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Genetic and Genomic Studies of Animal Welfare and Environmental Traits in Dairy Cattle

A thesis presented in partial fulfilment of the requirements
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Abstract

New Zealand dairy cows graze outdoors throughout the year and are exposed to a wide range of weather conditions also their excreta are dispersed in the paddock. There is an emerging interest in whether between-cow genetic variation could contribute to environmental sustainability and improved animal welfare. The initial objectives of the study were 1) to estimate genetic parameters of rectal temperature (RT) as an indicator of heat stress (HS), 2) to identify gene regions associated with HS through genome-wide association studies (GWAS), and 3) to estimate response of genetic selection for low HS on milk production using conventional selection in grazing dairy cows of New Zealand. The genetic variance of RT estimated in 776 mixed-breed cows at two Massey University experimental dairy farms was very low due to insufficient environmental stress on sampling days. This outcome diverted the topic of study to the genetic basis of milk urea concentration (MU) and efficiency crude protein utilisation (ECPU) and their potential role in improved environmental sustainability.

The new study was conducted considering MU as the trait of interest. Additionally, the possible reduction of urinary nitrogen (N) excretion by genetic selection for low milk urea N concentration (MUN) was determined. The heritability estimated for MU (from 0.27 to 0.49) and ECPU (from 0.02 to 0.41) using a random regression model in 634 mixed-breed cows indicated that these traits can be genetically manipulated by direct selection. Estimates of genetic correlations between MU and yield of milk, fat, crude protein, lactose, and liveweight (LWT) in each day of lactation were mostly positive and imply that selection for reduced MU would result in reduced production and LWT of cows. The GWAS identified six novel genes (GMDS, E2F7, SIAH1, SLC24A4, LGMN, ASS1) associated with MU that function in protein catabolism, urea cycle, ion transportation and N excretion. This suggests that genomic selection for MU is possible, however, the results should be validated with a larger sample size. Selection index theory was used to estimate correlated responses for different selection indices including MUN with different relative emphasis (RE). The predicted genetic responses per cow per year of the current index were 16.4 kg milk yield (MY), 2.0 kg fat yield (FY), 1.4 kg crude protein yield (CPY), -0.4 kg LWT and -0.05 mg/dl MUN. Including MUN in the selection index with 20% negative RE resulted in annual responses of 5.4 kg MY, 1.6 kg FY, 1.0 kg CPY, -1.1 kg LWT and -0.17 mg/dl MUN. The total N predicted to be excreted in the base year was 336.5 kg/ha. When stocking rate was adjusted by changes in milk production and cow feed requirements, 10 years of selection with a selection index not including MUN increased total N excretion to 338.9 kg/ha. A selection index with 20% negative RE for MUN increased total N excretion to 341.6 kg/ha, and genetic gain in production of milk protein per hectare was reduced, thereby reducing farm profits. The results of this thesis indicate that inclusion of MUN in a selection index with negative RE is not an effective pathway to reduce farm-level N leaching and carbon footprint.

Declaration

This thesis contains no material that has been accepted for a degree or diploma by the university or any other institution. To the best of my knowledge no material previously published or written by another person has been used, except where due acknowledgement has been made in the text.

This thesis has been written with chapters formatted as papers for publication. Therefore, there is some repetition of chapter introduction, material and methods; each chapter contains a full discussion, with the general discussion chapter providing a discussion of key findings of this thesis with their applications. Each chapter has been formatted for the New Zealand Journal of Agricultural Research and a complete list of references is provided at the end of the thesis.

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List of abbreviations

BV = Breeding value

BW = Breeding Worth

BCS = Body condition score

CP = Crude protein

CR = Correlated response

DIM = Days in milk

DMI = Dry matter intake

ECMY = Energy corrected milk yield

ECPU = Efficiency of crude protein utilization

EV = Economic value

F = Holstein-Friesian

FN = Faecal nitrogen

GHG = Greenhouse gas

GS = Genomic selection

GWAS = Genome-wide association studies

HS = Heat stress

HT = Heat tolerance

J = Jersey

LWT = Liveweight

ME = Metabolizable energy

MU = Milk urea

MUN = milk urea nitrogen

NDF = Neutral detergent fibre

NUE = Nitrogen utilization efficiency

OAD = Once a day

RE = Relative emphasis

RT = Rectal temperature

SCS = Somatic cell score

SNP = Single nucleotide polymorphism

SR = Stocking rate

TAD = Twice a day

THI = Temperature humidity index

UN = Urinary nitrogen

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Chapter 1

Introduction

The New Zealand dairy industry relies on cows that predominantly graze outdoors on ryegrass and white clover pasture at all times of the year. Spring calving (July-September) of cows aims to match the feed demand of the herd with pasture supply which is determined by a seasonal pattern of growth. Supplementary feeding with hay and silage is minimal and practised only when necessary to fulfil the pasture deficit either in summer droughts or winter where the pasture growth is lower than expected (Holmes et al., 2000). The spring calving enables maximum pasture utilization hence, greater farm profits. However, cows grazing outside all year around are exposed to solar radiation, wind, rain and variable temperature. Exposure of cows to higher heat, solar radiation, and humidity conditions that elevate the core body temperature above the normal body temperature (37.9 °C to 39.5 °C) (Wenz et al., 2011) can induce hyperthermic heat stress (HS). There are two types of mechanism which enable the cows to reduce body heat in order to maintain normal body temperature, namely; sensible and latent heat losses. Sensible heat loss depends on an elevated blood supply to the skin, so that the extra heat is effectively expelled to the environment, therefore, heart rate increases during the HS. Evaporation is the primary method in latent heat loss by which the cow cools the body itself through sweating, panting and breathing. Consequently, elevated rates of heart, sweating, panting and respiration become indirect indicators of HS. Degradation of consumed feed in the digestive tract is a thermogenic process, therefore, cows in HS will reduce feed intake causing substantial reduction in milk production and liveweight loss.

Providing artificial shade by plants or housing prevents animals from being exposed to direct solar radiation (Fisher et al., 2008). Improving evaporative and conventional heat loss through cooling with the aid of sprinklers and fans, respectively, are known to effectively alleviates the HS of indoor cows (Bucklin et al., 1991; Flamenbaum et al., 1995). These temporal advances of cooling are mostly achievable in off-paddock housing systems and are less feasible for outdoor grazing circumstances. An alternative approach is to utilize genetic tools for improving heat tolerance in New Zealand dairy cows.

The national selection objective for dairy cattle in New Zealand is called Breeding Worth (BW). This multi-trait objective includes estimated breeding values for lactation yields of milk, fat and protein, somatic cell score, cow liveweight and fertility, residual survival and body condition score, each weighted by an economic value. The economic values for BW index traits for the season 2018/19 were -$0.092/kg milk, $3.49/kg fat, $4.38/kg protein, -$1.30/kg liveweight, -$37.30 /unit of somatic cell score, $5.88/% of fertility, $0.112/day of residual survival and $96.30/unit of body condition score. The relative contribution of economic values toward the overall index were 13% for milk, 24% for fat, 17% for protein, 11% for liveweight, 6% for somatic cell score, 13% for fertility, 9% for residual survival, and 7% for body condition score (NZAEL, 2019). The BW index does not include tolerance to HS as a goal trait.

Studies that have evaluated the genetic basis of heat tolerance in New Zealand are scant, with the exception of the study published by Littlejohn et al. (2014) in which a genome-wide association study (GWAS) was performed to identify the genes associated with milk production, hair development and homeothermy in New Zealand *Bos taurus* cows with abnormally long and hairy coat. Therefore, the initial objective of this thesis was to evaluate the genetic basis of heat tolerance (HT) of free-grazing New Zealand dairy cows. The initial hypotheses were that high rectal temperature (RT) is an indication of HS, and that New Zealand dairy cows grazing outdoor in a typical summer show genetic variance for RT. Unfortunately, the estimated genetic variance of RT of trial cows was not different from zero, most likely caused by the mild ambient temperature on sampling days. This required the thesis objective to be diverted to evaluate the genetic basis of milk urea (MU) and efficiency of crude protein utilization (ECPU) in dairy cows.

Environmental sustainability in New Zealand includes managing the quality of air, fresh water, oceans, and waste (MfE, 2005c). The increasing demand for animal protein, together with less land allocated for farming, has enhanced the intensification of animal-based agriculture where genetic improvement, increased supplementary feeding, and fertilizer application to enhance plant growth play important roles (Macdonald et al., 2017). Fertilizers containing nitrogen (N) and phosphorus (P) are the most commonly applied fertilizers in New Zealand dairy farming (Statistics New Zealand, 2006). Animal effluents are the other source of P in New Zealand soils. The dissolved P that is not taken up by plants drains into groundwater and rivers causing eutrophication (Statistics New Zealand, 2006).

New Zealand emits unusually large quantities of greenhouse gasses (GHGs) (49% of the total emission) from agriculture (MfE, 2020) compared to nations with less important agricultural sectors. Agricultural GHGs in New Zealand are mainly comprised of nitrous oxide (N_2O) sourced by N fertilizer and livestock excreta and methane (CH_4) eructed by livestock (MfE, 2020). The N excreta which directly expels onto land by outdoor dairy cows and N fertilizer applied for improving pasture growth that ultimately reach the soil are decomposed into N_2O and ammonium (NH_4^+). The N_2O that is lost into the atmosphere is responsible for the global warming (Kroeze, 1994; Smith, 1997) while the NH_4^+ that is converted to nitrate leaches through the soil profile causing freshwater pollution (Choudhury and Kennedy, 2005; Smith and Schindler, 2009). This brings both animal welfare and environmental issues under a common umbrella, which are linked together by the growing global pressure to produce high quantities of animal proteins.

The greatest proportion of GHG emission in New Zealand originates from dairy cattle farming (22.9%) followed by sheep 11.9%, beef 8.1%, and other animal species 4.9%. From the total dairy emission, 18.9% is CH_4, and 4.1% is N_2O (MfE, 2020). Although the N_2O emission is only a smaller proportion compared to CH_4, the 100 years global warming potential of N_2O is 298 times greater than that of carbon dioxide while it is only 25 times greater than that of carbon dioxide for CH4 (IPCC, 2007). New Zealand dairy cows graze pasture that is rich in crude protein (>20%) but has less energy, which means cows grazing pasture excrete higher concentration of N in urine (urinary N, UN), compared with cows in indoor systems fed total mixed rations (DairyNZ, 2020a). Genetically reducing UN excretion is one option where the improvements are inherited from generation to the next hence, the benefits are long-term. Measuring of UN in free-grazing cows is not practical and instead milk urea N (MUN), which can be determined in routine herd-testing, has been proposed as an indicator of N excretion (Beatson et al., 2019) given the high positive phenotypic relationships found between MUN and UN in indoor cows feed on total mixed rations (Jonker et al., 1998; Kohn et al., 2002). New Zealand dairy cows show genetic variability for MUN (Lopez-Villalobos et al., 2018; Beatson et al., 2019) and therefore it can be considered as a candidate trait for indirect selection to reduce UN.

Cows with higher nitrogen utilization efficiency (NUE) capture more protein and convert into saleable products which minimize the proportion wasted in urine. Feeding plays an important role in improving NUE. The cows feed on total mixed rations are known to have greater NUE as they match the cow nutritional requirement (Hills et al., 2015). Feeding cows with less ruminally degradable proteins enables enzymatic protein digestion at the abomasum and small intestine, which is more efficient than digestion in the rumen, resulting in less UN (Castillo et al., 2000). Feeding of extra protein over the requirement of cow increases the wastage of protein as urea in urine while it induces inefficiency of protein feeding (Baker et al., 1995). Consequently, restricted protein feeding to satisfy only the requirements of cows is recommended. Therefore, management of feeding in order to improving NUE has become a short-term solution for reducing N leaching and carbon footprint.

As a heritable and easily measurable trait, MU can be used as an indicator trait of UN to enable the inclusion of UN into BW. Therefore, the primary hypotheses of the thesis were, firstly, that selecting for lower MU would reduce UN. Secondly, that due to the negative association between MU and NUE, cows selected for reduced MU would have higher ECPU. The main objectives of the thesis were the following:

1. To estimate the genetic parameters of MU and ECPU together with milk production, liveweight, and body condition score traits for each day of lactation.
2. To identify genomic regions or genes associated with MU and ECPU together with milk percentage traits through GWAS.
3. To evaluate the correlated response for milk production and liveweight traits in a selection index including MUN breeding values with different relative emphasis.

The results from this thesis were expected to provide information to the New Zealand dairy industry to evaluate MU as a potential indicator trait to genetically reduce the excretion of UN and to increase ECPU.

Chapter 2

Genetics of rectal temperature, milk urea, and efficiency of crude protein utilisation as indicators of animal welfare and environmental traits: Literature Review

At the start of the PhD thesis the main topic was the genetic basis of heat tolerance in New Zealand dairy cattle, but in the first experimental chapter of this thesis the estimated genetic variance of rectal temperature of dairy cows in two herds was not different from zero. This was most likely the result of exposing the cows to a mild ambient temperature of the sampling days. This ineffectual environmental circumstance enforced the overall thesis objective to be diverted to evaluate the genetic basis of milk urea and efficiency of crude protein utilization in dairy cows. For this reason, this chapter is a literature review on the genetics of animal welfare and environmental traits.

2.1 New Zealand dairy industry

New Zealand is the 7th largest global milk producer with just 3% of milk produced in the world (DairyNZ, 2019). About 95% of the milk produced in New Zealand is exported and, to June 2018, dairy was the country's largest export sector (DCANZ, 2018). Top four dairy export products include whole milk powder (39.4%), cheese (13.7%), skim milk powder (9.3%), and butter (11.6%), which are mostly exported to China, United States, United Arab Emirates, Australia, and Japan (DCANZ, 2018).

In the 2018-19 production season, milk processed by New Zealand dairy companies was 21.2 billion litres, comprising 1.88 billion kilograms of milksolids (fat+protein) (DairyNZ, 2019). Such production was achieved with a population of 4.95 million cows comprising 11,372 herds over 1.74 million effective hectares. The national herd was mainly composed of Holstein-Friesian (F) (33.1%), Jersey (J) (9.3%) and Holstein-Friesian × Jersey crossbred cows (F × J) (48.5%); Ayrshire (0.5%) and other breeds and crossed cows (8.6%) are also found to a lesser extent. The average milk production per cow was 4,290 litres or 381 kg milksolids (fat + protein) (DairyNZ, 2019).

As an export-oriented country, milk production is mostly focused on milksolids rather than volume, as water has to be evaporated to produce dairy products. This is reflected in the milk payment system adopted by dairy processing companies, which is called ‘A + B - C’ where A is the value per kg of fat, B is the value per kg of protein and C is the penalty value per litre of milk (Marshall, 1989). In 2018-19, the average price for one kilogram of milksolids paid to farmers was NZ$ 6.35 (DairyNZ, 2019).

A distinctive characteristic of New Zealand dairy industry is its reliance on pasture-based dairy farming (Harris, 2005). Temperate climate, fertile soil and abundance of clean water permit high production of high-quality pasture (Dillon et al., 2005). The geographical positioning of the country which acts as a natural barrier for pests and diseases, support outdoor gazing of dairy cattle year-round (DCANZ, 2018). Seasonal variation of pasture growth signifies that pasture availability can be high in spring but low in summer and winter. Farmers can introduce supplementary feeds and crops to the cows when pasture supply is not enough to satisfy the demand of the herd. Regardless of the level of supplements used, direct grazing of pasture year-round remains as the primary method of feeding cows in the majority of dairy farms in the country (Harris, 2005).

New Zealand pasture-based dairy cows are capable of producing milk at a relatively low cost compared to cows fed indoor or using total mixed rations systems available overseas (Macdonald et al., 2008). In New Zealand, pasture-based feeding is cost effective because importing supplementary feed has increased the cost of production of milk solids which on many farms cannot be supported with the current lower milk price (Back, 2017). Despite being economically profitable, direct grazing of pasture year-round creates many ecological and animal welfare problems including exposure of cows to extreme weather conditions (Fisher et al., 2008; Tucker et al., 2008), increase potential of greenhouse gases emission (GHGE), and nitrogen leaching (MfE, 2020).

2.2 Exposure to extreme weather

Outdoor grazing of pasture year-round increases the potential of cows' exposure to extreme weather. Extreme weather effects might include cold stress associated with ambient temperature-drops and heat stress (HS) associated with ambient temperature-rises. The global climate is predicted to change in the 21st century mainly due to human-related GHGE, therefore, raising ambient temperature and its associated HS effects on dairy cows are expected. The global average temperature is predicted to rise between 1.8 °C and 4.0 °C in the 21st century compared to the 20th century. However, changes in the temperature across the globe will vary among different regions and will also largely depend on the amount of GHGE that is projected for the region. The greatest increases in temperature are expected to be seen at high latitudes rather than low latitudes and tropics (IPCC, 2007).

Projections for New Zealand climate show an increment in temperature with every region of New Zealand being expected to be affected by elevated temperature conditions. The possible projections for future warming in New Zealand range from 0.2 to 1.7 °C by 2040, 0.1 to 4.6 °C by 2090, and 0.3 to 5.0 °C by 2110. In addition, climate change is expected to affect soil moisture availability. The east and north of the North Island and the South Island will be affected from the driest conditions, particularly during winter and spring while driest conditions are expected in summer for the west and central part of the North Island (MfE, 2018).

Several studies have been conducted to predict the effect of global climate change on different aspects of future agricultural (Cooper et al., 2008) and dairy production systems. For instance, Fodor et al. (2018) and Upadhyay et al. (2009) emphasized on the negative impact of climate change on dairy production, while Nesamvuni et al.

(2012) studied the effect of climate change on HS of farm animals and Guis et al. (2012) the spread of pest and diseases resulting from elevated temperatures. With the changing climate, a decrease in quality and quantity of pastures and an increase in spread of pests and diseases in New Zealand is expected (MfE, 2019). All these factors would disrupt the production of the cow influencing the economy of the country.

2.2.1 Phenotypes for heat stress

Heat stress is defined as the inability of an animal to effectively dissipate body heat and it represents a failure of the process of maintaining normal body temperatures through regular metabolism (i.e. thermoregulation). The range of ambient temperature (5 to 25 °C) at which animal maintains internal body temperatures with minimal metabolic regulation is known as thermoneutrality zone (Roenfeldt, 1998). The body temperature of a dairy cow at thermoneutral zone ranges from 37.9 °C to 39.5 °C (Wenz et al., 2011) and is the temperature at which cows achieve the maximum productivity (Johnson, 1987). Cow HS is driven by a number of factors including, solar radiation ambient temperature, ambient humidity, wind, and the heat produced by rumen fermentation during digestion. Increase in ambient temperatures over the upper limit of the thermoneutrality acts as a signal to induce hypothalamus of central nervous system of animal which subsequently activates physiological processes against heat stress (Baker et al., 1989). Rise in rectal temperature (RT) by 0.6 °C, over the thermoregulatory capacity is known to be coupled with reduction in thyroid activity and lower feed intake in hot weather which reduces digestion and metabolic rate (Johnson, 1980) and thereby depression of metabolic heat production (Yousef, 1987). The published reports on HS have noted its impacts on production (Reinemann et al., 1992; Bouraoui et al., 2002; Dikmen et al., 2014; Garcia et al., 2015), poor body condition (Lacetera et al., 1996), disease (Kadzere et al., 2002; Sanders et al., 2009; Jingar et al. 2014) and reproduction (Ahmad et al., 1995; Benyei et al., 2001; De Rensis and Scaramuzzi, 2003; Hansen, 2007).

There are numerous phenotypes in use to measure the degree of affectedness of cows by HS. Rectal temperature is a measure of core body temperature and an indicator of thermal balance (i.e. the amount of heat dispelled from the body and generated within the body), which can be used for measuring the adverse effects of HS on regular physiological processes of an animal (Johnson, 1980). Although measuring RT is labour intensive, it is the widely used phenotype in evaluating HS, probably due to the acceptance of it as a sensitive indicator of physiological responses to HS (McDowell et al., 1976). Infrared thermography, observing skin temperature using a camera, has recently become popular as a non-invasive method of generating phenotypes for evaluating HS (Daltro et al., 2017). Daltro et al. (2017) have identified a highly positive correlation between infrared thermography (lateral region of the udder) and RT suggesting that it is a reliable tool to identify HS in dairy cows. However, infrared thermography is technically demanding than measuring RT. Respiration (Collier et al., 1981; Littlejohn et al., 2014), heart (Muller et al., 1994) and sweating (Littlejohn et al., 2014) rate are all indirect measures of HS that could be used as phenotypes. However, obtaining these measures is difficult to perform in practice.

Changes in the behaviour of cows is another likely phenotype for studying the effect of HS (Fisher et al., 2008; Schütz et al., 2011). Cow behaviour was reported as a suitable mechanism by which cows counter the adverse effect of high temperature (Hafez et al., 1969). The degree of behavioural change can vary from one cow to another depending on many factors. Therefore, behavioural changes are hard to compare and are not precise measures of HS. Decline in production can be another indirect indicator of HS (Bryant et al., 2007). Although records on production decline can be easily obtained from farm databases, production decline can be attributed to various other reasons apart from HS. Therefore, production data alone may not produce accurate predictions on HS of cows and body temperature would be a useful indicator of HS.

2.3 Carbon footprint and nitrogen leaching

Another challenge for the New Zealand dairy industry is managing nitrogenous wastes from farms, which are a major source of greenhouse gases (GHG) leading to global warming and nitrogen (N) pollution of water. The N that is contained in urine, dung and excess fertilizer that reaches the soil breaks down to nitrous oxide (N_2O) and ammonia (NH_3) creating one of the main GHG (Kroeze, 1994; Smith, 1997) and N pollutant of freshwater (Choudhury and Kennedy, 2005; Smith and Schindler, 2009), respectively. The main GHGs related to agriculture are methane (CH_4) and N_2O, which are responsible for about a half of the total New Zealand GHG emissions (MfE, 2020). The level of GHG emissions from agriculture in New Zealand is greater than the emissions of other developed countries in the world (11%) (DairyNZ, 2020b). Methane emitted by the dairy industry is mainly produced as result of microbial forage digestion in the rumen of cows, which is difficult to control under either indoor or outdoor farming. The control over N_2O emission in outdoor farming is marginal but controllable via effluent treatment under indoor housing systems. The released NH_3 to the soil is converted to nitrate (NO_3^-) and is the key source of N pollutant of ground and surface waters in New Zealand. Freshwater pollution through excess of N from fertilizers is minor compared to pollution through excretion (PCE, 2018).

About 20% of the N consumed by a dairy cow is destined to produce milk protein (Mackle et al., 1996; Clement et al., 2016). From the 80% of excreted N, on average 13% is volatilised as NH_3 (Ball et al., 1979; Menneer et al., 2008), 2% is released as N_2O (Di and Cameron, 2006; Di and Cameron, 2008), 20% is leached as NO_3^- (Di and Cameron, 2007; Menneer et al., 2008), 41% is absorbed by pasture (Frase et al., 1994) and 26% is immobilised as organic nitrogen in soil microbes (Keeney and MacGregor, 1978; Clough et al., 1996). Apart from this, the N fertilizers that are applied to improve pasture growth also contribute to N leaching. Nitrogen fertilizer applied on pasture either promotes pasture productivity (Shepherd and Lucci, 2013) or promotes synthesis of crude protein (CP) within pasture (Moller et al., 1996). Either highly productive pastures or CP-rich pastures consumed by the cow increase the risk of N being excreted as urea in urine with increased dry matter intake associated with involuntary protein feeding above the requirements.

2.3.1 Phenotypes for carbon footprint and nitrogen leaching

The rumen degradable protein (RDP) available in dairy cow diets is digested by rumen microbes into amino acids and NH_3. Rumen microbes are also capable of converting non-protein nitrogen into NH_3. The produced amino acids are absorbed in the small intestine while NH_3 is absorbed by the rumen microbes in order to produce microbial proteins. However, if the diets comprised of lower energy (fermentable carbohydrates) in relation to proteins, the rumen microbes are not efficient in absorbing NH_3 (Baker et al., 1995). Ammonia is toxic to the cow and is absorbed by the bloodstream through the rumen wall and carries to the liver where it is detoxified by converting to urea. The protein synthesised by the rumen microorganisms and the ruminally undegradable dietary protein (RUDP) enter the small intestine and undergo enzymatic digestion converting protein into amino acids. Catabolism of these amino acids also releases NH_3 which will again be converted into urea in the liver. The produced urea is then released back to blood and passed to the kidney for excretion as urea. Most of the urea formed in a cow’s body can be released to the environment in urine. However, some urea is recycled back to the rumen via saliva and a small proportion can be diffused through the blood stream into milk (milk urea, MU) (Roseler et al., 1993). As a result, there is a high phenotypic correlation between milk urea nitrogen (MUN) and blood urea nitrogen (BUN) (Jonker et al., 1998). Milk urea nitrogen is a representation of dietary crude protein percentage to energy ratio in pasture (Jonker et al., 1998). Therefore, MUN can be used as an indicator of N utilization efficiency (NUE) in dairy cows (Baker et al., 1995; Hof et al., 1997; Schepers and Meijer, 1998). The negative effects of higher MUN on reproductive performance (Rajala-Schultz et al., 2001; Guo et al., 2004), cheese manufacturing (Guinot-Thomas, 1992) and lameness in dairy cows (Slovák et al., 2021) have been reported.

Excretion of urinary N (UN) is responsible for release of N_2O and NO_3^-. Therefore, UN is an indicator of both GHGE and N leaching. Measuring UN is expensive, but it is correlated to concentration of MUN and BUN. In carefully controlled trials, there has been shown to be a high positive phenotypic correlation between MUN and UN (Jonker et al., 1998; Kohn et al., 2002). Therefore, MUN is useful for predicting herd or individual UN excretion (Jonker et al., 1998; Kohn et al., 2002; Broderick, 2003; Hendriks, 2016). Milk urea nitrogen can be measured as another milk component at regular herd-testing for fat and crude protein percentages. The values of MU can be converted into MUN by multiplying MU with the factor of 0.47 where urea consists 47% of nitrogen (N). In New Zealand, urea surplus of milk is high in pasture-based systems where they fulfil more than 60% dietary requirements by pasture compared to cows fed total mixed rations that is balanced for CP and energy (Dairy NZ, 2020). Milk urea concentrations in New Zealand cows range from 28 to 32 mg/dl (Beatson et al., 2019) and can be elevated even beyond 50 mg/dl in spring where the pasture is very high in CP (>20%) (Dairy NZ 2020). Use of BUN records as a phenotype of selecting cows for low N excretion is possible due to its high phenotypic correlation with MUN. As animals can be measured for BUN at early stages of life even before they reach the sexual maturity rapid decision making on culling is possible. This eventually improves the rate of genetic gain in selection owing to reduced generation interval. However, MUN is the widely used phenotype in evaluating N leaching given that measuring BUN is practically intensive compared to measuring MUN. Inefficient N utilization causes cows to convert only a small proportion of dietary crude protein into milk protein while the majority of N is being converted to urea in urine (Burgos et al., 2001). Efficiency of N utilization can be estimated as either gross efficiency of CP (GECP) defined as ratio of CP yield (CPY) to CP intake (CPI), CP balance (CPB) defined as balance between CPI and CPY, or residual protein intake (RPI) defined as actual CPI and predicted CPI (Zamani et al., 2011). Apart from these three measures a recent Italian study has introduced three novel NUE indicators defined as CP to MUN ratio, casein to MUN ratio, and whey protein to MUN ratio (Bobbo et al., 2020).

2.4 Genetic basis of heat tolerance

Some progress has been achieved in reducing body temperature (Bucklin et al., 1991; Fisher et al., 2008) and increasing milk production (Bucklin et al., 1991; Flamenbaum et al., 1995; Fisher et al., 2008) and reproduction (Bucklin et al., 1991; Early et al., 1993; Thompson et al., 1996) of dairy cows through improved cooling using fans, sprinklers, and shades. However, the technical procedures require high capital investment and operating costs, besides, the effect is short-lived and the practicability to outdoor systems is minimum. The most effective method to New Zealand would be genetic approaches where the effects are long-term.

2.4.1 Crossbreeding for heat tolerance

Since there is a breed-wise difference in heat tolerance (HT), production and reproduction capabilities, crossbreeding is an important genetic alternative to combine the best characteristics from each breed and make a new animal better suited for a given production environment. Collectively, *Bos indicus* cattle breeds are known to be more heat tolerant than *Bos taurus* breeds. However, the production capabilities of *Bos indicus* are less compared to *Bos taurus*. Attempts have been taken to crossbreed the two breeds with the aim of obtaining a superior breed with the most beneficial characters. McDowell et al. (1996) demonstrated the improved growth rate, reproduction rate and milk yield in crossbred compared to purebred dairy cows at warm climates.

2.4.2 Selection for heat tolerance

Another genetic alternative to reduce the negative effect of HS on milk production and reproduction of dairy cows is genetic selection. If the RT of dairy cows under HS is heritable, then selection for low RT permits genetic improvement for thermo-tolerance. Estimates of heritability (h^2) and repeatability (t) of RT during heat stress are required for an effective animal evaluation. Evaluation of the genetic (r_G) and phenotypic (r_P) correlations between HT trait and milk production and fertility traits are required to define selection indexes that include production, reproduction, health, survival traits and heat tolerance traits. Rectal temperature is a heritable trait and the heritability reported in various studies (Table 2.1) was shown to vary from low (0.12) to moderate (0.31).

Table 2.1 Published estimates of heritability (h^2) and repeatability (t) for rectal temperature (RT) of cattle.

Study	Country	Breed (n)	Temp (°C)	RT (°C)	h^2	t
Seath et al., 1947	USA	Holstein (36), Jersey (16) Holstein (41), Jersey (27)	31.7	39.83 39.91	0.15 0.31	0.15 0.38
Turner, 1982	Australia	Hereford × Shorthorn (202) *Bos indicus* × *Bos taurus* (612)	22-32	39.80	0.25	0.34
Morris et al., 1989	New Zealand	Charolais × Murray Grey (334) Charolais × Simmenta (171) Charolais × Red Devon (106)	-	39.13	0.19	0.2
Prayaga and Henshall, 2005	Australia	British Sanga Zebu Zebu cross (2,540)[1]	>30.0	-	0.12	-
Dikmen et al., 2012	USA (Florida)	Holstein (1,695)	30.6	37.2-41.7	0.17	-
Otto et al., 2019	Netherlands	Gir × Holstein (341)	42.0	-	0.13	0.29

[1] Total number of cows measured from all the breeds collectively, n = number of animals measured, Temp = ambient temperature at which the RT was measured.

Some studies have reported the genetic correlation between RT and milk production traits. Most of these studies show that the genetic correlations between RT and milk production traits are positive but small whereas genetic correlations with most reproduction traits are negative and strong (Table 2.2).

Table 2.2 Published estimates of phenotypic (r_P) and genetic (r_G) correlations of rectal temperature with milk production and reproduction traits in cattle.

	Milk Traits				
	Milk yield	Fat yield	Protein yield	SCS	Productive life
r_P	-0.02[a]	0.07[a]	0.09[a]	-0.08[a]	-0.09[a]
r_G	0.09[a]	0.10[a]	0.10[a]	-0.01[a]	0.06[a]
	Reproduction Traits				
	Fertility	Calf birth weight			
r_P	0.032[a], 0.20[b]	0.49[b], 0.01[c]			
r_G	-0.013[a], -0.76[b]	-0.31[b], 0.01[c]			

[a]Dikmen et al. (2012); [b]Turner (1982); [c]Prayaga and Henshall (2005), SCS = somatic cell score.

The national selection index of the New Zealand dairy industry is known as the Breeding Worth (BW) index. This BW index uses eight traits which comprise four production efficiency traits and four robustness traits (Table 2.3). The BW index is calculated as a combination of breeding value (BV) of animals for each trait and the corresponding economic value (EV) of the trait. Animals are evaluated based on multiple traits simultaneously and ranked based on their overall performance (NZAEL, 2020). The New Zealand dairy industry is predominantly pasture based and greatly relies on the seasonal pasture supply, therefore, the best bulls and cows in New Zealand are the animals whose progeny will be the most efficient converters of feed into profit. The BW index ranks the animals by means of units of net profit in dollars per 5 tonnes of dry matter intake.

Table 2.3 The traits in the New Zealand Breeding Worth index and their economic values (EV) with their effective emphasis (EE) for 2019 production season.

Trait category	Trait (units)	EV	EE (%)
Production efficiency	Fat yield ($/kg)	3.49	24
	Protein yield ($/kg)	4.38	17
	Milk volume ($/kg)	-0.092	13
	Liveweight ($/kg)	-1.3	11
Robustness	Somatic cell score ($/SCS)	-37.3	6
	Fertility ($/CR42)	5.88	13
	Residual survival ($/day)	0.112	9
	Body condition score ($/unit)	96.3	7

CR42 = failure or success of calving within the first 42 days after the planned start of calving as percentage.

The literature reporting correlated response for traits in BW index including novel traits are scarce in New Zealand, except the efforts have been put in including liveweight (Spelman and Garrick, 1997), calving season day as a reproduction trait (Bowley et al., 2015), and lactose (Sneddon et al., 2016a). There is no published literature on estimation of correlated response of HS on either production or reproduction traits.

2.4.3 Genomics for heat tolerance

Genetic gain using the traditional selection approach based on phenotypes and pedigree information is usually slow given the long generation interval and less accuracy of estimated BVs (EBVs) calculated as the parent average using pedigree (Schefers and Weigel, 2012). Because of the greater negative impact of HS on production, reproduction and welfare of cows, immediate solutions for HS are desirable. Genomic approaches to improve HT include the use of genome-wide association studies (GWAS) and genomic selection (GS). Genome-wide association refers to the identification of genomic markers associated with HT and GS refers to the use of the identified genomic markers for selecting and breeding animals. Since

GWAS allows quick identification of genomic markers related to HT over the entire genome, estimation of genomic breeding values in young bulls and heifers are possible. This signifies that the rate of genetic gain of GS is greater than that of traditional progeny test selection scheme owing to the shorter generation interval (Schefers and Weigel, 2012).

Genome wide association studies are statistical tools for identifying genome-wide variants that show susceptibility and tolerance to HS. Various studies (Table 2.4) have identified single nucleotide polymorphisms (SNPs) and genes associated with thermoregulation phenotypes in cattle such as RT, rates of sweating and respiration, change in milk volume and constituents, fertility under HS through GWAS. The genes related to HS can be functionally categorised as genes associated with calcium signalling pathway, cellular response to HS, and oxidative stress response.

Table 2.4 Genome-wide association studies reporting candidate genes associated with rectal temperature (RT), rate of sweating (SR), and respiration (RR), coat colour (CC), feed intake (FI), milk yield (MY), percentage of milk fat (FP), and protein (PP), somatic cell score (SCS), and fertility (FT) in dairy cows under heat stress.

