THE GENETIC AND GENOMIC BACKGROUND OF METHANE EMISSION AND FEED EFFICIENCY IN DAIRY CATTLE

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List of abbreviations

£PLI	Profitable Lifetime Index
AIC	Akaike's Information Criterion
A-matrix	Genetic relationship matrix
BIC	Bayesian Information Criterion
CH_4	Methane
CO_2	Carbon-dioxide
Cov	Covariance
DMI	Dry Matter Intake
EBVs	Estimated Breeding Values
ES	Spain
FPCM	Fat and Protein Corrected Milk
FTIR	Fourier Transform Infrared
GHG	Greenhouse Gas
G-matrix	Genomic relationship matrix
GSires	Grand Sires
GGSires	Great Grand Sires
GWP	Global Warming Potential
H_2	Hydrogen
H-matrix	Pedigree-genomic relationship matrix
IPCC	Intergovernmental Panel on Climate Change
LCA	Life Cycle Assessment
LMD	Laser Methane Detector
LWT	Live Weight
MY	Milk Yield
NL	Netherlands
PCA	Principal Component Analysis
PME	Predicted Methane Emission
pnt	point breeding value

REML	Restricted Maximum Likelihood
RRM	Random Regression Model
s.e.	standard error
SF_6	Sulfur Hexafluoride
SNP	Single Nucleotide Polymorphism
st. dev.	Standard Deviation
UK	United Kingdom
WOL	Week of Lactation

Abstract

One of the biggest challenges facing modern society is climate change. Greenhouse gases, including methane (CH_4), play an important role in global warming. Dairy cattle produce 20% of global livestock sector emissions and reducing their impact will have an important influence on total CH_4 production. Few genetic parameters for enteric CH_4 production are currently available. This study obtained parameters for CH_4 production and milk yield (MY), life weight (LWT) and dry matter intake (DMI) for three research populations. Heritabilities, repeatabilities, genetic, and phenotypic correlations were obtained using restricted maximum likelihood models. Selection index theory was used to investigate the effect of including CH_4 production in national breeding goals of three European countries, under different scenarios. Life cycle assessment was performed to investigate change in CH_4 production for the whole sector when CH_4 related traits (calving index, lactation number, LWT, feed intake and MY) increased. Heritabilities derived for CH_4 production using pedigree based analyses varied from 0.03 to 0.45. Genetic correlations derived between CH_4 production and MY, LWT and DMI varied from -0.36 to 0.57, 0.00 to 1.10 and 0.05 to 0.98 respectively. Including CH_4 production in national breeding goals showed that CH_4 production is currently increasing. Imposing a restriction or economic value on CH_4 production reduced, but did not completely eradicate, the increase in genetic gain. Life cycle assessment showed a decrease in CH_4 production lost to air when increasing CH_4 related traits, except for live weight, which caused an increase. In conclusion, it is possible to genetically select cows for lower CH_4 production. Including CH_4 production in the national breeding goal but restricting CH_4 production results in a decrease in genetic gain but does not eradicate CH_4 production. Eradication of CH_4 production through genetics was not the goal of this study. Life cycle assessment analysis showed the importance of focus on sector level rather than cow level when reducing CH_4 production within the dairy sector.

1. GENERAL INTRODUCTION

1.1 INTRODUCTION

One of the biggest challenges facing modern society is climate change. Greenhouse gases, including methane (CH_4), play an important role in global warming. CH_4 has 28 times the warming potential of CO_2 (Myhre et al., 2013). Dairy cattle produce 20% of the global livestock sector GHG emissions, of which 46.5% is enteric CH_4 (Gerber et al., 2013). Therefore, reducing CH_4 production from dairy cattle will have an important influence on the total CH_4 production in the UK.

Ruminants have a digestive system that is adapted to utilise forages (Bauchop and Mountfort, 1981). This adaptation includes processing of the food by micro-organisms in the rumen, before entering the true stomach (Czerkawski, 1986). During this process bacteria and fungi convert food into energy and amino acids for microbial protein synthesis, and volatile fatty acids (principally acetate, propionate and butyrate), which are also nutritionally valuable to the animal (Czerkawski, 1986). During this 'digestion' CO_2 and H_2 are formed as waste products, with H_2 coming from synthesis of acetate (Ungerfeld, 2013). The animal cannot utilise these waste products and accumulation of H_2 can inhibit fermentation (McAllister and Newbold, 2008; Ungerfeld, 2013) as well cause a reduction in the amount of ATP produced (Ungerfeld, 2013). Methanogens in the rumen utilise CO_2 and H_2 by converting them into CH_4 (Czerkawski, 1986; Morgavi et al., 2010), which causes an energy loss of 2-12% of the gross energy intake of the animal (Ungerfeld, 2013). Enteric CH_4 and CO_2 are then removed from the rumen through eructation (Garnsworthy et al., 2012*a*).

1.2 FACTORS INFLUENCING CH_4 PRODUCTION

1.2.1 Diet composition

Nutrients are broken down and utilised in the rumen in different ways by different micro-organisms. It therefore is not surprising that different diet compositions lead to different amounts of CH_4 production (Ungerfeld, 2013; Cameron et al., 2018). Studies have found that maize or cereal silages give a lower CH_4 production than grass silage (Beauchemin et al., 2008). Most cows are fed a mixed diet (forage and concentrates) and there are indications that the ratio between the components plays a role in the amount of CH_4 produced. When the amount of concentrate in a diet is increased at the expense of roughage, CH_4 production goes down. This is caused by concentrates lowering the amount of acetate produced in the rumen (Kim et al., 2018). A change in digestibility of the forage takes place as well (Beauchemin et al., 2008). Specific additives can also influence the amount of CH_4 produced (Martin et al., 2009; Gomaa et al., 2018; Duthie et al., 2018). The way the feed is processed influences the nutrients available, and therefore influences the amount of CH_4 produced (Martin et al., 2009).

An important characteristic of ruminants is that they can utilise forages (Garnsworthy, 2012; Bauchop and Mountfort, 1981) unsuitable for human consumption, which they convert into products suitable for human consumption (Garnsworthy, 2012). This is a desirable characteristic and needs to be preserved when breeding for lower CH_4 production. However, since diet influences CH_4 production (Beauchemin et al., 2008; Martin et al., 2009), selecting for lower CH_4 production unintentionally might put selection pressure on the ability of utilising certain feed types. This could lead to selecting cows that indeed have lower CH_4 production but are less able to digest forage diets, which is undesirable (Huhtanen et al., 2016).

1.2.2 Feed intake and feed efficiency

Feed intake has a large influence on the amount of CH_4 produced during fermentation, although this relationship is not linear. Basarab et al. (2013) describe three hypotheses explaining how this influence takes place. i) Animals with a higher feed efficiency take in lower amounts of food, while maintaining the same levels of production and body maintenance. Less feed intake leads to less CH_4 production, while production and body processes are maintained at the same level as for cows with a lower feed efficiency. ii) Level of feed intake influences the time the feed stays in the rumen, as well as rumen volume. When rumen retention time goes down, the amount of CH_4 produced will be lower. iii) High feed efficiency has underlying differences in behaviour and digestion, causing a change in response in the microbial communities. This causes a shift from acetate into propionate, which will decrease the amount of hydrogen available for the formation of methane (Basarab et al., 2013).

Oliveira et al. (2018) assessed the relationship between feed efficiency (residual feed intake) and CH_4 production but could not confirm that higher feed efficiency leads to lower CH_4 production. Other studies have shown that improving feed efficiency will lower CH_4 production in cattle (Alford et al., 2006; Hegarty and McCorkell, 2007; Basarab et al., 2013). Huhtanen et al. (2016) using a simulation study found that CH_4 production increases when rumen retention time increases.

In literature feed efficiency is defined in many ways and the definition determines interpretation of the outcome. There are two ways of looking at efficiency, qualitatively and quantitatively. From a qualitative point of view, feed efficiency is defined with suitability for human consumption in mind (Garnsworthy, 2012). From a quantitative point of view, feed efficiency is the ratio of intake and output. Berry and Crowley (2013) reviewed definitions of quantitative feed efficiency and distinguish two groups of definitions; ratio traits and residual traits.

Ratio traits are, as the term implies, ratios of underlying variables. For example, Feed Conversion Ratio (FCR) is defined as Dry Matter Intake (DMI) divided by Average Daily Gain (ADG) (Berry and Crowley, 2013). Coleman et al. (2010) use the amount of milk solids divided by kg of body weight as a definition of efficiency.

Defined as a ratio trait, feed efficiency is calculated relatively easy, given the underlying variables are known, as is interpreting and comparing the results over different populations (Berry and Crowley, 2013). However, multiple disadvantages of ratio traits are known. Basarab et al. (2013) state that the selection response for ratio traits can be unpredictable, especially when the difference in variance between the underlying traits is too large. Berry and Crowley (2013) state that due to multiple underlying variables, the error variance of the trait is likely to increase. Also, ratio traits do not take into account differences in energy distribution between cows (e.g. the energy used for maintenance might be higher in some cows) (Berry and Crowley, 2013). Pryce et al. (2014) emphasise that absolute differences between animals are lost when only the ratio between variables is used.

Residual traits are based on the difference between predicted and observed energy intake of the cow. A residual trait often used is Residual Feed Intake (RFI, generally attributed to Koch et al. (1963)). RFI is generally defined as the difference between observed and predicted intake (Koch et al., 1963). With this definition cows with a low RFI are more efficient than cows with a high RFI. Another definition of efficiency as a residual trait is Residual Solids Production (RSP), defined by Coleman et al. (2010) as the regression of milk solids yield on year of measurement, total DMI, metabolic body weight, change in body weight and body condition score.

Residual traits can be calculated based on standardised values for energy costs or with the use of a least squares approach. When using a least squares approach, RFI for dairy cattle can be defined as the total DMI regressed on

year of measurement, fat yield, protein yield, lactose yield, metabolic body weight, change in body weight and body condition score (Coleman et al., 2010). When the least squares approach is used, a more individual estimation is made. This should cause better preservation of the true differences between cows. However, correlations can occur between the residual trait and the underlying variables (in this case the energy sinks). This could again cause problems when including the residual trait in a breeding index when underlying traits are included individually as well.

When choosing a definition to use for genetic analyses, it is important to ensure that the definition covers the trait of interest. At the same time, it is important to take into account the possible undesirable side effects of selection. Variables available are a limiting factor and comparability to other studies plays an important role as well.

In the current study the final objective was to include CH_4 production and feed efficiency in the national breeding goal. Zetouni et al. (2017) has shown that the highest genetic gain in multiple traits is obtained when using multi-trait selection, rather than including a ratio or residual trait in a selection index. Therefore, taking into account the problems that arise when selecting on ratio or residual traits and the findings by Zetouni et al. (2017), it was decided to focus on DMI as the trait of interest related to feed efficiency.

1.2.3 Fertility

Fertility influences CH_4 production in two ways, at an individual level and on herd level. Fertility traits (e.g. calving interval, pregnancy rate to first service, conception rate) are unfavourably correlated with milk yield (Oltenacu and Broom, 2010), causing fertility to decrease when milk yield increases. On an individual level this means that when fertility decreases, calving interval lengthens, which causes annual milk yield of the cow to become lower (Kossaibati and Esslemont, 1995). To compensate for this, more cows are

needed to produce a given amount of milk. On a herd level this means more CH_4 production, since all cows have a portion of CH_4 production originating from maintenance (Garnsworthy, 2004). An even bigger influence comes from the number of replacement heifers that are required to compensate for the loss in fertility, again causing CH_4 production to go up at herd level (Garnsworthy, 2004). This indicates that it is important to pay close attention to fertility traits (e.g. calving interval, calving to first service, non-return rate) while selecting for lower CH_4 production.

1.3 ESTIMATING CH_4 PRODUCTION

Several studies predict CH_4 production with the use of the IPCC Tier 2 method (Dong et al., 2006). For this method, firstly population and activity (obtaining food, water and shelter) data have to be obtained. Then, based on this information emission factors are estimated based on CH_4 conversion factor and gross energy intake. For this method information on diet, live weight, production level and farm management is needed. Obtaining this information is costly, but less expensive than measuring CH_4 production using e.g. respiration chambers (De Haas et al., 2011).

De Haas et al. (2011) used the IPCC Tier 2 method to obtain PME for 548 Holstein-Friesian heifers in their first lactation. Based on these emissions, genetic parameters were obtained. However, PME in this research is a prediction based on feed intake and diet composition. This will give the same PME for animals with the same characteristics, regardless whether there is a difference in actual CH_4 production or not due to genetic differences in efficiency of feed utilisation. Therefore, the estimated heritabilities and possible related SNPs are linked to feed intake and digestion efficiency, rather than direct CH_4 production. This emphasises the need for estimated CH_4 measurements on an animal level versus predicted CH_4 production before more

reliable parameters can be obtained and associated SNPs can be identified. Therefore, there is a need for developing methods that obtain reliable estimates of CH_4 production at animal level. Different methods have been developed to measure CH_4 production from ruminants.

For sheep, measurements in respiration chambers are feasible on a large scale (Pinares-Patiño et al., 2013). However, although respiration chambers gave reliable measurements, they inhibit natural feeding behaviour (Grainger et al., 2007; Garnsworthy et al., 2012*a*) of the animals which is likely to introduce bias in the results. Therefore, Lockyer (1997) housed sheep in a polythene tunnel to get as close as possible to natural conditions. This method was non-intrusive and expected to interfere as little as possible with natural behaviour. However, the polythene tunnel used in this experiment was too small to house a mature cow and enlarging it would cause other problems, e.g. with dry matter intake.

Respiration chambers have been in use for over 120 years and different types of respiration chambers exist. Generally, a respiration chamber is an enclosed area within which incoming and exhaust air are sampled for gas analyses and the airflow through the chamber is measured. From the airflow and the difference in concentration of gasses between incoming and exhaust air the gas production of the animal (e.g. CH_4 production) is calculated (Hammond et al., 2016).

When set up properly respiration chambers are able to give an exact measure of gasses produced by the animal in the chamber. Therefore, for a long time they have been seen as the gold standard (Hill et al., 2016) for measuring CH_4 production in cattle. However, besides inhibiting natural behaviour, respiration chambers are expensive to use (De Haas et al., 2011). Alternative methods have been suggested to estimate CH_4 production in cattle, while being less restraining towards natural behaviour.

Herd level

Griffith et al. (2008) used an N_2O tracer technique and an integrated horizontal flux technique (IHF) for measurements on 23 to 50 Friesian dairy cows. For the tracer technique N_2O was released from a cylinder placed on an upwind corner of the test area. Downwind of this area a meteorological mast, equipped with gas inlets, was placed. Gases measured at this mast were analysed with the use of FTIR (Fourier Transform Infrared) spectroscopy. From these measurements CH_4 production was calculated in two different ways. For the tracer technique the concentration of CH_4 was calculated from the ratio CH_4/N_2O and the total amount of N_2O released, while for the IHF technique the concentrations were calculated based on wind speed, gas concentration profiles (height of measurement and change in concentration) and width of the cluster of cows.

Kinsman et al. (1995) measured CH_4 concentration in air on 118 cows in a tie-stall barn. In the barn a ventilation system was installed, which through negative pressure drew air in. A multipoint gas sampling system drew 24 samples from different locations of this system, which were analysed by an infrared gas analyser in a monitoring room. This method did not interfere with the daily practise on this farm and was described as reliable and efficient. Additionally, this method is non-intrusive to the animal. However, the applicability of this method will depend on the farm and ventilation system that is in place.

Both the methods of Griffith et al. (2008) and Kinsman et al. (1995) are non-intrusive and do not inhibit natural behaviour. However, CH_4 emissions were estimated on herd level rather than on animal level and therefore are unsuitable for data collection with the purpose of genetic selection on animal level.

Cow level

Grainger et al. (2007) made use of sulfur hexafluoride (SF_6) as an internal tracer to measure methane emission and compared this with measurements in respiration chambers. Sixteen Holstein-Friesian cows were used for this comparison. For this method it is necessary to place a permeation tube with SF_6 in the rumen of the cow a few months before the experiment. During the experiment the cow wears a canister around her head, sampling air she breathes out. The air is then analysed with the use of gas chromatography. Grainger et al. (2007) state that this method was successful in 88% of the measurements, while failures were due to problems with air tubes. Total CH_4 production measured with this method was in agreement with measurements from respiration chambers. However, due to the placing of a permeation tube in the rumen, this technique is quite intrusive. Also, the canister placed around the head is likely to inhibit natural behaviour to a certain degree.

Hammond et al. (2015) used a GreenFeed system to measure CH_4 emissions and compared this to respiration chambers and the SF_6 tracer method. The GreenFeed system comprises of a sampling hood in which the animal positions its head to obtain concentrates as a reward. In this sampling hood sensors are placed which measure airflow, CH_4 and CO_2 concentrations. Concentrates are only released when the position of the cows' head is right to obtain measurements. Airflow was created with the use of an extractor fan. This method measures CH_4 production at a cow level and is non-intrusive. However, due to the equipment needed it is less suitable for use on commercial farms. Since use of the measuring equipment is voluntary, it is dependent on the diet composition and amount of concentrate provided whether cows will use this system.

Lassen et al. (2012) makes use of FTIR (Fourier Transform Infrared) spectroscopy, but measures this at individual level on 93 cows during milking in the milking robot. During milking a sampling tube was pointed at a cow's

head and every 20 seconds measurements were performed. Both CH_4 and CO_2 were measured and the ratio between the two was calculated, rather than total CH_4 production. This was done to obtain a more stable phenotype, since this method is dependent on the distance between the sampling tube and the head of the animal. From this ratio and heat production units (HPU) obtained for each cow, CH_4 production in $g^{-1}d$ was derived.

This method obtains CH_4 production on cow level, is non-intrusive, does not inhibit natural behaviour, is cost effective and suitable for use on commercial farms. However, when using the ratio between CH_4 and CO_2, it is more difficult to compare results with other studies. When CH_4 production is calculated based on HPU and the ratio between CH_4 and CO_2, it should be noted that HPU is an estimate rather than a true value. This could cause bias in the true CH_4 production.

Negussie et al. (2017b) made use of PAS (photoacoustic IR spectroscopy) technique implemented in F10 portable gas analysis equipment (PAS-F10) to measure CO_2 and CH_4 gas in the cow's breath. Using CO_2 as a tracer, the produced CH_4 ($l^{-1}d$) can be quantified using the ratio between CO_2 and CH_4 and HPU derived for each cow. This method is non-intrusive and concordance correlation coefficient between this method and respiration chamber was 0.84.

Chagunda et al. (2009) developed a method to measure CH_4 production on cow level, which was non-intrusive, does not inhibit natural behaviour and at the same time is cost effective and suitable for use on commercial farms. During this study CH_4 measurements were performed with the use of a Laser Methane Detector (LMD) on 110 Holstein-Friesian cows. This method was based on infrared absorption spectroscopy and measured column density of CH_4 containing gases at a distance. The laser of the LMD was pointed at the nostrils of the cows at a distance of 3m, to not disturb cow behaviour. A good link was found between measuring CH_4 emission in a respiration chamber using the LMD and using the chamber equipment simultaneously ($r=0.8$, $P<0.001$)

(Chagunda and Yan, 2011).

Pickering et al. (2015) further investigated this method and found that repeatability (se) for this trait was low (0.07 (0.08) within lactation and 0.03 (0.08) across lactations), probably due to the size of the dataset available. Therefore, more research needs to be done on this method before it can be used for genetic analysis.

Garnsworthy et al. (2012*a*) developed a method to measure CH_4 production on cow level, which is non-intrusive, does not inhibit natural behaviour, is cost effective, suitable for use on commercial farms and non-labour-intensive. During this study, 82 Holstein-Friesians cows were housed in a free-stall barn and milked in automatic milking stations. In these stations a tube sampling air was placed in the feeding bins. Analyses of these samples were performed with an infrared CH_4 analyser. Twelve of the cows from this experiment were also used to carry out CH_4 measurements in respiration chambers. Similar results were found between CH_4 production measured in the milking robot during milking and measured in respiration chambers (R^2=0.79). It was demonstrated that differences in CH_4 production between cows are indeed related to differences in CH_4 concentration eructed and frequencies of these eructations. This justifies the use of the CH_4 measurements obtained in the milking robot during milking as a measure for differences between cows in daily CH_4 production for further genetic and genomic analysis (Garnsworthy et al., 2012*a*).

Proxies

Several studies investigated using proxies to estimate CH_4 production. Different measures can be used as proxies (e.g. milk fatty acid profiles (Van Gastelen et al., 2018), milk mid-infrared (Kandel et al., 2017), microbial information (Auffret et al., 2018)). Although some proxies show promising results, generally still issues arise with the robustness of the use of proxies. As proxies will always remain an indirect method of estimating the amount of CH_4 produced,

they should be handled with care when genetic parameters derived from proxy estimations are used for breeding purposes (Negussie et al., 2017a).

1.4 DEFINITION OF GENETIC PARAMETERS

Genetic parameters of interest are heritabilities, repeatabilities, and genetic correlations between traits. Heritability shows in which extend genes transmitted from parents determine the phenotype of their offspring. It therefore is an important indicator of whether genetic selection on a trait of interest is possible. In the current study heritability is calculated in the following way (Falconer and Mackay, 1996):

$$h^2 = \frac{V_A}{V_P}, \tag{1}$$

where V_A is the additive genetic variance and V_P is the phenotypic variance.

Repeatability shows how reliable predictions for the future are when based on performance records obtained in the past. It also sets the upper limit to heritability. In the current study repeatability is calculated in the following way (Falconer and Mackay, 1996):

$$r = \frac{V_A + V_E}{V_P}, \tag{2}$$

where V_A is the additive genetic variance, V_E is the environmental variance and V_P is the phenotypic variance.

Genetic correlations show to which extend two traits are genetically the same trait (Falconer and Mackay, 1996), e.g. are influenced by the same genes. For example, when using two different measurement techniques for CH_4 production, a genetic correlation of 1 would indicate that these two traits measure exactly the same genetic trait. Whereas a genetic correlation lower than 1 would indicate that the two traits are not fully influenced by the same genes. When

the genetic correlation between traits is high, this means that when selection pressure is put on one trait the other trait will be influenced as well. In the current study genetic correlations are calculated in the following way (Falconer and Mackay, 1996):

$$r_{\mathrm{A}} = \frac{Cov_{\mathrm{x,y}}}{\sqrt{Var_{\mathrm{x}} Var_{\mathrm{y}}}} \tag{3}$$

where $\mathrm{Cov}_{\mathrm{x,y}}$ is the genetic covariance between trait x and trait y, $\mathrm{Var}_{\mathrm{x}}$ is the genetic variance of trait x and $\mathrm{Var}_{\mathrm{x}}$ is the genetic variance of trait y.

1.5 GENETIC SELECTION FOR LOWER CH_4 PRODUCTION

When genetically selecting for lower CH_4 emission, effects are cumulative and permanent over generations (De Haas et al., 2011). First, the question whether CH_4 production is influenced by the genes of the cow needs to be addressed. It can be argued that investigating the influence of the genes of the CH_4 producing micro-organisms is a more direct, and therefore possibly more effective way of investigating reduction of CH_4 production through genetic selection. However, indications are found that the genetics of mammals have an influence on the micro-organisms present in the gut (Hegarty and McEwan, 2010). This is supported by Weimer et al. (2010), who found that rumen bacteria return to previous populations over a period of 2 to 9 weeks after exchange of rumen contents. Goopy et al. (2014) showed it was possible to select sheep for high or low CH_4 yield and found selection for low CH_4 yield caused changes in the animal's nutritional physiology.

Therefore, since genomic data are available on host animals, but not (yet) on the inhabiting micro-organisms, this is the direction this study has taken.

When using breeding to reduce CH_4 production, there are several options. Direct selection on CH_4 production is possible, as well as indirect selection through feed and production efficiency. For some traits the effect of selection

on CH_4 production has to be taken into account at farm level rather than at animal level, e.g. for fertility traits. However, in the current study the focus was on direct selection at animal level for lower CH_4 production, feed- and production efficiency and the possible trade-offs between these traits and traits currently included in national breeding goal.

1.5.1 Genetic parameters

Available parameters at the start of the current study

With the use of the Tier 2 IPCC method (developed by the intergovernmental panel on climate change, IPCC) De Haas et al. (2011) found a variation in emission of 200-400 $g^{-1}d$, indicating that genetic selection seems possible. Heritability found for PME varied from 0.29 to 0.42, depending on the period of lactation the cow was in. The genetic correlation found for PME between first and last period of lactation was low (0.36), which might indicate that PME in early and late lactation are genetically different traits. Indications were found that certain chromosome regions have an influence on PME. However, even the largest SNP effect obtained had a very small influence on the total genetic variance. This gives an indication that selection on CH_4 production is possible. As mentioned before, there are some disadvantages to the IPCC Tier 2 method and these results and their interpretation should be handled with care (De Haas et al., 2011; Pickering et al., 2015). Pinares-Patino et al. (2013) used respiration chambers for direct CH_4 measurements on 1,225 sheep and found a heritability of 0.29 for CH_4 production ($g^{-1}day$), with a moderate repeatability (0.55, 14 days between measurements). Although respiration chambers do inhibit natural behaviour, this research indicates that CH_4 production in sheep is moderately heritable. Therefore, expectations are that CH_4 production in cattle will also be moderately heritable. Pinares-Patiño et al. (2011) found a genotype by environment (diet) interaction, indicating that it is important

to take into account diet composition and feed intake when analysing CH_4 production.

Garnsworthy et al. (2012*b*) obtained variation in MERm (methane emission rate during milking) ranging from 0.57mg to 3.6mg (CH_4^{-1}min), with a mean of 2.07g (CH_4^{-1}min). Variation could be explained by week of lactation, milk yield, parity and body weight. Repeatability of this trait was high over time (Garnsworthy et al., 2012*b*) and Garnsworthy et al. (2012*a*) found repeatability to be high across diets as well. Goopy et al. (2006) found CH_4 production to be persistent over time, showing that it indeed is worthwhile to reduce CH_4 emission through breeding. No heritabilities were estimated in these studies, but a significant sire effect was found, and estimated variation indicates that genetic selection is possible. Bell et al. (2014) focused on the source of variation in enteric CH_4 production. Estimated CH_4 production was measured as described by Garnsworthy et al. (2012*a*) on 1,964 animals. CH_4 production ranged from 0.6mg^{-1}l to 4.8 mg^{-1}l averaged over farm and a major part of this variation was explained by farm and animal factors. After CH_4 production was adjusted for fixed effects (farm and week of lactation), large variation remained, again indicating a possibility for genetic selection on CH_4 production. Table 1.1 shows a comparison of genetic parameters available at the start of the current study, obtained with different techniques of CH_4 measurements.

Table 1.1: Heritabilities and genetic variation of CH_4 production summarised from literature

Study	Measuring method	Number of animals	Species (breed)	Variation found (CH_4 per animal or % coefficient of variation)	Heritability
Hammond et al. (2015)	GreenFeed	4, 4 & 12	Dairy cattle (Holstein-) Friesian)	164-198 $g^{-1}d$	-
Pickering et al. (2015)	Tier 2 IPCC (Prediction)	1,305	Dairy cattle (Holstein-Friesian)	230 $g^{-1}d$	0.13 (average, with a peak of 0.30)
Pickering et al. (2015)	Laser Methane Detector	57	Dairy cattle (Holstein-Friesian)	180 $mg^{-1}kg$	0.05
Bell et al. (2014)	During-Milking	1,964	Dairy cattle (Holstein-Friesian)	0.6-4.8 $mg^{-1}l$	-
Pinares-Patiño et al. (2013)	Respiration chamber	1,225	Sheep	12.90%	0.29
Garnsworthy et al. (2012*a*)	During-Milking	12	Dairy cattle (Holstein-) Friesian)	14.4% (MERm[1])	-

[1] MERm is CH_4 emission rate during milking (g CH_4^{-1}min)

Table 1.1: Heritabilities and genetic variation of CH_4 production summarised from literature *(Continuation)*

Study	Measuring method	Number of animals	Species (breed)	Variation found (CH_4 per animal or % coefficient of variation	Heritability
Garnsworthy et al. (2012*a*)	Respiration chambers	12	Dairy cattle (Holstein-Friesian)	27% (MERm[1])	-
Lassen et al. (2012)	FTIR in AMS, CH_4:CO_2 ratio	50	Holstein		0.34 (repeatability)
Lassen et al. (2012)	FTIR in AMS, CH_4:CO_2 ratio	43	Jersey		0.33 (repeatability)
De Haas et al. (2011)	Tier 2 IPCC (Prediction)	548	Dairy cattle (Holstein-Friesian)	200-400 $g^{-1}d$	0.29-0.42
Chagunda et al. (2009)	Laser Methane Detector	110	Dairy cattle (Holstein-Friesian)	357 $g^{-1}d$	-
Griffith et al. (2008)	SF_6 tracer	23-50	Dairy cattle (Holstein-Friesian)	351-354 $g^{-1}d$	-

[1] MERm is CH_4 emission rate during milking (g CH_4^{-1}min)

Table 1.1: Heritabilities and genetic variation of CH_4 production summarised from literature *(Continuation)*

Study	Measuring method	Number of animals	Species (breed)	Variation found (CH_4 per animal or % coefficient of variation	Heritability
Griffith et al. (2008)	Integrated horizontal flux technique	23-50	Dairy cattle (Holstein-Friesian)	317 $g^{-1}d$	-
Grainger et al. (2007)	SF_6 tracer	16	Dairy cattle (Holstein)	19.6%	-
Grainger et al. (2007)	Respiration chambers	16	Dairy cattle (Holstein)	17.8%	-
Lockyer (1997)	Polythene tunnel + gas chromatograph with Flame Ionisation Detector	8	Sheep	3.8 - 29 $g^{-1}d$	-
Kinsman et al. (1995)	Ventilation system + infrared gas analyser	118	Dairy cattle (Holstein)	436 - 721 $L^{-1}24$ h	-

Available parameters at the final stage of the current study

Coinciding with the current study, other research groups were working on obtaining genetic and genomic parameters of CH_4 production. These parameters have been summarised in Table 1.2. Heritability for CH_4 production in cattle is currently expected to be between 0.21 to 0.30. This table also shows that currently few (significant) genetic correlations between CH_4 production and other traits are reported.

Table 1.2: Genetic parameters (se) of CH_4 production from literature published after the start of the current study

Study	Measuring method	Number of animals	Cattle breed	Heritability	Repeatability	Gen corr	Unit of CH_4
Zetouni et al. (2018)	FTIR in AMS	1,397	Holstein	0.25 (0.07)	-	-0.30 (0.13) to 0.28 (0.10) with conformation traits 0.07 (0.17) to 0.28 (0.21) with fertility traits -0.32 (0.16) to 0.06 (0.19) with health traits	$l^{-1}d$
Negussie et al. (2017b)	PAS-F10,with CO_2 as tracer method	115	Nordic Red cows	-	0.41	-	$l^{-1}d$
Pszczola et al. (2017)	FTIR in AMS CH_4:CO_2 ratio	483	Holstein-Friesian	0.23 (0.12) to 0.30 (0.08)	0.17 to 0.40	-	$g^{-1}d$
Donoghue et al. (2016)	Respiration chamber	1,060	Angus	0.27 (0.07)	0.16 to 0.91	0.84 (0.06) with DMI 0.50 (0.14) with MY	$g^{-1}d$
Hayes et al. (2016)	Respiration chamber	1,020[1]	Angus	0.27 (0.06) 0.28 (0.06)	-	-	$g^{-1}d$

[1] This study uses the same dataset as Donoghue et al. (2016).

Table 1.2: Genetic parameters (se) of CH_4 production from literature published after the start of the current study *(Continuation)*

Study	Measuring method	Number of animals	Cattle breed	Heritability	Repeatability	Gen corr	Unit of CH_4
Lassen and Løvendahl (2016)	FTIR in AMS CH_4:CO_2 ratio	1,745	Holstein	0.21 (0.06)	-	0.43 (0.10) with FPCM -0.18 (0.08) with LWT	$g^{-1}d$
Lassen et al. (2016)	FTIR in AMS CH_4:CO_2 ratio	339	Holstein	0.24 (0.15) to 0.25 (0.16)	-	-	$g^{-1}d$
Manzanilla-Pech et al. (2016a)	Respiration chamber	1,020	Angus	0.30 (0.06)	-	0.83 (0.05) with DMI	$g^{-1}d$
Manzanilla-Pech et al. (2016a)	SF_6 tracer technique	205	Holstein	0.23 (0.23)	-	-	$g^{-1}d$

1.6 OBJECTIVES OF THIS STUDY

Table 1.1 shows very few genetic parameters for CH_4 production were available at the start of the current study, but these parameters are essential for genetic and genomic selection on this trait.

Therefore, the objectives of the current study were to:

1) Obtain genetic and genomic parameters for CH_4 production.

2) Obtain correlations between CH_4 production and milk yield, live weight and dry matter intake.

3) Compare genetic and genomic parameters derived from different herds and CH_4 measurement techniques.

4) Investigate the effect of including CH_4 production in selection indexes and national breeding goals.

2. ANALYSIS OF THE NOTTINGHAM HERD

2.1 INTRODUCTION

To investigate the possibility of genetically selecting dairy cattle for lower CH_4 production, the heritability of CH_4 production and correlations with production traits need to be obtained. Different definitions of CH_4 phenotypes are currently in use (Herd et al., 2013). Methane production is defined as total production in grams or litres per day. Methane intensity is defined as grams or litres of CH_4 produced per kg of product. Methane yield is defined as grams or litres of CH_4 produced per kg of dry matter intake. And residual methane production is defined as the difference between actual CH_4 production and predicted CH_4 production (Herd et al., 2014; Negussie et al., 2017b) As each definition has its own strengths and weaknesses it depends on the aim of the study which definition is used. In this study CH_4 production ($g^{-1}d$) is used, as this definition is most suitable for the use in selection indexes, as will be discussed in chapter 6.

Up until now only a few studies have looked into the heritability of CH_4 production and correlations with other traits. De Haas et al. (2011) found a heritability for predicted methane emission (PME) of 0.35 (0.12). However, as discussed in Chapter 1, this is based on a prediction of CH_4 production rather than a direct measurement. Therefore, it reflects the heritability of the traits the prediction is based on, rather than the heritability of direct CH_4 production. Pickering et al. (2015) found a heritability of 0.05 with the use of a laser methane detector, which was not significant. Lassen and Løvendahl (2016) found a heritability of 0.21 for CH_4 production ($g^{-1}d$) measured using Fourier-transform infrared spectroscopy to measure CH_4 during milking. Pszczola et al. (2017) found a heritability of 0.27 measured using the same technique as Lassen and Løvendahl (2016) These studies show that there is

variation in the heritabilities found for CH_4 production. This could be caused by the different measurement techniques used, indicating that different techniques possibly measure a different CH_4 phenotype. In this chapter measurements of CH_4 production measured in the feeding bin of the milking robot during milking were analysed (Garnsworthy et al., 2012*a*).

Few studies have reported correlations between CH_4 and MY or LWT. De Haas et al. (2011) found a correlation of -0.66 to 0.70 (estimate of significance not provided) between PME and fat and protein corrected milk, depending on the week of lactation. The average for week 1 to 42 was 0.31. Lassen and Løvendahl (2016) found a significant correlation between CH_4 production and LWT of -0.18 and a significant correlation of 0.43 between CH_4 production and fat and protein corrected milk. More insight in these correlations is needed before including CH_4 production in the national breeding goal.

Therefore, the aims of this chapter were to estimate;

1) genetic parameters for CH_4 production measured in the milking robot during milking.

2) correlations between CH_4 production, milk yield and live weight.

2.2 METHODS

2.2.1 Data

Nottingham Dairy Centre

Data were available on 184 Holstein-Friesian cows housed at the Nottingham University Dairy Centre (Sutton Bonington, UK, average milk yield 11,000 L^{-1}cow per year), housed in a free stall barn under commercial conditions. Cows were born between May 2002 and June 2008. The pedigree was limited to three generations and consisted of 372 sires and 1,297 dams (pedigree analyses were performed with the CFC software package (Sargolzaei et al., 2006)). Historically herd replacements were bred using 4 sires a year, mainly

selecting for high milk yield, milk protein and udder shape. In 2008 ninety cows were bought in and included in the herd. All cows were fed the same diet of a partial mixed ration containing mainly grass, maize and whole crop silage and rapeseed meal supplied *ad libitum*, plus concentrates (Garnsworthy et al., 2012*b*). Milking took place individually, using automatic (robotic) milking stations. On average, cows voluntarily used the robot 2.8 times per day (Garnsworthy et al., 2012*a*).

Methane measurements

CH_4 production was measured in the milking robot during milking. An air sampling tube was placed in the feeding bins within the milking robots, sampling air at a rate of $1L^{-1}$minute. These samples were analysed with an infrared CH_4 analyser (Guardian Plus; Edinburgh Instruments Ltd., Livingston, UK). Results from this method were in agreement with results from measurements using respiration chambers ($r^2 = 0.79$, $P < 0.001$) (Garnsworthy et al., 2012*a*). Measurements were taken daily from December 2009 until April 2010. On average 13 observations were available per lactation per cow (min=1, max=19), after quality control (observations more than 3 times the standard error different from the trait average were removed as these were outside the 99.73% confidence interval). In total 2,456 observations on 184 cows were available on CH_4 production (CH_4Nottingham, $g^{-1}d$, weekly average).

Production traits

After quality control (observations more than 3 times the standard error different from the trait average were removed), 4,567 observations were available for milk yield (MY, $L^{-1}d$, weekly average) and 4,570 observations for live weight (LWT, kg, weekly average). Other variables available were cow age (months), age at first calving (months), age at last calving (months), calving season (4 seasons; Spring (Mar-May), Summer (Jun-Aug), Autumn

(Sep-Nov), Winter (Dec-Feb)), pregnancy status during experiment (pregnant Y/N), calving during experiment (Y/N), concentrate intake during milking ($g^{-1}d$), lactation number (limited to $\leqslant 4$, observations higher than 4 were removed (252 observations removed)) and week of lactation (limited to $\leqslant 52$, observations higher than 52 were removed from the dataset (4 observations removed)).

2.2.2 Statistical analyses

Pedigree based analyses (Henderson, 1975) was used to estimate genetic parameters including heritabilities, repeatabilities, genetic and phenotypic correlations. The model used for the analyses is (Schaeffer, 2004);

$$y = Xb + Z_1a + Z_2p + e, \tag{4}$$

where *y* is a vector of phenotypic observations, *X* is the design matrix indicating the relation between observation *y* and the fixed effects, *b* is a vector of fixed effects, Z_1 is the design matrix indicating the relation between observation and random additive genetic effects, *a* is the vector of random additive genetic effects with *a* distributed $N(0,A\sigma^2)$, Z_2 is the design matrix indicating the relation between observation and random permanent environmental effects, *p* is the vector of random permanent environmental effects with *p* distributed $N(0,I\sigma^2)$ and *e* is the vector of random error with *e* distributed $N(0,I\sigma^2)$. ASReml 3.0 software was used (Gilmour et al., 2009) to perform the analyses. Random regression models can be used to model genetic traits that change over time as they take into account a time dependent curve for the trait (Mrode, 2005; Kirkpatrick et al., 1994; Brotherstone et al., 2000; Jamrozik and Schaeffer, 1997). As the traits of interest in this study are largely longitudinal, random regression models were explored to examine if variance components, genetic or otherwise, varied over time. The random regression model was

deployed by fitting a fixed regression function to account for changes in the given trait at a population level across time, *b* in formula 4. A random regression function was used to account for changes in a given trait at an individual animal (genetic) level across time, (*a*) in formula 4 (Jamrozik and Schaeffer, 1997). In this study the time covariate used was weeks of lactation as the traits of interest show a change in expression over lactation.

The regression functions used in this study are Legendre polynomials. The use of Legendre polynomials for random regression models was first described by Kirkpatrick et al. (1990). Legendre polynomials used as orthogonal functions are suitable to analyse patterns of change in variance components, including the estimation of genetic parameters (Kirkpatrick et al., 1990; Pool et al., 2000). Where a trait varies over time the random regression model results in a smooth curve that statistically fits to the raw data allowing for a change in the expression of trait variance over time (Kirkpatrick et al., 1990). Furthermore, they are more accurate when data extrapolation occurs and converge more easily than conventional polynomials (Kirkpatrick et al., 1990; Pool et al., 2000).

In order to fit a random regression model with week of lactation as the vector of time covariates, it is important to have sufficient data across the whole lactation for each cow.

In many studies, including the current study, data on MY and LWT are frequently available across the whole lactation whereas more difficult to record traits, such as, CH_4 production are not always available. This could cause a large difference between the number of observations for the production traits and the number of observations for CH_4 production, which could lead to correlations being influenced by the trait with the higher number of observations. To reduce this influence, observations on milk yield (MY, $L^{-1}d$, weekly average), live weight (LWT, kg, weekly average) and dry matter intake (DMI, kg/d, weekly average) were limited to 4-week intervals coinciding with

the weeks for which there was a CH_4 observation for a particular cow. For example, when there are CH_4 observations for week *n* of lactation 1 and 2 for a certain cow, all weeks 1+4*n* and 2+4*n* for this cow were included in the dataset. In practise this meant that only 27 MY and LWT observations were removed from the dataset as CH_4 production was measured daily over 5 months.

Multivariate genetic analyses

MY, LWT and CH_4 production were analysed as traits of interest. Models were started using linear univariate models for each of these traits, followed by univariate random regression models to inform the final analyses using bivariate random regression models. Univariate models started with linear (CH_4 production) or repeatability model (MY and LWT) without fixed effects, to which each of the previously listed fixed effects were added one at a time. The Wald statistic was used to determine whether an effect was significant. Only significant fixed effects ($P < 0.001$) were kept in the model. When all individually significant fixed effects were included in a full model, fixed effects that were tested before but not found significant, were tested again. If they were significant in the full model they were kept in the model. Statistics (averages and standard errors per variable class, and distribution of number of observations over variable classes) of the raw dataset were examined to evaluate whether there was any confounding between fixed effects. Confounded effects were not fitted concurrently in the final model. When multiple models were possible, models were compared based on biological meaning, published results of similar analyses, and AIC (Akaike's Information Criterion) and BIC (Bayesian Information Criterion) (Kuha, 2004). AIC is defined as two times the number of parameters in the model minus two times the natural logarithm of the log likelihood value of the model. BIC is defined as the natural logarithm of the sample size multiplied by the number of parameters in the model minus two times the natural logarithm of the loglikelihood of the model.

