g/kg ruminal DOM[3]	73.3	58.3	44.1	41.9	3.88	< 0.01	0.06	0.15
g/kg milk	18.4	14.5	14.2	12.5	1.31	< 0.01	< 0.05	0.18
% of GE intake	7.20	5.12	4.90	4.36	0.304	< 0.01	< 0.01	< 0.05
Ruminal CO_2								
g/d	4056	3045	3880	3671	166.9	0.25	< 0.05	0.07
g/kg ruminal DOM	604	470	417	458	30.0	< 0.05	0.20	< 0.05
g/kg milk	152	117	135	138	13.6	0.87	0.15	0.09
Yield								
Milk (kg/d)	26.7	25.7	29.7	28.9	2.50	0.12	0.60	0.97
ECM (kg/d)	29.2	24.4	26.1	25.5	2.20	0.49	0.10	0.17
Fat (g/d)	1050	1076	1195	821	94.6	0.38	< 0.05	< 0.05
Protein (g/d)	901	823	1013	1012	60.9	< 0.05	0.42	0.43
Lactose (g/d)	1161	1122	1254	1215	120.8	0.29	0.64	1.00
Concentration (%)								
Fat	3.94	4.19	4.11	2.88	0.224	< 0.05	0.07	<0.05
Protein	3.38	3.23	3.48	3.67	0.152	0.11	0.89	0.29
Lactose	4.34	4.34	4.21	4.18	0.094	0.16	0.88	0.88

[1]Refers to diets based on high (H, 65:35) or low (L, 35:65) forage to concentrate ratio supplemented with 0.0 (O) or 50 (S) g/kg sunflower oil on dry matter basis.
[2] F, effect of forage to concentrate ratio; S, effect of sunflower oil supplements; F×S, interaction of F and S
[3] DOM, organic matter digested in the rumen

Dietary treatments had no effect ($P > 0.05$) on daily milk and ECM yields (Table 3). Supplements of SFO lowered milk fat concentration and yield on the high concentrate diet ($P < 0.05$ for forage ratio × SFO interaction), but not on the high forage diet. High starch-low fibre diets containing polyunsaturated fatty acids are known to induce milk depression as a consequence of alterations in the biohydrogenation of fatty acids in the rumen (Bauman and Griinari, 2001). Even though Ayrshire cows have a higher milk fat content than Holsteins, this experiment demonstrates that under certain conditions milk fat depression can occur in cows fed high quality grass silage.

Treatments had no effect ($P > 0.05$) on milk protein concentration, whereas daily milk protein yield was greater ($P < 0.05$) for L than H diets (Table 3). Milk protein concentrations for H and L diets containing SFO were 95.6% for H and 105.5% of the corresponding diets not supplemented with SFO, respectively. Energy intake is the main nutritional factor influencing milk protein content and increases in dietary starch content are often accompanied by an increase in milk protein (Wu and Huber, 1994; Lock and Shingfield, 2004). Conversely, milk protein concentration is often depressed on diets containing lipid supplements (Wu and Huber, 1994; Van Knegsel, 2007; Garnsworthy, 1997). Different responses to SFO supplements between the L and H diets, is probably best explained by a higher availability of glucogenic compounds on the high concentrate diet (Van Knegsel, 2007).

Conclusions

Decreases in the dietary forage:concentrate ratio from 65:35 to 35:65 lowered ruminal CH_4 production. Decreases in enteric CH_4 production were accompanied by alterations in rumen fermentation towards propionate at the expense of acetate, lower ruminal pH and decreased fibre digestion in the rumen. Sunflower oil supplements also lowered ruminal CH_4 production that was explained, at least in part, by less extensive digestion of organic matter in the rumen. There was no evidence that the effects of increases in concentrate and oil supplementation on methanogenesis were additive.

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WP 2. Genetics of environmental impact traits in dairy cows

This WP focuses on understanding the genetic basis of traits related to dairy system efficiency and methane production in dairy cows so as to develop practical tools for its mitigation. The WP has three different but interlinked work tasks.

Task 1. Collection of individual production, functional and efficiency traits

The main objective of this task was to collect phenotypes to build a rare and unique database that contains not only production traits but also feed efficiency, energy balance and dairy emission traits. Such collected phenotypes are instrumental in understanding the genetic mechanisms underlying the expression of several economically important traits.

Animals, feeding and sampling

In GreenDairy data, all the cows were Nordic Red dairy cattle MOET nucleus herd cows undergoing their first lactation. From these cows all available measurements between lactation days 2 – 280 are included in the data. Because of the continuous data collection the amount of available daily measurements for cows varies between 38 and 280. The cows in Rehtijärvi were housed in tie-stall barn, whereas in Minkiö the cows were housed in freestall barn. All cows had *ad libitum* feeding. Feeding of the animals and the procedures for data collection in different trials in Rehtijärvi data are explained by Mäntysaari et al. (2003; 2004; 2005; 2012). All cows in Minkiö data were fed grass silage and home blend concentrate mix. The concentrate was given from concentrate feeding stations. The amount of concentrates depended on the stage of lactation and the digestibility of the grass silage. When the digestibility of the organic matter of silage was between 680 – 700 g/kg dry matter (DM), the concentrates were offered so that the proportion of concentrate in the diet dry matter became 52 % during lactation days 1 – 150, 47 % during days 151 – 250 and 37 % thereafter. The amount of concentrate decreased or increased 2 %-units for each 10 g /kg DM increase or decrease in digestibility of grass silage. An average the proportion of concentrate in the diet in GreenDairy data was 48.3 %.

For the feed analysis, a sample of grass silage was taken twice a week. These sub-samples were combined to give a four-week sample for analysis. The samples were analyzed for DM, ash, crude protein, NDF, volatile fatty acids, lactic acid, water-soluble carbohydrates, ammonia-N and *in vitro* organic matter digestibility. Concentrate samples were collected once a week and combined to give a six-week sample for analysis. The concentrate samples were analysed for DM, ash, crude protein, ether extract and NDF. The analyses of grass silage and concentrate samples were performed using procedures previously described by Mäntysaari et al. (2007).

Data collection and calculations

In Minkiö herd the individual milk yield and feed intake was recorded daily. However, the feed intakes were not recorded during the pasture period. Milk protein, fat, lactose contents and somatic cell count were analysed once a month on test day. The daily milk composition was calculated as a linear change between measuring days. The body weight (BW) was weighted twice a day and the daily average weight was used in modelling. To correct the daily variation in BW the daily average weights were modelled by random regression model including Wilmink function (Wilmink, 1987) and second order polynomial term. The body condition scores 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) every fourth week. To calculate daily BCSs a cubic function on time for each animal was fitted. The daily BW and BCS were predicted from the individual fitted values.

Metabolizable energy (ME) content for grass silage was based on in vitro (Nousiainen et al., 2003) organic matter digestibility. The ME concentration of the concentrate was calculated from digestible nutrients (MAFF, 1975; MAFF, 1984). The digestibility coefficients for the components of the concentrates were taken from the Finnish feed tables (MTT, 2010). In the calculation of ME intake (MEI) the correction equation based on a large data set of production experiments (MTT, 2010) was used:

$$\text{Corrected MEI (MJ/d)} = \text{Uncorrected MEI (MJ/d)} - (-56.7 + 6.99 \times \text{MEm} + 1.621 \times \text{DMI} - 0.44595 \times \text{CP} + 0.00112 \times \text{CP}^2),$$

where DMI is dry matter intake (kg/d), MEm is uncorrected ME concentration of the diet (MJ/kg DM) and CP is the crude protein concentration of the diet (g/kg DM). The gross energy intake (GEI) was calculated based on dry matter intake (DMI) and the chemical composition (Jentsch et al. 2003):

GEI = DMI x [(23.6 x CP + 39.8 x ether extract + 18.9 x NDF + 17.3 x NFC)/1000],

where the dietary concentration of CP, ether extract, NDF and non-fiber carbohydrates (NFC) are expressed in g/kg DM and coefficients in MJ /kg DM. The NFC (g/kg of DM) was calculated according to NRC (2001):

NFC= 1000 –ash – CP –ether extract - NDF.