Trait	Candidate gene	Gene abbreviation	Reference	Chromosome	Function
RT	3-hydroxybutyrate dehydrogenase 2	BDH2	Dikmen et al., 2015	6	Cellular response to heat stress
RT	Calpastatin	CAST	Dikmen et al., 2015	7	Unknown
RT	Acetyl-coA acetyltransferase 2	ACAT2	Dikmen et al., 2015	9	Cellular response to heat stress
RT	Glucosaminyl (N-acetyl) transferase 3	GCNT3	Dikmen et al., 2015	10	Unknown
RT	Endothelial PAS domain protein 1	EPAS1	Dikmen et al., 2015	11	Oxidative stress-related hypoxic response
RT	DEP domain containing 7	DEPDC7	Dikmen et al., 2015	15	Cellular response to heat stress
RT	Progesterone receptor	PGR	Dikmen et al., 2015	15	Unknown
RT	DGCR8 microprocessor complex subunit	DGCR8	Otto et al., 2019	17	Cellular response to heat stress
RT	Leukemia inhibitory factor	LIF	Otto et al., 2019	17	Regulate of expression of heat shock protein
RT	Oncostatin M	OSM	Otto et al., 2019	17	Regulate of expression of heat shock protein
RT	Thioredoxin reductase 2	TXNRD2	Otto et al., 2019	17	Cell redox homeostasis
RT	Fibroblast growth factor 2	FGF2	Dikmen et al., 2015	17	Unknown
RT	NLR family pyrin domain containing 9	NLRP9	Dikmen et al., 2015	18	Unknown
RT	Golgin A4	GOLGA4	Dikmen et al., 2015	22	Unknown
RT	ADP ribosylation factor like GTPase 6 interacting protein 1	ARL6IP1	Dikmen et al., 2015	25	Cellular response to heat stress

Trait	Candidate gene	Gene abbreviation	Reference	Chromosome	Function
RT	Argininosuccinate lyase	ASL	Dikmen et al., 2015	25	Cellular response to heat stress
SR	Serpin family E member 2	SERPINE2	Dikmen et al., 2015	2	Cellular response to heat stress
SR	Phospholipase C beat 1	PLCB1	Li et al., 2020	13	Energy metabolism process
SR	Phospholipase C beat 4	PLCB4	Li et al., 2020	13	Energy metabolism process
SR	Heat shock transcription factor 1	HSF1	Li et al., 2020	14	Cellular response to heat
SR	Hermansky-pudlak syndrome 6	HPS6	Dikmen et al., 2013	26	Pigmentation
SR	Solute carrier family 18 member A2	SLC18A2	Dikmen et al., 2015	26	Cellular response to toxic substance
RR	FYN binding protein 2	FYB2	Dikmen et al., 2015	3	Unknown
RR	Acetyl-coA acetyltransferase 2	ACAT2	Dikmen et al., 2015	9	Energy metabolism process
RR	Dual specificity tyrosine phosphorylation regulated kinase 3	DYRK3	Dikmen et al., 2015	16	Unknown
RR	Calcium voltage-gated channel subunit alpha1 D	CACNA1D	Dikmen et al., 2015	22	Thermal sweating
RR	ADP ribosylation factor like GTPase 6 interacting protein 1	ARL6IP1	Dikmen et al., 2015	25	Unknown
CC	RAS oncogene family	RAB31	Li et al., 2020	24	trafficking, targeting, docking and fusion of melanosomes
FI	ATPase phospholipid transporting 8A1	ATP8A1	Li et al., 2020	6	Lipid transport
FI	SHC adaptor protein 3	SHC3	Li et al., 2020	8	Unknown
MY	Coiled-coil serine rich protein 1	CCSER1	Macciotta et al., 2017	6	Unknown

Trait	Candidate gene	Gene abbreviation	Reference	Chromosome	Function
MY	β-transducin repeat containing E3 ubiquitin protein ligase	BTRC	Macciotta et al., 2017	26	Mammary gland epithelial cell proliferation
FP	Rho GTPase activating protein 39	ARHGAP39	Macciotta et al., 2017	14	Regulation of transcription
FP	Diacylglycerol o-acyltransferase 1	DGAT1	Macciotta et al., 2017	14	Diacylglycerol metabolic process Triglyceride biosynthetic process Fatty acid homeostasis Long-chain fatty-acyl-CoA metabolic process Lipid storage
FP	Heat shock transcription factor 1 a protein	HSF1	Macciotta et al., 2017	14	Regulation of transcription
FP	Ribosomal protein L8	RPL8	Macciotta et al., 2017	14	Regulation of transcription
FP	Zinc finger protein 34	ZNF34	Macciotta et al., 2017	14	Regulation of transcription
PP	Malonyl-CoA-acyl carrier protein transacylase	MCAT	Macciotta et al., 2017	5	Fatty acid biosynthetic process
PP	Sorting and assembly machinery component	SAMM50	Macciotta et al., 2017	5	Cellular protein aggregation
SCS	Mitogen-activated protein kinase 15	MAPK15	Macciotta et al., 2017	14	Cellular response to DNA damage stimulus
FT	Tumor protein p63	TP63	Li et al., 2020	1	Embryonic limb morphogenesis

Trait	Candidate gene	Gene abbreviation	Reference	Chromosome	Function
FT	Mitogen-activated protein kinase kinase kinase 13	MAP3K13	Li et al., 2020	1	Unknown
FT	Bromodomain and WD repeat-containing 1	BRWD1	Sigdel et al., 2020	1	Meiotic chromosomal stability in the oocytes
FT	Protein tyrosine phosphatase non-receptor type 4	PTPN4	Li et al., 2020	2	Unknown
FT	Protein phosphatase 3 catalytic subunit gamma	PPP3CC	Li et al., 2020	8	Organogenesis
FT	Endothelial PAS domain-containing protein 1	EPAS1	Sigdel et al., 2020	11	Oxidative stress-related hypoxic response
FT	ADAMTS like 1	ADAMTSL1	Li et al., 2020	13	Extracellular matrix organization
FT	TAO kinase 3	TAOK3	Sigdel et al., 2020	17	Repair damage DNA in thermal stress
FT	Exonuclease 3'-5' domain containing 2	EXD2	Sigdel et al., 2020	21	Cellular response to DNA damage
FT	Solute carrier family 10 member 1	SLC10A1	Sigdel et al., 2020	21	Cellular response to nutrient levels
FT	Fibronectin type III and SPRY domain containing 2	FSD2	Sigdel et al., 2020	21	Protecting the cells from oxidative damage caused by heat stress
FT	Adaptor related protein complex 3 subunit beta 2	AP3B2	Sigdel et al., 2020	21	Intracellular removal of heat-induced protein aggregates

Genomic selection is a form of marker assisted selection where the BVs of individuals are calculated based on the genomic information (Meuwissen et al., 2001) rather than phenotypes and pedigree information (Schefers and Weigel, 2012). Most of the economically important traits of livestock are controlled by many genes and each gene may have a small effect on the trait of interest. Therefore, the BVs estimated using SNP-chips which cover the whole genome of the animal are called genomic EBVs (gEBVs).

Australia has taken efforts to include the gEBVs for HT into their national selection index to select HT animals for future breeding programs (Nguyen et al., 2016; 2017). In their study, Nguyen et al. (2016; 2017) used future climate predictions and calculated the heat exposure of a year in terms of temperature humidity index (THI) considering five consecutive day average THI units exceeding 60. Responses of cows to THI in relation to yield changes of milk, fat and protein have been calculated using a random regression model. Heat tolerance genomic breeding values of genotyped cows and bulls were calculated using genomic best linear unbiased prediction. Heat tolerance genomic breeding values for each milk component namely milk (gEBVhtm), fat (gEBVhtf) and protein (gEBVhtp) with their respective economic weights (EW) and total number of THI units exceeding 60 in a year (HL) were combined to make a heat tolerance breeding value (BVHT) in dollars as follow,

$$\mathrm{BVHT} = (\mathrm{EWm} \times \mathrm{gEBVhtm}) + (\mathrm{EWf} \times \mathrm{gEBVhtf}) + (\mathrm{EWp} \times \mathrm{gEBVhtp}) \times \mathrm{HL}$$

Thereby they have developed a balanced performance index-heat tolerance (BPI-HT) by combining the national dairy selection index (i.e. balanced performance index, BPI) with the BVHT. The new BPI-HT index expresses the breeding value for heat tolerance along with other economically important traits. The BVHT has been scaled using THI for each region so that the index can be used for any region under different THIs.

2.5 Genetics basis of milk urea and nitrogen utilization efficiency

Dietary CPI of cows manage under indoor systems has a significantly positive effect on the level of MUN and BUN (Jonker et al., 1998; Burgos et al., 2001). Feeding trials in New Zealand have shown that there is a possibility to reduce MUN and UN excretion and improve NUE by either feeding cows with diverse pasture containing herbs and legumes, besides perennial ryegrass (Totty et al., 2013; Edwards et al., 2015) or pasture containing at least 30% plantain dry matter (Minnee, 2017; Minnée et al., 2020). Although feeding management of cows seems a desirable option to cut down the carbon footprint and N leaching associated with dairying in New Zealand, it is greatly expensive compared to pasture feeding, suggesting that genetic alternatives would be better fit to the purpose.

2.5.1 Crossbreeding for milk urea and nitrogen utilization efficiency

Some evidence from overseas (Islam et al., 2015) and New Zealand (Lopez-Villalobos et al., 2018; Beatson et al., 2019) on breed difference in MUN suggests that crossbreeding could be a useful genetic tool to reduce the adverse effects of N excretion. A reduction of UN excretion in crossbreeding is apparently achieved due to reduced dry matter intake (DMI) and involuntary CPI of cows. For example, Jersey cows excrete less N compared to Friesian cows (Beatson et al., 2019) due to lower DMI. However, the benefit of crossbreeding will be diminished on per hectare level, given that on per hectare level, the stocking rate should be adjusted to balance the reduced DMI of crossbred herds. Apart from the non-significant ($P < 0.05$) difference in gross efficiency of crude protein (GECP) between J and F breeds reported by Lopez-Villalobos et al. (2018), no published reports are available on breed difference of NUE.

2.5.2 Selection for milk urea and nitrogen utilization efficiency

Milk urea nitrogen is a heritable trait which ranges from low (0.13) to moderately high (0.59) consequently, MUN can be reduced by selection and breeding for low MUN. The repeatability of MUN varies from low (0.22) to moderate (0.43) (Harper, 1994). Numerous studies (Table 2.5) have reported daily variability (Wood et al., 2003, Hossein-Zadeh and Ardalan, 2011) and parity-wise variability (Wood et al., 2003, Miglior et al., 2007, Hossein-Zadeh and Ardalan, 2011) in h^2 of MUN in lactating dairy cows. Mitchell et al. (2005) have observed a variation of h^2 and t estimates obtained from infrared spectroscopy and wet chemistry methods.

Genetic correlations between MUN and milk production, liveweight and reproduction traits are not consistent as shown in the studies presented in Table 2.6.

Table 2.5 Published estimates of heritability (h^2) and repeatability (t) for milk urea nitrogen of cattle.

Study	Country	Breed (n)	Lactation stage	Method	h^2	t
Wood et al., 2003	Canada	Holstein (6,102)	1st	WC	0.44	-
			2nd		0.59	
			3rd		0.48	
Mitchell et al., 2005	North Carolina	Holstein (46,951)	305 days	WC	0.15	0.36
		Holstein (26,540)		IR	0.22	0.40
Stoop et al., 2007	Netherlands	Holstein-Friesian (1,953)	305 days	IR	0.14	0.43
Miglior et al., 2007	Canada	Holsteins (5,022)	Average daily	IR	0.38-0.41	-
Bastin et al., 2009	Belgium	Holstein (72,468)	1st	IR	0.13	0.22
Mucha and Strandberg, 2011	Sweden	Holstein (76,959)	305 days	IR	0.17	-
Hossein-Zadeh and Ardalan, 2011	Iran	Holstein (57,301)	305 days	IR	0.14-0.21	-
Lopez-Villalobos et al., 2018	New Zealand	Holstein-Friesian (117) Jersey (58) Crossbred (293)	305 days	IR	0.24	0.42
Beatson et al., 2019	New Zealand	Holstein-Friesian (42,233) Jersey (14,150) Crossbred (77,241)	305 days	IR	0.22	-
Bobbo et al., 2020	Italy	Brown Swiss (10,827)	305 days	IR	0.20	-

n = number of cows measured, WC = wet chemistry, IR = infrared spectroscopy

Table 2.6 Published estimates of genetic correlation between milk urea nitrogen and milk production, liveweight, and reproduction traits.

Study	Milk production and liveweight										
	MY	FY	PY	LY	TS	FP	PP	LP	NEm	SCS	LWT
Wood et al., 2003											
Lactation 1	0.11	0.01	0.04	-	-	-	-	-	-	-	-
Lactation 2	0.17	0.32	0.22	-	-	-	-	-	-	-	-
Lactation 3	-0.05	0.20	0.06	-	-	-	-	-	-	-	-
Stoop et al., 2007	0.24	0.41	0.38	0.22	-	0.19	0.27	-0.09	0.21	0.85	-
Miglior et al., 2007	-0.094	-	-	-0.092	-	0.425	0.200	-0.041	-	-0.190	-
Mucha and Strandberg, 2011	0.22 – -0.15	0.22 – -0.15	0.22 – -0.15	-	-	-	-	-	-	-	-
Hossein-Zadeh and Ardalan, 2011	0.24	-	-	-	0.29	0.21	0.30	-0.01	0.31	-0.04	-
Lopez-Villalobos et al., 2018	0.38	-0.21	0.01	0.31	-	-0.80	-0.66	-0.76	-	0.20	0.31
Beatson et al., 2019	0.19	0.04	0.08	-	-	-0.15	- 0.20	-0.15	-	-	-
Bobbo et al., 2020	-0.03	-	-	-	-	0.07	-0.07	-	-	-0.06	-

	Reproduction										
	CFI	CLI	FLI	NINS	CI	PFI	DFS	DO	FSCR	NR56	NR90
Mitchell et al. 2005											
Lactation 1	-	-	-	-	-	-	0.14	0.21	-0.06	-	-
Lactation 2	-	-	-	-	-	-	0.18	0.41	0.01	-	-
König et al., 2008	0.29	-	-	-	-	-	-	-	-	-0.13	-0.12
Mucha and Strandberg, 2011	0 – -0.21	0 – -0.31	0 – -0.32	0 – -0.27	0.20 – 0.22	-0.10 – 0.28	-	-	-	-	-

MY = milk yield, FY = fat yield, PY = protein yield, LY = lactose yield, TS = total solids, FP = fat percentage, PP = protein percentage, LP = lactose percentage, NEm = net energy concentration in milk, SCS = somatic cell score, LWT = liveweight, CFI = interval from calving to first insemination, CLI = interval from calving to last insemination, FLI = interval from first to last insemination, NINS = number of inseminations to conception, CI = calving interval, PFI = pregnancy at first insemination, DFS = days to first service, DO = days open, FSCR = first-service conception rate, NR56 = liability to 56-d nonreturn, NR90 = liability to 90-d nonreturn.

Genetic variation of NUE has been rarely studied in dairy cattle. Nitrogen utilization efficiency is a heritable trait which varies from low; 0.1 (Li et al., 1998; Lopez-Villalobos et al., 2018; Bobbo et al., 2020) to moderate; 0.4 (Hayes and Ageeb, 2002; Zamani et al., 2011) while repeatability varies from 0.12 to 0.50 (Table 2.7). The values of the h^2 vary in different studies depending on the definition of the NUE, the stage of lactation, the breed of cattle studied, and the model used for analysis. Inefficient cows waste more NI as urea hence, less N is diverted into other pathways (Burgos et al., 2007) such as milk production, body weight gain, and embryo development, compared to more efficient cows. Therefore, genetic correlations between MUN and economically important traits (Table 2.5) can be considered as a measure of efficiency of cows. Some of these studies (Mucha and Strandberg, 2011; Lopez-Villalobos et al., 2018) have reported negative correlations between milk production, reproduction and liveweight traits and MUN suggesting that efficient cows convert more N and energy into production and reproduction traits and less N is wasted as urea. However, other studies either report positive (Mitchell et al., 2005) or weak relationships between production traits and MUN (Bobbo et al., 2020). The contradictory correlations among studies might attribute to the fact that some cows might be efficient in N utilisation at a standardised DMI; whereas others might be inefficient for N utilisation but simply have a greater genetic capacity to eat such that high MUN is associated with high CPI.

The greater correlations reported between MUN and milk percentage traits by Lopez-Villalobos et al., 2018 might have attributed to the use of multi-breed herds under pasture based production system instead of single-breed herds under intensive production systems, comparatively small sample size, and differences in the models used for parameter estimation.

Studying the correlation between MUN and production traits is an indirect approach to determine NUE. Alternatively, the relationship between milk production traits and NUE traits can be evaluated. Limited attempts have been made to estimate the genetic correlation between milk production and NUE traits (Table 2.8). However, the findings are not consistent on whether the cows selected for high milk production traits are also efficient in protein utilization. There are no previous studies on the genetic correlation between any NUE and reproduction traits, whereas the genetic correlations found between NUE traits and MUN reported in the literature are negative (Table 2.8).

To date there are no literature reporting the effect of including MUN as a trait in the selection index either in New Zealand or overseas. Since no attempts have been taken to include MUN in selection objective, there are no estimates of the economic value and correlated responses of MUN on the other traits. Therefore, there is no prior knowledge on the consequences of including MUN in selection objective.

The positive genetic correlations between MUN and yield of milk (MY), fat (FY), crude protein (CPY), lactose (LY), and average liveweight (LWT) reported in previous studies (Lopez-Villalobos et al., 2018; Beatson et al., 2019) suggest that the genetic selection for low MUN would result in cows with low genetic potential for milk production and LWT traits. However, the rate of change in response and the degree of impact of introducing MUN into the BW objective are unknown until the selection index is evaluated and correlated responses are predicted. The NUE is not directly measurable at herd-testing therefore, the potential of including them into BW index is limited. However, given the negative genetic correlations between NUE and MUN, NUE is expected to improve by selecting for low MUN.

Table 2.7 Published estimates of heritability (h^2) and repeatability (t) for nitrogen utilization efficiency of cattle.

Study	Country	Breed (n)	Lactation stage	Method	h^2	t
Li et al., 1998	China	Chinese Simmentals (151)	1-90 days	GECP	0.13	-
Hayes and Ageeb, 2002	Canada	Canadian Holsteins	90 days	GECP	0.10	-
			305 days		0.31	
Vallimont et al., 2011	Switzerland	Holstein (970)	1-60 days	GECP	0.21	-
Zamani et al., 2011	Iran	Holstein (501)	305 days	GECP	0.07	0.12
				CPB	0.40	0.40
				RPI	0.03	0.32
Lopez-Villalobos et al., 2018	New Zealand	Holstein-Friesian (117) Jersey (58) Crossbred (293)	305 days	GECP	0.11	0.50
Bobbo et al., 2020	Italy	Brown Swiss (10,827)	305 days	P/ MUN	0.15	-
				C/ MUN	0.15	-
				WP/ MUN	0.12	-

n = number of cows measured, GECP = gross efficiency of crude protein, CPB = crude protein balance, RPI = residual protein intake, P/MUN = protein to MUN ratio, C/MUN = casein to MUN ratio, WP/MUN = whey protein to MUN ratio, MUN = milk urea nitrogen.

Table 2.8 Published estimates of genetic correlations between nitrogen utilization efficiency and milk production traits of dairy cattle.

	Milk production and dry matter intake											
	MY	FY	PY	LY	FP	PP	LP	CSN	WP	SCS	MUN	DMI
Li et al., 1998												
GECP	0.23	0.01	0.62	-	-	-	-	-	-	-	-	-0.67
Hayes and Ageeb, 2002												
GECP	0.68 - 0.79	-	-	-	-	-	-	-	-	-	-	-
Vallimont et al., 2011												
GECP	-	-	0.90	-	-	-	-	-	-	-0.17	-	0.05
Zamani et al., 2011												
GECP	-0.29	0.32	-0.19	-	0.76	0.08	-	-	-	-	-	-0.72
CPB	0.64	-0.62	0.07	-	-0.88	-0.28	-	-	-	-	-	0.19
RPI	0.85	-0.04	0.87	-	-0.88	-0.79	-	-	-	-	-	0.96
Lopez-Villalobos et al., 2018												
GECP	0.01	0.63	0.59	-0.19	0.23	0.55	-0.73	-	-	0.76	-0.09	0.18
Bobbo et al., 2020												
P/MUN	-0.05	-	-	-	0.03	0.29	-	0.29	0.21	0.07	-0.65	-
C/ MUN	-0.05	-	-	-	0.04	0.29	-	0.30	0.18	0.06	-0.65	-
WP/ MUN	-0.07	-	-	-	0.02	0.27	-	0.24	0.30	0.11	-0.62	-

MY = milk yield, FY = fat yield, PY = protein yield, LY = lactose yield, FP = fat percentage, PP = protein percentage, LP = lactose percentage, CSN = casein percentage, WP = whey protein percentage, SCS = somatic cell score, MUN = milk urea nitrogen, DMI = dry matter intake, GECP = gross efficiency of crude protein, CPB = crude protein balance, RPI = residual protein intake.

2.5.3 Genomics for milk urea and nitrogen utilization efficiency

Milk urea and NUE are new traits for genetic and genomic studies relative to HS. The oldest estimates of genetic parameters of MUN and NUE were reported in 2003 (Wood et al., 2003), and 1998 (Li et al., 1998), respectively whereas reports for RT at HS in 1947 (Seath et al., 1947). To date no published literature reporting genes associated with either MUN or NUE and therefore, no reports on GS on either trait are available.

Numerous studies (Pimentel et al., 2011; Sanchez et al., 2017; Zhou et al., 2019) have identified candidate genes associated with milk production and composition. Studies reporting the candidate genes for MU or MUN are scarce. Although, few overseas studies (Strucken et al., 2012; Pegolo et al., 2018) have reported significant SNPs and genomic regions associated with MUN, the genes associated with the trait are unknown. Neither studies from overseas nor New Zealand have reported candidate genes for any NUE trait (NUE traits are described in section 2.2.1 of this chapter). Therefore, candidate genes associated with milk composition traits together with SNPs associated with MUN are presented in Table 2.9.

Table 2.9 Genome-wide association studies reporting genes and markers associated with percentage of milk fat (FP), protein (PP) and milk urea nitrogen (MUN) in dairy cows.

Trait	Candidate gene	Gene abbreviation	Reference	Chromosome	Function
FP	Cytochrome P450 oxidase	CYP27A1	Pimentel et al., 2011	2	Retinol metabolic process
	Isocitrate dehydrogenase1	IDH1	Pimentel et al., 2011	2	Regulation of phospholipid biosynthesis
	Microsomal glutathione S-transferase 1	MGST1	Littlejohn et al., 2016	5	Cellular response to lipid hydroperoxide
	Acyl-CoA oxidase 3, pristanoyl	ACOX3	Ibeagha-Awemu et al., 2016	6	Fatty acid metabolism
	Diacylglycerol O-acyltransferase 1	DGAT1	Nayeri et al., 2016, Wang et al., 2020	14	Diacylglycerol metabolic process Triglyceride biosynthetic process Fatty acid homeostasis Long-chain fatty-acyl-CoA metabolic process Lipid storage
	Maestro heat like repeat family member 1	MROH1	Wang et al., 2020	14	Unknown
	Cleavage and polyadenylation specific factor 1	CPSF1	Wang et al., 2020	14	Unknown
	Glycerol-3-phosphate acyltransferase 4	GPAT4	Wang et al., 2012, Xiang et al., 2017	15	Fatty acid metabolic process mammary gland development
	Myotubularin related protein 3	MTMR3	Pimentel et al., 2011	17	Lipid metabolic process

Trait	Candidate gene	Gene abbreviation	Reference	Chromosome	Function
	Mitogen-activated protein kinase kinase kinase 1	MAP3K1	Jiang et al., 2016	20	Unknown
	Lipase A, lysosomal acid type	LIPA	Pimentel et al., 2011	26	Lipid catabolic process
PP	Mitochondrial ribosomal protein L32	MRPL32	Pimentel et al., 2011	4	Translation
	Branched chain amino acid transaminase 1	BCAT1	Pimentel et al., 2011	5	Amino acid biosynthetic process Amino acid metabolic process
	Alpha-S1-casein	CSN1S1	Zhou et al., 2019, Sanchez et al., 2017	6	Stabilization of encoded proteins
	Beta-casein	CSN2	Zhou et al., 2019, Sanchez et al., 2017	6	Unknown
	Alpha-S2-casein	CSN1S2	Zhou et al., 2019, Sanchez et al., 2017	6	Unknown
	Kappa-casein	CSN3	Zhou et al., 2019, Sanchez et al., 2017	6	Stabilization of encoded proteins
	HECT and RLD domain containing E3 ubiquitin protein ligase family member 6	HERC6	Cohen-Zinder et al., 2005	6	Protein ubiquitination
	Leucine aminopeptidase 3	LAP3	Zhou et al., 2019	6	Protein metabolic process
	Transglutaminase 2	TGM2	Pimentel et al., 2011	13	Peptide cross-linking
	Diacylglycerol O-acyltransferase 1	DGAT1	Zhou et al., 2019	14	Lipoprotein particle assembly
	Heat shock transcription factor 1	HSF1	Zhou et al., 2019	14	Protein phosphorylation

Trait	Candidate gene	Gene abbreviation	Reference	Chromosome	Function
	Myotubularin related protein 3	MTMR3	Pimentel et al., 2011	17	Protein dephosphorylation
	Oligoadenylate synthetase 1	OAS1	Pimentel et al., 2011	17	Regulation of ribonuclease activity
	Golgi SNAP receptor complex member 2	GOSR2	Vijayakumar et al., 2019	19	Protein transport
	Mitogen-activated protein kinase kinase kinase 1	MAP3K1	Jiang et al., 2016	20	Protein phosphorylation
	Serpin peptidase inhibitor, clade A member 14	SERPINA14	Pimentel et al., 2011	21	Negative regulation of endopeptidase activity
	AT-rich interaction domain 5B	ARID5B	Pimentel et al., 2011	28	Negative regulation of transcription
MUN		BTA-71368-no-rs	Pegolo et al., 2018	4	Unknown
		Hapmap44167-BTA-95489	Pegolo et al., 2018	5	Unknown
		ARS-BFGL-NGS-108308	Pegolo et al., 2018	13	Unknown
		Hapmap60738-rs29023086	Strucken et al., 2012	13	Unknown
		BTA-62484-no-rs	Strucken et al., 2012	27	Unknown

2.6 Conclusions and gaps in knowledge

Dairying plays a crucial role in New Zealand economy. Pastoral-based farming systems practiced in New Zealand are coupled with animal welfare and environmental pollution issues. Outdoor farming increases the potential of cows' exposure to high environmental temperatures in the summer. Moreover, the negative effects of rising temperatures are expected to increase in future given the predicted future global warming due to GHGE. In addition, direct urine excretion on the paddock increases the chance of GHGE by means of N_2O volatilisation and increases N leaching in comparison to the cows fed indoors. The cows with greater NUE are predicted to allocate more dietary N into production therefore less N is excreted in urine, consequently reducing the potential of GHGE and N leaching.

There are some studies carried out in New Zealand that demonstrate the effect of elevated ambient temperatures on production performance of dairy cattle (Bryant et al. 2007) and the importance of use of management practices to minimize the adverse effect of summer temperatures (Fisher et al., 2008). However, studies on the genetic basis of heat tolerance have been limited in New Zealand with the exception of the finding of dominant genetic mutations responsible for abnormally long and hairy coats which increases heat stress in *Bos taurus* cattle (Littlejohn et al., 2014). To date, no studies have been done in New Zealand to estimate genetic parameters, genes associated with HS and effect of including a breeding value for HT in the BW index for dairy cattle.

Estimation of genetic parameters for MUN have been reported in several dairy cattle populations and the number of genetic studies on nitrogen metabolism is increasing due to environmental concerns caused by N excretion, high cost of protein feeding, and future food security. Genes associated with either MUN or NUE are not yet discovered therefore, GS is not possible for these traits.

No studies on correlated responses of including either RT or MUN in selection indexes or breeding objectives using selection index theory were found in this review. Therefore, the intention of this study was to estimate genetic parameters, perform GWAS and estimate correlated response in selection indexes on HT or MUN and NUE in mix-breed dairy cows in New Zealand pasture-based systems.

Chapter 3

Estimation of genetic parameters for rectal temperature, milk production traits, liveweight, and somatic cell score in dairy cows

The genetic parameters of rectal temperature of New Zealand dairy cows in heat stress were evaluated. The low ambient temperatures of sampling days were the likely reason for the estimated very low genetic variance of rectal temperature.

3.1 Abstract

The objective of this study was to estimate the heritability of rectal temperature (RT) and, if the trait is heritable, to estimate genetic correlations with milk production traits (milk yield: MY; fat yield: FY; crude protein yield: CPY), somatic cell score (SCS), and liveweight (LWT). The study utilized RT as an indicator of heat stress (HS) in 776 New Zealand dairy cows. Variance components were obtained using a mixed-model that included fixed effects of breed-lactation number, proportion of Friesian breed, heterosis of Friesian and Jersey crossbreds (F × J), a regression on the deviation from median calving date, and random effect of animal. Breed and lactation number affected milk production traits but not RT. Although the estimated (h^2) for RT was zero, the h^2 estimates for MY (0.27), FY (0.23), CPY (0.24) SCS (0.14) and LWT (0.69) were similar to reported values in literature. The zero h^2 in the current study was most likely due to low ambient temperatures on sampling days. New Zealand dairy cows are likely to experience HS at some point during summer, but it may last only for a few days making it difficult to capture data. Long-term monitoring of body temperatures using internal temperature-loggers can be suggested for studying HS in New Zealand dairy cows.

Keywords: dairy cows; rectal temperature; heat stress; production traits; heritability; thermoneutral zone

3.2 Introduction

Heat stress (HS) is one of the greatest global challenges faced by dairy farmers as it affects both production and reproduction of cattle in many parts of the world (Fuquay, 1981). Lactating dairy cows produce a large quantity of metabolic heat in the process of production of milk and they gain radiant heat from the environment. Cooling mechanisms which dissipates accumulated heat in the body are compromised by environmental conditions such as high ambient temperatures and relative humidity causing the cows to become heat stressed (Finch, 1986). The consequent reduction of dry matter intake causes a negative energy balance (West, 1999) which affects both milk production and fertility. The intensity of HS affecting the cows can be measured using temperature-humidity index (THI) proposed by Kibler (1964).

Dairying plays an important role in the New Zealand economy. Milk production systems are seasonal and based on pastures grazed by cows. The typical dairy cow management system means cows experience maximum average ambient temperature ranges between 20 and 30 °C during the summer (NIWA, 2018) making them vulnerable to HS. The effect of HS can be severe as considerable proportion of the national herd (33.4%) is Holstein-Friesian cows (DairyNZ, 2017). Holstein-Friesian breed is known to be more susceptible to HS than the Jersey breed (Seath and Miller, 1947; Harris et al., 1960). Studies by Bryant et al. (2007) have shown that daily milk and milk solid productions in New Zealand dairy cows started to reduce as a response to increase in three-day average THI by one unit at ambient temperature as lower as 21 and 25 °C in Holstein-Friesian and Jersey cows, respectively with demonstrated breed difference in adaptability to thermal environments. Fisher et al.

(2008) and Quartermain (1960) have observed that New Zealand gazing dairy cows were seeking for shade at average ambient temperature of 25 °C (range = 20.2 - 27.0 °C) and 26.1 °C (range = 21.6 - 29.7 °C) where the daytime average vaginal (Fisher et al., 2008) and rectal temperatures (RT) (Quartermain, 1960) reached 39.0 °C and 38.9 °C, respectively. Therefore, there is a possibility that HS may occur in New Zealand dairy cattle during similar thermal conditions in the summer. This raises the importance of developing intervention tools for New Zealand dairy cattle to reduce the effect of HS during summer, thereby to prevent economic losses.

Several strategies are being used worldwide to reduce the effect of HS such as use of heat abatements at cattle barns and traditional selection for a heat tolerant phenotype. However, the applicability of the former method to New Zealand is limited where cows are managed outdoors. Instead, a more appropriate method to New Zealand might be selection for heat tolerance phenotype where, estimates of genetic parameters including heritability ($h^{2)}$, genetic (r_G), and phenotypic (r_P) correlations between traits are required. Estimation of r_P and r_G correlations are important as selection for a phenotype without considering the associations with other traits may involuntarily affect other economically important traits.

Estimates of genetic parameters for RT and correlations with milk production and fertility traits in New Zealand dairy cattle are scarce. Although many such studies have been done worldwide, the applicability of those findings to New Zealand is limited due to variation in environmental conditions compared to the rest of the world. Therefore, the aim of this study was to estimate the heritability of RT in New Zealand Holstein-Friesian and Jersey cows and their crosses and if the RT is heritable, then to estimate the genetic and phenotypic correlations between RT and milk production traits, somatic cell score and liveweight, by considering the RT as an indicator of ability of cows to cope with HS.

3.3 Material and methods

3.3.1 Animals and measuring rectal temperatures

The data were originated from cows available at two Massey University experimental farms; Dairy 1 and Dairy 4, Palmerston North. Both experimental stations comprised of mixed-breed herds of Holstein-Friesian (F), Jersey (J) and crossbred (F × J) cows. Altogether, 776 cows were measured for RT including 227 cows from Dairy 1 where once a day (OAD) milking is practiced and 549 cows from Dairy 4 where twice a day (TAD) milking is practiced and utilized in this study. Breed information of cows measured at two farms are presented in Table 3.1.

Table 3.10 Breed information of cows used for measuring rectal temperatures at Massey University dairy farms.

Breed	Dairy 1	Dairy 4
Holstein-Friesian	58	199
Jersey	54	04
Crossbred	115	346
Total	227	549

Rectal temperature was recorded in cows on two hot sunny days (where expected ambient temperatures at least reached 25°C) between 14 Feb 2018 and 15 Feb 2018 starting from 12.30 h (day 1) and 11.30 h (day 2), respectively. The sampling was proceeded for an hour and two hours at Dairy 1 and Dairy 4, respectively. Sampling dates were selected based on predicted ambient temperature by National Institute of Water and Atmospheric Research (NIWA, 2018) and the expected ambient temperatures of selected dates reached at least 25°C. Rectal temperatures of cows were measured using digital thermometers (SureTemp® Plus 690) and were recorded to the nearest 0.1 °C. Each cow was measured once by insertion of a thermometer probe approximately 9.5 cm into the rectum from the external anal sphincter of the cow. Some cows were measured an additional time when the thermometer probe lost tissue connection within the rectum of cow which was detected as the thermometer beeped and displayed lost tissue connection. On the days of data collection, cows were yarded especially for the purpose after morning milking. All the cows were kept in direct sunlight until they were measured, and Dairy 1 cows were allowed into cowshed for measuring whereas Dairy 4 cows were measured at the race. Climatological data were obtained from the national climate database, station number 21963 (the station located within Massey University) including maximum, minimum, average temperatures and relative humidity. Ambient temperature reported at the starting point of measuring cows was considered as the consistent ambient temperature of the sampling day.

3.3.2 Descriptive statistics and fixed effects

Accumulated yields of milk (MY), fat (FY), crude protein (CPY) and liveweight (LWT) for 2017/18 production season up to 28th Feb 2018 were extracted from the farm databases for each cow. Average somatic cell score (SCS) was obtained from individual herd-tests for each cow, where SCS was calculated for each test as SCS = Log_2(somatic cell count).

Calving date, lactation number, breed composition and pedigree information were available for each cow. Cows in lactation 3 and above were considered as one lactation group (+3). Energy corrected milk yield (ECMY) was calculated using the following equation (Bernard, 1997):

$$\text{ECMY} = (0.325 \times \text{kg milk}) + (12.86 \times \text{kg fat}) + (7.04 \times \text{kg crude protein}).$$

Temperature humidity index (THI) was calculated using the following equation (Kibler, 1964):

$$\text{THI} = 1.8\text{Ta} - (1 - \text{RH})(\text{Ta} - 14.3) + 32,$$

where Ta is the ambient temperature in centigrade and RH is the relative humidity, both measured on the sampling day.

Rectal temperature data were tested for normality using the UNIVARIATE procedure in SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). Since the data were not normally distributed ($P < 0.005$), two extra measures were taken to test for normality, 1) the data were log transformed, 2) the deviation from a basal body temperature (37 °C or 38 °C) as discussed by Turner, 1982; 1984. The GLM procedure of SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) was used to identify the significant fixed effects on RT, MY and ECMY. A linear model that included the fixed effects of breed, lactation number, interaction between breed and lactation number and a covariate of deviation from herd median calving date were used. The analysis was performed for each farm separately. None of the fixed effects tested were significantly associated with RT however, were included in the model as they were strongly associated with MY and ECMY ($P < 0.05$).The analysis for Dairy 4 did not consider J cows as there were only four cows of that breed on this farm. Effect of RT on ECMY was evaluated by including RT in the model as a covariate.

3.3.3 Variance components and heritabilities

Variance components required for estimating the h^2 of RT, LWT, MY, FY, CPY and SCS were derived from a univariate animal model using the statistical package ASReml (Gilmour et al., 2009). The model for MY and ECMY included fixed effects of lactation number, proportion of F, the F × J heterosis, deviation from median calving date, interaction effect of lactation number and proportion of F, and the random effects of animal and residual error. The model for RT included the same fixed and random effects as the above-mentioned model excluding the effects of lactation number, proportion of F, and the interaction effects, since they were not significantly ($P > 0.05$) associated on trait. The pedigree file included animal, parents and grandparents with complete breed information. Heritability was calculated as $h^2 = \sigma^2_a / \sigma^2_p$ where σ^2_a, and σ^2_p are the animal and phenotypic variances respectively and σ^2_p is the sum of σ^2_a and residual variances (σ^2_e).

3.4 Results

3.4.1 Descriptive statistics and fixed effects

Neither the raw RT data (Appendix 1) nor transformed (log transformed and deviation from basal body temperature) data were normally distributed, therefore, results were presented only for raw RT data in this chapter. The climate data for day 1 and day 2 and RT measurements of cows are presented in Table 3.2. The mean RT of cows at Dairy 1 (39.09 °C) was significantly higher ($P < 0.05$) than the mean RT of cows at Dairy 4 (38.91 °C).

Table 3.2. Ambient temperatures at the beginning of the two sampling days and rectal temperatures of dairy cows at Massey University dairy farms.

Day	Ambient Temperature (°C)	RH (%)	THI	Rectal Temperature (°C)		
				Min	Max	Mean (SD)
Day 1	25.2	92	80.4	36.3	40.8	39.1 (±0.49)
Day 2	24.6	79	78.0	36.7	40.2	38.9 (±0.35)

RH = relative humidity, THI = temperature humidity index

Holstein-Friesian and F × J cows produced higher ECMY and MY on Dairy 1 than Dairy 4. ECMY and MY were not different between F and F × J on both farms. Cows in lactation 3+ produced more ECMY and MY than lactation 1 and 2 cows on either Dairy 1 or Dairy 2. On Dairy 1, lactation 2 cows produced more ECMY and MY than lactation 1 cows (Table 3.3). There was no significant effect of interaction between breed and lactation number on any trait studied. The regression coefficients of ECMY on RT were 200 kg (±97; $P = 0.041$) and -72 kg (±73; $P = 0.327$) at Dairy 1 and Dairy 4, respectively.

3.4.2 Variance components and heritabilities

Estimates of h^2 for LWT (0.69) was high, MY (0.27), FY (0.23) and CPY (0.24) were moderate, whereas h^2 estimate for SCS (0.14) was low. Estimate of h^2 for rectal temperature of the current study was zero (Table 3.4).

Table 3.3. Least squares means (±SEM) for milk production and temperature of dairy cows at Massey University dairy farms.

		Dairy 1			Dairy 4		
		RT (°C)	ECMY (kg)	MY (kg)	RT (°C)	ECMY (kg)	MY (kg)
Breed	Lactation number						
F		39.16±0.06	3,776^{a}±131	3,174^{a}±110	38.95 ±0.03	4,192±62	3,659±54
F × J		39.09±0.05	3,800^{a}±94	2,965 ^{a}±78	38.88 ±0.03	4,219±58	3,515±51
J		39.09±0.07	3,351^{b}±148	2,402^{b}±124			
P value		0.6798	0.0314	<.0001	0.0779	0.7540	0.0554
	1	39.13±0.09	3,051^{a}±176	2,418 ^{a} ±146	38.89 ±0.04	3,767^{a} ±87	3,167 ^{a}±76
	2	39.13±0.06	3,320^{a}±114	2,562 ^{a}±95	38.96 ±0.04	4,117^{b}±82	3,530 ^{b}±72
	+3	39.08±0.03	4,557^{b}±64	3,560 ^{b}±53	38.90 ±0.02	4,733^{c}±43	4,064^{c}±38
P value		0.7137	<.0001	<.0001	0.3423	<.0001	<.0001
F	1		3,169^{acd}±272	2,687^{acd}±228		3,746^{a}±115	3,216^{a} ±101
	2		3,360 ^{acd}±257	2,839 ^{ac}±215		4,099^{d}±126	3,571^{b}±111
	+3		4,800^{b}±117	3,995 ^{e}±98		4,733^{c}±72	4,191^{c}±63
F × J	1		3,228^{acd}±232	2,537^{bc} ±194		3,789^{ab} ±131	3,119^{a}±115
	2		3,622 ^{d}±134	2,763^{c} ±112		4,136 ^{d}±107	3,489 ^{b} ±94
	+3		4,548 ^{e}±83	3,594^{f}±69		4,733^{c}±48	3,938 ^{d} ±42
J	1		2,755 ^{c}±385	2,030^{bd}±322			
	2		2,975 ^{acd}±181	2,084^{b}152			
	+3		4,321 ^{e}±125	3,092^{a}±104			
P value of interaction			0.3951	0.7164		0.9657	0.4568

RT = rectal temperature, ECMY = energy corrected milk yield, MY = milk yield, F = Holstein-Friesian, J = Jersey, F × J = Holstein-Friesian × Jersey crossbred. ^{a,b,c} Means with different superscripts within the same trait and effect are significantly different ($P < 0.05$).