For all traits a Legendre polynomial was fitted on week of lactation, to account for the natural change within a trait over a lactation period. When fitted as a fixed effect, this polynomial adds a lactation curve for the whole population to the model (Meyer, 1998). The order of the Legendre polynomial used for the interaction with the fixed effect was set to 2 and was increased as long as the effect of the highest order was significant, with a maximum of order 5. For MY the fixed regression polynomial interacted with lactation number.

These univariate fixed regression models were used to construct univariate random regression models by adding an interaction between a Legendre polynomial on week of lactation and the random animal additive genetic effect. This allows for individual deviations from the population curve fitted by fitting a Legendre polynomial on week of lactation as a fixed effect (Meyer, 1998). The order of the Legendre polynomial was set to 1, which was sufficient to model the deviation of the population curve. It was not possible to fit an interaction between a Legendre polynomial fitted on week of lactation and the permanent environmental effect, due to the size of the dataset. However, as the permanent environment is not expected to change over lactation, this isn't a problem. Distribution of residuals was examined by plotting residuals against week of lactation. Residuals were uniform over time and therefore only one residual class was used. To obtain correlations between traits, bivariate random regression models were fitted based on the best univariate random regression model for each trait. Due to the size of the dataset it was not possible to run a trivariate model. Therefore, bivariate models were constructed between each combination of the three traits. Fixed effects for each trait were kept identical

between models. The following models were constructed;

$$MY = \mu + lact.WOL_leg5 + rec_wk + b1 * age + \quad (1)$$
$$WOL_leg.An + AnPE + e;$$

$$LWT = \mu + WOL_leg4 + lact + calv_seas4 + rec_wk + b1 * age + \quad (2)$$
$$WOL_leg.An + AnPE + e;$$

$$CH_4 = \mu + WOL_leg1 + rec_wk + \quad (3)$$
$$WOL_leg.An + AnPE + e;$$

Where μ is the general mean; lact.WOL_leg5 is the interaction between number of lactation and a Legendre polynomial of order 5 on week of lactation as a fixed effect; WOL_leg4 is a Legendre polynomial of order 4 on week of lactation as a fixed effect; WOL_leg1 is a Legendre polynomial of order 1 on week of lactation as a fixed effect; lact is the fixed effect of lactation number; rec_wk is the fixed effect of the week number the measurements took place; calv_seas4 is the effect of calving season (4 seasons); age is the covariate that describes the effect of age at measurement; WOL_leg.An is the interaction between a Legendre polynomial of order 1 on week of lactation and the additive genetic effect of animal as a random effect; AnPE is the permanent environment effect; and e is the random residual.

From these models genetic variance parameters for each Legendre polynomial order for each week of lactation, permanent environment and residual variance parameters were estimated with the use of restricted maximum likelihood (REML) using ASReml 3.0 software (Gilmour et al., 2009). These genetic variances were used to derive genetic variances for each week of lactation

using the method described by Kirkpatrick et al. (1990). From these genetic, permanent environment and residual variances, heritabilities, repeatabilities and correlations between traits were derived using formulae 1, 2 and 3 in Chapter I. Standard errors were manually derived as described in Gilmour et al. (2009).

2.3 RESULTS

Heritabilities and repeatabilities from bivariate genetic analyses

Heritabilities and repeatabilities obtained can be found in Table 7.16. Although heritabilities and repeatabilities for the same trait varied between models, estimates were in agreement. This showed that bivariate models were sufficient to explore these parameters.

Table 2.1: Heritabilities (s.e.) (bold), repeatabilities (s.e.) (bold, italic), genetic (s.e.) (below diagonal) and phenotypic correlations (s.e.) (above diagonal) for, and between, milk yield (MY), live weight (LWT) and CH_4 production (CH_4) obtained from bivariate random regression models for the Nottingham herd

Bivariate Nottingham		MY	LWT	CH_4	Repeatabilities
MY	LWT	**0.18 (0.27) - 0.65 (0.12)**[1]	-0.26 (0.34) - -0.11 (0.18)[2]		***0.80 (0.42) - 0.91 (0.21)***[3]
	CH_4	**0.16 (0.27) - 0.65 (0.12)**[1]		0.13 (0.34) - 0.30[4] (0.14)	***0.78 (0.42) - 0.91 (0.19)***[4]
LWT	MY	-0.23 (0.59) - -0.02 (0.17)[2]	**0.48 (0.27) - 0.67 (0.18)**[5]		***0.93 (0.46) - 0.96 (0.30)***
	CH_4		**0.40 (0.27) - 0.64 (0.17)**[6]	0.13 (0.19) - 0.20 $(0.32)_2$	***0.93 (0.47) - 0.96 (0.29)***[7]
CH_4	MY	0.38 (0.42) - 0.57 (0.52)[8]		**0.12 (0.16) - 0.45 (0.11)**[9]	***0.50 (0.23) - 0.69 (0.16)***
	LWT		0.01 (0.43) - 0.02 $(0.65)_2$	**0.12 (0.16) - 0.40 (0.11)**[10]	***0.50 (0.23) - 0.66 (0.17)***

1 Significant for week of lactation 33 to 52
2 Not significant throughout lactation
3 Significant for week of lactation 1 to 4 and 22 to 52
4 Significant for week of lactation 52
5 Significant for week of lactation 24 to 52
6 Significant for week of lactation 28 to 52
7 Significant for week of lactation 14 to 52
8 Significant for week of lactation 35 to 52
9 Significant for week of lactation 39 to 52
10 Significant for week of lactation 42 to 52

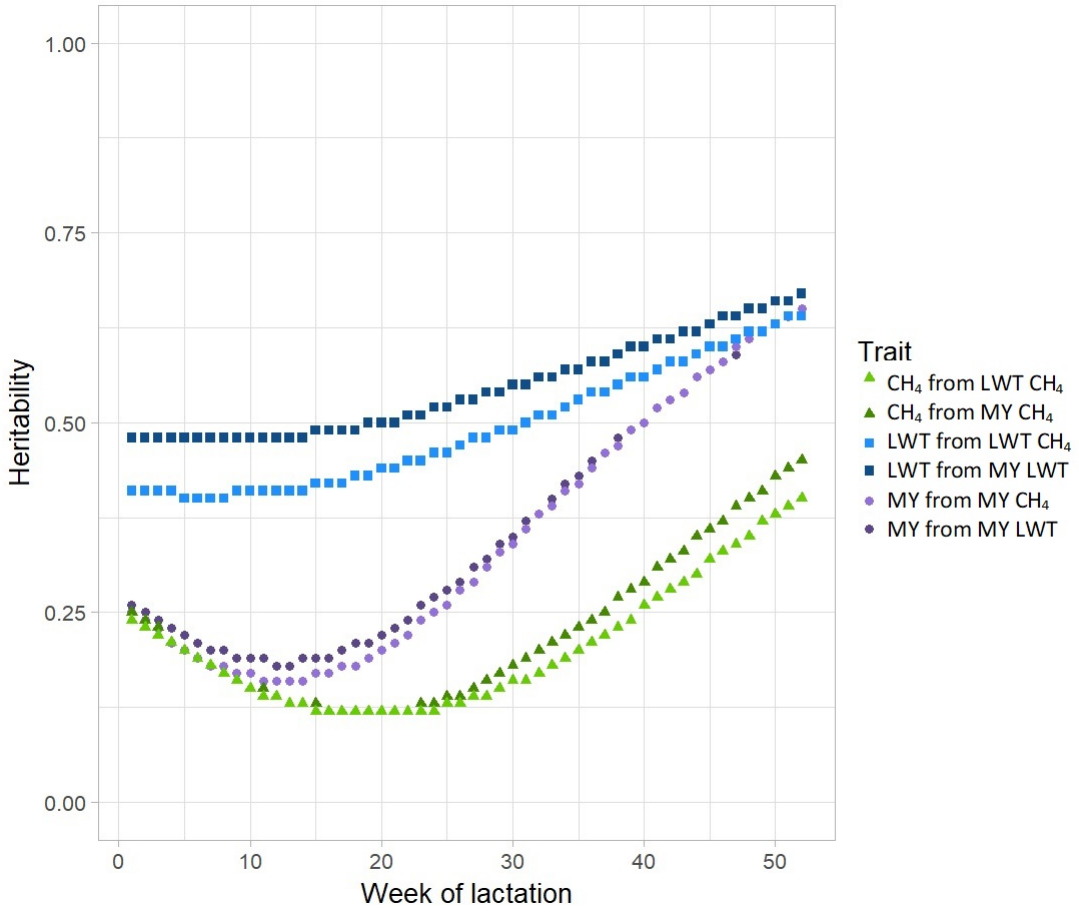


Figure 2.1: Heritabilities of milk yield (MY), live weight (LWT) and CH_4 production (CH_4) over weeks of lactation resulting from bivariate analyses for the Nottingham herd

Heritabilities (Figure 2.1) for MY were significant for weeks of lactation 33 to 52 for both bivariates. When only taking into account weeks of lactation with a significant estimate, heritability started at 0.40 in week 33 of lactation and went up to 0.65 in week 52 for the bivariate with LWT. For the bivariate with CH_4 production heritability started at 0.39 in week 33 of lactation and went up to 0.65 in week 52.

Repeatabilities for MY were significant for weeks of lactation 1 to 4 and 22 to 52 for both bivariates. For the bivariate with LWT repeatability started at 0.82 in week 1 of lactation and went down to 0.81 in week 2 to 4. For week 22 to 23 of lactation repeatability started at 0.81 and went up to 0.91

in week 50 to 51. For the bivariate with CH_4 repeatability started at 0.80 in week 1 to 2 of lactation and went down to 0.79 for week 3 to 4. For week 22 repeatability started at 0.79 and went up to 0.91 in week 52. Heritabilities for LWT were significant for weeks of lactation 24 to 52 for the bivariate with MY and significant for weeks 28 to 52 of lactation for the bivariate with CH_4 production. When only taking into account weeks of lactation with a significant estimate, heritability started at 0.52 in week 24 of lactation and went up to 0.67 in week 52 of lactation for the bivariate with MY. For the bivariate with CH_4 production heritability started at 0.48 in week 28 of lactation and went up to 0.64 in week 52 of lactation.

Repeatabilities for LWT were significant for the whole lactation for the bivariate with MY and significant for weeks 14 to 52 of lactation for the bivariate with CH_4 production. For the bivariate with MY repeatability started at 0.93 in week 1 of lactation and went up to 0.96 in week 52. For the bivariate with CH_4 production repeatability started at 0.93 in week 14 to 27 of lactation and went up to 0.96 in week 52. Heritabilities for CH_4 production were significant for weeks of lactation 39 to 52 for the bivariate with MY and significant for weeks 42 to 52 of lactation for the bivariate with LWT. When only taking into account weeks of lactation with a significant estimate, heritability started at 0.28 in week 39 of lactation and went up to 0.45 in week 52 of lactation for the bivariate with MY. For the bivariate with LWT heritability started at 0.28 in week 42 of lactation and went up to 0.40 in week 52 of lactation.

Repeatabilities for CH_4 production were significant throughout lactation for both bivariates. For the bivariate with MY repeatability started at 0.57 in week 1 of lactation, went down to 0.50 in week 14 to 24, after which it went up to 0.69 in week 52. For the bivariate with LWT repeatability started at 0.57 in week 1 of lactation, went down to 0.50 in week 15 to 25 after which it went up to 0.66 in week 52.

Phenotypic and genetic correlations from bivariate analyses

Phenotypic and genetic correlations between MY, LWT and CH_4 are in Figure 2.2.

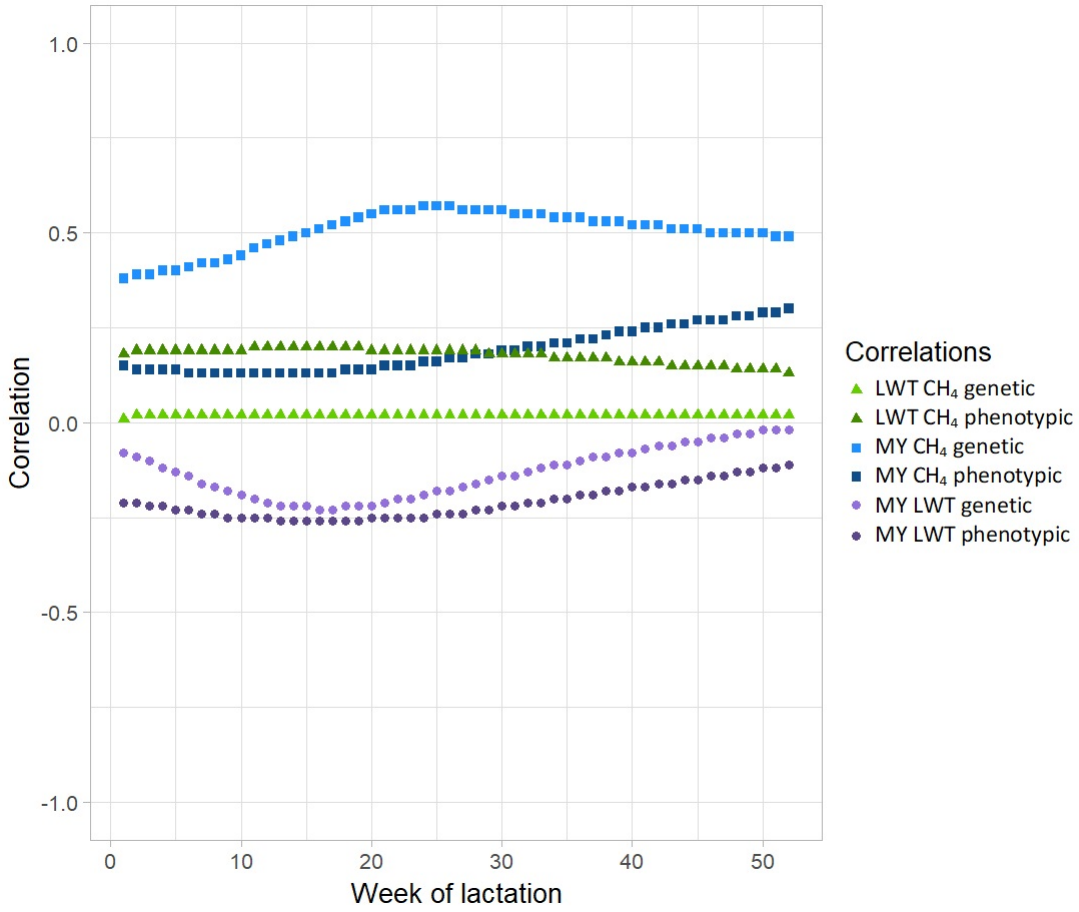


Figure 2.2: Phenotypic and genetic correlations between milk yield (MY), live weight (LWT) and CH_4 production (CH_4) for the Nottingham herd

The phenotypic correlation between MY and LWT started at -0.21 in week 1 and 2 of lactation, went down to -0.26 in week 13 to 19, after which it went up to -0.11 in week 52. The correlation was not significant at any point of lactation.

The phenotypic correlation between MY and CH_4 started at 0.15 in week 1 of lactation, went down to 0.13 in week 6 to 17, after which it went up to 0.30 in week 52. Only the correlation for week 52 is significant.

The phenotypic correlation between LWT and CH_4 started at 0.18 in week 1 of lactation, went up to 0.20 in week 11 to 19, after which it went down to

0.13 in week 52. The correlation was not significant at any point of lactation. The genetic correlation between MY and LWT started at -0.08 in week 1 of lactation, went down to -0.23 in week 16 to 17, after which it went up to -0.02 in week 50 to 52. Correlations were not significant at any point in lactation.

The genetic correlation found between MY and CH_4 started at 0.38 in week 1 of lactation, went up to 0.57 in weeks 24 to 26 after which it went up to 0.49 in week 51 and 52. Only correlations for week 35 to 52 of lactation were significant and were 0.49 to 0.54.

The genetic correlation between LWT and CH_4 production started at 0.01 in week 1 of lactation and remained 0.02 for week 2 to 52. Correlations were not significant at any point in lactation.

2.4 DISCUSSION

Heritabilities from bivariate analysis

Significant heritabilities for MY varied from 0.39 to 0.65 for both bivariate analyses. Jamrozik and Schaeffer (1997) found heritabilities for test day MY for first lactation varying from 0.40 to 0.59 for the whole lactation, with heritabilities ranging from 0.41 to 0.48 between 205 and 364 days in milk. Jakobsen et al. (2002) found a heritability for test day MY for first lactation varying from 0.19 to 0.37. Heritability found for day 285 in milk was 0.31. This shows that heritabilities found in the current study were similar, but higher than expected in late lactation.

Significant heritabilities for LWT varied from 0.48 to 0.67 for both bivariate analyses. Estimates found for the current study were similar to estimates reported with the use of random regression models. Berry et al. (2003*a*) found that genetic variance for body weight continuously increased from day 63 in milk, with the associated heritability ranging from 0.48 at day 0 in milk to 0.61 at day 294 in milk. Genetic variance found by Berry et al. (2003*a*) followed a

curve in which variance went down in early lactation, to increase a lot in later lactation. Koenen and Veerkamp (1998) found a significant heritability of 0.54 to 0.56 (0.06 to 0.10) for live weight for week 20 to 25 of lactation, which is similar to the heritability found in the current study. Hüttmann et al. (2009) found a heritability of 0.31 for live weight for day 151 to 180 of lactation, which is lower than heritability found in the current study.

Significant heritabilities for CH_4 were; 0.28 in week 39 of lactation going up to 0.45 in week 52 of lactation (bivariate with MY), and 0.28 in week 42 of lactation going up to 0.40 in week 52 of lactation (bivariate with LWT).

As described in the introduction, literature shows a range of heritabilities for CH_4 production between 0.05 (not significant) (Laser methane detector; Pickering et al. (2015)) to 0.35 (Predicted methane emission; De Haas et al. (2011)) depending on the measurement technique used. The differences in heritabilities seem partly due to the method used to measure CH_4 production. Lassen and Løvendahl (2016) used a similar measurement method as used in the current study. They found a significant heritability of 0.21 for CH_4 production ($g^{-1}d$) measured using Fourier-transform infrared spectroscopy in the feeding bin in the milking robot during milking. CH_4 production was then estimated from the ratio of CH_4 and CO_2 concentration in the breath of the cow. Lassen and Løvendahl (2016) made use of a linear animal model to obtain genetic parameters. Pszczola et al. (2017) found an average significant heritability of 0.27 measured using the same technique as Lassen and Løvendahl (2016). When looking at the whole lactation, heritability ranges from 0.23 to 0.30 obtained with the use of a random regression model. These heritabilities were close to the heritabilities found in the current study, except for late lactation where the current study shows higher heritabilities. Significant repeatabilities for CH_4 production varied from 0.50 to 0.69 for both bivariate analyses. Pszczola et al. (2017) found an average repeatability of 0.25 and Negussie et al. (2017b) found a repeatability ranging from 0.40 to 0.46 for CH_4 ($l^{-1}day$). These repeatabilities

were (much) lower than repeatabilities found in the current study. For many studies using random regression models, estimates of significance were not provided.

Table 2.2 shows a summary of study sizes of literature used for comparison above. For the Nottingham herd 4,567 observations for MY, 4,570 observations for LWT and 2,456 observations for CH_4 production were available from 184 cows. Compared to literature the number of observations on MY and LWT in the current study is low, but the number of CH_4 production observations is very reasonable taking into account the measurement period (5 months) and the number of cows available.

Table 2.2: Overview of the study sizes of literature used to compare to heritabilities found in the current study

Study	Trait	Nr obs	Nr cows	Nr herds
Jamrozik and Schaeffer (1997)	Milk yield	50,412	6,516	158
Jakobsen et al. (2002)	Milk yield	64,994	8,075	57
Berry et al. (2003*a*)	Body weight	91,937	8,499	76
Koenen and Veerkamp (1998)	Body weight	469	469	1
Hüttmann et al. (2009)	Live weight	17,716	289	1
Pickering et al. (2015)	CH_4 production	173	57	1
Lassen and Løvendahl (2016)	CH_4 production	-	1,745	20
Pszczola et al. (2017)	CH_4 production	443,533	483	2
Negussie et al. (2017b)	CH_4 production	-	115	1

Genetic correlations from bivariate analyses

The genetic correlation between MY and LWT was not significant at any point of lactation. Estimates vary from -0.08 (0.50) in week 1 of lactation, go down to -0.23 (0.58 to 0.59) in week 16 to 17, after which they go up to -0.02 (0.16 to 0.17) in week 50 to 52. Veerkamp and Thompson (1999) found genetic correlations ranging from -0.06 to 0.02 for weeks 3 to 15 of lactation, with the use of a bivariate multi-trait covariance function model. Although the correlations were similar at the start of lactation, the correlation in the current study becomes moderately negative towards week 16 of lactation. Hüttmann et al. (2009) found genetic correlations ranging from -0.38 (day 11 to 30

of lactation) to -0.04 (day 151 to 180 of lactation), with a trough of -0.41 (day 31 to 60 of lactation). Although the correlations found by Hüttmann et al. (2009) were negative and of the same magnitude for some parts of the lactation, the shape of the curve over lactation is opposite to the curve found in the current study. Due to the negative energy balance a strong negative correlation in early lactation is as expected. In this period cows will utilise stored energy to produce milk as the energy from the feed intake is not enough to provide for the level of MY (Veerkamp, 2000), causing the cows to lose weight. A significant genetic correlation (se) between MY and CH_4 was found for week 35 to 52 of lactation for which estimates varied from 0.49 (0.11 to 0.12) to 0.54 (0.25 to 0.26). De Haas et al. (2011) found correlations ranging from -0.66 to 0.70 for week 1 to 30, but no correlations for week 35 to 52 of lactation. When comparing the whole lactation, De Haas et al. (2011) found a correlation which started negative and keeps increasing throughout lactation. In the current study, the correlation started positive and increased towards mid lactation, after which it decreased slowly. Lassen and Løvendahl (2016) found a significant correlation of 0.43 with the use of a linear animal model. This is similar to the correlations found in the current study.

From a biological point of view these correlations were as expected. At the beginning of lactation cows enter a negative energy balance. Body energy is utilised for milk production as energy from DMI is not sufficient. Therefore, in early lactation the correlation is expected to be negative. Later in lactation energy from DMI for milk production is sufficient, causing a positive correlation between DMI and CH_4. Genetic correlations between LWT and CH_4 started at 0.01 in week 1 of lactation and remained 0.02 for week 2 to 52 but were not significant at any point of lactation. Lassen and Løvendahl (2016) found a significant correlation of -0.18 with the use of a linear animal model. In the current study the correlation did not become negative at any point of lactation, however estimates were not significant. From a biological point of view both

a positive and a negative correlation are possible. When looking at the whole lifetime of the cow, initial LWT was low and increased up to a certain level after which it remained stable. DMI and CH_4 production move in the same direction as heavier cows need more food for maintenance and therefore have more substrate to produce CH_4. This results in a positive correlation between LWT and CH_4. When looking at within-lactation level, due to the negative energy balance in the beginning of lactation, LWT decreased at the beginning of lactation, after which it will increase again towards the end of lactation. CH_4 follows the same curve as DMI, which started low at the beginning of lactation, peaks in early lactation and then slowly declines towards the end of lactation. This results in a negative correlation between LWT and CH_4. The current study found a correlation that is almost zero, which might indicate that these two mechanisms are cancelling each other out in this herd.

Again, for many studies using random regression models, estimates of significance were not provided.

Legendre Polynomials

All studies mentioned in the comparison for these traits used random regression models to analyse their data (except for CH_4 production), although not all of them made use of orthogonal polynomials. As mentioned before, it is known that Legendre polynomials overestimate measurement errors in early and late lactation (Brotherstone et al., 2000) when there are less observations available in the dataset. It is likely that this effect has a bigger impact in a small dataset. The dataset available for this study was smaller than the datasets used in the aforementioned studies.

2.5 CONCLUSION

For the Nottingham herd CH_4 production was measured in the milking robot during milking. Data were analysed using bivariate random regression repeatability animal models. Heritability (s.e.) for CH_4 production obtained from this analysis was 0.12 (0.16) to 0.45 (0.11) (significant for week 39 to 52 of lactation) for the bivariate with MY, and 0.12 (0.16) to 0.40 (0.11) (significant for week 42 to 52 of lactation) for the bivariate with LWT. Repeatability (s.e.) for CH_4 production resulting obtained from this analysis was 0.50 (0.23) to 0.69 (0.16) (significant throughout lactation) for the bivariate with MY and 0.50 (0.23) to 0.66 (0.17) (significant throughout lactation) for the bivariate with LWT. Heritability obtained for this dataset indicates that CH_4 production is low to moderately heritable and genetic selection on this trait is possible. Repeatability obtained for this dataset shows that weekly averages of measurements taken over 5 consecutive months are moderate to high in agreement with each other. This indicates that it is possible to use past records to predict future CH_4 production (Falconer and Mackay, 1996).

Genetic correlations (s.e.) with CH_4 production resulting from this analysis were 0.38 (0.42) to 0.57 (0.52) (significant for week 35 to 52 of lactation) with MY, and 0.01 (0.43) to 0.02 (0.65) (not significant at any point of lactation) with LWT. This indicates that CH_4 production increases with increasing MY. The correlation obtained between CH_4 production and LWT was not significant at any point in lactation. If this correlation would have been significant, this would indicate that CH_4 production and LWT do not influence each other on a genetic level.

3. ANALYSIS OF THE LANGHILL HERD

3.1 INTRODUCTION

Methane is produced as a waste product of enteric fermentation in ruminants, when acetate originating from proteins and carbohydrates is broken down to CH_4 gas (Qiao et al., 2014; Marchaim, 1992). Therefore, the amount of DMI utilised (as well as diet ingredients and feed types) directly influences the amount of CH_4 produced. Accordingly, the genetic correlation between DMI and CH_4 production is expected to be strong and positive. This means that when selecting for lower CH_4 production, DMI will reduce as well. As the correlation between MY and DMI is positive (Hüttmann et al., 2009), selecting for lower CH_4 production will lead to lower MY, which is undesirable.

A way to cope with this is to select on feed efficiency instead. More efficient cows produce the same amount of milk on a lower DMI and therefore less CH_4 is produced (Basarab et al., 2013). As described in chapter 1, many definitions of feed efficiency are currently in use. A distinction is made between ratio traits and residual traits. Ratio traits are ratios of underlying traits whereas residual traits are based on the difference in predicted energy (calculated based on underlying traits) and true energy needs. Therefore, feed efficiency is always dependent on underlying traits, which makes it difficult to predict effects of genetic selection on feed efficiency (Basarab et al., 2013). It is therefore preferred to select directly on these underlying traits instead.

In order to do so, both DMI and CH_4 production have to be included in a selection index simultaneously with production, health and fertility traits. This means correlations between CH_4 production and DMI, production, health and fertility traits are needed. Since the start of the current study only Manzanilla-Pech et al. (2016a) has published a (significant) genetic correlation between DMI and CH_4 production, of 0.83. To affirm this correlation, it is important that more studies obtain this correlation with different datasets.

For the current study DMI measurements were available for the Langhill herd, in which CH_4 production was measured using a laser methane detector (LMD) (a larger dataset was available than was available for Pickering et al. (2015), including measurements for 2014 for which better standardisation of the measurement method had taken place). As described in chapter 1, CH_4 measurements obtained with different techniques might be measuring different traits. Therefore, it is important to obtain and compare genetic parameters for CH_4 production derived from analyses using different CH_4 measurement methods.

The aims of this chapter were to derive;

1) genetic parameters for CH_4 production measured using a laser methane detector,

2) correlations between CH_4 production, milk yield, live weight and DMI.

3.2 METHODS

3.2.1 Data

Data were available on 521 Holstein-Friesian cows housed at SRUC Dairy Research Centre (Dumfries, Scotland). Cows were born between February 2004 and April 2012 and originated from two genetic lines; a selection line for high milk solids, and a control line kept close to average UK commercial herds for milk solids. The pedigree was limited to 3 generations and consisted of 246 sires and 1,235 dams (pedigree analyses were performed with CFC (Coancestry, inbreeding (F) and Contribution) software (Sargolzaei et al., 2006)). During the years of the experiment cows were also part of a feed trial. Until September 2011 cows were either fed a high forage or a low forage diet, providing them with a low energy or a high energy diet respectively. After this date high forage diet was replaced by a diet based on home grown forage, while low forage diet was replaced by a diet based on by products. The difference in energy levels between the diets remained.

Methane measurements

CH_4 production was measured using a laser methane detector (LMD). This method is based on infrared absorption spectroscopy and measures column density of CH_4 containing gases at a distance. This gives CH_4 concentration in ppm m, which was converted into $g^{-1}d$ as described by Chagunda et al. (2009). Measurements were taken in 2010, 2011 and 2014, on 198 cows. Measurements were taken once daily for 1 to 3 days in a row. For initial analyses of the Langhill herd these measurements were analysed as daily values (770 observations ($g^{-1}d$), after quality control (observations more than 3 times the standard error different from the trait average were removed)), but for the subsequent analysis of the Langhill herd they were averaged by calendar week (206 observations, $g^{-1}d$ weekly average). For some cows this resulted in data available for subsequent weeks. The structure and size of the dataset did not allow for fitting a repeatability model for CH_4 production. This could have caused genetic variance to be overestimated due to permanent environmental variance being assigned to genetic variance. This would then lead to an overestimated heritability. Therefore, out of the consecutive weeks only the week with the highest number of underlying measurements was kept. When multiple weeks had the highest number of measurements, the first of these weeks was kept.

Production traits

For cows for which there were CH_4 observations available data on milk yield (MY, $L^{-1}d$, weekly average), live weight (LWT, kg, weekly average) and dry matter intake (DMI, $kg^{-1}d$, weekly average) were limited to 4-week intervals coinciding with the weeks for which there was a CH_4 observation for a particular cow, as described in chapter 2. This was done to avoid correlations being strongly influenced by the production traits, as more observations were available for these traits. For initial analyses only, data on cows for which a CH_4 observation were available were analysed. Therefore 8,520 observations

on MY, 8,353 observations on LWT and 6,612 observations on DMI were included.

For the subsequent analysis data were added on cows for which no CH_4 observations were available. For these cows, number of observations used on MY, LWT and DMI would not influence the overall correlation with CH_4 production. Therefore, all observations between February 2010 and April 2014 were used when an observation on all three traits were available. This resulted in 30,026 observations on MY, 29,164 observations on LWT and 23,895 observations on DMI, after quality control (observations more than 3 times the standard error different from the trait average were removed).

Variables available were record week (calendar week, starting on Monday; 1 to 54 weeks) record year (2010 to 2014), record year group (2010 to 2013 (58 CH_4 observations) and 2014 (148 CH_4 observations)), cow age (months), age at first calving (months), age at last calving (months), calving season (4 seasons; Spring (Mar-May), Summer (Jun-Aug), Autumn (Sep-Nov), Winter (Dec-Feb)), diet group (two groups, a high energy and a low energy diet), genetic line (two lines, one line selected for high milk protein and fat and one line kept close to the UK average commercial herd), lactation number (limited to $\leqslant 3$, observations higher than 3 were set to 3 for initial analyses and removed for the subsequent analysis (55 observations)) and week of lactation (limited to $\leqslant 52$, observations higher than 52 were set to 52 (7 observations).

3.2.2 Statistical analyses

Multivariate genetic analysis

Initial analyses

Pedigree based analyses was used to estimate genetic parameters including heritabilities, repeatabilities, genetic and phenotypic correlations (see chapter 2 for a description).

Data on MY, LWT, DMI and CH_4 production were analysed. Models were investigated in the same way as for the Nottingham herd (chapter 2). The Wald statistic was used to determine whether an effect was significant. Only significant fixed effects ($P < 0.001$) were kept in the model. Exceptions were made for genetic group and feed group, which were kept in the model regardless, due to the experimental design.

As described in chapter 2, a fixed regression function was interacted with week of lactation to account for the lactation curve on population level. A random regression function was interacted with week of lactation and the animal genetic effect to allow for individual deviations from this curve.

For the Langhill dataset it was not possible to use a random regression function for CH_4 production or the permanent environmental effect. For genetic variation of CH_4 production this meant that expression of the trait over lactation did not deviate from the lactation curve for the population. This gave a point estimate of genetic variance, rather than a range over lactation. For permanent environmental variance this meant that it was assumed that there was no change in variance across lactation. As the permanent environment is not expected to change, this assumption is justified.

Distribution of residuals was examined by plotting residuals against week of lactation. Residuals were uniform over time and therefore only one residual class was used. Bivariate models were constructed between each combination of the four traits, and fixed effects for each trait were kept identical between models.

The following models were constructed;

$$MY = \mu + lact.WOL_leg5 + genline + feedgr + rec_yr + calv_seas4 +$$
$$b1 * age + WOL_leg.An + AnPE + e$$

$$LWT = \mu + WOL_leg4 + lact + genline + feedgr + rec_yr + calv_seas4 +$$
$$b1 * age + WOL_leg.An + AnPE + e$$

$$DMI = \mu + lact.WOL_leg3 + genline + feedgr + rec_yr + calv_seas4 +$$
$$b1 * age + WOL_leg.An + AnPE + e$$

$$CH_4 = \mu + WOL_leg2 + genline + feedgr + rec_yr + calv_seas4 +$$
$$An + e$$

Where μ is the general mean; lact.WOL_leg5 is the interaction between number of lactation and a Legendre polynomial of order 5 on week of lactation as a fixed effect; lact.WOL_leg3 is the interaction between number of lactation and a Legendre polynomial of order 3 on week of lactation as a fixed effect; WOL_leg4 is a Legendre polynomial of order 4 on week of lactation as a fixed effect; WOL_leg2 is a Legendre polynomial of order 2 on week of lactation as a fixed effect; lact is the fixed effect of lactation number; genline is the fixed effect of genetic line; feedgr is the fixed effect of feed group; rec_yr is the fixed effect of the year the measurements took place; calv_seas4 is the effect of calving season (4 seasons); age is the covariate that describes the effect of age at measurement; WOL_leg.An is the interaction between a Legendre of order 1 on week of lactation and the additive genetic effect of animal as a random effect; An is the additive genetic effect of animal as a random effect; AnPE is the permanent environment effect; and e is the random residual.

Subsequent analysis

For the subsequent analysis weekly averages of CH_4 production were used as well as observations on cows for which no CH_4 observations were available. Models were started based on the final models used for initial analyses and based on models used by Coffey et al. (2002) for analyses of the Langhill data. Again, the Wald statistic was used to determine whether an effect was significant. Genetic group and feed group were kept in the model regardless, due to the structure of the experiment. As the number of observations on CH_4 production decreased (770 to 206 observations), fixed effects that were significant for CH_4 production in initial analyses were kept in the model even if they were no longer significant for the subsequent analysis. The effect of the levels within a fixed effect (for variables on a discrete scale) was investigated and where fixed effects had a level which had a deviating effect compared to other levels, levels were grouped together in a logical way. For CH_4 production the order of the Legendre term fitted on week of lactation as a fixed effect was based on a visual examination of the raw data.

A 4-trait model was constructed between the four traits;

$$MY = \mu + lact.WOL_leg5 + genline + feedgr + rec_wk + rec_yr + calv_seas4 + b1 * aac + b2 * aac2 + WOL_leg.An + AnPE + e$$

$$LWT = \mu + lact + WOL_leg5 + genline + feedgr + rec_yr + calv_seas4 + b1 * aac + b2 * aac2 + WOL_leg.An + AnPE + e$$

$$DMI = \mu + lact.WOL_leg5 + genline + feedgr + rec_wk + rec_yr + calv_seas4 + b1 * aac + b2 * aac2 + WOL_leg.An + AnPE + e$$

$$CH_4 = \mu + MOL_leg3 + genline + feedgr + rec_wk + rec_yrgr + calv_seas2 + An + e$$

Where μ is the general mean; lact.WOL_leg5 is the interaction between number of lactation and a Legendre polynomial of order 5 on week of lactation as a fixed effect; WOL_leg5 is a Legendre polynomial of order 5 on week of lactation as a fixed effect; MOL_leg3 is a Legendre polynomial of order 3 on Month of lactation as a fixed effect; lact is the fixed effect of lactation number; genline is the fixed effect of genetic line; feedgr is the fixed effect of feed group; rec_wk is the fixed effect of the calendar week the measurements took place; rec_yr is the fixed effect of the year the measurements took place; rec_yrgr is the fixed effect of the grouped record years the measurement took place; calv_seas2 is the effect of calving season (2 seasons); calv_seas4 is the effect of calving season (4 seasons); aac is the covariate that describes the effect of age at calving; aac2 is the covariate that describes the squared effect of age at calving; WOL_leg.An is the interaction between a Legendre of order 1 on week of lactation and the additive genetic effect of animal as a random

effect; An is the additive genetic effect of animal as a random effect; AnPE is the permanent environment effect; and e is the random residual.

For both analyses genetic, permanent environment and residual variances, and heritabilities, repeatabilities, correlations and standard errors were derived as described in chapter 2.

3.3 RESULTS

Heritabilities and repeatabilities

Heritabilities and repeatabilities obtained for initial analyses (CH_4 production as daily measurements, and cows without CH_4 observations not included) are in Tables 3.1 and 3.2. Although heritabilities and repeatabilities for the same trait varied between bivariate models, estimates were in agreement. Heritabilities and repeatabilities obtained from the subsequent analysis (weekly averages of CH_4 production and including MY, LWT and DMI data on cows for which no CH_4 observations were available) are in Table 3.3. For initial analyses heritability for MY was significant throughout lactation for the bivariate analysis with LWT, significant for weeks 1 to 12 and 25 to 52 of lactation for the bivariate analysis with DMI and significant for weeks 1 to 9 and 26 to 52 of lactation for the bivariate analysis with CH_4 production. When only taking into account weeks of lactation with a significant estimate, for the bivariate analysis with LWT heritability started at 0.63 in week 1 of lactation, went down to 0.39 in weeks 17 and 18 of lactation, after which it went up to 0.84 in week 52. For the bivariate analysis with DMI heritability started at 0.58 in week 1 of lactation and went down to 0.36 in week 12. In week 25 of lactation heritability started at 0.37 and went up to 0.81 in week 52 of lactation. For the bivariate analysis with CH_4 production heritability started at 0.52 in week 1 of lactation and went down to 0.31 in week 9. In week 26 of lactation heritability started at 0.33 and went up to 0.80 in week 52 of lactation.

Repeatabilities were significant throughout lactation for all bivariate analyses. For the bivariate analysis with LWT repeatability started at 0.85 in week 1 of lactation, went down to 0.75 in weeks 14 to 20 of lactation, after which it went up to 0.93 in week 49 to 52. For the bivariate analysis with DMI repeatability started at 0.85 in week 1 of lactation, went down to 0.75 in week 15 to 20, after which it went up to 0.93 in week 51 and 52. For the bivariate analysis with CH_4 production repeatability started at 0.83 in week 1, went down to 0.72 in week 14 to 20, after which it went up to 0.93 in week 51 and 52 of lactation. For the subsequent analysis heritability and repeatability found for MY were significant throughout lactation. Heritability started at 0.35 in week 1 of lactation, went down to 0.29 for week 17 to 26 of lactation, after which it went up to 0.41 in week 52 of lactation. Repeatability started at 0.54 in week 1 of lactation, went down to 0.50 for week 16 and 27 of lactation, after which it went up to 0.58 for week 51 and 52 of lactation.

For initial analyses heritability for LWT was significant throughout lactation for all three bivariate analyses. For the bivariate with MY heritability started at 0.73 in week 1 of lactation, went down to 0.62 in weeks 23 to 29 of lactation, after which it went up to 0.73 in week 52. For the bivariate with DMI heritability started at 0.77 in week 1 of lactation and went down to 0.66 in week 22 to 33, after which it went up to 0.75 in week 52 of lactation. For the bivariate with CH_4 production heritability started at 0.77 in week 1 of lactation and went down to 0.67 in week 24 to 27, after which it went up to 0.78 in week 52 of lactation.

Repeatabilities were significant throughout lactation for all bivariate analyses. For the bivariate with MY repeatability started at 0.93 for week 1 to 4 of lactation, went down to 0.90 in weeks 22 to 30 of lactation, after which it went up to 0.93 in week 48 to 52. For the bivariate with DMI repeatability started at 0.94 in week 1 of lactation, went down to 0.91 in week 15 to 41, after which it went up to 0.93 in week 49 and 52. For the bivariate with CH_4

production repeatability started at 0.93 in week 1, went down to 0.90 in week 15 to 35, after which it went up to 0.93 in week 49 and 52 of lactation. For the subsequent analysis heritability and repeatability for LWT were significant throughout lactation. Heritability started at 0.68 in week 1 of lactation, went down to 0.67 for week 9 to 17 of lactation, after which it went up to 0.72 for week 49 to 52 of lactation. Repeatability started at 0.80 in week 1 of lactation, went down to 0.79 for week 7 to 22 of lactation, after which it went up to 0.82 for week 46 to 52 of lactation.

For initial analyses heritability for DMI was significant throughout lactation for all three bivariate analyses. For the bivariate with MY heritability started at 0.82 in week 1 of lactation, went down to 0.61 in weeks 22 to 25 of lactation, after which it went up to 0.87 in week 52. For the bivariate with LWT heritability started at 0.84 in week 1 of lactation and went down to 0.66 in week 22 to 27, after which it went up to 0.87 in week 52 of lactation. For the bivariate with CH_4 production heritability started at 0.83 in week 1 of lactation and went down to 0.61 in week 21 to 25, after which it went up to 0.88 in week 52 of lactation.

Repeatabilities were significant throughout lactation for all bivariate analyses. For the bivariate analysis with MY repeatability started at 0.89 for week 1 of lactation, went down to 0.75 in weeks 23 to 24 of lactation, after which it went up to 0.92 in week 52. For the bivariate analysis with LWT repeatability started at 0.90 in week 1 of lactation, went down to 0.78 in week 23 to 26, after which it went up to 0.92 in week 52. For the bivariate analysis with CH_4 production repeatability started at 0.89 in week 1, went down to 0.75 in week 20 to 26, after which it went up to 0.92 in week 51 and 52 of lactation.