The energy-corrected milk (ECM) was calculated according to Sjaunja et al. (1990). The energy balance (EB, MJ ME/d) was calculated for each cow using the feed intake, milk production and body weight data:

EB =energy intake (MJ ME/d) – energy required for milk and maintenance (MJ ME/d).

The ME (MJ) used for ECM and for maintenance was calculated based on the official Finnish requirements (MTT, 2010):

ME_{milk} MJ= 5.15 x ECM

$ME_{maintenance}$ MJ=$BW^{0,75}$ x 0,515

Energy efficiency was estimated with energy conversion efficiency (ECE; ECM kg/ME MJ) and by two alternative residual energy intake (REI; ME MJ/d):

ECE = ECM /MEI;

REI= energy intake (MJ ME/d) – energy required for milk, maintenance and BW gain or loss (MJ ME/d).

In REI_1 the energy used based on the official Finnish energy requirements (MTT, 2010) for milk, maintenance and BW gain or loss were subtracted from the actual total energy intake. In calculation of REI_2, the requirements were estimated from the current data; i.e. a multiple linear first order regression including daily energy output in milk, BW and piecewise regressions of BW gain and BW loss, was used to model the total energy intake, and thereafter the prediction equation residual was defined to be REI_2 for the cow and each day.

Feed intake and production

The average ECM yield for the cows in GreenDairy data was 27.8 kg/d, the total intake of DM and ME are 18.6 kg/d and 206 MJ/d, respectively (Table 1). Because all cows were in their first lactation, the milk and DM intake curves were flat, as is typical for primiparous cows (Figure 1). The development of calculated EB is presented in Figure 1. It was the lowest on the fourth week of lactation, being -25.2 ME MJ/d. Energy balance turned positive on week 11.

Table 1. Mean, standard deviation and range (min, max) of cow-wise average milk production, feed intake and body weight and condition during lactation weeks 2-40.

Item	Mean	SD	Min	Max
Milk yield, kg/d	27.0	3.70	14.3	37.6
ECM yield, kg/d	27.8	3.54	16.2	39.5
Milk composition, %				
Fat	4.22	0.377	3.20	5.68
Protein	3.52	0.191	3.01	4.06
Lactose	4.61	0.118	4.13	5.00
Intake				
Forage, kg DM/d	9.6	1.28	5.7	14.3
Concentrate, kg DM/d	9.0	1.17	4.9	12.3
Total intake, kg DM/d	18.6	2.31	10.5	26.6
Energy, ME MJ/d	205.5	23.4	123.1	284.7
Crude protein, kg/d	3.23	0.46	1.73	4.45
Body weight, kg	587	50.5	443	752
Body weight change, kg/d	0.185	0.251	-1.761	0.678
Body condition score	3.09	0.27	2.27	4.07

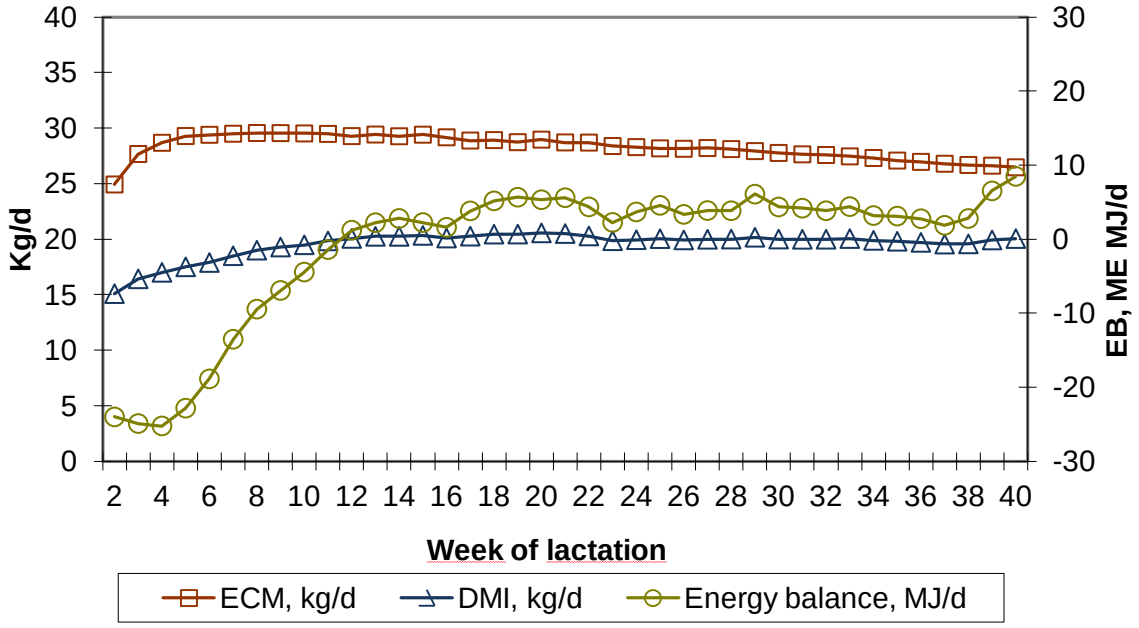

Figure 1. Daily average ECM yield (□) and DMI (Δ) and EB (○) by week of lactation of the primiparous cows in GreenDairy data.

The average BW of the cows was 587 kg varying from 443 kg to 752 kg. The cows lost BW during the first 5 weeks of the lactation, after which BW increased very slowly until week 14 and little faster thereafter (Figure 2). The total BW loss in the beginning of lactation was only modest. During the first 40 weeks the cows increased their BW by 51 kg on average. The changes in BCS agree with the changes in BW (Figure 2). At calving the BCS was 3.18, on average, reaching the nadir (2.99) in the lactation week 12.

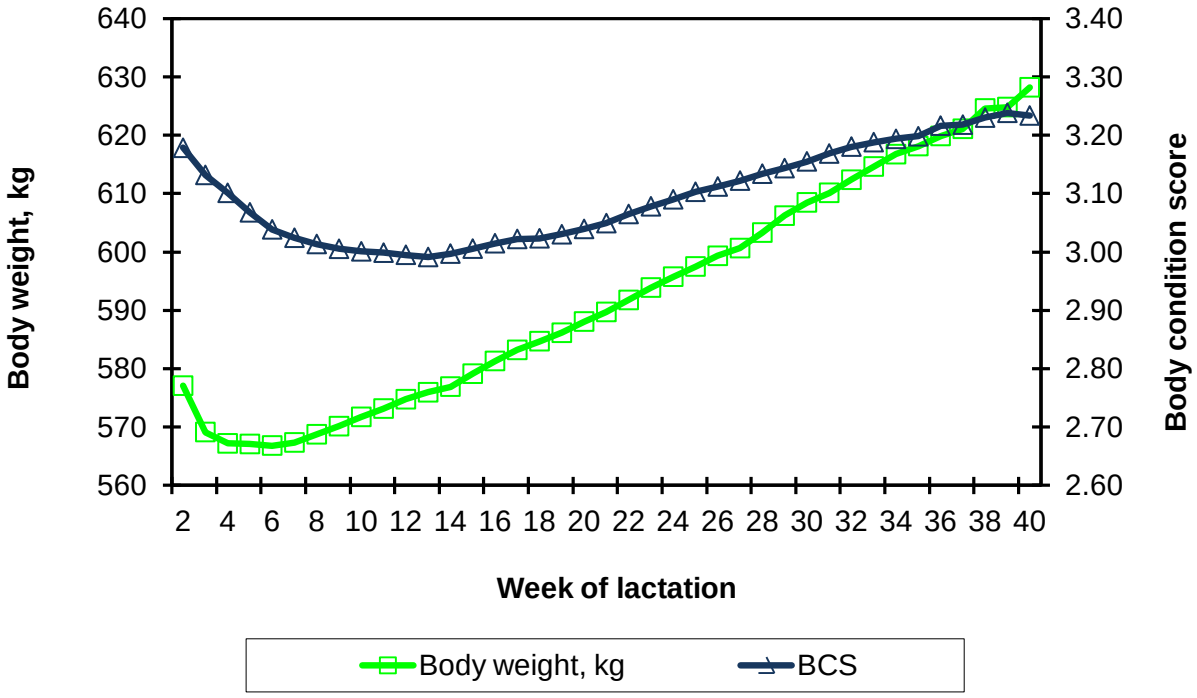

Figure 2. Mean BW (□) and BCS (Δ)(○) by week of lactation of the primiparous cows in GreenDairy data.