Table 3.4. Estimates of heritability for production traits, somatic cell score and rectal temperature in dairy cows at Massey University dairy farms.

Trait	σ^2_e	σ^2_a	σ^2_p	h^2 (±SEM)
MY	271,959	102,452	374,411	0.27 (±0.07)
FY	599.62	174.84	774.46	0.23 (±0.07)
CPY	347.77	110.56	458.33	0.24 (±0.07)
LWT	737.14	1,625.94	2,363.08	0.69 (±0.08)
SCS	1.63	0.26	1.89	0.14 (±0.09)
RT	0.14	1.30E-03	0.14	0.00

σ^2_e = residual variance, σ^2_a = genetic variance, σ^2_p = phenotypic variance, h^2 (SEM) = heritability (standard error of the mean), MY = milk yield, FY = fat yield, CPY = crude protein yield, LWT = liveweight, SCS = somatic cell score, RT = rectal temperature.

Since no genetic variation in RT was detected in the current study, r_G and r_P for RT with other traits were not estimated.

3.5 Discussion

The range (36.3-40.8 °C), mean (38.9-39.1 °C), and phenotypic standard deviation (0.35-0.49 °C) of RT of the cows in two herds in the current study are comparable with the estimates reported in New Zealand and overseas studies. The range of RT (from 38.59 to 39.61 °C), the herd average RT (39.13 °C) reported in the study of Morris et al. (1989) in 611 New Zealand Charolais × Murray Grey, Charolais × Simmental and Charolais × Red Devon cattle measured over a period of four years are comparable with the estimates of the current study, except they reported a greater phenotypic standard deviation in RT (2.23°C) in their study. Dikmen et al. (2012) and Turner (1982) reported the corresponding estimates of RT of Holsteins in North Florida and Hereford × Shorthorn and *Bos indicus* × *Bos taurus* in Australia, respectively. The reported range (37.20-41.70 °C), average (39.00-39.80 °C) and phenotypic standard deviation (0.24-0.60 °C) of RT in their studies are also comparable with the values reported in the current study.

The method we used in this study to capture the body temperature of cows was RT and it has been widely used as reliable tool for estimating genetic parameters in dairy cows (Turner, 1982; Morris et al., 1989; McMillan et al., 2007; Dikmen et al., 2012; Riley et al., 2012). However, the RT recorded in the current study had a skewed distribution (Graph A.1 of the Appendix One). This might be an indication that the cows were not heat stressed when the measurements were taken and/or a reflective of error in how the temperatures were measured and/or the sensitivity of the thermometers used.

The highest milk production recorded from F cows in this study is consistent with what previously reported by Abubakar et al. (1986) and Prendiville et al. (2010). This advantage in MY is the reason for greater ECMY from F cows. Although the MY of F × J cows is lower than in F, the ECMY of both F × J and F cows was similar on both farms. Similar fat and protein yields in milk of both F and F × J were reported by Lembeye et al. (2016a) and this should be the reason for comparable ECMY of the breeds. In each breed group MY and ECMY improved with increasing lactation number as expected because of the relationship between lactation number and MY (Lembeye et al., 2016a; Costa et al., 2019).

The significant positive regression of ECMY on the RT of cows in the Dairy 1 indicates that the cows that produced high volumes of milk and milk solids are more likely to have higher RT than less productive cows. This relationship was recorded only in the cows from Dairy 1 and this probably due to significantly greater RT of cows at Dairy 1 compared to the cows in dairy 4 owing to slightly higher ambient temperature in day 1 compared to day 2. This observation indicates the short-lived nature of environmental stresses in the summer of New Zealand advocating the long-term monitoring of body temperature for studying HS under New Zealand weather conditions. Alternatively, this could be due to greater physiological stress owing to greater milk volumes at the morning milking in OAD milking cows at dairy 1 than the TAD cows.

Previous studies which focused on estimating the genetic parameters were based on larger data sets compared to the current study. For example, Sneddon et al. (2016a) analysed 4310 cows and Ahlborn and Dempfle (1992) analysed over 10000 cows for estimating genetic parameters for milk production and body size. The size of the dataset (776 cows) in the current study is quite small compared to previous studies. The smaller sample size could be a possible reason for non-significant association of the fixed effects with RT. However, the fixed effects were significantly associated with the traits other than the RT, therefore, the current study seems to contribute advances on knowledge on traits other than RT.

The mean RT of cows from Dairy 1 was higher than the mean RT of cows from Dairy 4. This could possibly be due to high THI on the day of data collection at Dairy 1 (day 1) compared to day 2 and or ambient temperature that is slightly higher than the upper limit of thermoneutral zone at day 1.

In this study, between breed differences for RT were not found. This result is in contrast to some studies reporting that Friesian cows have higher RT than Jersey cows in the United States (Seath and Miller, 1947; Harris et al., 1960). However, the experimental design in Harris et al. (1960) involved only six Holstein cattle and six Jersey cattle which was a too small sample size to draw a precise estimate. Breed differences in body temperatures from cows in those studies is understandable where the climate is much hotter and humid compared to summer in New Zealand. Moreover, the US cows are capable of producing more milk compared to New Zealand cows and RT is reported to be higher in high milk producers than low producers owing to greater metabolic heat production (Finch, 1986). In the study by Bryant et al., 2007, a reduction greater than 10 g of milk solids/day per unit increase in 3-day average THI, started to occur at 3-day average THI of 68 in HF cows, where THI 68 approximately equivalent to ambient temperature of 21 °C. This observation indicates that, it is possible to observe a breed difference in the milk solid production even at lower ambient temperatures as 21 °C.

In the current study, phenotypes of the accumulated yields along with single point-in-time RT measurements were utilized. The accumulated lactation yields are reflective of the seasonal effects, whereas the single point-in-time RT measurements reflect what was occurring in the cow at the time of measurement only. This indicates that any short-lived effect, perhaps effects related to any stages of lactation, and/or that reflected a short-term change in any given cow (especially on the day RT was measured), may have been masked or not detected. This made the conclusion of the point-in-time RT measurements should be analysed with daily records of yields in order to detect the effect of HS on milk production. For this to be fulfilled, the measuring of daily RT should be carried out in-line with daily measurements of milk yield and composition, SCS and LWT. However, measuring of these traits are not carried out on the daily basis at the farms making it is difficult to detect the short-lived effects of RT on milk production.

Lactation number had no significant effect on RT of cows. This result was unexpected as previous studies demonstrated that RT increased with increasing lactation number (Bernabucci et al., 2014) and according to McDowell (McDowell et al., 1976) THI in-between 75 and 78 is stressful. A possible reason for this result was the low ambient temperatures of sampling days. Although the THI was high as the relative humidity was greater on both sampling days, the ambient temperatures might not have been sufficient to make the cows heat stressed as they were within the range of thermoneutral zone for lactating dairy cows (Ambient temperature: 5 to 25 °C, RH:50%, THI $\geq$ 72) (Roenfeldt, 1998). The level of heat production of animals is unaffected by temperature changes within the thermoneutral zone (Webster, 1979) and this would have at least partially attributed for not detecting breed and lactation number effects on rectal temperature in the current study.

Heritabilities for MY, FY and CPY were higher than the estimates by (Lopez-Villalobos, 2013) in F, J and F × J crossbred cows in New Zealand. However, the estimated heritabilities for FY and CPY were similar to the estimates of Hossein-Zadeh and Ardalan (2011) in Iranian Holstein dairy cows. Heritability estimate of SCS found in this study had comparable values to the estimate reported by Welper and Freeman (1992) and lower than estimates of Lopez-Villalobos, (2013) and Hossein-Zadeh and Ardalan (2011). Heritability of LWT reported by Svendsen et al. (1994) and Tveit et al. (1991) were comparable with estimates of the current study. The heritability estimates for milk traits, LWT and SCS suggested that adequate genetic variation exists in the population analysed in the current study for the traits other than the RT.

The estimated h^2 for RT in this study was extremely low compared to estimates reported in literature. Morris et al. (1989) reported h^2 estimates of 0.19 in 611 New Zealand Charolais × Murray Grey, Charolais × Simmental and Charolais × Red Devon cattle. However, there is no mention about ambient temperatures at which the RT of cows were measured in the study of Morris et al. (1989), otherwise it would be possible to get an inference about the likely ambient temperature thresholds for obtaining genetic variation in RT under New Zealand conditions. Dikmen et al. (2012) and Turner (1982) reported h^2 of 0.17 and 0.25, respectively for RT. However, the genetic standard deviation reported in the current study was lower than the estimates (0.06-0.37 ˚C) reported in other studies (Turner 1982; Morris et al., 1989; Dikmen et al., 2012). Therefore, the low h^2 of RT recorded in this study was most likely due to the very low genetic variance in RT. This can be at least partially attributed to low ambient temperatures on the sampling days which were not adequate to HS the cows. The h^2 estimate of Dikmen et al., 2012 is understandable as RT of cows were recorded at greater average ambient temperature conditions (30.6 ˚C) at which cows are known to be heat stressed (Roenfeldt, 1998). The upper thermoneutrality boundary for cattle is widely accepted as being 25 ˚C (Robinson et al., 1986), and in this study that limit was only just exceeded (25.2 ˚C) on day 1. Regardless the published ambient temperatures by NIWA on the days of the measuring, there may had been a micro-climate effects on the farms. These micro-climate effects might have had higher or lower than temperatures reported by NIWA and humidities on the sampling days and locations which has not taken into account in the current study. On the other hand, the greater ambient temperatures as Dikmen et al. (2012) observed in North Florida are unlikely to frequently observe in New Zealand. Therefore, use of data loggers which logs a couple of body temperature records per every hour (Fisher et al., 2008) would be a better option compared to actively measuring it on hot days defined based on the predicted ambient temperatures.

3.6 Conclusions

The results of the current study showed no effect of breed or lactation number on RT of cows. This could be due to low variation of body temperature, as the RT of the cows were mostly remained within the thermoneutral zone during the sample collection and or this could be a result of smaller sample size. The estimated heritability of RT in this study was zero which is in contradiction to the estimates found in the literature. However, this study confirmed the findings of the previous studies that LWT has a high h^2 and MY, CPY and FY have moderate heritabilities while SCS has a low $h^2$and this proves that the cows of the current study possess enough genetic variation for traits other than RT. This negative result can primarily be attributed to mild weather conditions present during the measurement period such that cows were not sufficiently heat stressed to exhibit heat-induced variations in RT. This indicates that the cows in the Palmerston North area are unlikely to experience HS for extended period of time. Future studies to investigate HS in New Zealand need to ensure cows are either naturally (using Ergovaline to chemically induces body temperature) or artificially (using heat chambers) heat stressed to increase the variation in RT or should use long-term monitoring systems such as vaginal temperature loggers.

Appendix one

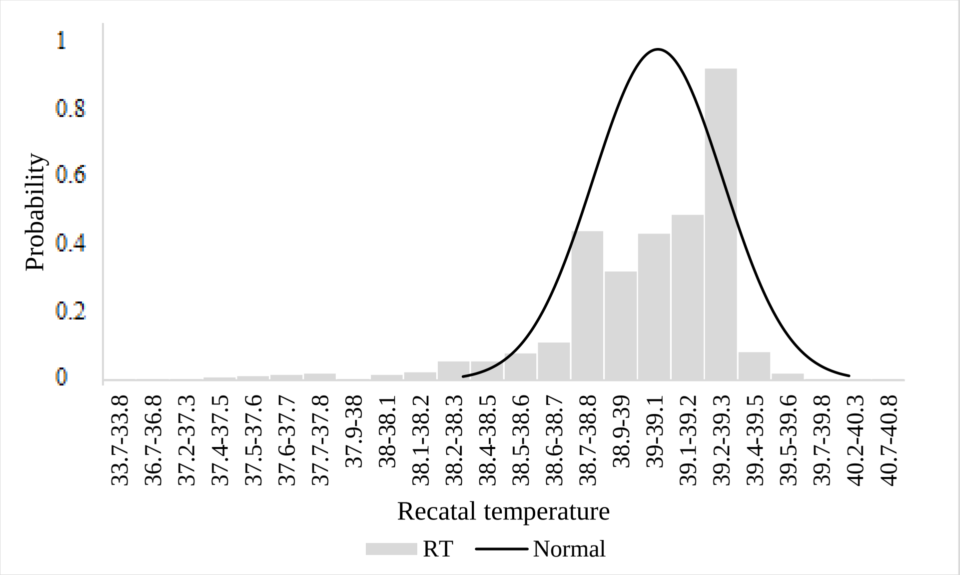

Figure A.1 Distribution of the rectal temperature (RT, ℃) of dairy cows at Massey University farms.

Chapter 4

Genetic parameters for efficiency of crude protein utilization and its relationship with production traits across lactations in grazing dairy cows

Due to zero genetic variance of rectal temperature of the analysed cows, the objective of the thesis was diverted to estimate the genetic basis of environmental traits. This paper was published as

Ariyarathne HBPC, Correa-Luna M, Blair HT, Garrick DJ, Lopez-Villalobos N. 2020. Genetic parameters for efficiency of crude protein utilisation and its relationship with production traits across lactations in grazing dairy cows. New Zealand Journal of Agricultural Research 63: 1-21. Doi: 10.1080/00288233.2020.1726414.

4.1 Abstract

The objective of the study was to estimate genetic parameters of efficiency of crude protein utilization (ECPU) and its genetic correlation (r_G) with other production traits across lactation in grazing dairy cows. Data were originated from 467 (2016-17 season) and 460 cows (2017-18 season) representing Holstein-Friesian (F), Jersey (J) and their crosses (F × J), from two New Zealand farms. Ratio between crude protein yield in milk and crude protein intake is defined as ECPU. (Co)variance components between ECPU and other traits were estimated using bivariate random regression models that included fixed effects of herd-test-date, parity, calving date, proportion of F, and F × J heterosis, with random effects of animal additive genetic, and within-lactation permanent environmental. Estimates of heritability of daily ECPU ranged from 0.02 to 0.41. Estimates of r_G between daily ECPU and milk yield traits were strong and positive throughout lactation suggesting that selection for milk production traits improves ECPU of dairy cows. Genetic correlations between ECPU and mid-infrared predictions of milk urea (MU) concentration at each day of lactation were mostly negative confirming that cows with high ECPU had genetically lower MU. Estimates of genetic relationships between ECPU and fertility traits are required to assess implications of including ECPU in breeding objective.

Key words: Dairy cows; efficiency of crude protein utilization; milk urea; genetic parameters; random regression model; genetic selection

4.2 Introduction

Crude protein plays an important role in milk payment systems of most countries (Sneddon et al., 2013) and is a determinant of feeding costs. Providing protein in the ration for cows in indoor systems is expensive and therefore, higher farm profitability is achieved when more feed protein is converted into milk protein. The proper balance between protein and energy in feed is crucial for efficient conversion of protein (Kebreab et al., 2002). Therefore, feeds offered to cows are normally balanced for protein and energy in indoor systems where they are fed on mixed rations aiming to optimize the use of feed nutrients.

Controlling the composition of diet is difficult to implement under New Zealand farm management systems where cows freely graze on pasture in outdoor conditions. In those circumstances the balance between protein and energy in feed is variable throughout the year (Correa-Luna et al., 2018). Baker et al. (1995) defined efficiency of crude protein utilization (ECPU) as the proportion of crude protein yield (CPY) in milk relative to the estimated crude protein intake (CPI) and they found that ECPU decreases when cows are fed with low energy diets relative to crude protein, resulting in a higher proportion of the consumed protein being excreted as urea in urine. A strong positive ($R^2 = 0.98$) phenotypic correlation (r_P) has been reported between urinary nitrogen (UN) and milk urea nitrogen (MUN) in carefully controlled trials under indoor systems (Jonker et al., 1998), and MUN has been suggested as an indicator of ECPU (Baker et al., 1995) and as an estimate of energy balance of diets (Oltner and Wiktorsson, 1983). Improving ECPU would be beneficial in increasing farm profits and reducing environmental nitrogen excretion of animals.

Zamani et al. (2011) described three definitions of protein utilization efficiency: the ECPU previously mentioned, the crude protein balance (CPB) defined as the difference between CPI and CPY, and the residual protein intake (RPI) defined as the difference between actual CPI and predicted CPI. Genetic improvement of protein utilization of dairy cows has been seldom in the literature and estimates of genetic parameters for protein utilization are scarce. Zamani et al. (2011) reported low to moderately high heritability (h^2) for ECPU (0.12), CPB (0.40) and RPI (0.32) whereas Li et al. (1998) and Hayes and Ageeb (2002) reported h^2 estimates of 0.13 and 0.10 (at 90 days in milk) and 0.31 (at 305 days in milk) for ECPU, respectively. Genetic correlations (r_G) between protein utilization efficiency and milk production traits were not consistent among these studies. We are unaware of published estimates of day-by-day genetic parameters of ECPU. The aim of this study was to estimate genetic parameters for ECPU and milk urea (MU), and their relationships with other milk production traits, liveweight (LWT), liveweight change (LWTc), and body condition score (BCS) by day of lactation in New Zealand grazing dairy cows.

4.3 Material and methods

4.3.1 Data collection

Test-day records from 467 cows measured in 2016-17 production season and 460 cows measured in 2017-18 production season at the Massey University Dairy 1 and Dairy 4 experimental farms in Palmerston North, New Zealand were used for this study. The herds on both farms comprised mixed-breed cows of Holstein-Friesian (F), Jersey (J) or their crosses (F × J). The dataset contained 257 cows from Dairy 1 including 66 F, 55 J and 136 F × J and 210 cows from Dairy 4 including 51 F, 3 J and 156 F × J for season 2016-17. Records for season 2017-18 included 251 cows from Dairy 1 including 62 F, 58 J and 131 F × J and 209 cows from Dairy 4 including 105 F, 4 J and 100 F × J.

Dairy 1 practices a once-a-day milking system with low levels of supplementary feeding with low stocking rate (2.1 cows/ha) and the feed strategy includes fresh ryegrass (Lolium perenne)/white clover (Trifolium repens) pasture as the main diet component with restricted supplementation and grazing crops used sporadically in summer. Dairy 4 is managed as a high intensity production system with cows milked twice-daily throughout the season, with higher stocking rate (2.8 cows/ha). Cows in Dairy 4 are also fed with ryegrass/white clover as main feed source but, higher supplementation level is included throughout the year. More details of cow management and feeding can be found in Correa-Luna et al. (2018).

Monthly herd-test records of daily yield of milk (MY), fat percentage (FP), crude protein percentage (CPP), and somatic cell count (SCC) were collected for each cow. Fat yield (FY) and CPY were calculated using FP and CPP multiplied by the corresponding milk MY obtained during the herd-tests. Somatic cell count was log transformed to calculate somatic cell score (SCS = Log_2 of SCC).

Milk urea concentration and lactose percentage (LP) were determined for each cow only for three of the herd-tests per season, representing early (September), mid (December) and late (March) lactation. Sample collection for measuring MU and LP was independent from regular herd-tests of the farms and was greatly dependent on funds available for covering costs associated with sampling and laboratory testing for milk components and feed quality. Milk samples were measured for MU and LP at MilkTestNZ (Hamilton, NZ) by the CombiFoss$^{\text{TM}}$ 7 instrument (Foss Electric, Hillerød, Denmark) which uses mid-infrared technique to measure milk components. The accuracy of predicted MU concentration using mid-infrared technique has been evaluated with the reference method of wet-chemistry and was found to be 0.97 (Arunvipas et al., 2003). Lactose yield (LY) was calculated as the product of LP and MY at the corresponding herd-test. Body condition score of all cows was collected in synchrony with each herd-test assigned by a single technician using a 10-point scale and liveweight measurements were generated daily using an automatic walk-over scale. Daily estimates of milk production traits (MY, FY, CPY, LY, FP, CPP, LP, SCS and MU), LWT, BCS for each lactation were obtained by modelling the lactation curves of the traits using orthogonal polynomials of order 3. Liveweight change was calculated over the lactation as the difference in LWT at consecutive weightings.

Pasture and crop samples were collected from the field, and along with samples of supplementary feed offered to the cow were analysed using near infrared spectroscopy in order to obtain dietary metabolizable energy (DME) content and dietary CPP (DCPP) available in the diet fed to cows. The DME (Hu et al., 2019) and DCPP (Marchesini et al., 2018) were reported to be predicted with a high accuracy using near infrared spectroscopy. Total ME requirements of each cow on each day of lactation was calculated as the sum of estimated metabolizable energy requirements for maintenance, pregnancy, lactation and liveweight change in each lactation as described by Lopez-Villalobos (2008). Apparent dry matter intake (DMI) was calculated as the estimated total metabolizable energy requirement of each cow divided by total DME content. Crude protein intake of the cow was determined as DCPP available in any diet offered to the cow in product with estimated DMI. Daily ECPU was estimated as the proportion of CPY and estimated CPI.

4.3.2 Data editing and descriptive statistics

Cows with a minimum of three herd-test records from lactations of not less than 150 days were included in the analysis. Since MU records were available until 240 days in milk (DIM) of the lactation, genetic parameters were estimated from 1 to 240 DIM. Data from day 1 to day 240 were separated into 5 stage of lactation (SOL) classes according to DIM. The intervals were defined as 1-50, 51-100, 101-150, 151-200 and 201-240 days. Descriptive statistics for the traits were obtained using the MEANS procedure of the statistical package SAS 9.4 (SAS Institute Inc., Cary, NC, USA).

4.3.3 Estimation of (co)variance components

Estimates of (co)variance components were obtained by the restricted maximum likelihood (REML) procedure in ASReml 4.1 (Gilmour et al. 2015). A univariate random regression test-day animal model was used to estimate variance components for animal additive genetic, within-lactation cow permanent environmental, across-lactation cow permanent environmental and residual effects. The model included fixed effects of herd-test-date (HTD), parity, regression on the deviation of calving day from median calving date, regression on proportion of F, and regression on F × J heterosis. The model for LWT and BCS included herd-year-month instead of HTD as the contemporary group. Cows in parities 1, 2 and 3 were considered as separate parity groups whereas cows in parity 4 and above were not discriminated by parity. The random effects included in the model were animal additive genetic, within-lactation cow permanent environmental, across-lactation cow permanent environmental and residual error. The animal additive genetic effects were modelled using second order orthogonal polynomials for all traits, except for MY which had a better fit from a third order orthogonal polynomial. Within lactation cow permanent effect was modelled using a first order orthogonal polynomial for BCS, DMI and ECPU, a second order orthogonal polynomial for SCS and LWT, and the intercept for the other traits. Across-lactation cow permanent environmental effect was modelled as a constant for all the traits. Heterogeneity in the residual variance was modelled by assigning each SOL with a different residual variance for each trait. The most parsimonious RRM for each trait analysed was determined using Akaike information criterion (Akaike, 1988) by comparing models with different order of polynomials for fixed, additive genetic and within lactation permanent environmental effects and selecting the model that reached the lowest criterion.

Additive genetic (**C**) and permanent environmental (**B**) (co)variances for each day of lactation were estimated using a covariance function from the following equations:

$$\mathbf{C} = \mathbf{\Phi}\ \mathbf{G}\ \mathbf{\Phi}'$$

and

$$\mathbf{B} = \mathbf{\Phi}\ \mathbf{P}\ \mathbf{\Phi}'$$

where **G** and **P** are (co)variance matrices of the random regression coefficients for additive genetic and within-permanent environmental effects and **Φ** is the matrix of orthogonal polynomial coefficients.

Bivariate RRM were used to estimate the matrix of variances of each trait and covariances between two traits at the same and different days in milk with the following equations:

$$\begin{bmatrix} \mathbf{C_{11}} & \mathbf{C_{12}} \\ \hline \mathbf{C_{21}} & \mathbf{C_{22}} \end{bmatrix} = \begin{bmatrix} \Phi\mathbf{G_{11}}\Phi' & \Phi\mathbf{G_{12}}\Phi' \\ \hline \Phi\mathbf{G_{21}}\Phi' & \Phi\mathbf{G_{22}}\Phi' \end{bmatrix}$$

where $\mathbf{G}_{mn}$ is the matrix of (co)variances of the random regression coefficients for additive genetic effects between trait *m* and trait *n*.

4.3.4 Estimation of genetic parameters

Heritability and repeatability (t) for each trait at the *i*th DIM was estimated as

$$h^2_i = \sigma^2_{ai} / (\sigma^2_{ai} + \sigma^2_{p(w)i} + \sigma^2_{p(a)} + \sigma^2_{ei})$$

and

$$t_i = (\sigma^2_{ai} + \sigma^2_{p(w)i} + \sigma^2_{p(a)}) / (\sigma^2_{ai} + \sigma^2_{p(w)i} + \sigma^2_{p(a)} + \sigma^2_{ei})$$

where σ^2_{ai}, $\sigma^2_{p(w)i}$, and σ^2_{ei} are estimated additive genetic, within lactation permanent environmental and residual error variances at the ith DIM respectively, and $\sigma^2_{p(a)}$ is the across lactation permanent environmental variance which was the same for the entire lactation trajectory.

Genetic correlations between specific DIM (1, 100 and 240 DIM) and the rest of the DIM over the lactation for ECPU and MU were calculated as the ratio between the genetic covariance and the product of the genetic standard deviations at the corresponding days. The same approach was used to calculate genetic correlations between two traits at different DIM.

The potential to change the lactation profile of ECPU and MU traits by selection was assessed using eigenfunctions (Kirkpatrick et al., 1990) derived from the eigenvectors and eigenvalues of the matrix **G** for ECPU and MU.

4.4 Results

4.4.1 Descriptive statistics

Descriptive statistics for the traits are presented in Table 4.1. Daily MY ranged from 0.70 to 40.4 kg/d with an average of 17.5 kg/d. High values of FP (9.77) and CPP (7.66) were observed. The coefficient of variation for FP and CPP was greater than the coefficient of variation for LP. The average ECPU was 24.4% and ranged from 4.0 to 45.5%. The average MU was 25.6 mg/dl (corresponding to an average MUN = 12.03 mg/dl) with a range from 6.08 to 61.7 mg/dl.

Table 4.1. Number of observations (N), mean, standard deviation (SD), coefficient of variation (CV), minimum (Min) and maximum (Max) values of milk traits, liveweight, body condition score, dry matter intake and protein efficiency in grazing dairy cows at Massey University Dairy 1 and Dairy 4.

Trait	N	Mean	SD	CV	Min	Max
Milk yield, kg/day	6,618	17.5	6.51	37	0.70	40.4
Fat yield, kg/day	6,618	0.86	0.27	31	0.10	2.50
Crude protein yield, kg/day	6,618	0.69	0.20	29	0.10	1.40
Lactose yield, kg/day	2,306	0.92	0.34	37	0.20	2.36
Fat percentage, %	6,618	5.07	0.92	18	1.77	9.77
Crude protein percentage, %	6,618	4.05	0.55	14	2.72	7.66
Lactose percentage, %	2,306	5.02	0.17	3	0.34	5.96
Somatic cell score[1]	6,618	5.54	1.65	30	1.00	12.7
Body condition score	7,112	4.38	0.52	12	2.50	6.50
Liveweight, kg	8,149	490	64.6	13	320	77.0
Liveweight change, kg/day	2,306	0.09	0.21	-	-1.19	1.52
Dry matter intake, kg/day	5,229	14.7	2.74	19	5.10	22.1
Milk urea, mg/dl	1,866	25.6	8.31	32	6.08	61.7
ECPU, %	1,866	24.4	6.65	27	4.00	45.5

[1] Somatic cell score = $\log_2$ somatic cell count, ECPU = efficiency of crude protein utilization, BCS = body condition score in 1 - 10 scale.

Positive F × J heterosis effect was observed for both MU and ECPU. The effect of parity was positive for MU where the lowest MU was reported in first lactation cows while the effect was not different from zero for ECPU.

4.4.2 Variances of efficiency of crude protein utilization and milk urea

Genetic, permanent environmental and residual variances for ECPU and MU estimated from 1 DIM to 240 DIM are shown in Figure 4.1. Genetic variances were higher at the beginning and at the end of lactation for both ECPU and MU traits, and lowest around 60 DIM for ECPU and 100 DIM for MU. Permanent environmental variances were lower than the genetic variances throughout the lactation for ECPU and MU. However, permanent environmental variances were higher than genetic variances around 60 DIM for MU where the genetic variance was the lowest. Residual variances were the highest among all the variances for both ECPU and MU throughout the lactation where estimates were a little below the genetic variances at the end of lactation. Stepwise appearance of the residual variances in both traits was due to the assumed heterogeneity which was classified into five stages of the lactation.

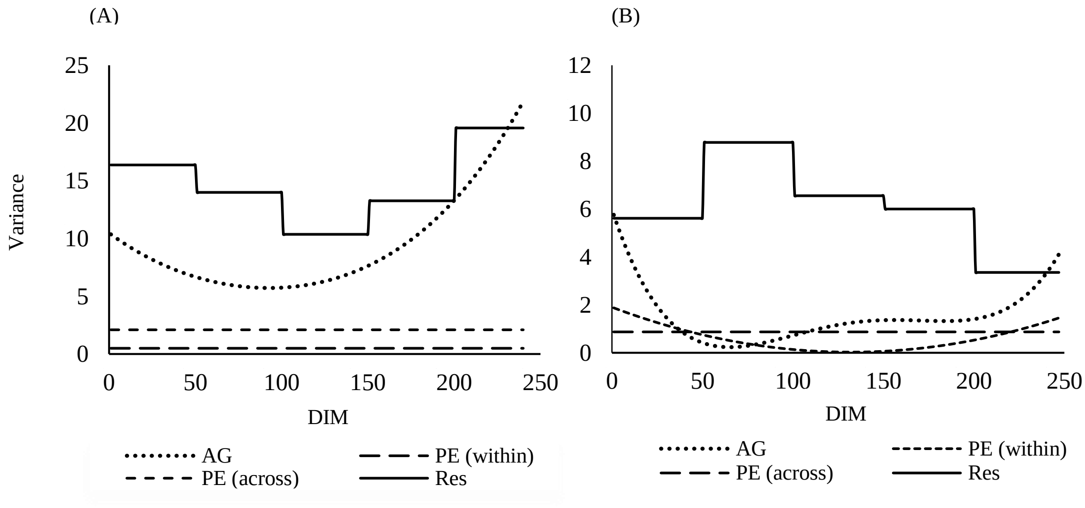

Figure 4.1. Additive genetic (AG), within lactation permanent environmental (PE(within)), across lactation permanent environmental (PE(across)) and residual (res) variances for efficiency for crude protein utilization (A) and milk urea (B) over days in milk (DIM) in grazing dairy cows at Massey University Dairy 1 and Dairy 4.

4.4.3 Heritability and repeatability estimates

The h^2 and t estimates of all the traits varied over the lactation. Point estimates of specific stages of the lactation are included in Table 4.2. Curves drawn for h^2 of each trait by the day of lactation are shown in Figure 4.2. Dry matter intake, LWT and CPP were among the highly heritable traits whereas SCS, LWTc and ECPU showed the lowest. Highly heritable traits were also highly repeatable however, t for LY, FP, CPP, LP, SCS and BCS were also high. Permanent environmental effects account for a higher variation in SCS than genetic effects throughout the lactation demonstrating greater values for t compared to h^2. The effect of genetics was higher for CPP and LWTc compared to effects of permanent environmental effects, therefore, the difference between t and h^2 became less. However, permanent environmental effects accounted for as much of the variation as genetic effects for the other traits.

Table 4.2. Estimates of heritability and repeatability for milk traits, liveweight, body condition score, dry matter intake and protein efficiency in grazing dairy cows at Massey University Dairy 1 and Dairy 4

DIM	MY	FY	CPY	LY	FP	CPP	LP	SCS	BCS	LWT	LWTc	DMI	MU	ECPU
Heritability														
1	0.27	0.21	0.18	0.55	0.37	0.62	0.42	0.17	0.35	0.60	0.39	0.73	0.35	0.41
60	0.22	0.21	0.17	0.41	0.37	0.58	0.21	0.17	0.45	0.65	0.12	0.56	0.27	0.02
120	0.28	0.21	0.20	0.39	0.35	0.56	0.26	0.15	0.54	0.75	0.15	0.60	0.32	0.12
180	0.29	0.19	0.21	0.33	0.36	0.61	0.37	0.11	0.56	0.73	0.06	0.52	0.39	0.16
240	0.21	0.23	0.21	0.46	0.41	0.67	0.45	0.16	0.46	0.78	0.49	0.36	0.49	0.38
Repeatability														
1	0.47	0.34	0.36	0.64	0.54	0.64	0.67	0.68	0.56	0.70	0.40	0.88	0.44	0.60
60	0.40	0.34	0.33	0.54	0.53	0.60	0.64	0.69	0.63	0.85	0.14	0.87	0.38	0.16
120	0.52	0.35	0.41	0.61	0.50	0.58	0.69	0.73	0.68	0.93	0.18	0.85	0.35	0.23
180	0.54	0.33	0.44	0.64	0.51	0.64	0.78	0.66	0.72	0.90	0.07	0.87	0.49	0.29
240	0.45	0.36	0.42	0.75	0.60	0.70	0.74	0.73	0.73	0.95	0.51	0.75	0.55	0.62

MY = Milk yield, FY = fat yield, CPY = crude protein yield, LY = lactose yield, FP = fat percentage, CPP = crude protein percentage, LP = lactose percentage, SCS = somatic cell score ($\log_2$ somatic cell count), BCS = body condition score, LWT = liveweight, LWTc = liveweight change, DMI = dry matter intake, MU = milk urea, ECPU = efficiency of crude protein utilization.

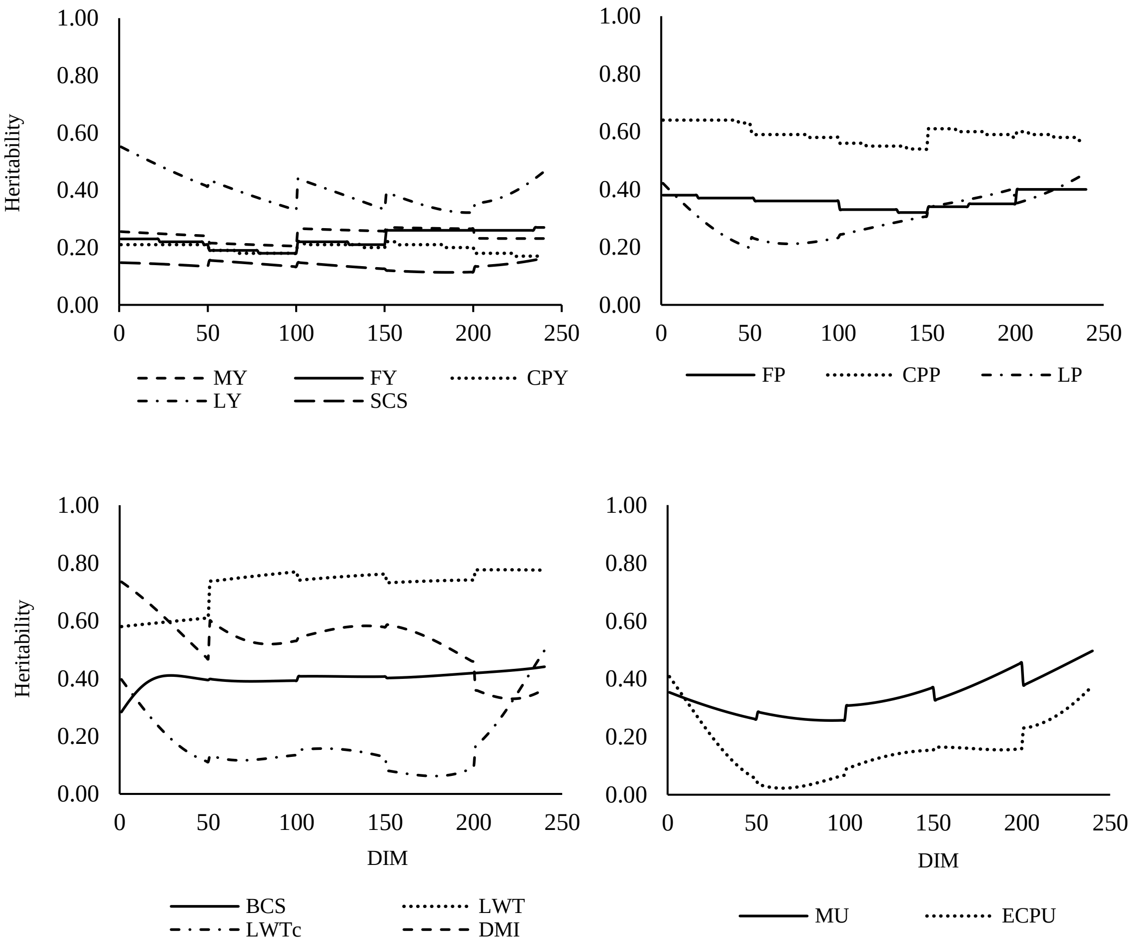

Figure 4.2. Daily heritabilities of yields of milk (MY), fat (FY), crude protein (CPY), lactose (LY), somatic cell score (SCS), percentage of fat (FP), crude protein (CPP), lactose (LP), body condition score (BCS), liveweight (LWT), liveweight change (LWTc) and dry matter intake (DMI), milk urea (MU) and efficiency of crude protein utilization (ECPU) over days in milk (DIM) in grazing dairy cows at Massey University Dairy 1 and Dairy 4.