For the subsequent analysis heritability and repeatability for DMI were significant throughout lactation. Heritability started at 0.24 in week 1 of lactation, went down to 0.10 for week 25 and 36 of lactation, after which it went up to 0.18 for week 52 of lactation. Repeatability started at 0.36 in week 1 of lactation,

went down to 0.23 for week 28 to 33, after which it went up to 0.30 for week 51 and 52 of lactation.

For initial analyses heritability for CH_4 production was 0.01, for the subsequent analysis heritability for CH_4 production was 0.22. Neither was significant. The increase in heritability between the two analyses was as expected, as averaging observations can reduce measurement errors. No repeatability was estimated for CH_4 production. It should be noted that as no Legendre polynomial was fitted on weeks of lactation in interaction with genetic animal variance, only one heritability was estimated for each analysis.

Table 3.1: Heritabilities (s.e.) (bold), genetic (s.e.) (below diagonal) and phenotypic (s.e.) (above diagonal) correlations for, and between, milk yield (MY), live weight (LWT), dry matter intake (DMI) and CH_4 production (CH_4) obtained from bivariate random regression models

Bivariate Langhill		MY	LWT	DMI	CH_4
MY	LWT	**0.39 (0.20)-0.84 (0.06)**	-0.33 (0.09)-0.49(0.12)[1]		
	DMI	**0.30 (0.19)-0.81 (0.06)**[2]		0.59 (0.04)-1.21 (0.11)	
	CH_4	**0.20 (0.19)-0.80 (0.05)**[3]			0.03 (0.37)-0.12 (0.78)[4]
LWT	MY	-0.39 (0.07)-0.85 (0.06)[5]	**0.62 (0.18)-0.73 (0.14)**		
	DMI		**0.66 (0.20)-0.77 (0.14)**	0.17 (0.11) - 0.66 (0.12)[6]	
	CH_4		**0.67 (0.21)-0.78 (0.15)**		0.00 (0.73)-0.01 (0.79)[4]
DMI	MY	0.37 (0.08)-0.82 (0.08)		**0.61 (0.18)-0.87 (0.06)**	
	LWT		0.18 (0.09)-0.87 (0.06)	**0.66 (0.16)-0.87 (0.07)**	
	CH_4			**0.61 (0.18)-0.88 (0.06)**	0.07 (0.55)-0.17 (0.79)[4]
CH_4	MY	-0.55 (0.75)-0.06 (0.59)[4]			**0.01 (0.02)[4]**
	LWT		-0.09 (0.63)-0.02 (0.62[4]		**0.01 (0.02)[4]**
	DMI			-0.79 (0.18)-0.42 (0.31)[7]	**0.01 (0.02)[4]**

[1] Significant for week of lactation 1 to 11
[2] Significant for week of lactation 1 to 14 and 25 to 52
[3] Significant for week of lactation 1 to 9 and 26 to 52
[4] Not significant throughout lactation
[5] Significant for week of lactation 1 to 20 and 37 to 52
[6] Significant for week of lactation 1 to 41
[7] Significant for week of lactation 1 to 13

Table 3.2: Repeatabilities (s.e.) for milk yield (MY), live weight (LWT) and dry matter intake (DMI) obtained from bivariate random regression models for the Langhill herd

Bivariate Langhill		Repeatabilities
MY	LWT	0.75 (0.29)-0.93 (0.09)
	DMI	0.75 (0.29)-0.93 (0.09)
	CH_4	0.72 (0.28)-0.93 (0.08)
LWT	MY	0.90 (0.29)-0.93 (0.22)
	DMI	0.91 (0.32)-0.94 (0.22)
	CH_4	0.90 (0.33)-0.93 (0.25)
DMI	MY	0.75 (0.26)-0.92 (0.09)
	LWT	0.79 (0.24)-0.92 (0.10)
	CH_4	0.75 (0.26)-0.92 (0.09)

Figure 3.1 shows heritabilities of MY, LWT and DMI over weeks of lactation for the three bivariate models they are included in for initial analyses. Heritabilities of MY, LWT and DMI over weeks of lactation for the 4-trait model for the subsequent analysis are in Figure 3.2.

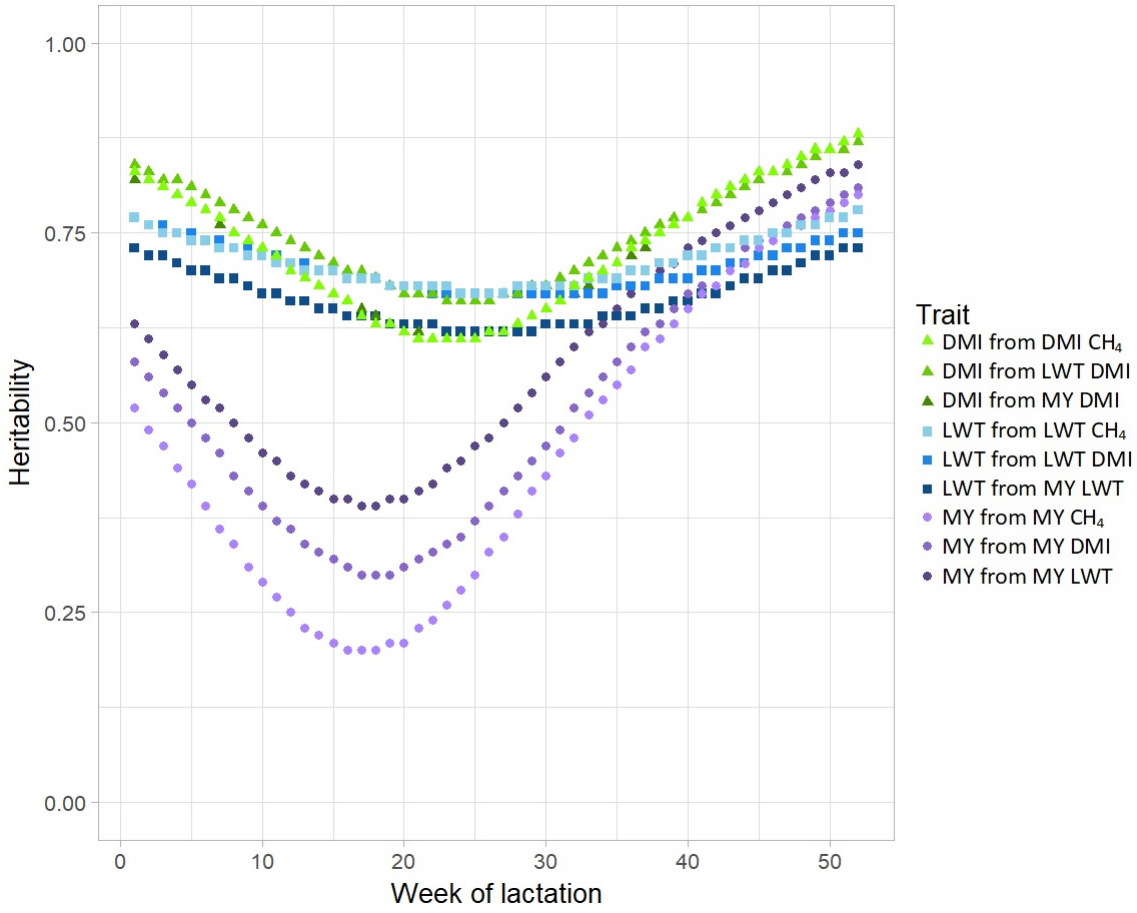


Figure 3.1: Heritabilities of milk yield (MY), live weight (LWT) and dry matter intake (DMI) resulting from bivariate models for the Langhill herd

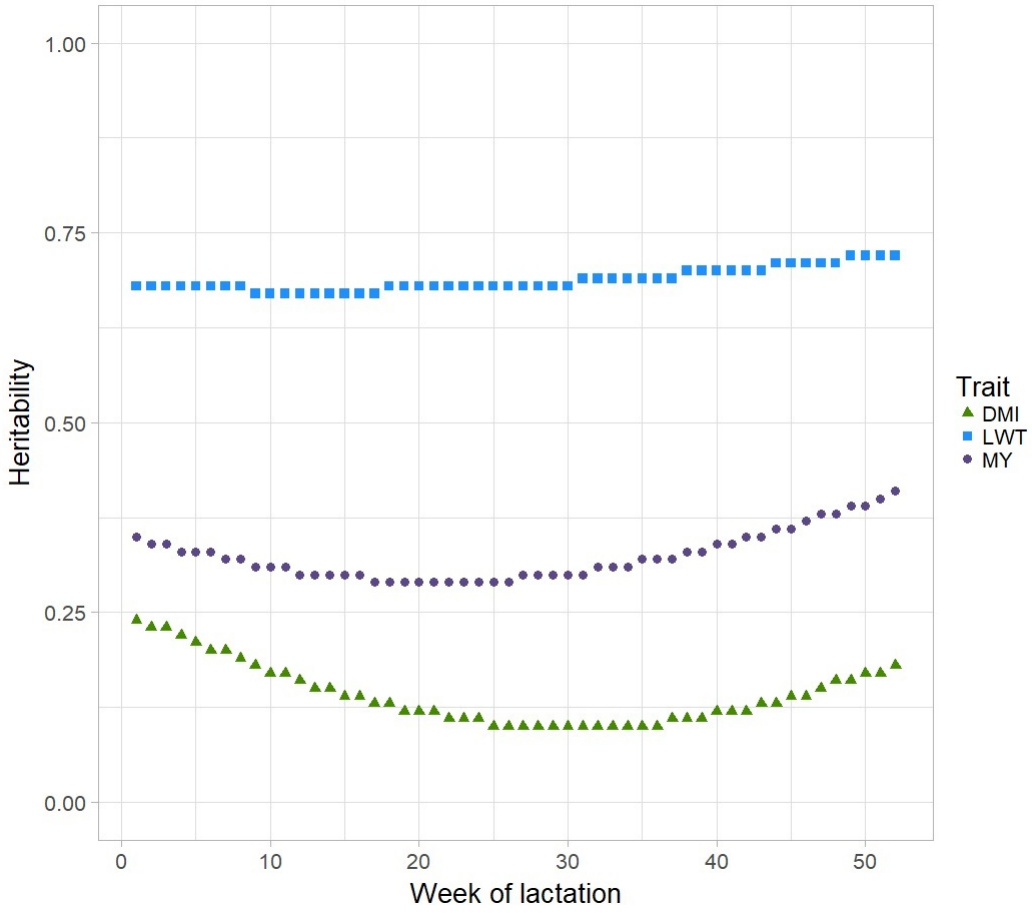


Figure 3.2: Heritabilities of milk yield (MY), live weight (LWT) and dry matter intake (DMI) resulting from the 4-trait model for the Langhill herd

Table 3.3: Heritabilities (s.e.) (bold), repeatabilities (s.e.) (bold, italic), genetic (s.e.) (below diagonal) and phenotypic (s.e.) (above diagonal) correlations for, and between, milk yield (MY), live weight (LWT), dry matter intake (DMI) and CH_4 production (CH_4) resulting from the 4-trait model for the Langhill herd

4-trait Langhill	MY	LWT	DMI	CH_4	Rep
MY	**0.29 (0.07)-0.41 (0.05)**	-0.26 (0.07)–0.05 (0.01)	0.33 (0.10)-0.36 (0.07)	0.27 (0.09)-0.33 (0.08)	***0.50 (0.07)-0.58 (0.06)***
LWT	-0.60 (0.06)–0.25 (0.10)	**0.67 (0.09)-0.72 (0.08)**	-0.06 (0.20)-0.01 (0.20)	0.00 (0.21)-0.03 (0.22)	***0.79 (0.11)-0.82 (0.10)***
DMI	0.13 (0.20)-0.29 (0.09)[1]	0.36(0.09)-0.59 (0.10)	**0.10 (0.04)-0.24 (0.03)**	0.02 (0.24)-0.03 (0.24)	***0.23 (0.04)-0.36 (0.03)***
CH_4	-0.36 (0.21)–0.09 (0.22)[2]	0.00 (0.21)- 0.09 (0.26)[2]	0.05 (0.27)-0.11 (0.35)[2]	**0.22 (0.15)[2]**	

[1] significant for week 1 to 8 and 50 to 52 of lactation

[2] not significant throughout lactation

Phenotypic and genetic correlations between production traits

Phenotypic and genetic correlations can be found in Tables 3.1 and 3.2 for initial analyses and Table 3.3 for the 4-trait analysis. Genetic correlations between production and efficiency traits for the Langhill herd for initial analyses are in Figure 3.3 and for the subsequent analysis in Figure 3.4.

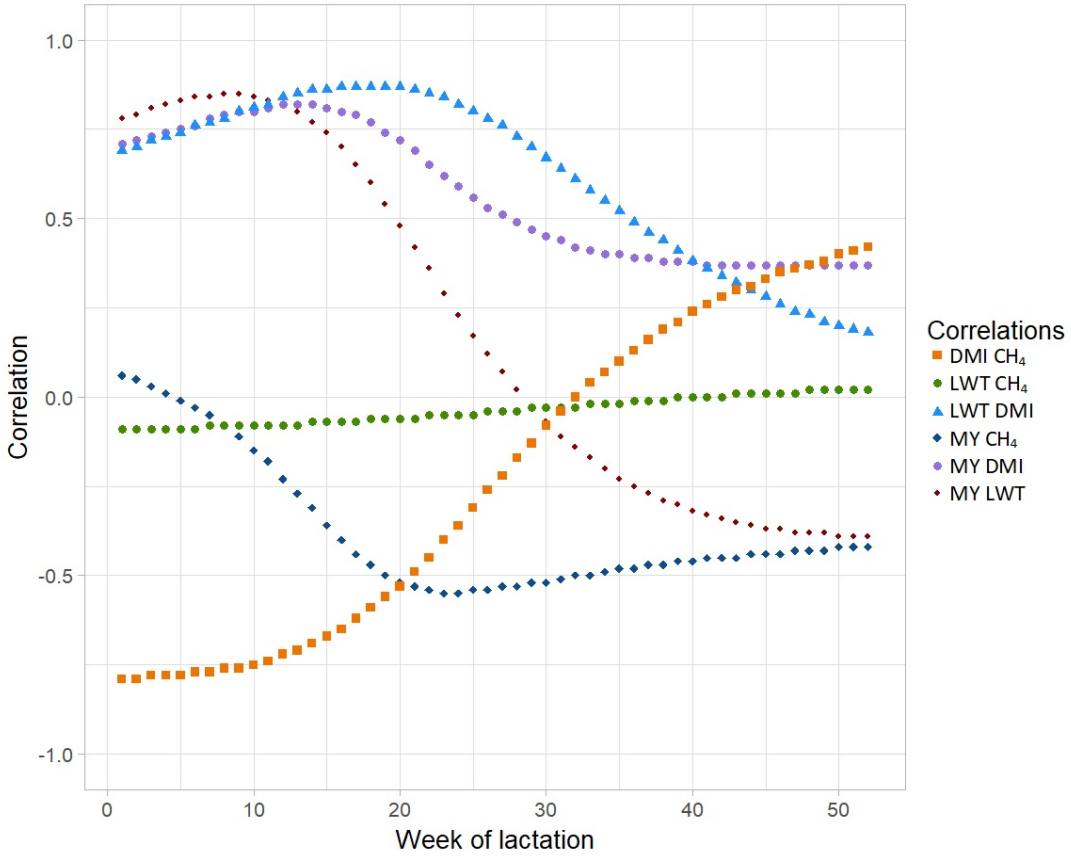


Figure 3.3: Genetic correlations between milk yield (MY), live weight (LWT), dry matter intake (DMI) and CH_4 production (CH_4) for initial analyses for the Langhill herd

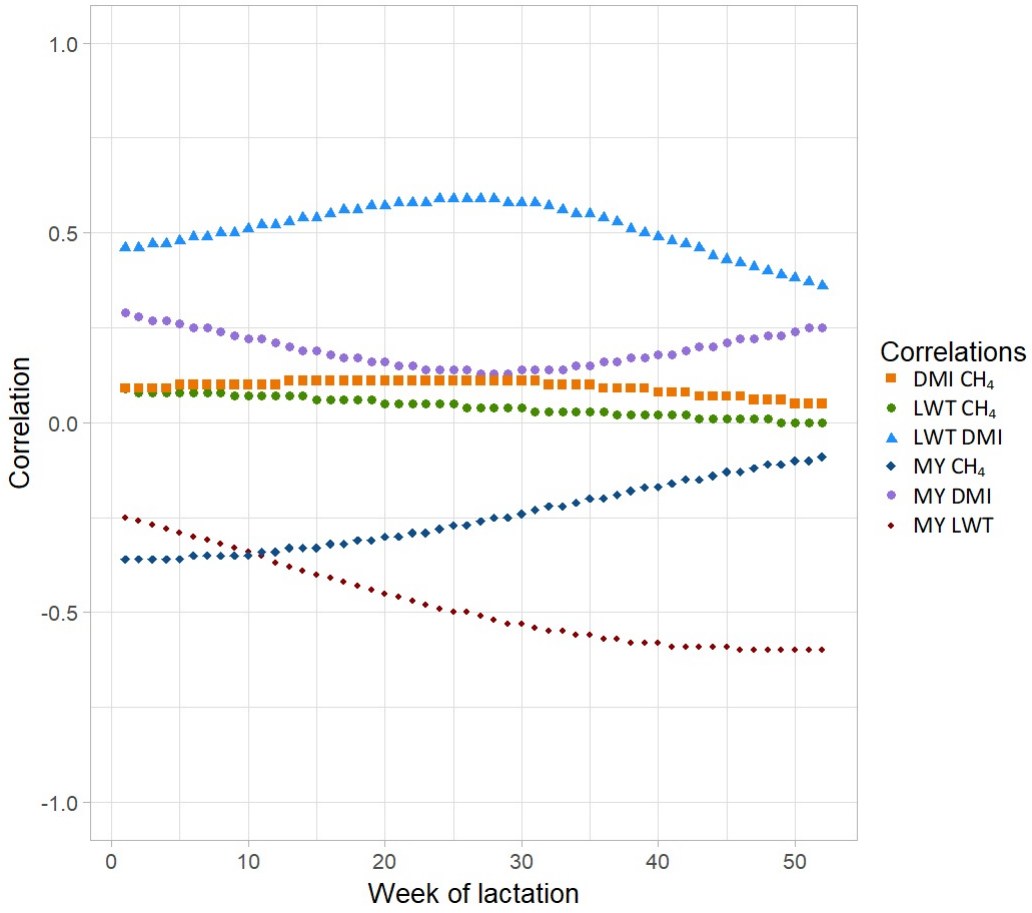


Figure 3.4: Genetic correlations between milk yield (MY), live weight (LWT), dry matter intake (DMI) and CH_4 production (CH_4) for the subsequent analysis for the Langhill herd

For initial analyses genetic correlation between MY and LWT started positive at 0.78 in week 1 of lactation, went up to 0.85 in week 8 to 9 of lactation, after which it went down to 0.02 in week 28 of lactation. From week 29 of lactation onward the correlation turned negative from -0.03 to -0.39 in week 52 of lactation. Correlations were significant for week 1 to 20, for which they range from 0.48 to 0.85 and for week 37 to 52 of lactation, for which they range from -0.27 to -0.39.

For the subsequent analysis genetic correlation between MY and LWT started at -0.25 in week 1 of lactation and went down to -0.60 for week 46 to 52. Correlations were significant throughout lactation.

For initial analyses genetic correlation between MY and DMI started at 0.71 in week 1 of lactation, went up to 0.82 for week 12 to 14 of lactation, after which it went down to 0.37 for week 41 to 52 of lactation. Correlations were significant throughout lactation.

For the subsequent analysis genetic correlation between MY and DMI started at 0.29 in week 1 of lactation, went down to 0.13 for week 27 to 29 of lactation, after which it went up to 0.25 for week 51 and 52 of lactation. Correlations were significant for week 1 to 8, for which they ranged from 0.24 to 0.29 and for week 50 to 52 of lactation, for which they ranged from 0.24 to 0.25.

For initial analyses genetic correlation between LWT and DMI started at 0.69 in week 1 of lactation, went up to 0.87 for week 16 to 20 of lactation, after which it went down to 0.18 for week 52 of lactation. Correlations were significant throughout lactation.

For the subsequent analysis genetic correlation between LWT and DMI started at 0.46 in week 1 of lactation, went up to 0.59 for week 24 to 28 of lactation, after which it went down to 0.36 in week 52 of lactation. Correlations were significant throughout lactation.

Correlations between production traits and CH_4 production

Correlations between production traits and CH_4 production for the Langhill herd for initial analyses are in Figure 3.3 and for the subsequent analysis in Figure 3.4.

For initial analyses genetic correlation between MY and CH_4 production started at 0.06 and from week 4 of lactation became negative towards -0.55 for week 23 and 24 of lactation, after which it went up to -0.42 in week 52 of lactation. However, standard errors ranged from 0.27 to 1.06, indicating that correlations were not significant at any point in lactation. As standard errors are expected to be within the range of 0 to 1, a standard error of 1.06 indicates a problem in the modelling.

For the subsequent analysis genetic correlation between MY and CH_4 production started at -0.36 in week 1 of lactation and went up to -0.09 in week 52 of lactation. Correlations were not significant at any point in lactation. For initial analyses genetic correlation between LWT and CH_4 production started negative at -0.09 in week 1 of lactation, became 0.00 for week 39 to 42 of lactation, after which it went up to 0.02 in week 48 to 52 of lactation. Correlations were not significant at any point in lactation.

For the subsequent analysis genetic correlation between LWT and CH_4 production started at 0.09 in week 1 of lactation and went down to 0.00 for week 49 to 52 of lactation. Correlations were not significant at any point in lactation.

For initial analyses correlation between DMI and CH_4 production started negative at -0.79, went up to 0.00 in week 32 of lactation, after which it went up to 0.42 in week 52 of lactation. Correlations went significant for week 1 to 13, for which they ranged from -0.79 to -0.71.

For the subsequent analysis correlation between DMI and CH_4 production started at 0.09 in week 1 of lactation, went up to 0.11 in week 13 to 31 of lactation, after which it went down to 0.05 for week 50 to 52 of lactation. Correlations were not significant at any point in lactation.

3.4 DISCUSSION

Heritabilities for Langhill

Heritability for MY ranged from 0.20 to 0.84 for initial analyses and from 0.29 to 0.41 for the subsequent analysis. Repeatability ranged from 0.72 to 0.93 for initial analyses and 0.50 to 0.58 for the subsequent analysis.

When comparing the initial and subsequent analyses it was seen that the range of heritability for MY became smaller and no longer went up to extreme values towards end of lactation for the subsequent analysis. The range of standard

errors became smaller as well and heritability became significant throughout lactation. Heritabilities found were different from literature using random regression models. Jakobsen et al. (2002) found a heritability for test day MY for first lactation starting at 0.19 for day 5 in milk going up to 0.37 for day 165 in milk after which heritability went down to 0.31 for day 285 in milk. Although heritability for 165 days in milk was close to heritabilities from initial analyses and higher from the subsequent analysis for the same moment in lactation, the overall curve was different. Jamrozik and Schaeffer (1997) found heritability for test day MY for first lactation vary from 0.40 to 0.59, without a clear trend over time. Heritabilities were similar to heritabilities found for the current study but did not reflect the trough (Figure 3.1 and 3.2) that was found for both the initial and subsequent analyses. Hüttmann et al. (2009) found heritabilities for energy corrected milk for 11 to 180 days in milk ranging from 0.22 to 0.47, which is similar to heritabilities from the subsequent analysis. Heritability for LWT ranged from 0.66 to 0.78 for initial analyses and from 0.67 to 0.72 for the subsequent analysis. Repeatability ranged from 0.90 to 0.94 for initial analyses and from 0.79 to 0.82 for the subsequent analysis.

When comparing the initial and subsequent analyses it was seen that the range of heritabilities was similar, but standard errors became lower for the subsequent analysis. This was as expected as there is less variation in LWT than in MY, which meant the effect of adding in more data were smaller. Repeatability was lower for the subsequent analysis than for initial analyses, with lower standard errors.

Heritabilities derived in the current study were higher than heritabilities found in literature. Berry et al. (2003*a*) found genetic variance for body weight continuously increased from day 63 in milk, with associated heritability ranging from 0.48 at day 0 in milk to 0.61 at day 294 in milk. This was lower than heritabilities for both the initial and subsequent analyses of the current study. Veerkamp and Thompson (1999) found a heritability between 0.29 and 0.47

for live weight for week 1 to 15 of lactation. The curve of heritabilities over time Veerkamp and Thompson (1999) was relatively flat and heritabilities were lower than heritabilities found in the current study. Koenen and Veerkamp (1998) found heritabilities for week 0 to 25 of lactation varying from 0.43 to 0.56, starting at 0.43 and going up in time. Standard errors ranged from 0.06 to 0.10. This was opposite to heritabilities found in the current study, which started off high in early lactation and went down towards mid-lactation for initial analyses and remained constant from the beginning of lactation towards mid-lactation for the subsequent analysis. Hüttmann et al. (2009) found heritabilities for days 11 to 180 in milk ranging from 0.26 to 0.45. Heritabilities started off high early in lactation, went down around 91 to 150 days of lactation and went up again from 151 days onwards, which gave a similar curve as in the current study. Heritabilities found were lower than heritabilities found in the current study for initial analyses and higher than found for the subsequent analysis.

Heritability for DMI ranged from 0.61 to 0.88 for initial analyses and from 0.10 to 0.24 for the subsequent analysis.

When comparing both analyses it was seen that heritability became lower, as did standard errors. These estimates were different than expected from other studies that make use of random regression models. Veerkamp and Thompson (1999) found heritabilities for week 1 to 15 of lactation to range from 0.13 to 0.54, with no clear curve in time. Koenen and Veerkamp (1998) found heritabilities for week 3 to 25 of lactation to range from 0.18 to 0.37, going up until week 15 after which heritabilities slowly decreased. Standard errors ranged from 0.06 to 0.10. Heritabilities found in the current study were higher for this period of lactation. Hüttmann et al. (2009) found heritabilities for days 11 to 180 in milk ranging from 0.06 to 0.12, which was lower than heritabilities found in the current study. No literature was found on repeatabilities for DMI obtained using random regression models.

Heritability for CH_4 production was 0.01 (not significant) for initial analyses and 0.22 (not significant) for the subsequent analysis.

When compared to literature, heritability for CH_4 production was expected to be in the range of 0.05 (not significant) (LMD; Pickering et al. (2015)) to 0.35 (prediction, s.e. of 0.12; De Haas et al. (2011)). However, this depends on measurement technique used. Pickering et al. (2015) used the same dataset and measurement technique as used for the current study and found a heritability of 0.05 (not significant) using a linear mixed model. This was close to heritability from initial analyses, however, it was also not significant. Lassen and Løvendahl (2016) found a significant heritability of 0.21 for CH_4 production ($g^{-1}d$) in dairy cattle. Pszczola et al. (2017) found an average significant heritability of 0.27 for CH_4 production ($g^{-1}d$). Both heritability found by Lassen and Løvendahl (2016) and Pszczola et al. (2017) were close to heritability found for the subsequent analysis in the current study.

Heritability found for CH_4 production when using point estimates and bivariate models was lower than expected, but in agreement with Pickering et al. (2015) using the same dataset. The low heritability might be due to measurements being taken over different years, by different people. There might be genetic variance for CH_4 production, but this likely is masked by the influence of the fixed effects fitted in the model. For initial analyses CH_4 production was analysed as point estimates. This was done because the available dataset was small and averaging data would reduce number of observations, as well as causing a loss in variation. However, when using weekly averages instead of point estimates, this can reduce measurement errors and therefore possibly give a more precise estimate. When comparing variances found for initial analyses and the subsequent analysis, indeed a large reduction in residual variance was found, whereas genetic animal variance only slightly increased. It should be taken into account that the models differed as well.

All studies mentioned in the literature comparison for these traits use random

regression models to analyse their data, although not all of them make use of orthogonal polynomials. Also, almost all studies only include data on cows in first lactation, while in the current study cows with a lactation number up to 3 were present in the Langhill dataset. This might have caused more variation to be present in the dataset even though lactation number was fitted in the models as a fixed effect. As for chapter 2, for many studies using random regression models estimates of significance were not provided.

Genetic correlations between production traits

Genetic correlation between MY and LWT ranged from -0.39 to 0.85 (not significant for any week of lactation) for initial analyses and from -0.25 to -0.60 for the subsequent analysis. When comparing results from both analyses it was seen that the direction of the correlation was different in early lactation and stronger negative in late lactation for the subsequent analysis than for initial analyses. When compared to literature the correlation found for the subsequent analysis was more similar than for initial analyses. Veerkamp and Thompson (1999) found genetic correlations ranging from -0.06 to -0.24 for weeks 3 to 9 of lactation. Hüttmann et al. (2009) found genetic correlations ranging from -0.04 to -0.41 (day 11 to 180 in milk), where correlations were highly negative early in lactation and approaching zero around 180 days in milk. Correlations found for the subsequent analysis were negative in early lactation and became even stronger negative towards mid-lactation.

Genetic correlation between MY and DMI ranged from 0.37 to 0.82 for initial analyses and 0.13 to 0.29 for the subsequent analysis. When comparing results from both analyses it was seen that the magnitude of the correlation and shape of the curve were different, but both correlations were positive. When compared to literature, Hüttmann et al. (2009) found a genetic correlation ranging from 0.42 to 0.93 for 11 to 180 days in milk. However, in Hüttmann et al. (2009) the correlation was moderate in early lactation and went up

towards 180 days in milk, while for initial analyses in the current study the correlation went down after week 14 of lactation. For the subsequent analysis the correlation went down from early towards mid-lactation.

Genetic correlation between LWT and DMI ranged from 0.17 to 0.87 (not significant for any week of lactation) for initial analyses and 0.36 to 0.59 for the subsequent analysis. When comparing results from both analyses it was seen that the correlation found for initial analyses started higher and increased more from early to mid-lactation compared to the subsequent analysis, however towards late lactation the correlation remained stronger for the subsequent analysis. Veerkamp and Thompson (1999) found correlations ranging from 0.65 to 0.84 for weeks 3 to 15 of lactation. This was similar to correlations found for initial analyses but higher than for the subsequent analysis. Both correlations found in the current study were in the same direction as found by Veerkamp and Thompson (1999).

Genetic correlations found for initial and subsequent analyses show differences. For the correlation between MY and LWT and between MY and DMI the curve over lactation was quite different and for MY and LWT sometimes even in a different direction. These differences can have multiple causes. The dataset used for initial analyses only consisted of 198 cows with 8,520 observations for MY, 8,353 for LWT and 6,612 for DMI. The dataset used for the subsequent analysis consisted of 521 cows with 30,026 observations for MY, 29,164 for LWT and 23,895 for DMI. It is possible that the dataset used for initial analyses was not representative for the whole herd. The dataset used for the subsequent analysis was larger and observations were only selected based on whether a MY, LWT and DMI observation were available for a certain week.

This does not explain differences between correlations between production traits and CH_4 production as the same number of observations was available for both analyses. For initial analyses multiple bivariate models were analysed, while for the subsequent analysis one 4-trait model was used. This meant that

for the subsequent analysis all four dependent variables were taken into account simultaneously, whereas for initial analyses dependent variables were analysed pairwise. This could have led to differences in partitioning of variances.

Finally, it should be taken into account that the fixed effects in the models were different between the initial and subsequent analyses. The biggest change was using age at calving and age at calving squared instead of age at measurement. Initially age at measurement was used, as age at calving was confounded with lactation number. However, age at calving is widely used in literature and is more appropriate to use as it directly affects expression of the traits.

As more data were available for the subsequent analysis and models used were more appropriate, results from the subsequent analysis are seen as more reliable.

For the subsequent analysis correlation between MY and LWT was negative and became stronger towards late lactation. From a biological point of view this was as expected as these traits are competitors for available energy. Genetic correlations found between MY and DMI for the subsequent analysis were positive throughout lactation. From a biological point of view this was as expected as cows with a higher milk production require a higher energy intake (Veerkamp, 2000). Genetic correlations found between LWT and DMI for the subsequent analysis were positive throughout lactation. From a biological point of view this was as expected, as cows with a higher LWT are expected to need more DMI for maintenance (Veerkamp, 1998).

Again, for many studies using random regression models estimates of significance were not provided.

Genetic correlations between production traits and CH_4 production

Genetic correlation between MY and CH_4 production was -0.55 to 0.06 for initial analyses and -0.09 to -0.36 for the subsequent analysis (correlations were not significant at any stage of lactation for either of the analyses).

When comparing both analyses it was seen that correlations found were different. Correlation found for initial analyses started off small but positive and then became large and negative. Correlation found for the subsequent analysis starts of large and negative and became smaller over lactation but did not become positive at any point. When compared to literature, De Haas et al. (2011) found a correlation of -0.66 for week 1 to 5, -0.18 for week 6 to 10, 0.42 for week 11 to 15, 0.67 for week 16 to 20, 0.70 for week 21 to 25 and 0.60 for week 26 to 30. Lassen and Løvendahl (2016) found a significant correlation of 0.43 using a linear animal model. These correlations were as expected from a biological point of view. At early lactation cows enter a negative energy balance. Therefore, although milk is produced, body energy is utilised as well as energy from DMI. Therefore, in early lactation the correlation is expected to be negative. Later in lactation energy for milk production from DMI is sufficient and therefore a positive correlation between DMI and CH_4 production is expected.

Genetic correlation between LWT and CH_4 production was -0.09 to 0.02 for initial analyses and 0.00 to 0.09 for the subsequent analysis (correlations were not significant at any stage of lactation for either of the analyses).

When comparing both analyses it was seen that correlations were close to zero for both analyses, but correlations move in a different direction. When compared to literature, Lassen and Løvendahl (2016) found a significant correlation of -0.18 using a linear animal model. From a biological point of view both a positive and a negative correlation were possible. When looking at the whole lifetime of the cow, LWT started off low and increased up to a certain level after which it remained stable. DMI and CH_4 production move in the same direction as heavier cows need more food for maintenance and therefore have more substrate to produce CH_4. This results in a positive correlation between LWT and CH_4 production. When looking at lactation level, due to negative energy balance in early lactation LWT decreased in early

lactation after which it increases again towards late lactation. CH_4 production follows the same curve as DMI, which starts low in early lactation, peaks in early lactation and then slowly declines towards late lactation. This results in a negative correlation between LWT and CH_4 production. For the analyses in the current study it is possible that these two mechanisms cancel each other out.

Genetic correlation between DMI and CH_4 production was -0.79 to 0.42 (not significant for any week of lactation) for initial analyses and 0.05 to 0.11 (not significant at any point of lactation) for the subsequent analysis.

When comparing initial and subsequent analyses it was seen that magnitude and curve of correlations were different. Correlation for initial analyses started off large and negative and became moderately positive towards late lactation. Correlation derived from the subsequent analysis remained low and positive throughout lactation. Manzanilla-Pech et al. (2016a) found a significant correlation of 0.83 between DMI and CH_4 production. From a biological point of view a strong positive correlation was expected as DMI is the main driver behind CH_4 production.

As discussed in chapter 2, it should be noted that Legendre polynomials can overestimate measurement errors in early and late lactation (Brotherstone et al., 2000), due to less observations being available for these weeks of lactation. Again, for many studies using random regression models estimates of significance were not provided.

It should be noted that no Legendre polynomial was fitted on WOL in interaction with genetic animal variance for CH_4 production for the Langhill herd due to the size of the dataset, and that therefore the correlations with production traits were based on a genetic variance that was constant over lactation for CH_4 production. In practise it is expected that the genetic variance of CH_4 production varies over weeks of lactation as well. Not taking this into account will have influenced the correlations obtained between CH_4 production and the production traits. However, CH_4 production has been

corrected for WOL and therefore these results do give a first indication of genetic correlations with CH_4 production.

3.5 CONCLUSION

For the Langhill herd CH_4 production was measured using LMD. Final analyses were performed with a 4-trait random regression repeatability (MY, LWT and DMI) and linear (CH_4) animal model. Heritability (s.e.) for CH_4 production obtained from this analysis was 0.22 (0.15). Heritability obtained was not significant, which is likely due to the size of the dataset (for discussion on this, see Chapter 7 section 2). If this heritability was significant, this would indicate heritability for CH_4 production is low. Genetic selection on this trait would be possible, but improvement would be slow over generations.

Genetic correlations (s.e.) with CH_4 production resulting from this analysis were -0.36 (0.21) to 0.09 (0.22) with MY, 0.00 (0.21) to 0.09 (0.26) with LWT, and 0.05 (0.27) to 0.11 (0.35) with DMI. Neither correlation found was significant at any point in lactation (for discussion on this, see Chapter 7 section 2). If these correlations would have been significant, this would have indicated the following; Increasing MY would be accompanied by decreasing CH_4 production in early lactation and accompanied by increasing CH_4 production from mid to late lactation. CH_4 production and LWT do not influence each other on a genetic level. DMI and CH_4 production only slightly positively influence each other on a genetic level.

4. COMBINED ANALYSIS OF THE NOTTINGHAM AND LANGHILL HERD

4.1 INTRODUCTION

When new traits like CH_4 production are investigated, one of the challenges is to obtain a large enough dataset to find significant results. Initial studies are often small, and as different research groups are working in the same research areas, methods and techniques used will differ. As described in chapter 1 this is also the case for CH_4 production. Currently it is questioned whether the different measurement techniques that are in use, are actually measuring the same genetic trait. To confirm this, the genetic correlation between CH_4 traits measured with different techniques needs to be obtained. Ideally this would be done by measuring the same cow with the same measuring technique. This is not always possible, due to labour, logistical and financial constraints. However, when measurements with different measuring techniques are performed on cows that are genetically related to each other, it is possible to obtain a genetic correlation between the traits these measuring techniques investigate.

Therefore, the aims of this chapter were to estimate;

1) genetic parameters for CH_4 production measured based on a combined analyses of the Nottingham and Langhill herd, where CH_4 production was measured with the use of the laser methane detector and in the milking robot during milking respectively.

2) the correlation between CH_4 production measured using the laser methane detector and measured in the milking robot during milking.

4.2 METHODS

The datasets used in chapter 2 and 3 were combined into one dataset. Variables that were available from both datasets and were measured in the same way (e.g. age at measurement, calving season, lactation number and LWT) were merged to one variable. CH_4 production was measured in different ways, and therefore was kept in the dataset as two different variables. MY was measured in different ways for the two herds as well. In the Nottingham herd cows have 24-h access to the milking robot, which resulted in 3.5 milkings per day on average. In the Langhill herd cows were milked in the milking robot three times a day at set times. This difference in recording systems could mean that a different milk yield trait has been measured in the two herds. However, literature shows no evidence was found that this is the case when investigating once daily milking compared to twice daily milking (Lembeye et al., 2018). It therefore was assumed that measuring milk yield with an automated system is not different from measuring milk yield three times a day and MY observations were merged to one variable.

A pedigree was composed with all cows from both datasets included in the base population. The pedigree was limited to three generations and consisted of 514 sires and 2,243 dams (pedigree analyses were performed with the CFC software package (Sargolzaei et al., 2006)). CH_4 production was measured with the use of a laser methane detector (LMD) for the Langhill herd (see chapter 2) and in the milking robot during milking for the Nottingham herd (see chapter 3). The interest lies in whether these two measurement techniques are measuring the same genetic trait. As the measurement technique used was dependent on the herd the cow belonged to, no cows were measured with both techniques. Therefore, obtaining the genetic correlation between CH_4 production measured with these two methods is based on the genetic linkage between the two herds due to common ancestral sires. To gain initial insight in how linked the two herds are, the number of sires, paternal grand sires (GSires) and paternal great

grand sires (GGSires) the two herds have in common was examined (Table 4.1). Each ratio shows number of males in the pedigree of that herd which are also in the pedigree of the other herd, divided by number of males in the pedigree of that herd. For example, there were 43 GSires in the Nottingham pedigree, and 32 of those males were also in the Langhill pedigree.

Table 4.1: Number of sires, paternal grand sires (GSires) and paternal great grand sires (GGSires) in the Nottingham and Langhill herd that are present in the other herd, as a ratio of total sires in this herd

	Nottingham	Langhill
Sires	0/67	4/51
GSires	32/43	28/37
GGSires	16/19	22/29

To investigate this linkage, a principal component analysis (PCA) was performed on the genetic relationship matrix (A-matrix) using the "princomp" function of the R software package (R Core Team, 2016). From Figure 4.1 it can be seen that the cows from both herds are related with an overlap between the individual Langhill lines with the overall Nottingham population. This justifies the merging of the datasets and the analysis of traits measured in a similar manner in both herds as one trait.