Energy efficiency

Energy efficiency was described either by ECE or by REI_1 calculated with partial efficiencies for milk, maintenance and growth estimated on population level, or by REI_2 calculated using the requirements derived from the current data. Thus, the two REI values denote the difference in the utilization of energy by the cows in the data relative to the population or current group mean. If the studied group of cows would represent the population well, both the REI values should be equal. In our data the average of REI_2 was, as expected, close to zero being 0.09±26.0 ME MJ/d. The residual energy intake calculated based on official energy requirements (MTT, 2010) was -9,3±27.5 ME MJ/d. The negative value of RFI_1 indicates that the cows in our data used energy more efficiently than the cows on average. It is possible that the high genetic merit cows in our data were more efficient energy user than the average cows, but it is also possible that the favorable environment and good management in research herd may have influence the efficiency. The energy conversion efficiency (mean±SD) was during the lactation weeks 2-40 an average 0.133±0,025 kg ECM/ME MJ.

The changes in energy efficiency during lactation are shown in Figure 3. The ECE varied considerably, being highest during the first weeks of lactation and decreasing slowly thereafter. Also the mean REI was lower (better efficiency) in the beginning of lactation than in the later lactation. In the case of ECE this is easily explained by the increased use of body reserves during the first weeks of lactation. In the calculation of REI the use of body reserves is taken into account. However, the differences in REI during lactation may be related to the difficulties in estimating the BW change and its composition during the first weeks after calving (Tamminga et al., 1997). It can also be speculated that there are differences in ME utilization for separate functions during lactation. The milk fat content is higher during the first weeks of lactation, which may influence the utilization of ME for the production (Chwalibog, 1991). These findings indicate that when comparing REI values of different cows, the values should either be based on the same period of lactation or in modeling a reliable estimation for the effect of stage of lactation is needed.

Table 2. Mean, standard deviation (SD), the within animal standard deviation (SE) and between animals variance as a proportion of total variance (C^2) for energy efficiency and energy balance during lactation weeks 2-40.

Item	Mean	SD	SE	C^2
ECM/ME, kg/ME MJ	0.133	0.025	0.018	0.48
REI_1, ME MJ/d[1]	-9.3	27.5	20.8	0.43
REI_2, ME MJ/d[2]	0.09	26.0	19.	0.46
Energy balance, ME MJ/d	-1.9	28.8	21.1	0.46

[1] Residual energy intake calculated with official Finnish energy requirements (MTT, 2010)

[2] Residual energy intake calculated with energy requirements from current data

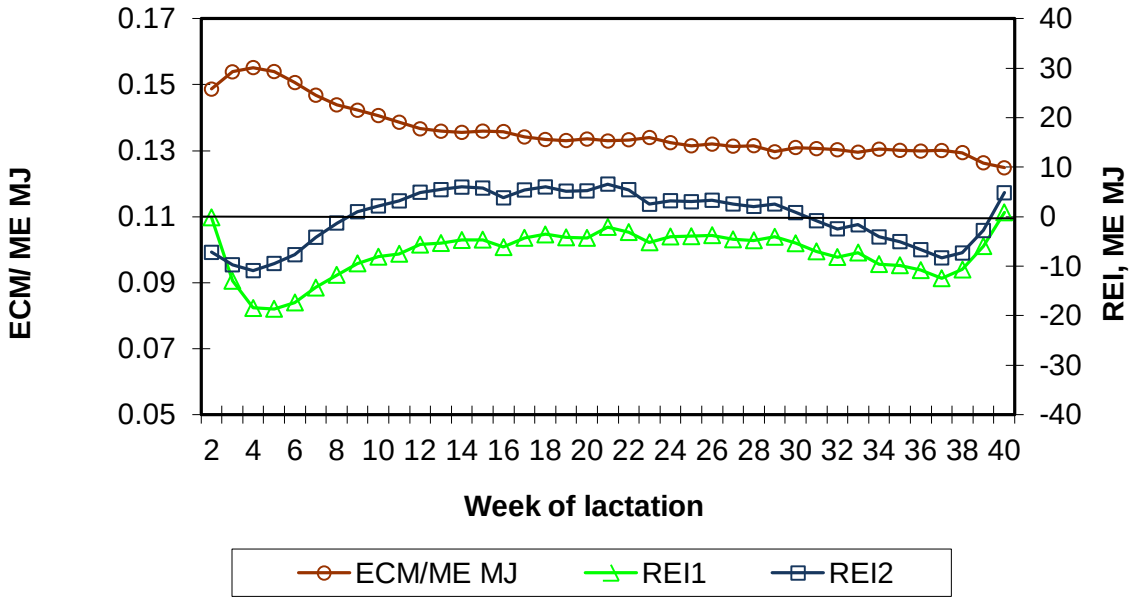

Figure 3. Mean energy conversion efficiency (○), residual energy intake based on official Finnish energy requirements (REI_1; Δ), residual energy intake based on requirements from current data (REI_2: □) by week of lactation of the primiparous cows in GreenDairy data.

Although the average efficiency measurements were very close to what was expected, there was a notable variation between the cows for each measure. The proportion of total variance due to animals (C^2) was 0.43 for REI_1, 0.46 for REI_2 and 0.48 for ECE (Table 2). In the case of ECE the variation arises partly from its inability to distinguish between the energy used for maintenance, milk and BW loss or gain. The energy needed for the maintenance is related to BW, which varied significantly in our data. Also the partitioning of energy for milk and body tissue varies between cows. The genetic selection based on ECE could therefore lead to favoring of cows with long and deep energy deficiency in the beginning of lactation. With REI the use of energy for different functions is considered and the use of body reserves is taken into account. Nonetheless, a large variation in REI was observed among the cows. Partly the variation in REI can be associated with errors in the measures of production, intake or BW, even if the current data was collected accurately in experimental conditions, but partly it reflects the true differences in the energetic efficiency between cows. In GreenDairy data the proportion of total variance due to animals for REI_2 was 0.46, which corresponds to 12 MJ/d. This is about 6 % of the average daily energy intake of the cows. Our findings indicate that there is true phenotypic between-animal variation in the energy efficiency among Nordic Red Dairy Cattle cows. Thus, phenotypic grounds for the selection on energetic efficiency exist.

The Data set

The data set built in the project included individual production, functional and efficiency traits of the primiparous cows at MTT Minkiö herd collected from November 2009 till June 2013 (Minkiö data) and individual records of primiparous cows collected in Rehtijärvi herd (Rehtijärvi data) during previous projects, including measurements from 1998 till 2009, were added to the database. This compound data (=GreenDairy data) include 436 cows with 43735 daily measurements.

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Task 2. Genetics of feed efficiency traits: Residual feed intake in dairy cows

Genetic selection for more feed-efficient cows, e.g., animals with low residual feed intake (RFI), would offer a feasible and long-term means of reducing feed costs and enteric methane emissions of dairy cattle. Understanding its genetic basis is therefore essential in developing genetic tools for its improvement. The main aim of this task was to estimate genetic parameters for feed efficiency traits and quantify its associations with other production and functional traits.

The genetic analyses of the feed efficiency traits were on data from ASMO cows kept at MTT Rehtijärvi and Minkiö herds. It included records on feed intake, milk production and body measurements. The final data set used in the analyses had a total of 10 374 observations from lactation 2 - 30 weeks from a total of 400 cows which were descendants of 114 sires. The feed efficiency traits studied included residual energy intake, and energy conversion efficiency. Residual energy intake was defined as the difference between total energy intake of each animal, and the energy required for milk, maintenance and body weight change. Residual energy intake was expressed as weekly averages in ME MJ/d. Energy conversion efficiency was defined as the ratio of weekly average ECM yield to the weekly average ME intake in MJ. The other traits included in the analyses were weekly averages of energy corrected milk yield kg/d, dry matter intake kg/d, body weight kg, body condition score (on the scale of 1 lowest to 5 highest), and energy balance ME MJ/d. Detailed description of the data used is given in Table 1.