During the lactation, h^2 of ECPU ranged from low to moderately high (from 0.02 to 0.41) whereas repeatability ranged from moderately low to high (from 0.16 to 0.62). The lowest h^2 and t of ECPU was found at 60 DIM and the highest at the extremes of the lactation profile. MU was moderately heritable (from 0.27 to 0.49) and moderately repeatable (from 0.35 to 0.55) trait and a drop in h^2 and t were observed between 60 and 120 DIM, however, both components increased thereafter with highest estimates at the end of lactation. Heritability of MY (from 0.21 to 0.29), FY (from 0.19 to 0.23), CPY (from 0.17 to 0.21), FP (from 0.35 to 0.41) and CPP (from 0.56 to 0.67) were relatively stable over the lactation and t were moderately high for all those traits and followed the same pattern as h^2 over DIM. Lactose yield (from 0.33 to 0.55) and LP (from 0.21 to 0.45), however showed a fluctuation from moderate to moderately high h^2 and a high t, ranging from 0.54 to 0.75 for LY and from 0.64 to 0.78 for LP. Body condition score was a moderately highly heritable trait ranging from 0.35 to 0.56 and increased throughout the lactation with slight reduction towards the end of lactation however, t increased with DIM. Both h^2 and t of LWT ranged from 0.60 to 0.78 and from 0.70 to 0.95, respectively with the highest estimates at 180 DIM for h^2 and 240 DIM for t. Heritability of LWTc was highly variable (from 0.06 to 0.49) showing the lowest estimates at 180 DIM. Dry matter intake was highly heritable and highly repeatable at the beginning of the lactation and lowest at the end of lactation.

4.4.4 Genetic correlation estimates

4.4.4.1 Within trait genetic correlation

Genetic correlations within a trait between specific DIM are illustrated in Figure 4.3 for ECPU and MU. Genetic correlations of selected DIM with the days of the rest of lactation were variable for ECPU. Strongly positive r_G was found with subsequent DIM whereas, r_G became either weak or negative when the interval between days increased. Correlation of 1 DIM with consecutive days was strongly positive until 30 DIM for ECPU and it rapidly reduced after 70 DIM. Strongest negative r_G (-0.69) of 1 DIM was reported between 125-140 DIM, however, it reached moderately positive r_G by the end of lactation. Similarly, 100 DIM showed highest r_G with adjacent DIM and reduced the correlation with the days at extremes of the lactation. Highly negative r_G was found between 100 DIM and first 5 DIM of the lactation.

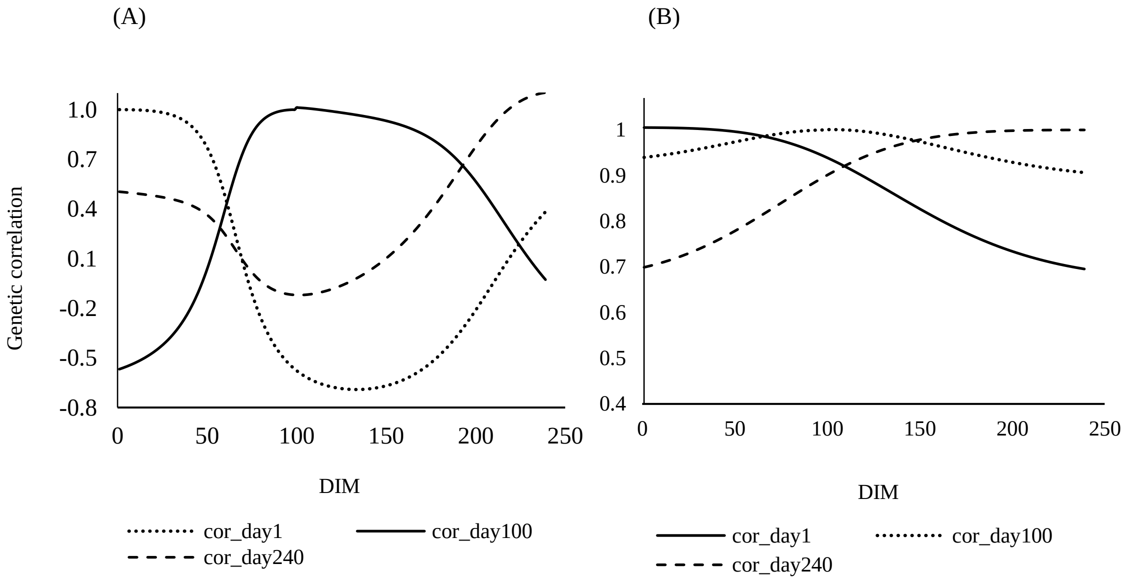

Figure 4.3. Genetic correlations across specific days in milk (DIM) for efficiency of crude protein utilization (A) and milk urea (B) in grazing dairy cows at Massey University Dairy 1 and Dairy 4.

Day 240 also showed a similar r_G to 1 DIM with the rest of the days in the lactation, however, moderately high positive correlations (0.5) was observed with the days of the early lactation (1-20 DIM). All three selected DIM were highly correlated to the rest of the DIM for MU. Genetic correlation of 1 DIM with another DIM of the lactation ranged between 1 to 0.7 where r_G were very high, closer to unity, until 93 DIM. From all the selected DIM, 100 DIM of MU showed the highest r_G with the rest of DIM and was variable only between 1 and 0.91. Comparatively low r_G were recorded between 240 DIM with the days at beginning of the lactation where lowest r_G was observed with 1 DIM (0.69), however, the r_G was very strong between 158 and 240 DIM.

Estimated eigenvalues and associated eigenvectors for ECPU and MU are shown in Table 4.3. The largest eigenvalue explained 76.81% of the total variation of the ECPU while the first two eigenvalues collectively were responsible for more than 97% variation of the trait. The largest eigenvector showed the highest variation at the quadratic part of the lactation curve whereas eigenvectors of second and third eigenvalues showed the highest variation at the constant part of the lactation curve. The largest eigenvalue of MU explained 82% of the variation whereas the first two eigenvalues collectively were responsible for 100% variation. First and second eigenvectors of MU showed the greatest variations at the constant and linear parts of lactation curve, respectively.

Estimated eigenfunctions for ECPU and MU over DIM are shown in Figure 4.4. The first eigenfunction which associated with largest eigenvalue of ECPU was positive at the beginning of the lactation, negative after 65 DIM and turned positive again at the end of lactation. The second eigenfunction of ECPU was slightly curvilinear and remained positive throughout the lactation. The first eigenfunction associated with largest eigenvalue of MU was consistently negative. The second eigenfunction of MU was positive at the beginning and linearly reduced over time, turning negative after 150 DIM.

Table 4.3. Estimates of eigenvalues with propotion of variance explained by each eigenvalue and associated eigenvectors for efficiency of crude protein utilization (ECPU) and milk urea (MU) in grazing dairy cows at Massey University Dairy 1 and Dairy 4

	Eigenvalues				
	ECPU			MU	
	1	2	3	1	2
	3.33	0.89	0.12	9.70	2.09
Variance explained (%)	76.8	20.5	2.7	82	18
	Eigenvectors				
(constant)	-0.04	0.72	0.69	-0.92	0.31
1(linear)	-0.43	0.62	-0.66	-0.31	-0.95
2(quadratic)	0.90	0.32	-0.29	-0.25	0.05

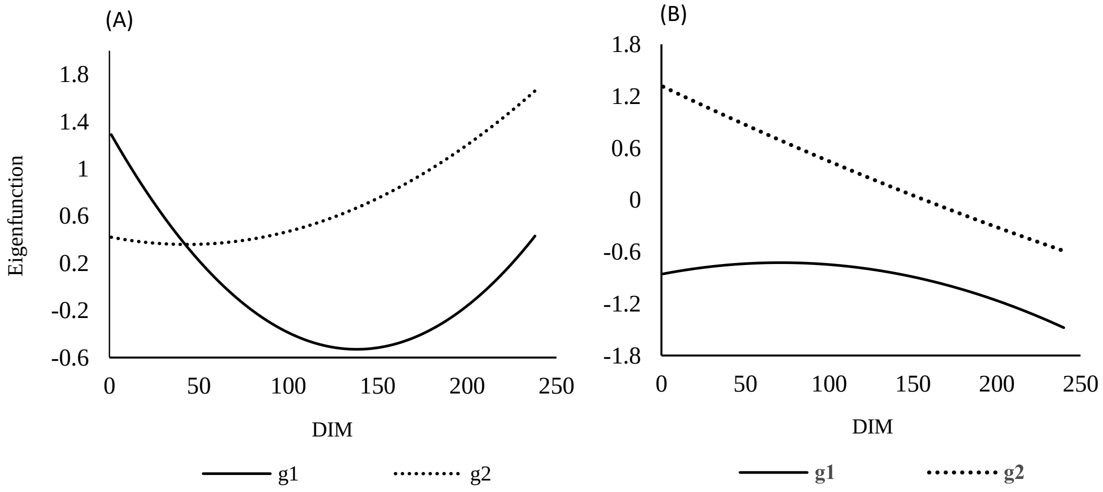

Figure 4.4. First (g1) and second (g2) eigenfunctions of the trajectory of additive genetic variance for efficiency of crude protein utilization (A) and milk urea (B) throughout the lactation in grazing dairy cows at Massey University Dairy 1 and Dairy 4.

4.4.4.2 Genetic correlation with efficiency of crude protein utilization

Genetic correlations between ECPU and other traits at each day of the lactation are presented in Figure 4.5 and point estimates of these r_G were presented in Table 4.4. The r_G between ECPU and all the milk production traits analysed were positive throughout the lactation except the weakly negative r_G recorded with LP and SCS. Strongly positive r_G of ECPU were observed with MY (from 0.01 to 0.79), FY (from 0.53 to 0.80) and PY (from 0.41 to 0.98) from 60 to 180 DIM where the r_G of ECPU with MU became negative. However, the r_G with LY was not different from zero for most of the lactation. FP showed a moderately negative to strong positive r_G (from -0.44 to 0.61) whereas CPP showed a weak negative to strong positive r_G (from -0.09 to 0.66), however, r_G with FP and CPP were mostly positive during the lactation. A negative r_G throughout the lactation was observed with BCS where the r_G was strongest at the 60 DIM. Both LWT and LWTc had negative r_G with ECPU throughout the lactation. The r_G was strong until 60 DIM for LWT and it turned not different form zero after 180 DIM. Dry matter intake had a moderately negative r_G at the beginning of the lactation and it became a comparatively strong and positive by the 60 DIM and turned to a weak r_G thereafter.

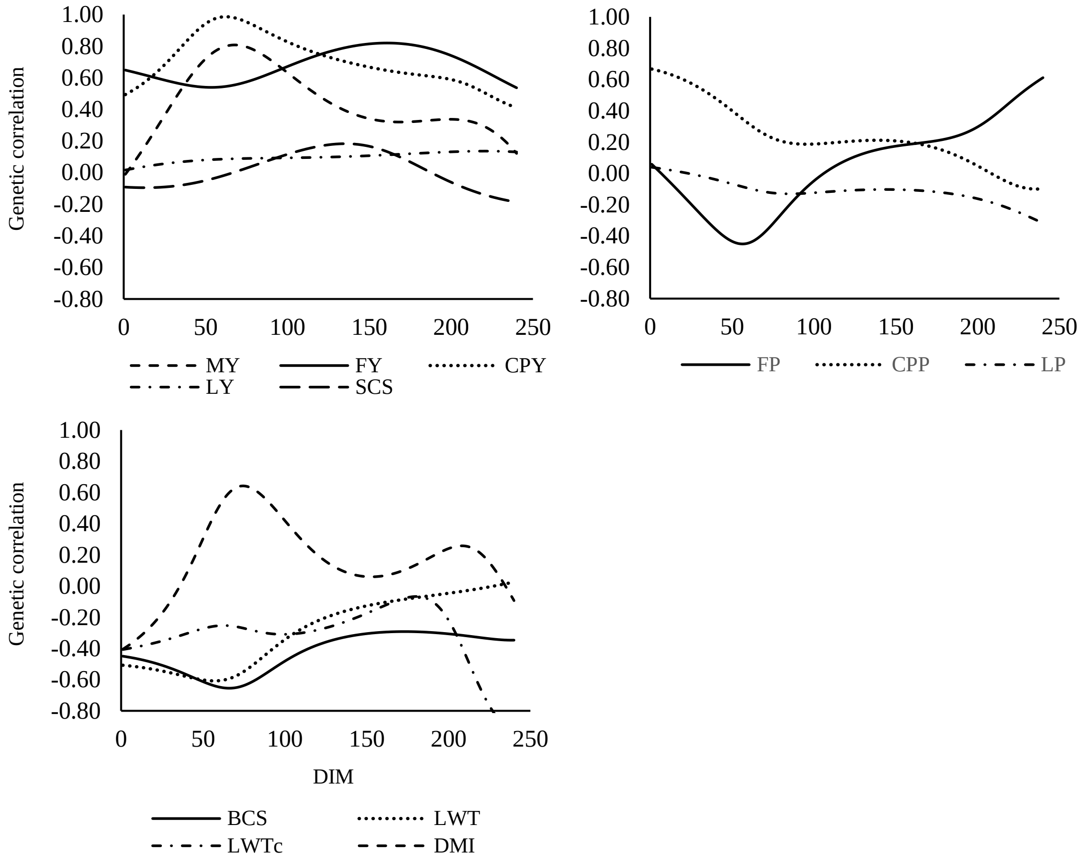

Figure 4.5. Daily genetic correlation between ECPU and yield of milk (MY), fat (FY), crude protein (CPY), lactose (LY), percentage of fat (FP), crude protein (CPP), lactose (LP), somtic cell score (SCS), body condition score (BCS), liveweight (LWT), liveweight change (LWTc) and dry matter intake (DMI) over days in milk (DIM) in grazing dairy cows at Massey University Dairy 1 and Dairy 4.

Table 4.4. Estimates of genetic correlations for efficiency of crude protein utilization and milk urea with milk traits, liveweight, body condition score, and dry matter intake in grazing dairy cows at Massey University Dairy 1 and Dairy 4

Genetic correlation with ECPU

DIM	MY	FY	CPY	LY	FP	CPP	LP	SCS	BCS	LWT	LWTc	DMI
1	0.01	0.64	0.49	0.01	0.05	0.66	0.03	-0.09	-0.44	-0.50	-0.40	-0.40
60	0.79	0.54	0.98	0.08	-0.44	0.31	-0.09	-0.02	-0.64	-0.60	-0.25	0.51
120	0.48	0.74	0.78	0.09	0.08	0.20	-0.10	0.16	-0.37	-0.22	-0.28	0.19
180	0.32	0.80	0.61	0.12	0.21	0.14	-0.12	0.04	-0.29	-0.07	-0.06	0.13
240	0.12	0.53	0.41	0.13	0.61	-0.09	-0.31	-0.18	-0.34	0.02	-0.94	-0.09

Genetic correlation with MU

DIM	MY	FY	CPY	LY	FP	CPP	LP	SCS	BCS	LWT	LWTc	DMI	ECPU
1	0.29	0.06	0.27	-0.002	-0.36	-0.15	-0.25	0.001	-0.16	0.092	-0.006	0.29	0.25
60	0.16	-0.16	-0.04	0.09	-0.23	-0.13	-0.26	-0.05	-0.28	0.190	-0.05	0.28	-0.23
120	0.18	-0.14	0.02	0.16	-0.29	-0.17	-0.26	-0.04	-0.30	0.215	-0.01	0.21	-0.46
180	0.21	0.17	0.16	0.16	-0.27	-0.18	-0.21	-0.04	-0.30	0.183	0.002	0.20	-0.11
240	0.29	0.55	0.64	0.17	-0.28	-0.09	-0.09	-0.0004	-0.07	0.092	-0.003	0.08	0.81

MY = Milk yield, FY = fat yield, CPY = crude protein yield, LY = lactose yield, FP = fat percentage, CPP = crude protein percentage, LP = lactose percentage, SCS = somatic cell score ($\log_2$ somatic cell count), BCS = body condition score, LWT = liveweight, LWTc = liveweight change, DMI = dry matter intake, MU = milk urea, ECPU = efficiency of crude protein utilization.

4.4.4.3 Genetic correlation with milk urea

Genetic correlations between MU and other traits in each day of lactation are presented in Figure 4.6 and point estimates of correlations were presented in Table 4.4. The r_G between MU and other traits varied from moderately positive to moderately negative and point estimates of r_G at specific stages of lactation are shown in the Table 4.4.

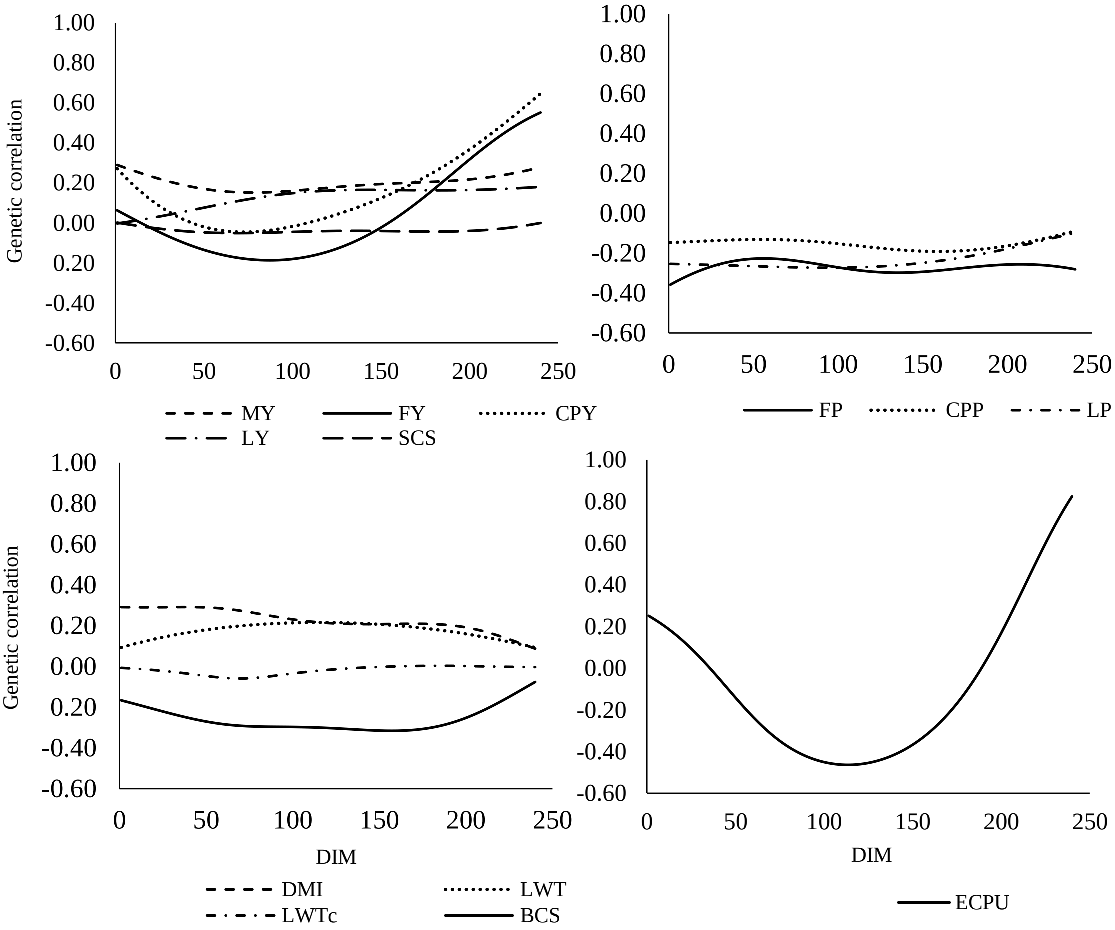

Figure 4.6. Daily genetic correlations between MU and yield of milk (MY), fat (FY), crude protein (CPY), lactose (LY), percentage of fat (FP), crude protein (CPP), lactose (LP), somatic cell score (SCS), body condition score (BCS), liveweight (LWT), liveweight change (LWTc), dry matter intake (DMI) and efficiency of crude protein utilization (ECPU) over days in milk in grazing dairy cows at Massey University Dairy 1 and Dairy 4.

The r_G between MU and ECPU was moderately positive at the beginning and highly positive at end of the lactation, however, the r_G became moderately negative (-0.46) by mid lactation. Genetic correlations between MU and MY were positive throughout the lactation and ranged from 0.16 to 0.29 with fewer fluctuations within the lactation. Genetic correlations with FY (from -0.14 to 0.55), CPY (from -0.04 to 0.64) and LY (from 0.00 to 0.17) were low, except for moderate to high r_G found at the end of lactation for FY and CPY. All three yield traits fluctuated from negative to positive whereas trends of fluctuation of FY and CPY were similar to MY. Negative r_G throughout the lactation were observed with percentages of fat (from -0.23 to -0.36), crude protein (from -0.09 to -0.18) and lactose (from -0.09 to -0.26) and were rather constant over the lactation. Genetic correlations with SCS were not different from zero. BCS showed a negative r_G throughout, however, the r_G became moderately negative from 60 DIM to 180 DIM. A moderate positive r_G with LWT was found in this study, however, r_G with LWTc were not different from zero. A positive, constant r_G (from 0.20 to 0.29) with DMI was recorded until 240 DIM and thereafter r_G was not different from zero.

4.5 Discussion

4.5.1 Descriptive statistics

The definition of ECPU on milking cows has varied among published studies. Zamani et al. (2011) estimated protein utilization efficiency similarly to this study and the average protein utilization efficiency in their study was 27% which was slightly higher than the average ECPU (24.4%) observed in the current study. The higher average protein utilization efficiency recorded in Zamani et al. (2011) could be attributed to the feed type offered to milking cows. In their study, cows were fed total mixed rations (TMR) which were balanced for CP and energy therefore, a higher ECPU was expected. Nitrogen utilization efficiency (Milk N/N intake) reported for Finnish dairy cow in Huhtanen et al. (2008) ranged from 16.4 to 40.2% and the average efficiency of their study was 27.7%. The range reported in Huhtanen et al. (2008) was within the range (4.0 to 45.5%) of ECPU reported in this study.

The range of MU concentration (from 6.08 to 61.7 mg/dl) found in the current study was within the range (1.20 to 78.6 mg/dl) previously reported by Godden et al. (2001) in Ontario Holstein dairy cows. Mean MU concentrations reported by Rzewuska and Strabel (2013) ranged from 22.6 to 23.03 mg/dl over three parities of Polish Holstein-Friesians and a range of 24.66 to 25.57 mg/dl was reported by Bastin et al. (2009), which were comparable with the mean MU concentration (25.6 mg/dl) observed in the current study. However, the average MU concentration (30.43 mg/dl) observed in a recent New Zealand study (Beatson et al., 2019) in mixed-breed herds of dairy cows was a little higher than the average concentration (25.6 mg/dl) in the current study. The MU concentrations were measured using IR spectroscopy in all of the above studies.

The positive first cross F × J heterosis effects on MU and ECPU were previously reported by Lopez-Villalobos et al. (2018). The positive effect of parity on MU might attribute to higher DMI of mature cows hence higher CPI due to greater milk production of mature cows compared to younger cows (Lembeye et al., 2016a).

4.5.2 Variances of efficiency of crude protein utilization and milk urea

All the traits except SCS, CPP and LWTc showed an equal contribution from both genetic and permanent environmental variances in determining the total variances of the traits. The contribution of permanent environmental variance for SCS was high whereas it was negligible for CPP and LWTc.

Estimates of additive genetic variances for daily MU were lower at mid lactation and higher at the extremes of the lactation. Similar observations have been reported by Rzewuska and Strabel (2013) reporting lowest variance at mid lactation, however, Miglior et al. (2007) and Bastin et al. (2009) observed the lowest variability at early lactation. In all three studies the genetic variances were greater than permanent environmental variances and the highest variances were observed from residual variance by Bastin et al. (2009) and Rzewuska and Strabel (2013). Residual variance is an indication of the degree of model fit to the data, higher residual variances would be a consequence of measurement errors as well as all the other effects that were not included in the model due to unavailability of information (Rzewuska and Strabel, 2013). To our knowledge, there are no information available on the variability of daily variance components for ECPU in the literature.

4.5.3 Heritability and repeatability estimates

Heritability estimates for daily ECPU ranged from 0.02 to 0.41 in the current study with higher estimates occurring towards the beginning and end of lactation. Hayes and Ageeb (2002) also reported a similar variability where h^2 was low at 90 DIM (0.10) and comparatively high at 305 DIM (0.31). However, the accuracy of estimates at the extremes of the lactation could be low due to few number of records at peripheries of the lactation therefore, these results should be used with caution in selection. Although the point estimates of h^2 for ECPU showed high values at the extremes of the lactation, the trait was lowly heritable for much of the lactation and comparable with the overall h^2 estimated for the entire lactation by Zamani et al. (2011) (0.07) and Li et al. (1998) (0.13). Van Arendonk et al. (1991) observed a much higher overall estimate of 0.37 for ECPU in lactating dairy heifers in the Netherlands. In the study of Van Arendonk, they estimated gross feed efficiency as the ratio between energy intake and fat and protein corrected milk, whereas in other studies it was estimated as the ratio between milk protein and intake protein. However, the estimated h^2 of ECPU in indoor cows fed with total mixed rations should be considered with caution when comparing with estimates obtained from cows in pastoral systems like in this study. There were no studies in the literature reporting day-to-day variability of h^2 in ECPU.

The current study reported higher h^2 estimates of MU at the beginning and end of lactation and lowest at 60 DIM. A similar shape of the lactation curve was reported by Rzewuska and Strabel (2013) for Polish Holstein-Friesians, however, they observed the lowest h^2 by 194 DIM. The estimated h^2 in this study were within the range (0.13-0.44) reported by previous studies (Wood et al., 2003; Stoop et al., 2007;

Bastin et al., 2009; Rzewuska and Strabel; 2013 Beatson et al., 2019). Repeatability estimates in the current study were within the reported range from 0.22 (Bastin et al., 2009) to 0.43 (Stoop et al., 2007). The inconsistencies among studies could be attributed to the method used for measuring MU concentration as Mitchell et al. (2005) observed differences in h^2 and t estimated using IR spectroscopy compared to wet chemistry which were referred to as genetically different traits. Variability could also be attributed to various other effects such as animal effects, model used and definition of h^2 (where the variance of HTD may have been included in the denominator).

Estimates of h^2 for yield and percentage traits (MY, FY, CPY, LY, FP, CPP, LP) obtained in this study were similar to estimates reported in New Zealand by Ahlborn and Dempfle (1992), Lopez-Villalobos et al. (2014), and Sneddon et al. (2015). Estimates of h^2 for LWT were similar to estimates reported in New Zealand (Spelman and Garrick, 1997) and Ireland (Berry et al., 2002; 2003). Estimates of heritability for DMI were comparable to the estimates reported by Veerkamp and Brotherstone (1997) in the United Kingdom.

4.5.4 Genetic correlation estimates

4.5.4.1 Within trait genetic correlation

Results of the current study indicate within trait r_G of ECPU were either low or strongly negative between different DIM. Therefore, selection for ECPU should be based on records from the complete lactation, otherwise selection based on records of one phase of lactation might negatively affect another phase. There are no previous studies reporting day-to-day variability of within lactation genetic correlations for ECPU.

The total variance explained by first and second eigenvalues was nearly 97% for ECPU and this means that eigenvectors associated with these two eigenvalues are responsible for the majority of the variance. The eigenfunction associated with largest eigenvalue altered its sign across DIM indicating weak r_G of ECPU at different phases of lactation and the potential to genetically improve the lactation profile for ECPU in these cows by changing the shape of the lactation curve.

The largest eigenvalue and second largest eigenvalue showed greatest variations in the quadratic and constant effect of lactation for ECPU, respectively. This means that two independent components related to ECPU are acting independently on different phases of lactation and might be associated with different genes or two different parts of the genome. The dominant eigenvalue demonstrated the greatest variation in the quadratic effect for ECPU indicating a better opportunity to improve the ECPU at the quadratic part of the lactation curve. The shape of the lactation curve of ECPU is curvilinear for New Zealand dairy cows with greater protein efficiency in early lactation (Correa-Luna, 2019), it gradually reduces towards mid-lactation with the lowest efficiency at the end of lactation profile. Lower ECPU at the end of lactation along with the higher variation of quadratic part of lactation in the eigenfunction is associated with the largest eigenvector suggesting that there is potential to improve the ECPU at the end of lactation. Selecting cows for different shapes of the lactation curve gives an opportunity for New Zealand farmers to obtain optimum farm profit by synchronizing demand for DMI for milk production of cows with the pasture growth pattern of the region. There are differences in average pattern of pasture growth due typical regional climates of New Zealand where depressed pasture yields (kg DM/ha/day) of North Island was observed in autumn with higher yields in spring while pasture growth is less liable to affect by the climate in Southland region (Baars et al., 1990). Under these circumstances cows with more peak lactation milk yields are more suitable for North Island whereas persistent cows for milk production are more suitable for South Island. However, improving production at the end of lactation could compromise the reproductive performance of cows due to higher metabolic demand for nutrients of the growing foetus during this period (Lean et al., 1989). Therefore, extra caution should be paid when decision making on selecting cows for different shapes of lactation curves.

Estimates of r_G of MU at different DIM found in this study contradict the trends reported by Rzewuska and Strabel (2013), where they observed relatively low correlation between MU at different DIM. The reason for low r_G in Rzewuska and Strabel (2013) could be attributed to comparatively higher variability in daily σ^2_{ai} variances in their study. The observed high r_G between different DIM in this study suggests that selection for MU could be possible even in the absence of records for complete lactations given the strong correlation between different phases of the lactation profile.

First two eigenvalues explained 100% variation of MU and eigenvectors associated with those two eigenvalues were responsible for all the variation of MU. However, the eigenfunction associated with dominant eigenvalue of MU remained consistently negative throughout lactation demonstrating the greater r_G between different phases of lactation. The largest eigenvalue also showed the highest variation in the constant effect for MU indicating that selection for this factor would improve MU over all the stages of lactation.

4.5.4.2 Genetic correlation with efficiency of crude protein utilization

The current study reported positive r_G between ECPU and MY, FY and PY throughout the lactation profile. The r_G of these traits with ECPU became strongly positive from 60 to 180 DIM and may reflect greater efficiency of the cows during this period. Positive r_G with MY and FY reported in this study were in agreement with the previous findings by Li et al. (1998) and Zamani et al. (2011), respectively. Hayes and Ageeb (2002) have also recorded high positive r_G with MY at 90 DIM (0.68) and 305 DIM (0.79). The positive r_G between ECPU and milk production traits indicate these cows are capable of converting more protein from feed into milk components. The cows may achieve this high efficiency by allocating a greater proportion of consumed nutrients to milk production while reducing the nutrient allocation to maintenance, demonstrating negative r_G with ECPU and BCS (Vallimont et al. 2011), LWT (Persaud et al. 1991), and LWTc.

Mostly positive r_G during the lactation were observed with FP and CPP in this study. However, the negative correlation with FP at the 60 DIM is likely to be a result of a greater demand for consumed feed protein during that period for milk protein production and increased allocation of feed protein for milk protein production rather than milk fat production where fat synthesis does not require a nitrogen source directly. Positive correlations between ECPU and FP and CPP were also reported by Zamani et al. (2011).

The negative correlations of ECPU with BCS, LWT and LWTc traits were understandable. This is because even though cows are provided with energy rich diets, they cannot consume enough feed to sustain the high level of production determined by their genetic merit. Cows in early stages of postpartum do not consume their maximum capacity of dry matter, as a result cows usually receive a lower level of energy than they require (Coppock, 1974; Drackley et al., 2001). A recent study in New Zealand demonstrated the feed offered to cows is not well balanced for energy and CP ratio (Correa-Luna et al., 2018). Under these circumstances, higher yielding cows are genetically driven to mobilize body tissues, hence they lose body reserves to sustain milk production during this period of negative energy balance (Herdt, 2000), consequently reducing LWT and BCS. The r_G of ECPU with LWT (-0.60) and BCS (-0.64) around 60 DIM were highly negative in this study. This indicates that lactating cows become more efficient during the peak lactation period, reducing LWT and BCS to a greater extent. Moreover, r_G between ECPU and MY (0.79), FY (0.54) and PY (0.98) around 60 DIM were strong and positive, suggesting that the cows might utilize energy and protein produced through body tissue mobilization during peak lactation for production of milk and milk solids.

The r_G between ECPU and DMI increased from -0.40 to 0.60 during 0 to 60 DIM. This strong shift is genetically driven by the increase in production of milk, fat and protein accompanied by increased DMI besides protein originated from the body tissue mobilization. The cows at day 60 of lactation had the highest ECPU with the highest DMI. Mostly positive correlations between ECPU and DMI during the lactation found in this study, however, contradict the findings of Zamani et al. (2011) of -0.716 and Li et al. (1998) of -0.67 where they reported a highly negative r_G. In Zamani et al. (2011) the data were originated from cows fed with TMR whereas in Li et al. (1998) data were originated from cows available at five farms, where four farms out of five were managed as half confined systems (grazing+supplement concentrate and roughage indoor) and the other farm as a confined system (no grazing). As TMR are balanced for protein and energy, the nutrient requirements of the cows can be fulfilled by consuming a lesser ration of diet in order to achieve the production driven by their genetic merit. The feed that these cows consume over the requirement would not contribute in milk protein production and eventually reducing ECPU with additional feed intake. However, the cows under pastoral systems need to consume a higher ration of diets compared to the cows fed with TMR in order to fulfil their requirements. Therefore, cows under pastoral systems maximize their protein intake by increasing feed intake, subsequently, this would result in higher ECPU.

4.5.4.3 Genetic correlation with milk urea

The r_G between MU and ECPU was moderately positive at the beginning of lactation and it became moderately negative in peak lactation. The r_G between MU and ECPU remained negative for a long period of DIM and became moderately positive in late lactation with the highest estimate at the end of lactation. This negative r_G for most of lactation indicates cows that produce higher concentrations of MU are less efficient in protein utilization. This is because less efficient cows lose a greater amount of consumed feed protein through excretion as urea in urine and therefore, a lesser amount of consumed protein would be converted into milk protein. The negative r_G between MU and ECPU enables selection of cows with higher MY due to greater positive r_G with CPY (Lopez-Villalobos et al., 2018) but reduced MU concentration. However, ECPU and concentrations of MU in lactating dairy cows in New Zealand are variable over the lactation and the lactation curve of the ECPU shows the lowest efficiency of cows towards the end of lactation where the highest production of MU is also reported (Correa-Luna et al., 2018), demonstrating the negative phenotypic correlation between MU and ECPU. Another possible reason for the negative r_G between MU and ECPU for much of the lactation is the cows' ability to utilize protein and energy from body reserves rather than capturing them from feed. Feed digestion requires energy and the protein digestion process in the rumen is coupled with ammonia production as a by-product. However, when cows utilize nutrients and energy directly from body-reserves they do not need to pay the extra costs of energy for feed digestion and prevent ammonia production in rumen. Therefore, cows use of body reserves for production purposes benefit from saving of energy and protein thereby improve ECPU through reduced CPI and reduce the production of MU.

However, the high positive correlation between MU and ECPU at the end of the lactation could be an artefact of the mathematical properties of polynomial random regression due to the lesser number of herd-test records towards the end of lactations reflecting that the estimates should be used with caution in selection.

Although the correlations found with milk yield traits (FY, PY and LY) were weakly positive, fairly constant and moderately high negative genetic correlations were observed with milk percentage traits in this study. The negative relationship with FP (-0.15), PP (-0.20) and LP (-0.15) found in Beatson et al. (2019) were comparable with estimates reported in this study. However, the estimated r_G with LP by Stoop et al., 2007 (-0.09), Miglior et al., 2007 (-0.041) and Hossein-Zadeh and Ardalan, 2011 (-0.01) were lower than the estimates in the current study. The comparable estimates of r_G between MU and milk percentage traits of this study and Beatson et al. (2018) is likely because both studies used New Zealand grazing cows whereas estimates of other studies originated from cows under indoor conditions.

The consistent positive r_G between MU and MY over the lactation found in the current study was comparable with estimates reported by Stoop et al. (2007) in indoor Dutch cows and Hossein-Zadeh and Ardalan (2011) in indoor Iranian cows. However, r_G was higher than the observed correlation by Beatson et al. (2019) (0.19) in New Zealand grazing cows. Estimates of r_G correlations of MU with FY (0.41), PY (0.38) and LY (0.24) by Stoop et al. (2007) were moderately positive and higher than the estimates of this study. However, the estimates found in the current study were higher than the estimates of Beatson et al. (2019) for FY (0.04) and PY (0.08) of mixed-breed herds of dairy cows in New Zealand.

The positive r_G of MU with yield traits could be partly explained by higher feeding of crude protein and energy associated with higher DMI and studies have shown that higher milk production is associated with higher protein and energy intake (Ferguson and Chalupa, 1989). Conversely, cows may increase milk production by increasing the efficiency of utilization of absorbed nutrients (Chalupa, 1984). The negative r_G correlation between MU with FY around 60 DIM (r_G = -0.16) found in this study indicate that cows producing more milk fat had lower MU contents during peak lactation.