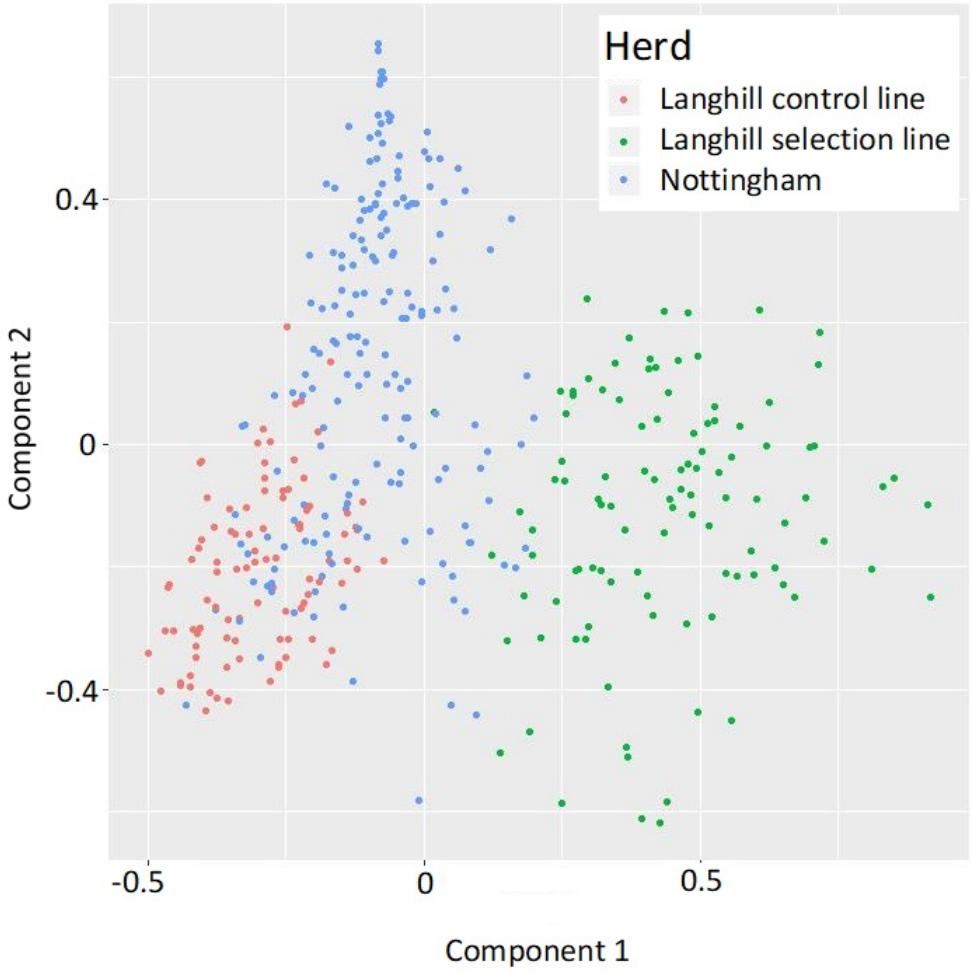


Figure 4.1: Principal component analysis (PCA) of the genetic (pedigree) relationship matrix of Langhill and Nottingham cows with component 1 and 2 explaining 9.6% and 2.7% of the total variation

4.2.1 STATISTICAL ANALYSES

Models

As the genetic linkage between the herds is based on common ancestral sires, sire models were used to obtain the genetic parameters. Univariate linear (repeatability) sire models were started based on the final animal models obtained in chapter 2 and 3. Models were continued as multivariate linear (repeatability) sire models, up to a 5-trait linear (repeatability) sire model with MYcomb, LWTcomb, DMILang, CH_4Notts and CH_4Lang as the dependent

variables;

$$MYcomb = \mu + herd + lact.WOL_leg5 + genline + feedgr + rec_wk + aac + aac2 + Sire + AnPE + e$$

$$LWTcomb = \mu + herd + lact + WOL_leg5 + genline + feedgr + rec_yr + rec_wk + calv_seas4 + Sire + AnPE + e$$

$$DMILang = \mu + lact.WOL_leg5 + genline + feedgr + rec_yr + rec_wk + calv_seas4 + Sire + AnPE + e$$

$$CH_4Nottingham = \mu + WOL_leg5 + rec_yr + Sire + AnPE + e$$

$$CH_4Langhill = \mu + MOL_leg3 + genline + feedgr + ryCH4 + rec_wk + calv_seas2 + Sire + AnPE + e$$

Where μ is the general mean; herd is the research herd the cow is housed in; lact.WOL_leg5 is the interaction between lactation number and a Legendre polynomial of order 5 on week of lactation as a fixed effect; lact is the fixed effect of lactation number; WOL_leg5 is a Legendre polynomial of order 5 interacted with week of lactation as a fixed effect; MOL_leg3 is a Legendre polynomial of order 3 interacted with month of lactation as a fixed effect; genline is the fixed effect of genetic line; feedgr is the fixed effect of feed group; rec_yr is the fixed effect of the year the measurement took place; ryCH4 is the fixed effect of the record year group the CH_4 measurement took place (two groups); rec_wk is the fixed effect of the week the measurements took place; calv_seas4 is the effect of the season in which calving took place (4 seasons); calv_seas2 is

the effect of the season in which calving took place (2 seasons); aac is the covariate that describes the effect of age at calving at time of measurement; aac2 is the squared effect of aac; Sire is the genetic effect of sire; AnPE is the permanent environment effect of the animal; and e is the random residual.

To be able to use a sire model for the analyses only cow observations originating from sires with 50 or more daughter observations and 3 or more daughters in the dataset were used (1,279 observations were removed). Cow observations for which the sire was unknown were removed (265 observations, from 12 cows). As DMI observations were only available for the Langhill herd and CH_4 production was recorded using different methods in each herd, the only way to run this model was to fix the covariance between these traits to a set value, chosen by the user. Therefore, the correlation calculated between these traits was strongly influenced by the user and does not reflect the true correlation between those traits. When these covariances were not fixed by the user, singularities in the average information matrix occurred, causing the analysis to abort.

Therefore, both a trivariate linear (repeatability) and a 4-trait linear repeatability sire model were used instead. Only dependent variables from the same herd or variables combined for both herds were fitted in the model at the same time. Fixed effects were kept identical between the two models for MYcomb and LWTcomb and were only kept in the model if significant for both models. Exceptions were made for herd, genetic group and feed group, which were kept in the models regardless, due to the design of the experiments.

For the CH_4 production traits the fixed effects used were the same as used for the previous chapters, even if they were not significant in the current models. This was done as it was already shown that these variables have a significant effect given the structure of the individual datasets. It is assumed that if these variables do not have a significant effect in the analyses of the combined

dataset this is due to the increased size of the dataset,as the underlying structure of the CH_4 dataset has not changed. The following models were used;

Trivariate model between MYcomb, LWTcomb and CH_4Notts;

$$MYcomb = \mu + herd + lact.WOL_leg5 + genline + feedgr + rec_wk + aac + aac2 + Sire + AnPE + e$$

$$LWTcomb = \mu + herd + lact + WOL_leg5 + genline + feedgr + rec_yr + rec_wk + YOB + calv_seas4 + aac + aac2 + Sire + AnPE + e$$

$$CH_4Nottingham = \mu + WOL_leg1 + rec_wk + Sire + AnPE + e$$

4-trait model between MYcomb, LWTcomb, DMILang and CH_4Langh;

$$MYcomb = \mu + herd + lact.WOL_leg5 + genline + feedgr + rec_wk + aac + aac2 + Sire + AnPE + e$$

$$LWTcomb = \mu + herd + lact + WOL_leg5 + genline + feedgr + rec_yr + rec_wk + YOB + calv_seas4 + aac + aac2 + Sire + AnPE + e$$

$$DMILang = \mu + lact.WOL_leg5 + genline + feedgr + rec_wk + rec_yr + calv_seas4 + Sire + AnPE + e$$

$$CH_4Langhill = \mu + MOL_leg3 + gengr + feedgr + rec_wk + ryCH4 + calv_seas2 + Sire + e$$

Where μ is the general mean; herd is the research herd the cow is housed in; lact.WOL_leg5 is the interaction between lactation number and a Legendre polynomial of order 5 on week of lactation as a fixed effect; lact is the fixed effect of lactation number; WOL_leg5 is a Legendre polynomial of order 5 interacted with week of lactation as a fixed effect; MOL_leg3 is a Legendre polynomial of order 3 interacted with month of lactation as a fixed effect; genline is the fixed effect of genetic line; feedgr is the fixed effect of feed group; rec_yr is the fixed effect of the year the measurement took place; ryCH4 is the fixed effect of the record year group the CH_4 measurement took place; rec_wk is the fixed effect of the week the measurement took place; calv_seas4 is the effect of the season in which calving took place (4 seasons); calv_seas2 is the effect of the season in which calving took place (2 seasons); aac is the covariate that describes the effect of age at calving at time of measurement;

aac2 is the squared effect of aac; Sire is the genetic effect of sire; AnPE is the permanent environment effect of the animal; and e is the random residual.

From these models variance parameters were estimated with the use of restricted maximum likelihood (REML) using ASReml 3.0 software (Gilmour et al., 2009). These variances were used to derived heritabilities using the following formula (Falconer and Mackay, 1996);

Heritability:

$$h^2 = \frac{V_S * 4}{V_P}, \tag{5}$$

where V_S is the genetic sire variance and V_P is the phenotypic variance.

Correlations within models were derived with formula 4 described in chapter 2. All calculations were performed with ASReml 3.0 (Gilmour et al., 2009).

4.2.2 Covariances and correlations

From the results of the trivariate and 4-trait models the covariance between CH_4Lang and CH_4Notts was calculated with the use of the following formula (adapted from Mrode (2005) by team members of EGENES):

$$Cov_{y,z} = \frac{Cov_{x,z} * Cov_{x,y}}{Var_x}, \tag{6}$$

where *y* and *z* are the traits between which the covariance is to be calculated, and *x* is a trait for which the covariance with *y* and *z* is known. Here *y* and *z* are CH_4Lang and CH_4Notts. The covariance was calculated with both MYcomb and LWTcomb used as trait *x* and results were compared to see whether there is consistency in the calculated covariance. The estimated covariance was then used to calculate the genetic correlation between CH_4Lang and CH_4Notts with

Formula 4 from chapter 2.

When obtaining the (co)variances from the trivariate model between MYcomb, LWTcomb and CH_4Notts, it was noticed that the (co)variances and correlations between MYcomb and CH_4Notts, and LWTcomb and CH_4Notts vary between the bivariate and trivariate models. To gain insight in how the correlations between these traits are influenced by other traits, conditional correlations were obtained and compared between bivariate and trivariate models both for the combined dataset and the Nottingham-only dataset. The models used for this comparison for the combined dataset were the trivariate model described above and bivariate models based on this model while dropping one dependent variable at a time. For the Nottingham-only dataset the bivariate models in chapter 2 were rewritten into linear repeatability models and the trivariate model used was based on these bivariate models;

$$MYNottingham = \mu + lact.WOL_leg5 + rec_wk + age + Animal + AnPE + e$$

$$LWTNottingham = \mu + WOL_leg4 + lact + calv_seas4 + rec_wk + age + Animal + AnPE + e$$

$$CH_4Nottingham = \mu + MOL_leg1 + rec_wk + An + AnPE + e$$

Where μ is the general mean; lact.WOL_leg5 is the interaction between lactation number and a Legendre polynomial of order 5 on week of lactation as a fixed effect; lact is the fixed effect of lactation number; WOL_leg4 is a Legendre polynomial of order 4 interacted with week of lactation as a fixed effect; MOL_leg1 is a Legendre polynomial of order 1 interacted with month of lactation as a fixed effect; rec_wk is the fixed effect of the week the measurement took place; calv_seas4 is the effect of the season in which

calving took place (4 seasons); age is the covariate that describes the effect of age at measurement at time of measurement; An is the additive genetic effect of animal as a random effect; AnPE is the permanent environment effect of the animal as a random effect; and e is the random residual.

The following formulae were used to calculate the conditional (co)variances (Pryce et al., 2002). Formula 7 shows how the variance for MYcomb is adjusted for the variance of LWTcomb;

$$\sigma^2 MYcomb^* = \sigma^2 MYcomb - \frac{\sigma^2 LWTcomb, MYcomb}{\sigma^2 LWTcomb}, \quad (7)$$

where, $\sigma^2 MYcomb^*$ is the variance adjusted for MYcomb, $\sigma^2 LWTcomb$, *MYcomb* is the squared unadjusted covariance between LWTcomb and MYcomb, and $\sigma^2 LWT$ is the unadjusted variance for LWTcomb.

In the same way the variance of LWTcomb was adjusted for the influence of MYcomb, and the variance of CH_4Notts was adjusted for the influence of MYcomb, and LWTcomb.

Formula 8 shows how the covariance between MYcomb and CH_4Notts is adjusted for LWTcomb;

$$\sigma MYcomb^*, CH_4Notts^* =$$

$$\sigma MYcomb, CH_4Nottingham - \quad (8)$$

$$\frac{\sigma LWTcomb, MYcomb * \sigma LWTcomb, CH_4Notts}{\sigma^2 LWTcomb},$$

where, $\sigma MYcomb^*, CH_4Notts^*$ is the covariance between MYcomb and CH_4Notts adjusted for LWTcomb; $\sigma MYcomb, CH_4$ *Nottingham* is the obtained

covariance between MYcomb and CH_4Notts; $\sigma LWTcomb,MYcomb$ is the covariance between LWTcomb and MYcomb; $\sigma LWTcomb,CH_4Notts$ is the covariance between LWTcomb and CH_4Notts; and $\sigma^2 LWTcomb$ is the variance of LWTcomb. In the same way the covariance between $\sigma LWTcomb^*,CH_4\ Notts$ was adjusted for the influence of LWTcomb.

4.3 RESULTS

Multivariate analyses

Results of the trivariate analyses of MYcomb, LWTcomb and CH_4Notts, and the 4-trait of MYcomb, LWTcomb, DMILang and CH_4Lang are in Tables 4.2 and 4.3.

Table 4.2: Heritabilities (s.e.) (on-diagonal, bold), genetic (s.e.) (below diagonal) and phenotypic (s.e.) (above diagonal) correlations obtained from trivariate analyses of milk yield combined (MYcomb), live weight combined (LWTcomb) and CH_4 production Nottingham (CH_4Notts)

Trivariate	MYcomb	LWTcomb	CH_4Notts
MYcomb	**0.40 (0.11)**	0.07 (0.03)	0.23 (0.05)
LWTcomb	0.29 (0.21)	**0.56 (0.15)**	0.15 (0.06)
CH_4Notts	0.23 (0.43)	0.50 (0.50)	**0.22 (0.19)**

Table 4.3: Heritabilities (s.e.) (on-diagonal, bold), genetic (s.e.) (below diagonal) and phenotypic (s.e.) (above diagonal) correlations obtained from 4-trait analyses of milk yield combined (MYcomb), live weight combined (LWTcomb), dry matter intake Langhill (DMILang) and CH_4 production Langhill (CH_4Lang)

4-trait	MYcomb	LWTcomb	DMILang	CH_4Lang
MYcomb	**0.40 (0.11)**	0.07 (0.03)	0.48 (0.02)	0.06 (0.06)
LWTcomb	0.28 (0.21)	**0.55 (0.15)**	0.32 (0.02)	0.07 (0.05)
DMILang	0.71 (0.12)	0.70 (0.14)	**0.20 (0.07)**	0.01 (0.08)
CH_4Lang	-0.31 (0.43)	0.85 (0.41)	0.48 (0.44)	**0.28 (0.25)**

From the tables it can be seen that the parameters for MYcomb and LWTcomb

were similar between the two models. For MYcomb heritability was 0.40 for both models. For LWTcomb heritability was 0.55 to 0.56. For DMILang heritability was 0.20. For CH_4Notts heritability was 0.22. For CH_4Lang heritability was 0.28.

The genetic correlation between MYcomb and LWTcomb was 0.28 to 0.29. The phenotypic correlation between these traits was 0.07. The genetic correlation between MYcomb and DMILang was 0.71, the phenotypic correlation was 0.48. The genetic correlation between LWTcomb and DMILang was 0.70, the phenotypic correlation was 0.32. The genetic correlation between LWTcomb and DMILang was 0.70, the phenotypic correlation was 0.32. The genetic and phenotypic correlations between the two CH_4 traits, and MYcomb and LWTcomb ware different. The genetic correlation between MYcomb and CH_4Notts was 0.23, whereas the genetic correlation between MYcomb and CH_4Lang was -0.31. The phenotypic correlation between MYcomb and CH_4Notts was 0.23, whereas the phenotypic correlation between MYcomb and CH_4Lang was 0.06. Only the correlation between MYcomb and CH_4Notts was significant. The genetic correlation between LWTcomb and CH_4Notts was 0.50, whereas the genetic correlation between MYcomb and CH_4Lang was 0.85. The phenotypic correlation between LWTcomb and CH_4Notts was 0.15 but the phenotypic correlation between LWTcomb and CH_4Lang was 0.07. Only the correlation between LWTcomb and CH_4Notts was significant.

The genetic correlation between DMILang and CH_4Lang was 0.48, the phenotypic correlation was 0.01.

Covariances and correlations

Calculated correlation between the two CH_4 traits

The covariance between CH_4Langhill and CH_4Notts was obtained with Formula 7 and 8 where both MYcomb and LWTcomb were used as trait *x*. When MYcomb was used, the covariance found was -0.22 and when LWTcomb was

used the covariance was 0.28. The correlations calculated from this were -0.15 and 0.20, when trait x was MYcomb or LWTcomb respectively.

Conditional correlations

Conditional correlations were calculated between MYcomb and CH_4Notts, and LWTcomb and CH_4Notts. For both the combined dataset and the Nottingham-only dataset bivariate and trivariate models were analysed.

Table 4.4: Obtained and conditional correlations for bivariate (bivar) and trivariate (trivar) analyses of milk yield (MY), milk yield combined (MYcomb), live weight (LWT), live weight combined (LWTcomb) and CH_4 production (CH_4) for the Nottingham-only dataset and the combined Nottingham and Langhill dataset

	Obtained correlations		Conditional correlations	
	MY,CH_4	LWT,CH_4	MY,CH_4[1]	LWT,CH_4[2]
Bivar comb	0.46	0.12	0.50	-0.02
Trivar comb	0.71	0.43	0.68	0.35
Bivar Notts	0.99	0.19	NE[3]	NE[3]
Trivar Notts	0.78	0.16	0.77	0.92

[1]Corrected for LWTcomb
[2]Corrected for MYcomb
[3]Not estimable

The results for the trivariate analyses showed that the obtained correlation between MYcomb and CH_4Notts was similar for both datasets (0.71 for the combined dataset and 0.78 for the Nottingham-only dataset), as was the conditional correlation adjusted for LWTcomb (0.68 for the combined dataset and 0.77 for the Nottingham-only dataset).

When looking at the obtained correlation between LWTcomb and CH_4Notts there was a difference between the two datasets. For the combined dataset the correlation was 0.43 whereas for the Nottingham-only dataset the correlation was 0.16. The correlation between LWTcomb and CH_4Notts corrected for MYcomb was different between both datasets as well (0.35 for the combined

dataset and 0.92 for the Nottingham-only dataset) and the difference with the obtained correlation was in the opposite direction. Where the adjusted correlation was lower than the obtained correlation for the combined dataset, for the Nottingham-only dataset the conditional correlation went up by 475%, from 0.16 to 0.92.

This indicates that for the combined dataset the correlation between LWTcomb and CH_4Notts is positively influenced by MYcomb as the corrected correlation is lower than the obtained correlation. For the Nottingham-only dataset the correlation between LWTcomb and CH_4Notts is negatively influenced by MYcomb as the corrected correlation is higher than the obtained correlation.

4.4 DISCUSSION

Multivariate analyses

Results have shown that the heritabilities, genetic and phenotypic correlations found for MYcomb and LWTcomb were similar between the analyses of the trivariate and 4-trait models. This was as expected as both traits contain data combined from both herds and therefore both analyses contained largely the same information.

Heritabilities for production traits

Heritability for MYcomb was 0.40, which was as expected from literature. Veerkamp et al. (2011) found a heritability of 0.47 for MY (weekly) with the use of a linear animal model and Kadarmideen et al. (2003) found a heritability of 0.28 for 305-day MY with the use of a linear repeatability animal model, which was lower than heritability found in the current study.

Heritability for LWTcomb was 0.55 to 0.56, which was similar to heritabilities found in literature. Berry et al. (2003*b*) found a heritability for average body weight (day 5 to 180 in milk, 4 measuring points) of 0.60 and Veerkamp

et al. (2011) found a heritability of 0.59 for body weight (weekly) with the use of a linear animal model. However, Köck et al. (2018) found a lower heritability of 0.31 using a linear animal model. Heritability for DMILang was 0.20, which was lower than most heritabilities found in literature. De Haas et al. (2012) found a heritability of 0.37 for DMI (both predicted averages and measured average DMI) for the UK, the Netherlands and Australia combined using a linear animal model. Veerkamp et al. (2011) found a heritability of 0.78 for daily recorded DMI with the use of a linear animal model using pedigree information. Although literature estimates of heritability for direct DMI were higher than the heritability found in the current study, literature shows there is a wide variation in obtained heritabilities.

Genetic correlations between production traits

The genetic correlation between MYcomb and LWTcomb was 0.28 to 0.29. When compared to literature, the correlation found in the current study was higher than found by Veerkamp et al. (2011) who found a genetic correlation of 0.19 between MY (weekly) and body weight (weekly) using a linear animal model. However, other studies have found a negative correlation between MY and LWT. For example, Berry et al. (2003) found a correlation of -0.01 (estimates of significance were not provided) between MY (cumulative to day 240 in milk) and average body weight (day 5 to 180 in milk, 4 measuring points). Manzanilla-Pech et al. (2016) found a correlation of -0.09 between MY (weekly) and body weight (weekly) for the Netherlands and -0.29 between MY (daily) and body weight (daily, weekly or biweekly) for the United States (neither estimate was significant). From a biological point of view both a positive and a negative correlation are possible. When a cow utilises body fat to obtain energy for milk production, a negative correlation is expected. When a cow produces milk from feed intake, it is expected that bigger cows have a higher feed intake. Part of the energy obtained from feed will go towards body

maintenance (depending on the stage of lactation), while the other part of the energy will go towards milk production. Depending on whether this balance tips in favour of maintenance or production, a negative correlation or a positive correlation is expected respectively.

The genetic correlation between MYcomb and DMILang was 0.71, which is higher than correlations found in literature. Manzanilla-Pech et al. (2016b) found a correlation of 0.44 between MY (weekly) and DMI (weekly) for the Netherlands, and a correlation of 0.36 between MY (daily) and DMI (daily) for the United States, using a linear animal model for both countries. Veerkamp et al. (2011) found a genetic correlation of 0.44 between MY (weekly) and DMI (daily) using a linear animal model.

The genetic correlation between LWTcomb and DMILang of 0.70 was similar to the correlation of 0.75 found by Veerkamp et al. (2011) between LWT (weekly) and DMI (daily) using a linear animal model. However, Manzanilla-Pech et al. (2016b) found a correlation of 0.55 between body weight (weekly) and DMI (weekly) for the Netherlands, and a correlation of 0.46 between body weight (daily, weekly or biweekly) and DMI (daily) for the United States. These correlations were lower than correlations found in the current study.

Heritabilities for CH_4 production

Heritabilities of 0.22 for CH_4Notts and 0.28 for CH_4Lang were as expected from literature. De Haas et al. (2011) found a heritability between 0.29 to 0.42 (0.10 to 0.12) for predicted CH_4 production and Lassen et al. (2016) found a heritability of 0.25 (0.16, not significant) for CH_4 production obtained from a ratio between CH_4 and CO_2 analysed with the Fourier transform infrared method. The heritability for CH_4Lang was not significant, which was as expected. Heritability was not significant in the Langhill only analyses and combining the datasets did not increase the number of observations on CH_4Lang. Heritability for CH_4Notts was significant for part of the lactation

and therefore it was expected that a significant heritability was found for the analyses of the combined dataset, even though the number of observations for CH_4Notts did not increase either.

Genetic correlations between production traits

As mentioned in the results section, genetic and phenotypic correlations found between the CH_4 traits, and MYcomb and LWTcomb were different for the two herds. The genetic correlation between MYcomb and CH_4Notts was 0.23 and between MYcomb and CH_4Lang was -0.31. Lassen and Løvendahl (2016) found a correlation of 0.43 between FPCM (fat and protein corrected milk) and CH_4 production obtained from the ratio of CH_4 and CO_2 concentration with the use of a linear animal model. This was higher than the correlation found between MYcomb and CH_4Notts but in the same direction. The negative correlation found between MYcomb and CH_4Lang was not as expected as from a biological point of view cows producing more milk have a higher feed demand. As CH_4 is a product of rumen fermentation, a higher feed intake will lead to a higher production of CH_4, assuming that the extra intake gets utilised. However, in early lactation many cows utilise body energy next to energy from feed intake to produce milk. This can lead to a negative correlation between MYcomb and CH_4Lang. De Haas et al. (2011) found a correlation of -0.66 for week 1 to 5, -0.18 for week 6 to 10, 0.42 for week 11 to 15, 0.67 for week 16 to 20, 0.70 for week 21 to 25 and 0.60 for week 26 to 30 (no standard errors were given) when using a random regression animal model. This shows that indeed in early lactation the genetic correlation between MYcomb and CH_4Lang can be negative.

The genetic correlation between LWTcomb and CH_4Notts was 0.50, and between LWTcomb and CH_4Lang was 0.85. When compared to literature Lassen and Løvendahl (2016) found a correlation of -0.18 (0.08, significant) between LWT (measured at milking) and CH_4 production obtained from the

ratio of CH_4 and CO_2 concentration. This correlation was in the opposite direction than the correlations found in the current study. As already discussed in chapter 2 and above, from a biological point of view both a positive and a negative correlation were possible.

The genetic correlation between DMILang and CH_4Lang was 0.48. Manzanilla-Pech et al. (2016a) found a correlation of 0.83 (0.05, significant) between CH_4 production and DMI, which is higher than the correlation found in the current study. From a biological point of view a strong positive correlation was expected as DMI is the main driver behind CH_4 production.

Correlation between CH_4 production traits

As it was not possible to include CH_4Notts and CH_4Lang in the same model simultaneously, the covariance between these traits was obtained with Formula 6. Based on this covariance the correlation was calculated. The conditional covariance was based on the covariance between the CH_4 traits and either MYcomb or LWTcomb. When MYcomb was used the conditional correlation was -0.15 and when LWTcomb was used the conditional correlation was 0.20. Although the direction of the correlations was opposite, both results showed a weak genetic correlation between CH_4Notts and CH_4Lang. This showed that CH_4Notts and CH_4Lang were genetically different traits.

Conditional correlations

As can be seen from Table 5.3 problems arose obtaining conditional correlations based on the results of the analyses using bivariate models of the Nottingham dataset, leading to negative variances which are not possible. These negative variances indicate that the bivariate models are less robust than the trivariate model where no negative variances were found.

For the analyses of the trivariate model the small difference between the obtained correlation and the conditional correlation between MYcomb and

CH_4Notts shows that LWTcomb has almost no influence on this correlation.

When looking at the obtained correlations between LWTcomb and CH_4Notts the difference in results between the two datasets is unexpected. Although the combined dataset includes more observations on *LWT*, these extra observations were not linked to any CH_4 observations and therefore were not expected to influence the correlation.

However, as for the combined analyses a sire model was used, observations for which the sire was unknown were removed, as were observations originating from sires with less than 50 daughter observations or less than 3 daughters in the dataset. Therefore, the combined dataset included fewer observations on the Nottingham herd than the Nottingham-only dataset. For the Nottingham-only dataset analyses the pedigree was based on the Nottingham cows only, for the combined analyses the pedigree was based on cows from both herds. As the herds are genetically linked this will have influenced the analyses as well, as has the use of sire models versus animal models.

When looking at the conditional correlations between LWTcomb and CH_4Notts it was seen that this correlation was weaker than the obtained correlation for the combined dataset, but becomes stronger for the Nottingham-only dataset. This disagreement was unexpected, but can be explained by the differences mentioned before. From a biological point of view the correlation between LWT and CH_4 production is driven by DMI and hence indirectly by MY. Therefore, it was expected that the conditional correlation between LWTcomb and CH_4Notts corrected for MYcomb was lower than the obtained correlation. This was in agreement with the results from the trivariate analyses of the combined dataset.

4.5 CONCLUSION

For the combined analyses of the Nottingham and Langhill herd, CH_4 production measurements were treated as individual traits. For the analysis of

CH_4 production for the Nottingham herd a trivariate linear repeatability sire model was used. For the analysis of CH_4 production for the Langhill herd a 4-trait linear (CH_4) repeatability (MY, LWT and DMI) sire model was used. Heritability (s.e.) for CH_4 production for the Nottingham herd obtained from this analysis was 0.22 (0.19). Heritability (s.e.) for CH_4 production for the Langhill herd obtained from this analysis was 0.28 (0.25). Heritability found for each herd was not significant. If heritabilities were significant, this would indicate heritability for CH_4 production is low. Genetic selection on this trait would be possible, but improvement would be slow over generations.

The correlation between CH_4 production for the Nottingham herd and CH_4 production for the Langhill herd was calculated based on the correlations each of these traits had with MYcomb, and with LWTcomb. Correlations calculated between CH_4 production for the Nottingham herd and CH_4 production for the Langhill herd were -0.15 (based on MYcomb) and 0.20 (based on LWTcomb). This indicates that CH_4 production as measured in the Nottingham herd and as measured in the Langhill herd are genetically not the same trait.

5. ANALYSIS OF THE ELLINBANK AND LANGHILL HERD

5.1 INTRODUCTION

When datasets are limited by the number of observations available, it is important to use the available data as effectively as possible. Although pedigrees are recorded extensively, sometimes links between animals are overlooked. Introducing genotype data to the analyses might reveal more links between animals aiding the analyses of the phenotypic data. In this chapter results are described and compared between analyses based on pedigree information only, genotype information only (G-matrix analyses), and pedigree and genotype information combined (H-matrix analyses). When performing G-matrix analyses (Henderson, 1975) a G-matrix is used which contains relationship coefficients between animals based on their genotype. This matrix can be calculated as described by VanRaden (2008). When performing H-matrix analyses an H-matrix is build combining the pedigree and genotype information in one matrix, as described by Legarra et al. (2014). These matrices are then included in the analyses in the same way as the A matrix is included for pedigree based analyses (see chapter 2), resulting in genomic parameters as heritabilities and genomic correlations.

Gaining insight in correlations between traits is important as selecting on one trait inevitably has an effect on other traits. Therefore, when constructing a selection index, heritabilities and correlations between traits need to be known for all traits included in this index. Next to obtaining direct correlations between traits it is important to gain insight in the mechanisms underlying these correlations (Pryce et al., 2002). This insight will be useful in fully understanding how traits influence each other.

Therefore, the aims of this chapter were to;

1) estimate genetic and genomic parameters for CH_4 production based on pedigree information, genotype information and a combination of both.

2) quantify the relationship between dry matter intake and CH_4 production independent of the influence of milk yield or live weight.

5.2 METHODS

5.2.1 Data

Data used in this chapter originated from two herds, the Langhill herd (see chapter 3) and the Ellinbank herd.

5.2.2 Ellinbank

The Ellinbank herd is housed at the DEDJTR (Department of Economic Development, Jobs, Transport, and Resources) Ellinbank research centre (Victoria, Australia). Data were available on 476 Holstein cows born between August 1995 and September 2013. Cows originated from one population, which was kept close to the average genetic Australian commercial herd. On average there were 2.4 generations in the pedigree and consisted of 438 sires and 1,456 dams (Pedigree analyses were performed with Sargolzaei et al. (2006)). All cows were housed at a testing facility where milking took place using a herringbone milking platform. Cows were all fed the same diet of alfalfa supplemented with crushed wheat.

Methane measurements

CH_4 production was measured with the use of the SF_6 (sulfur hexafluoride) tracer technique (Grainger et al., 2007). For this technique a permeation tube with SF_6 was placed in the rumen of the cows a few months before the start of the experiment. During the experiment the cow was fitted with a halter with

a sampling tube connected to a PVC canister placed around the neck of the cow. Air from around the nostrils was continuously drawn into the tube over a 24-h period, after which the canister was replaced. Sampled air was then analysed with the use of gas chromatography. CH_4 production was calculated from the ratio with SF_6 and the known release rate of the permeation tube. Deighton et al. (2013) found a concordance correlation coefficient between the SF_6 tracer technique and respiration chambers of 0.84. This shows that the SF_6 tracer technique is a reliable technique to measure CH_4 production. CH_4 measurements were taken in 3 batches each spring from 2013 to 2015, for 3 to 5 days in a row. In total 353 observations (1 per cow) were obtained, after quality control (observations more than 3 times the standard error different from the trait average were removed).

Production traits

A total of 620 observations (one to three per cow) were available on milk yield (MY, $kg^{-1}d$, point estimate), 618 observations (one to three per cow) on live weight (LWT, kg, point estimate) and 616 observations (one to three per cow) on dry matter intake (DMI, $kg^{-1}d$, point estimate), after quality control (observations more than 3 times the standard error different from the trait average were removed).

Other variables available were age at recording (months, ranging from 23 to 229), age at first calving (months, ranging from 20 to 33), age at last calving (months, ranging from 22 to 227), calendar year of calving (2010 to 2015), calving season (3 seasons: May-July, August-October, November-January), CH_4 experimental batch (batch to account for the recording window for the CH_4 measurements), CH_4 experiment year (year the experiment batch was measured in, ranging from 2011 to 2015), lactation (limited to lactation 4, lactation numbers higher than 4 were set to 4), weeks in milk (ranging from 3 to 64), fat and protein corrected milk (FPCM) and feed efficiency (calculated

as FPCM/DMI). As CH_4 production was measured as point estimates and MY, LWT and DMI were only available as point estimates either, all data were used as opposite to using whole lactation data with 4-week intervals.

Genomic Data

When performing genomic analyses all animals in the dataset need to have a genotype available, therefore only data on 314 genotyped cows were included in the genomic analyses. Four-hundred-and-ten observations (one to three per cow) were available on MY, 409 observations (one to three per cow) on LWT, 407 observations (one to three per cow) on DMI and 308 observations (one per cow) on CH_4 production. Other variables available (where different from the full dataset) were age at measurement (months, ranging from 23 to 91), age at first calving (months, ranging from 20 to 33) and age at calving (months, ranging from 22 to 88).

5.2.3 Langhill

For the Langhill herd analyses using a G-(genomic) and an H-(pedigree and genomic) relationship matrix were performed. Again, for G-matrix analyses only genotyped animals were included, where for H-matrix the same dataset was used as for the earlier pedigree based analyses (chapter 3). Where this subset of the original dataset differs from the full dataset described in chapter 3, differences are described here. Phenotypic data were available on 177 cows housed at the SRUC Dairy Research Centre (Dumfries, Scotland). Cows were born between October 2004 and February 2012. One-hundred-eighty-three CH_4 ($g^{-1}d$, weekly average) observations were available on 175 cows (one to two observations per cow), measured in 2010, 2011 and 2014. For the production traits 8,348 observations were available on MY ($kg^{-1}day$, weekly average), 8,181 on LWT (kg, weekly average) and 6,475 on DMI ($kg^{-1}day$, weekly

average), recorded between February 2010 and April 2014. Other variables available were cow age (months, ranging from 22 to 72), age at first calving (months, ranging from 21 to 30), age at calving (months, ranging from 21 to 70), calving season (Feb to Aug and Sep to Jan or, Feb to Apr, May to Jul, Aug to Oct and Nov to Jan), diet group (two groups), genetic line (two lines), lactation number (limited to 3, observations higher than 3 were removed) and week of lactation (limited to 52, observations higher than 52 were set to 52).

5.2.4 Genotypes Ellinbank and Langhill

Genotypes for both herds were obtained and data prepared as part of a collaboration within the Methagene COST action group. Genotyping was performed with the Illumina BovineSNP50 BeadChip v01 (Illumina Inc., San Diego, CA; (www.illumina.com/agriculture)) and lower density chips were imputed to a common 50K SNP chip. Quality control of SNPs included removing mitochondrial, Y chromosome and unmapped or mismapped SNPs, SNPs with a GenCall <0.6, SNP with a minor allele frequency of less than 10 copies of an allele in the population, SNP with Mendelian inconsistencies and SNP with excess heterozygosity (Erbe et al., 2012). After editing 44,963 SNPs were available.

These pre-edited genotypes resulted in genotypes on 178 Langhill phenotyped animals and 314 Ellinbank animals. One of the Langhill animals was removed for final analysis based on QA of phenotype information but the genotype was included in the construction of the G-matrix.

5.3 STATISTICAL ANALYSES

Pedigree based analyses and G-matrix analyses were used to estimate genetic parameters for the Ellinbank herd and G- and H-matrix analyses for the Langhill

herd. Both for the pedigree based analyses and the H-matrix analyses all available data were used. For the G-matrix analyses only data on cows for which a genotype was available were used. For a description of pedigree based analyses see chapter 2.

G-matrix analyses (Henderson, 1975) and H-matrix analyses (Legarra et al., 2014) were used to estimate genetic parameters including heritabilities, repeatabilities, and genetic and phenotypic correlations. The model used for the G- and H-matrix analyses was the same as for the pedigree based analyses (chapter 2), except that the A-matrix was replaced by a G-matrix or H-matrix respectively. The A-matrix and inverse A-matrix were calculated by ASReml based on the pedigree, the G- and H-matrix were calculated separately.

The model used for the G- and H-matrix analyses is (Henderson, 1975; Legarra et al., 2014);

$$y = Xb + Z_1a + Z_2p + e, \tag{9}$$

where y is a vector of phenotypic observations, X is the design matrix indicating the relation between observation y and the fixed effects, b is a vector of fixed effects, Z_1 is the design matrix indicating the relation between observation and random additive genetic effects, a is the vector of random additive genetic effects based on the genotypes (G-matrix analyses) or genotypes and pedigree (H-matrix analyses) with a distributed N(0,$\mathbf{G}\sigma^2$) for G-matrix analyses and N(0,$\mathbf{H}\sigma^2$) for H-matrix analyses, Z_2 is the design matrix indicating the relation between observation and random permanent environmental effects, p is the vector of random permanent environmental effects with p distributed N(0,Iσ^2) and e is the vector of random error with e distributed N(0,Iσ^2).

ASReml 3.0 software was used (Gilmour et al., 2009) to perform the analyses.

G-matrix

The G-matrix contains the genomic relationship coefficients between animals based on their genotypes (Forni et al., 2011). The genotype data file (VCF tools format) was obtained from the Methagene COST action group and a quality control was already performed (see earlier in this chapter). This data file was converted into PLINK software (Purcell et al., 2007) output files, including the following final quality control steps: The maximum number of alleles missing per SNP, and per person was set to 0.05, the Hardy-Weinberg disequilibrium threshold was set to a p value of 0.001, the minor allele frequency was set to a maximum of 0.0005, the Mendel error rate threshold per SNP was set to 0.05 and per family to 1. The PLINK output files were used as input files for GCTA (Yang et al., 2011) which calculated the G-matrix. This matrix was used for the analyses in ASReml, the G inverse matrix was calculated by ASReml.

H-matrix

The H inverse matrix is a hierarchical matrix combining information from the A inverse matrix (pedigree information) and the G inverse matrix (genotype information) in the following way (Legarra et al., 2014);

$$\boldsymbol{H^{-1}} = \boldsymbol{A^{-1}} + \begin{pmatrix} 0 & 0 \\ 0 & \boldsymbol{G^{-1}} - \boldsymbol{A_{22}^{-1}}, \end{pmatrix}$$

where, $\boldsymbol{A^{-1}}$ is the inverse A matrix including all animals, $\boldsymbol{G^{-1}}$ is the inverse G-matrix including all animals that are genotyped and $\boldsymbol{A_{22}^{-1}}$ is the inverse A matrix only including animals that are genotyped.

The H-matrix was generated from the A- and G-matrices in this study using the processing pipeline for UK and international genomic evaluations in the UK (EGENES, 2018; Mrode, 2005).

5.3.1 Genetic and genomic analyses for Ellinbank

Genetic analyses

MY, LWT, DMI and CH_4 $g^{-1}d$ were analysed as dependent variables. The interest lies in parameters for the individual traits as well as in the relationships between traits. As not all traits are measured on the same scale, LWT and CH_4 were scaled by dividing the values of the phenotypic observations by 10. This caused the variances for all traits to be of the same magnitude. This will not change the individual parameters but is expected to give more robust covariances between the traits.

Models were started from simple univariate towards multivariate linear models. Due to the size of the dataset it was not possible to build models with more than three dependent variables without having to remove fixed effects, as otherwise no residual variation was left. Therefore, trivariate models were constructed between each combination of the four traits. Fixed effects for each trait were kept identical between models. Only fixed effects that were significant for all trivariate models were kept. An exception was made for exp_bat included as a fixed effect for MY. This was significant for the trivariates between MY, LWT and DMI, and MY, DMI and CH_4, but not for the trivariate between MY, LWT and CH_4 (p=0.055). However, removing exp_bat from the first two trivariate models resulted in no or negative residual variation and therefore was kept in the model.Both age (months) and age at first calving (afc, months) were significant as a fixed effect. However, as age was confounded with lactation number, age at first calving was favoured over age. As not enough repeated observations were available, the permanent environment effect was not included in the model.

The following models were constructed;

$$MY = \mu + lact.WOL + afc + exp_bat + An + e$$

$$LWT = \mu + lact + WOL + afc + exp_bat + An + e$$

$$DMI = \mu + lact + WOL + exp_yr + An + e$$

$$CH_4 = \mu + WOL + exp_yr + An + e,$$

where μ is the overall mean, lact is lactation number, WOL is week of lactation, lact.WOL is the interaction between lact and WOL, afc is age at first calving (months), exp_bat is experimental batch, exp_yr is experimental year, An is the random animal effect based on the pedigree and e is the random residual.

Genomic analyses

MY, LWT, DMI and CH_4 were analysed as dependent variables. Again, phenotypic observations of LWT and CH_4 were scaled by dividing by 10.

Each of the traits were first analysed with a univariate linear model to inform starting variance parameters for multi-trait models (up to 4-trait linear models);

$$MY = \mu + lact.WOL + afc + exp_bat + An + e$$

$$LWT = \mu + lact + WOL + afc + exp_bat + An + e$$

$$DMI = \mu + leg_WOL5 + exp_yr + An + e$$

$$CH_4 = \mu + leg_WOL5 + exp_bat + An + e,$$

where μ is the overall mean, lact is lactation number, WOL is week of lactation, lact.WOL is the interaction between lact and WOL, leg_WOL5 is WOL fitted as a Legendre polynomial of order 5, afc is age at first calving (months), exp_bat is experimental batch, exp_yr is experimental year, An is the random animal effect based on the genomic relationship matrix and e is the random residual.

From these models heritabilities and genetic and residual correlations were calculated with the formulae described in chapter 2. As no permanent environmental effect was fitted in the model, due to not enough repeated observations being available, no phenotypic correlations and repeatabilities were obtained.

Conditional covariances

Conditional covariances were calculated based on the correlations obtained from the G-matrix analyses of the Ellinbank data. Calculations were performed

using formula 4 and 5 from chapter 4. conditional covariances were obtained for the correlation between DMI and CH_4 corrected for the influence of MY, and for the influence of LWT.