Table 1**.** Descriptive statistics of the studied traits in the final GreenDairy data set

Trait	**Unit**	**No. animals**	**No. obs**	**Mean**	**SD**	**Min**	**Max**
Energy corrected milk	kg/d	400	10 363	28.06	4.34	6.43	45.62
Dry matter intake	kg/d	400	7 828	18.61	3.22	6.43	32.18
Live weight	kg	400	10 343	579.10	51.24	421.48	770.21
Body condition score	Scale 1-5[§]	400	10 320	3.05	0.30	1.75	7.72
Energy balance	MJ ME/kg	400	7 801	-3.77	30.70	-115.70	136.60
Residual energy intake	MJ ME/kg	400	7 762	0.01	26.73	-111.20	128.20
Energy conversion efficiency	kg/MJ ME	400	7 821	0.14	0.03	0.05	0.32

[§] Scale of 1 thinnest to 5 fattest

The data was analyzed by fitting various genetic models including fixed and random regression models as well as a multitrait model. In the fixed regression model the data were treated as repeated observations over the whole 2-30 week period. In random regression models the data from each lactation week was treated as a separate observation as before, and in the multitrait models lactation weeks were first grouped and averaged in lactation months (wk 2-4, 5-8, 9-12 etc.), and consequently each month was treated as a separate trait in the analyses.

Results from the fixed regression model were better for traits which had more or less linear heritability estimates over the studied lactation period, but yielded low heritability estimates for energy traits (Table 2). This was probably due to the parabolic heritability estimate patterns over the studied lactation periods, which were confirmed by both random regression and multitrait models (Figure 1). The standard errors from the multitrait models were high due to the small number of observations in each month class. Consequently, the genetic correlations between the traits were only estimated with fixed and random regression models.

Table 2. Estimates of genetic parameters over the whole period of lactation weeks 2 – 30 for energy corrected milk production (ECM), dry matter intake (DMI), live weight (LW), body condition score (BCS), energy balance (EB), residual energy intake (REI) and energy conversion efficiency (ECE) in Nordic Red dairy heifers (fixed regression model) †

Traits	ECM	DMI	BW	BCS	EB	REI	ECE
ECM	**0.38±0.06**	0.71±0.06	0.01±0.04	-0.01±0.05	-0.32±0.11	**0.86±0.06**	0.60±0.09
DMI		**0.27±0.04**	0.68±0.08	0.34±0.07	0.43±0.15	0.70±0.10	-0.12±0.17
BW			**0.60±0.06**	0.59±0.04	0.87±0.10	0.23±0.25	-0.76±0.08
BCS				**0.39±0.01**	0.42±0.13	0.16±0.14	-0.39±0.11
EB					**0.08±0.03**	-0.24±0.28	-0.94±0.02
REI						**0.09±0.02**	0.44±0.26
ECE							**0.16±0.04**

†Heritabilities (±s.e.) on the diagonal and genetic correlations above the diagonal

The heritability estimates for residual energy intake and energy conversion efficiency were low from fixed regression models (0.09±0.02 and 0.16±0.04, respectively, Table 2), while from random regression and multitrait models they were similar to those obtained earlier with RR models from smaller data sets. From RR and MT models the heritability estimates were moderate in the beginning of lactation (0.20 - 0.4), dropped close to zero after lactation week 10, and started to rise again towards mid- to late lactation (up to 0.2 - 0.3) (Figure 1). The heritability estimates of other studied traits were similar to those obtained earlier with smaller data sets (estimates from FR models in Table 2 and RR models in Figure 2).

Figure 1. Heritability estimates for residual energy intake and energy conversion efficiency from random regression models (lactation weeks 2-30) and multitrait models (each lactation month treated as a separate trait).

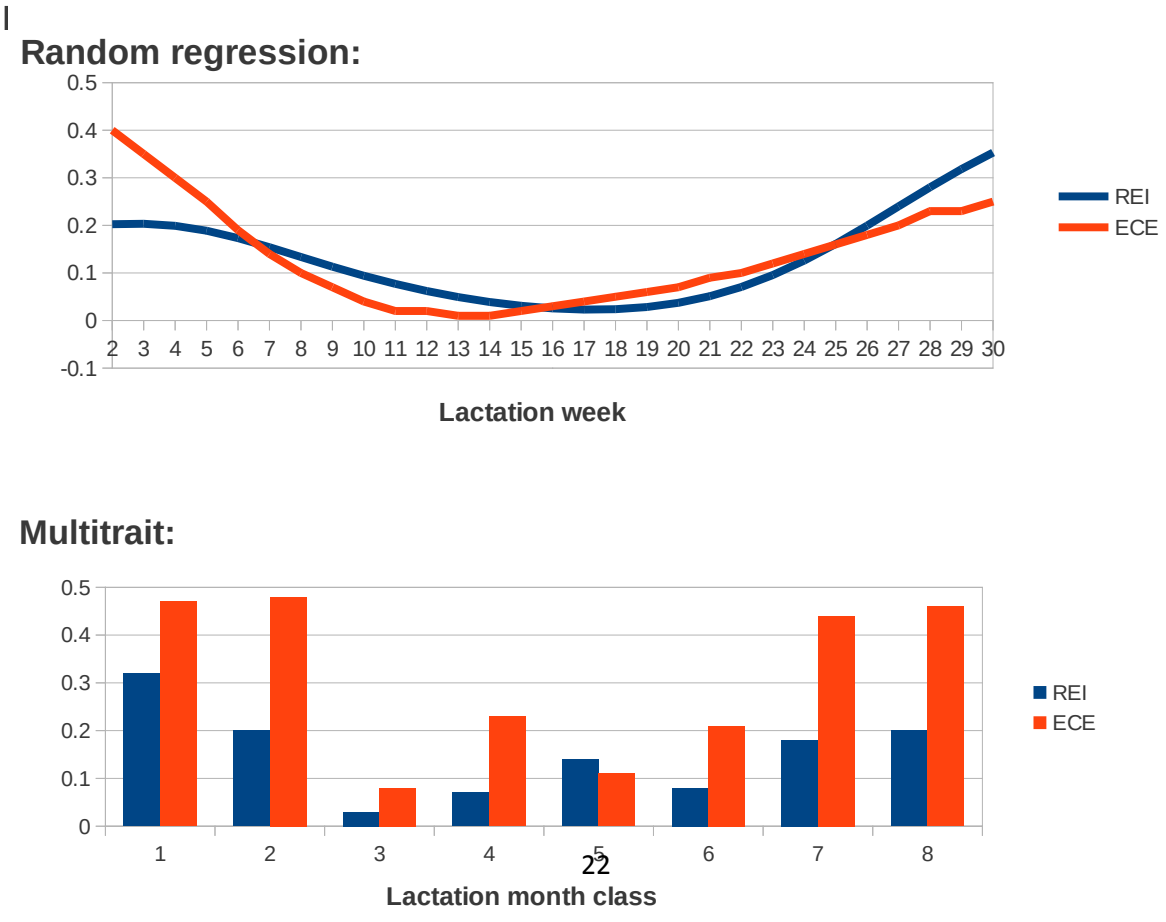
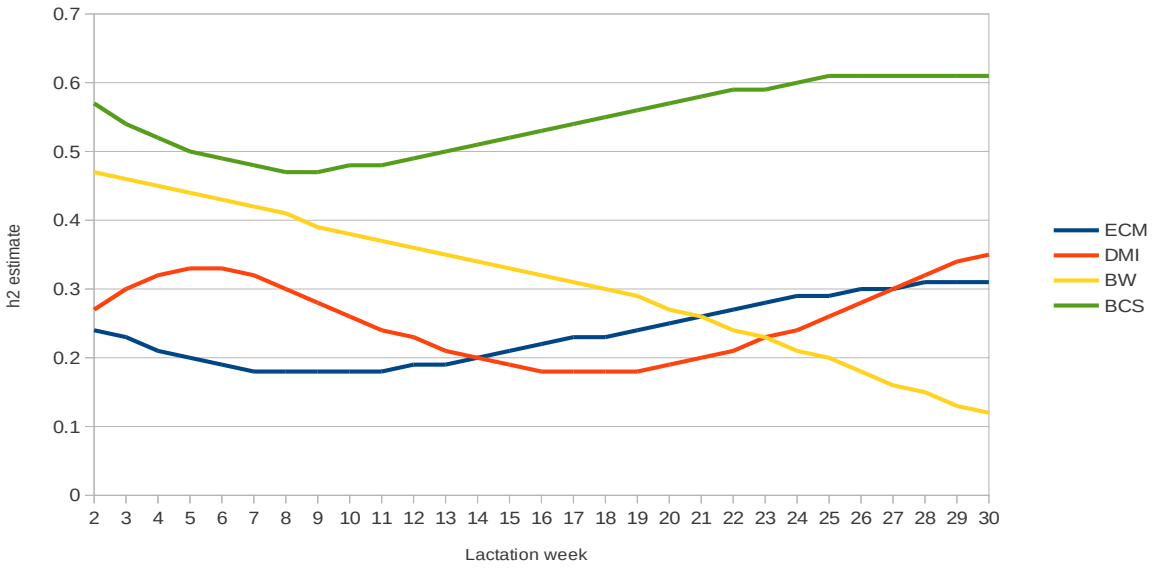