The estimates of r_G of MU with FP, CPP and LP were moderately negative throughout lactation, indicating that cows producing milk with higher FP, CPP and LP would produce milk with lower MU content. High concentrations of MU indicate those cows also produce greater amounts of ammonia in the rumen during protein digestion. Ammonia is toxic to the cow and it is detoxified in the liver by converting it to urea which is eliminated from the body through urine. This is an energy (Vandehaar, 1998) and protein (Van Soest, 2018) demanding process, therefore, a cow with a greater amount of ammonia production tends to waste more protein and energy on detoxification than cows with lower production of ammonia. Consequently, the deficiency of protein and energy within the cow will limit production of milk components. The reported negative r_G between MU and percentage traits (FP, CPP and LP) suggest that genetic selection for higher percentage traits would reduce MU hence mitigate the environmental impact through less ammonia loss.

Fairly low positive r_G from the early to mid-lactation and moderately positive r_G towards the end of lactation between yield traits (FY, PY and LY) and MU suggest that genetic selection for low MU would result in only a slight reduction in yields from early to mid-lactation however, the effect on yield traits would be comparatively higher towards the end of lactation.

The current study showed no correlation of MU with SCS and this finding agrees with the reported averaged across lactations r_G between these traits (-0.04) by Hossein-Zadeh and Ardalan (2011). This finding indicates that the selection for mastitis resistance based on SCS would not affect the concentration of MU.

When cows expel more consumed protein as MU, the amount of protein allocated into muscle growth and BCS would be limited, therefore, the positive r_G found between MU and LWT in this study was unexpected. Oltner et al. (1985) reported a negative relationship between LWT and MU. The moderately low and positive correlation with LWT reported in this study is likely due to high involuntary CPI associated with high DMI. However, BCS is negatively correlated with MU concentration, possibly affected by the restricted phenotypic correlation between LWT and BCS (Morris et al., 2002).

The positive r_G between MU and DMI was likely due to the higher involuntary protein intake enhanced by DMI. Higher dietary CP relative to less energy content in low quality pastures are coupled with greater production of MU (Kebreab et al., 2002). On the other hand, the excess of amino acids supplied to the body tissues can result in deamination of unused amino acids and are eventually converted to urea (Lapierre et al., 2005) which causes elevated MU levels. Relatively higher r_G between MU and DMI from 1 DIM to 60 DIM was estimated immediately after calving where quite low DMI is usually observed as cows mainly utilize mobilized body tissues as the source of protein and energy for several weeks post-partum. However, conversion of amino acids originated from body tissue mobilization into glucose in the liver is also coupled with production of urea (Kuhla et al., 2011) and this could be the reason for positive r_G between MU and DMI in early lactation.

4.6 Conclusions

The moderate estimates of h^2 for daily ECPU and MU suggest these traits can be genetically manipulated by direct selection. In addition, the eigenfunction analysis for ECPU suggests it can be improved by altering the lactation profile at late lactation.

Improving ECPU is beneficial in many aspects, however, the trait cannot be measured directly at herd-testing and estimating ECPU is costly and labour intensive. Moderate to strong positive r_G between ECPU and MY, FY and PY observed during different stages over the lactation in the current study suggest that selection for milk, fat and protein yields would improve ECPU of lactating dairy cows. If ECPU is genetically improved then the concentration of MU will be reduced, mostly in peak lactation given that the r_G between MU and ECPU is strongly negative during this period. Unlike ECPU, MU can be measured in regular herd-testing and can be incorporated into a selection index for reducing MU. However, the low positive r_G from early to mid-lactation and moderately positive r_G towards the end of lactation between MU and MY, FY and CPY warn that selection for low MU could result in reduced milk production to different extents depending on the stages of lactation. Inauspicious r_G between MU and milk production creates a conflict of selection for low MU and improved ECPU.

Further studies are needed to assess the relationship of MU and ECPU traits with fertility traits in order to evaluate the usefulness of these traits into a selection index for New Zealand dairy cows.

Chapter 5

Identification of genomic regions associated with concentrations of milk fat, protein, milk urea, or efficiency of crude protein utilization in grazing mixed-breed dairy cows

As genetic variances for environmental traits were detected, genes associated with those traits were identified. This paper was published as

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5.1 Abstract

The objective of the current study was to identify genomic regions associated with mid-infrared predictions of milk fat percentage (FP), crude protein percentage (CPP), milk urea concentration (MU) or efficiency of crude protein utilisation (ECPU: ratio between crude protein yield in milk and dietary crude protein intake) using grazing mixed breed dairy cows in New Zealand. Phenotypes from 634 Holstein-Friesian, Jersey or crossbred cows were obtained from two herds at Massey University. A subset of 490 of these cows was genotyped using Bovine Illumina 50K SNP-chips. Two genome-wide association studies (GWAS) approaches were used, a single-locus model fitted to data from 490 cows and a single-step Bayes C model fitted to data from 634 cows. The single-locus analysis was performed with the Efficient Mixed-Model Association eXpedited model as implemented in the SVS package. Single nucleotide polymorphisms (SNPs) with genome-wide association P-values $\leq 1.11\times10^{-6}$ were considered as putative quantitative trait loci (QTL). The Bayes C analysis was performed with the JWAS package 0.7.3 (Cheng et al., 2018) and 1-Mb genomic windows containing SNPs that explained > 0.37% of the genetic variance were considered as putative QTL. Candidate genes within 100 kb from the identified SNPs in single-locus GWAS or the 1-Mb windows were identified using gene ontology, as implemented in the Ensemble Genome Browser. The genes detected in association with FP (MGST1, DGAT1, MROH1, RHPN1, GPAT4, and ACOX3) and CPP (DGAT1, CSN1S1, GOSR2, HERC6, and IGF1R) were identified as candidates. Gene ontology revealed six novel candidate genes (GMDS, E2F7, SIAH1, SLC24A4, LGMN, and ASS1) significantly associated with MU that function in protein catabolism, urea cycle, ion transportation and N excretion. One novel candidate gene was in association with ECPU (MAP3K1) that functions in

post-transcriptional modification of proteins. The findings should be validated using a larger population of New Zealand grazing dairy cows.

Key words: genome-wide association studies; candidate gene; single nucleotide polymorphism; efficiency of crude protein utilization; milk urea concentration; dairy cows

5.2 Introduction

Milk fat and protein play a vital role in New Zealand’s milk payment scheme (Sneddon et al., 2013). Protein utilization efficiency, measured as the proportion of dietary protein that is converted to protein in milk or protein in muscles, is influenced by gut microbes and highly sensitive to the ratio of protein and fermentable energy in the diet (Baker et al., 1995). If the diet is high in protein and deficient in energy, such as most fresh pasture, surplus dietary protein results in the production of ammonia in the rumen and this is then converted to urea in the liver after which it circulates in the blood supply. Urea in the blood is then transported into the kidney and excreted in urine or diffuses into the mammary gland as a component of milk. The concentration of urea in urine is phenotypically highly correlated to the milk urea (MU) (Jonker et al., 1998) and is an indicator of protein utilization efficiency (Baker et al., 1995). The latter can be defined in several ways (Zamani et al., 2011); 1) efficiency of crude protein utilisation (ECPU): the ratio of milk crude protein yield (CPY) to dietary crude protein intake (CPI); 2) the crude protein balance (CPB): the difference between CPI and CPY, or 3) the residual protein intake (RPI): the difference between actual CPI and predicted CPI. The most widely used measure of protein efficiency is ECPU (Li et al., 1998; Huhtanen et al., 2008; Zamani et al., 2011).

The heritabilities in New Zealand dairy cows for percentage of milk fat (from 0.62 to 0.66) and milk crude protein (0.67) (Lembeye et al., 2016b) are moderate to high while MU (0.22) (Beatson et al., 2019) is moderate whereas ECPU (0.11) (Lopez-Villalobos et al., 2018) is low. These estimates indicate that genetic changes for these traits are expected if these traits are placed under selection pressure. Changes in fat (FP) and crude protein (CPP) percentages will directly influence farm profitability if they are not associated with differences in milk yield, whereas changes in MU and ECPU might indirectly affect farm profitability through their impact on animal efficiency and nitrogen emissions.

Genome-wide association studies (GWAS) scan the entire genome of an organism to discover associations between genetic markers and phenotypes. This is a crucial step in understanding the genes associated with the phenotype. There are two major methods of GWAS depending on how the association between the marker and the trait is being tested. Single-locus GWAS is the simplest form of association testing and involves markers being fitted one at a time as fixed effects in a statistical model used to estimate the additive effect of the marker alleles on the trait. The method relies on linkage disequilibrium (LD) between the markers being fitted and the causal mutation to find any evidence of association, unless the marker panel includes all sequence variants and other genomic features that represent the causal mutation. Testing the association with phenotype of multiple markers fitted simultaneously as random effects is the other approach of GWAS. That approach takes advantage of the fact that linear functions of the multiple-markers being tested are in greater LD with the quantitative trait loci (QTL) in comparison to the single-locus GWAS approach and consequently, this increases the power of the experiment. Bayesian regression is one method of testing associations of multiple markers simultaneously with the phenotype.

Many studies have reported candidate genes for FP (Pimentel et al., 2011; Strucken et al., 2012; Cecchinato et al., 2014) and CPP (Pimentel et al., 2011; Schopen et al., 2011; Strucken et al., 2012; Cecchinato et al., 2014) while a few have identified genomic regions associated with MU in dairy cattle fed total mixed rations in indoor circumstances (Bouwman et al., 2010; Strucken et al., 2012; Pegolo et al., 2018). Few GWAS have identified candidate genes for FP or CPP in New Zealand dairy cows (Lehnert et al., 2015; Littlejohn et al., 2016; Burborough et al., 2018). There are no published GWAS studies in New Zealand that have identified candidate genes associated with MU nor any publication of GWAS on dairy cattle protein utilization efficiency, worldwide.

The objective of the current study was to identify genes/QTLs associated with FP, CPP, MU, or ECPU using either a single-locus approach or a multi-locus single-step Bayes C approach.

5.3 Material and methods

5.3.1 Animals and phenotypes

Two herds comprising a total of 634 cows, from Massey University Dairy 1 and Dairy 4 experimental farms, Palmerston North, New Zealand were used for this study. Test-day records from 467 cows milked in 2016-17 and an overlapping group of 451 cows milked in the 2017-18 production seasons were collected. These cows had a minimum of three herd-test records from lactations of not less than 150 days. Both herds comprised mixed-breed Holstein-Friesian (F) and Jersey (J) cows or their crosses (F × J) (Table 5.1).

Table 5.1. Cows from each farm, breed group and production season used for the study

		Production season			
Farm	Breed	Only 2016-17	Only 2017-18	Both Seasons	Total
Dairy 1	Friesian	17	13	49	79
	Jersey	15	18	40	73
	Crosses	31	26	105	162
Dairy 4	Friesian	28	76	23	127
	Jersey	1	2	2	5
	Crosses	91	32	65	188

Only 2016-17 = number of cows found only in season 2016-2017, only 2017-18 = number of cows found only in season 2017-2018, Both seasons = number of cows found in both seasons.

Cows at Dairy 1 are managed in a once-a-day milking system with low levels of supplementary feeding and a low stocking rate (2.1 cows/ha). In contrast, cows in Dairy 4 are milked twice-a-day, with high levels of supplementary feeding and a higher stocking rate (2.8 cows/ha).

Herd-test records on FP and CPP of each cow were collected on a monthly basis and stored in farm databases. Daily CPY was estimated at each month as the product of daily CPP and corresponding daily milk yield. The MU concentration for each cow was predicted by mid-infrared spectral data from additional milk samples collected only for three of the herd-tests per season due to limited funds available. The herd-tests for MU were carried out in September, December, and March to represent early, mid, and late lactations, respectively. Milk samples were measured for MU at MilkTestNZ (Hamilton, NZ) using the CombiFossTM 7 instrument (Foss Electric, Hillerød, Denmark). A daily composite sample of morning and afternoon milking followed by weighting according to morning and afternoon milk yields were used for estimating daily MU when twice a day milking was practiced, whereas the raw sample was used when once a day milking was practiced on the sampling days.

Dietary metabolizable energy (DME) and dietary crude protein percentage (DCPP) were estimated by analysing pasture, crop, and supplementary feed offered to the cows using near-infrared spectroscopy. The total metabolizable energy (ME) requirements of each cow on each day of lactation was calculated as the sum of the estimated ME requirements for maintenance, pregnancy, lactation, and liveweight change, as described by Lopez-Villalobos et al. (2008). Apparent daily dry matter intake was calculated as the estimated daily total ME requirement of each cow divided by total DME offered to the cow on the corresponding day. Daily crude protein intake (CPI) of the cow was determined as DCPP available in any diet offered to the cow multiplied by the estimated daily dry matter intake. Daily ECPU was estimated as the proportion of CPY in relation to estimated CPI. Daily estimates of milk yield, FP and CPP were predicted by modelling the herd-test records of cows using third order orthogonal polynomials for each lactation.

5.3.2 Genotypes and quality control

Phenotypes were collected from a total of 634 cows but only a subset of 490 cows were genotyped because sample collection for genotyping was done after the 2017-18 production season and 114 of the cows milking in 2016-17 had already been culled. DNA was extracted from the ear punch tissue samples which were then genotyped using Bovine Illumina 50K SNP-chips. SNP & Variation Suite (SVS 8.8) was used for initial analysis and quality control (QC) steps. The genotypes recorded in an A/B allele format in Illumina were converted to 0, 1, or 2, depending on the number of B alleles present in each locus. Loci with a call rate ≤ 80% or minor allele frequency ≤ 0.01, as well as animals with a call rate ≤ 80% were excluded from the data set. After these QC steps, a total of 45,062 SNPs were available for association analysis.

5.3.3 Descriptive statistics

Descriptive statistics for milk composition traits (FP, CPP, MU) and ECPU, were performed for 634 cows using the MEANS procedure of SAS package 9.4 (SAS Institute Inc. 2013, Cary, NC, USA).

5.3.4 Parameter estimation

All the 634 cows with phenotypes were used for estimating variance components. Variance components for animal additive genetic variance (σ^2_a), within lactation permanent environmental variance (σ^2_{pw}), across lactation permanent environmental variance (σ^2_{pa}) and residual effects variance (σ^2_e) were estimated by fitting the following repeatability mixed animal model as implemented in the JWAS package:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Zu} + \mathbf{Wp} + \mathbf{Kc} + \mathbf{e} \quad (1)$$

where **y** is the vector of test-day phenotypes, **X** is the design matrix of fixed effects including contemporary group defined by herd-test-date (HTD), parity, days in milk nested within each HTD, regression on proportion of F, and regression on F × J heterosis effects, **b** is the vector of fixed effects, **Z** is the design matrix of random animal additive genetic effects (**u**), **W** is the design matrix of within lactation permanent environmental effects (**p**), **K** is the design matrix of across lactation permanent environmental effects (**c**) and **e** is the vector of random residual errors. It was assumed that the animal additive genetic effects were distributed as $\mathbf{u}\sim N(0, \mathbf{A}\sigma^2_a)$ where **A** is the numerator relationship matrix, $\mathbf{p}\sim N(0, \mathbf{I_1}\sigma^2_{pw})$ where $\mathbf{I_1}$ is an identity matrix of order equals to the number of interactions between cows and seasons; $\mathbf{c}\sim N(0, \mathbf{I_2}\sigma^2_{pa})$ where $\mathbf{I_2}$ is an identity matrix of order equals to the number of cows with records, and $\mathbf{e}\sim N(0, \mathbf{I_3}\sigma^2_e)$ where $\mathbf{I_3}$ is an identity matrix of order equal to the total number of test-day records.

Heritabilities (h^2) and repeatabilities (t) of the investigated traits were calculated using the estimated variance components. The heritability is the proportion of phenotypic variance explained by breeding values and the repeatability is the proportion of phenotypic variance explained by σ^2_a, σ^2_{pw}, and σ^2_{pa} where the phenotypic variance is the sum of σ^2_a, σ^2_{pw}, σ^2_{pa}, and σ^2_e. The estimated variance components, heritabilities, and repeatabilities for milk traits and ECPU are presented in Table 5.2.

Table 5.2. Estimates of variance components, heritabilities and repeatabilities of milk percentage traits, milk urea and efficiency of crude protein utilization using a univariate repeatability animal model in grazing dairy cows at Massey University Dairy 1 and Dairy 4.

Trait	σ^2_a	σ^2_{pw}	σ^2_{pa}	σ^2_e	σ^2_p	h^2	t
FP	0.13	0.00	0.06	0.18	0.37	0.35	0.51
CPP	0.05	0.00	0.00	0.03	0.08	0.62	0.63
MU	6.77	0.68	1.73	15.29	24.47	0.28	0.38
ECPU	0.57	0.00	0.49	26.18	27.25	0.02	0.04

FP = fat percentage, CPP = crude protein percentage, MU = milk urea concentration, ECPU = efficiency of crude protein utilization, σ^2_a = additive genetic variance, σ^2_{pw} = within lactation permanent environmental variance, σ^2_{pa} = across lactation permanent environmental variance, σ^2_e = residual variance, σ^2_p = phenotypic variance ($\sigma^2_a + \sigma^2_{pw} + \sigma^2_{pa} + \sigma^2_e$), h^2 = trait heritability (σ^2_a / σ^2_p), t = repeatability ($[\sigma^2_a + \sigma^2_{pw} + \sigma^2_{pa}] / \sigma^2_p$).

A single-step Bayes C linear mixed model (genomic data), as implemented in the JWAS package, was used to account for variance explained by SNP markers. The model used for estimating individual SNP effects was same as the model (1), other than the inclusion of random SNP effects that were fitted simultaneously using Bayes C priors (model 1). There is a model equation corresponding to genotyped animals and another one for non-genotyped animals. The following model equation was fitted for genotyped animals to estimate marker effects:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Zu} + \mathbf{Wp} + \mathbf{Kc} + \mathbf{Ms} + \mathbf{e} \qquad (2)$$

where **M** is the design matrix of genotypes corresponding to the number of genotyped loci (each coded as 0,1,2), and **s** is a vector of marker effects. It was assumed that the animal additive genetic effects are distributed as $\mathbf{u} \sim N(0, \mathbf{A}\sigma^2_g)$ where **A** is the numerator relationship matrix for polygenic effects and σ^2_g is the additive-genetic variance not explained by markers, representing some fraction (1-d) of σ^2_a from the pedigree-based best linear unbiased prediction model.

The priors for marker effects have identical and independent mixture distributions, where each has a point mass at zero with probability π (marker effect is null) and a univariate-normal distribution with probability 1-π and a null mean and variance σ^2_s such that the scalar variance of the vector of genomic breeding values, i.e. var(**Ms**) reflects the additive-genetic variance explained by markers (i.e. $d\sigma^2_a$). The mixed model used to estimate marker effects for the non-genotyped individuals was similar to model 2, except, **M** contains the imputed genotypes of non-genotyped individuals in this analysis instead of actual genotypes for genotyped individuals. Since this model deals with imputed genotypes, the model equation includes another matrix, **H** and vector **m,** where **H** is the incidence matrix corresponding to the imputation residuals and **m** is a vector of imputation residual effects.

Pre-estimated σ^2_a, σ^2_{pw}, σ^2_{pa} and σ^2_e using a repeatability mixed animal model are presented in Table 5.4 and were considered as prior variances in estimating marker effects. All the SNPs with non-zero effects in Bayes C have a common variance (Kizilkaya et al., 2010) denoted σ^2_s whose prior was estimated as described by Fernando and Garrick (2013):

$$\sigma^2_s = \frac{d\sigma^2_a}{k\,(1-\pi)\,2pq}$$

where σ^2_a is the additive genetic variance of the trait, d is the proportion of additive genetic variance assumed to be explained by markers, k is the number of markers in the SNP-chip, π is the probability that the markers have null effects, 2pq is the average heterozygosity across all genotyped loci.

The assumed proportion of SNPs with zero effect on the traits (π) was 0.997 (Hanna et al., 2014), corresponding to about 135 SNPs (1 - 0.997 = 0.003 of 45,062 SNPs) fitted per iteration. The value for π was taken to be 0.997 to fit fewer SNPs in the model than number of animals (Ros-Freixedes et al., 2014) and results in less shrinkage of marker effects towards zero than would occur when more markers are fitted (Fernando and Garrick, 2013). Estimating π requires a larger dataset but single-step Bayesian methods are less sensitive to assumed π in comparison to Bayesian methods that include only genotyped animals (Lee et al., 2017). The SNP genotypes with sampled non-zero effects on the traits were fitted in the model along with phenotypes and pedigrees in a single-step in any particular sample of the Markov chain.

In Bayesian methods, prior assumptions about parameters together with the data are used to estimate posterior distributions in order to make inference about the unknown parameters (Sorensen and Gianola, 2002). For this, information from samples is usually obtained using the Markov chain Monte Carlo (MCMC) technique. In this study, the chain included 50,000 MCMC iterations and marker effects from each 50th iteration was used to estimate genomic merit of all animals. The genomic merit was sampled in each MCMC iteration by multiplying the matrix $\mathbf{M}$ by the corresponding sample of marker effects $\mathbf{s}$ for that MCMC iteration. The posterior mean of variances (σ^2_m) of the sampled genomic breeding value for each iteration across all cows was used to estimate the genomic heritability (s^2) which is the proportion of phenotypic variance explained by the additive effects of the SNPs (i.e. σ^2_m is $d\sigma^2_a$). After including SNP effects in the model, heritabilities and repeatabilities of the traits were estimated by replacing σ^2_a in the numerator and the denominator of the ratio with $\sigma^2_g + \sigma^2_m$. A complete pedigree was included for three generations in all the models to infer covariances between animals for the additional polygenic effect and imputation residual effects.

5.3.5 GWAS

5.3.5.1 Single-locus method

Cows (n = 490) with both phenotypes and genotypes were used for single-locus GWAS. Residuals were produced for each test-day record and trait, separately, using a mixed model in ASReml package 4.1 (Gilmour et al., 2015). The model included fixed effects of HTD, parity, days in milk nested within each HTD, regression on proportion of F, and regression on F × J heterosis. Random effects included within and across lactation permanent environmental effects, and residual error. The evidence of association of the adjusted phenotypes with the genotypes was calculated in the SVS software using an additive, single locus, mixed model called Efficient Mixed-Model Association eXpedited (EMMAX), where an association was tested for each SNP fitted separately as a fixed effect. A genomic relationship matrix was computed in the form of identity-by-state using the same genotypes analysed and fitted as a random effect in the model to account for structure due to relatedness among animals.

The estimated associations were presented as Manhattan plots in which $-\log_{10}$(P-values) were plotted against the genomic locations of the markers using the annotation for bovine assembly UMD3.1 in SVS software. In the single-locus method the marker's associations are tested separately, therefore, there is a risk of detecting false positive results associated with multiple testing. In order to overcome the problem of multiple testing, marker associations were corrected for multiple testing using Bonferroni corrections (Bland and Altman, 1995). The Bonferroni correction is known to be very conservative (Johnson et al., 2010), therefore, a P-value threshold for suggestive association was also computed. The suggestive association is less conservative and implies one false positive result across the genome (Lander and Kruglyak, 1995). The genome-wide threshold uses a nominal P-value of 0.05 (0.05/number of SNPs), and the suggestive threshold uses a nominal P-value of one (1/number of SNPs). The Bonferroni corrected P-value threshold for genome-wide significant levels was 1.11×10^{-6} (0.05/45,062) which corresponded to 5.95 on a -log10(P-value) scale. The P-value threshold for suggestive associations was 2.22×10^{-5} (1/45,062) which corresponded to a -log10(P-value) of 4.65. The SNPs that surpass the Bonferroni adjusted genome-wide P-value were described and their genomic position, allele substitution effect, and closest gene were reported. The SNPs that surpass the suggestive P-value were described and their genomic position and allele substitution effect were reported as supplementary material.

5.3.5.2 Single-step Bayesian method

Genome-wide association analyses were performed separately for each of the milk composition and ECPU traits using a Bayes C linear mixed model (genomic data), as implemented in the JWAS package including both genotyped (n = 490) and non-genotyped (n = 144) cows in a single step model (model 2). The Bayes C method was used rather than Bayes A or Bayes B, because posterior distributions in Bayes C models are not as reliant on prior assumptions made by the user but are more driven by information contained within the data. Bayes C shrinks QTLs with small effects less than Bayes B allowing it to capture QTLs with small effects. The MCMC included 50,000 iterations. The statistical models used were similar to model 2 (including the same fixed and random effects). Variances (σ^2_a, σ^2_{pw}, σ^2_{pa}, σ^2_e and σ^2_m) presented in Table 5.4 were taken to be the prior variances in this analysis. The probability that SNPs would have zero effect on the trait was taken to be 99.7% (π = 0.997). The SNP genotypes with non-zero effects in any particular iteration were simultaneously fitted in the model in that iteration along with phenotypes, and pedigree in a single step.

The effect of a QTL could be distributed across a large number of markers that are in LD with the QTL therefore, the resultant posterior mean of individual markers will underestimate the real QTL effect. Accordingly, marker effects were collectively used to predict the genomic merit (Onteru et al., 2011; Oliveira et al., 2018) of cows in chromosomal regions that include non-overlapping 1-Mb windows based on the physical map order. Gibbs samples of marker effects within each 1-Mb window were used every 50th iteration to sample the genomic merit of all cows for every window. The window genomic merit was sampled by multiplying the genotype matrix related to number of minor alleles for each marker within each 1-Mb window by their corresponding marker effects of each MCMC iteration. The variance associated with genomic merit of each 1-Mb window across all cows was expressed as a proportion of variance associated with whole-genomic prediction samples to identify the most informative genomic regions. There were 2,676 unique SNP windows across the whole genome including 31 chromosomes (29 autosomes, mitochondrial, X chromosome). The expected proportion of variance explained by each 1-Mb window was 3.74×10^{-2} % (1/2,676 SNPs) and the window explained at least 0.19% of genetic variance, which corresponds to five times the expected proportion of variance (3.74×10^{-2} % × 5 = 0.19 %) was considered as a suggestive significance level (Sollero et al., 2017) while the SNP windows that explained 0.37 % of genetic variance, which corresponds to ten times the expected proportion of variance (3.74×10^{-2} % × 10 = 0.37%) were considered to reach genome-wide significance level. The

SNP windows that surpass the genome-wide significance level were considered as putative QTL (used for further analysis) and their genomic position, number of SNPs in each window, proportion of variance explained (PVE), window posterior probability of association (WPPA): the posterior probability that marker regression coefficient is non-zero for at least one SNP in a window, and associated genes were reported. The SNP windows that surpassed the suggestive significance level were presented as additional information in supplementary material. The estimated proportion of genetic variance explained by each 1-Mb window was plotted against genomic locations of the markers using the qqman package in R software (Turner, 2014).

5.3.5.3 Candidate genes and functional analysis

The individual SNPs that were highly associated ($P < 1 \times 10^{-6}$) with traits were examined to locate the closest genes within 100 kb upstream and downstream from the identified SNPs in the single-locus method. The 1-Mb genomic windows that explained PVE ≥ 0.37% were examined to identify the closest genes within 100 kb upstream and downstream from the identified window. All genes were identified using the Bos taurus reference genome in Ensembl (Ensembl release 94) using the UMD v3.1 assembly (https://www.animalgenome.org/cgi-bin/QTLdb/index). The biological functions of significant genes were identified using the GO (Ashburner et al., 2000) gene ontology tool as implemented in Ensembl (Ensembl release 94).

5.4 Results

5.4.1 Descriptive statistics

Descriptive statistics for the test-day milk composition and ECPU are presented in Table 5.3.

Table 5.3. Number of observations (N), mean, standard deviation (SD), coefficient of variation (CV), minimum (Min) and maximum (Max) values of milk percentage traits, milk urea concentration and efficiency of crude protein utilization in grazing dairy cows at Massey University Dairy 1 and Dairy 4.

Trait	N	Mean	SD	CV	Min	Max
FP, %	6,618	5.07	0.92	18	1.77	9.77
CPP, %	6,618	4.05	0.55	14	2.72	7.66
MU, mg/dl	1,866	25.6	8.31	32	6.08	61.7
ECPU, %	1,866	24.4	6.65	27	4.00	45.5

FP = fat percentage, CPP = crude protein percentage, MU = milk urea concentration, ECPU = efficiency of crude protein utilization.

5.4.2 Parameter estimation

The estimated variance components, heritabilities and repeatabilities of the traits applying the univariate single-step Bayesian (Bayes C, π = 0.997) linear mixed model are presented in Table 5.4. The s^2 of traits vary from zero to moderately high; s^2 of ECPU was not different from zero, s^2 of MU was moderately low and s^2 of milk percentage traits were moderately high.

Table 5.4. Estimates of variance components of milk yield percentage traits, milk urea concentration and efficiency of crude protein utilization using a univariate single-step Bayesian (Bayes C, $\pi = 0.997$) linear mixed model in grazing dairy cows.

Trait	σ^2_g	σ^2_m	σ^2_{pw}	σ^2_{pa}	σ^2_e	σ^2_p	s^2	h^2	t
FP	0.07	0.13	0.00	0.04	0.18	0.42	0.31	0.48	0.57
CPP	0.02	0.03	0.00	0.00	0.03	0.08	0.38	0.63	0.63
MU	6.64	3.37	0.41	0.53	15.58	26.53	0.13	0.38	0.41
ECPU	0.17	0.02	0.00	0.58	25.16	25.93	0.00	0.01	0.03

FP = fat percentage, CPP = crude protein percentage, MU = milk urea concentration, ECPU = efficiency of crude protein utilization, σ^2_g = additional polygenic variance, σ^2_m = additive genetic variance explained by the markers, σ^2_{pw} = within lactation permanent environmental variance, σ^2_{pa} = across lactation permanent environmental variance, σ^2_e = residual variance, σ^2_p = phenotypic variance ($\sigma^2_g + \sigma^2_m + \sigma^2_{pw} + \sigma^2_{pa} + \sigma^2_e$), s^2 = genomic heritability (σ^2_m / σ^2_p), h^2 = trait heritability ($[\sigma^2_g + \sigma^2_m] / \sigma^2_p$), t = repeatability ($[\sigma^2_g + \sigma^2_m + \sigma^2_{pw} + \sigma^2_{pa}] / \sigma^2_p$).

5.4.3 GWAS

5.4.3.1 Single-locus method

Manhattan plots resulting from the single-locus EMMAX method are presented in Figures 5.1-5.4 and details of the SNPs that surpass the genome-wide significance threshold P-value are presented in Table 5.5.

Two SNPs from chromosome 14 were found to be significantly associated ($P < 2.22 \times 10^{-5}$) with FP (Figure 5.1) at the genome-wide significant threshold ($P < 1 \times 10^{-6}$). The SNPs that surpass the suggestive p-value for FP were described and their genomic position, allele substitution effect, and genes were reported in Appendix 2 (Table A.1).

None of the SNPs were found to be significantly associated with CPP, MU, and ECPU either at genome-wide significant threshold or the suggestive threshold and are presented in Figure 5.1-5.4 respectively.

5*.4.3.2 Candidate genes and functional analysis*

All the significant SNPs for FP harboured good candidate genes located in chromosome 14 (Table 5.5). Two candidate genes were identified: diacylglycerol O-acyltransferase 1 (DGAT1) and solute carrier family 52 member 2 (SLC52A2).

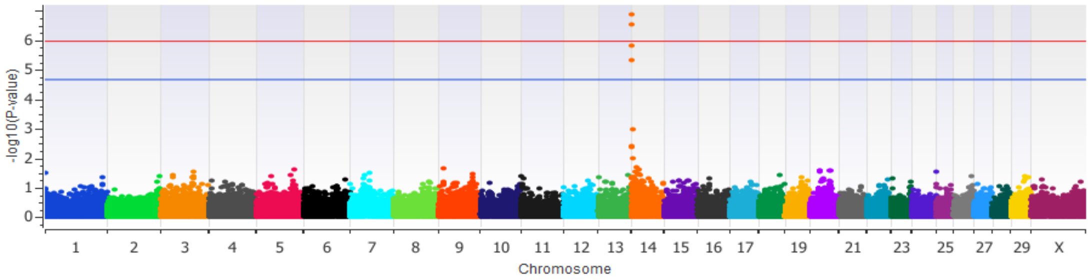

Figure 5.1. Manhattan plot of genome-wide association for fat percentage. The red line shows the genome-wide significant level at -log10(P-value) = 5.95 and the blue line shows the suggestive association significant level at -log10(P-value) = 4.65.

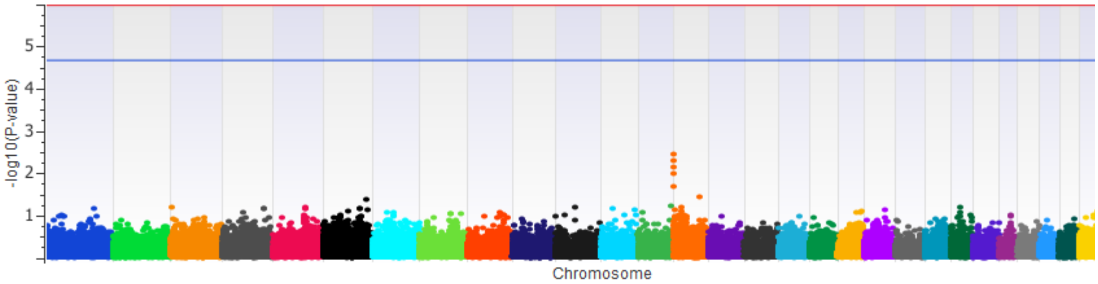

Figure 5.2. Manhattan plot of genome-wide association for crude protein percentage. The red line shows the genome-wide significant level at -log10(P-value) = 5.95 and the blue line shows the suggestive association significant level at -log10(P-value) = 4.65.

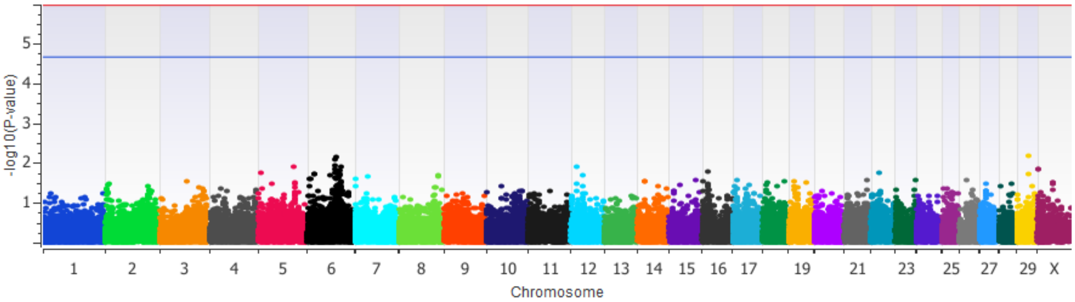

Figure 5.3. Manhattan plot of genome-wide association for milk urea concentration. The red line shows the genome-wide significant level at -log10(P-value) = 5.95 and the blue line shows the suggestive association significant level at -log10(P-value) = 4.65.

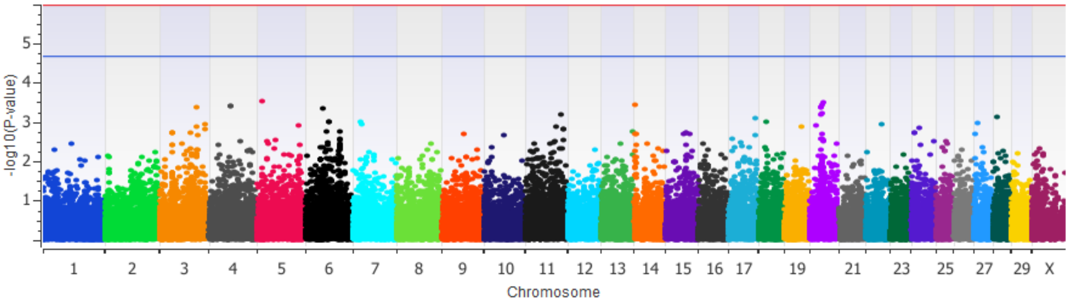

Figure 5.4. Manhattan plot of genome-wide association for efficiency of crude protein utilization. The red line shows the genome-wide significant level at -log10(P-value) = 5.95 and the blue line shows the suggestive association significant level at -log10(P-value) = 4.65.

Table 5.5. The SNPs which reached significance in single-locus association for fat percentage at genome-wide threshold ($P < 1 \times 10^{-6}$).

Trait	Locus	Chr	Position	P-Value	Effect	Effect SE	Ref	MA	MAF	Gene
FP	rs109421300	14	1,801,116	4.31E-08	-0.06	0.01	C	T	0.43	DGAT1
	rs137071126	14	1,765,835	5.45E-08	-0.06	0.01	G	C	0.45	SLC52A2

FP = fat percentage, Chr = chromosome, Ref = reference allele, MA = minor allele, MAF = minor allele frequency, DGAT1 = diacylglycerol O-acyltransferase 1, SLC52A2 = solute carrier family 52 member 2.

5.4.3.3 Single-step Bayesian method

The Manhattan plots based on a single-step Bayesian method are presented in Figures 5.5-5.8. Details of the 1-Mb SNP windows that surpass the genome-wide significance threshold (PVE ≥ 0.37%) are presented in Tables 5.6. The SNP windows that surpassed the suggestive significance level were presented as additional information in Appendix 2 (Table A.2). The number of SNPs included in a 1-Mb window varied from 1 to 99 and averaged 17 SNPs per window (±5.73).

Eight 1-Mb SNP windows from chromosome 5, 6, 14, 15, 20, and 27 were significantly (PVE ≥ 0.37%) associated with FP (Figure 5.5). The proportion of genetic variance explained by the most significant 1-Mb window was 38% and all the windows that were significantly associated with FP at the genome-wide threshold collectively explained 44% of the total genetic variance (Table 5.6).

Twenty 1-Mb SNP windows from 14 different chromosomes including; 3, 4, 5, 6, 8, 9, 13, 14, 17, 19, 21, 23, 24, and 26 were found to be significantly (PVE ≥ 0.37%) associated with CPP (Figure 5.6). The proportion of genetic variance explained by the QTLs varied from 0.38 to 9.83% and all the windows that were significantly associated with CPP at genome-wide threshold collectively explained approximately 39% of the total genetic variance (Table 5.6).