5.3.2 Genomic analyses for Langhill

MY, LWT, DMI and CH_4 production were analysed as dependent variables. Again, phenotypic observations of LWT and CH_4 production were scaled by dividing by 10. For the G-matrix analyses a 4-trait linear repeatability model was based on previous univariate linear models. Univariate models were based on the final 4-trait model of the genetic analyses (pedigree based analyses) of the Langhill herd (Chapter 3). As this dataset was smaller than the dataset used for the pedigree based analyses, including polynomials in the fixed effects lead to overfitting of the model. Not including polynomials in the fixed effects meant that it was not possible to fit a random regression model as no population curve was fitted.

The following model was obtained;

$$MY = \mu + lact.WOL + genline + feedgr + rec_yr + calv_seas4 + aac + aac2 + An + AnPE + e$$

$$LWT = \mu + lact + WOL + genline + feedgr + rec_yr + calv_seas4 + aac + aac2 + An + AnPE + e$$

$$DMI = \mu + WOL + genline + feedgr + rec_wk + rec_yr + calv_seas4 + An + AnPE + e$$

$$CH_4 = \mu + MOL + genline + feedgr + rec_wk + rec_yrgr + calv_seas2 + An + e,$$

where μ is the general mean; lact is the fixed effect of lactation number; WOL is week of lactation as a fixed effect; lact.WOL is the interaction between number of lactation and week of lactation; genline is the fixed effect of genetic line; feedgr is the fixed effect of feed group; rec_wk is the fixed effect of the calendar week the measurements took place; rec_yr is the fixed effect of the year the observations took place; rec_yrgr is the fixed effect of the grouped record years the measurement took place; calv_seas4 is the effect of calving season (4 seasons); aac is the covariate that describes the effect of age at calving; aac2 is the squared covariate that describes the effect of age at calving; An is the random animal effect based on the genomic relationship matrix; AnPE is the permanent environment effect; and e is the random residual.

For the H-matrix analyses the final 4-trait random regression model for the pedigree based analyses was used, as this model was more complete than the final model used for G-matrix. The following model was used;

$$MY = \mu + lact.WOL_leg5 + genline + feedgr + rec_wk + rec_yr + calv_seas4 + aac + aac2 + WOL_leg.An + AnPE + e$$

$$LWT = \mu + lact + WOL_leg5 + genline + feedgr + rec_yr + calv_seas4 + aac + aac2 + WOL_leg.An + AnPE + e$$

$$DMI = \mu + lact.WOL_leg5 + genline + feedgr + rec_wk + rec_yr + calv_seas4 + aac + aac2 + WOL_leg.An + AnPE + e$$

$$CH_4 = \mu + MOL_leg3 + genline + feedgr + rec_wk + rec_yrgr + calv_seas2 + An + e$$

Where μ is the general mean; lact.WOL_leg5 is the interaction between number of lactation and a Legendre polynomial of order 5 on week of lactation as a fixed effect; WOL_leg5 is a Legendre polynomial of order 5 on week of lactation as a fixed effect; MOL_leg3 is a Legendre polynomial of order 3 on Month of lactation as a fixed effect; lact is the fixed effect of lactation number; genline is the fixed effect of genetic line; feedgr is the fixed effect of feed group; rec_wk is the fixed effect of the calendar week the measurements took place; rec_yr is the fixed effect of the year the measurements took place; rec_yrgr is the fixed effect of the grouped record years the measurement took place; calv_seas2 is the effect of calving season (2 seasons); calv_seas4 is the effect of calving season (4 seasons); aac is the covariate that describes the effect of age at calving (months); aac2 is the covariate that describes the

squared effect of age at calving (months); WOL_leg.An is the interaction between a Legendre of order 1 on week of lactation and the additive genetic effect of animal as a random effect; An is the random animal effect based on the genomic relationship matrix; AnPE is the permanent environment effect; and *e* is the random residual.

From these models heritabilities and genetic and phenotypic correlations were calculated with the formulae described in chapter 2.

5.4 RESULTS

Genetic and genomic analyses for Ellinbank

Results of the genetic analyses using trivariate models of MY, LWT, DMI and CH_4 can be found in Table 5.1.

Table 5.1: Heritabilities (s.e.) (bold), genetic (s.e.) (below diagonal) and residual (s.e.) (above diagonal) correlations for, and between, milk yield (MY), live weight (LWT), dry matter intake (DMI) and CH_4 production (CH_4) obtained from trivariate linear animal models for the Ellinbank herd using pedigree analyses

Trivariates Ellinbank		MY	LWT	DMI	CH_4
MY	LWT DMI	**0.36 (0.07)**	0.16 (0.08)	0.30 (0.06)	-
	LWT CH_4	**0.35 (0.07)**	0.12 (0.08)	-	0.17 (0.10)
	DMI CH_4	**0.37 (0.07)**	-	0.30 (0.06)	0.09 (0.10)
LWT	MY DMI	0.40 (0.10)	**0.72 (0.04)**	0.54 (0.05)	-
	MY CH_4	0.43 (0.10)	**0.76 (0.04)**	-	0.10 (0.12)
	DMI CH_4	-	**0.73 (0.04)**	0.54 (0.05)	0.26 (0.11)
DMI	MY LWT	0.95 (0.16)	0.80 (0.12)	**0.14 (0.06)**	-
	MY CH_4	1.05 (0.22)	-	**0.10 (0.06)**	0.48 (0.07)
	LWT CH_4	-	0.86 (0.13)	**0.14 (0.06)**	0.42 (0.07)
CH_4	MY LWT	0.48 (0.49)	1.10 (0.57)	-	**0.09 (0.10)**
	MY DMI	0.45 (1.09)	-	0.60 (1.21)	**0.03 (0.10)**
	LWT DMI	-	0.99 (1.09)	0.98 (1.05)	**0.04 (0.10)**

Heritabilities for the same trait vary only between models, correlation estimates vary more between models. Some correlations and standard errors are outside the expected range (-1 to 1 for correlations and 0 to 1 for standard errors). This could indicate that the use of trivariate pedigree based models is not the optimal way of analysing this small dataset, when only pedigree information is used to determine relationships between animals.

Heritabilities were 0.35 to 0.37 for MY, 0.72 to 0.76 for LWT, 0.10 to 0.14 for DMI and 0.03 to 0.09 for CH_4. All heritabilities were significant except for CH_4, and DMI analysed with a trivariate model with MY, DMI and CH_4. The genetic correlation between MY and LWT was 0.40 to 0.43, between MY and DMI was 0.95 to 1.05, and between LWT and DMI was 0.80 to 0.86. All genetic correlations between production traits were significant, however the correlation between MY and DMI obtained from the trivariate model with MY DMI and CH_4 was larger than 1. The genetic correlations between CH_4 and the production traits were 0.45 to 0.48 with MY, 0.99 to 1.10 with LWT and 0.60 to 0.98 with DMI. None of the genetic correlations between the production traits and CH_4 were significant. The residual correlation between MY and LWT was 0.12 to 0.16, between MY and DMI was 0.30 and between LWT and DMI was 0.54. All residual correlations between production traits were significant, except for the correlation between MY and LWT obtained from the trivariate model with MY, LWT and CH_4. The residual correlations between CH_4 and the production traits were 0.09 to 0.17 with MY, 0.10 to 0.16 with LWT and 0.42 to 0.48 with DMI. All residual correlations between CH_4 and production traits were significant, except for the correlations between CH_4 and MY, and the correlation between CH_4 and LWT obtained from the trivariate model with MY, LWT and CH_4.

Heritabilities, genetic and residual correlations obtained from the G-matrix analyses of the Ellinbank data can be found in Table 5.2. All parameters obtained were in the expected range.

Table 5.2: Heritabilities (s.e.) (bold, on-diagonal), genetic (s.e.) (below diagonal) and residual (s.e.) (above diagonal) correlations for, and between, milk yield (MY), live weight (LWT), dry matter intake (DMI) and CH_4 production (CH_4) obtained from a 4-trait linear animal model for the Ellinbank herd using a G-matrix

4-trait variate	MY	LWT	DMI	CH_4
MY	**0.29 (0.09)**	0.22 (0.10)	0.40 (0.07)	0.11 (0.12)
LWT	0.51 (0.13)	**0.79 (0.04)**	0.53 (0.07)	0.31 (0.14)
DMI	0.78 (0.14)	0.77 (0.08)	**0.32 (0.08)**	0.53 (0.09)
CH_4	0.27 (0.27)	0.44 (0.16)	0.34 (0.22)	**0.32 (0.15)**

Heritabilities were 0.29 for MY, 0.79 for LWT, 0.32 for DMI and 0.32 for CH_4. All heritabilities were significant. The genetic correlation between MY and LWT was 0.51, between MY and DMI was 0.78 and between LWT and DMI was 0.77. All genetic correlations between production traits were significant. The genetic correlations between CH_4 and the production traits were 0.27 with MY, 0.44 with LWT and 0.34 with DMI. Only the genetic correlation between CH_4 and LWT was significant. The residual correlation between MY and LWT was 0.22, between MY and DMI was 0.40 and between LWT and DMI was 0.53. All residual correlations between production traits were significant. The residual correlations between CH_4 and the production traits were 0.11 with MY, 0.31 with LWT and 0.53 with DMI. Only the residual correlation between CH_4 and MY was not significant.

Conditional covariances

Conditional covariances obtained from the G-matrix analyses of the Ellinbank herd and calculated conditional covariances between DMI and CH_4 can be found in Table 5.3.

Table 5.3: Obtained and conditional correlations, corrected for milk yield (MY) and live weight (LWT), between dry matter intake (DMI) and CH_4 production (CH_4) obtained from the G-matrix analyses of the Ellinbank herd

Correlation	Obtained	Corrected for MY	Corrected for LWT
Phenotypic	0.47 (0.05)	0.46	0.35
Genomic	0.34 (0.22)	0.22	0.00

The obtained phenotypic correlation between DMI and CH_4 was 0.47. When correcting this correlation for the influence of MY, the conditional correlation is 0.46. When correcting for the influence of LWT, the conditional correlation is 0.35. The obtained genomic correlation between DMI and CH_4 was 0.34. When correcting this correlation for the influence of MY, the conditional correlation is 0.22. When correcting for the influence of LWT, the conditional correlation is 0.00.

Genomic analyses for Langhill

Results of the G-matrix analyses using a 4-trait model including MY, LWT, DMI and CH_4 can be found in Table 5.4.

Table 5.4: Heritabilities (s.e.) (bold, on-diagonal), genetic (s.e.) (below diagonal) and phenotypic (s.e.) (above diagonal) correlations for, and between, milk yield (MY), live weight (LWT), dry matter intake (DMI) and CH_4 production (CH_4) obtained from a 4-trait linear animal model for the Langhill herd using a G-matrix

4-trait variate	MY	LWT	DMI	CH_4
MY	**0.28 (0.10)**	0.20 (0.05)	0.58 (0.03)	0.00 (0.09)
LWT	0.36 (0.23)	**0.42 (0.13)**	0.43 (0.04)	0.04 (0.08)
DMI	0.86 (0.08)	0.67 (0.14)	**0.42 (0.10)**	-0.03 (0.10)
CH_4	-0.57 (0.70)	0.28 (0.51)	-0.09 (0.43)	**0.07 (0.14)**

Table 5.5: Repeatabilities (s.e.) for milk yield (MY), live weight (LWT) and dry matter intake (DMI) obtained from a 4-trait linear animal model (with CH_4 production) for the Langhill herd

4-trait variate	Repeatability
MY	0.53 (0.03)
LWT	0.77 (0.02)
DMI	0.54 (0.03)

Heritabilities, genetic and phenotypic correlations obtained from the G-matrix analyses of the Langhill herd can be found in Table 5.4. Heritabilities were 0.28 for MY, 0.42 for LWT, 0.42 for DMI and 0.07 for CH_4 production. All heritabilities were significant except the heritability for CH_4 production. Repeatabilities (Table 5.5) were 0.53 for MY, 0.77 for LWT and 0.54 for DMI, no repeatability was obtained for CH_4 production. All repeatabilities were significant. The genetic correlation between MY and LWT was 0.36, between MY and DMI was 0.86 and between LWT and DMI was 0.67. All genetic correlations between production traits were significant. The genetic correlations between CH_4 production and the production traits were -0.57 with MY, 0.28 with LWT and -0.09 with DMI. None of the genetic correlations between CH_4 production and the production traits were significant. The phenotypic correlation between MY and LWT was 0.20, between MY and DMI was 0.58 and between LWT and DMI was 0.43. All phenotypic correlations between production traits were significant. The phenotypic correlations between CH_4 and the production traits were 0.00 with MY, 0.04 with LWT and -0.03 with DMI. None of the phenotypic correlations between CH_4 and the production traits were significant.

Results of the H-matrix analyses using a 4-trait model including MY, LWT, DMI and CH_4 can be found in Table 5.6.

Table 5.6: Heritabilities (s.e.) (bold, on-diagonal), genetic (s.e.) (below diagonal) and phenotypic (s.e.) (above diagonal) correlations for, and between, milk yield (MY), live weight (LWT), dry matter intake (DMI) and CH_4 production (CH_4) obtained from the 4-trait random regression model for the Langhill herd using an H-matrix

4-trait variate Langhill	MY	LWT	DMI	CH_4
MY	**0.27 (0.07)-0.39 (0.05)**	-0.25 (0.08)–0.04 (0.09)[1]	0.34 (0.10)-0.36 (0.07)	-0.06 (0.23)-0.01 (0.21)[2]
LWT	-0.63 (0.06)–0.25 (0.10)	**0.64 (0.09)-0.69 (0.08)**	0.27 (0.09)-0.33 (0.09)	0.00 (0.23)-0.03 (0.24)[2]
DMI	0.12 (0.21)-0.28 (0.10)[3]	0.34 (0.11)-0.58 (0.09)	**0.09 (0.04)-0.24 (0.03)**	0.01 (0.20)-0.02 (0.27)[2]
CH_4	-0.43 (0.23)–0.11 (0.23)[2]	0.01 (0.22)-0.09 (0.24)[2]	0.04 (0.21)-0.09 (0.40)[2]	**0.16 (0.13)[4]**

[1]Significant for week 36 to 52 of lactation
[2]Not significant throughout lactation
[3]Significant for week 1 to 7 and 50 to 52 of lactation
[4]Not significant

Table 5.7: Repeatabilities (s.e.) for milk yield (MY), live weight (LWT) and dry matter intake (DMI) obtained from the 4-trait random regression model (including CH_4 production) for the Langhill herd

4-trait variate Langhill	Repeatability
MY	0.50 (0.07)-0.58 (0.06)
LWT	0.79 (0.11)-0.82 (0.10)
DMI	0.23 (0.04)-0.36 (0.03)

Heritabilities and genetic correlations obtained from the H-matrix analyses of the Langhill herd can be found in Table 5.7 and Figures 5.1 and 5.2.

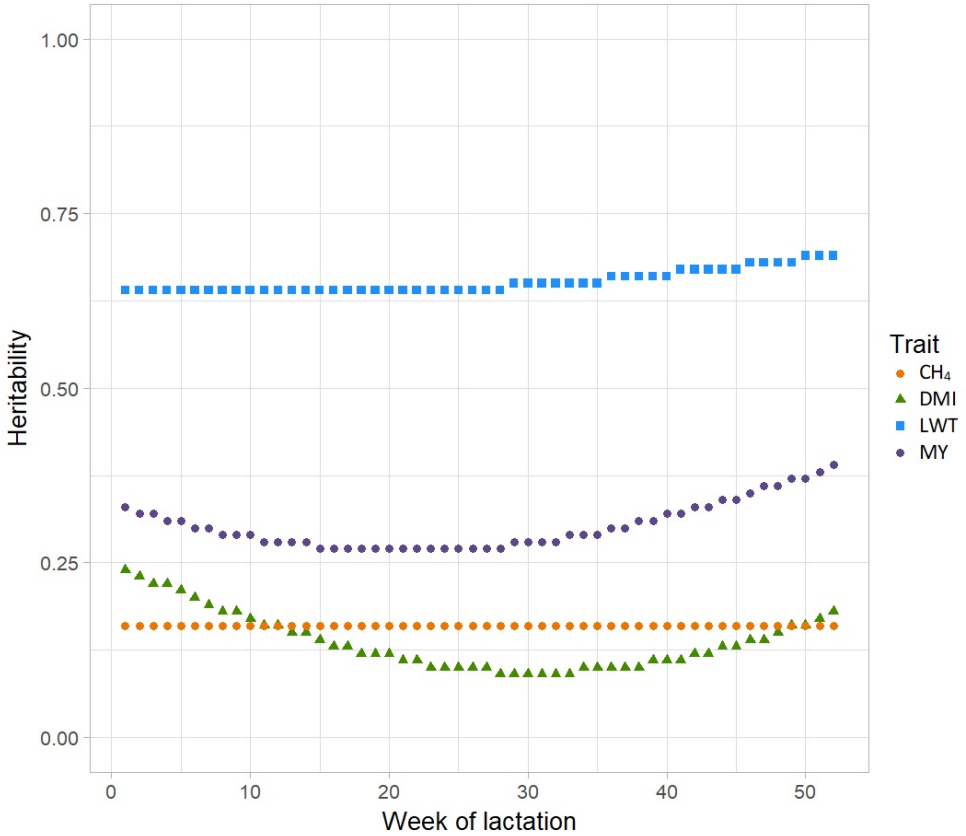


Figure 5.1: Heritabilities of milk yield (MY), live weight (LWT), dry matter intake (DMI) and CH_4 production (CH_4) obtained from H-matrix analyses for the Langhill herd

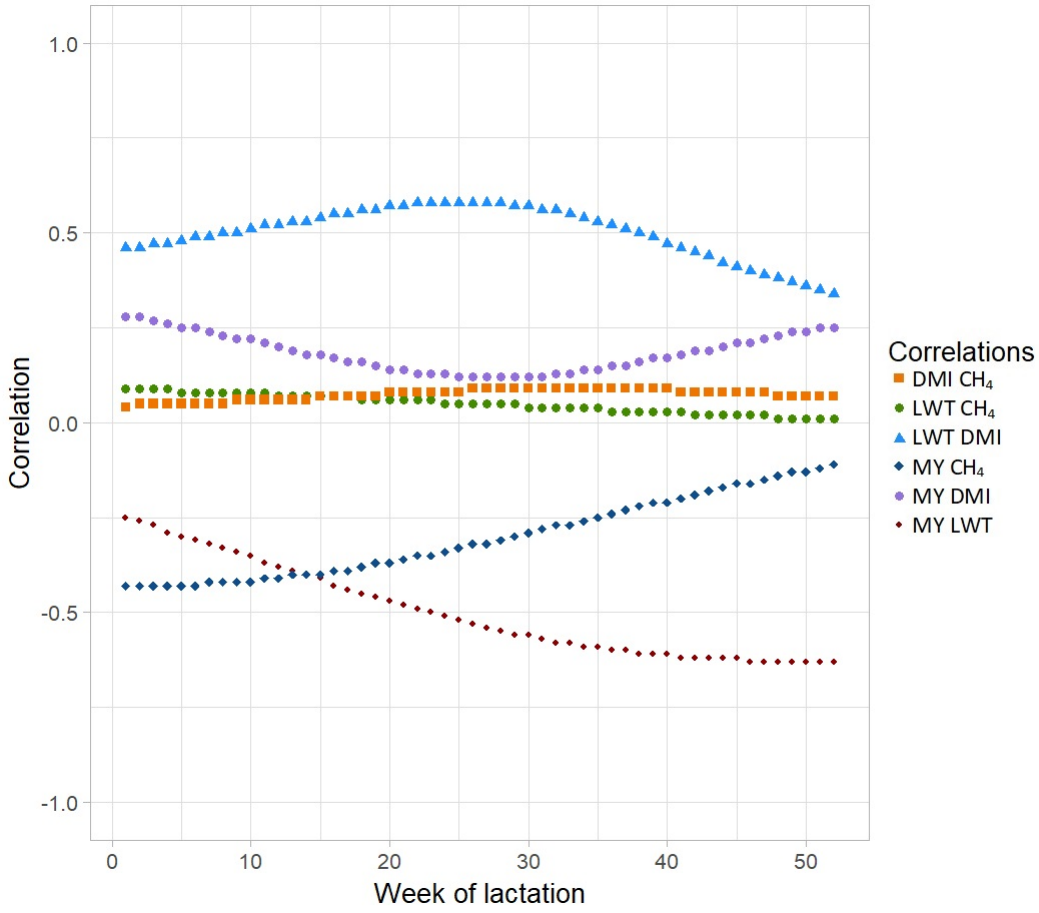


Figure 5.2: Genetic correlations between milk yield (MY), live weight (LWT), dry matter intake (DMI) and CH_4 production (CH_4) obtained from H-matrix analyses for the Langhill herd

Heritability for MY started at 0.33 in week 1 of lactation, went down to 0.27 in week 15 to 28 of lactation, after which it went up to 0.39 in week 52 of lactation. Heritability for LWT started at 0.64 in week 1 to 28 of lactation and went up to 0.69 in week 50 to 52 of lactation. Heritability for DMI starts at 0.24 in week 1 of lactation, went down to 0.09 in week 28 to 33 of lactation, after which it went up to 0.18 in week 52 of lactation. Heritabilities were significant for all weeks of lactation. Heritability for CH_4 was 0.16 and was not significant. This was a point estimate as no random regression term could be fitted on CH_4 production.

Repeatability for MY started at 0.54 in week 1 of lactation, went down to 0.50

in week 13 to 30 of lactation after which it went up to 0.58 in week 51 and 52 of lactation. Repeatability for LWT started at 0.79 in week 1 to 26 of lactation and went up towards 0.82 in week 47 to 52 of lactation. Repeatability for DMI started at 0.36 in week 1 of lactation, went down to 0.23 in week 28 to 33 of lactation, after which it went up to 0.30 in week 51 and 52. All repeatabilities obtained were significant. No repeatability was obtained for CH_4 production.

The genetic correlation between MY and LWT started at -0.25 in week 1 of lactation and went down to -0.63 in week 46 to 52. The correlation was significant for the whole lactation. The genetic correlation between MY and DMI started at 0.28 in week 1 and 2 of lactation, went down to 0.12 in week 26 to 31 of lactation, after which it went up to 0.25 in week 51 and 52 of lactation. Only correlations for week 1 to 7 and 50 to 52 of lactation were significant. The genetic correlation between LWT and DMI started at 0.46 in week 1 and 2 of lactation, went up to 0.58 in week 22 to 28 of lactation, after which it went down to 0.34 in week 52 of lactation. The correlation was significant for the whole lactation.

The genetic correlation between MY and CH_4 production started at -0.43 in week 1 to 6 of lactation and went up to -0.11 in week 52 of lactation. The genetic correlation between LWT and CH_4 started at 0.09 in week 1 to 4 of lactation and went down to 0.01 in week 48 to 52 of lactation. The genetic correlation between DMI and CH_4 production started at 0.04 in week 1 of lactation, went up to 0.09 in week 26 to 39 of lactation, after which it went down to 0.07 in week 48 to 52 of lactation. None of the genetic correlations between CH_4 production and the production traits were significant.

5.5 DISCUSSION

5.5.1 Genetic and genomic analyses for Ellinbank

Heritabilities

Heritability for MY was 0.35 to 0.37 for the pedigree based analyses and 0.29 for the G-matrix analyses. Heritability found for the G-matrix analyses was lower than for the pedigree based analyses. Veerkamp et al. (2011) found a heritability of 0.47 for MY (weekly) with the use of a linear animal model using pedigree information. When using a G-matrix Veerkamp et al. (2011) found a heritability of 0.44. This is higher than heritabilities found in the current study. Heritability for LWT was 0.72 to 0.76 for the pedigree based analyses and 0.79 for the G-matrix analyses. Berry et al. (2003*b*) found a heritability for average body weight (day 5 to 180 in milk, 4 measuring points) of 0.60. This was lower than the heritability found in the current study. Veerkamp et al. (2011) found a heritability of 0.59 for body weight (weekly) with the use of a linear animal model using pedigree information. When using a G-matrix Veerkamp et al. (2011) found a heritability of 0.47. This is lower than heritabilities found in the current study. Heritability for DMI was 0.10 to 0.14 for the pedigree based analyses and 0.32 for the G-matrix analyses. De Haas et al. (2012) found a heritability of 0.37 for DMI for the UK, the Netherlands and Australia combined using pedigree information and a heritability of 0.34 when using G-matrix analyses. Both analyses were performed using a linear animal model. These heritabilities are similar to the heritability found in the current study for the G-matrix analyses. Veerkamp et al. (2011) found a heritability of 0.78 for DMI (daily) with the use of a linear animal model using pedigree information. When using a G-matrix Veerkamp et al. (2011) found a heritability of 0.65. These heritabilities are higher than the heritability found in the current study. Heritability for CH_4 was 0.03 to 0.09 for the pedigree based analyses and 0.32 for the G-matrix analyses. No literature was found that included the heritability of CH_4 production measured with the use of the SF_6 tracer technique. De Haas

et al. (2011) found a heritability between 0.29 to 0.42 for predicted CH_4 and Lassen et al. (2016) found a heritability (s.e.) of 0.25 (0.16, not significant) (pedigree based analyses) and 0.24 (0.15, not significant) (G-matrix analyses) for CH_4 production obtained from a ratio between CH_4 and CO_2 analysed with the Fourier transform infrared method. The heritability found in the current study for the G-matrix analyses is similar to heritabilities found in literature. The heritabilities for DMI and CH_4 production found in the current study for the pedigree based analyses were lower than expected from literature and not in agreement with the results of the G-matrix analyses of the current study.

Genetic and genomic correlations between production traits

Genetic correlations between MY and LWT were 0.40 to 0.43 for the pedigree based analyses and 0.51 for the G-matrix based analyses. Berry et al. (2003*b*) found a correlation of -0.01 (no estimate of significance given) between MY (cumulative to day 240 in milk) and average body weight (day 5 to 180 in milk, 4 measuring points). Manzanilla-Pech et al. (2016b) found a correlation of -0.09 between MY (weekly) and body weight (weekly) for the Netherlands and -0.29 between MY (daily) and body weight (daily, weekly or biweekly) for the United States (neither estimate was significant). Both studies found a (small) negative correlation, while the current study found a positive correlation. Veerkamp et al. (2011) found a genetic correlation of 0.19 between MY (weekly) and body weight (weekly) using a linear animal model using pedigree information. When using a G-matrix Veerkamp et al. (2011) found a correlation of 0.25 between MY (weekly) and body weight (weekly) (neither estimate was significant). Although these correlations were lower than correlations found in the current study, they were in the same direction.

As described in chapter 4, from a biological point of view both a positive and a negative correlation are possible.

Genetic correlations between MY and DMI were 0.95 to 1.05 (which is outside

the expected range) for the pedigree based analyses and 0.78 for the G-matrix based analyses. Manzanilla-Pech et al. (2016b) found a correlation of 0.44 between MY (weekly) and DMI (weekly) for the Netherlands, and 0.36 between MY (daily) and DMI (daily) for the United States, using a linear animal model for both countries. Veerkamp et al. (2011) found a genetic correlation of 0.44 between MY (weekly) and DMI (daily) using a linear animal model using pedigree information. When using a G-matrix Veerkamp et al. (2011) found a correlation of 0.40 between MY (weekly) and DMI (daily). These correlations are lower than correlations found in the current study.

Genetic correlations between LWT and DMI were 0.80 to 0.86 for the pedigree based analyses and 0.77 for the G-matrix analyses. Manzanilla-Pech et al. (2016b) found a correlation of 0.55 between body weight (weekly) and DMI (weekly) for the Netherlands, and a correlation of 0.46 between body weight (daily, weekly or biweekly) and DMI (daily) for the United States. These correlations are lower than correlations found in the current study. Veerkamp et al. (2011) found a genetic correlation of 0.75 between LWT (weekly) and DMI (daily) using a linear animal model using pedigree information. This is lower but similar to the correlation found in the current study. When using a G-matrix Veerkamp et al. (2011) found a correlation of 0.75 between LWT (weekly) and DMI (daily). This is similar to the correlation found in the current study.

Genetic correlations between CH_4 and production traits

Genetic correlations between MY and CH_4 were 0.45 to 0.48 for the pedigree based analyses and 0.27 for the G-matrix based analyses. Lassen and Løvendahl (2016) found a correlation of 0.43 between FPCM (fat and protein corrected milk) and CH_4 obtained from the ratio of CH_4 and CO_2 concentration with the use of a linear animal model. This is similar to the correlation found in the current study for the pedigree based analyses. From a biological point of

view a positive correlation is expected as cows that produce more milk, need a higher feed intake. As CH_4 is a product of rumen fermentation, a higher feed intake will lead to a higher production of CH_4, assuming that the extra intake gets utilised. Genetic correlations between LWT and CH_4 were 0.99 to 1.10 (which was outside of the expected range) for the pedigree based analyses and 0.44 for the G-matrix based analyses. Lassen and Løvendahl (2016) found a correlation of -0.18 between LWT (measured at milking) and CH_4 production obtained from the ratio of CH_4 and CO_2 concentration. This correlation is in the opposite direction than the correlation found in the current study. From a biological point of view both a positive and a negative correlation are possible, see chapter 2. Genetic correlations between DMI and CH_4 were 0.60 to 0.98 for the pedigree based analyses and 0.34 for the G-matrix based analyses. Manzanilla-Pech et al. (2016a) found a correlation of 0.83 (0.05, significant) between CH_4 production and DMI, which is similar to the genetic correlations found in the current study when using pedigree based analyses. From a biological point of view a strong positive correlation is expected as DMI is the main driver behind CH_4 production.

It can be seen that the genetic correlations found for the pedigree based analyses and G-matrix analyses are different. Some correlations (and standard errors, see Table 5.1) were outside the expected range of -1 to 1. This shows that the pedigree based analyses are less robust than the G-matrix analyses. This is likely due to weaker linkage between the animals through the pedigree than through the G-matrix. Differences in results between the pedigree based analyses and G-matrix analyses can be explained by differences in the datasets used. As G-matrix analyses require all animals to have a genotype available, the dataset available for G-matrix analyses was smaller (314 cows) than for the pedigree based analyses (476 cows). As the pedigree based analyses only uses relationship information based on the pedigree and the G-matrix analyses only uses relationship information based on genotypes, it is expected that

the relationship coefficients between animals differ for the two analyses. The pedigree was restricted to three generations due to computational limitations. Therefore, it is possible that animals are more related to each other based on genotypes, due to common ancestors earlier in the pedigree passing on these genotypes but not being present in the current pedigree. It of course should also be noted that errors occur both in pedigree recording and in genotyping. These errors could cause differences between the relationship coefficients for the two analyses although these errors do not reflect true differences.

Conditional covariances

When correcting the phenotypic correlation between DMI and CH_4 for the influence of MY, the correlation became only slightly lower. This indicates that MY has almost no effect on this correlation. When correcting the phenotypic correlation for the influence of LWT, the correlation between DMI and CH_4 became lower. This indicates that LWT has a positive influence on the phenotypic correlation between DMI and CH_4. When correcting the genomic correlation between DMI and CH_4 for the influence of MY, the correlation became lower. This indicates that MY has a positive effect on this correlation. When correcting the genomic correlation for the influence of LWT, the correlation between DMI and CH_4 became zero. This indicates that LWT completely drives the genetic correlation between DMI and CH_4.

5.5.2 Genomic analyses for Langhill

Heritabilities

Heritability for MY was 0.28 for the G-matrix analyses. Veerkamp et al. (2011) found a heritability of 0.44 for MY (weekly) with the use of a linear animal model using a G-matrix. When using an H-matrix Veerkamp et al. (2011) found a heritability of 0.42 (not significant). This was higher than heritabilities

found in the current study.

For the H-matrix analyses heritability for MY started at 0.33, went down to 0.27 in early to mid-lactation, after which it went up to 0.39 at the end of lactation. Jakobsen et al. (2002) found a heritability for test day MY for first lactation starting at 0.19 for day 5 in milk going up to 0.37 for day 165 in milk after which heritability went down to 0.31 for day 285 in milk. Veerkamp and Thompson (1999) found heritabilities for MY (weekly) for weeks 1 to 15 of lactation ranging from 0.11 to 0.35, without a clear trend over time. These heritabilities are similar to the heritabilities found in the current study, although for some parts of the lactation a lower heritability was found. The curve of the heritabilities is different. Hüttmann et al. (2009) found heritabilities for energy corrected milk for 11 to 180 days in milk starting at 0.47, went down to 0.22, after which it went up to 0.31. Although heritability started off higher at the beginning of lactation, estimates and curve over lactation were similar to the results of the current study. All studies used to compare to the H-matrix analyses results of the current study made use of random regression models.

Heritability for LWT was 0.42 for the G-matrix analyses. Berry et al. (2003*b*) found a heritability of 0.60 for average body weight which is higher. Veerkamp et al. (2011) found a heritability of 0.47 for body weight (weekly) with the use of a linear animal model using a G-matrix. When using an H-matrix Veerkamp et al. (2011) found a heritability of 0.50. This is similar to heritabilities found in the current study.

For the H-matrix analyses heritability found for LWT started at 0.64 and went up to 0.69 towards the end of lactation. Veerkamp and Thompson (1999) found heritabilities between 0.29 and 0.47 for live weight (weekly) for week 1 to 15 of lactation. Although the curve over lactation is similar, the heritabilities found are lower than heritabilities found in the current study. Hüttmann et al. (2009) found heritabilities for LWT (measured at milking) days 11 to 180 in milk ranging from 0.26 to 0.45. Heritabilities start off high early in lactation,

drop around 91 to 150 days of lactation after which they go up again from 151 days onwards. These heritabilities are lower than found for the current study and follow a different curve over lactation. Koenen and Veerkamp (1998) found heritabilities for LWT (weekly) for week 0 to 25 of lactation varying from 0.43 to 0.56, starting at 0.43 and going up in time. These heritabilities are lower than found in the current study, but the curve over lactation is similar. This is as expected as Koenen and Veerkamp (1998) used data from the Langhill herd (collected between 1982 and 1996) for their analyses. All studies used to compare to the H-matrix analyses results of the current study make use of random regression models.

Heritability for DMI was 0.42 for the G-matrix analyses. De Haas et al. (2012) found a heritability of 0.34 for DMI when using G-matrix analyses, which was lower than heritability found for the G-matrix analysis of the current study. Veerkamp et al. (2011) found a heritability of 0.65 for DMI (daily) with the use of a linear animal model using a G-matrix. When using an H-matrix Veerkamp et al. (2011) found a heritability of 0.65. These heritabilities were higher than the heritability found in the current study.

For the H-matrix analyses heritability found for DMI started at 0.24, went down to 0.09 after mid-lactation, after which it went up to 0.18 at the end of lactation. Veerkamp and Thompson (1999) found heritabilities for DMI (weekly) for week 1 to 15 of lactation ranging from 0.13 to 0.54, with no clear curve in time. This was higher than heritabilities found in the current study. Koenen and Veerkamp (1998) found heritabilities for DMI (measured on four consecutive days each week) for the Langhill herd for week 3 to 25 of lactation. Heritability ranged from 0.18 to 0.37, going up until week 15 and then slowly decreasing. This is higher than heritabilities found in the current study. Hüttmann et al. (2009) found heritabilities for DMI ($kg^{-1}d$) for days 11 to 180 in milk ranging from 0.06 to 0.12, which was lower than heritabilities found in this study. All studies used to compare to the H-matrix analyses

results of the current study make use of random regression models.

Heritability for CH_4 was 0.07 for the G-matrix analyses and 0.16 for the H-matrix analyses, neither was significant. Pickering et al. (2015) found a heritability of 0.05 (not significant) for CH_4 production measured with the use of a laser methane detector. CH_4 production was measured on the same herd as for the current study, however for the current study more CH_4 measurements were available. Heritability found was similar between the two studies, which was as expected as data were obtained from the same herd. De Haas et al. (2011) found a heritability between 0.29 to 0.42 for predicted CH_4 and Lassen et al. (2016) found a heritability of 0.24 (not significant) (G-matrix analyses) for CH_4 obtained from a ratio between CH_4 and CO_2. Heritability found in the current study from the G-matrix analyses was lower and not significant.

For the H-matrix analyses repeatability found for MY started at 0.54, went down to 0.50 mid-lactation after which it went up to 0.58 at the end of lactation. Løvendahl and Chagunda (2011) found a repeatability for MY (kg^{-1}day) starting at 0.82 for day 5 in milk, going up to 0.93 for day 305 in milk. Gebreyohannes et al. (2016) found a repeatability for monthly test-day milk yield starting at 0.84 for 30 days in milk, going up to 0.94 for 300 days in milk. Both studies found higher repeatabilities than found in the current study. Repeatability for LWT started at 0.79 at the beginning of lactation and went up to 0.82 towards the end of lactation. Repeatability for DMI started at 0.36 at the beginning of lactation, went down to 0.23 after mid-lactation after which it went up to 0.30 at the end of lactation. All studies used to compare to the H-matrix analyses results of the current study make use of random regression models. No literature was found on repeatabilities for LWT or DMI obtained with the use of random regression models.

For many studies using random regression models, estimates of significance were not provided.

Genetic correlations between production traits

The genetic correlation found between MY and LWT was 0.36 for the G-matrix analyses. Berry et al. (2003*b*) found a correlation of -0.01 between MY and average body weight and Manzanilla-Pech et al. (2016b) found a correlation of -0.09 for the Netherlands, and a correlation of -0.29 for the United States between MY and LWT (neither estimate was significant). Again, the current study found a positive correlation. As described above, from a biological point of view both a positive and a negative correlation are possible. Veerkamp et al. (2011) found a genetic correlation of 0.25 (not significant) between MY (weekly) and body weight (weekly) using a linear animal model using a G-matrix. When using an H-matrix Veerkamp et al. (2011) found a correlation of 0.10 (not significant) between MY (weekly) and body weight (weekly). Although these correlations are lower than correlations found in the current study, they are in the same direction.

The genetic correlation found between MY and LWT for the H-matrix analyses started at -0.25 and went down to -0.63 at the end of lactation. Veerkamp and Thompson (1999) found a genetic correlation between MY (weekly) and LWT (weekly) ranging from -0.24 to 0.06 for weeks 3 to 15 of lactation. Correlations start off slightly negative at the beginning of lactation, become more strongly negative around week 9 of lactation, after which they become positive towards week 13 of lactation. Although the correlation found by Veerkamp and Thompson (1999) is negative for some weeks of lactation, the negative correlation found in the current study is stronger. Hüttmann et al. (2009) found a genetic correlation between energy corrected milk and LWT (measured at milking) ranging from -0.04 to -0.41 (day 11 to 180 in milk), with correlations being highly negative early in lactation and approaching zero around 180 days in milk. Although this correlation is more similar to the correlations found in the current study, the curve over lactation moves in the opposite direction. All studies used to compare to the H-matrix analyses results

of the current study make use of random regression models.

The genetic correlation found between MY and DMI was 0.86 for the G-matrix analyses. As mentioned before Manzanilla-Pech et al. (2016b) found a correlation of 0.44 for the Netherlands, and a correlation of 0.36 for the United States. Veerkamp et al. (2011) found a genetic correlation of 0.40 between MY (weekly) and DMI (daily) using a linear animal model using a G-matrix. When using an H-matrix Veerkamp et al. (2011) found a correlation of 0.35 between MY (weekly) and DMI (daily). These correlations are lower than correlations found in the current study.

The genetic correlation found between MY and DMI for the H-matrix analyses started at 0.28 at the beginning of lactation, went down to 0.12 after mid-lactation, after which it went up to 0.25 at the end of lactation. Hüttmann et al. (2009) found a genetic correlation between energy corrected milk and DMI ($kg^{-1}d$) ranging from 0.42 to 0.93 for 11 to 180 days in milk, with a slight peak around 121 to 150 days in milk. The correlation found here is stronger than the correlation found in the current study and the curve over lactation shows a peak rather than a trough. All studies used to compare to the H-matrix analyses results of the current study make use of random regression models.

The genetic correlation found between LWT and DMI was 0.67 for the G-matrix analyses. As mentioned before Manzanilla-Pech et al. (2016b) found a correlation of 0.55 for the Netherlands, and a correlation of 0.46 for the United States between body weight and DMI. These correlations were lower than the correlations found in the current study. Veerkamp et al. (2011) found a genetic correlation of 0.75 between LWT (weekly) and DMI (daily) using a linear animal model using a G-matrix. When using an H-matrix Veerkamp et al. (2011) found a correlation of 0.72 between LWT (weekly) and DMI (daily). These correlations were stronger than the correlations found in the current study.

The genetic correlation found between LWT and DMI for the H-matrix analyses

started at 0.46 at the beginning of lactation, went up to 0.58 mid-lactation, after which it went down to 0.34 at the end of lactation. Veerkamp and Thompson (1999) found a correlation between LWT (weekly) and DMI (weekly) ranging from 0.65 to 0.84 for weeks 3 to 15 of lactation, without a clear curve over time. These correlations are stronger than correlations found in the current study. All studies used to compare to the H-matrix analyses results of the current study make use of random regression models.

Genetic correlations between CH_4 and production traits

The genetic correlation found between MY and CH_4 was -0.57 for the G-matrix analyses. As mentioned before Lassen and Løvendahl (2016) found a correlation of 0.43 between FPCM and CH_4 obtained from the ratio of CH_4 and CO_2 concentration.

The genetic correlation found between MY and CH_4 for the H-matrix analyses started at -0.43 at the beginning of lactation and went up to -0.11 at the end of lactation. No literature was found where the correlation between MY and CH_4 was obtained with the use of a random regression model. Both the results from the G-matrix and the H-matrix analyses show a negative correlation. From a biological point of view a positive correlation was expected, as cows producing more milk will have a higher energy requirement and therefore a higher feed intake. If more feed is processed, more CH_4 is produced. The genetic correlation found between LWT and CH_4 was 0.28 for the G-matrix analyses. As mentioned before, Lassen and Løvendahl (2016) found a correlation of -0.18 between LWT and CH_4 obtained from the ratio of CH_4 and CO_2 concentration. Again, this correlation is in the opposite direction than the correlation found in the current study. As noted before (see chapter 2), from a biological point of view both a positive and a negative correlation are possible.

The genetic correlation found between LWT and CH_4 for the H-matrix analyses started at 0.09 at the beginning of lactation and went down to 0.01 towards the

end of lactation. No literature was found where the correlation between LWT and CH_4 was obtained with the use of a random regression model. From a biological point of view both a positive and a negative correlation are possible, see chapter 2.