Figure 2. Heritability estimates for energy corrected milk (ECM), dry matter intake (DMI), body weight (BW) and body condition score (BCS) over lactation weeks 2-30 from the random regression model.



Genetic correlations of residual energy intake and energy conversion efficiency were strong and positive with energy corrected milk estimated with fixed regression model (Table 2). Residual energy intake was also positively correlated with dry matter intake, while energy conversion efficiency had strong negative correlations with body weight and energy balance.

From RR models, the genetic correlations of energy balance and residual energy intake with energy corrected milk yield were negative in the beginning of the lactation, but changed into moderate and positive later on (Figure 3). The genetic correlations between energy balance and residual energy intake were high and positive as well as those of the two traits with dry matter intake, and moderate and positive with body weight and body condition score (Figures 3 and 4). Energy conversion efficiency was correlated moderate to strong and positively with milk yield and had moderate to low negative genetic correlations with dry matter intake, body weight and body condition score as could be expected from its component traits (Figures 3 and 4).

Modeling energy efficiency traits is difficult especially in the beginning of the lactation when there is fast changes occurring in the milk production and energy balance status of the cows. Modeling would benefit especially from more detailed information on the body composition changes, which could not be modeled sufficiently with the information on body composition score available in this data. However, the results could give some suggestion that in the early and mid-lactation periods residual energy intake and energy conversion efficiency describe partially different physiological traits with differing genetic mechanisms acting behind them (Figure 5). The differences observed in the genetic correlations of the traits measured at different time periods might be at least partly due to the strong influence of reproduction on the cow performance in the beginning and the end of lactation with the current calf and the growing new foetus, respectively. In addition, the GreenDairy cows were measured in their first lactation so they also continue their own growth during the data collection period. In general, these results highlight the need to carefully consider stage of lactation when estimating genetic correlations among traits related to feed intake and efficiency.

Figure 3. Genetic correlations of energy balance, residual energy intake and energy conversion efficiency with energy corrected milk and dry matter intake from random regression models.

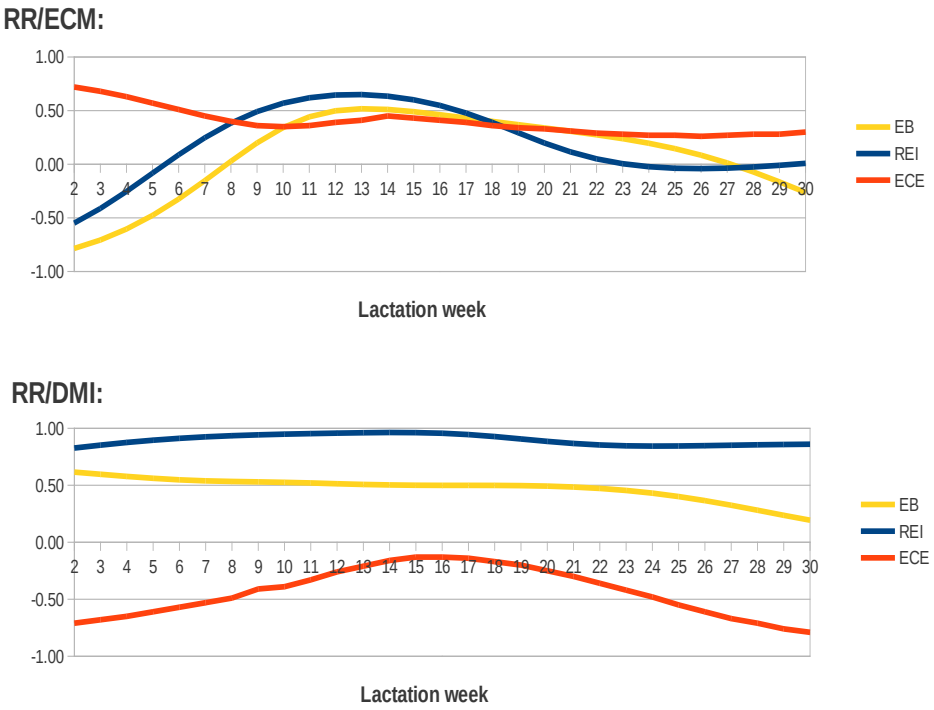
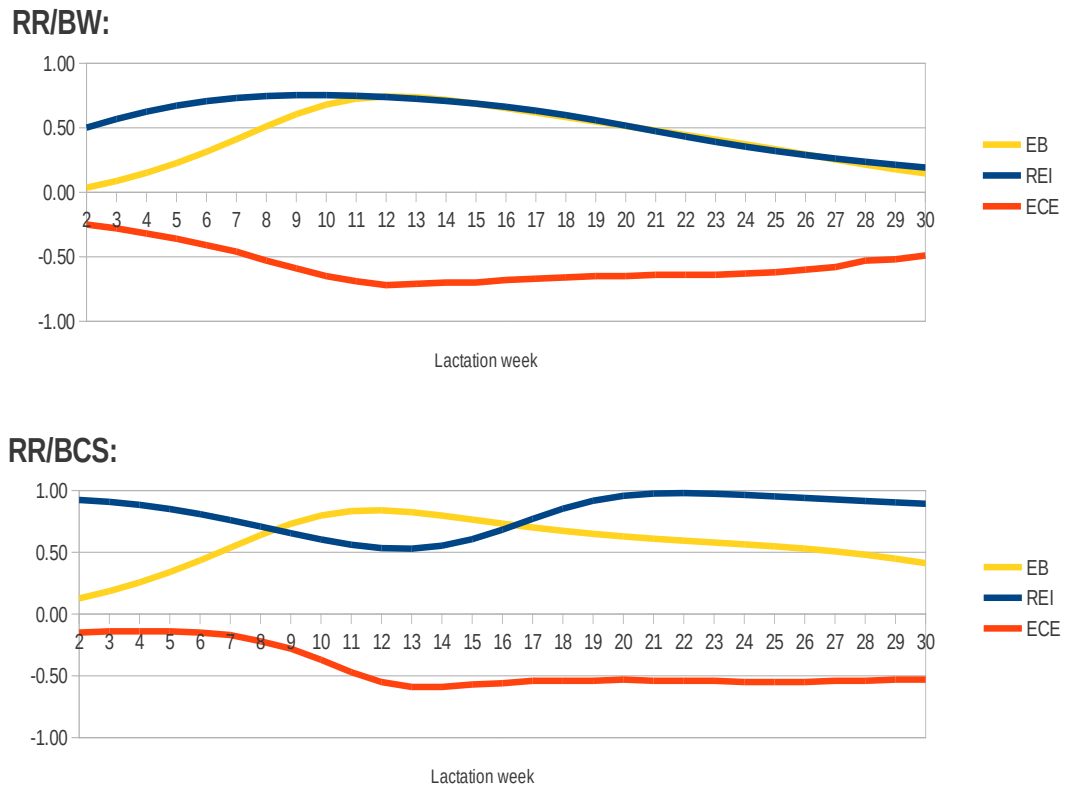


Figure 4. Genetic correlations of energy balance, residual energy intake and energy conversion efficiency with body weight and body condition score from random regression models.