Eighteen 1-Mb SNP windows from 14 different chromosome including; 3, 5, 6, 11, 12, 16, 17, 21, 22, 23, 25, 27, and 29 were found to be significantly (PVE ≥ 0.37%) associated with MU (Figure 5.7). The top QTL spans in chromosome 23 (WPPA = 0.7), alone explained 3.25% of total genetic variance of MU. All the QTLs that were significantly associated with MU at the genome-wide threshold collectively explained only 14% of the total genetic variance (Table 5.6).

Only one 1-Mb SNP window from chromosome 20 was significantly (PVE ≥ 0.37%) associated with ECPU (Figure 5.8). The PVE explained by significant QTL was only 0.41% (Table 5.6).

5.4.3.4 Candidate genes and functional analysis

The significant genomic windows harboured good candidate genes for FP including; DGAT1, glycerol-3-phosphate acyltransferase 4 (GPAT4), microsomal glutathione S-transferase 1 (MGST1), acyl-CoA oxidase 3, pristanoyl (ACOX3), and CCAAT enhancer binding protein delta (CEBPD). These genes were located at chromosome 6, 14, and 27 (Table 5.6). A suggestive gene reported to be associated with FP was glutamic-pyruvic transaminase 2 (GPT2) (Table A.2).

Most of the significant genomic windows contained good candidate gene for CPP, including; DGAT1, alpha-S1-casein (CSN1S1), beta-casein (CSN2), alpha-S2-casein (CSN1S2), kappa-casein (CSN3), Bos taurus ribosomal protein S12 (RPS12), glutamate ionotropic receptor NMDA type subunit 2C (GRIN2C), eukaryotic translation initiation factor 3 subunit D (EIF3D), ADAM metallopeptidase domain 11 (ADAM11), the golgi SNAP receptor complex member 2 (GOSR2), HECT and RLD domain containing E3 ubiquitin protein ligase family member 6 (HERC6), and insulin like growth factor 1 receptor (IGF1R). These genes were located on chromosome 5, 6, 9, 14, 19, and 21 (Table 5.6). Two genes associated with CPP atsuggestive level were tripartite motif containing 45 (TRIM45) and ubiquitin protein ligase E3 component n-recognin 5 (UBR5) (Table A.2).

Six good candidate genes for MU were found over 18 genomic windows and were located in chromosome 5, 11, 12, 21, and 23 (Table 5.6). The candidate genes found to be associated with MU were GDP-mannose 4,6-dehydratase (GMDS), E2F transcription factor 7 (E2F7), solute carrier family 52 member 2 (SIAH3), solute carrier family 24 member 4 (SLC24A4), legumain (LGMN), and argininosuccinate synthase 1 (ASS1). Twenty genes were identified to be associated with MU at suggestive significant level (Table A.2).

The 1-Mb genomic windows that significantly associated with ECPU harboured one good gene in chromosome 20 (Table 5.6). Mitogen-activated protein kinase kinase 1 (MAP3K1) gene was identified as a candidate gene for ECPU. Sixteen genes were identified to be associated with ECPU at suggestive significant level (Table A.2). These genes include, mitogen-activated protein kinase kinase kinase kinase 4 (MAP4K4), golgi-associated, γ adaptin ear containing, ARF binding protein 3 (GGA3), tripartite motif containing 63 (TRIM63) and CDP diacylglycerol synthase 1 (CDS1).

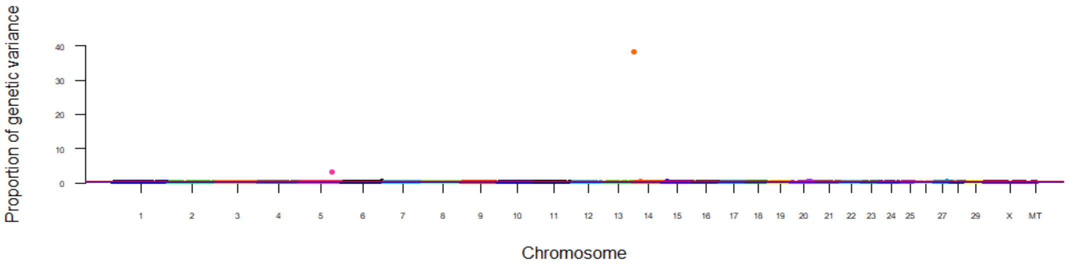

Figure 5.5. Manhattan plot of genome-wide association for fat percentage. The red line indicates the genome-wide significant level at proportion of variance explained = 0.37% and the blue line indicates the suggestive association significant level at proportion of variance explained = 0.19%.

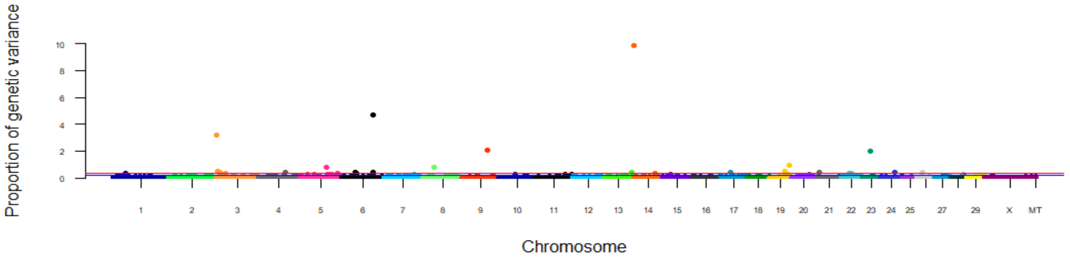

Figure 5.6. Manhattan plot of genome-wide association for crude protein percentage. The red line indicates the genome-wide significant level at proportion of variance explained = 0.37% and the blue line indicates the suggestive association significant level at proportion of variance explained = 0.19%.

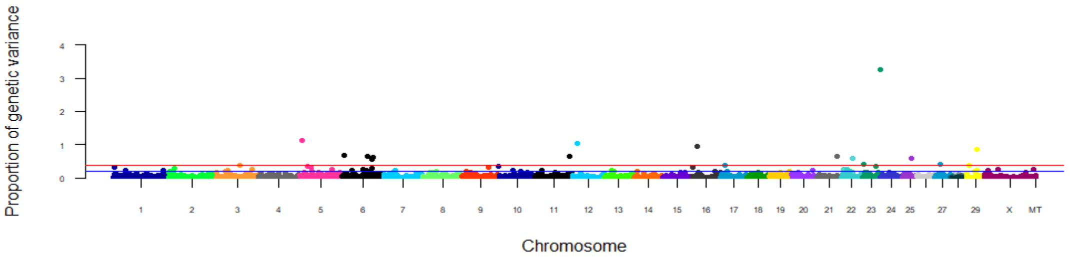

Figure 5.7. Manhattan plot of genome-wide association for milk urea concentration. The red line indicates the genome-wide significant level at proportion of variance explained = 0.37% and the blue line indicates the suggestive association significant level at proportion of variance explained = 0.19%.

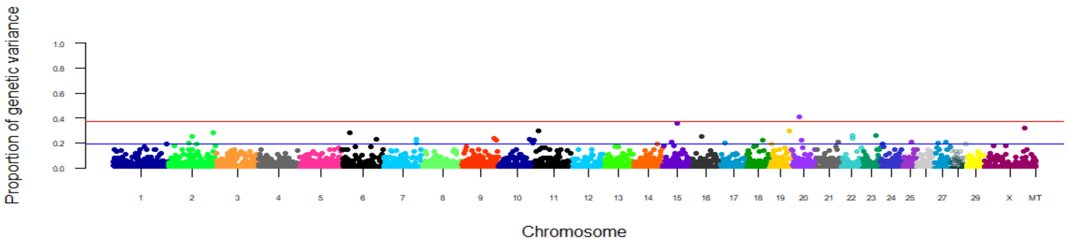

Figure 5.8. Manhattan plot of efficiency of genome-wide association for crude protein utilization. The red line indicates the genome-wide significant level at proportion of variance explained = 0.37% and the blue line indicates the suggestive association significant level at proportion of variance explained = 0.19%.

Table 5.6. The 1-Mb SNP windows with highest proportion of genetic variance (PVE) and window posterior probability of association (WPPA) for milk composition and fat (FP) and crude protein (CPP) percentages, milk urea (MU), and efficiency of crude protein utilization (ECPU).

Trait	Window	Chr	Start-end window (Mb)	Start SNP	End SNP	No. of SNP	PVE (%)	WPPA	Gene
FP	1506	14	1 - 2	1,118,964	1,971,143	22	38.12	1.00	DGAT1
	633	5	9.3 - 9.4	93,005,014	93,995,487	34	3.29	0.86	MGST1
	1602	15	1.2 - 1.3	12,020,185	12,928,912	18	0.53	0.30	-
	2418	27	3.6 - 3.7	36,075,350	36,959,262	16	0.53	0.18	GPAT4
	776	6	11.4 - 11.5	114,134,405	114,987,655	15	0.52	0.30	ACOX3
	2015	20	50 - 51	50,067,711	50,999,239	22	0.44	0.22	-
	2017	20	52 - 53	52,011,466	52,897,607	13	0.41	0.28	-
	1524	14	19 - 20	19,001,879	19,993,440	20	0.37	0.22	CEBPD
CPP	1506	14	1 - 2	1,118,964	1,971,143	22	9.83	1.00	DGAT1
	749	6	87 - 88	87,022,091	87,996,364	43	4.66	0.90	CSN1S1, CSN2, CSN1S2, CSN3
	299	3	2 - 3	2,120,827	2,903,624	15	3.20	0.68	-
	1080	9	71 - 72	71,021,904	71,970,552	19	2.08	0.56	RPS12
	2193	23	22 - 23	22,080,127	22,916,565	14	2.03	0.54	-
	1957	19	57 - 58	57,128,225	57,980,697	20	0.95	0.36	GRIN2C
	615	5	75 - 76	75,013,265	75,993,374	26	0.79	0.44	EIF3D
	928	8	33 - 34	33,060,034	33,937,052	17	0.79	0.46	-
	1945	19	45 - 46	45,094,650	45,989,813	18	0.50	0.20	ADAM11, GOSR2

Trait	Window	Chr	Start-end window (Mb)	Start SNP	End SNP	No. of SNP	PVE (%)	WPPA	Gene
	301	3	4 - 5	4,151,051	4,996,345	19	0.46	0.20	-
	700	6	38 - 39	38,019,605	38,939,012	49	0.45	0.30	HERC6
	498	4	79 - 80	79,008,823	79,904,993	15	0.43	0.20	-
	1499	13	79 - 80	79,008,708	79,991,041	20	0.41	0.22	-
	1787	17	29 - 30	29,008,821	29,936,157	19	0.41	0.24	-
	2045	21	8 - 9	8,031,396	8,955,497	19	0.41	0.18	IGFR1
	2347	26	17 - 18	17,042,328	17,986,547	21	0.41	0.24	-
	309	3	12 - 13	12,112,945	12,941,656	20	0.40	0.24	-
	750	6	88 - 89	88,049,208	88,958,861	29	0.40	0.18	-
	2263	24	39 - 40	39,013,392	39,987,594	24	0.40	0.22	-
	647	5	107 - 108	107,190,274	107,946,258	17	0.38	0.22	-
MU	2222	23	51 - 52	51,058,024	51,991,897	29	3.25	0.70	GMDS
	546	5	6 - 7	6,013,434	6,976,839	16	1.12	0.40	E2F7
	1343	12	15 - 16	15,017,263	15,988,893	22	1.04	0.36	SIAH1
	1689	16	13 - 14	13,015,545	13,976,725	19	0.95	0.28	-
	2505	29	30 - 31	30,075,012	30,986,595	17	0.86	0.30	-
	666	6	4 - 5	4,068,561	4,868,243	18	0.67	0.26	-
	2094	21	57 - 58	57,094,715	57,948,571	86	0.65	0.42	SLC24A4, LGMN
	735	6	73 - 74	73,015,703	73,990,002	21	0.64	0.30	-
	1320	11	100 - 101	100,018,300	100,942,106	18	0.64	0.20	ASS1
	749	6	87 - 88	87,022,091	87,996,364	43	0.62	0.44	-

Trait	Window	Chr	Start-end window (Mb)	Start SNP	End SNP	No. of SNP	PVE (%)	WPPA	Gene
	2314	25	27 - 28	27,028,950	27,987,306	23	0.6	0.30	-
	2140	22	31 - 32	31,020,826	31,942,134	16	0.59	0.24	-
	746	6	84 - 85	84,035,488	84,986,240	28	0.57	0.24	-
	2397	27	15 - 16	15,006,739	15,961,806	18	0.41	0.18	-
	2175	23	4 - 5	4,016,329	4,980,889	17	0.40	0.18	-
	364	3	67 - 68	67,096,213	67,987,280	14	0.39	0.22	-
	1770	17	12 - 13	12,057,061	12,986,781	17	0.39	0.16	-
	2481	29	6 - 7	6,141,144	6,976,219	13	0.39	0.14	-
ECPU	1987	20	22 - 23	22,006,676	22,960,402	99	0.41	0.30	MAP3K1

Chr = chromosome, DGAT1 = diacylglycerol O-acyltransferase 1, GPAT4 = glycerol-3-phosphate acyltransferase 4, MGST1 = microsomal glutathione S-transferase 1, ACOX3 = acyl-CoA oxidase 3 pristanoyl, and CEBPD = CCAAT enhancer binding protein delta, CSN1S1 = alpha-S1-casein CSN2 = beta-casein, CSN1S2 = alpha-S2-casein, CSN3 = kappa-casein, RPS12 = Bos taurus ribosomal protein S12, GRIN2C = glutamate ionotropic receptor NMDA type subunit 2C, EIF3D = eukaryotic translation initiation factor 3 subunit D, ADAM11 = ADAM metallopeptidase domain 11, GOSR2 = golgi SNAP receptor complex member 2, HERC6 = HECT and RLD domain containing E3 ubiquitin protein ligase family member 6, IGF1R = insulin like growth factor 1 receptor, GMDS = GDP-mannose 4,6-dehydratase, E2F7 = E2F transcription factor 7, SIAH3 = solute carrier family 52 member 2, SLC24A4 = solute carrier family 24 member 4, LGMN = legumain, ASS1 = argininosuccinate synthase 1, MAP3K1 = mitogen-activated protein kinase kinase 1.

5.5 Discussion

5.5.1 Descriptive statistics

The range of FP (from 1.77 to 9.77%) and PP (from 2.72 to 7.66%) found in the current study were within the range (1.84 to 11.32% and 2.76 to 6.77%) previously reported by Sneddon et al. (2015). The average MU concentration (29.96 mg/dl) observed by Beatson et al (2019) in mixed-breed New Zealand dairy cows was somewhat higher than the average MU (25.6 mg/dl) observed in the current study. Beatson et al. (2019) analysed cows from 540 herds located throughout New Zealand for four lactations, therefore, their MU predictions should be a better representative of average MU in New Zealand dairy cows than our predictions based on 634 cows under experimental conditions. Moreover, the twice a day milking herd in the current study is more suplimentary fed than a New Zealand average herd does (Correa-Luna, 2019), which might have improved the protein utilization efficiency of those cows ultimately resulting in the lower average MU. However, the reported average MU in the this study was comparable with the range (22.6 to 25.57 mg/dl) found in overseas studies (Bastin et al., 2009; Rzewuska and Strabel, 2013). The average ECPU (24.4%) observed in this study is consistent with the average protein utilization efficiency (27%) and nitrogen utilisation efficiency (27.7%) reported by Zamani et al. (2011) and Huhtanen et al. (2008), respectively. Therefore, the data obtained for other milk traits except for MU from these herds appear representative of cows in New Zealand. However, there are no studies on ECPU in dairy cows of New Zealand to compare with.

5.5.2 Parameter estimation

In this chapter genetic parameters were estimated using a repeatability animal model. The estimates of h^2 and t were consistent with the literature for FP and CPP (Lembeye et al., 2016b), for MU (Stoop et al., 2007; Beatson et al., 2019), and for ECPU (Zamani et al., 2011). The s^2 reported for FP (0.31) in this study is comparable with the estimate (0.35) found in another New Zealand study using a much larger dataset comprising of mixed-breed cows (Littlejohn et al., 2016). The greater s^2 range reported for CPP (from 0.59 to 0.62) by Kemper et al. (2015) in multi-breed cows of Australia using a nonlinear Bayesian method in comparison to the estimated s^2 in this study (0.38), could be due to benefit of using high-density arrays (777 K SNPs) for genotyping their cows. The estimated s^2 in the current study for MU (0.13) is comparable to estimated s^2 (0.14) by Bouwman et al. (2010) in Dutch Holstein-Friesian cows.

The estimates of all the traits indicated that the markers explained considerable proportion of phenotypic variance of all the traits, except for ECPU. The current study used only 45K SNPs and they should be spread over the genome therefore, the number of SNPs within causative loci is probably limited. However, the higher proportion of genetic variance explained by markers in the current study despite the small sample size, advises that there is a considerable linkage disequilibrium among SNPs and causative genes. The s^2 for milk percentage traits were moderate, indicating good marker predictions for FP and CPP traits, s^2 for MU was low, indicating comparatively low marker prediction for MU and s^2 of ECPU was zero, indicating poor marker prediction for ECPU.

5.5.3 GWAS

5.5.3.1 Single-locus method

Two SNPs in chromosome 14 were significantly associated with the FP and two of them reached the genome-wide significant threshold suggesting that, these two SNPs might be in linkage disequilibrium with the QTL for FP. None of the SNPs for CPP, MU, and ECPU reached significance at the genome-wide threshold. This observation suggests that these traits are under control of many genes (polygenic), each with smaller effects which could not be detected by either considering single-locus method or with the number of animals available in this study.

5.5.3.2 Candidate genes and functional analysis

The single-locus method (EMMAX) identified two candidate genes associated with FP. They were DGAT1and SLC52A2. The association of DGAT1 with FP is widely reported (Strzałkowska et al., 2005; Banos et al., 2008; Nayeri et al., 2016; Wang et al., 2020) and the gene has assigned biological functions of diacylglycerol metabolic process (GO:0046339), fatty acid homeostasis (GO:0055089), lipid storage (GO:0019915), long-chain fatty-acyl-CoA metabolic process (GO:0035336) and triglyceride biosynthetic process (GO:0019432). The DGAT1 is also known to associate with very-low-density lipoprotein particle assembly in the liver (GO:0034379). The aggregation of lipoprotein in the liver subsequently causes fatty liver in lactating dairy cows (Van den Top et al., 1995). This process may avoid the mobilised fatty acids being used efficiently by the cow in an attempt to meet the demands of either maintenance by being oxidised or milk fat production (Wensing et al., 1997). The gene SLC52A2 has an assigned function of transportation of riboflavin (GO:0032218). Riboflavin is involved in cellular metabolism of fats and proteins therefore, the activity of this gene may affect the utilization of feed fats and proteins, and ultimately their conversion into constituents in milk. The relationships of SLC52A2 gene with FP of Chinese Holstein cows were reported recently by Wang et al. (2020) in a GWA study.

Two suggestive genes associated with FP were reported. The gene CPSF1 has the biological function of mRNA polyadenylation (GO:0006378) and its association with milk production in Canadian Holstein-Friesians was reported by Nayeri et al. (2016). The MROH1 genes has no known biological functions related to FP in milk but its relationships with FP of Chinese Holstein cows were reported recently by Wang et al. (2020) in a GWAS.

5.5.3.3 Single-step Bayesian method

Collectively, the windows significantly associated with FP at the genome-wide threshold explained about 43% of the total genetic variance, this confirms the initial idea that a small number of genes have an important role in regulating FP. The top-most windows in this method and the top-most significant SNP in single-locus method were located within the DGAT1 gene. This indicates that both methods perform well when the effect of gene is high. However, more genomic regions were identified by the single-step Bayesian method for FP than for single-locus method. No SNPs reached the level of significance for CPP, MU, and ECPU traits when the association was tested using single-locus method. However, some genomic windows for CPP, and MU, and one genomic window for ECPU, which harboured good candidate genes, became significant at the genome-wide level when the associations were tested using the Bayesian method. This was likely due to the greater power of detection of associations when the markers are fitted simultaneously as random effects in the model and therefore, more genetic variation is captured by the markers compared to single-locus methods (Fernando and Garrick, 2013). The PVE by the significant window of ECPU was only 0.41%, suggesting again that the trait is polygenic with no genes of major effect. In a previous study (Bolormaa et al., 2013), feed efficiency has been identified as a polygenic trait in multi-breed cow herds in Australia, which had a moderately high h^2 (0.36) and was under control of larger number of SNPs.

5.5.3.4 Candidate genes and functional analysis

Under the assumptions applied on single-step Bayes C method in the current study, five, twelve, six, and one gene associated with FP, CPP, MU, and ECPU were identified, respectively. Gene DGAT1, MGST1, GPAT4, ACOX3, and CEBPD were identified important for FP. The functions of DGAT1were described earlier. Another important gene associated with FP is MGST1, which is related to cellular response to lipid hydroperoxide (GO:0071449). This gene has been previously identified as a causative gene for FP in New Zealand mixed-breed dairy cows, although the functional relationship of the gene with FP has not been revealed yet (Littlejohn et al., 2016). The gene GPAT4 is known to be involved in fatty acid metabolic process (GO:0006631) and mammary gland development (GO:0030879). Genome-wide association studies have shown that this gene is highly polymorphic and highly significantly associated with FP in German Holstein-Friesian cows (Wang et al., 2012; Xiang et al., 2017). The ACOX3 gene is also involved with fatty acid metabolic process (GO:0006631) and GWAS has shown its relationship with fatty acids synthesis in Canadian Holstein cows (Ibeagha-Awemu et al., 2016). The gene, CEBPD is related with fat cell differentiation (GO:0045444), a process that enhances synthesis and storage of fat. This gene has been identified with its function in body fat composition and distribution in beef cattle (Ihara et al., 2003) and influence on carcass and meat quality traits in Korean cattle (Shin et al., 2007). The suggestive gene GPT2 has a biological function related to regulation of biosynthesis (GO:0009058), therefore, the gene might be important in milk and milk component production. However, its association with FP in dairy cows has not yet been revealed through GWAS. Most of the genes associated with FP in the current study have previously been reported as candidate genes for FP; this gives confidence that the current findings could be applied in the selection of New Zealand dairy cows.

Twelve candidate genes including DGAT1, CSN1S1, CSN1S1, CSN1S2, CSN3, RPS12, GRIN2C, EIF3D, ADAM11, GOSR2, HERC6, and IGFR1 were identified as candidate genes for CPP. The casein cluster genes CSN1S1-CSN2-CSN1S2-CSN3 encode αs1, β, αs2, and κ caseins, respectively. Casein is the most common type of protein in bovine milk and these genes are known to significantly affect the physical, chemical and the nutritional quality of milk (Martin et al., 2002). The genes in the casein family are over-expressed in the mammary gland compared to other tissues (Chamberlain et al., 2015). Also, CSN1S1 (GO:0050821) and CSN3 (GO:0050821) genes are involved in the stabilization of encoded proteins by preventing degradation. The association of these genes with milk protein composition has been reported in lactating dairy cows in many studies including Zhou et al. (2019) and Sanchez et al. (2017). The RPS12 gene encodes a ribosomal protein (GO:0006412), which is a component of the 40S subunit of the ribosome (GO:0022627). Ribosomes are organelles that synthesize protein within the cell. This gene plays a vital role by coding for a structural constituent of the ribosome, which makes it highly likely to be an important candidate gene for milk protein synthesis. Gene GRIN2C has a known biological function of negative regulation of protein catabolic process (GO:0042177). The gene EIF3D codes for subunit 3D of eukaryotic translation initiation factor, which initiates the translation through mTOR signalling pathway (LeFebvre et al., 2006). This pathway is known to positively control the milk protein synthesis in ruminants (Bionaz and Loor, 2011). The ADAM11 gene is related with proteolysis (GO:0006508), the hydrolysis of proteins into smaller polypeptides and/or amino acids by cleavage of their peptide bonds. Proteolysis has been reported to affect the cheese making properties (Watkinson et al., 2001). The GOSR2 gene has a biological function related to protein transport (GO:0015031). This gene has been identified as a candidate gene for milk protein percentage in a meta-analysis in lactating cows (Vijayakumar et al., 2019). The HERC6 is a gene related to protein ubiquitination (GO:0016567). This gene has been suggested as candidate gene for protein yield (Cohen-Zinder et al., 2005) and lactation persistency in Canadian Holstein cattle (Do et al., 2017). The gene, IGF1R is involved with protein phosphorylation (GO:0016310). This gene is associated with milk protein yield in Simmental cows in Poland (Szewczuk, 2016). Both suggestive genes; TRIM45 and UBR5 have biological function related to protein ubiquitination (GO:0016567). Some of the genes identified in association with CPP in this study were previously reported as good candidate genes using GWAS. This study identified additional genes associated with CPP in New Zealand dairy cows which can be potential candidates for the trait.

The genes reported in association with MU, namely GMDS, E2F7, SIAH1, SLC24A4, LGMN, and ASS1 are closely related with protein metabolism and many steps in urea cycle and excretion, including protein catabolism, converting ammonia to urea, transportation of ammonia through blood stream and disposal of NH_3. The SIAH1 gene is related to ubiquitin-dependent protein catabolic process

(GO:0006511), i.e. pathways resulting in the breakdown of unusable cellular proteins into amino acids and ammonia allowing the cells to utilize the amino acids to generate vital proteins or energy (Doherty et al., 2002). The gene SLC24A4 has an assigned function in ion transport (GO:0006811) which involves movement of charged molecules into and out of cell. Ammonia exists as ammonium ion ($NH4^+$) at the physiological pH. The regulation of this gene, therefore, is vital for efficient removal of $NH4^+$ formed inside cells as a result of protein digestion. This process facilitates efficient transportation of $NH4^+$ molecules into the liver where they can be converted to urea which is toxic otherwise. The E2F7 gene has a function related to hepatocyte differentiation (GO:0070365). Hepatocytes are the functional units of the liver and this gene regulates the generation of specialized hepatocytes from unspecialized cells. The process of hepatocyte differentiation is vital for efficient conversion of NH_3 to urea in the liver. ASS1 is known to regulate the urea cycle of animals (GO:0000050). The overall reaction involves conversion of NH_3 into urea and the urea cycle primarily takes place in the liver. A mutation in the ASS1gene is associated with citrullinemia, which is an autosomal recessive urea cycle disorder that causes ammonia to accumulate in the blood causing lethal reaction in newly born Holstein-Friesian calves (Harper et al., 1986). The disease was disseminated throughout the Australian Holstein-Friesian population following insemination of cows with semen imported from an American-born Holstein bull, Linmack Kriss King (Healy, 1996) which was a carrier of the disease. Fifty percent of the Australian national Friesian herds and thirty percent of bulls in artificial insemination centres were descendants of this carrier (Healy et al., 1991). Citrullinemia has also reported in Indian Holstein cattle (Kotikalapudi et al., 2014). Diez-Fernandez et al. (2017) reported a mutation of this gene to be associated with life-threatening neonatal hyper-ammonemia in human. The GMDS gene is involved with GDP-mannose metabolic process (GO:0019673). One of the key purposes of the cellular metabolism is to eliminate nitrogenous waste from the body. Variations in this gene are associated with milk fatty acid traits in Canadian Holstein dairy cows (Ibeagha-Awemu et al., 2016), however, no association was found with milk urea production. The legumain gene has a biological function related to renal system process which is linked to disposal of nitrogenous waste products through the kidney (GO:0003014). The suggestive genes associated with MU have similar biological functions as genes associated with the trait at genome-wide significant level. None of the candidate genes for MU found in this study were reported before. However, Bouwman et al. (2010) reported that the QTL's in chromosome 1, 6, 21, and 23 were associated with MU and MU yield in Dutch Holstein-Friesian cows. Strucken et al. (2012) demonstrated that the markers in chromosome 3, 13, and 27 were in association with milk urea nitrogen (MUN) and MUN content in German Holstein-Friesian cows. Pegolo et al. (2018) showed that markers at chromosome 4, 5, and 13 were associated with MUN in Italian Brown Swiss cows. The chromosomes found to be associated with MU in the current study concur with the results of the previous studies. Genes identified in the current study as being associated with MU are related to genes regulating protein metabolism and N excretion. These findings suggest that MU can be genetically manipulated by controlling genes related to different stages of protein cycle and functions of the organs associated with excretory system.

The gene MAP3K1, which was identified as a candidate gene associated with ECPU, is involved with protein phosphorylation (GO:0006468). Phosphorylation is a post-translational modification that proteins undergo, is responsible for their stability and is a process that can alter the mechanical properties of milk (Fang et al., 2016). Jiang et al. (2016) identified MAP3K1 as a promising candidate gene affecting yield of milk, fat, protein, and percentages of fat and protein in Chinese Holstein cows through GWAS. Genome-wide association studies also found that the MAP3K1 gene is associated with human breast cancer (Shan et al., 2012). Therefore, this gene probably plays an important role in keeping the bovine mammary gland function healthy (Jiang et al., 2016). The suggestive genes associated with ECPU have biological functions related to production, modification, metabolism and transportation of either milk protein or fat. However, only few of the genes have previously been reported in GWA studies. MAP4K4 gene has been reported to be associated with milk yield, protein percentage, and mastitis susceptibility in Chinese Holstein cattle (Bhattarai et al., 2017). Although the association of GGA3 gene with feed use efficiency in dairy cows has not been previously reported, its association with average daily gain and average daily feed intake has been reported in chickens (Ye et al., 2019). The TRIM63 gene has been identified with its association to lactation persistency in Canadian Holstein cattle (Cohen-Zinder et al., 2005) while the CDS1 gene is known to affect the yields of milk and fat and percentage of protein in Italian sheep (Di Gerlando et al., 2019). Although this study reports some potential candidate genes for protein utilization efficiency in lactating dairy cows, no literature was found reporting candidate genes for ECPU.

Milk urea is a by-product of protein metabolism and is an indicator of inefficient protein use. In the current study it was demonstrated that genes related to protein metabolism were associated with MU and that genes related to milk protein production were associated with ECPU. Efficiency of crude protein utilization is related to protein intake and to milk protein yield. The observations made in this study provide evidence that the known phenotypic relationships between MU, ECPU, and CPP traits are likely to be at least partially driven by genetic differences among cows.

In chapter four, highly positive, genetic correlations were estimated between ECPU, and yields of milk, crude protein, and fat throughout the lactation. That finding, together with the observations in this chapter that genes associated with ECPU are also related to yields of milk, crude protein, and fat, suggest that there are good candidate genes for marker assisted selection for production efficiency in New Zealand dairy cows.

Further analysis should consider the use of random regression models to evaluate if the association of the candidate genes found in this study are the same across the lactation. Recently, Lu and Bovenhuis (2019) showed that GWAS for genotype by lactation stage interaction offers new possibilities to identify QTL involved in milk production. In particular, the authors have reported that separate GWAS for specific lactation stages might lead to the detection of new QTL that would not been detected when using models assuming genetic effects are constant over time. Therefore, the aforementioned approach will be tested for further studies, increasing the number of records and animals.

5.6 Conclusions

The present study perfomed GWAS using 50K SNP-chips and reported some QTLs and genes for FP, CPP, MU and ECPU which showed moderate to very low heritabilities using single-locus and single-step Bayesian methods. The identification of the associations of traits in the current study with the single-locus analysis was likely limited by the small sample size however, Bayesian method was more sensitive and capable of detecting small associations. The study reports novel QTLs and genes affecting MU and ECPU and confirms the previously reported candidate genes and QTLs for FP and CPP. The novel genes found in the study could be potentially important as commercial molecular markers for marker assisted selections. Selection for ECPU would have substantial impact on economy and wellbeing of the cows and selection for MU would ensure the environmental sustainability. However, validation of the results of the study in a larger dataset containing New Zealand dairy cow is essential in order to confirm the findings before implementing into breeding programs.

Appendix two

Table A.1 The single nucleotide polymorphisms which reached significance in single-locus association for fat percentages at suggestive threshold (P < 2.22 × 10-5).

Trait	Locus	Chr	Position	P-Value	Effect	Effect SE	Ref	MA	MAF	Gene
FP	rs137787931	14	1,880,378	$4.27E^{-07}$	-0.06	0.01	T	C	0.42	MROH1
	rs134432442	14	1,736,599	$8.69E^{-07}$	0.06	0.01	C	T	0.49	CPSF1

FP = fat percentage, Chr = chromosome, Ref = reference allele, MA = minor allele, MAF = minor allele frequency, MROH1 = maestro heat like repeat family member 1, CPSF1 = cleavage and polyadenylation specific factor 1.

Table A.2 The 1-Mb windows surpass suggestive significance level that is proportion of genetic variance (PVE) at 0.19 % and their window posterior probability of association (WPPA) for percentages of milk fat (FP) and crude protein (CPP), milk urea (MU) and efficiency of crude protein utilization (ECPU).

Trait	Window	Chr	Start-end window (Mb)	Start SNP	End SNP	No. of SNP	PVE (%)	WPPA	Gene
FP	1849	18	15-16	15,039,844	15,954,290	21	0.31	0.22	GPT2
CPP	323	3	26-27	26,020,004	26,925,312	18	0.36	0.22	TRIM45
	1567	14	62-63	62,081,472	62,960,995	17	0.34	0.12	UBR5
MU	563	5	23-24	23,019,369	23,949,571	19	0.34	0.08	UBE2N
	1084	9	75-76	75,026,578	75,935,285	19	0.33	0.14	TNFAIP3
	1677	16	1-2	1,033,239	1,972,109	24	0.32	0.14	ATP2B4, REN
	4	1	3-4	3,079,342	3,987,104	19	0.31	0.18	UBR1
	399	3	102-103	102,000,000	103,000,000	20	0.25	0.1	ATP6V0B
	2125	22	16-17	16,024,708	16,983,046	16	0.23	0.12	VHL
	145	1	144-145	144,000,000	145,000,000	21	0.22	0.14	TRPM2
	1440	13	20-21	20,006,828	20,982,977	17	0.22	0.14	ARL5B
	2503	29	28-29	28,078,118	28,887,293	17	0.22	0.12	PANX3
	573	5	33-34	33,078,266	33,991,103	12	0.21	0.16	SLC38A4, SLC38A2, SLC38A1
	1177	10	62-63	62,036,795	62,853,400	18	0.21	0.1	SLC12A1
	1775	17	17-18	17,027,443	17,947,446	13	0.2	0.1	UCP1
	1956	19	56-57	56,104,218	56,944,557	84	0.2	0.18	NUP85, OTOP2,

Trait	Window	Chr	Start-end window (Mb)	Start SNP	End SNP	No. of SNP	PVE (%)	WPPA	Gene
MU	1956	19	56-57	56,104,218	56,944,557	84	0.2	0.18	OTOP3, GRIN2C, SLC9A3R1
ECPU	1230	11	10-11	10,037,282	10,985,610	15	0.3	0.08	MAP4K4, MRPS9, TGFBRAP1
	1956	19	56-57	56,104,218	56,944,557	84	0.3	0.16	GGA3, MRPS7
	287	2	127-128	127,000,000	128,000,000	77	0.28	0.2	TRIM63
	2210	23	39-40	39,021,955	39,980,694	80	0.26	0.22	RNF144B
	229	2	69-70	69,014,623	69,939,162	13	0.25	0.06	INSIG2
	2141	22	32-33	32,008,861	32,990,568	19	0.24	0.04	UBA3
	761	6	99-100	99,028,913	99,992,455	18	0.23	0.08	CDS1
	1106	9	97-98	97,022,238	97,944,712	19	0.22	0.06	PRKN
	1207	10	92-93	92,011,190	92,935,729	17	0.22	0.06	SEL1L
	152	1	151-152	151,000,000	152,000,000	14	0.19	0.04	PIK3R4
	1907	19	7-8	7,004,150	7,972,717	26	0.19	0.06	GDF6, TRIM25
	2473	28	45-46	45,096,626	45,997,628	16	0.19	0.08	COG2

Chr = chromosome, GPT2 = glutamic-pyruvic transaminase 2, TRIM45= tripartite motif containing 45, UBR5 = ubiquitin protein ligase E3 component n-recognin 5, UBE2N = ubiquitin conjugating enzyme E2N, TNFAIP3 = TNF alpha induced protein 3, ATP2B4 = ATPase plasma membrane Ca2^{+} transporting 4, REN = renin, UBR1 = ubiquitin protein ligase E3 component n-recognin 1, ATP6V0B = ATPase H+ transporting V0 subunit b, VHL = von Hippel-Lindau tumor suppressor, TRPM2 = transient receptor potential cation channel subfamily M member 2, ARL5B = ADP ribosylation factor like GTPase 5B, PANX3 = pannexin 3, SLC38A4 = solute carrier family 38 member 4, SLC38A2 = solute carrier family 38 member 2, SLC38A1 = solute carrier family 38 member 1, SLC12A1= solute carrier family 12 member 1, UCP1 = uncoupling protein 1, NUP85 = nucleoporin 85, OTOP2 = otopetrin 2, OTOP3 = otopetrin 3, GRIN2C = glutamate ionotropic receptor NMDA type subunit

2C, SLC9A3R1 = SLC9A3 regulator 1, MAP4K4 = mitogen-activated protein kinase kinase kinase kinase 4, MRPS9 = mitochondrial ribosomal protein S9, TGFBRAP1 = transforming growth factor beta receptor associated protein 1, GGA3 = golgi-associated, gamma adaptin ear containing, ARF binding protein 3, MRPS7 = mitochondrial ribosomal protein S7, TRIM63 = tripartite motif containing 63, RNF144B = ring finger protein 144B, INSIG2 = insulin induced gene 2, UBA3 = ubiquitin like modifier activating enzyme 3, CDS1 = CDP diacylglycerol synthase 1, PRKN = parkin RBR E3 ubiquitin protein ligase, SEL1L = SEL1L adaptor subunit of ERAD E3 ubiquitin ligase, PIK3R4 = phosphoinositide-3-kinase regulatory subunit 4, GDF6 = growth differentiation factor 6, TRIM25 = tripartite motif containing 25, COG2 = component of oligomeric golgi complex 2.