The genetic correlation found between DMI and CH_4 was -0.09 for the G-matrix analyses. The genetic correlation found for the H-matrix analyses started at 0.04 at the beginning of lactation, went up to 0.09 after mid-lactation, after which it went down to 0.07 towards the end of lactation. This is much lower than expected from literature and from a biological point of view. Manzanilla-Pech et al. (2016a) found a significant correlation of 0.83 between DMI and CH_4 production. From a biological point of view a strong positive correlation is expected as DMI is the main driver behind CH_4 production.

Again, for many studies using random regression models, estimates of significance were not provided.

Comparison of herds

When comparing the results of the G-matrix analyses of both herds, heritability found for MY was similar. Heritability found for LWT was higher for the Ellinbank herd and for DMI was higher for the Langhill herd. Heritability for CH_4 was higher for the Ellinbank herd than for the Langhill herd. However, it should be noted that CH_4 was measured in a different way in both herds. This again might indicate that CH_4 observations measured with different measurement techniques are genetically different traits.

5.6 CONCLUSION

Genetic parameters obtained for the Ellinbank herd using bivariate models and pedigree information were not significant and sometimes out of range, as already discussed in section 5.5 (see also chapter 7 section 2). Heritability

(s.e.) obtained for the Ellinbank herd using a 4-trait model and genotype information was 0.32 (0.15). Genomic correlations (s.e.) for CH_4 production obtained using a 4-trait model and genotype information were 0.27 (0.27) with MY, 0.44 (0.16) with LWT and 0.34 (0.22) with DMI. Heritability obtained for this dataset indicates that CH_4 production is moderately heritable and genetic selection on this trait is possible. The genomic correlation obtained between CH_4 production and LWT indicates that cows with a higher LWT produce more CH_4 than cows with a lower LWT. The genomic correlations obtained between CH_4 production and MY, and CH_4 production and DMI were not significant. If these correlations would have been significant, this would have indicated that CH_4 production increases with increasing MY, and CH_4 production increases with increasing DMI.

Heritability (s.e.) for CH_4 production obtained for the Langhill herd using a 4-trait model and genotype information was 0.07 (0.14) and using a 4-trait model and combined pedigree and genotype information was 0.16 (0.13). Genetic correlations (s.e.) obtained using a 4-trait model and genotype information for CH_4 production were -0.57 (0.70) with MY, 0.28 (0.51) with LWT, and -0.09 (0.43) with DMI. Genomic correlations (s.e.) obtained using a 4-trait model and combined pedigree and genotype information were -0.43 (0.23) to 0.11 (0.23) with MY, 0.01 (0.22) to 0.09 (0.24) with LWT, and 0.04 (0.21) to 0.09 (0.40) with DMI. Heritabilities obtained were not significant. If heritabilities were significant, this would indicate that heritability for CH_4 production is low. Genetic selection on this trait would be difficult, and improvement, if any, would be very slow. None of the correlations found were significant. If these correlations would have been significant, this would have indicated that increasing MY would be accompanied by decreasing CH_4 production in early lactation and accompanied by increasing CH_4 production from mid to late lactation. CH_4 production and LWT do not influence each other on a genetic level. DMI and CH_4 production only slightly positively

influence each other on a genetic level. For a discussion on significance of results and out of range estimates, see chapter 7 section 2.

For the Ellinbank herd, the relationship between DMI and CH_4 production independent of the influence of MY or LWT was quantified. The genomic correlation between DMI and CH_4 production went down from 0.34 to 0.22 when corrected for MY, indicating that the correlation between DMI and CH_4 production is positively influenced by MY. The genomic correlation between DMI and CH_4 production went down from 0.34 to 0.00 when corrected for LWT, indicating that the between DMI and CH_4 production is completely driven by LWT.

6. SELECTION INDEX THEORY AND NATIONAL BREEDING GOALS

6.1 INTRODUCTION

The previous chapters focused on obtaining the genetic parameters of, and between, production and efficiency traits and CH_4 production traits. Although parameters obtained do vary, the current study does show that it is possible for breeders to select cows that produce less CH_4. It also became clear that it is important to make sure no negative effects occur in the production traits due to the direction and magnitude of the genetic correlations between these traits and CH_4 production. Selection index theory (Hazel, 1943) is often used to quantify the effects selection on traits in the selection objective have on traits in the selection criterion. When one standardised economic value is assigned to all traits, this causes the output of the selection index to give insight in the biological mechanisms underlying the influence traits have on each other. When an economic value is assigned to the traits, the selection index gives an indication of the expected gain in profit per generation. Interest lies in the change in profit when CH_4 production is added to the selection index, as well as in the change in CH_4 production due to selection pressure on correlated traits.

When looking at the effect of selection on CH_4 production it is important to take into account not only the CH_4 directly produced by the animals. CH_4 production originating from material flows as feed, manure, transport and housing, and activities as calving, herd size, herd replacements and milk yield production level should be taken into account as well when trying to reduce CH_4 production. Life Cycle Assessments (LCA) are commonly used to model the influence of these material flows and activities. LCAs give the opportunity to examine the effect of changes occurring within a livestock sector on outputs resulting from the sector, like greenhouse gases produced.

Therefore, the aims of this chapter were to investigate;

1) the correlated responses to selection for milk yield, live weight, dry matter intake and CH_4 production based on the genetic and phenotypic parameters derived from the analyses of the Ellinbank herd (chapter 5).

2) the changes in the national breeding goals and the correlated responses of the traits included, under the following scenarios;

a) including CH_4 production in the current breeding goals, without any restriction or economic value on this trait.

b) including CH_4 production in the current breeding goals, but restricting the genetic change in CH_4 production to zero.

c) including CH_4 production in the current breeding goals, but assigning a low, medium or high economic value to CH_4 production.

d) estimate the index value in kg CH_4 production, kg CO_2e and British pounds, when calving interval, lifespan, live weight, milk yield and dry matter intake increase with one-unit (1 day, 0.1 lactation, 1 kg, 1% and 1% respectively) in the Life Cycle Assessment (LCA) analyses.

6.2 METHODS

6.2.1 Ellinbank selection index theory analyses

Selection index theory (Hazel, 1943) as described by (Cameron, 1997) was used to obtain the correlated responses in MY, LWT, DMI and CH_4 production (measured traits: selection criterion) when selecting for MY, DMI and/or CH_4 production (traits to select on: selection objective). Multiple scenarios were analysed where selection took place on one, two or all three traits at the same time. To build the selection index, genetic and phenotypic parameters derived during the analyses of the Ellinbank data (chapter 5) were used. These parameters were used as for the Ellinbank herd parameters were obtained over

lactation rather than per week of lactation. Selection using selection indexes generally is performed per lactation (305 d).

The correlated response (CR) was calculated as;

$$CR = i\frac{b'G}{\sqrt{b'Pb}}, \tag{10}$$

where *i* is the standardised selection differential, or selection intensity (set to 1), *b* is the selection criterion coefficient, *b'* is the transpose of vector *b*, *G* is the genetic covariance matrix between the traits in the selection objective and the selection criterion, and *P* is the phenotypic variance covariance matrix between the traits in the selection criterion.

The vector of selection criterion coefficients (*b*) (including all traits in the selection criterion) was calculated as;

$$b = P^{-1}Ga, \tag{11}$$

where *G* is the genetic covariance matrix between the traits in the selection objective and the selection criterion, *P* is the phenotypic variance covariance matrix between the traits in the selection criterion and *a* is a vector of economic values of all traits in the selection criterion.

For traits not included in the selection objective the economic value was set to \$0 AUD. For traits included in the selection objective the economic value was set to \$1 AUD (MY) or -\$1 AUD (DMI, CH_4 production), depending on the desired direction of change. The economic values of \$1 AUD or -\$1 AUD do not reflect the true economic values of the traits. These values were chosen so that biological mechanisms underlying responses to selection were not influenced by economic values. This was done as the aim of this analysis was to gain insight into effects of biological mechanisms on correlated responses in

traits.

The phenotypic variance-covariance (symmetric) (P-)matrix includes traits which are in the selection criterion. This matrix was built based on phenotypic (co)variances. Matrix elements between selection animals were σ^2_{P} (phenotypic variance) within traits and σ_{P} (phenotypic covariance) between traits. Matrix elements between selection animals and progeny were calculated as;

$$t * \sigma^2{}_{\mathsf{P}}, \tag{12}$$

within traits, and;

$$a * \sigma_{\mathsf{A}} + \sigma_{\mathsf{C}}, \tag{13}$$

between traits, where σ^2_{P} is the phenotypic variance within a trait in the selection criterion, σ_{A} is the genetic covariance between traits in the selection criterion, σ_{C} is the genetic covariance between traits in the selection objective and the selection criterion, *a* is the relationship coefficient between selection animals and their progeny (0.5) and *t* is between animal correlation, where *t* is;

$$\frac{1}{2} * \sigma^2{}_{\mathsf{A}}, \tag{14}$$

for selection animal-progeny relationships, where $\sigma^2{}_{\mathsf{A}}$ is the genetic variance of the trait of interest.

Matrix elements for the *P* matrix between progeny were calculated as;

$$\left(t + \frac{1-t}{n}\right) \sigma^2{}_{\mathsf{P}}, \tag{15}$$

within traits, and;

$$\frac{\sigma_{\mathsf{P}}}{n} + \frac{n-1}{n} \left(a * \sigma_{\mathsf{A}} + \sigma_{\mathsf{C}}\right), \tag{16}$$

between traits, where n is the number of progeny observations for the trait, σ^2_P is the phenotypic variance within a trait in the selection criterion, σ_P is the phenotypic covariance between traits in the selection criterion, σ_A is the genetic covariance between traits in the selection criterion, σ_C is the genetic covariance between traits in the selection objective and the selection criterion, a is the relationship coefficient between progeny (half-sibs, 0.25) and t is between animal correlation, where t is;

$$\frac{1}{4} * h^2, \tag{17}$$

for half-sib (between progeny) relationships. h^2 is the heritability of the trait of interest.

The genetic covariance (non-symmetric) (G-)matrix was built based on the genotypic variances (within traits) and covariances (between traits) between traits in the selection criterion and the selection objective, for selection animals and progeny. The matrix elements between selection animals were the genetic (co)variances. The matrix elements between selection animals and progeny were calculated as the genetic (co)variances multiplied by the relationship coefficient between selection animals and progeny (0.5).

The genetic variance-covariance (non-symmetric) (C-)matrix only contained information on traits in the selection objective for measured animals and therefore matrix elements were the genetic variances (on-diagonal) and covariances (off-diagonal) of and between these traits. All formulae used were taken from Cameron (1997). Tables 6.1 and 6.2 show the formulae for the P- and G-matrices when the selection objective includes two traits (MY and CH_4 production) and the selection criterion includes two traits (MY and LWT).

Table 6.1: Phenotypic variance covariance matrix including all traits in the selection criterion where MY is milk yield and LWT is live weight

	MY	LWT	$\underline{\text{MY}}$	$\underline{\text{LWT}}$
MY	$\sigma^2_{P_{MY}}$	$\sigma_{P_{MY,LWT}}$	$t_{MY} * \sigma^2_{P_{MY}}$	$a * \sigma_{A\ MY,LWT}$
LWT	$\sigma_{P_{MY,LWT}}$	$\sigma^2_{P_{LWT}}$	$a * \sigma_{A\ MY,LWT}$	$t_{LWT} * \sigma^2_{P_{LWT}}$
$\underline{\text{MY}}$	$t_{MY} * \sigma^2_{P_{MY}}$	$a * \sigma_{A\ MY,LWT}$	$\left(t_{MY} + \frac{1-t_{MY}}{n}\right)\sigma^2_{P_{MY}}$	$\frac{\sigma_{P_{MY,LWT}}}{n} + \frac{n-1}{n}\left(t * \sigma_{A_{MY,LWT}}\right)$
$\underline{\text{LWT}}$	$a * \sigma_{A\ MY,LWT}$	$t_{LWT} * \sigma^2_{P_{LWT}}$	$\frac{\sigma_{P_{MY,LWT}}}{n} + \frac{n-1}{n}\left(t * \sigma_{A_{MY,LWT}}\right)$	$\left(t_{LWT} + \frac{1-t_{LWT}}{n}\right)\sigma^2_{P_{LWT}}$

Table 6.2: Genetic covariance matrix between traits in the selection objective and selection criterion where MY is milk yield, LWT is live weight and CH_4 is CH_4 production

	MY	CH_4
MY	σ^2_{MY}	σ_{MY,CH_4}
LWT	$\sigma_{MY,LWT}$	σ_{LWT,CH_4}
$\underline{\text{MY}}$	$t * \sigma^2_{MY}$	$t * \sigma_{MY,CH_4}$
$\underline{\text{LWT}}$	$t * \sigma_{MY,LWT}$	$t * \sigma_{LWT,CH_4}$

One-hundred progeny observations per animal were assumed for MY and LWT and ten progeny observations for DMI and CH_4 production. This was assumed as observations on MY are likely to be more widely available and therefore numerous and observations on LWT are more and more routinely recorded in the milking robot during milking. Observations on DMI and CH_4 production are not currently routinely measured on commercial farms as it is labour intense to obtain these measurements.

Different scenarios were analysed, with one (MY, DMI or CH_4 production), two (MY and DMI, MY and CH_4 production or DMI and CH_4 production) or three (MY, DMI and CH_4 production) traits in the selection objective.

Correlated responses for scenarios without traits restricted were calculated as described above. Correlated responses for scenarios with one (MY, LWT, DMI or CH_4 production) or two (all combinations of the aforementioned four traits) traits restricted to no genetic change, were calculated using the method for restricted selection indexes as described by Cameron (1997). When one trait was restricted to no genetic change, one extra column and row were added to the P matrix. Off-diagonal the matrix elements for this row and column were the same as the column elements of the G matrix corresponding to the trait that was restricted. On-diagonal the matrix element was set to zero. When two traits were restricted to no genetic change, two columns and rows were added to the P matrix. Matrix elements shared by two restricted traits were set to zero. This resulted in a correlated response of zero for the restricted trait(s) and influenced the correlated responses of the traits that were not restricted.

6.2.2 National breeding goals for the Netherlands, the UK and Spain

Selection Index Theory analyses

The current breeding index for the Netherlands (NVI, from CRV) was supplied by Yvette de Haas (received June 2017 as the current breeding index) and

for Spain (ICO, from CONAFE) was supplied by Oscar Gonzales (received June 2017 as the current breeding index) as part of the Methagene COST action collaboration. The current breeding index for the UK (£PLI) was made available by Marco Winters (personal communication, received August 2017 as the current breeding index) from the Agriculture and Horticulture Development Board (AHDB, UK). All breeding indexes included heritabilities, repeatabilities and correlations as currently used by these countries. Number of observations used for each trait were taken from literature on the national breeding goal of the Netherlands (CRV, 2002). Number of observations for traits that were not in the national breeding goal for the Netherlands but were in the national breeding goal for Spain or the UK, were based on comparable traits in the national breeding goal of the Netherlands. The number of observations used for CH_4 production was set to 50 observations, which was $\frac{2}{3}$ of the lowest number of observations of traits already in the breeding goals.

Scenarios tested were;

1) Including CH_4 production in the current selection indexes. No restriction or economic value was placed on CH_4 production.
2) Including CH_4 production in the current selection indexes and restricting the genetic change in CH_4 production to zero. No economic value was placed on CH_4 production.
3) Including CH_4 production in the current selection indexes and assigning a low, medium or high economic value to CH_4 production.
4) Obtaining the index value in kg CH_4 production, kg CO_2e and British pounds, when calving interval, lifespan, live weight, milk yield and dry matter intake increase with one (1 day, 0.1 lactation, 1 kg, 1% and 1% respectively) in the Life Cycle Assessment (LCA) analyses.

Scenario 1, 2 and 3

The first three scenarios were modelled using an Excel spreadsheet programmed

by Julius van der Werf (Van der Werf, 2018) which calculated the correlated response in all traits in the selection criterion.

Genetic parameters used in the breeding goals

Heritabilities, repeatabilities, phenotypic standard deviations and correlations used for non-CH_4 traits were kept as they are in the current index for each country (see Appendix I.). Genetic parameters for and with traits not currently included in the national breeding goals were obtained from as few sources as possible, as genetic parameters are population specific and therefore can vary between different populations of the same breed. As not all parameters needed for these analyses were obtained in the current study, values from literature were used. Where it was not possible to obtain parameters from the same literature source, parameters from the current study were used where appropriate.

For CH_4 production the heritability was set to 0.21 and the phenotypic standard deviation to 36.2 $g^{-1}d$. The genetic correlation between CH_4 production and production traits was set to 0.43 with milk yield (MY), 0.37 with milk fat and 0.77 with milk protein. These parameters were obtained from Lassen et al. (2016) and Lassen and Løvendahl (2016), where the value for FPCM (fat and protein corrected milk) was used for MY. Lassen et al. (2016) and Lassen and Løvendahl (2016) have not included an estimate on repeatability. Therefore, repeatability in this study was set to 0.210001 as due to computational limitations repeatability used has to be higher than heritability. LWT is not currently in the national breeding indexes. As CH_4 production is influenced by LWT and LWT is widely measured it was decided to include LWT without assigning an economic value to it. Genetic parameters used were 0.35 for the heritability, 0.350001 for the repeatability and 68 for the phenotypic standard deviation (Lassen and Løvendahl, 2016). Genetic correlation between LWT and MY was taken from the Ellinbank analyses (chapter 5) and was

0.50. Genetic correlations between LWT and body condition score, foot angle and locomotion were obtained from Berry et al. (2004) and were 0.5, -0.02 and 0.04 respectively. It is currently not clear from research whether the correlation between CH_4 production and LWT is positive or negative. From a biological point of view both are possible (as discussed in chapter 2, section 4). Therefore, separate analyses were performed with a value of -0.18 (Lassen and Løvendahl, 2016) and 0.42 (obtained in chapter 5). No correlation was available between CH_4 production and other traits in the selection indexes.

Economic value of CH_4 production

The economic value of CH_4 production used for the selection indexes was based on the shadow price of CO_2. Low, medium and high economic values for CH_4 production were calculated. The medium economic value was based on the expected shadow price for CO_2 for 2025 obtained from literature (DBEIS, 2017). Low and high economic values were also calculated to investigate what would happen to the value of the breeding indexes if the future economic value for CH_4 production turns out to be much lower or much higher than expected. The shadow price for CO_2 used for the calculation of the medium shadow price of CH_4 production was obtained from the UK Government report on "Updated short-term traded carbon values used for modelling purposes" (DBEIS, 2017). This was £32.38/tCO2e (or €36.19/tCO2e, current exchange rate at time of analyses (July 2017)). A value of £8.95/tCO2e (€10/tCO2e) was chosen to calculate the low economic value and £89.47/tCO2e (€100/tCO2e) was chosen to calculate the high economic value for CH_4 production. These values were agreed upon within a working group of the Methagene COST action collaboration and were used as extreme values compared to the literature value. The following formula was then used to calculate the economic values of CH_4

production;

$$Economic\ value = -1\ *\ (shadow\ price\ of\ CO_2\ *\ 28/1,000,000)\ *\ 305, \quad (18)$$

where the multiplication by -1 was due to the direction of selection on CH_4 production being negative, shadow prices of CO_2 were low, medium and high, as described above, 28 represents the global warming potential of CH_4 (Myhre et al., 2013) and was used to convert from CO_2 shadow price to CH_4 shadow price, division by 1,000,000 converts from tonnes to grams, and multiplication by 305 (lactation length in days) was because the index calculates gain per year and CH_4 data are in grams per day.

This resulted in a low economic value of -£0.08 g^{-1}d per y (-€0.09 g^{-1}d per y), a medium economic value of -£0.28 g^{-1}d per y (-€0.31 g^{-1}d per y) and a high economic value of -£0.76 (-€0.85).

Index value calculations

The index values were then calculated within the Excel spreadsheet programmed by Julius van der Werf, based on selection index theory (Hazel, 1943) as described earlier. For scenario 1, CH_4 production was included in the breeding goals, without an economic value assigned to it. For none of the traits was genetic gain restricted to zero. This obtained the current index values and the genetic gain in CH_4 production currently resulting from the national breeding goals. For scenario 2, the genetic gain in CH_4 production was restricted to no genetic change. No other trait was restricted, and no economic value was assigned to CH_4 production. This obtained the index values and the effect on the genetic gain of other traits if the genetic gain in CH_4 production would be halted with immediate effect. For scenario 3, CH_4 production was included in the breeding goals, without a restriction on the change in genetic gain. All

three economic values described above were used to analyse the effect on the genetic gain in CH_4 production as well as in the other traits in the breeding goals. The index values were obtained in two ways, 1) the economic value of CH_4 production was included in the calculation of the genetic change in all traits and included in the overall index value, or 2) the economic value of CH_4 production was included in the calculation of the genetic change in all traits but excluded from the overall index value. This was done as currently it is not clear whether it is the farmer who will end up paying for the cost assigned to CH_4 production, or companies in the supply chain or the final customer.

For all three scenarios the index values were obtained as absolute values. For scenarios 2 and 3 the index values were expressed as the absolute difference and the relative difference (in percentage) relative to scenario 1. The genetic gain for the traits in the breeding goals were obtained as absolute values and absolute difference compared to scenario 1. As traits were measured in different units and the magnitude and variation in traits was different, comparing direct values might give a biased view. Therefore, the relative genetic gain for each trait was calculated as the genetic gain of the trait divided by the phenotypic standard deviation of the trait multiplied by 100%.

Scenario 4

For scenario 4 a model of Life Cycle Assessment (LCA) of the agricultural sector (Williams et al., 2015) was used. As this model was specific for the agricultural sector in England and Wales, only the UK breeding goal was analysed for this aim. Within this model different sectors (e.g. beef production, milk production and egg production) of the agricultural sector were modelled on national level. For the current study changes were only made within the dairy sector. Interest lay in change in GHG impact under different scenarios of the LCA. GHG impact was expressed as CH_4 production lost to air and change in global warming potential (GWP) expressed in kg CO_2e. GWP was modelled

based on a timescale of 20, 100 and 500 years.

Traits included for the dairy sector were; 'percentage of autumn calving herds', 'percentage of maize silage', 'percentage of milk production as organic', 'percentage of low yielding cows', 'percentage of mid yielding cows', 'non-organic calving index in days', 'organic calving index in days', 'non-organic average number of lactations in herd', 'organic average number of lactations in herd', 'non-organic proportion housed on straw', 'weight of mature dairy cow for average milk yield relative to standard value', 'voluntary feed intake relative to standard value', 'increase in litres of yield for medium yielding cows non-organic', 'increase in litres of milk yield for medium yielding cows organic', 'spread in milk yield system distribution of low, medium and high yielding cows in litres per year', 'increase in lifetime milking days with increasing annual milk yield in days per 1000 litres', 'change in ratio between live weight and milk yield as a power function', 'percentage butterfat concentration', 'percentage protein concentration' and 'percentage farmyard manure versus slurry'.

Traits for which the value was changed to analyse the effect of change were 'non-organic calving index in days', 'non-organic average number of lactations in herd', 'weight of mature dairy cow for average milk yield relative to standard value', 'voluntary feed intake relative to standard value', and 'increase in litres of milk yield for medium yielding cows non-organic'. For these traits the value used in the LCA was changed by one-unit at a time for 20 units (1 day, 0.1 lactation, 1 kg, 1% and 1% respectively for the above traits), one trait at a time. These changes were plotted against the units of change to obtain the slope of the change. This gave average change in CH_4 production lost to air and average change in global warming potential (GWP_{20}, GWP_{100} and GWP_{500}) when the trait increased by one-unit.

Average change per unit in CH_4 production lost to air and GWP_{20}, GWP_{100} and GWP_{500} was then included in the national breeding goal for the UK. As traits included in the LCA and the breeding goal are not exactly the same,

traits were assigned to traits closely related. The change in 'non-organic calving index in days' was assigned to 'calving interval'. Change in 'non-organic average number of lactations in herd' was assigned to 'lifespan'. Change in 'weight of mature dairy cow for average milk yield relative to standard value' was assigned to 'LWT'. Change in 'increase in litres of milk yield for medium yielding cows non-organic' was assigned to 'MY'. Currently DMI is not included in the national breeding goal of the UK, as this trait is not widely recorded and labour intensive to obtain. Scenario 1, 2 and 3 are real life scenarios, therefore DMI was not included in these analyses. As CH_4 production and DMI are moderately correlated and feed production is an important part of LCA, DMI was included in the national breeding goal for scenario 4. Genetic parameters (heritability, repeatability, genetic and phenotypic correlations) used in the national breeding goal for DMI for scenario 4 were taken from chapter 4. Therefore, 'voluntary feed intake relative to standard value' was assigned to 'DMI'. To include the effect of changing the traits of interest with one-unit, economic values in the selection index were set to zero. For the trait of interest, the economic value was then set to the change per unit, in kg CH_4 production or kg CO_2e. The resulting index value now reflected the change in kg CH_4 production or kg CO_2e when the trait of interest increased by one-unit. Next to this, the selection index values were obtained when all five traits change with one-unit simultaneously. To do so, the change per unit (in kg CH_4 production or kg CO_2 equivalent (kg CO_2e)) of all five traits was included in the selection index instead of the economic values. For all other traits the economic value was set to zero. From this the genetic gain in all traits was calculated. The index value (in £) was then calculated by multiplying the genetic gain per trait by the original economic value for each trait and summing the result over all traits. This was done for all combinations of GHG impact (CH_4 lost to air, GWP_{20}, GWP_{100} and GWP_{500}), economic prices assigned to CH_4 production (low, medium and high) and both the positive (0.42) and

negative (-0.18) genetic correlation between LWT and CH_4 production.

6.3 RESULTS

6.3.1 Ellinbank selection index theory analyses

The correlated responses for MY, LWT, DMI and CH_4 production when selecting for MY, DMI or CH_4 production while restricting none, one or two traits to no genetic gain are shown in Tables 6.3, 6.4 and 6.5. When selecting for increased MY (Table 6.3) the genetic gain in all traits increased. When restricting LWT to no genetic gain while selecting for MY, the response in MY and DMI still increased but less so than without any restriction. The genetic gain in CH_4 production became negative. When DMI was restricted to no genetic gain, the response in MY and CH_4 still increased but less so than without any restriction or with a restriction on LWT. When CH_4 was restricted to no genetic change, the genetic gain in MY still increased as did the genetic gain in LWT and DMI.

When restricting both LWT and DMI, LWT and CH_4, or DMI and CH_4 to no genetic change, it was still possible to increase the genetic gain in MY but less so than without any restrictions on the traits in the selection criterion. When restricting both LWT and DMI to no genetic change, the genetic gain in CH_4 production still increased.

Table 6.3: Correlated responses for milk yield (MY), live weight (LWT), dry matter intake (DMI) and CH_4 production (CH_4) when selecting for increased milk yield with none, one or two traits restricted to no genetic change

Goal trait	MY						
Restricted	None	LWT	DMI	CH_4	LWT, DMI	LWT, CH_4	DMI, CH_4
MY (kg)	0.93	0.46	0.27	0.74	0.24	0.46	0.27
LWT (kg/10)	1.64	0.00	-0.26	1.04	0.00	0.00	-0.31
DMI (kg)	0.73	0.20	0.00	0.58	0.00	0.20	0.00
CH_4 (g/10)	0.59	-0.04	0.11	0.00	0.36	0.00	0.00

When selecting for lower DMI (Table 6.4) without restricting any traits, the genetic gain in all traits decreased. When restricting MY to no genetic change, the genetic gain in the other traits still decreased but less so than without any restriction. The genetic gain in CH_4 was $\approx$ zero. When restricting LWT to no genetic change, the genetic gain in MY and DMI still decreased but the genetic gain in CH_4 increased. When restricting CH_4 production to no genetic change, genetic gain decreased in all traits, but less so than without any restriction. When restricting MY and LWT to no genetic change, the genetic gain in DMI decreased but the genetic gain in CH_4 increased. When MY and CH_4, or LWT and CH_4 were restricted to no genetic change, genetic gain in all other traits decreased.

Table 6.4: Correlated responses for milk yield (MY), live weight (LWT), dry matter intake (DMI) and CH_4 production (CH_4) when selecting for decreased dry matter intake with none, one or two traits restricted to no genetic change

Goal trait	DMI						
Restricted	None	MY	LWT	CH_4	MY, LWT	MY, CH_4	LWT, CH_4
MY (kg)	-0.89	0.00	-0.39	-0.69	0.00	0.00	-0.41
LWT (kg/10)	-1.79	-0.77	0.00	-1.24	0.00	-0.74	0.00
DMI (kg)	-0.76	-0.22	-0.24	-0.62	-0.12	-0.22	-0.22
CH_4 (g/10)	-0.58	-0.06	0.27	0.00	0.45	0.00	0.00

When selecting for lower CH_4 production (Table 6.5) without restricting any

traits, the genetic gain in all traits decreased. When restricting MY to no genetic change, the genetic gain in all traits decreased but less so than without any restriction. The genetic gain in DMI was ≈ zero. When restricting DMI to no genetic change, the genetic gain in MY decreased and the genetic gain in CH_4 production decreased but less so than without a restriction on genetic gain for any trait. When restricting MY and LWT to no genetic change, the genetic gain in DMI increases slightly. The genetic gain in CH_4 production decreased, but less so than without any restriction. When restricting MY and DMI to no genetic change, CH_4 production decreased but less so than without a restriction on genetic gain in any trait. This decrease was likely due to a decrease in maintenance costs as the genetic gain in LWT decreased as well. When restricting LWT and DMI to no genetic change, the genetic gain in MY and CH_4 production decreased, but less so than without any restriction.

Table 6.5: Correlated responses for milk yield (MY), live weight (LWT), dry matter intake (DMI) and CH_4 production (CH_4) when selecting for decreased CH_4 production with none, one or two traits restricted to no genetic change

Goal trait	CH_4						
Restricted	None	MY	LWT	DMI	MY, LWT	MY, DMI	LWT, DMI
MY (kg)	-0.55	0.00	0.03	-0.04	0.00	0.00	-0.14
LWT (kg/10)	-1.34	-0.46	0.00	-0.35	0.00	-0.39	0.00
DMI (kg)	-0.45	-0.02	0.09	0.00	0.08	0.00	0.00
CH_4(g/10)	-0.98	-0.79	-0.69	-0.79	-0.69	-0.78	-0.63

Table 6.6 shows the correlated responses for MY, LWT, DMI and CH_4 production when selecting for two or three traits simultaneously while restricting none or one trait to no genetic change. When selecting for increased MY and decreased CH_4 production simultaneously without any restriction, the genetic gain in MY increased and the genetic gain in CH_4 production decreased as expected. When DMI was restricted to no genetic gain, the genetic gain in CH_4 production decreased further but there was almost no increase in the genetic gain in MY. When LWT was restricted to no genetic

change, the genetic gain in MY still increased, but less so than without any restrictions. The genetic gain in CH_4 production decreased more than without any restrictions.

When selecting for increased MY and decreased DMI simultaneously without any restriction, both the genetic gain in MY and CH_4 production increased. However, the genetic gain in DMI increased as well. This increase in genetic gain in DMI was likely due to the low genetic additive variance present for DMI, which was lower than the genetic additive variance present for MY, as well as the strong positive correlation between the traits. When LWT was restricted to no genetic change, the increase in genetic gain in all other traits still increased, but less so than without any restrictions. When restricting CH_4 production to no genetic gain, the genetic gain in MY and DMI still increased, but less so than without any restrictions. The genetic gain in LWT decreased.

When selecting for MY, DMI and CH_4 production simultaneously without any restrictions, the genetic gain in all traits decreased. When restricting LWT to no genetic change, the genetic gain in both MY and DMI increased but the genetic gain in CH_4 production decreased.

Table 6.6: Correlated responses for milk yield (MY), live weight (LWT) and dry matter intake (DMI) when selecting for two or three traits (milk yield, live weight and/or CH_4 production (CH_4)) with none or one trait (live weight, dry matter intake or CH_4 production) restricted to no genetic change

Goal traits	MY, CH_4			MY, DMI			MY, DMI, CH_4	
Restricted	None	DMI	LWT	None	LWT	CH_4	None	LWT
MY (kg)	0.38	0.06	0.28	0.49	0.40	0.36	-0.34	0.19
LWT (kg/10)	0.24	-0.44	0.00	0.23	0.00	-0.16	-1.22	0.00
DMI (kg)	0.28	0.00	0.19	0.19	0.10	0.07	-0.35	0.14
CH_4 (g/10)	-0.49	-0.75	-0.58	0.29	0.21	0.00	-0.91	-0.63

6.3.2 National breeding goals for the Netherlands, the UK and Spain

The change in correlated response for each trait for each country for the first three scenarios can be found in appendix II. The index values for the first three scenarios for each country can be found in Table 6.7 (assuming a negative correlation between LWT and CH_4 production) and Table 6.8 (assuming a positive correlation between LWT and CH_4 production). The top of each Table shows the absolute values and the bottom of each Table shows the percentage change in index value relative to scenario 1. For scenario 3 both the index value including the economic value and the index value excluding the economic value (in brackets) of CH_4 production are shown.

Table 6.7: Index values for each scenario for each country (Netherlands (NL), Spain (ES) and United Kingdom (UK)) when assuming a negative correlation between live weight (LWT) and CH_4 production (CH_4)

Index values	Scenario 1	Scenario 2	Scenario 3 low (excl.)	Scenario 3 lit (excl.)	Scenario 3 high (excl.)
NL (€)	228.25	221.02	227.90 (228.25)	227.15 (228.27)	225.35 (227.94)
ES (€)	91.79	81.76	91.28 (91.85)	89.90 (91.73)	87.09 (91.19)
UK (£)	85.15	72.30	84.25 (84.92)	82.60 (84.81)	79.39 (84.50)
Absolute change		Scenario 2	Scenario 3 low (excl.)	Scenario 3 lit (excl.)	Scenario 3 high (excl.)
NL (€)		-7.23	-0.35 (0.00)	-1.10 (0.02)	-2.90 (-0.31)
ES (€)		-10.03	-0.51 (0.06)	-1.89 (-0.06)	-4.70 (-0.60)
UK (£)		-12.85	-0.90 (-0.23)	-2.55 (-0.34)	-5.77 (-0.65)

Table 6.8: Index values for each scenario for each country (Netherlands (NL), Spain (ES) and United Kingdom (UK)) when assuming a positive correlation between live weight (LWT) and CH_4 production (CH_4)

Index values	Scenario 1	Scenario 2	Scenario 3 low (excl.)	Scenario 3 lit (excl.)	Scenario 3 high (excl.)
NL (€)	228.46	221.54	228.18 (228.51)	227.38 (228.47)	225.60 (228.11)
ES (€)	92.28	82.74	91.79 (92.35)	90.43 (92.25)	87.54 (91.56)
UK (£)	83.28	71.61	82.52 (83.17)	81.02 (83.15)	77.86 (82.67)
Absolute change		Scenario 2	Scenario 3 low (excl.)	Scenario 3 lit (excl.)	Scenario 3 high (excl.)
NL (€)		-6.92	-0.28 (0.06)	-1.08 (0.01)	-2.85 (-0.35)
ES (€)		-9.54	-0.49 (0.08)	-1.84 (-0.03)	-4.74 (-0.72)
UK (£)		-11.68	-0.77 (-0.12)	-2.27 (-0.13)	-5.42 (-0.61)

The correlated response in CH_4 production for the first three scenarios for each country can be found in Figure 6.1 (assuming a negative correlation between LWT and CH_4 production) and 6.2 (assuming a positive correlation between LWT and CH_4 production).

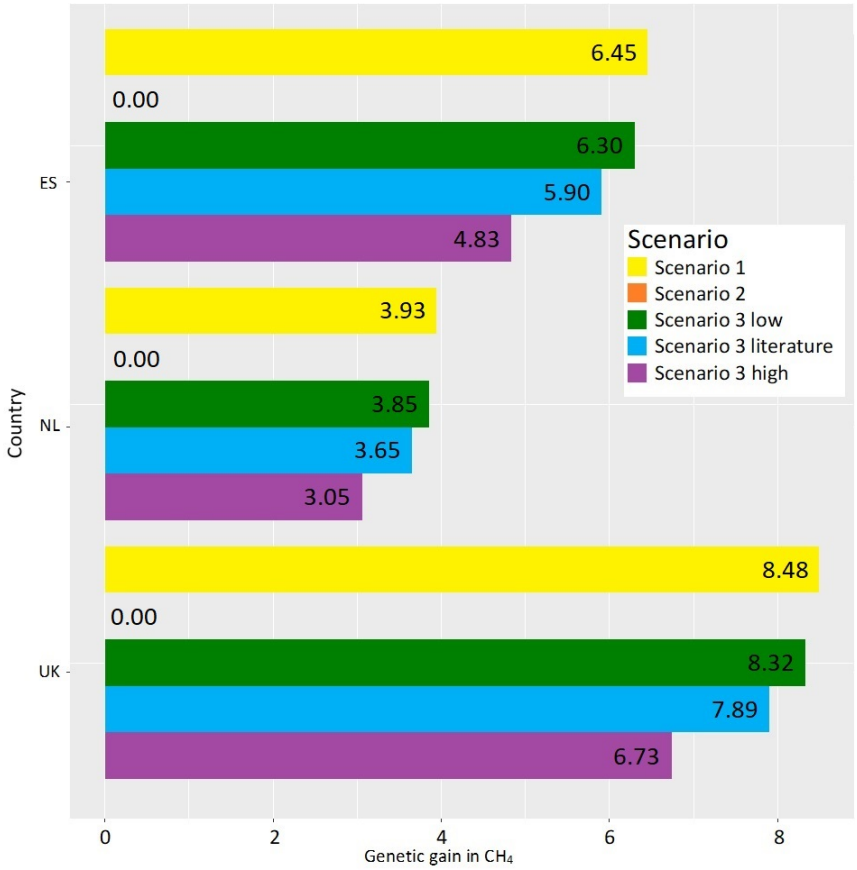


Figure 6.1: Genetic gain of CH_4 production (CH_4) for the Netherlands (NL), Spain (ES) and the United Kingdom (UK) assuming a negative correlation between live weight and CH_4 production, where Scenario 1: current breeding goals including CH_4 production without restrictions or economic values, Scenario 2: current breeding goals including CH_4 production with a restriction on the genetic gain in CH_4 production, and Scenario 3: current breeding goals including CH_4 production with a low, literature, or high economic value assigned to CH_4 production

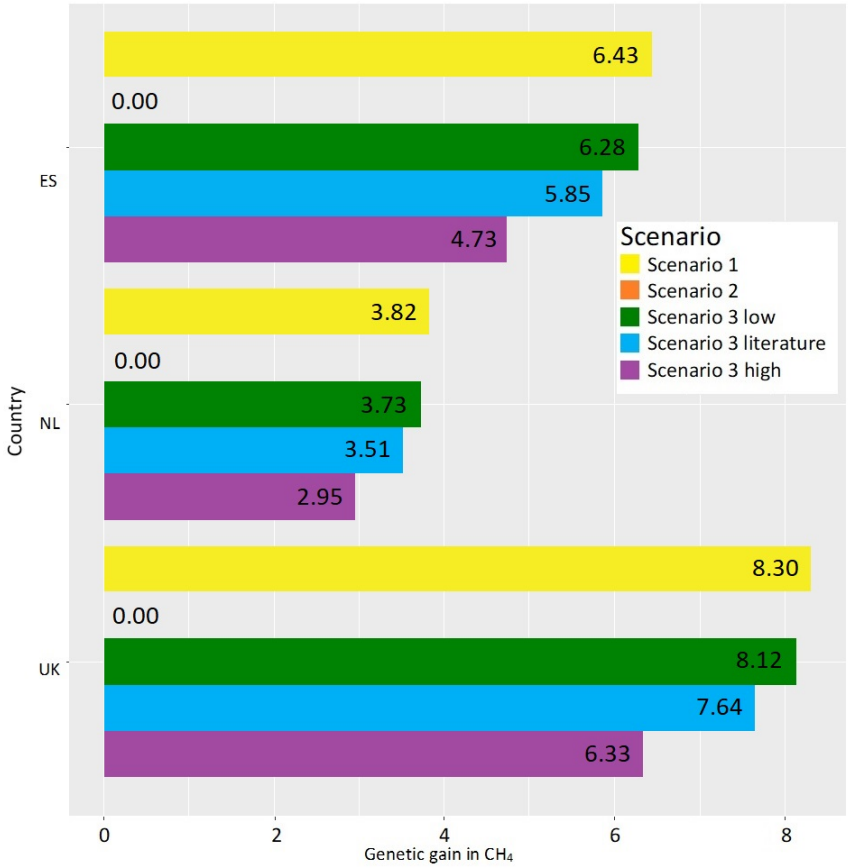


Figure 6.2: Genetic gain of CH_4 production (CH_4) for the Netherlands (NL), Spain (ES) and the United Kingdom (UK) assuming a positive correlation between live weight and CH_4 production, where Scenario 1: current breeding goals including CH_4 production without restrictions or economic values, Scenario 2: current breeding goals including CH_4 production with a restriction on the genetic gain in CH_4 production, and Scenario 3: current breeding goals including CH_4 production with a low, literature, or high economic value assigned to CH_4 production

Tables 6.9 and 6.10 show the absolute and relative change in CH_4 production relative to scenario 1. The top of each Table shows the absolute change in index value and the bottom of the Table shows the percentage change in index value relative to scenario 1.