The definition of feed efficiency traits for dairy cows remains still problematic on the whole. Residual energy intake is genetically similar to energy balance despite of being uncorrelated with milk yield and body weight on phenotypic level. As a consequence its genetic correlations with energy balance status, energy corrected milk yield and body weight are at least partly unfavorable with respect of the biologically meaningful dairy cattle breeding goals. On the other hand, energy conversion efficiency is easy to understand as a concept, but as it is strongly correlated genetically to its component traits it is not clear if it actually brings any new information.

The general problem with the currently defined energy efficiency measures is that they all rely to some extent on prediction of energy requirements from pre-defined feeding norms, and thus it is important to search for direct biologically relevant indicator traits for feed utilization efficiency in dairy cows in the future.

Summary

This part of the study focused on better understanding the genetic basis of feed efficiency in dairy cows. The genetic analyses were done with data obtained from 400 first lactation ASMO cows at MTT Rehtijärvi and Minkiö herds between 1998 and 2012, resulting in a total of 10 374 observations from lactation weeks 2 - 30. The studied feed efficiency traits included residual energy intake and energy conversion efficiency, and in addition traits related to milk production and body measurements. Various genetic models were tested on the data including fixed and random regression models as well as a multitrait model.

The heritability estimates and genetic correlations obtained in the study suggest that the feed efficiency traits are moderately heritable, but have partially different physiological backgrounds and differing genetic mechanisms in different lactation stages. Thus, applying either feed efficiency trait in practical breeding selection is not straightforward. Modeling energy efficiency especially in the beginning of the lactation would benefit from more detailed information on the body composition changes.

The definition of feed efficiency for dairy cows remains problematic, as both residual energy intake and energy conversion efficiency rely to some extent on prediction of cow energy requirements from pre-defined feeding norms. Thus, in the future it would be profitable to search for directly biologically relevant indicator traits for feed utilization efficiency in dairy cows.

Task 3. Building capacity for large scale individual cow methane measurements to elucidate the genetic basis of methane emissions from dairy systems

Background

Any attempt to reduce the ecological foot print of milk production via selection requires a sound understanding of the genetic basis of methane emission. However, the lack of fast and reliable techniques for the measurement of methane output from large number of individual cows has been a hindrance to this. So far, many of the available measurement techniques are either slow, expensive, labour intensive and are unsuitable for large scale measurements which is a prerequisite for genetic studies. The objective of this task was to test and develop a simple, fast and non-invasive system for the measurement of methane output from individual cows on a large scale.

Methane measurement equipment

The need for rapid and reliable monitoring of Greenhouse gasses, air pollutants and hazardous gases is constantly growing and the use of ***photoacoustic spectroscopy*** provides an efficient technology for gasseous measurement and monitoring purposes (GASERA, 2010manual). For this, F10 equipment that is Multi-gas Analyzer based on the principle of Photoacoustic Infrared Spectroscopy (PAS) was procured from the Finnish company GASERA (GASERA Ltd, Turku, Finland).

Photoacoustic principle and advantages of PAS equipment

F10 is based on the principle of the photoacoustic spectroscopy (PAS) with high sensitivity in gas detection. It utilizes Gasera's novel optical cantilever microphone, which allows below ppb limits of detection to be reached particularly when combined with laser light sources. In PAS the absorption is measured directly, not relative to the background as in other infrared absorption techniques. This means that it is a zero-background technology and the zero-point stability of the system is extremely good. Furthermore, the response of the optical cantilever microphone is extremely stable. This means that very low amount of drift occurs and calibration interval is very long. Dynamic measurement range of over 100 000 times the detection limit is possible even with only one point span calibration. This allows simultaneous analysis of very high and low concentrations without any range adjustments.

Most important advantages of F10 include very low sample volume, only a few milliliters, is required to achieve similar sensitivity compared to multipath gas cells of several meters and several liters in volume in other IR techniques. This is particularly useful when only a small amount of sample gas is available for analysis, e.g. in headspace measurements. In addition, due to the short optical path length the response is highly linear over a wide dynamic range. This is advantageous in compensating for the effect of other gases in the sample gas mixture. F10 multigas analyzer is particularly useful when analyzing wet gases like the measurement of difficult gas mixtures (e.g., with high humidity) and it requires no consumables (Figure 1).

One of the typical applications of F10 is in the measurement and monitoring of Greenhouse gasses. Particularly its high sensitivity with low sample volume (only few millilitres), suitability for the measurement of difficult gas mixtures (e.g., With high humidity) and the fact that it requires no consumables (Figure 1) made it a suitable choice for use in the GreenDairy project. Therefore, this technique was developed and adapted for the measuremnt and monitoring of CH_4, CO_2, NH_3 and Acetone from the breath sample of inividual cows.

Figure 1. F10 Multi-gas analyzer (GASERA Ltd. Turku, Finland).

In this task, the main objective was to be able to measure methane output from individual cows on a large scale. For this, Madsen et al. (2010) has presented a simple, fast, reliable and cheap method to estimate the CH_4 output of animals based on CH4 and CO2 concentrations in air near the animals

combined with an estimation of the total CO2 production from information on intake of metabolizable energy or heat producing units. This requires the measurement of concentrations of CH_4 and CO_2 and their ratio (CH_4:CO_2) in the cow breath samples. By using F10, the concentration of CH4 and CO2 gasses and CH4:CO2 ratios in the breath of cows were quantified and from these the proportion of the carbon that is not metabolized to CO2, but excreted as CH4 was calculated for each individual cows as per the equation in Madsen et al. (2010).

$$CH_4\ produced\ (l/day) = a * (b - d)/(c - e)$$

Where:
a CO2 produced by the animal, l/day
b the concentration of CH4 in air mix, ppm
c the concentration of CO2 in air mix, ppm
d the concentration of CH4 in backgroud air, ppm
e the concentration of CO2 in background air, ppm

Methane measurement technique

Using the F10 multi-gas analyzer, individual cow methane, carbon dioxide, acetone, ammonia outputs were measured continuously at the MTT experimental dairy farm. In the barn the multi-point F10 gas analyzer was fitted to two feeding kiosks (sampling points). A two-point sampling method was used to measure individual cow methane (CH_4), carbon dioxide (CO_2) and acetone outputs from the breath sample of cows via sampling tubes fitted to two separate individual concentrate feeding kiosks. The feeding kiosks are visited by cows several times during the day. During each visit the breath of a cow was sampled several times and analyzed for the contents of the different gases and the ID, date, time and its measurements were recorded automatically. Repeated daily F10 measurements of the gases were used to calculate the daily mean CH_4:CO_2 ratio for each cow. The CH_4:CO_2 ratios were then used to estimate the daily methane output of cows using the method by Madsen *et al.* (2010). Methane output per day was described as total output in gram/day (CH4g) or per unit of product or intake as: CH4g/kg milk (CH4mk), CH4g/kg DM intake (CH4dm) or feed energy lost as methane as percentage of gross energy intake (CH4GE). Figure 2 shows the description of the two-point F10 multi-gas analyzer set-up and a cow in the feeding kiosk getting her concentrate supplement while the sampling and measurement of the gases are carried out.

Figure 2. A two-point F10 Multi-gas analyzer set-up in operation with a cow in feeding kiosk getting her concentrate supplement while the gas sampling and measurements are made at the MTT Minkiö dairy barn.