Chapter 6

Effect of including selection for or against milk urea nitrogen concentration on nitrogen excretion and genetic change in Breeding Worth of New Zealand dairy cattle

The correlated responses for milk production and liveweight traits to different selection objectives and indices including milk urea nitrogen were evaluated. The effect of selection for low milk urea nitrogen on mitigating total nitrogen excretion on a per cow and hectare bases was evaluated. This paper was published as

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6.1 Abstract

The objectives of this study were two-fold. Firstly, to estimate the correlated responses in milk urea nitrogen (MUN) concentration, lactation yields of milk (MY), fat (FY) and crude protein (CPY) and mature cow liveweight (LWT) under three selection scenarios which varied in relative emphasis (RE) for MUN; 0% RE ($MUN_{RE\ 0\%}$: equivalent to current New Zealand Breeding Worth index), and sign of the economic value (EV); 20% RE positive selection ($MUN_{RE\ +20\%}$), and 20% RE negative selection ($MUN_{RE\ -20\%}$). Secondly, to estimate for these three scenarios the likely change in urinary nitrogen (UN) excretion under pasture based grazing conditions in New Zealand dairy cows. The predicted genetic responses per cow per year of the current index were 16.4 kg MY, 2.0 kg FY, 1.4 kg CPY, -0.4 kg LWT and -0.05 mg/dl MUN. Including positive selection on MUN resulted in annual responses of 23.7 kg MY, 2.0 kg FY, 1.4 kg CPY, 0.6 kg LWT and 0.10 mg/dl MUN, while negative selection on MUN resulted in annual responses of 5.4 kg MY, 1.6 kg FY, 1.0 kg CPY, -1.1 kg LWT and -0.17 mg/dl MUN. The $MUN_{RE\ -20\%}$ scenario reduced both MUN and cow productivity whereas the $MUN_{RE\ +20\%}$ scenario increased MUN, milk production and LWT. Per cow dry matter intake was increased in all three scenarios as production changed, therefore stocking rate (SR) needed to be adjusted to maintain pasture cover. Paradoxically, ten years of selection with SR adjusted to maintain annual feed demand under the $MUN_{RE\ +20\%}$ scenario actually reduced per ha UN excretion by 3.54 kg with increased 63 kg MY, 26 kg FY and 16 kg CPY compared to the base year. Ten years of selection on the $MUN_{RE\ 0\%}$ index was predicted to have a greater reduction of 10.45 kg UN and 30 kg MY with increase 32 kg FY and 21 kg CPY per ha whereas $MUN_{RE\ -20\%}$ index reduced 14.06 kg UN and 136 kg MY with increased 32 kg FY and 18 kg CPY. All three scenarios increased partitioning of nitrogen excreted as faeces. Selection indices excluding MUN are economically beneficial over selection indices including MUN. There is no substantial benefit from an environmental point of view of including MUN to the BW index, because N leaching is more sensitive to SR rather than individual cow UN excretion. Attention needs to be paid to the whole system consequences of selection for environmental outcomes in pastoral grazing circumstances.

Key words: genetic selection; four-pathways of selection; progeny testing; milk urea nitrogen; urinary nitrogen; dairy cows

6.2 Introduction

Dairying in New Zealand is pasture-based with management of stocking rate and mating dates chosen to achieve concordance between predicted pasture supply and feed demand of the herd. The feeding system is highly dependent on seasonal pasture supply and therefore, the stocking rate of cows is managed to achieve the optimal pasture utilization for producing milk and milk solids (Clark et al., 2006). The level of supplementary feeding is low (Wales and Kolver, 2017) compared to the levels of supplementary feeding found in indoor systems such as in North America and Europe (Harris, 2005). Crops may be used for offsetting pasture shortages in winter and any summer months with drought. Fresh pasture contains high quantities of crude protein relative to energy, therefore cows grazing pastures without energy supplementation involuntarily consume excessive quantities of protein, greatly exceeding their requirements (Kolver and Muller, 1998). When the consumed pasture is processed in the rumen, ruminally degradable proteins are broken down to amino acids and ammonia. The ammonia is transported to the liver and converted to urea which enters the blood stream and a small proportion is diffused into the milk, some is recycled back to the rumen via saliva, while the bulk of the urea is transported to the kidneys for excretion in urine (Roseler et al., 1993).

Milk urea nitrogen (MUN) concentration has been proposed as a diagnostic tool for protein relative to energy feeding of animals (Hof et al., 1997; Schepers and Meijer, 1998). Protein feeding of cows is an expensive component of the diet relative to energy in indoor systems. Further, the process of converting rumen derived ammonia to urea in the liver is energy (Vandehaar, 1998) and protein (Van Soest, 2018) demanding. Some researchers have pointed out direct negative effects of MUN on reproductive performance (Rajala-Schultz et al., 2001; Guo et al., 2004) as a result of increased blood urea nitrogen concentrations and decreased uterine pH (Elrod and Butler, 1993). Guinot-Thomas (1992) reported that cheese manufacturing can also be compromised given the greater coagulation time required for milk with higher MUN concentration. A major concern from the environmental point of view is that MUN may be positively associated with the amount of urinary nitrogen (UN) excreted by the cow (Gustafsson and Palmquist, 1993). Nitrogen (N) that enters the environment as UN breaks down to ammonia and nitrous oxide at the site of the urine patch making it a major source of water and air pollution in New Zealand. Averaged across the year, 20% of the UN load is typically leached through the soil (Selbie et al., 2015).

There are several options that could contribute to reduce MUN. Feeding cows with a diet balanced for protein and energy content is an appealing option if energy supplementation is cost-effective and practical. In a balanced diet, ammonia produced during protein metabolism in the rumen can be readily captured by rumen microbes and utilized for microbial protein synthesis which generates usable protein for the cow. Microbial protein synthesis is highly sensitive to the amount of fermentable energy in the diet (Oltner et al., 1985). If the diet contains insufficient fermentable energy then the rumen microbes are inefficient in capturing the ammonia, hence the produced ammonia is converted into urea in the liver and excreted in urine as an un-utilized form of protein. In New Zealand pasture systems, a balanced diet would require energy supplementation which is expensive and intensive in terms of increasing demand for labour, feed storage facilities and feeding equipment.

Another option is the retention of replacement heifers sired by bulls selected for low MUN (Beatson et al., 2019). This presumably improves N utilization efficiency of cows, enabling cows to partition more of their dietary N into milk protein (Baker et al., 1995), ultimately reducing the amount of N exported from the cow as MUN. However, reduced MUN per cow might physiologically be accomplished by reducing voluntary dry-matter intake, and therefore reducing metabolizable energy (ME) available above maintenance, and consequently reducing productivity per cow (Roche et al., 2016).

Beatson et al. (2019) discussed the environmental benefits of selecting for low MUN in cows managed under New Zealand grazing conditions. They proposed that MUN and UN excretion in cows could be reduced by breeding replacement heifers from sires with low MUN breeding values. This assumed that New Zealand cows competing with each other for voluntary intake of pasture had the same relationship between MUN and UN as overseas cows fed with total mixed rations (TMR) and assumed that reducing MUN through breeding has a similar effect on UN to reducing MUN through feeding.

The national breeding objective in New Zealand is to breed dairy cows that are able to efficiently convert feed into profit. To achieve that objective, cows and bulls are selected based on the Breeding Worth (BW) index, which is calculated as the sum of the product of the estimated breeding values and respective economic value of each of the traits under selection. The BW index ranks the animals in units of net profit expressed in dollars per 5 tonnes of dry matter intake. The calculation of BW includes eight traits, namely lactation yields of milk, fat and protein, cow liveweight, fertility, longevity, body condition score, and somatic cell score (NZAEL, 2020). Since MUN is associated both with N leaching and N utilization efficiency (Baker and Ferguson, 1993), MUN may be considered as a candidate trait to be included in the selection criteria for New Zealand dairy cattle to improve environmental sustainability and food security.

There are no published studies that have considered the response to selection from using MUN as a trait in the breeding objective. The objectives of the this study were 1) to evaluate the correlated responses in lactation yields of milk (MY), fat (FY), crude protein (CPY), or average mature cow liveweight (LWT) and MUN for three different selection indices in New Zealand dairy cows milked twice a day based on a conventional progeny test (PT) selection scheme, and 2) to evaluate the likely correlated phenotypic responses of production and UN per cow and per hectare for the three selection indices.

6.3 Material and Methods

6.3.1 Base cow

Average whole lactation MY, FY, CPY and average LWT of the New Zealand national herd were obtained from New Zealand Dairy Statistics 2018-19 (LIC and DairyNZ, 2019) to parameterise the base cow comprising; annual yields of 4,290 kg milk, 214 kg fat, 167 kg protein, and 456 kg LWT. Average MUN concentration during the lactation was assumed to be 14 mg/dl (Beatson et al., 2019). Milk urea yield (MUY) was calculated as milk urea (MU) × MY and MU was calculated as MUN × 2.14.

6.3.2 Lactation curves

Herd-test records of 634 cows with MY and percentages of fat (FP) and crude protein (CPP) were collected monthly from Dairy 1 and Dairy 4 experimental farms at Massey University. FY and CPY were calculated from FP and CPP multiplied by the corresponding MY obtained during the herd-tests. Milk urea concentration was determined indirectly for each cow for three of the herd-tests per season given the limited funds available, representing early (September), mid (December) and late (March) lactations. Milk samples were assessed for MU at MilkTestNZ (Hamilton, NZ) by the CombiFossTM 7 instrument (Foss Electric, Hillerød, Denmark) using a mid-infrared technique and not by wet chemistry. A daily composite sample of morning and afternoon milking followed by weighting according to morning and afternoon milk yields were used for estimating daily MUN concentration when twice a day milking was practiced, whereas the raw milk sample was used when once a day milking was practiced on the sampling days. Liveweight measurements were generated at every herd-test using an automatic walkover weigh scale. Liveweight change (LWTc) was calculated over the lactation as the difference in LWT at consecutive weightings. The Wilmink function (Wilmink, 1987) was used to model the lactation curves for MY, FY, CPY, LWT, and MUN as follow:

$$y_t = a + be^{-kt} + ct$$

where y indicates the predicted value at day *t* of the lactation, a, b, and c indicate the production (or liveweight) after calving, the slope associated with production (or liveweight) before the peak lactation, and the slope associated with production (or liveweight loss) after the peak lactation, respectively. The parameter k is associated with the time of peak lactation and was assumed to be 0.05 in this study, e is the base of the natural logarithm.

The regression coefficients: a, b, and c estimated from the 634 cows using the Wilmink function were modified to obtain the total lactation yields of milk, fat, protein, and average across lactation MUN and LWT of cows under each scenario using Solver in Excel so that the sum of the squared differences between the predictions and desired productions were minimized. Daily ME requirements for maintenance and lactation of the cow were estimated following the guidelines of the advisory manual prepared by the AFRC technical committee (AFRC, 1993). Apparent daily dry matter intake (DMI) was calculated as the estimated daily total ME requirement of cow divided by total dietary ME content (DME) of the pasture fed to cows that particular day. Daily crude protein intake (CPI) of the cow was determined as dietary crude protein percentage (DCPP) available in any diet offered to the cow multiplied by the estimated daily DMI. Daily N utilization efficiency (NUE) was estimated as the proportion of N in CPY and estimated daily N intake (IN). Stocking rate (SR), defined as number of cows grazing per hectare, was estimated given an annual consumption of 12,000 kg of dry matter (DM) per hectare (Lopez-Villalobos, 2000) divided by the estimated per cow annual requirement of DM. The annual per hectare production was estimated as per cow annual production multiplied by SR.

The daily UN excretion (g/d) for the lactation period (from day 1 to day 270) was estimated using the following equation developed by Huhtanen et al. (2015):

$$\mathrm{UN} = -29 + (4.3 \times \mathrm{DMI}) + (4.3 \times \mathrm{MUN}) + (0.14 \times \mathrm{LWT})$$

and faecal N (FN, g/d) for the lactation period (from day 1 to day 270) was estimated as the N balance between intake and outputs (urine, milk, retention) using the following equation:

$$\mathrm{FN} = \mathrm{IN} - \mathrm{UN} - \mathrm{MN} - \mathrm{RN}$$

where IN indicates grams of N intake, estimated as CPI/6.25 (N percentage in protein), MN indicates grams of milk N, estimated as CPY/6.25, and RN indicates grams of retained N, estimated as LWTc × 0.16 (each kilogram of LWT change contains 0.16 kg of CP ; Huhtanen et al., 2015).

The equations for estimating UN and FN rely on MN, consequently, those equations are not directly suitable for use during the non-lactating winter period. The daily UN excretion from day 271 to day 365 was estimated by rearranging the above equation and with MN = 0 as described by Reed et al. (2015):

$$\mathrm{UN} = \mathrm{IN} - \mathrm{FN} - \mathrm{RN}$$

where IN, FN, and RN as defined above.

In order to estimate the UN excretion using the above equation, the FN from day 271 to day 365 must be known and that was estimated using the following equation:

$$FN = 72.7 - (11.8 \times ME) - (0.4 \times NDF) + (3.5 \times DCPP) + (0.2 \times ForR) + (9.3 \times DMI)$$

where ME, NDF, and DCPP are megajoules (MJ) of metabolizable energy per kg of DM, percentage of neutral detergent fibre, percentage of crude protein content of diet, respectively. ForR is the percentage of forage in the ration, which was assumed to be forage only (100%) at each day of lactation.

The annual UN and FN excretions per cow were estimated as the sum of daily excretion (from day 1 to 365) of UN and FN, respectively, and the total N excreted was estimated as the sum of UN and FN excretions. The annual per hectare excretions were estimated as per cow annual excretions multiplied by SR. The total across New Zealand excretion was estimated as the annual per hectare excretion times the total effective hectares of dairy land in New Zealand: 1.74 million ha (DairyNZ, 2019), assuming that the effective land under dairy would not change in future. The across New Zealand annual change in N excretion was estimated as the difference between total across New Zealand excretion of the base year and across New Zealand excretion under each scenario, divided by ten (the time lag between base year and calculation for each scenario was ten years).

Cows were assumed to be solely fed a pasture diet throughout the year, mainly containing ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*). The DME

(Litherland and Lambert, 2007), DCPP (Litherland and Lambert, 2007), and neutral detergent fibre (NDF) (Moller et al., 1996) contents of the pasture were taken from published literature as representative of New Zealand pasture and used for estimating DMI and CPI of cows. Published literature reported the monthly averages of DME, DCPP, and NDF, therefore, the quality of the pasture changed each month but was assumed to remain unchanged within each month (Table 6.1).

Table 6.1. Assumed quality of the pasture including dietary metabolizable energy (DME), crude protein percentage (DCPP), and neutral detergent fibre (NDF) used for estimating dry matter intake, protein utilization efficiency and faecal nitrogen excretion in the current study.

	Pasture quality		
Month	DME[1] (MJ ME/kg DM)	DCPP[1] (%)	NDF[2] (%)
Jun	11.0	24	35
Jul	11.0	24	35
Aug	11.1	22	34
Sep	11.4	24	35
Oct	11.4	23	37
Nov	11.4	21	41
Dec	11.3	19	42
Jan	10.7	21	44
Feb	10.5	22	44
Mar	10.4	21	41
Apr	11.0	23	38
May	11.0	22	37

[1]Litherland and Lambert, 2007, [2]Moller et al., 1996

6.3.3 Breeding scheme

The breeding scheme modelled in the study does not represent the current New Zealand breeding scheme, which uses genomic selection to select bulls and bull mothers. Instead, this study models a traditional progeny test methodology to provide an insight into the direction of responses for traits if MUN is included as a trait of interest in the selection index.

The contributions of each bull and cow selection pathway were separately evaluated to obtain the overall rate of genetic gain using four pathways of selection proposed by Rendel and Robertson (1950), using the differences in the generation intervals and sources of information between males and females. This study assumed the New Zealand national herd comprised only one breed although in practice the New Zealand national herd comprised 4.95 million cows mainly made of two breeds and their crosses: F, J and F × J (DairyNZ, 2019). From the national herd of 4.95 million cows, 90% were selected for producing cow replacements in cows to breed cows (CC) pathway. The total bulls available for progeny testing were assumed to be 300. From the total cow population, 1 million cows were assumed to be potential bull mothers. The number of cows selected as active bull mothers in cows to breed bulls (CB) pathway were 2,100 assuming seven contract matings are required to produce one bull entering progeny testing (7 × 300). The best 10% bulls are selected for producing replacement cows whereas 3% of progeny tested elite bulls were selected for producing bull replacements in bulls to breed cows (BC) and bulls to breed bulls (BB) pathways, respectively. Cows were evaluated based on one of their own lactation records and bulls were evaluated assuming 75 half-sib progeny records. The generation intervals (L) were 4.8 (CC), 4.0 (CB), 6.6 (BC), and 6.3 (BB) years for each pathway. Generation interval for CC pathway, was estimated assuming replacement heifers from all age groups were retained for milking and majority of cows being selected in CB pathway are four years old. Generation intervals for bulls in BC and BB pathways were assumed considering the time required for obtaining progeny records from 75 daughters. Intensity of selection (i) and generation interval of each pathway are presented in Table 6.2.

Table 6.2. Assumptions made for performing four pathways of selection for improving genetic gain of New Zealand dairy cows.

Path	Population	Number selected	Proportion selected	I	L	NR	NP
CC	4,900,000	4,410,000	0.90	0.20	4.8	1	0
CB	1,000,000	2,100	0.002	3.20	4.0	1	0
BC	300	30	0.10	1.76	6.6	0	75
BB	300	9	0.03	2.27	6.3	0	75

CC = cows to breed cows, CB = cows to breed bulls, BC = bulls to breed cows, BB = bulls to breed bulls, i = intensity of selection, L = generation interval (years), NR = number of records, NP = number of progenies.

6.3.4. Breeding objective and Selection index

The current New Zealand breeding objective comprises eight traits. This study evaluated the effect of inclusion of selection for or against MUN on a simpler selection index comprised only of four key traits: MY, FY, PY, and LWT.

The EVs for MY, FY, PY, and LWT were as published in DairyNZ (2019). An EV for MUN is not available as it is not included into the current breeding objective of New Zealand. Therefore, two economic values for MUN were assumed compared to zero (equivalent to the current national selection index in New Zealand) and these were 20% relative emphasis (RE) with either a positive ($MUN_{RE\ +20\%}$) or a negative ($MUN_{RE\ -20\%}$) EV on MUN. Relative emphasis on traits was calculated by multiplying the absolute EV by genetic standard deviation of traits. Accordingly, three selection scenarios were performed: scenario 1 was to select with zero RE for MUN ($MUN_{RE\ 0\%}$), and thus genetic response for MUN was obtained from the genetic covariances with other traits, whereas scenario 2 and 3 were with 20% RE for MUN, but positive ($MUN_{RE\ +20\%}$) or negative ($MUN_{RE\ -20\%}$) EV, respectively. The EVs and RE on the traits under three scenarios are presented in Table 6.3.

Table 6.3. Economic values (EV) and relative emphasis (RE) for traits in three different selection scenarios that included milk urea nitrogen (MUN) with different RE in the breeding objective: zero ($\mathrm{MUN}_{\mathrm{RE}\ 0\%}$) and twenty percent with positive ($\mathrm{MUN}_{\mathrm{RE}\ +20\%}$) and negative ($\mathrm{MUN}_{\mathrm{RE}\ -20\%}$) EV.

	Breeding objective					
	$\mathrm{MUN}_{\mathrm{RE}\ 0\%}$		$\mathrm{MUN}_{\mathrm{RE}\ +20\%}$		$\mathrm{MUN}_{\mathrm{RE}\ -20\%}$	
Trait[1]	EV[2]	RE[3]	EV[2]	RE[3]	EV[2]	RE[3]
MY, kg	-0.09	20.22	-0.09	16.18	-0.09	16.18
FY, kg	3.49	31.66	3.49	25.33	3.49	25.33
CPY, kg	4.38	29.24	4.38	23.39	4.38	23.39
LWT, kg	-1.30	18.87	-1.30	15.10	-1.30	15.10
MUN, mg/dl	0.00[a]	0.00	24.35[a]	20.00	-24.35[a]	20.00

[1] MY = milk yield, FY = fat yield, CPY = crude protein yield, LWT = liveweight, MUN = milk urea nitrogen.

[2] EV= economic value obtained from the DairyNZ, 2019.

[3] RE = relative emphasis calculated by multiplying the absolute economic value of each trait by corresponding genetic standard deviations.

[a] Economic value calculated based on the RE assigned to MUN in the breeding objective.

6.3.5 Calculation of selection index and prediction of breeding objective

The selection index coefficients for each pathway were calculated using the following equation:

$$\mathbf{b} = \mathbf{P}^{-1}\mathbf{G}\,\mathbf{a}$$

where **P** is a 5 × 5 matrix of the phenotypic (co)variance of the traits in selection index, **G** is a 5 × 5 matrix of the genetic covariance between traits in the objective and those used for selection index, and **a** is a 5 × 1 vector of economic values.

The matrices **P** and **G** for cows were estimated separately to bulls as described by Cameron (1997). The diagonal elements of the matrix **P** contain the phenotypic variances (P_{ii}) of the traits and the off-diagonal elements contain the phenotypic covariances (P_{ij}) between trait *i* and *j*.

The P_{ij} elements were estimated using knowledge of phenotypic variances and phenotypic correlations between traits *i* and *j*.

The elements of matrix **P** for cows' pathways were as follows:

$$P_{ii} = \sigma^2_{pii}$$

and

$$P_{ij} = \sigma_{pij}$$

The diagonal elements of the matrix **G** contain the genetic variances (G_{ii}) of the traits and the off-diagonal elements contain the genetic covariances (G_{ij}) between traits in breeding objective and selection index and G_{ij} were estimated using genetic correlation and heritabilities of traits *i* and *j* as presented in Table 6.4.

The environmental variances (σ^2_{eii}) and covariances (σ_{eij}) between trait *i* and *j* were estimated as:

$$\sigma^2_{eii} = \sigma^2_{pii} - \sigma^2_{gii}$$

and

$$\sigma_{eij} = \frac{r_{pij} - (h_i h_j r_{gij})}{1 - h_i h_j}$$

where r_{pij}, r_{gij} and h are phenotypic and genetic correlations between traits *i* and *j* and the square root of heritability, respectively.

The elements of the matrix **P** for bulls' pathways evaluated using n number of daughters were constructed as:

$$P_{ii} = \frac{((1 + (n - 1)\ t\ \sigma^2_{gii})}{n} + \frac{\sigma^2_{eii}}{n}$$

and

$$P_{ij} = \frac{((1 + (n - 1)\ t\ \sigma_{gij})}{n} + \frac{\sigma_{eij}}{n}$$

where t is the correlation among individuals of the progeny which is 0.25 for half-sib progeny. The elements of **G** matrix for bulls' pathways were estimated as:

$$G_{ii} = k\ \sigma^2_{gii}$$

and

$$G_{ij} = k\ \sigma_{gij}$$

where k indicates relationship between sire and progeny which is 0.5 for bull to daughter.

Genetic parameters required for constructing **P** and **G** matrices were extracted from published literature (Spelman and Garrick, 1997; Lopez-Villalobos et al., 2018; Beatson et al., 2019; DairyNZ, 2019) and are presented in Table 6.4. The constructed **P** and **G** matrices met the criterion for positive definiteness therefore, bending of the matrices was not performed.

Table 6.4. Estimates of genetic parameters[1] for yields of milk (MY), fat (FY), crude protein (CPY), and for liveweight (LWT) and milk urea nitrogen (MUN) as used in selection index.

				Correlations[1]				
Trait	h^2	t	σ_p	MY	FY	PY	LWT	MUN
MY	0.28[a]	0.60[a]	519.52[a]		0.80[a]	0.90[a]	0.20[a]	0.11[b]
FY	0.22[a]	0.60[a]	22.61[a]	0.60[a]		0.90[a]	0.18[a]	0.02[b]
CPY	0.25[a]	0.60[a]	16.64[a]	0.80[a]	0.70[a]		0.22[a]	0.05[b]
LWT	0.24[a]	0.65[a]	34.64[a]	0.39[a]	0.34[a]	0.37[a]		0.02[c]
MUN	0.22[b]	0.35[b]	2.340[b]	0.19[a]	0.04[b]	0.08[b]	0.31[c]	

h^2 = heritability, t = repeatability, σ_p = phenotypic standard deviation.

[1] Genetic correlations below diagonal and phenotypic correlations above diagonal.

[a] Spelman and Garrick (1997), [b] Beatson et al. (2019), [c] Lopez-Villalobos et al. (2018).

The overall response to selection is the sum of the selection responses in each individual trait. The correlated response (CR) of each trait to selection on BW was calculated using the following equation:

$$CR_j = i_I \frac{\mathbf{b}'\mathbf{G}_j}{\sqrt{\mathbf{b}'\mathbf{P}\mathbf{b}}}$$

where CR_j is CR of the *j*th trait and $\mathbf{G}_j$ is the *j*th column of the **G** matrix and i_I is the standardised selection differential in the selection index.

Total CR was calculated as the sum of CR in each pathway and each trait and then the total CR of each trait was divided by the sum of generation intervals of each pathway to estimate the annual rate of genetic gain in the BW. The total economic response of the breeding objective (R_H) is the sum of individual economic responses of each trait in the index. The R_H for each trait (MY, FY, CPY, LWT) was calculated as individual trait selection response multiplied by the respective relative economic value.

However, MUN was not considered for estimating the R_H as MUN does not currently have a direct EV. The accuracy of selection index (r_{HI}) was calculated as:

$$r_{HI} = \frac{\sqrt{\mathbf{b}'\mathbf{P}\mathbf{b}}}{\sqrt{\mathbf{a}'\mathbf{G}\mathbf{a}}}.$$

The CR, total CR, and r_{HI} for each trait under each scenario were estimated separately using four pathways of selection. The genetic gain of cows under each scenario for a period of ten years was expressed relative to the base cow. Therefore, the estimated genetic gain from each scenario was multiplied by ten, assuming genetic gain is steady over generations and added to the performance of the genetic base cow to express the expected average performance of selected cows after ten years of selection.

In this study the genetic response to selection is taken at the asymptotic point. However, after the mating of desired dams and bulls, there will be at least three years of lag with no improvement in milking cow phenotype. The new-born calves which are genetically capable of producing less MUN will be included into the age group zero after calving and will produce milk with less MUN when they calve for the first time at two years of age. This means there will be at least three years of lag with no improvement in milking cow phenotype therefore immediate results cannot be expected through selection.

6.4 Results

The predicted CRs per cow per year in the assumed dairy cow population under three different scenarios based on the progeny testing scheme are presented in Table 6.5. The predicted annual responses of the current selection index on a per cow basis were 16.4 kg MY, 2.0 kg FY, 1.4 kg CPY, -0.4 kg LWT, and -0.05 mg/dl MUN respectively. The predicted increase of MUN for the positive selection index ($MUN_{RE\ +20\%}$) was 0.1 mg/dl with correlated increase of MY 23.7 kg/year, FY 2 kg/year, PY 1.4 kg/year, LWT 0.6 kg/year. Applying a negative selection ($MUN_{RE\ -20\%}$) in the index resulted in a reduction of 0.17 mg/dl MUN per cow, with increased MY 5.4 kg/year, FY 1.6 kg/year, PY 1.0 kg/year, and reduced LWT 1.1 kg/year. Selection of cow under positive EV on MUN reduced the overall annual economic response of cow by 15% (NZ$ 1.80/cow) whereas a negative EV reduced the overall annual economic response by 11% (NZ$ 1.30/cow) when compared to the cow selected under the current index ($MUN_{RE\ 0\%}$).

Table 6.5. Annual correlated responses of five traits using different selection scenarios that include milk urea nitrogen (MUN) with different relative emphasis (RE) in the breeding objective: zero ($MUN_{RE-0\%}$) and twenty percent with positive ($MUN_{RE\ +20\%}$) and negative ($MUN_{RE\ -20\%}$) economic values.

	Breeding objective		
Trait[1]	$MUN_{RE\ 0\%}$	$MUN_{RE\ +20\%}$	$MUN_{RE\ -20\%}$
MY, kg	16.4	23.7	5.4
FY, kg	2.0	2.0	1.6
CPY, kg	1.4	1.4	1.0
LWT, kg	-0.4	0.6	-1.1
MUN, mg/dl	-0.05	0.1	-0.17
R_H	$12.30	$10.50	$11.00

MY = milk yield, FY = fat yield, CPY = crude protein yield, LWT = liveweight, MUN = milk urea nitrogen, RE = relative emphasis, R_H = overall economic response; sum of individual trait genetic gain multiplied by their corresponding relative economic values only for milk, fat, protein and liveweight.

The simulated performance for milk traits, liveweight and MUN after 10 years of selection under the three selection scenarios on a per cow and per hectare basis are presented in Table 6.6. The daily concentrations of MUN for cows in the base year and cows selected under positive and negative scenarios are shown in Figure 6.1.

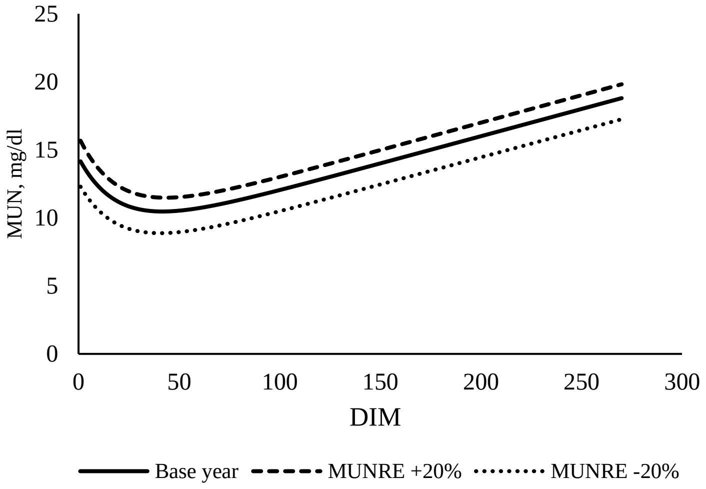

Figure 6.1. Concentration of milk urea nitrogen (MUN) at each day in milk (DIM) for cows in the base year (——) and after ten years of selection with 20% relative emphasis and positive ($\mathrm{MUN}_{\mathrm{RE}\ +20\%}$) (- - - -) or negative ($\mathrm{MUN}_{\mathrm{RE}\ -20\%}$) (……) economic values for MUN.

After ten years of selection with the current selection index ($\mathrm{MUN}_{\mathrm{RE}\ 0\%}$), there would be an increase of MY (164 kg), FY (20 kg), PY (14 kg), a decrease of LWT (4 kg) with a marginal decrease in MUN (0.55 mg/dl) per cow, compared to the base year. Ten years of selection under positive scenario would increase MY (237 kg), FY (20 kg), PY (14 kg), LWT (6 kg) and MUN (0.9 mg/dl) per cow, compared to the base year. The selection based on negative scenario for ten years would decrease MUN (1.67 mg/dl), compared to the base year, with per cow increase in MY (54 kg), FY (16 kg), PY (10 kg), and decrease of LWT (11 kg).

Table 6.6. Expected average lactation yields of milk (MY), fat (FY), crude protein (CPY), liveweight (LWT) and milk urea nitrogen (MUN) in cows of base year and different selection scenarios after 10 years of selection for MUN with different relative emphasis (RE) in the breeding objective: zero (MUN$_{RE\ 0\%}$), and twenty percent with positive (MUN$_{RE\ +20\%}$) and negative (MUN$_{RE\ -20\%}$) economic values.

Trait	Base year	Breeding objective		
		MUN$_{RE}$ 0%	MUN$_{RE}$ +20%	MUN$_{RE}$ -20%
Per cow[1]				
MY, kg	4,290	4,454	4,527	4,344
FY, kg	214	234	234	230
CPY, kg	167	181	181	177
LWT, kg	456	452	462	445
DMI, kg	4,024	4,188	4,226	4,119
MUN, mg/dl[1]	14.00	13.45	14.90	12.33
MUY, kg	1.2	1.2	1.4	1.1
IN during lactation[*], kg	125	131	132	128
IN, kg	142	148	149	145
MN, kg	26.7	29.0	29.0	28.7
FN, kg	56.3	63.1	62.0	64.2
UN, kg	56.5	55.2	58.1	53.0
Total N excreted, kg	112.8	118.3	120.1	117.2
NUE, %	21.4	22.2	22.1	22.3
Per ha[2]				
SR, cows	2.982	2.865	2.840	2.914
MY, kg	12,792	12,762	12,855	12,656
FY, kg	638	670	664	670
CPY, kg	498	519	514	516
MUY, kg	3.5	3.5	4.0	3.2
IN, kg	423	423	423	423
MN, kg	79.6	83.2	82.3	83.5
FN, kg	167.9	180.8	176.1	187.0
UN, kg	168.6	158.2	165.1	154.5
Total N excreted, kg	336.5	338.9	341.0	341.6
Across New Zealand[3]				
Total N excreted, m kg	585.5	589.8	593.4	594.3
Change in N excretion, m kg/year		+0.4	+0.8	+0.9

DMI = day matter intake, MUY = kilograms of milk urea yield, IN = nitrogen intake, MN = milk nitrogen, FN = faecal nitrogen, UN = urinary nitrogen, Total N excreted = sum of FN and UN, NUE = nitrogen utilization efficiency, change in N excretion = across country annual total N excreted in base year - across country annual total N excreted in each selection scenario.

[1]estimated using correlated response, [2]estimated as per cow estimate × SR, [3]estimated as per ha estimate × 1.74 million hectares (number of effective hectares of dairy lands)
[*]sum of IN only during the lactation period (from 1 - 270 days in milk).

A loss of production of fat and crude protein and LWT under negative selection ($\text{MUN}_{\text{RE -20\%}}$) resulted in a reduction in ME requirements and a subsequent reduction in per cow DMI compared to the $\text{MUN}_{\text{RE 0\%}}$ scenario. The reduction in DMI per cow led to a reduction in SR from 2.982 (base year) to 2.914 cows/ha for optimal utilization of seasonal pasture supply. Even though a similar production of fat and crude protein was observed under both $\text{MUN}_{\text{RE 0\%}}$ and $\text{MUN}_{\text{RE +20\%}}$ scenarios, the increased MY and LWT increased the ME requirement which is reflected in the DMI of the cow in the latter scenario. This led to a need to reduce the SR under $\text{MUN}_{\text{RE +20\%}}$ scenario relative to SR of the base year.

Ten years of selection based on a selection index with no emphasis on MUN will result in cows excreting 1.3 kg less UN and 6.7 kg more FN compared to the cows in the base year. The total N excreted (UN+FN) by the cows in base year is 112.8 kg which is predicted to be increased by 5.4 kg per cow as consequence of $\text{MUN}_{\text{RE 0\%}}$ selection scenario relative to cows in the base year. On a per ha basis the annual UN excretion is 168.6 kg and total N excreted is 336.5 kg in the base year and UN was estimated to reduce by 10.4 kg while N excreted was estimated to increase by 2.5 kg for cow selected with no emphasis on MUN.

The cow selected under the $\text{MUN}_{\text{RE +20\%}}$ scenario for ten years would excrete 1.6 kg more UN, 5.7 kg more FN and 7.3 kg more total N compared to the cow in the base year. On a per hectare basis, there will be a reduction of 3.5 kg of UN excretion with an increase of 4.6 kg of total N excretion compared to the base year.

After ten years of selection, a cow in $MUN_{RE\ -20\%}$ scenario would have a reduced UN of 3.5 kg, with an increase of 7.9 kg in FN compared to cow in base year. This selection will also be responsible for 14.1 kg less UN but 5.1 kg more total N excretion on a per hectare basis compared to the base year. The total N excretion by cows selected under the $MUN_{RE\ +20\%}$ scenario was slightly lower than that of the cow selected under $MUN_{RE\ -20\%}$.

Across New Zealand (over 1.74 m of effective hectares of dairy lands) the annual total N excretion (UN+FN) of cows in the base year was 585.5 m kg and was predicted to annually increase by 0.4 m kg, 0.8 m kg, and 0.9 m kg for cows selected under $MUN_{RE\ -0\%,}$ $MUN_{RE\ +20\%,}$ and $MUN_{RE\ -20\%}$ scenarios, respectively.

The across lactation average estimates of N allocation among the different N pools (MN, UN, FN) and NUE for cows in the base year and each selection scenario are presented in Table 6.6. A greater daily N allocation for MN and FN but lower daily N allocation for UN was predicted in the cow selected under the $MUN_{RE\ -20\%}$ scenario (Figure 6.2), compared to the base year, whereas the N allocation for all three pools were higher in $MUN_{RE\ +20\%}$ scenario compared to the base year. There is only a slight difference between NUE between cow selected under $MUN_{RE\ +20\%}$ and $MUN_{RE\ -20\%}$ scenarios. The NUE of cows reduces with increased NI.

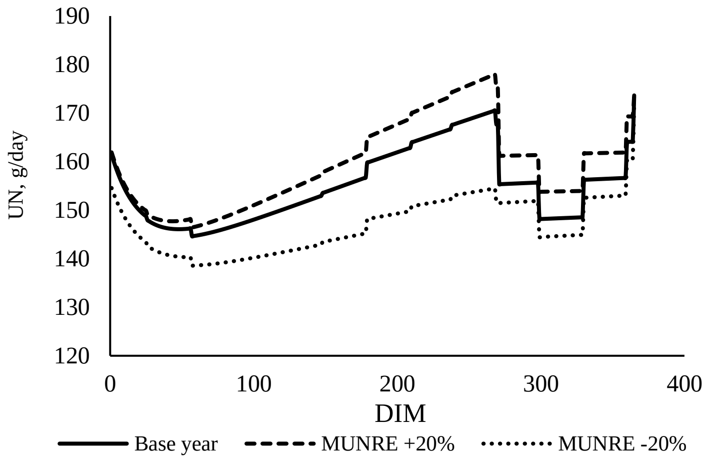

Figure 6.2. Nitrogen allocation for urinary nitrogen (UN) at each day in milk (DIM) in cows of base year (▬▬) and cows selected for ten years with 20% relative emphasis (RE) and positive (MUN$_{RE\ +20\%}$) (- - - -) and negative (MUN$_{RE\ -20\%}$) (……) economic values for milk urea nitrogen (MUN).