Table 6.9: Genetic gain in CH_4 production (CH_4) for each scenario for each country (Netherlands (NL), Spain (ES) and United Kingdom (UK)) assuming a negative correlation between live weight (LWT) and CH_4 production (CH_4)

Absolute change (g^{-1}d per y)	Scenario 2	Scenario 3 low	Scenario 3 literature	Scenario 3 high
NL	-3.93	-0.08	-0.31	-0.88
ES	-6.45	-0.15	-0.55	-1.62
UK	-8.48	-0.16	-0.59	-1.75
% change	Scenario 2	Scenario 3 low	Scenario 3 literature	Scenario 3 high
NL	-100	-2.04	-7.89	-22.39
ES	-100	-2.33	-8.53	-25.12
UK	-100	-1.89	-6.96	-20.64

Table 6.10: Genetic gain in CH_4 production (CH_4) for each scenario for each country (Netherlands (NL), Spain (ES) and United Kingdom (UK)) assuming a positive correlation between live weight (LWT) and CH_4 production (CH_4)

Absolute change (g^{-1}d per y)	Scenario 2	Scenario 3 low	Scenario 3 literature	Scenario 3 high
NL	-3.82	-0.09	-0.31	-0.87
ES	-6.43	-0.15	-0.58	-1.70
UK	-8.30	-0.18	-0.66	-1.97
% change	Scenario 2	Scenario 3 low	Scenario 3 literature	Scenario 3 high
NL	-100	-2.36	-8.12	-22.77
ES	-100	-2.46	-9.12	-26.55
UK	-100	-2.17	-7.95	-23.73

Scenario 1

Index values

For scenario 1 CH_4 production was included in the current breeding goals, without an economic value assigned or any restriction imposed. The index values found for the different countries vary considerably (Tables 6.7 and 6.8). The index values were €228.25 for the Netherlands, €91.79 for Spain and £85.15 for the UK when the correlation between LWT and CH_4 was -0.18. When the correlation between LWT and CH_4 was 0.42, the index values were €228.46 for the Netherlands, €92.28 for Spain and £83.28 for the UK.

Tables 6.9 and 6.10 show that when CH_4 production was added to the current selection indexes, CH_4 production increased each year for all countries (both when a negative or a positive correlation was assumed between LWT and CH_4 production).

For the Netherlands CH_4 production increased by 3.93 $g^{-1}d^{-1}y$, for Spain by 6.45 $g^{-1}d^{-1}y$ and for the UK by 8.48 $g^{-1}d^{-1}y$ when the correlation between LWT and CH_4 was -0.18. When the correlation between LWT and CH_4 was 0.42, CH_4 production increased by 3.82 $g^{-1}d^{-1}y$ for the Netherlands, 6.43 $g^{-1}d^{-1}y$ for Spain and 8.30 $g^{-1}d^{-1}y$ for the UK.

Scenario 2

Index values

For scenario 2 CH_4 production was included in the current breeding goals, without an economic value assigned. The genetic change in CH_4 production was restricted to zero. Table 6.7 shows the reduction in index value under scenario 2 compared to scenario 1, when a negative correlation was assumed between LWT and CH_4 production. For the Netherlands the index value decreased from €228.25 to €221.02, a decrease of €7.23 or 3.17%. The biggest change in the NVI came from the economic change in milk protein (-€7.38), milk fat (-€3.53) and milk lactose (€3.45).

For Spain the index value decreased from €91.79 to €81.76, a decrease of €10.03 or 10.93%. The biggest change in the ICO came from the economic change in milk protein (-€10.29), SCC (€6.37) and milk yield (-€5.57).

For the UK the index value decreased from £85.15 to £72.30, a decrease of £12.85 or 15.09%. The biggest change in the £PLI came from the economic change in milk protein (-£27.35), milk yield (£9.09) and lifespan (£5.64).

Table 6.8 shows the reduction in index value under scenario 2 compared to scenario 1, when a positive correlation was assumed between LWT and CH_4 production. For the Netherlands the NVI decreased from €228.46 to €221.54, a decrease of €6.92 or 3.03%. The biggest change in the NVI came from the economic change in milk protein (-€7.13), milk fat (-€3.49) and milk lactose (€3.48).

For Spain the index value decreased from €92.28 to €82.74, a decrease of €9.54 or 12.02%. The biggest change in the ICO came from the economic change in milk protein (-€8.99), SCC (€5.81) and milk yield (-€5.75).

For the UK the index value decreased from £83.28 to £71.61, a decrease of £11.68 or 14.02%. The biggest change in the £PLI came from the economic change in milk protein (-£22.83), milk yield (£8.45) and lifespan (£3.08).

For all countries LWT did not directly influence the index values as currently no economic value is assigned to LWT.

Relative change in traits

For the Netherlands the largest change in relative genetic gain happened in milk protein (69.95% to 60.47%) and milk fat (45.00% to 39.00%), when the correlation between LWT and CH_4 was -0.18. When the correlation between LWT and CH_4 was 0.42, the biggest change in relative gain happened in milk protein (70.37% to 61.21%), milk fat (45.04% to 39.11%) and LWT (-0.03% to -3.37%).

For Spain the biggest change in relative gain happened in milk protein (from

28.68% to 11.21%) and milk yield (27.51% to 19.27%), when the correlation between LWT and CH_4 was -0.18. When the correlation between LWT and CH_4 was 0.42, the biggest change in relative gain happened in milk protein (31.05% to 15.79%), milk yield (27.05% to 18.55%) and LWT (11.28% to 1.72%).

For the UK the biggest change in relative gain happened in milk protein (from 36.26% to 12.26%) and milk yield (from 13.77% to -0.75%), when the correlation between LWT and CH_4 was -0.18. When the correlation between LWT and CH_4 was 0.42, the biggest change in relative gain happened in milk protein (39.96% to 19.93%), LWT (-13.46% and -29.49%) and milk yield (13.88% to 0.4%).

Traits related to CH_4 production

Traits in the indexes that are indirectly linked to CH_4 production are fertility traits (like calving interval and days open), LWT and lifespan/longevity.

For the UK the genetic gain in calving interval (days) decreased from -1.16% to -2.40%, when the correlation between LWT and CH_4 production was -0.18. When the correlation between LWT and CH_4 production was 0.42, the genetic gain in calving interval decreased from -1.51% to -2.38%. The genetic gain in LWT (kg) increased from -13.94kg to -9.93kg, when the correlation between LWT and CH_4 production was -0.18. When the correlation between LWT and CH_4 production was 0.42, the genetic gain in LWT decreased from -13.46% to -29.49%. The genetic gain in lifespan (score, based on underlying traits) increased from 18.85% to 22.7%, when the correlation between LWT and CH_4 production was -0.18. When the correlation between LWT and CH_4 production was 0.42, the genetic gain in lifespan increased from 13.62% to 15.71%.

For the Netherlands the gain in calving interval (point breeding value, pnt) decreased from -16.32% to -16.77%, when the correlation between LWT and CH_4 production was -0.18. When the correlation between LWT and

CH_4 production was 0.42, the genetic gain in calving interval decreased from -16.10% to -16.55%. The genetic gain in LWT (kg) increased from 0.06kg to 1.29kg, when the correlation between LWT and CH_4 production was -0.18. When the correlation between LWT and CH_4 production was 0.42, the genetic gain in LWT decreased from -0.03% to -3.37%. The genetic gain in longevity (pnt, point breeding value) increased from 16.30% to 16.61%, when the correlation between LWT and CH_4 production was -0.18. When the correlation between LWT and CH_4 production was 0.42, the genetic gain in longevity increased from 16.28% to 16.60%.

For Spain the gain in days open (days) increased from -1.30% to -0.30%, when the correlation between LWT and CH_4 production was -0.18. When the correlation between LWT and CH_4 production was 0.42, the genetic gain in days open increased from -0.10% to 1.00%. The genetic gain in LWT (kg) increased from 10.66kg to 18.62kg, when the correlation between LWT and CH_4 production was -0.18. When the correlation between LWT and CH_4 production was 0.42, the genetic gain in LWT decreased from 11.28% to 1.72%. The genetic gain in longevity (days) increased from 6.72% to 8.18%, when the correlation between LWT and CH_4 production was -0.18. When the correlation between LWT and CH_4 production was 0.42, the genetic gain in longevity increased from 6.62% to 7.70%.

Scenario 3

Index values

From Table 6.9 and 6.10 it can be seen that for all three countries the increase in CH_4 production per year went down when an economic value was assigned to CH_4 production. The higher the economic value assigned to CH_4 production, the lower the increase in CH_4 production per year became. However, CH_4 production was still increasing for all countries for all economic values analysed. The reduction in increase of CH_4 production $g^{-1}d$ per year was

similar between the countries for the three economic values analysed. When a low economic value was assigned to CH_4 production the correlated response in CH_4 production decreases by 2% relative to scenario 1 for all countries. When the economic value obtained from literature was assigned to CH_4 production the correlated response in CH_4 production decreased by 7 to 9%. When a high economic value was assigned to CH_4 production the correlated response in CH_4 production decreased by 21 to 27%. The results were similar between the use of a negative or a positive correlation between LWT and CH_4 production.

The economic values of the indexes in scenario 3 were calculated based on the shadow price of CH_4 production having a direct effect on the economic value of the indexes (directly included in the economic value) and having an indirect effect on the economic value of the indexes (the economic value was included in the selection index but not in the economic value of the index). From Tables 6.7 and 6.8 it can be seen that the index values including the shadow price for CH_4 for all countries decreased when an economic value was assigned to CH_4 production. This decrease in index values became larger when the economic value became higher.

When a low economic value was assigned to CH_4 production the index value decreased by 0 to 1% relative to scenario 1 for all countries. When the economic value obtained from literature was assigned to CH_4 production the index value decreased by 0 to 3%. When a high economic value was assigned to CH_4 production the index value decreased by 1 to 7%. The smallest decreases occurred for the Netherlands. When the economic value put on CH_4 production was excluded from the economic value of the index, the economic value of the index only decreased by 0 to 1% for all countries. Again, the results were similar for the use of a negative or a positive correlation between LWT and CH_4 production.

Production traits

When looking at production traits, for all three countries the increase in milk yield, milk fat and milk protein per year became lower when an economic value was put on CH_4 production. Again, the higher the economic value put on CH_4 production the lower the increase in milk traits became. For the UK the change in milk traits was biggest for milk protein (-4.59% when the genetic correlation between LWT and CH_4 production was -0.18 and -4.22% when the genetic correlation between LWT and CH_4 production was 0.42) when the highest economic value was assigned to CH_4 production. For Spain this change in milk traits was biggest for milk protein (-4.10% when the genetic correlation between LWT and CH_4 production was -0.18 and -3.68% when the genetic correlation between LWT and CH_4 production was 0.42) when the highest economic value was assigned to CH_4 production. For the Netherlands this change in milk traits was biggest for milk protein (-1.74% for both possible correlations between LWT and CH_4 production) when the highest economic value was assigned to CH_4 production. For all countries and all milk traits the absolute change (in unit of product) per year remained positive.

Traits related to CH_4 production

Table 6.11 shows the genetic gain as a percentage of the phenotypic standard deviation for calving interval/days open, LWT and lifespan/longevity for the different economic values used for CH_4 production under scenario 3.

Table 6.11: Genetic gain as a percentage of the phenotypic standard deviation for traits related to CH_4 production (CH_4) under scenario 3 (scen3) relative to scenario 1 (scen1) assuming a positive or negative correlation between CH_4 production and live weight (LWT)

Country	Trait	Corr LWT-CH_4	Value Scen 1	Scen 3 low	Scen 3 literature	Scen 3 high
UK	Calving	-	-1.16	-1.18	-1.27	-1.47
	interval	+	-1.51	-1.53	-1.60	-1.78
	LWT	-	-13.94	-13.90	-13.78	-13.41
		+	-13.46	-13.91	-15.10	-18.10
	Lifespan	-	18.85	18.85	19.20	20.25
		+	13.62	13.62	13.97	14.66
NL	Calving	-	-16.32	-16.32	-16.32	-16.32
	interval	+	-16.10	-16.10	-16.32	-16.32
	LWT	-	0.06	0.07	0.15	0.34
		+	-0.03	-0.12	-0.31	-0.81
	Longevity	-	16.30	16.31	16.36	16.46
		+	16.28	16.30	16.34	16.44
Spain	Days open	-	-1.30	-1.30	-1.20	-1.10
		+	-0.10	-0.10	0.00	0.20
	LWT	-	10.66	10.90	11.53	13.07
		+	11.28	11.04	10.43	8.78
	Longevity	-	6.72	6.77	6.92	7.26
		+	6.62	6.66	6.79	7.07

From Table 6.11 it can be seen that for the UK the genetic gain in calving interval (days) decreased with an increasing economic value assigned to CH_4 production. This happened both for the positive and negative correlation between LWT and CH_4 production. For the Netherlands the genetic gain in calving interval did not change when the genetic correlation between LWT and CH_4 production was positive. When this correlation was negative, the genetic gain in calving interval changed when a literature or high economic value was assigned to CH_4 production. For Spain the genetic gain in days open (days) increased with an increasing economic value assigned to CH_4 production, both for the positive and negative correlation between LWT and CH_4 production.

For all countries the genetic gain in LWT (kg) increased when the genetic correlation between LWT and CH_4 production was negative. When this

correlation was positive, the genetic gain in LWT decreased for all countries. For the UK the genetic gain in lifespan only increased when a literature or high economic value was assigned to CH_4 production. This happened for both the positive and negative correlation between LWT and CH_4 production. For the Netherlands and Spain, the genetic gain in longevity (point breeding value and days, respectively) increased with an increasing economic value assigned to CH_4 production. This happened both for the positive and negative correlation between LWT and CH_4 production.

Scenario 4

Table 6.12 shows the average change in CH_4 production lost to air (kg^{-1}year), GWP_{20}, GWP_{100} and GWP_{500} (kg CO_2e) when 'non-organic calving index in days', 'non-organic average number of lactations in herd', 'weight of mature dairy cow for average milk yield relative to standard value', 'voluntary feed intake relative to standard value' and 'increase in litres of milk yield for medium yielding cows non-organic' changed with one-unit.

Table 6.12: The average change in CH_4 production lost to air (CH_4, kg) and GWP_{20}, GWP_{100} and GWP_{500} (kg CO_2e)

	CH_4	GWP_{20}	GWP_{100}	GWP_{500}
Calving index	-0.01	-0.58	-0.31	-0.17
Lactation number	-0.24	-25.96	-14.85	-7.53
Live weight	0.05	3.37	1.41	0.51
Feed intake	-0.03	-1.43	0.04	-0.70
Milk yield	-0.22	-18.91	-8.66	-3.67

From Table 6.12 it can be seen that a one-unit (day) increase in 'calving index' caused a decrease of 0.01 kg of CH_4 production lost to air. For GWP_{20}, GWP_{100} and GWP_{500} this was a decrease of -0.58, -0.31 and -0.17 kg CO_2e respectively. A one-unit (0.1 lactation) increase in 'number of lactations in the herd (non-organic)' caused a decrease of -0.24 kg of CH_4 production lost to air. For GWP_{20}, GWP_{100} and GWP_{500} this was a decrease of -25.96, -14.85

and -7.53 kg CO_2e respectively. A one-unit (1 kg) increase in 'weight of mature dairy cow for average milk yield (non-organic)' caused an increase of 0.04 of CH_4 production lost to air. For GWP_{20}, GWP_{100} and GWP_{500} this was an increase of 3.37, 1.41 and 0.51 kg CO_2e respectively. A one-unit (1 kg) increase in 'voluntary feed intake relative to standard value (non-organic)' caused a decrease of 0.03 of CH_4 production lost to air. For GWP_{20} and GWP_{500} this was a decrease of 1.43 and 0.70 kg CO_2e respectively. For GWP_{100} the impact increased by 0.04 kg CO_2e. A one-unit (1 kg) increase in 'increase in litres of milk yield for medium yielding cows (non-organic)' caused a decrease of 0.22 of CH_4 production lost to air. For GWP_{20}, GWP_{100} and GWP_{500} this was a decrease of 18.91, 8.66 and 3.67 kg CO_2e respectively.

Table 6.13: The change in CH_4 production lost to air (CH_4 kg) and GWP_{20}, GWP_{100} and GWP_{500} (kg CO_2e) when the one-unit change in the traits of interest (CI, calving index; LN, lactation number; LWT, live weight; FI, feed intake; and MY, milk yield) is included in the selection index calculations

Correlation	CH_4		GWP_{20}		GWP_{100}		GWP_{500}	
LWT CH_4	-	+	-	+	-	+	-	+
CI	0.04	0.04	3.87	3.87	2.11	2.11	1.14	1.14
LN	0.17	0.15	18.30	16.27	10.47	9.31	5.31	4.72
LWT	1.66	1.67	132.03	132.68	55.12	55.38	19.93	20.02
FI	0.87	0.86	45.29	45.08	1.41	1.40	22.30	22.19
MY	123	123	10,391	10,386	4,759	4,756	2,015	2,014

Table 6.13 shows the increase in index value in kg of CH_4 production lost to air and in kg CO_2e for GWP. It can be seen that for all traits an increase of one-unit results in a higher loss of CH_4 production to air or an increase in GWP. The increase in CH_4 production to air is small for all traits (0.04 to 1.67 $kg^{-1}year$), except for MY which showed an increase of 123 $kg^{-1}year$. For all traits the increase in GWP is highest for GWP_{20} and lowest for GWP_{500}, except for feed intake, where GWP_{100} has the lowest increase. The highest increase in GWP occurs for MY, with an increase of 10,391 and 10,386 kg CO_2e for GWP_{20}, 4759 and 4756 kg CO_2e for GWP_{100}, and 2015 and 2014 kg

CO_2e for GWP_{500}. The lowest increase in GWP occurs for calving index, with an increase of 3.87 kg CO_2e for GWP_{20}, 2.11 kg CO_2e for GWP_{100} and 1.14 CO_2e for GWP_{500}. It should be noted that the increase in GWP_{100} for calving index is higher than the increase in GWP_{100} for feed intake. There is almost no difference between the results using a positive or negative correlation between LWT and CH_4 production.

Table 6.14 shows the absolute index values when the five traits of interest simultaneously change with one-unit. It can be seen that the index value is negative for all combinations tested. For all economic values for CH_4 production and all GHG impacts tested (CH_4 lost to air, GWP_{20}, GWP_{100} and GWP_{500}) the index value is lower when the genetic correlation between LWT and CH_4 production is negative. When comparing the GHG impact within the economic value for CH_4 production and genetic correlation between LWT and CH_4 production, it can be seen that the selection index value becomes lower starting at scenario CH_4 lost to air, moving on to GWP_{20} and GWP_{100}. However, the economic value for GWP_{500} is the least negative of all GHG impacts within all economic values for CH_4 production and genetic correlation between LWT and CH_4 production.

Table 6.14: Selection index values (in £) when all five traits (milk yield, calving interval, lifespan, live weight and dry matter intake) change with one-unit simultaneously, for CH_4 lost to air (CH_4, kg), GWP_{20}, GWP_{100} or GWP_{500} (kg CO_2e), assuming a low, literature or high economic value for CH_4 production (CH_4) when assuming a positive or negative correlation (corr) between live weight (LWT) and CH_4 production (CH_4)

Economic value CH_4	Corr LWT CH_4	CH_4	GWP_{20}	GWP_{100}	GWP_{500}
Low	+	-17.89	-17.97	-18.12	-17.70
	-	-18.56	-18.64	-18.79	-18.37
literature	+	-16.46	-16.55	-16.70	-16.28
	-	-17.16	-17.25	-17.40	-16.98
High	+	-12.99	-13.08	-13.23	-12.80
	-	-13.76	-13.85	-14.00	-13.58

6.4 DISCUSSION

6.4.1 Ellinbank selection index theory analyses

The correlated responses in MY, LWT, DMI and CH_4 production were obtained when selecting and restricting these traits in different combinations. First, the correlated responses when selecting for one trait at a time were investigated. When selecting for higher MY, CH_4 production increased simultaneously, unless only LWT was restricted to no genetic gain. This was as expected due to the positive correlation between MY and CH_4 production. When selecting for lower DMI, CH_4 production decreased as well, unless at least LWT was restricted to no genetic gain. This was as expected due to the positive correlation between DMI and CH_4 production and due to the biological connection between DMI and CH_4 production as CH_4 is a by-product of rumen fermentation. When directly selecting for lower CH_4 production, CH_4 production decreased, but genetic gain in MY did not increase anymore and in some cases even decreased. The analyses selecting for genetic gain in one trait at a time show the relationships these four traits have with each other and show how complex it is to directly decrease CH_4

production without resulting in an undesired change in other traits. This shows the importance of selecting for multiple traits simultaneously.

The analyses on selection for multiple traits simultaneously showed that it is possible to simultaneously select for increased genetic gain in MY and decreased genetic gain in CH_4 production. As can be seen from the results, this led to an increase in the genetic gain of DMI. It is possible to restrict the genetic gain in DMI to no genetic change, but this results in a very small genetic gain in MY. When the genetic gain in DMI increases this likely leads to an increase in feed costs. This does not have to be a problem if this is covered by an increase in profit from MY. However, if on sector level the production of more feed indirectly causes higher CH_4 production, then on sector level progress made in the reduction of greenhouse gases is not maximised.

An important aspect of selection index theory is to identify traits that will become important in the future (Miglior et al., 2017). These analyses identify CH_4 production as an important trait to include in selection indexes in future, due to the interaction with important production traits as MY, LWT and DMI. It should be noted that more traits play a role in the national breeding goal, as the following analyses have shown. Especially the effect selection on milk yield and CH_4 production has on fertility traits needs to be taken into account. If a reduction of CH_4 production at cow level would reduce fertility, this could cause an increase in CH_4 production at herd level when taking into account herd replacements (Garnsworthy, 2004).

6.4.2 National breeding goals for the Netherlands, the UK and Spain

Scenario 1, 2 and 3

The index values of the different countries under scenario 1 show that the increase in profit was the largest in the Netherlands. This is likely due to the relatively high economic values assigned to milk protein and milk fat and no direct selection on milk yield. The genetic gain in CH_4 production was lowest

for the Netherlands when CH_4 production is included in the current breeding goals. This might be due to the relative small variation between economic values the Netherlands has assigned to the traits in the selection index.

Under scenario 2 the biggest decrease in index values for all countries was caused by a decrease in milk protein. This is as expected as the genetic correlation between CH_4 production and milk protein is 0.77. The index value for all countries became lower when the genetic change in CH_4 production was restricted to zero. For the Netherlands the index value decreased with only 3%. However, as the Netherlands already had a lower genetic gain in CH_4 production under scenario 1, less change was needed to achieve a genetic gain of zero. The decrease in index values for Spain was 10% to 11% and for the UK was 14% to 15%. Under scenario 3 the decrease in genetic gain in CH_4 production became bigger when the economic value assigned to CH_4 production became higher.

For the economic value based on the literature shadow price for CH_4 production, the decrease (relative to scenario 1) in genetic gain in CH_4 production varied from 0.31 to 0.66 $g^{-1}d/y$. This means CH_4 production still increased by 3.51 to 7.89 $g^{-1}d/y$. For the economic value based on the high shadow price for CH_4 production, the decrease (relative to scenario 1) in genetic gain in CH_4 production varied from 0.87 to 1.97 $g^{-1}d/y$. This means CH_4 production still increased by 2.95 to 6.73 $g^{-1}d/y$. This shows that even with the high shadow price used for CH_4 production, CH_4 production still increased for all countries.

When looking at the countries individually, the Netherlands had the lowest genetic gain for CH_4 production for each scenario, including scenario 1. This might be due to the economic values the Netherlands has assigned to the traits for which a correlation with CH_4 production is available in the index. The Netherlands did not assign a direct value to milk yield, while the values assigned to milk fat and protein were higher than those used in Spain. The UK assigned higher values to milk fat and milk protein than Spain as well, and for

milk protein assigned a higher value than the Netherlands. The UK however assigned a small negative value to milk yield, while Spain did not. The high economic values the Netherlands assigned to the milk traits might have limited the reduction in the index value for scenario 2 and 3. Of course the economic values of traits that were included in the selection indexes without a direct correlation with CH_4 production will have had an influence on the change in CH_4 production as well, through their direct correlations with the traits CH_4 production was directly correlated with. Looking at the countries individually also showed that the absolute change in CH_4 production was lowest in the Netherlands for all scenarios. This might be due to the variation in economic values for the non-CH_4 traits, which is smaller for the Netherlands than for the UK and Spain. This seems to make the NVI more robust against changes. However, as CH_4 production was already lowest in the Netherlands under scenario 1, the required changes in all traits in the index under the different scenarios was already smaller than for the other countries.

Scenario 4

Change in GHG impact

The change in GHG impacts (CH_4 production lost to air (kg), GWP_{20}, GWP_{100} and GWP_{500} (kg CO2e)) was obtained when 'non-organic calving index in days', 'non-organic average number of lactations in herd', 'weight of mature dairy cow for average milk yield relative to standard value', 'voluntary feed intake relative to standard value' and 'increase in litres of milk yield for medium yielding cows non-organic' changed with one unit in the LCA.

A one-unit increase in 'non-organic calving index in days' caused a decrease in CH_4 production lost to air (kg) and GWP_{20}, GWP_{100} and GWP_{500} (kg CO2e). This was not as expected. As discussed in chapter 1, a longer calving interval causes a lower annual milk yield per cow (Kossaibati and Esslemont, 1995), which on a herd level means more cows are needed to keep the production

level up. More cows will lead to higher CH_4 production as all cows have a portion of CH_4 production originating from maintenance (Garnsworthy, 2004). To compensate for the loss in fertility it was expected that the number of herd replacements went up, also increasing CH_4 production at herd level (Garnsworthy, 2004). However, these analyses have shown that when all material flows and activities in the LCA were taken into account, the net effect of increasing calving index was a decrease in GHG impacts. When looking at the processes influencing the LCA value, the increase in CH_4 production lost to air (kg) is caused by a decrease in farmyard manure (4kg $N^{-1}t$), a decrease in organic milk herds (low, medium and high yielding cows) and a shift in non-organic calving from high yielding cows towards low yielding cows. In addition to the changes in these material flows and activities, the change in GWPs was caused by a decrease in concentrates fed, lowland grazing and silage, slurry (3kg and 5kg $N^{-1}t$), use of small delivery vehicles, diesel and herd replacements, and an increase in maize silage and wheat straw fed, and farmyard manure (6kg $N^{-1}t$, only for GWP 20). The shift in non-organic calving now included medium yielding cows to low yielding cows. This shows that, opposite to the expectation from literature, the number of herd replacements on a sector level decreases when calving index increases.

A one-unit increase in 'non-organic average number of lactations in herd' caused a decrease in CH_4 production lost to air (kg), GWP_{20}, GWP_{100} and GWP_{500} (kg CO2e). This was as expected as a longer herd life means less replacement heifers are needed. This results in lower CH_4 production originating from the unproductive first years of a cow's life.

When looking at the processes influencing the LCA value, the decrease in CH_4 production lost to air (kg) was caused by a decrease in lowland silage, slurry (5 kg $N^{-1}t$), farmyard manure (4 kg $N^{-1}t$) and herd replacements and an increase in farmyard manure (6 kg $N^{-1}t$). In addition to these changes, the change in GWPs was also caused by a decrease in concentrates fed, lowland grazing,

slurry (3 kg $N^{-1}t$), use of small delivery vehicles, cattle housing and diesel use, and an increase in wheat straw fed. This shows that indeed an increase in herd life causes a reduction in replacement heifers needed.

A one-unit increase in 'weight of mature dairy cow for average milk yield relative to standard value' caused an increase in CH_4 production lost to air (kg) and GWP_{20}, GWP_{100} and GWP_{500} (kg CO2e). This was as expected as heavier cows need more energy for maintenance and therefore produce more CH_4.

When looking at the processes influencing the LCA value, the decrease in CH_4 production lost to air (kg) is caused by an increase in lowland silage (non-org), slurry (5kg $N^{-1}t$, non-org), farmyard manure (4kg $N^{-1}t$, non-org), non-organic calving (low, medium and high yielding cows), herd size organic herds (low, medium and high yielding cows) and herd replacements.

In addition to these changes, the change in GWPs was also caused by an increase in lowland grazing (org and non-org), lowland silage (org), slurry (3kg $N^{-1}t$, non-org), farmyard manure (6kg $N^{-1}t$, non-org), farmyard manure (org; GWP_{20}: 4 and 6kg $N^{-1}t$, GWP_{100}: 4kg $N^{-1}t$), use of small delivery vehicles and cattle housing, and a decrease in concentrates (org and non-org), maize silage and wheat straw fed. This shows that a change in weight of mature cows leads to a shift in feed products used. As discussed in chapter 1, it is known that diet composition has an influence on the amount of CH_4 produced. Literature has shown that grass and grass silage lead to a higher CH_4 production than concentrates and maize silage (Beauchemin et al., 2008). It is therefore not surprising that a shift away from concentrates and maize silage results in a higher CH_4 production.

A one-unit increase in 'voluntary feed intake relative to standard value' caused a decrease in CH_4 production lost to air (kg), GWP_{20} and GWP_{500} (kg CO2e) and an increase in GWP_{100}. This was not as expected. It was expected that an increase in utilised feed would lead to an increase in CH_4 production. It was

also expected that the GWP time frames would all move in the same direction. When looking at the processes influencing the LCA value, the decrease in CH_4 production lost to air (kg) was caused by an increase in lowland grazing and silage (non-org) and a decrease in concentrates fed (non-org), herd size (organic, low and medium yielding cows) and herd replacement (non-organic). A shift occurred in non-organic calving from low and mid yielding cows to high yielding cows. In addition to these changes, the change in GWPs was also caused by an increase in lowland grazing and silage (org), maize silage and wheat straw fed (GWP_{20} and GWP_{100}), and a decrease in concentrates fed (org), slurry (3 and 5kg $N^{-1}t$), farmyard manure (4 and 6kg $N^{-1}t$), small vehicle use, cattle housing, diesel use, herd size (org, high yield), herd replacements (org, GWP_{20} and GWP_{100}), non-organic calving (low, medium and high yielding cows, GWP_{500}). A shift occurred in non-organic autumn calving from low to medium and high yielding cows (GWP_{20} and GWP_{100}) and in non-organic spring calving from low and medium to high yielding cows (GWP_{20} and GWP_{100}). The decrease in GHG impact when voluntary feed intake increased therefore seems due to a change in diet composition rather than an increase in intake of the current diet, as well as a shift towards smaller herds with higher yielding cows.

A one-unit increase in 'litres of yield for medium yielding cows non-organic' caused a decrease in CH_4 production lost to air (kg), GWP_{20}, GWP_{100} and GWP_{500} (kg CO2e). This was as expected as an increase in milk yield per cow means fewer cows are needed to reach the same level of milk production. Therefore, on a herd level this reduces the CH_4 production coming from feed utilised for body maintenance.

When looking at the processes influencing the LCA value, the decrease in CH_4 production lost to air (kg) is caused by an increase in maize silage (non-org) and a decrease in lowland silage fed (non-org), slurry (3 and 5kg $N^{-1}t$, non-org), farmyard manure (4 and 6kg $N^{-1}t$, non-org), non-organic calving (low, medium

and high yielding cows), herd size (organic, low, medium and high yielding cows) and replacements needed (org and non-org). In addition to these changes, the change in GWPs was also caused by an increase in concentrates (org) and wheat straw (non-org) fed, and a decrease in concentrates (non-org) fed, lowland grazing (org and non-org) and silage (org), slurry (3 and 5kg $N^{-1}t$, org), farmyard manure (4kg $N^{-1}t$, org; 6kg $N^{-1}t$, org, GWP_{20}), small delivery vehicle use and diesel use. This shows that an increase in milk production per cow at sector level indeed leads to a decrease in non-organic calving and herd replacements.

Change in index value

The absolute index values of the selection index were obtained when MY, calving interval, lifespan, LWT and DMI simultaneously change with one-unit in the LCA. It was found that for all combinations of GWP impacts and economic values assigned to CH_4 production the selection index value in pound sterling became negative. The largest loss in index value was caused by a reduction in profit for milk fat and protein. When comparing the index values obtained using a positive or negative genetic correlation between LWT and CH_4 production, the index value is always lower when the negative correlation is used. For all combinations of GHG impact and economic value assigned to CH_4 production this was mainly caused by the difference in change in lifespan. For all combinations of GHG impact and economic value assigned to CH_4 production the economic gain from lifespan was positive when assuming a positive correlation and negative when assuming a negative correlation between LWT and CH_4 production. Other traits that contributed to a lower index value when using the negative correlation between LWT and CH_4 production are milk fat and protein, calving interval and CH_4 production.

When comparing the change in GHG impacts from Table 6.12 with the index values obtained when all five traits of interest change simultaneously (Table

6.14) it was seen that a decrease in GHG impact is not necessarily linked to an increase in the index value. When comparing the index values between GHG impacts, it was found that index values were highest for GWP_{500} and lowest for GWP_{100}. This was not as expected as it was expected that the GHG impact would increase when the projection time frame of the potential to warm the planet increased. Although Table 6.12 shows that the reduction in GHG impact indeed decreases when the time frame increases, the change in selection index value does not follow this trend. This can be explained by the influence the underlying correlations between traits in the selection index have on each other, as well as the desired direction of change of a trait and the magnitude of the economic value assigned to a trait. When looking at the index values for the individual traits, the highest overall index values for GWP_{500} and the lowest overall index values for GWP_{100} can be explained by the index values for milk fat and protein. For all GHG impacts the index values for these traits reduces, but the reduction is smallest for GWP_{500} and largest for GWP_{100}.

It should be noted that for all analyses using the national breeding goals the genetic and phenotypic correlations between CH_4 production and most traits in the selection indexes (including fertility traits) are currently unknown, and therefore were set to zero. It is unlikely that these correlations are indeed zero and therefore the results of the selection index analyses will change when these parameters become available. Correlations obtained in this study were included.

6.5 CONCLUSION

Correlated responses for MY, LWT, DMI and CH_4 production were obtained for the Ellinbank herd, to investigate the biological mechanisms underlying response to selection. These analyses have shown that it is possible to simultaneously increase MY and reduce CH_4 production at cow level. When doing so DMI simultaneously increases. This does not have to be a problem if the profit in

MY compensates for the extra cost in feed requirements. However, it needs to be taken into account that a higher feed requirement could increase CH_4 production elsewhere in the sector.

The effect of including CH_4 production into the national breeding goals for the Netherlands, Spain and the United Kingdom was investigated. These analyses have shown that when including CH_4 production into the current breeding goals (without restrictions or economic values assigned), CH_4 production is currently increasing in all three countries. When assigning a low, medium or high (negative) economic value to CH_4 production, the genetic gain in CH_4 production still increases for all three countries. This indicates that only assigning an economic value to CH_4 production is not enough to halt the increase in genetic gain in CH_4 production. When restricting the genetic gain in CH_4 production to zero, the economic index values (€ or £) decrease. This indicates that halting the increase in genetic gain in CH_4 production will bring an economic cost. This could cause difficulties for implementing a breeding strategy for halting the increase in genetic gain in CH_4 production in practise.

LCA analyses has shown that on a sector level a one unit increase in calving index, lactation number, feed intake and MY will cause a decrease, and in LWT will cause an increase, in CH_4 production lost to air (kg). These analyses have shown that it is important to perform an LCA instead of extrapolating the effect the increase of a trait has from cow level to herd or sector level. For the current study this is especially noticeable for calving index, where on cow level literature suggests that an increase in calving index will cause an increase in CH_4 production. Results from the LCA analyses show that actually the number of herd replacements decreases on sector level, resulting in lower CH_4 production lost to air (kg).

7. GENERAL DISCUSSION

7.1 THESIS OVERVIEW

7.1.1 Genetic and genomic parameters

The first two objectives of this study were to obtain genetic and genomic parameters for CH_4 production and to obtain correlations with MY, LWT and DMI. Table 7.1 shows the heritabilities obtained using pedigree analyses for all model types and herds and Table 7.2 shows the heritabilities obtained using G-matrix analyses for the Langhill and Ellinbank herd.

Table 7.1: Heritabilities obtained using pedigree analyses over all model types and herds for milk yield (MY), live weight (LWT), CH_4 production (CH_4), dry matter intake (DMI), milk yield combined (MYcomb), live weight combined (LWTcomb), DMI Langhill (DMILang), CH_4 production Nottingham (CH_4Notts) and CH_4 production Langhill (CH_4Lang)

Herd	Model type	Variate	Trait	Heritability
Nottingham	RR[1] repeatability	Bivariate	MY	0.18-0.65[2]
	animal			0.16-0.65[2]
			LWT	0.48-0.67[2]
				0.40-0.64[2]
			CH_4	0.12-0.45[2]
				0.12-0.40[2]
Langhill	RR[1] repeatability	4-trait variate	MY	0.29-0.41[2]
	animal		LWT	0.67-0.72[2]
			DMI	0.10-0.24[2]
	linear animal	4-trait variate	CH_4	0.22
Notts&Lang[3]	linear repeatability	4-trait variate	MYcomb	0.40
	sire	trivariate	MYcomb	0.40
		4-trait variate	LWTcomb	0.55
		trivariate	LWTcomb	0.56
		4-trait variate	DMILang	0.20
		trivariate	CH_4Notts	0.22
	linear sire	4-trait variate	CH_4Lang	0.28
Ellinbank	linear animal	trivariate	MY	0.35-0.37[4]
			LWT	0.72-0.76[4]
			DMI	0.10-0.14[4]
			CH_4	0.03-0.09[4]

[1]Random Regression
[2]Heritabilities ranging over lactation
[3]Nottingham and Langhill combined analyses
[4]Range of heritabilities obtained with separate trivariate models

Table 7.2: Heritabilities obtained using G-matrix analyses for the Langhill and Ellinbank herd for milk yield (MY), live weight (LWT), dry matter intake (DMI) and CH_4 production (CH_4)

Herd	Model type	Variate	Trait	Heritability
Langhill	linear repeatability animal	4-trait variate	MY	0.28
			LWT	0.42
			DMI	0.42
	linear animal		CH_4	0.07
Ellinbank	linear animal	4-trait variate	MY	0.29
			LWT	0.79
			DMI	0.32
			CH_4	0.32

From these tables it can be seen that the heritability for CH_4 production varies from 0.03 to 0.45. This shows that heritability for CH_4 production is low to moderate and genetic selection is possible, but will be slow. The genetic correlation obtained between CH_4 production measured in the milking robot during milking and measured using an LMD was -0.15 to 0.20 (see chapter 4), indicating that these two CH_4 production traits are genetically different traits. This explains the wide range in heritabilities obtained in this study.

Table 7.3 shows a comparison of genetic correlations over all herds and models using pedigree based analyses and table 7.4 shows a comparison of genetic correlations obtained using G-matrix analyses for the Langhill and Ellinbank herd.

Table 7.3: Genetic correlations (Gen corr) obtained using pedigree analyses over all model types and herds for milk yield (MY), milk yield combined (MYcomb), live weight (LWT), live weight combined (LWTcomb), dry matter intake (DMI), dry matter intake Langhill (DMILang), CH_4 production (CH_4), CH_4 production Nottingham (CH_4Notts) and CH_4 production Langhill (CH_4Lang)

Herd	Model type	Variate	Trait	Gen corr
Nottingham	RR[1] repeatability	bi	MY LWT	-0.23-0.02[2]
	animal		MY CH_4	0.38-0.57[2]
			LWT CH_4	0.01-0.02[2]
Langhill	RR[1] repeatability	4-trait	MY LWT	-0.60-0.25[2]
	animal		MY DMI	0.13-0.29[2]
			LWT DMI	0.36-0.59[2]
	combined[3]	4-trait	MY CH_4	-0.36-0.09[2]
			LWT CH_4	0.00-0.09[2]
			DMI CH_4	0.05-0.11[2]
Notts&Lang[4]	linear repeatability	4-trait	MYcomb LWTcomb	0.28
	sire	tri	MYcomb LWTcomb	0.29
		4-trait	MYcomb DMILang	0.71
		tri	LWTcomb DMILang	0.70
		tri	MYcomb CH_4Notts	0.23
		4-trait	LWTcomb CH_4Notts	0.50
	combined[5]	4-trait	MYcomb CH_4Lang	-0.31
		4-trait	LWTcomb CH_4Lang	0.85
		4-trait	DMILang CH_4Lang	0.48
Ellinbank	linear animal	tri	MY LWT	0.40-0.43[6]
			MY DMI	0.95-1.05[6]
			LWT DMI	0.80-0.86[6]
			MY CH_4	0.45-0.48[6]
			LWT CH_4	0.99-1.10[6]
			DMI CH_4	0.60-0.98[6]

[1]Random Regression
[2]Correlations ranging over lactation
[3]Combination of random regression repeatability and linear animal model
[4]Nottingham and Langhill combined analyses
[5]Combination of linear repeatability and linear sire model
[6]Range of correlations obtained with separate trivariate models

Table 7.4: Genetic correlations (Gen corr) obtained using G-matrix analyses for the Langhill and Ellinbank herd for milk yield (MY), live weight (LWT), dry matter intake (DMI) and CH_4 production (CH_4)

Herd	Model type	Variate	Traits	Gen corr
Langhill	linear repeatability	4-trait variate	MY LWT	0.36
			MY DMI	0.86
			LWT DMI	0.67
	combined[1]		MY CH_4	-0.57
			LWT CH_4	0.28
			DMI CH_4	-0.09
Ellinbank	linear animal	4-trait variate	MY LWT	0.51
			MY DMI	0.78
			LWT DMI	0.77
			MY CH_4	0.27
			LWT CH_4	0.44
			DMI CH_4	0.34

[1]Combination of linear repeatability and linear animal model

From these tables it can be seen that both a positive and negative correlation was found between MY and CH_4 production. From a biological point of view both correlations are possible. In early lactation cows enter a negative energy balance. During this period energy utilised for milk production was largely body energy, rather than energy from DMI. Therefore, in early lactation the correlation is expected to be negative. Later in lactation energy for milk production is utilized from DMI, causing a positive correlation between MY and CH_4 production. This is in agreement with De Haas et al. (2011), who found a correlation of -0.66 for week 1 to 5, -0.18 for week 6 to 10, 0.42 for week 11 to 15, 0.67 for week 16 to 20, 0.70 for week 21 to 25 and 0.60 for week 26 to 30 between MY and predicted CH_4 production. All correlations found between LWT and CH_4 production in the current study were positive, although small in some cases. From a biological point of view both a positive and a negative correlation were possible, as already discussed in Chapter 2. When looking at the whole lifetime of the cow, LWT starts off low and increases up to a certain level after which it remains stable. DMI and CH_4 production

move in the same direction as heavier cows need more feed for maintenance and therefore ingest more substrate from which CH_4 can be produced. This results in a positive correlation. On lactation level, due to negative energy balance in early lactation, LWT decreases in early lactation after which it increases again towards late lactation. Methane production follows the same curve as DMI, which starts low in early lactation, peaks in early/mid lactation and then slowly declines towards late lactation. This results in a negative correlation. Currently Lassen and Løvendahl (2016) have published a genetic correlation (SE) between LWT and CH_4 production, which was -0.18 (0.08). Genetic correlations found between DMI and CH_4 production were moderately high to high, except for the G-matrix analyses of the Langhill herd. From a biological point of view this is as expected as DMI is the source for rumen microbes to produce CH_4 production from. This is also in agreement with literature as Donoghue et al. (2016) found a correlation (SE) of 0.84 (0.06) and Manzanilla-Pech et al. (2016a) found a correlation (SE) of 0.83 (0.05).
These results show that care must be taken when including CH_4 production into national breeding goals. The positive correlation between MY and CH_4 production could lead to a decrease in MY when the increase in genetic gain for CH_4 production is actively restrained. The positive correlation between DMI and CH_4 production could also lead to a decrease in MY when the genetic gain for CH_4 production is restrained, due to the positive correlation between DMI and MY.