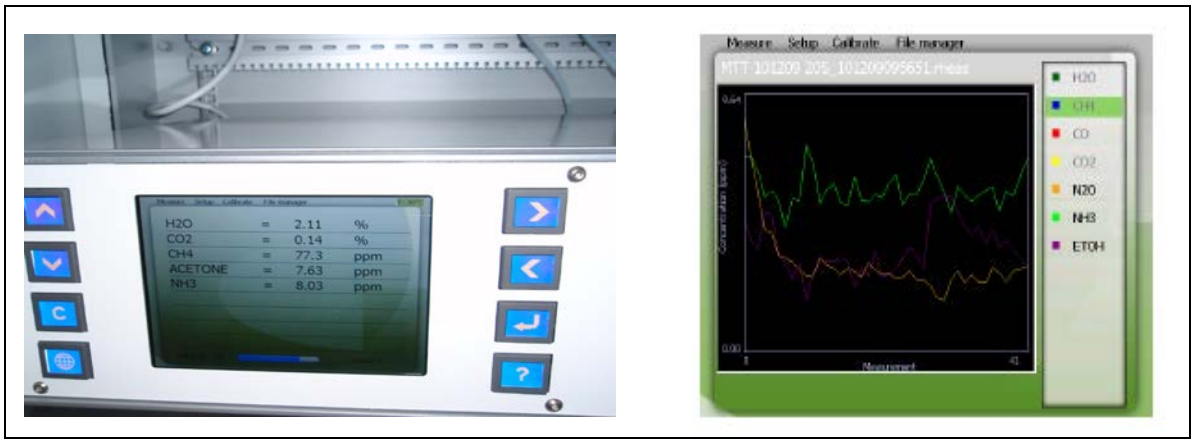


Figure 3. Description of quantitative measurements of gasses by the F10 equipment

Methane and other GHG Database

The project has built a database of methane and other greenhouse gasses since 2011 with several hundred thousand records in the database. The data will be very good resources for scientific study and policy development regarding GHG emission from agricultural systems. Table 1 shows the data table in the greenhouse gas monitoring and data collection database.

Table 1. Description of greenhouse gas data table in the database

ID	Date	Time	H2O	CO2	CH4	ACETONE	NH3
168	29/08/2011	03:45:11	16996.63	5290.255	429.9038	1.908915	1.433957
168	29/08/2011	03:47:44	17178.41	5855.193	524.7892	3.65796	0.289056
168	29/08/2011	08:14:58	18344.31	3089.106	189.6908	3.967466	1.015362
168	29/08/2011	08:17:30	18292.28	4962.747	425.2347	2.438153	0
168	29/08/2011	14:01:30	16903.32	2829.761	139.0584	6.723155	0
168	29/08/2011	14:04:03	17213.79	1595.577	48.00489	5.132166	0.38195
168	29/08/2011	14:06:35	17192.05	4135.073	302.6347	0.536232	0
168	30/08/2011	05:09:05	16747.72	4221.432	159.9475	4.097175	0.738285
168	30/08/2011	05:11:40	17128.6	3531.223	312.4493	2.628282	1.126755
168	30/08/2011	08:16:50	18089.37	5665.886	299.0479	5.141232	1.405721
168	30/08/2011	12:38:47	19214.78	3406.083	393.2244	4.545047	0.317881
168	30/08/2011	12:41:21	19207.69	3795.06	142.8746	5.452518	0.289933
168	30/08/2011	14:44:32	19208.93	3476.334	194.6148	8.848724	3.198337
168	30/08/2011	14:47:05	19076.93	4321.217	444.8734	0	2.404873

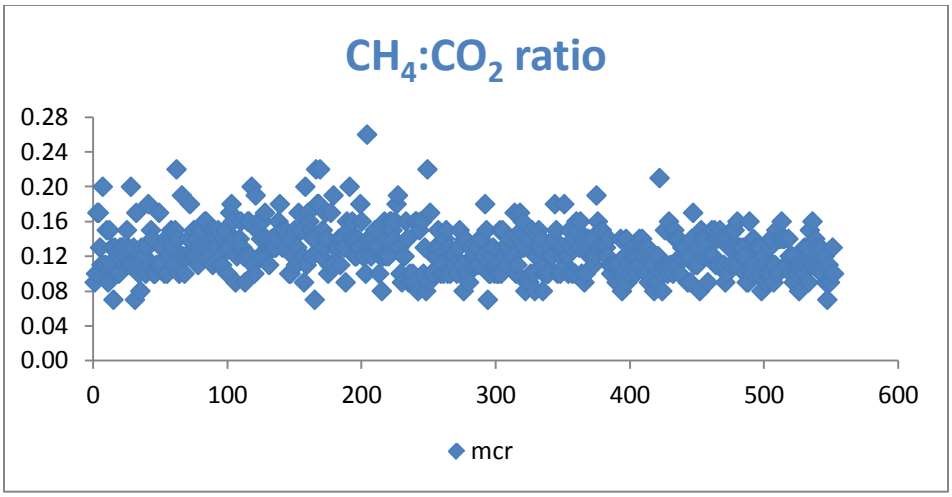


Figure 4. Methane to carbon dioxide ratio in the greenhouse gas database

WP 3. Between-animal variation in methane output and new breeding goals that include environmental impact traits.

Task 1 and 2. Quantify between-animal variations in methane output & identify environmental impact traits

Background

Understanding the genetic basis of GHG emissions from the dairy systems is essential to reduce the ecological footprint of milk production. Genetics is a powerful tool that could be used to make cumulative and long term improvements in animals and thereby lower methane emissions from the dairy systems. However, the scope of lowering methane emission form the dairy systems depends on the amount of variation that the trait exhibits and what proportion of this variation is heritable. So far there is no much information on between-animals variations in GHG emission and its heritability from the dairy systems. In addition, the most suitable environmental impact traits (methane phenotype) that are stable, repeatable and easy to record from the dairy production systems are largely not known. The main objectives of this WP were to estimate the between-animal variation in methane emissions and identify environmental impact traits that could be included in the developments of new breeding goals that include not only production traits but also environmental impact and other functional traits. In this work package two different tasks were included. The first task was directed towards quantifying the between-animal and environmental sources of variations in methane emission whilst the second task was directed at identifying environmental impact traits that would help develop new breeding goals that include environmental impact traits.

Basically the development new breeding goals require accurate estimates of genetic parameters for emission traits as well as quantifying the association with the various production and functional traits. For this the availability of large and quality data is very essential. However, because of the nature of the data collection for this trait, the data so far collocated by the project was only from 107 cows. **The data so far collected is too small for any meaningful variance component estimation to address all of the planned activities for task2. Therefore our report covers task 1 activity and part of task2 activities. Some parts of task 2 activities will be addressed in the newly started feed efficiency project when enough data are collected which allows reasonable estimation of genetic parameters.**

Between-animal and environmental sources of variations in methane emission from the dairy systems

Efforts to reduce the ecological foot print of milk production require a sound understanding of the sources of variation in enteric methane output of dairy cows. Particularly understanding the between and within-individual variations in methane output using a technique that is suitable for large scale measurement of methane is an essential requisite for developing genetic tools for its mitigation. To address this, data collected during the last two years of the project was used.

Data. The data was from first-lactation Nordic Red cows of the MTT Agrifood Research Finland experimental dairy herd. The dataset contained records on milk yield, dry matter (DM) intake, body weight and other production and energy efficiency and energy balance variables from a total of 429 cows from 1998 onwards and of which about 107 cows had part to whole lactation measurements of daily methane output. The cows were managed similarly and were fed on a similar diet that is composed of grass silage and concentrate as indicated in wp2 task 1.

Methane measurement. The methane output of each individual cow was monitored continuously in the barn using F10 multi-gas analyzer equipment (GASERA Ltd. Turku, Finland). A two-point sampling method was used to measure individual cow methane (CH_4), carbon dioxide (CO_2) and acetone outputs from the breath sample of cows via sampling tubes fitted to two separate individual concentrate feeding kiosks as described in WP2 task3. Repeated daily F10 measurements of the gases were used to calculate the daily mean CH_4:CO_2 ratio for each cow and the resulting CH_4:CO_2 ratios were then used to estimate the daily methane output of cows using the method by Madsen *et al.* (2010).