Differences between herds

Differences in genetic parameters found between the herds can be explained by differences in the data collection, management systems and location of the herds. Data for the Nottingham herd were collected on a research farm that is run as a commercial farm. Data for the Ellinbank and Langhill herds were collected on research herds that are run as research facilities. For the

Langhill herd, management systems related to feeding and diet were different between the two feed groups, as described in chapter 3. Methane production was measured in a different way in each herd and experiments were set up in different ways. For the Nottingham herd CH_4 production was measured for five consecutive months, for the Langhill herd CH_4 production was measured as point estimates during consecutive days for multiple years. For both herds data on other traits were available for the whole lactation length for most cows. For the Ellinbank herd CH_4 production was measured in 9 batches over 3 years, where each batch was measured for 3 to 5 days in a row. Data on other traits were measured between November 2010 and November 2015 and were available as point estimates as well. Data on other traits were measured both on cows for which a CH_4 production measurement was available and on cows for which this was not the case.

Different diets were fed on the three farms, which could have influenced the genetic parameters obtained. During the experiment the Langhill herd was fed on high forage/homegrown forage and low forage/by products depending on the diet group the cow was in. The Nottingham herd was fed *ad libitum* on a partial mixed ration and concentrates during milking. The Ellinbank herd was fed on alfalfa pellets supplemented with crushed wheat at the time of milking. These differences in diets could have caused differences in for example DMI, feed utilisation, energy available for milk production and energy available to maintain LWT. Also, as different nutrients are broken down by different pathways in the rumen, different diets can lead to different levels of CH_4 production (Ungerfeld, 2013).

7.1.2 Pedigree, G-matrix and H-matrix analyses

Literature has shown that in general heritabilities based on genotype information are lower than heritabilities obtained using pedigree information.

This gap between heritability estimates originating from genotype or from pedigree information is often referred to as the 'missing heritability problem' (Turkheimer, 2011). Bourrat and Lu (2016) describe that heritability obtained in G-matrix analyses is exclusively based on genetic variance coming from variance caused by the DNA variants which they refer to as "DNA-based narrow-sense heritability" (h^2_{DNA}). Heritability obtained from traditional quantitative methods, like pedigree based analyses, includes the variance caused by the DNA variants as well as the variance caused by epigenetic factors and extra components that represent non-additive genetic variance (e.g. dominance variance and environmental correlation between relatives). Bourrat and Lu (2016) refer to this as "broader-sense heritability" (h^2_b). "Narrow-sense heritability" is defined as $h^2 = \frac{V_A}{VP}$ (Falconer and Mackay, 1996), where V_A includes the additive genetic effect coming from DNA variants as well as from epigenetic factors (Bourrat and Lu, 2016). When comparing h^2_b to h^2, h^2_b is overestimated, where h^2_{DNA} is underestimated compared to h^2. This is an explanation for the difference in heritabilities resulting from pedigree based and G-matrix analyses.

In the current study some heritabilities resulting from G-matrix analyses are higher than heritabilities resulting from pedigree based analyses. As the extra information in h^2_b (epigenetic factors and non-additive genetic variance) compared to h^2_{DNA} comes from the pedigree, it is likely that the quality and the size of the pedigree has a large influence on h^2_b. In the current study, due to calculation limitations, the pedigrees were limited to a maximum of three generations. This might not have been enough information to fully cover the genetic variance coming from the epigenetic factors and non-additive genetic variance (see also section 7.4, subsection pedigree and genotypes). As genotypes include information on relationships between animals regardless of generation information, it is likely that more relationships between animals are covered by the genotypes than by the three generations in the pedigree. This

could cause the genetic additive variance coming from DNA variants ($V_{A_{DNA}}$) obtained by G-matrix analyses to be higher than the $V_{A_{DNA}}$ obtained by the pedigree based analyses. This explains why some analyses in the current study result in a higher heritability for G-matrix analyses than for pedigree based analyses.

When comparing genetic correlations obtained using pedigree based analyses with correlations obtained using G-matrix analyses, for the Ellinbank herd all correlations found using G-matrix analyses were lower than correlations found using pedigree based analyses. However, only the correlation found between LWT and CH_4 production obtained with G-matrix analyses was significant. Most of the correlations obtained with pedigree based analyses were out of range, which is likely due to limitations caused by the size of the dataset. For the Langhill herd the genetic correlations obtained using pedigree based and H-matrix analyses were very similar. This was as expected as both analyses include pedigree information. The genetic correlations obtained using G-matrix analyses between MY and CH_4 production, and LWT and CH_4 production were stronger than correlations found with the other analyses methods. The genetic correlation obtained using G-matrix analyses between DMI and CH_4 production was similar but in the opposite direction than correlations found using the other analyses methods. None of the genetic correlations found for the Langhill herd were significant.

7.1.3 Selection indexes and national breeding goals

Selection index theory analyses have shown that from a biological point of view it is possible to increase the genetic gain in MY and decrease the genetic gain in CH_4 production simultaneously. In order to gain insight in the biological mechanisms underlying the influence the traits have on each other, the economic values assigned were standardised for all traits. In practise

however, economic values of traits will not be standardised, and traits deemed more valuable, or more detrimental will get a larger (either positive or negative) value assigned. This will have an influence on the resulting genetic gains. It should be emphasised that selection index theory analyses the effects and impacts traits have on each other at cow level, rather than on herd or sector level.

In the current study the point value heritability for CH_4 production obtained for the Ellinbank herd was used, rather than heritabilities obtained using random regression models for the Langhill and Nottingham herd. This was done as selection indexes are generally expressed over lactation, rather than e.g. over week of lactation. As in the current study the selection index was expressed over lactation and interest was in genetic gain per trait, a point value heritability estimate was sufficient. If interest would lie in e.g. changing milk production in early lactation, it could be advantageous to include milk yield in early lactation as a separate trait in the index. To do so, the corresponding heritability for that period of lactation needs to be used, as well as corresponding correlations with other traits in the index. Meyer (2004) found that using genetic parameters obtained using random regression models included in selection indexes can improve the accuracy of breeding values obtained. Another application for the implementation of parameters obtained from random regression models into selection indexes is to investigate the effects of environmental sensitivity of traits, as done by Calus and Veerkamp (2003).

The analyses of the national breeding goals of the UK, Spain and the Netherlands have shown that CH_4 production is currently increasing in all three countries. This emphasises the need of implementing CH_4 production into the current breeding goals as soon as feasible. The results of the analyses of scenario 2 showed that when restricting CH_4 production to no genetic gain, all index values decrease (3 to 15%). Although the genetic gain in CH_4 production is restricted to zero, this does not mean there is no CH_4 production at all. It

merely means CH_4 production remains on the same level as for the previous generation. The results of the analyses of scenario 3 showed that even with an unrealistically high shadow price assigned to CH_4 production, CH_4 production still increases (3 to 7 $g^{-1}d$). This indicates that assigning a negative economic value to CH_4 production in the national breeding goals is not sufficient to halt the increase in CH_4 production originating from current breeding goals.

For scenario 4 a life cycle assessment (LCA) was performed to examine the effects of changes within traits on the GHG outputs of the dairy sector. The most important conclusion from this analysis is that it is not always possible to extrapolate cow or herd level results to sector level. For example, at cow level it was expected that an increase in calving index would lead to a decrease in annual milk yield, which would lead to an increase in the number of cows on herd level. This was expected to increase the number of herd replacements needed (see chapter 1 for a more extensive description). However, the results of the LCA showed a decrease in herd replacements needed on sector level when calving index increased. Similar results were found for an increase in voluntary feed intake. At cow level it was expected that an increase in feed intake leads to an increase in CH_4 production as *Methanogens* in the rumen gain substrate to feed on. The results of the LCA however, showed that increased voluntary feed intake leads to a change in diet and herd composition. These results emphasise the importance of LCA on herd level rather than extrapolation from cow level results. This by no means indicates that LCA can replace selection index theory, as both methods obtain very different parameters. It does however, show the importance of combining different information sources to gain the best outcome possible.

7.1.4 Previous work

Genetic and genomic parameters

Since the start of the current study more heritabilities on CH_4 production became available. Currently heritability for CH_4 production is expected to lie within the range of 0.05 (Pickering et al., 2015) and 0.30 (Pszczola et al., 2017). The current study has shown that CH_4 production measured with the use of a laser methane detector and CH_4 production measured in the milking robot during milking are different traits. Only a few heritability estimates are available yet to directly compare heritabilities originating from the same measurement techniques.

Pickering et al. (2015) found a heritability (se) of 0.05 (0.07) using LMD, which is similar to the heritability found for the initial analyses of the Langhill herd but lower than for the subsequent analyses. As both studies use data on the same research herd and measuring technique, a similar result was expected. For the initial analyses of the Langhill herd, CH_4 production was analysed as daily values. For the subsequent analyses CH_4 production was analysed as a weekly average in grams per day over calendar weeks. Pickering et al. (2015) used a weekly average in grams per day using week of lactation. Differences between the analyses in Pickering et al. (2015) and the subsequent analyses of the current study were the number of observations available and the fixed effects used in the model. Pickering et al. (2015) had data available on 57 cows, whereas the current study had data available on 198 cows. The difference in obtained heritability can be explained by the difference in fixed effects used in the model. Pickering et al. (2015) did not correct for the genetic line and feed group cows were assigned for, nor did they fit a lactation curve. This is likely due to the limited size of the dataset available. Not correcting for genetic line and feed group could lead to more noise in the dataset which, if assigned to the environmental variance, can result in a lower heritability. This shows the importance of adequately sized datasets when investigating new traits.

Whether the size of a dataset is reasonable depends on the statistical power and precision with which estimates (e.g. heritability) can be obtained from the dataset. When animals in a dataset are related to each other, calculating the effective sample size is complicated (Raffa and Thompson, 2016). Blangero et al. (2013) describe how to obtain the statistical power to detect heritability in a given dataset.

Lassen and Løvendahl (2016) found a heritability (se) of 0.21 (0.06) using the ratio between CH_4 and CO_2 concentration (ppm) measured in the milking robot during milking, multiplied by heat-producing units and converted to grams per day of CH_4 production. This is within the range of heritabilities obtained using random regression models for the Nottingham herd when CH_4 production was measured in the milking robot during milking. The heritability for CH_4 production for the Nottingham herd obtained using a linear repeatability sire model was very similar to this heritability as well. Manzanilla-Pech et al. (2016a) found a heritability (SE) of 0.23 ($\approx$0.23) for CH_4 production measured with the SF_6 tracer technique using G-matrix analyses. This is slightly lower but similar to the heritability found for the G-matrix analyses of the Ellinbank herd. This was as expected as the datasets used for these studies partly overlap.

7.2 LIMITATIONS AND STRENGTHS

Analyses to obtain genetic and genomic parameters

As described above, both random regression models and linear (repeatability) models were used to obtain genetic parameters. The advantage of the use of random regression models is their suitability for the analyses of longitudinal traits. As longitudinal traits are known to change gradually over time (Mrode, 2005), random regression models show the change in expression of genetic variance over time where linear models do not. A disadvantage of random regression models is that they tend to overestimate genetic parameters at the

extremes of lactation, as there is no data available before the first measurement and after the last measurement to extrapolate to. This effect increases when fewer observations are available for the beginning and end of lactation (Brotherstone et al., 2000).

The datasets available for the genetic and genomic analyses were collected before the start of the current study and were small. This resulted in difficulties obtaining significant results. As for some herds data were not available for the whole lactation length, it was not always possible to fit a random regression model, although this is the model of choice for longitudinal traits (Mrode, 2005). This meant that results between models were only indirectly comparable as estimates obtained from these analyses statistically have slightly different meanings.

Some of the correlation estimates and standard errors obtained were outside the expected range (-1 to 1 for correlations and 0 to 1 for standard errors). This occurred for the initial analyses of the Langhill data, and the pedigree based analyses of the Ellinbank data. As no problems arose with the subsequent analysis of the Langhill data, it is likely that the models chosen for the initial analyses were not suitable for the size of the dataset and the data structure for CH_4 production (daily values). For the Ellinbank analyses problems with estimates being out of range only arose for the pedigree based analyses. This was as expected as the dataset was small and the pedigree information available was limited. Therefore, for both herds it is likely that the out of range estimates were caused by over fitting of the models given the data (and data structure) available.

As not all animals were genotyped, the G-matrix analyses included less information than the pedigree based and H-matrix analyses. Again, this meant that a direct comparison between analyses was influenced by this difference in information.

For the datasets available CH_4 production was measured in different ways. This

presented the opportunity to compare measurement techniques and genetic parameters based on data obtained with these techniques. When the current study started, no heritabilities for or correlations with CH_4 production measured on dairy cattle were available. The current study contributed heritability and correlation estimates with MY, LWT and DMI for CH_4 production obtained through three different measurement techniques to the field of animal genetics and sustainability research.

It would have been beneficial to have CH_4 production measured with different techniques on the same cow to investigate whether the different techniques measure the same trait. However, due to the genetic linkage between the Langhill and Nottingham herd it was still possible to obtain a correlation between the CH_4 production traits measured for these herds. This correlation showed that these CH_4 production traits are genetically different, even though heritabilities were in agreement. Therefore, the current study does contribute to a first exploration into the genetic background of the different measurement techniques for CH_4 production.

As discussed in chapter 1, next to direct measures of CH_4 production, when direct measurements of CH_4 production are not available, it is possible to make use of proxies. Negussie et al. (2017a) reviewed the current *status quo* and described 5 main groups of CH_4 proxies; 1) feed intake and behaviour, 2) rumen characteristics, 3) milk characteristics, 4) hindgut and faeces, 5) measurements at animal level (LWT, conformation traits, animal type and lactation stage). However, they conclude that currently still problems arise with the robustness of proxies and suggest using a combination of two or more proxies.

It is becoming easier to obtain direct measurements of CH_4 production and labour intensity is going down. As CH_4 production and DMI are strongly correlated, this rises opportunities to use CH_4 production as a proxy for DMI as DMI still is labour intense to obtain.

In chapter 4 it became apparent that CH_4 production measured in the milking robot during milking and using an LMD are genetically different traits (e.g. the genetic correlation between the traits is lower than unity, Robertson (1959) suggests a threshold of 0.8). MY was measured in different ways for both herds. For the Nottingham herd MY was measured in an automatic milking system available to the cows 24h a day, 7 days a week. For the Langhill herd MY was also measured using milking robots, but cows were measured at fixed times each day. The difference between these two methods causes cows from the Langhill herd to have a limit on their milk production imposed by the farmer, but cows from the Nottingham herd can reach their full genetic potential. There might be different genes involved in full milk yield potential and milk yield whilst milking is restricted. If indeed different genes are involved, even when this is only partially, this means the traits are genetically different. The implication of this is that genetic parameters as heritability and correlations with other traits can be different. In practise this means that when data is used from different sources one needs to make sure traits measured in a different way are genetically the same trait, e.g. by checking the genetic correlation between the traits obtained. This can occur with any trait when measured in a different way, and therefore the measurement method will always need to be taken into account. Some literature is already available on this topic, e.g. Lembeye et al. (2018) showed that MY from cows milked once a day is genetically the same trait as MY from cows milked twice a day (genetic correlation (SE): 0.87 (0.08) to 0.96 (0.04)) for dairy cattle in New Zealand.

Analyses on selection indexes and national breeding goals

It is important to keep in mind that more traits than just MY, LWT, DMI and CH_4 production play a role in the national breeding goal. As not all correlations between traits of interest (e.g. health, fertility) are currently known, it is important that these correlations are obtained in the near future.

It is also important that these correlations are robust. This means that they have to be representative for the whole country of interest and have a high reliability (low standard error). However, the current study does indicate that it is possible to reduce CH_4 production by including this trait in a selection index and simultaneously increase milk production.

When analysing the national breeding goals of the Netherlands, Spain and the UK, an economic value was assigned to CH_4 production. As mentioned above, even the highest negative economic value assigned to CH_4 production did not halt the increase in CH_4 production. At the moment no policies are in place on how to directly reduce CH_4 production originating from the agricultural sector. Currently, two approaches are considered by scientists; 1) farmers producing more than the threshold to be set for CH_4 production will be penalised, or 2) farmers producing below the threshold to be set for CH_4 production get subsidised. However, as it currently is unknown if, how, and when policies are to be implemented this limits the accuracy of the outcome of current analyses. Another limitation is that the future shadow price for CH_4 production is not known, although estimates have been obtained. The current study takes both scenarios into account by using three underpinned shadow prices for CH_4 production and looked at including and excluding the economic value for CH_4 production from the overall index value. This gives a first indication of what to expect for the future.

7.3 CONCLUSIONS AND IMPLICATIONS

Genetic and genomic parameters

The current study has shown that CH_4 production is moderately heritable. This means that it is possible to genetically select cows for lower CH_4 production and therefore that progress made will be passed on to next generations. However, as heritability is moderate, progress will be slow. To maximise the reduction in CH_4 production, a breeding strategy should be combined with management

strategies including optimising the diet.

The genetic correlation (-0.15 to 0.20) obtained between CH_4 production measured with the use of a laser methane detector and CH_4 production measured in the feeding bin in the milking robot during milking shows that these traits are genetically different. Heritabilities for these traits were in agreement. In practise this means that care must be taken when comparing genetic parameters of CH_4 production obtained in different ways, even when they appear similar.

Selection index theory and national breeding goals

The final objectives of the current study were to investigate the effects of including CH_4 production into the national breeding goals. The current study has shown that when including MY, LWT, DMI and CH_4 production in a selection criterion, it is possible to reduce CH_4 production and increase MY simultaneously. This resulted in an increase in the genetic gain for DMI, which could be a problem if the demand for higher DMI would cause a higher production of CH_4 elsewhere in the sector.

The analyses of the national breeding goals showed that under the current goals CH_4 production is increasing. Even when a high shadow price was assigned to CH_4 production this was the case. The implication of this is that if CH_4 production originating from the dairy sector is to be reduced (partly) by selective breeding, an incentive needs to be created for the farmer to actively breed for lower CH_4 production. This could be done e.g. through regulations put in place by the government, pressure from consumers for higher sustainability during the production of the products they consume, or active recommendation of breeding animals with EBVs for low CH_4 production by breeding companies.

Greenhouse gas reduction is high on the political agenda of many countries. However, no country has yet implemented a penalty or rewards system for the

agricultural sector. Whether a penalty or rewards system will be implemented will have a huge impact on the farmer and will influence the way the national breeding goal can be adapted. As more traits play a role in the national breeding goals, it is vital that these correlations are obtained in order to make a reliable prediction of the effects of including CH_4 production in the national breeding goals.

Novel findings

At the start of this study very few heritabilities and no genetic correlations with production traits were available for CH_4 production. This study contributed to the knowledge on heritability of CH_4 production obtained in the milking robot during milking and obtained using LMD. Currently there is uncertainty whether CH_4 production measured using different techniques indeed obtains the same genetic trait. This study has shown that for CH_4 production obtained in the milking robot during milking and obtained using LMD this is not the case.

As mentioned before, to be able to include CH_4 production into national breeding goals, genetic correlations with traits currently in the breeding goals need to be available. Within this study correlations between CH_4 production and MY, LWT and DMI were obtained for different herds. Obtaining these genetic correlations has contributed to the understanding of the genetic background of CH_4 production and its relationship with production traits, as well as contributed to moving towards inclusion of CH_4 production into national breeding goals.

This study made a first effort to include CH_4 production into the breeding goals of three European countries. Currently, this is the only study investigating the change in CH_4 production under current breeding goals, investigating the effect on breeding index values when restricting CH_4 production to no genetic gain, and investigating the influence of assigning a shadow price to CH_4 production on genetic gain in CH_4 production as well as breeding index values. The results

of these analyses are important to gain an understanding of how changes in the genetic management of CH_4 production will genetically influence other traits in the index. These results can inform governments, breeding organisations and consumers in decision making with regards to legislation and consumer spending behaviour.

This study concluded with an LCA, which thus far has not been used to quantify the direct effect on CH_4 production when correlated traits change. Within this study it was shown that results on animal level cannot just be extrapolated to sector level, and therefore shows the importance of examination of CH_4 production on sector level as well as on animal level.

7.4 UNLIMITED RESOURCES AND FUTURE RESEARCH

Experimental set up

When unlimited resources are available, a long-term (5+ years) experiment should be set up in which multiple dairy herds in different locations are measured using multiple different CH_4 measurement techniques (respiration chambers, sniffer and tracer techniques) on the same cows. These techniques should be used simultaneously if possible. For some techniques this will not be possible, e.g. for the SF_6 tracer technique and sampling CH_4 production from the feeding bin in the milking robot. For these techniques cows should be divided in two groups which are measured simultaneously with a different measurement technique, after which the techniques are switched between the groups. This will eliminate the environmental differences during measurements as much as possible.

Traits measured

Traits measured should include production traits, fertility traits, efficiency traits

(including DMI) and health/longevity traits. For future research it is important that genetic correlations between CH_4 production and these traits become available.

Use of proxies

Milk samples will be taken and used to investigate MIR spectral data as a proxy for CH_4 production. Dehareng et al. (2012) found an R^2_{cv} of 0.73 between milk MIR spectral data and CH_4 production, showing that the use of milk MIR spectral data as a proxy for CH_4 production is feasible. Milk MIR spectral data as a proxy for CH_4 production therefore, is an efficient method to obtain CH_4 production data on a large scale from commercial farms as milk samples are already taken routinely. Measurements on DMI will also be used to investigate and improve the use of DMI as a proxy for CH_4 production, as the correlation between DMI and CH_4 production and the biological link between the traits is favourable to do so. Ellis et al. (2007) found an R^2 of 0.64 between DMI and CH_4 production in dairy cattle based in the northern United States and Canada. Different diets should be investigated to see the effect this has on the usability of DMI as a proxy. Rumen microbial information will also be collected and investigated as a proxy (Auffret et al., 2018).

Pedigree and genotypes

As discussed in section 7.2, due to the pedigrees in this study being limited to three generations the estimated additive genetic variance could have been underestimated. With unlimited resources pedigree will be traced back as far as possible. However, this will increase computation requirements, both for power and speed. Not much is known yet on the ideal depth of pedigrees for the estimation of additive genetic variance, but Yang and Guosheng (2016) showed that a pedigree consisting of three to five generations with phenotypes available plus one to three ancestral generations leads to maximised reliability

of estimated breeding values in pigs. This seems a good starting point and with a full pedigree available, the effects of limiting pedigree information can be investigated.

All cows will be genotyped using a 50K SNP chip. This data will be used to update and validate the pedigree, e.g. by performing parent offspring checks and filling in gaps in the pedigree based on genotype matches. Genotype data will also be used combined with pedigree information to build an H-matrix with genetic and genomic relationship coefficients between animals. When using H-matrix relationship coefficients, both the information from alleles identical by descent (pedigree information) and identical by state (genotype information) will be utilized. To do so it might be necessary to update the current SNP-chips. As current SNP-chips are often based on minor allele frequency, they might already be out of date for traits under selection pressure. For these traits the mutations that affected them when the current SNP-chips were designed might no longer be present or might be fixed (Meuwissen et al., 2014). Therefore, these mutations will no longer be informative and updates SNP-chips are needed.

Breed choices and cross-breeding

Little is known yet about the effects of cross-breeding on CH_4 production. However, Goopy et al. (2014) found that sheep with low CH_4 yield have smaller rumen and a shorter rumen retention time. Physiological and behavioural differences between breeds will likely also play a role in CH_4 production in cattle. Roehe et al. (2016) found a significant difference in daily CH_4 production between Aberdeen Angus and Limousin cattle, caused by higher feed intake of Aberdeen Angus cattle. However, Duthie et al. (2017) reports no breed effect in CH_4 yield when comparing Charolais cross and purebred Luing cattle. To my knowledge no study has yet investigated the effect of cross-breeding on CH_4 production in dairy cattle. Therefore, with unlimited

resources CH_4 production measurements will be obtained for multiple dairy breeds and cross-breeds to gain insight in the differences between breeds and the advantages of cross-breeding on reducing CH_4 production in dairy cattle.

Genotype-by-diet interactions

Indications have been found showing that the microbiome composition in the rumen is influenced by the genetics of the host (Hegarty and McEwan, 2010). It is also known that diet composition influences the amount of CH_4 produced (Ungerfeld, 2013; Cameron et al., 2018) due to different digestion pathways (see chapter 1, subsection 1.2.1). It is therefore possible that genotype-by-diet interactions exist with regards to CH_4 production. The Langhill dataset used in the current study consists of two genetic lines and two diet groups. Initial analysis did not show a genotype-by-diet interaction on MY, LWT, DMI and CH_4 production. However, as the dataset was small, the number of observations in each genetic line by diet group were too small to draw any significant conclusions. Roehe et al. (2016) found that the effect of a genotype (breed type) on CH_4 emission is not influenced by a change in diet. They note further studies are needed to confirm this. Therefore, with unlimited resources two genetic lines should be developed. One line should be selected for low CH_4 production and one for high CH_4 production. Cows from both genetic lines should be present at the dairy herds in different locations. At each location both lines should be divided into diet groups and different diets should be investigated.

National breeding goals

From the analyses on the national breeding goals it became clear that the increase in CH_4 production differs between countries. Currently the increase in CH_4 production is lowest in the Netherlands. As described in chapter 6 this is likely due to the economic values the Netherlands assigned to the traits in

the breeding goal. Especially the small variation in economic values seems to make the Dutch breeding goal robust against changes in overall value. Next to this the high number of traits in the breeding goal (13 for the Netherlands, not including LWT or CH_4 production), could cause moderation in the genetic change that is possible for each trait individually. This indicates that for future research it is beneficial to compare national breeding goals to gain more insight in factors (economic values assigned, number of traits, type of traits included) contributing to a lower genetic gain in CH_4 production.

Appendix

I. Genetic parameters for the Netherlands, Spain and the UK as currently in use

Table 7.5: Genetic parameters and economic values for the Netherlands (received June 2017 as current breeding index) where Her is heritability and Rep is repeatability

	Trait	Phenotypic St. dev.	Her	Rep	Economic value (€)
1	Lactose	687.10	0.55	0.550001	0.30
2	Milk fat	28.00	0.58	0.580001	2.10
3	Milk protein	19.00	0.5	0.500001	4.10
4	Longevity	270.00	0.12	0.120001	0.09
5	Udder health	4.47	0.089	0.089001	5.20
6	Interval first last calving	4.47	0.08	0.080001	0.52
7	Calving interval	4.47	0.15	0.150001	0.52
8	Udder conformation	4.47	0.34	0.340001	5.20
9	Feet and leg	4.47	0.17	0.170001	6.00
10	Direct calving ease	4.47	0.068	0.068001	0.08
11	Maternal calving ease	4.47	0.048	0.048001	0.08
12	Direct vitality	4.47	0.038	0.038001	0.55
13	Maternal vitality	4.47	0.085	0.085001	0.83

For all above traits 140 progeny observations were assumed

Table 7.6: Genetic parameters and economic values for Spain (received June 2017 as current breeding index) where Her is heritability and Rep is repeatability

	Trait	Phenotypic St. dev.	Her	Rep	Economic value (€)
1	Milk yield	563	0.28	0.5	0.120
2	Milk fat	13	0.28	0.5	0.660
3	Milk protein	19	0.28	0.5	3.100
4	Udder health & conf	1	0.39	0.390001	0.000
5	Feet and leg	10	0.16	0.16	0.000
6	Longevity	100	0.115	0.115	0.170
7	Somatic cell score	10	0.175	0.37	-18.740
8	Days open	10	0.043	0.07	-1.890

For all above traits 140 progeny observations were assumed

Table 7.7: Genetic parameters and economic values for the UK (received August 2017 as current breeding index) where Her is heritability and Rep is repeatability

	Trait	Phenotypic St. dev.	Her	Rep	Economic value (£)
1	Lameness	0.19	0.02	0.020001	0.000
2	Milk yield	895	0.4	0.400001	-0.066
3	Milk fat	35	0.391	0.391001	1.290
4	Milk protein	27	0.39	0.390001	4.220
5	Condition score	1.4	0.27	0.270001	0.000
6	Calving interval	45	0.03	0.030001	-3.660
7	Lifespan	2.864	0.06	0.060001	51.300
8	Mastitis	0.28	0.038	0.038001	0.000
9	Somatic cell count	0.65	0.12	0.120001	-0.780
10	NR56[1]	0.48	0.018	0.018001	4.170
11	Feet and leg	4.57	0.179	0.179001	6.440
12	MCI[2]	4.85	0.315	0.315001	6.780
13	Foot angle	1.21	0.112	0.112001	0.000
14	Locomotion	1.26	0.105	0.105001	0.000
15	Condition score 120d	1.49	0.48	0.480001	0.000

[1]First service non return rate to 56 days

[2]Mammary composite index

For all above traits 140 progeny observations were assumed

Table 7.8: Correlations used between traits for the Netherlands

Trait	1	2	3	4	5	6	7	8	9	10	11	12	13
1	1	0	0	0	0	0	0	0	0	0	0	0	0
2	0.38	1	0	0	0	0	0	0	0	0	0	0	0
3	0.88	0.58	1	0	0	0	0	0	0	0	0	0	0
4	0.36	0.35	0.42	1	0	0	0	0	0	0	0	0	0
5	-0.06	-0.02	-0.06	0.36	1	0	0	0	0	0	0	0	0
6	-0.34	-0.24	-0.29	0.25	0.27	1	0	0	0	0	0	0	0
7	-0.44	-0.33	-0.37	0.11	0.21	0.85	1	0	0	0	0	0	0
8	-0.08	-0.04	-0.1	0.11	0.27	-0.05	0	1	0	0	0	0	0
9	0.02	0.04	0.05	0.25	0.21	0	0	0.35	1	0	0	0	0
10	0.07	0.15	0.11	0.24	0.15	0.20	0.24	0	0	1	0	0	0
11	0	0	0	0.16	0.09	0.25	0.24	0.10	0.10	0.19	1	0	0
12	0.05	0.09	0.02	0.14	0.05	0.10	0.14	0	0	0.60	0.14	1	0
13	-0.04	-0.07	0.03	0.16	0.07	0.32	0.24	0	0	0.11	0.34	-0.16	1

Trait number corresponds with trait number in Table 7.5

Table 7.9: Correlations used between traits for Spain

Trait	1	2	3	4	5	6	7	8
1	1	0.73	0.93	0.20	0.20	0.20	-0.04	0.11
2	0.55	1	0.84	0.20	0.20	0.21	-0.01	0.02
3	0.85	0.67	1	0.20	0.20	0.22	0.04	0.08
4	0.41	0.32	0.40	1	0.10	0.40	0	0
5	0.30	0.25	0.31	0.41	1	0.40	0	0
6	-0.23	-0.11	-0.26	0.13	0.07	1	0	0
7	0.11	0.01	0.12	-0.06	0.03	-0.40	1	0
8	0.32	0.16	-0.15	0.08	0.08	-0.23	0.14	1

Trait number corresponds with trait number in Table 7.6

Table 7.10: Correlations used between traits for the UK

Trait	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	1	0.02	0.02	0.01	0	0	0	0.01	0	0	0	0	0	-0.9	0
2	0.27	1	0.80	0.93	-0.42	0.20	0	0.03	-0.12	-0.12	0	0.17	0	0.08	-0.42
3	0.32	0.67	1	0.85	-0.24	0.18	0	0.02	-0.11	-0.12	0	0.14	0	0.07	-0.24
4	0.37	0.73	0.80	1	-0.27	0.18	0	0.02	-0.11	-0.14	0	0.16	0	0.09	-0.27
5	0	-0.38	-0.33	-0.39	1	0.01	0.08	0	-0.02	0.01	-0.09	-0.22	0.10	-0.02	0.90
6	0	0.53	0.49	0.48	-0.40	1	0	0.03	0.12	-0.34	0	0.04	0	0	0.01
7	0.25	0	0	0	-0.11	-0.51	1	0.40	-0.06	0	0.08	0.11	0.01	0.06	0.08
8	0.44	0.35	0.27	0.36	-0.36	0.28	-0.22	1	0.20	-0.02	0	-0.10	0	0	0
9	0	0.06	0.14	0.09	0	0.28	-0.27	0.70	1	-0.08	-0.01	-0.09	0	-0.02	-0.02
10	0	-0.42	-0.47	-0.43	-0.30	-0.45	0.23	-0.21	-0.40	1	-0.01	0	0	0	0.02
11	0	0	0	0	-0.32	0	0.50	0	0.01	-0.21	1	0.29	0.31	0.78	-0.09
12	0	0.12	0.01	0.04	-0.44	0.14	0.19	-0.70	-0.21	0	0.45	1	0.06	0.19	-0.22
13	0	0	0	0	0	0	0.06	0.06	-0.15	0	0.48	-0.04	1	0.23	0.10
14	-1	0.03	0.13	0.11	-0.22	0	0.65	0	-0.03	0	0.98	0.37	0.53	1	-0.02
15	0	-0.26	-0.20	-0.26	0.90	-0.35	0.19	-0.36	-0.22	-0.30	-0.32	-0.44	0	-0.22	1

Trait number corresponds with trait number in Table 7.7

II. Change in correlated response for each trait for each country for the first three scenarios

Table 7.11: Change in correlated response for each trait for the Netherlands when a positive correlation is assumed between live weight (LWT) and CH_4 (CH_4 production) where Scen is scenario and pnt is point breeding value

	Unit	Scen 1	Scen 2	Scen 3 low	Scen 3 literature	Scen 3 high
Lactose	kg	474.88	486.48	475.47	476.96	480.19
Milk fat	kg	12.61	10.95	12.58	12.50	12.29
Milk protein	kg	13.37	11.63	13.34	13.26	13.04
Longevity	days	43.95	44.82	44.00	44.13	44.39
Udder health	pnt	0.02	0.03	0.02	0.02	0.02
IFLC[1]	pnt	-0.39	-0.40	-0.39	-0.39	-0.39
Calving interval	pnt	-0.72	-0.74	-0.72	-0.73	-0.73
Udder conformation	pnt	0.01	0.02	0.01	0.01	0.01
Feet and leg	pnt	0.19	0.20	0.20	0.20	0.20
Direct calving ease	pnt	0.13	0.13	0.13	0.13	0.13
Maternal calving ease	pnt	0.02	0.03	0.02	0.02	0.03
Direct vitality	pnt	0.06	0.07	0.06	0.06	0.06
Maternal vitality	pnt	-0.03	-0.04	-0.03	-0.03	-0.04
CH_4 production	g/d	3.82	0.00	3.73	3.51	2.95
Live weight	kg	-0.02	-2.29	-0.08	-0.21	-0.55

[1]Interval first last calving

Table 7.12: Change in correlated response for each trait for the Netherlands when a negative correlation is assumed between live weight (LWT) and CH_4 (CH_4 production) where Scen is scenario and pnt is point breeding value

	Unit	Scen 1	Scen 2	Scen 3 low	Scen 3 literature	Scen 3 high
Lactose	kg	475.38	486.88	475.98	477.48	480.72
Milk fat	kg	12.60	10.92	12.57	12.49	12.29
Milk protein	kg	13.29	11.49	13.26	13.18	12.96
Longevity	days	44.00	44.84	44.05	44.18	44.44
Udder health	pnt	0.02	0.03	0.02	0.02	0.02
IFLC[1]	pnt	-0.39	-0.40	-0.39	-0.39	-0.40
Calving interval	pnt	-0.73	-0.75	-0.73	-0.73	-0.73
Udder conformation	pnt	0.01	0.02	0.01	0.01	0.01
Feet and leg	pnt	0.19	0.20	0.19	0.20	0.20
Direct calving ease	pnt	0.13	0.13	0.13	0.13	0.13
Maternal calving ease	pnt	0.02	0.03	0.02	0.02	0.03
Direct vitality	pnt	0.06	0.07	0.06	0.06	0.06
Maternal vitality	pnt	-0.04	-0.04	-0.04	-0.04	-0.04
CH_4 production	g/d	3.93	0.00	3.85	3.62	3.05
Live weight	kg	0.04	0.88	0.05	0.10	0.23

[1]Interval first last calving

Table 7.13: Change in correlated response for each trait for Spain when a positive correlation is assumed between live weight (LWT) and CH_4 (CH_4 production) where Scen is scenario and pnt is point breeding value

	Unit	Scen 1	Scen 2	Scen 3 low	Scen 3 literature	Scen 3 high
Milk yield	kg	152.29	104.41	151.40	148.91	141.83
Milk fat	kg	2.89	2.01	2.87	2.82	2.696
Milk protein	kg	5.90	3.00	5.84	5.67	5.196
Udder h&c[1]	pnt	0.21	0.24	0.22	0.22	0.226
Feet and leg	pnt	0.73	0.80	0.73	0.74	0.76
Longevity	days	6.62	7.70	6.66	6.79	7.07
Somatic cell score	cells/ml	-2.81	-3.12	-2.83	-2.87	-2.96
Days open	days	-0.01	0.10	-0.01	0.00	0.02
Methane	g/d	6.43	0.00	6.28	5.85	4.73
Live weight	kg	7.67	1.17	7.51	7.09	5.97

[1]Udder health & conformation

Table 7.14: Change in correlated response for each trait for Spain when a negative correlation is assumed between live weight (LWT) and CH_4 (CH_4 production) where Scen is scenario and pnt is point breeding value

	Unit	Scen 1	Scen 2	Scen 3 low	Scen 3 literature	Scen 3 high
Milk yield	kg	154.89	108.49	154.11	151.94	145.67
Milk fat	kg	2.86	1.94	2.84	2.798	2.671
Milk protein	kg	5.45	2.13	5.38	5.187	4.668
Udder h&c[1]	pnt	0.22	0.24	0.22	0.221	0.228
Feet and leg	pnt	0.74	0.82	0.75	0.76	0.78
Longevity	days	6.72	8.18	6.77	6.92	7.26
Somatic cell score	cells/ml	-2.83	-3.17	-2.85	-2.89	-2.99
Days open	days	-0.13	-0.03	-0.13	-0.12	-0.11
Methane	g/d	6.45	0.00	6.30	5.90	4.83
Live weight	kg	7.25	12.66	7.41	7.84	8.89

[1]Udder health & conformation

Table 7.15: Change in correlated response for each trait for the UK when a positive correlation is assumed between live weight (LWT) and CH_4 (CH_4 production) where Scen is scenario and pnt is point breeding value

	Unit	Scen 1	Scen 2	Scen 3 low	Scen 3 literature	Scen 3 high
Lameness	binary	0.01	0.01	0.01	0.01	0.01
Milk yield	kg	124.25	3.55	121.66	114.61	95.69
Milk fat	kg	11.30	8.50	11.27	11.18	10.88
Milk protein	kg	10.79	5.38	10.69	10.42	9.65
Condition score	score	-0.28	-0.36	-0.28	-0.29	-0.31
Calving interval	days	-0.68	-1.07	-0.69	-0.72	-0.80
Lifespan	score	0.39	0.45	0.39	0.40	0.42
Mastitis	binary	-0.01	-0.01	-0.01	-0.01	-0.01
Somatic cell count	cell/ml	-0.03	-0.04	-0.03	-0.03	-0.04
NR56[1]	binary	-0.01	0.00	-0.01	-0.01	-0.01
Feet and leg	score	0.75	0.88	0.76	0.77	0.81
MCI[2]	score	0.74	0.89	0.75	0.76	0.80
Foot angle	score	0.03	0.04	0.03	0.04	0.04
Locomotion	score	0.19	0.21	0.20	0.20	0.21
Condition score 120d	score	-0.05	-0.05	-0.05	-0.05	-0.05
CH_4 production	g/d	8.30	0.00	8.12	7.64	6.33
Live weight	kg	-9.15	-20.05	-9.46	-10.27	-12.31

[1]Mammary composite index

Table 7.16: Change in correlated response for each trait for the UK when a negative correlation is assumed between live weight (LWT) and CH_4 (CH_4 production) where Scen is scenario and pnt is point breeding value

	Unit	Scen 1	Scen 2	Scen 3 low	Scen 3 literature	Scen 3 high
Lameness	binary	0.02	0.02	0.02	0.02	0.02
Milk yield	kg	123.22	-6.68	120.77	114.10	96.23
Milk fat	kg	11.16	8.11	11.13	11.04	10.77
Milk protein	kg	9.79	3.31	9.68	9.37	8.55
Condition score	score	-0.45	-0.52	-0.45	-0.46	-0.48
Calving interval	days	-0.52	-1.08	-0.53	-0.57	-0.66
Lifespan	score	0.54	0.65	0.54	0.55	0.58
Mastitis	binary	0.01	0.01	0.01	0.01	0.01
Somatic cell count	cell/ml	-0.05	-0.06	-0.05	-0.05	-0.05
NR56[1]	binary	-0.03	-0.03	-0.03	-0.03	-0.03
Feet and leg	score	0.69	0.82	0.70	0.71	0.74
MCI[2]	score	0.67	0.80	0.67	0.69	0.72
Foot angle	score	0.04	0.05	0.04	0.05	0.05
Locomotion	score	0.22	0.25	0.22	0.22	0.23
Condition score 120d	score	-0.13	-0.16	-0.13	-0.14	-0.14
CH_4 production	g/d	8.48	0.00	8.32	7.89	6.73
Live weight	kg	-9.48	-6.75	-9.45	-9.37	-9.12

[1]Mammary composite index

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