The daily methane output of a cow was described as total output in gram/day (CH4g) or per unit of a product or intake as: CH4g/kg milk (CH4mk), CH4g/kg DM intake (CH4dm) or as the feed energy lost as methane

as a percentage of the gross energy intake (CH4GE). Finally, for each animal, these variables were merged with the corresponding records of feed intake, production and energy efficiency (Energy Conversion Efficiency (ECE) and Residual Energy Intake(REI)) and energy balance traits for statistical analysis.

Statistical analysis. To assess the sources of variations in methane output phenotypes, the data was analyzed fitting mixed linear model. Fixed effects in the model included age, time of measurement, production year month, stages of lactation with random effects of animal and residual. The repeatability of methane output traits at different stages of lactation and associations within methane output traits as well as associations with other production and efficiency traits were estimated by fitting univariate and bivariate random regression models. The models fitted included quadratic Legendre polynomial plus the Wilmink term to model the fixed lactation curve and individual animal effects in the data. Variance estimates of the regression coefficients were then used to estimate within and between-animal variations and calculate phenotypic associations between traits, $r_{p_{(ti,tj)}}$ at day t_i and t_j and repeatability for a trait, r_{ti} as a ratio of between animal to total variation as follows:

$$r_{p_{(ti,tj)}} = \frac{\phi'_{(ti)}\mathbf{P}\phi_{(tj)}}{\sqrt{\phi'_{(ti)}\mathbf{P}\phi_{(ti)} \cdot \phi'_{(tj)}\mathbf{P}\phi_{(tj)}}}$$

$$r_{ti} = \left(\phi'_{(ti)}\mathbf{G}\phi_{(ti)}\right)/\left(\phi'_{(ti)}\mathbf{P}\phi_{(ti)}\right)$$

Where **G** is the genotypic variance plus the general environmental variance (V_G+V_{EG}) and **P** is the phenotypic variance (V_P) (Falconer, 1981). Statistical analyses were made using DMU package (Madsen and Jensen, 2010).

Results and Discussion

Sources of variation

Table 1 shows the mean and SD of the different enteric methane output traits as well as dairy cow production and energy efficiency traits. For methane phenotypes, feed intake and time of the day had highly significant (P<0.001) effects on estimated daily methane output of cows. Increase in the intake of fibrous feed such as silage increased the methane output. During the day, the time when CH_4 was measured showed high variability with three marked peaks observed at 08:00, 17:00 and 20:00 hrs. Particularly, among others, the stages of lactation were one of the main sources of variation. With the progression of lactation and with the increase in the level of DM intake corresponding increases in methane output was observed. During lactation, the highest daily methane output was observed around mid lactation when the animals start to gain weight and condition. Working on 665 Holstein-Friesian heifers, de Haas et al. (2011) also reported a similar trend for predicted methane output during lactation that followed a standard feed intake curve during lactation.

Table 1. Across lactation mean and standard deviations and correlations between enteric methane output traits CH4g (g/day), CH4mk(g/kg), CH4dm(g/kg), CH4GE (%) and feed efficiency traits REI (MJ of ME/d), ECE (kg ECM/MJ of ME) traits in Nordic Red cattle.

			Correlation with (r_p)	
			ECE	REI
Traits	Mean	SD		
Ch4g	330.0	58.0	-0.62	0.60
Ch4mk	13.5	3.8	-0.68	0.47
Ch4dm	17.0	3.7	-0.52	0.38
Ch4GE	5.70	0.9	-0.52	0.38
ECE	0.13	0.01	1.00	-0.70
REI	0.95	14.2	-0.70	1.00

[§] ECM=Energy corrected milk; ME= Metabolizable energy, MJ= Mega joule.

Associations between traits

Across lactation, correlations between methane output traits and ECE or REI ranged from (-0.52 to -0.62) and (0.38 to 0.60), respectively (Table 1). De Haas et al. (2011) also reported a correlation of 0.72 between Residual feed intake (RFI) and predicted methane output. For instance in Table 1, the high and positive correlation between CH4g and REI indicates that cows with lower REI (high FE) also tend to have lower methane output. This could be because animals with improved feed utilization efficiency (lower REI) tend to have lower DM intake and have improved feed conversion ratio and hence lower enteric CH4 emission than their high REI counterparts at a relatively similar levels of production. This is in line with the results of Nkrumah et al. (2006) who also reported similar results working on beef cattle. This suggests that selection for FE traits could be an alternative strategy for lowering methane output of cows.

In dairy cows daily feed intake and milk production vary during lactation. Methane output is a function of these variables and hence understanding the variation in these traits at different stages of lactation is important. Our estimates of within trait phenotypic correlations for CH4g varied during lactation. Correlations between DIM 30 and 60, between DIM 30 and 150 were 0.62 and 0.10, respectively whilst that between DIM 30 and 240 was 0.35. These correlations of less than unity indicate CH4g in early lactation is different than in mid lactation and also different than CH4g in late lactation underscoring the need for daily or frequent monitoring and recording. On the other hand, across-traits phenotypic correlations between CH4g-REI and CH4mk-REI during lactation are in Table 2. Here the associations between the FE and methane phenotypes were relatively lower in early lactation than mid to late lactation. The reason for this may be related to the lower feed intake in early lactation and hence relatively lower methane output despite the relatively more milk production due partly to body reserve mobilization. However, the correlation started to rise after peak lactation at a time when feed intake also begins to rise slightly and cows start to gain weight and condition. This trend follows the DM intake curve during lactation which rises shortly after the beginning and increases towards peak lactation until it starts to decline gradually in late lactation. A similar trend of intake has been reported by Mäntysaari et al. (2012) for Nordic Red cows.

Table 2. Across lactation phenotypic correlation between CH4g and REI (below diagonal) and between CH4mk and REI (above diagonal) for selected days in milk.

	Days in Milk					
DIM	30	60	90	120	180	240[&]
30		0.14	0.14	0.12	0.05	0.01
60	0.29		0.13	0.18	0.17	0.05
90	0.26	0.41		0.21	0.24	0.09
120	0.21	0.42	0.52		0.28	0.13
180	0.14	0.38	0.50	0.56		0.22
[‡]240	0.10	0.19	0.28	0.35	0.51	

[&]Above diagonal is phenotypic correlation between CH4mk and REI at selected days in milk, [‡]Below diagonal is phenotypic correlation between CH4g and REI at selected days in milk.

Between-animal variation in methane output traits

Marked variability was observed in the size of between-animal variation of the different methane output phenotypes during lactation (Table 3). Repeatability, as a ratio of between-animal to total variation indicates the potentially available animal variation which will predict the scope of improvement in these traits via selection. Our estimates of repeatability for the three different methane phenotypes are in Table 3. The result shows that between-animal variations for these traits are higher in early lactation and moderate in mid and started to rise again towards late lactation. Of all the three methane phenotypes, the repeatability was higher for CH4GE ranging from 0.2 to 0.74 during lactation. Repeatabilities of these traits from dairy cows and across the different stages of lactation are rarely reported.

Table 3. Daily repeatability (between-animal variation /total variation) estimates of methane output traits for selected days in milk.

	Days in Milk				
Traits	30	60	120	180	240
CH4g	0.65	0.39	0.35	0.27	0.38
CH4mk	0.73	0.28	0.11	0.08	0.04
CH4GE	0.74	0.40	0.30	0.19	0.30

Conclusion

Available between-animal genetic variation determines the scope of lowering methane output via selection strategies. For the three different methane phenotypes, our estimates of repeatability ranged from 0.2 to 0.7 during lactation. This indicates that there is potential genetic variation suggesting selection for lower methane output should be considered as one mitigation strategy.

Relationships between FE and methane output traits varied during lactation. The analysis of divergent FE groups showed that the high FE group had relatively lower feed intake and hence lower daily methane output at relatively similar production level. They also have lower fraction of energy lost as methane per kg DM suggesting underlying innate differences in methane output between the divergent FE groups. Our results indicate a potential of selection for feed efficiency traits as an alternative to reduce the carbon foot print of milk production systems particularly when large scale measurements of methane phenotypes are difficult or impossible. However the superiority of the high FE group should be validated at different stages of lactation and the consequences of selection on energy efficiency traits on other production and functional traits needs to be validated.

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