6.5 Discussion

The objective of the current study was to evaluate the effect of including MUN into the BW index in order to reduce the UN excretion at cow and per hectare basis.

The predicted annual UN excretion per cow of the base year (56.5 kg) in the current study was lower than the range (74.8-91.6 kg) reported by Box et al. (2017) in pasture-fed New Zealand dairy cows. The annual FN production of New Zealand dairy cows estimated using collected faecal samples in pasture-based feeding trials varies from 41.6 kg (Harkin, 2014) to 48.5 kg (Hendriks, 2016) and are lower than the estimated FN excretion in the base year (56.3 kg/cow) of the current study, which was estimated as the balance between the dietary N intake and the other N outputs. Comparatively lower annual UN excretion predicted in this study is understandable given the fact that the prediction equations used for estimations were derived in indoor cows feed on TMR and under such circumstances UN excretion of those cows are lower than cows feed on pasture as in this study. The level of annual FN estimated with such lower levels of UN as a balance between IN and outputs (MN, UN, RN) in cows was expected to be greater than actually observe under pasture based production systems. The simulated annual MN production of the cow in base year in the current study was 26.7 kg and is within the range of MN production of pasture fed New Zealand dairy cows reported by Mackle et al., 1996 (25.1-27.3 kg) and was comparable with values reported by Totty et al., 2013 (27.3-28.1 kg). MPI (2010) reported the annual total N excretion per dairy cow in 2007 as 113.6 kg and the total annual N excreted by the New Zealand dairy cattle population (5.26 million) as 598 m kg. The annual total N excretion per cow and across New Zealand total excretion based on the SR and effective hectares for milk production in the 2018/19 production season (over 1.74 m ha) in the base year were 112.8 kg/cow and 586.5 m kg/ha, respectively. The estimated annual NI (142 kg) of the base cow based on the predicted DMI of this study is in agreement with the NI (166 kg) estimated using measured DMI in 24 Jersey and Friesian cows in Mackle et al. (1996). Since, the predictions of N partitioning for MN, DMI, NI and total N excretions of the predicted base cow of the current study appears representative of a New Zealand cow, the predicted base cow is an appropriate reference point to compare different selection scenarios for those traits.

The simulated CR of the $MUN_{RE\ 0\%}$ selection scenario for FY and CPY in the current study were comparable with the estimated CR in Sneddon et al. (2016b) and Spelman and Garrick (1997). The simulated CR of $MUN_{RE\ 0\%}$ for MY and LWT in the current study were comparable with the simulated CR by Spelman and Garrick (1997). The slight differences in CR reported in this study in comparison to the published literature were likely to result from using slightly different phenotypic and genetic correlations in the current study due to differences in traits of interest in each study.

Negative selection for MUN was responsible for the greatest reduction in MUN concentration, however, that scenario had unfavourable CR for FY and CPY. This resulted in less improvement of profit compared to the current index as payment of milk in the New Zealand dairy industry is based on the A + B - C multiple component pricing system where A and B are the values per kilogram of fat and protein and C is the penalty on kg of MY (Sneddon et al., 2013). Selection for increased MUN ($MUN_{RE\ +20\%}$) did not decrease milk solid production compared to the $MUN_{RE\ 0\%}$ scenario. As yield of milk solids are highly sensitive to the negative EV on MUN, selection against MUN should be considered with caution.

The number of milking cows in the 2018-19 production season was 4.95 million (DairyNZ, 2019) and if the national herd size remains unchanged, NZ$ 8.91 million (NZ$ 1.8 × 4.95) or NZ$ 6.4 million (NZ$ 1.3 × 4.95) income loss is expected annually after ten years of selection for the $MUN_{RE\ +20\%}$ and $MUN_{RE\ -20\%}$ scenarios, respectively compared to the cows selected under $MUN_{RE\ 0\%}$ scenario. The reduced economic response in $MUN_{RE\ -20\%}$ scenario will be caused by reduced genetic gains of FY and CPY which had positive economic values. The increased genetic gains of MY and LWT are the likely reason for the reduced economic response in $MUN_{RE\ +20\%}$ scenario.

Although, fertility traits have not been included into the selection index in this study due to unavailability of variance-covariance information, König et al. (2008) reported negative genetic and phenotypic correlations between MUN and 56-day and 90-day nonreturn rates in German Holstein cows, indicating that cow fertility would be improved if MUN is reduced. However, König et al. (2008) reported that the genetic correlations between MUN and nonreturn rates were too weak to justify the use of MUN as an indicator trait in genetic selection for improved fertility. Therefore, correlations between MUN and reproduction traits need to be estimated for New Zealand dairy cows to define the selection index with production, reproduction, and environmental traits.

The annual per hectare UN excretion of cows estimated per cow UN excretion and current SR were 168.6 kg in base year, 164.60 kg in $MUN_{RE\ 0\%}$ (55.2 kg × 2.982), 173.3 kg in $MUN_{RE\ +20\%}$ (58.1 kg × 2.982), and 158.2 kg in $MUN_{RE\ -20\%}$ (53.0 kg × 2.982) scenario. This would result in an annual per hectare change of UN excretion of -4.0 kg in $MUN_{RE\ 0\%}$, 4.7 kg in $MUN_{RE\ +20\%}$, and -10.4 kg in $MUN_{RE\ -20\%}$ scenarios compared to the base year. However, the cows selected under all the three scenarios

($MUN_{RE\ 0\%}$, $MUN_{RE\ +20\%}$, $MUN_{RE\ -20\%}$) had greater production of milk volume and milk solids compared to cows in the base year. This required the selected cows to have a higher DMI and consequently there would be a lower SR than for cows in the base year. The $MUN_{RE\ 0\%}$ and $MUN_{RE\ -20\%}$ scenarios allowed the cows to excrete even lesser UN at the per hectare basis (-10.4 kg and -14.1 kg, respectively) than cows in the base year.

Cows selected for higher MUN ($MUN_{RE\ +20\%}$) that produced higher MUN on a per cow basis also excreted less UN (-3.5 kg) on a per hectare basis due to the reduced SR in comparison to the base scenario. This means that, the cow in base year with less MUN at per cow basis (14 mg/dl) excreted higher UN (168.6 kg) at per hectare basis compared to the cow with higher MUN per cow basis (14.9 mg/dl) that selected for higher MUN and UN (165.1 kg). The simulated result of the current study indicates that the increased MUN at per cow basis is not necessarily responsible for reduced UN in per hectare basis but, lower SR plays an important role in reducing UN at the per hectare basis. A recent New Zealand study (Kennett et al., 2020) has indicated that there is a less opportunity to reduce N leaching by genetic selection for urine traits when SR and IN of cows are high. The results from this simulation study however contradict the points made by Roche et al. (2016) who indicated that N leaching can be reduced with increased SR with minimum supplementary feeding in the context of the whole farm system experiment. The reduced N excretion in their study is reasonable given the per cow reduction of DMI hence, reduced N intake with increased SR, whereas in the current study DMI is adjusted by change in SR.

Selected cows in all three scenarios excreted higher FN compared to the cows in the base year. These cows also had greater N allocation for MN compared to the cows of the base year. These simulated results are in agreement with Marshall et al. (2020)

who reported that cows with low MUNBV had increased milk protein percentage throughout lactation and FN in late lactation. The higher N allocation for other N pools, apart from urine, is likely be linked with higher involuntary N intake associated with higher DMI of improved cows. The increase of FN production of improved cows that excrete less UN might be an artefact of the method used to estimate FN in this study but not due to a difference in physiology of the improved cows. However, the increased FN allocation of improved cows made cows selected in all three scenarios equivalent in terms of the total N excreted, despite the RE and direction of selection for MUN.

The simulated daily reduction of UN as reported by Beatson et al. (2019), was 18 g/cow per year of selection in the progeny of the low MUN breeding value (BV = -2.4) bulls compared to the progeny of BV MUN 0 bulls. Low MUN cows had a mean phenotype of 14.0 mg/dl (New Zealand average) for MUN. This reduction in UN corresponds to an annual reduction of 6.6 kg/cow and this estimation is further supported by the study of Marshall et al. (2020) using measured urinary urea N excretions in 58 multiparous lactating Holstein-Friesian cows. Beatson et al. (2019) calculated that this reduction in UN excretion corresponded to an annual reduction of 42 million kg N from the 6.5 million dairy animals farmed across New Zealand. They used published prediction equations for estimating UN based on MUN. According to the estimates in this study, the CR of MUN after ten years of selection in the negative scenario ($MUN_{RE\ -20\%}$), and using a combination of prediction equations of Huhtanen et al. (2015) and Reed et al. (2015), would be reduced by only 17.3 m kg of UN over the 4.95 million of milking cows (3.5 kg/cow/ten years × 4.95 m cows). Following the same assumptions, 7.9 m kg (1.6 kg/cow/ten years × 4.95 m cows) of increase in UN excretion, after ten years of selection under $MUN_{RE\ +20\%}$

scenario was predicted in the current study. This indicates that the selection for high MUN has relatively slow genetic response than selection for low MUN.

A positive relationship between N intake and urinary N outputs in New Zealand dairy cows has been reported (Mackle et al., 1996; Hendriks, 2016). New Zealand pasture on average contains around 200 g of CP/kg DM (Holmes et al., 2002) but is even richer in CP (265 g/kg DM) during the spring (Lincoln University, 2016). The current study evaluated the possible improvement of UN excretion using a PT based selection under the current feeding management (feeding cows with diet in which CP varies from 190 to 240 g/kg DM) but the UN excretion is prone to incline in any circumstance at which CP of diet increase over the upper limit of the study despite the genetic improvement gain through selection. This suggests that diet management is a promising alternative to control MUN and associated N excretion.

Most of the prediction equations for MUN and UN in the literature were derived under indoor conditions where cows were fed with TMR (Jonker et al., 1998; Kauffman and St-Pierre, 2001; Kohn et al., 2002; Nousiainen et al., 2004). However, under New Zealand outdoor grazing conditions, DMI is controlled at the herd level, meaning that individual cows will vary in voluntary feed intake, given the same access to pasture. Accordingly, the N intake of cows in the same herd will exhibit variation that contributes to between cow variation in MUN and UN. Milk urea N is affected by various factors besides genetic makeup, including level of production, level of protein feeding, and stage of the lactation and therefore, there are subsequent effects of those factors on UN. Given that MUN is a multifactorial trait, the prediction equations containing additional dependent variable other than MUN were used for estimation of UN in the current study. In this study, UN was estimated using a combination of equations predicted by Huhtanen et al. (2015) and Reed et al.

(2015). Huhtanen et al. (2015) have developed a prediction equation for UN in lactating cows that were fed indoors with forage, concentrates and TMR. In their equation, UN was estimated as a function of DMI, LW, and MUN where, DMI is a representative of both level of production and level of CPI, LWT is a measure of level of production, and MUN is a measure of N partitioning on excretion. Therefore, the estimates of UN in their study was an indirect measure of balance between the N intake and utilization and output. In indoor feeding trials, Reed et al. (2015) derived a prediction equation for UN independent to MUN, directly as the balance between N intake, and N utilization and outputs (faecal N, N in milk, retention of N in the body, estimated as a function of liveweight gain or loss). The partitioning of dietary nutrients for different processes is genetically driven (Friggens and Newbold, 2007) and specific to each animal, therefore, it seems more appropriate to use these equations for estimating UN, as performed in our study, rather than a direct conversion of MUN into UN, as assumed by Beatson et al. (2019).

Some authors have reported a negative relationship between MUN and NUE (Jonker et al., 1998) and a negative relationship between NUE and IN (Castillo et al., 2000; Kebreab et al., 2001). Therefore, the cows selected in the index with a negative EV for MUN would result in improved NUE. Although, the NUE increased in the $\mathrm{MUN}_{\mathrm{RE}\ -20\%}$ scenario at reduced NI, the level of production including volume and constituents of milk also reduced compared to cow selected under $\mathrm{MUN}_{\mathrm{RE}\ 0\%}$ scenario. The reduction of yields of milk and milk constituents are explained by the strong positive genetic correlations between yields of milk constituents and volume of milk (Sneddon et al., 2015; Lopez-Villalobos et al., 2018) and positive genetic correlation between MUN and volume of milk (Lopez-Villalobos et al., 2018; Beatson et al., 2019). This attenuation of milk production is not desirable in terms of farm profitability, because price of milk in New Zealand is a function of milk constituents.

Considering the overall economic impact to the New Zealand dairy industry, neither the negative ($\text{MUN}_{\text{RE -20\%}}$) nor positive ($\text{MUN}_{\text{RE +20\%}}$) selection scenarios were favourable compared to the $\text{MUN}_{\text{RE 0\%}}$ scenario owing to the reduced production of milk constituents and increased MY and LWT, respectively. However, the EV of MUN was not considered when estimating the overall economic responses under the selection scenarios due to uncertainty of the true EV for MUN. The true EV for MUN should be estimated as a combination of benefits (reduced N leaching and greenhouse gas emission) and costs (supplementary feeding and evaluation of sires and dams) associated with reduced MUN.

The current selection scheme for the genetic improvement of New Zealand dairy cattle combines genomic selection and progeny test based on both own performance and genomic information of animals for all traits including in the aggregate breeding value. Schefers and Weigel (2012) documented the advantages of including genomic selection as a selection strategy. According to their estimates, the genetic change per year under genomic selection is double in comparison to the genetic change per year achieved through a conventional progeny testing selection scheme. However, setting up the **P** and **G** matrices for combined breeding goal comprised with multiple traits, considering the covariance between performance records and genotypes for all the traits is very complex (Konig and Swalve, 2009) and was not implemented in the current study. Instead, standard selection index theory (Hazel, 1943) with a conventional progeny test was simulated to determine responses to various selection indices including MUN. Although the CRs might have been improved if genotypic information were incorporated due to the reduced generation interval, the direction of response to selection is unlikely to change as it depends on genetic variation of the trait and the genetic correlation with other traits in the index (Komlósi et al., 2010).

6.6 Conclusion

New Zealand dairy cows can be selected to reduce MUN through a conventional breeding programme. The correlated response when applying a negative EV for MUN is not favourable for fat and protein yields, and the income of the farmers would be negatively affected due to reduced milk constituents in attempting to reduce UN. Compared to the current selection index, the sequential selection of bulls for low MUN breeding values would cause the New Zealand national dairy herd to comprise cows with lower Breeding Worth, affecting future milk fat and protein production of the country. Cows with the genetic potential to produce greater MUN do not necessarily increase N leaching on a per hectare basis. This is because N leaching is positively associated with SR and SR needs to be reduced with increased DMI of improved cows to ensure the cows receive adequate ME. Consequently, the UN excretion of cows selected with positive EV for MUN is reduced on a per hectare basis. However, the predicted reduction in UN excretion per cow is marginal even under greater negative relative emphasis for MUN and also results are not realised until several years into the future. Therefore, other options such as feeding should get more attention in controlling N excretion rather than relying on genetic selection for reduced MUN.

Chapter 7

General discussion and conclusions

The major findings, limitations, and directions for future research for animal welfare traits together with environmental traits were discussed.

The selection index for New Zealand dairy cows is called Breeding Worth (BW), it is economically oriented with little emphasis on animal welfare and environmental traits (NZAEL, 2019). However, because of the seasonal pastoral-based outdoor management practiced in New Zealand, there are welfare and environmental issues which could be addressed in future selection indices.

Dairy cows fed outdoors are liable to be exposed to extreme weather conditions (Bryant et al., 2007; Robinson, 2015). There is evidence for attenuated milk production (Bryant et al., 2007; Fisher et al., 2008) and liveweight loss (Morris et al., 1989) in cattle of New Zealand in summer months, suggesting that New Zealand cows may experience heat stress (HS) in the summer. Environmental temperatures of New Zealand are expected to increase in the 21st century in response to global climate change as a result of greenhouse gas (GHG) emissions (MfE, 2018). Despite the predicted rise in environmental temperatures, and published literature on the negative effects of environmental stress on dairy cattle production under New Zealand pastoral-based systems, not much attention has been paid on evaluating the genetic basis of HS of dairy cows in New Zealand.

New Zealand dairy cows feed on pasture containing crude protein (CP) levels ≥20% excrete a higher proportion of nitrogen (N) in urine (UN) compared to the cows feed on total mixed rations (TMR) due to an imbalance of CP to fermentable carbohydrates (DairyNZ, 2020). The direct expulsion of excreta (urine plus faeces) by cows grazed outdoors, together with the excessive application of N fertilizer on pasture (McElroy et al., 1976), are closely associated with the release of nitrous oxide (N_2O) and N leaching. Nitrous oxide is one of the main GHG which is responsible for ozone layer depletion (Ravishankara et al., 2009) and global warming

(Kroeze, 1994). The evaluation of possible genetic tools for reducing the environmental impact of UN excretion has begun in the New Zealand dairy industry (CRV Ambreed, 2020; DairyNZ, 2020), given that feeding cows with TMR is expensive and the controlled distribution of excreta in outdoor systems is unlikely.

The aim of this thesis was, initially, to evaluate the genetic basis of heat tolerance (HT) of New Zealand dairy cows managed under pasture-based outdoor conditions. The main hypotheses were that the high rectal temperature (RT) is reflective of HS, and that there is sufficient genetic variance in RT of cows during typical summer conditions in New Zealand to enable selection for HT cows. The initial specific objectives were:

1. To estimate the genetic parameters of RT under HS along with other milk production, somatic cell score, and liveweight traits.
2. To identify genomic regions or genes associated with RT at heat stress through genome-wide association studies (GWAS).
3. To evaluate the correlated response in milk production and liveweight traits when the selection index included HT breeding values.

To address these objectives, 776 mixed-breed cows at two Massey University experimental dairy farms were measured for RT on two hot summer days during which the maximum day-time temperatures were expected to be ≥25℃. The estimated genetic variance of RT of these cows was very low because the environmental temperature was not high enough to produce HS in the cows. Due to the failure of this experiment, the overall aim of the thesis was diverted to evaluate the genetic basis of milk urea (MU) and efficiency of crude protein utilization

(ECPU) in lactating dairy cows. To achieve this aim, the following specific objectives were proposed:

1. To estimate genetic parameters of MU and ECPU along with other milk production, liveweight, and body condition score traits for each day of lactation.
2. To identify genomic regions or genes associated with MU and ECPU together with milk percentage traits through GWAS.
3. To evaluate the correlated response in milk production and liveweight traits in a selection index including milk urea nitrogen breeding values with different relative emphasis.

This chapter presents a concise summary of findings of the thesis together with applications of the findings, limitations of the study and directions for future studies to determine the animal welfare and environmental traits under New Zealand circumstances. Finally, the main conclusions drawn from the thesis are presented.

7.1 Major findings and their applications

The use of a random regression model in chapter 4 allowed variation in environmental and genetic effects that influence traits over the lactation period to be accounted for. The identification of days in milk with different genetic effects demonstrates the potential to change the shape of the lactation curve to be suitable for the particular farming system. The estimated strong and positive genetic correlations between ECPU and milk yield traits throughout lactation signify that selection for milk yield traits would improve ECPU of cows. Mostly negative genetic correlation between MU and ECPU for much of the lactation period indicates that selection for less MU also would improve ECPU of cows. The average across lactation estimates of genetic parameters recorded in this study were comparable with the estimates in published reports (Li et al., 1998; Sneddon et al., 2015; Beatson et al., 2019).

The majority of the genes identified in chapter 5, using either single-locus or multi-locus Bayes C approaches, that were associated with percentages of fat (FP) and crude protein (CPP) had been identified as good candidate genes in earlier GWAS (Cohen-Zinder et al., 2005; Ibeagha-Awemu et al., 2016; Wang et al., 2020). Although the CEBPD gene was not reported in previous GWAS in relation to FP, the gene is known to enhance synthesis and storage of fat (GO:0045444). The genes RPS12, GRIN2C, EIF3D, and ADAM11 were associated with CPP in the current study but were not identified in earlier GWAS. However, these genes are reported to be functionally associated with either protein synthesis or catabolism. Multi-locus Bayes C GWAS identified several genes associated with MU which were functionally related to the physiological regulation of protein metabolism, the urea cycle and urea excretion. Genes associated with ECPU had previously been identified as good candidates for yields of milk, fat, protein, and percentages of fat and protein (Jiang et al., 2016). An absence of GWAS for MU and ECPU meant it was not possible to corroborate findings from the current study.

Chapter 6 explored the correlated responses for milk production, milk urea nitrogen (MUN) and UN on per cow and per ha bases in selection indexes including MUN with different relative economic weights. There was only a slight difference in the annual kg of UN excreted per hectare between cows selected for less MUN and not selected for MUN. The annual per hectare excretion provided a holistic overview of N leaching, as it accounted for changes in stocking rate. This outcome suggested that alternative approaches, such as feeding management of cows and application of N inhibitors on soil to stabilize fertilizer and improve fertilizer use efficiency of pasture are required to reduce the negative environmental impacts of N leaching.

7.2 Limitations of the study

7.2.1 Inadequate ambient stresses

The h^2 estimate for RT for cows analysed in the current study was very low because of the almost nil genetic variation in RT. This result may have been greatly affected by the ambient temperatures during the sampling days. The aim was to measure RT when the cows were experiencing some level of HS, and this was not achieved in this experiment. Future studies examining the effect of HS on the production and reproduction traits of dairy cows in New Zealand should ensure that there is enough environmental stress on the cows to make them heat-stressed. Induction of stressful conditions artificially using methods such as heat chambers could be an option for this, however, the feasibility of this method largely depends on the funds available as the method is costly. Furthermore, the number of animals that can be studied under this experimental design will likely be limited which will affect the statistical power of the study.

7.2.2 Genome-wide association studies with small sample size

The cattle populations examined in the current study were small for a GWAS study. However, this was determined by the number of cows in the Massey University herds and the funding available. A power analysis estimates the effective sample size required for each trait of interest and should be undertaken during the trial design phase.

In Bayesian regression, the marker effects are estimated from the samples taken from the posterior distribution which is a function of user defined prior assumptions on markers and information derived from the data. However, the prior assumptions are known to be less influential when the sample size is large enough (Garrick and Fernando, 2013). This reveals the importance of a large sample size when the intention is to carry out GWAS for novel traits where the prior distribution of marker effects is unknown. Loci with larger effects can be detected with several hundred genotyped animals (Garrick and Fernando, 2013). Testing for associations of common loci (minor allele frequency ≥ 0.25-0.5) with the phenotype can be done with a smaller sample size compared to associations of less common loci (minor allele frequency ≤ 0.05) (Hong and Park, 2012) Therefore, the general recommendation is to use at least 1,000 genotyped animals in GWAS (Garrick and Fernando, 2013). This is because the effect size of a quantitative trait is defined as the proportion of phenotypic variance attributed to loci for traits of interest. The total variance might distribute over a large number of loci with each locus contributing only a minute proportion of the phenotypic variance. Therefore, only a large sample size would allow the detection of the majority of the associated loci, which will increase the proportion of phenotypic variance explained. The requirement of a larger sample size to detect loci with small effect is the most likely reason for the limited success in identifying genes or genomic regions for MU and ECPU traits in the current study. The identification of genes related to FP and CPP in this study, however, signifies that the sample size was good enough for identification of regions affecting traits other than MU and ECPU.

7.3 Future work

7.3.1 The economic value for MUN

Breeding values for milk yield (MY) and liveweight (LWT) are included in the current New Zealand dairy industry selection index with negative economic values (EV). Increased MY will cause greater costs associated with transportation and processing of milk for extraction of milk solids. Similarly, the heavier the cow the higher the feed costs for maintenance and grazing activity of the cow. Higher MY and heavier LWT of cows are associated with extra costs that need to be covered by the industry and/or the farmer. Similarly, there are environmental and lost opportunity costs associated with N excretion but, because there is currently no cost to farmers, there is no pressure on them to decrease N excretion by their cows. One way to address this is a charge to be imposed on farmers for the amount of N excreted onto their land as already practiced in some European countries including Finland, Sweden, Norway and Austria (Rougoor and van der Weijden, 2001). By estimating economic importance through calculated lost opportunity cost related to N excretion and tax to impose on amount of N excreted, the environmental traits could be considered as economic trait.

In order to select animals that are superior for production while being less environmentally hostile, the BW index should ideally be modified to include a breeding value for UN with an appropriate EV. However, given the fact that measuring UN of cows is very intensive and difficult to measure under the free-grazing conditions in New Zealand, MUN can be used in a selection index as an indicative trait assuming a high genetic correlation between MUN and UN owing to the known highly positive phenotypic correlation.

An EV for UN should be estimated based on all important costs and benefits associated with the expression of the trait. The cost of conversion of ammonia to urea in the liver is known to be 0.05 MJ ME/g of excess N excreted (Van Soest, 2018) which equivalent to a loss of 50 MJ ME/kg of excretion. The cost of 1 kg pasture dry matter in New Zealand can be assumed at NZ$ 0.18 with a content of 11 MJ ME, therefore, the value of 1 MJ ME is assumed to be NZ$ 0.016. The estimated lost opportunity cost associated with per kg of N excretion was NZ$ 0.80 (50 MJ ME/kg × NZ$ 0.016/MJ ME).

The Ministry for the Environment (2005b) suggested a tax of NZ$ 19.00 per tonne of carbon dioxide equivalent nitrous oxide (CO_2e N_2O) emission for the year of 2021 in New Zealand. The average, annual, total emissions of methane (CH_4) and N_2O from a dairy cow excreta are 2.5 t CO_2e (MfE, 2005b) and the average, annual emission of N_2O from excreta of dairy cow is approximately 0.5 t CO_2e, based on one fifth of the total emission ($CH_4 + N_2O$) being contributed by N_2O (MfE, 2020). Therefore, the per cow/year taxation associated with N_2O emissions based on González-Recio et al. (2020) is equivalent to NZ$ 9.5 (NZ$ 19.00 per t × 0.5 t). The average annual UN production of a cow in the base year is 56.5 kg (estimated in chapter 6), therefore, the tax estimated per kg of UN excreted is NZ$ 0.17/kg (NZ$ 9.5/56.5 kg). Assuming the average SR to be 2.8 cow/ha, the tax per hectare N excretion was calculated to be NZ$ 26.9/ha/yr (NZ$ 0.17/kg × 2.8/ha × 56.5 kg/yr).

Different to New Zealand, the European Union has imposed a tax aiming to reduce N pollution from N fertilizer use by farmers on their dairy lands (Rougoor et al., 2001). The tax is imposed as a percentage of per kg of N fertilizer price and rates vary among countries from 10% to 72% depending on how much reduction in N fertilizer use is desired by each country (Rougoor and van der Weijden, 2001). The price of kg of N fertilizer (ammonium nitrate) is € 0.2 (AHDB, 2021) and assuming 50% tax per kg of N fertilizer price is imposed, then the estimated tax per kg of N fertilizer was estimated to be € 0.1/kg (€ 0.2 × 0.5) which is equivalent to NZ$ 0.17/kg (€ 1 = NZ$ 1.67). The estimated tax on per kg of N fertilizer use is equivalent to the tax estimated per kg of UN excreted by New Zealand dairy cows in this study. Assuming use of 150 kg of N fertilizer per hectare/year (Rougoor and van der Weijden, 2001) the tax per hectare of N fertilizer use was calculated to be NZ$ 25.5/ha/yr (NZ$ 0.17/kg × 150 kg/ha/yr) which is comparable with the tax per hectare N excretion by New Zealand dairy cows calculated (NZ$ 26.9/ha/yr) in this study.

These figures indicate that there is a substantial cost associated with lost opportunity and environmental impact via UN excretion and taxes that have already being used by other countries are comparable with estimated taxes in the current study, suggesting that a higher relative emphasis should be allocated when estimating the EV for UN. This justifies the higher relative emphasis imposed on EV of MUN in the current study.

If MUN is included into the selection index with negative EV as carried out in this study, the overall farm revenue declines due to a reduction in fat and protein production (chapter 6). Suppose that, there are policies for taxation of farmers for the possible environmental impacts associated with N leaching, then there is a price to pay for the milk with higher MUN concentrations. In this situation, the loss of profit associated with reduced milk solids by including MUN in the selection objective and index results in a trade-off with the reduction of cost associated with taxation for N leaching. However, this is realistic only if there is legal pressure imposed by the government on controlling N leaching. In addition it requires a breeding objective with MUN added along with an appropriate EV and an accepted system for evaluating of MUN concentrations for all dairy farms in New Zealand.

7.3.2 Other approaches of reducing nitrogen leaching

The results of this study suggest that selection for low MUN concentration will only very slowly reduce N leaching at the per hectare level, therefore, dairy farmers will require non-genetic solutions in the immediate future. A recent New Zealand study (Kennett et al., 2020) elaborated that the genetic selection for urine traits is a less effective tool for reducing N leaching where the stocking rate and N intake of cows are high. However, they have not estimated the correlated response using selection index in their simulation. Rather, they have modelled total urine volume and volume per urination to quantify the impact of stocking rate on N leaching. The current study however is not in full agreement with their findings whether increased N intake linearly increase the N leaching. This is because, UN excretion does not totally depend on N intake, but represents the balance between N intake and outputs (milk, LWT, faeces) and is unique to each cow; efficient cows convert more N consumed into saleable products whereas inefficient cows divert more N intake into UN (Burgos et al., 2007).

Positive outcomes of feeding management have long been discussed as having a potential of reducing N excretion by improved nitrogen utilization efficiency (NUE) (Baker at al.,1995; Jonker et al.,1998; Castillo et al., 2000) and numerous studies have evolved in New Zealand over the last few years. Feeding cows with pasture mixtures including herbs and legumes besides ryegrass grass and white clover only diets has been identified as an option (Totty et al., 2013; Edwards et al., 2015). The use of plantain in proportions greater than 30% of the total dry matter of the diet has been widely studied (Minnee, 2017; Minnée et al., 2020) and has been suggested as a solution for reducing N leaching. Selective feeding of pastures might be less expensive than concentrate feeding, but this is also less practical.

Roche et al. (2016) have elaborated the influence of increased stocking rate on reducing UN excretion which was achieved through restricted voluntary feed intake and involuntary crude protein intake (CPI). However, under the management they proposed in their study, the reduction of N excretion at herd level was a trade-off in attenuated days in milk and milk production at the cow level, offering an opportunity to decide if reducing UN was vital over the profit from milk production.

Another concept that has been recently put forward by LIC (2020a) is that herds comprised of a higher proportion of 5th lactation cows excrete less N and emit less methane while increasing milk production compared to younger cows that are in 4th lactation. Herds with low replacement rates, reduce the replacement cost because the financial inputs on rearing replacement heifers is minimized. Moreover, the low replacement herds increase the yields of milksolids (fat+protein) production as the mature cows produce more milksolids than young heifers in high replacement herds, leading to higher farm profitability in low replacement herds (Lopez-Villalobos and Holmes, 2010).

7.3.3 The relationship between milk urea nitrogen and urinary nitrogen

In this study the UN excretion of cows was estimated either as a function of dry matter intake (DMI), LWT and MUN or as a balance between N intake and N outputs (milk, faeces, liveweight). The N intake was estimated as a measure of daily DMI and daily CPI. However, neither daily DMI nor daily CPI was measured directly, instead daily DMI was estimated and crude protein of the diet was taken from published reports in New Zealand and daily UN excretion was estimated assuming that cows were fed with pasture only. However, cows competing each other in free grazing may not consume sufficient amount of dry matter to fulfil their metabolizable energy requirement. Given the fact that the estimated DMI might be biased, cows in reality might excrete a different level of UN than predicted based on estimated DMI. Even though, grazing remains the major method of feeding for New Zealand dairy cows, they are supplemented with crops and concentrates during winter and summer months to a certain extent (Harris, 2005). A greater NUE is expected in cows that are fed with a mixture of diet, due to the higher ratio of fermentable carbohydrates to dietary crude protein percentage (Baker et al., 1995) and higher level of ruminally undegradable protein (Minnée et al., 2020) available in concentrates and crops, respectively. Therefore, the cows might excrete lower UN than they are expected to excrete when fed with pasture only diets as assumed in this study. This may also be responsible for higher rates of fecal N (FN) excretions when FN was estimated as the balance between the N intake and N outputs including estimated UN as implemented in this study but higher FN in these cows does not necessarily indicate lower digestibility of N from pasture intake.

The UN excretion of the cows under each scenario was estimated using the phenotypic relationships estimated by Huhtanen et al. (2015) and Reed et al. (2015). Spek (2013) presented another prediction equation to estimate UN in dairy cows using dietary crude protein, independent to MUN. Figure 7.1 shows the daily UN excretion of the base cow estimated using the prediction equation of Huhtanen et al. (2015) and Reed et al. (2015) in comparison to that estimated using the relationship presented by Spek (2013). The daily estimates of UN excretions produced in the prediction equation derived by Spek (2013) were subsequently greater than that of combined prediction equations of Huhtanen et al. (2015) and Reed et al. (2015).

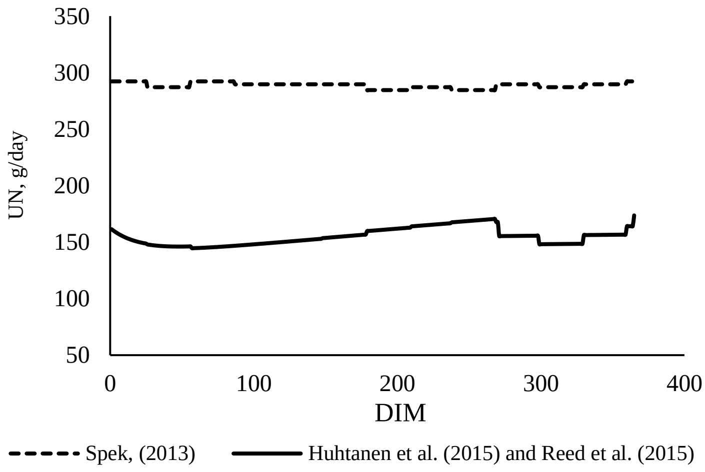

Figure 7.1. Urinary nitrogen (UN) excretion at each day in milk (DIM) estimated using the combined prediction equations of Huhtanen et al. (2015) and Reed et al. (2015) (——) versus Spek (2013) (- - - -) for cows in the base year.

If the relationship presented by Spek (2013) was used instead of a combination of Huhtanen et al. (2015) and Reed et al. (2015) equations, the level of N excretions (urine+faeces) under each scenario in this study would have been about 100% higher than the estimates used due to the substantial difference in daily UN estimates predicted by two methods. It is noteworthy to mention that each prediction equation is a representation of a herd analysed in the corresponding study under that genetic background, management, and environmental circumstances, therefore, use of a prediction equation estimated in one production system and used in another production system is likely problematic. Moreover, the current study assumed that reducing MUN by feeding has a similar effect on UN to reducing MUN by breeding as Beatson et al. (2019) assumed in their modelling to predict the reduction of N leaching in replacement heifers bred to Low N Sires. However, greater phenotypic correlation reported between MUN and UN (Jonker et al., 1998; Kohn et al., 2002; Broderick, 2003; Hendriks, 2016) does not necessarily indicate a greater genetic correlation between MUN and UN. Therefore, the genetic correlation between MUN and UN is required to estimate the actual reduction of UN excretion through selection for low MUN. Marshall et al. (2020) reported that cows of high MUN breeding values had higher UN excretion, which suggest that the genetic correlation between the two traits is positive in New Zealand dairy cows, therefore, selection for low MUN enables reducing UN excretion.

The genetic correlation between UN and milk production and reproduction traits are needed for an effective evaluation of the impact of selecting for low N excretion on production and reproduction in dairy cows. DairyNZ in collaboration with New Zealand Animal Evaluation Limited (NZAEL) has initiated a research programme to perform a genetic analysis of UN and N utilization. The amount of urine and concentration of UN will be measured using automated urine sensor technology, together with blood urea N and MUN over 1,000 grazing cows. Identification of quantitative trait loci and estimation of genomic breeding values for these traits are among other aims of their research (DairyNZ, 2020c). Livestock improvement corporation (LIC, 2020b) has also implemented a new index called “hoofprint” where dairy bulls are ranked on the UN excretion and methane emission. These research projects provide a glimpse where the BW indices are heading.

7.4 General conclusions

The hypothesis raised in chapter 3 was that the New Zealand dairy cows experience heat stress during the typical summer. Milk urea and ECPU traits were heritable as hypothesized in chapter 4, therefore, genes associated with these environmental traits were investigated and correlated responses for milk production and LWT by selection for MUN were evaluated in the subsequent chapters. The main conclusions were:

1. Dairy cows in the Manawatu area are unlikely to be affected by severe heat stress for prolonged periods during the typical summer conditions. Results may be different if a heat stress study is conducted in the summer of the Northland area, in which maximum temperatures reach 30℃ during the hottest days of summer (December to February).
2. Both MU and ECPU traits are heritable and the degree of heritability is variable through the lactation providing breeders an opportunity to change the shape of the trait lactation curve as optimum for the agricultural system.
3. Selection of cows for higher milksolid yields is predicted to improve the ECPU due to its highly positive genetic correlation with yields of fat and crude protein.
4. Genes identified to be significantly associated with MU are functionally related with protein utilization and N excretion. If these associations are confirmed in larger studies, there is an opportunity to provide a commercial

genetic marker service to improve the protein utilization and reduce N excretion of dairy cows by genomic selection.

5. Genetic approaches to reducing N leaching are less effective at the herd level as stocking rate has a greater influence. Reducing the intensification of dairy farming, reducing crude protein intake of cows by feeding them with improved pasture and crops are more effective alternatives of reducing N leaching and carbon footprint in the immediate future.

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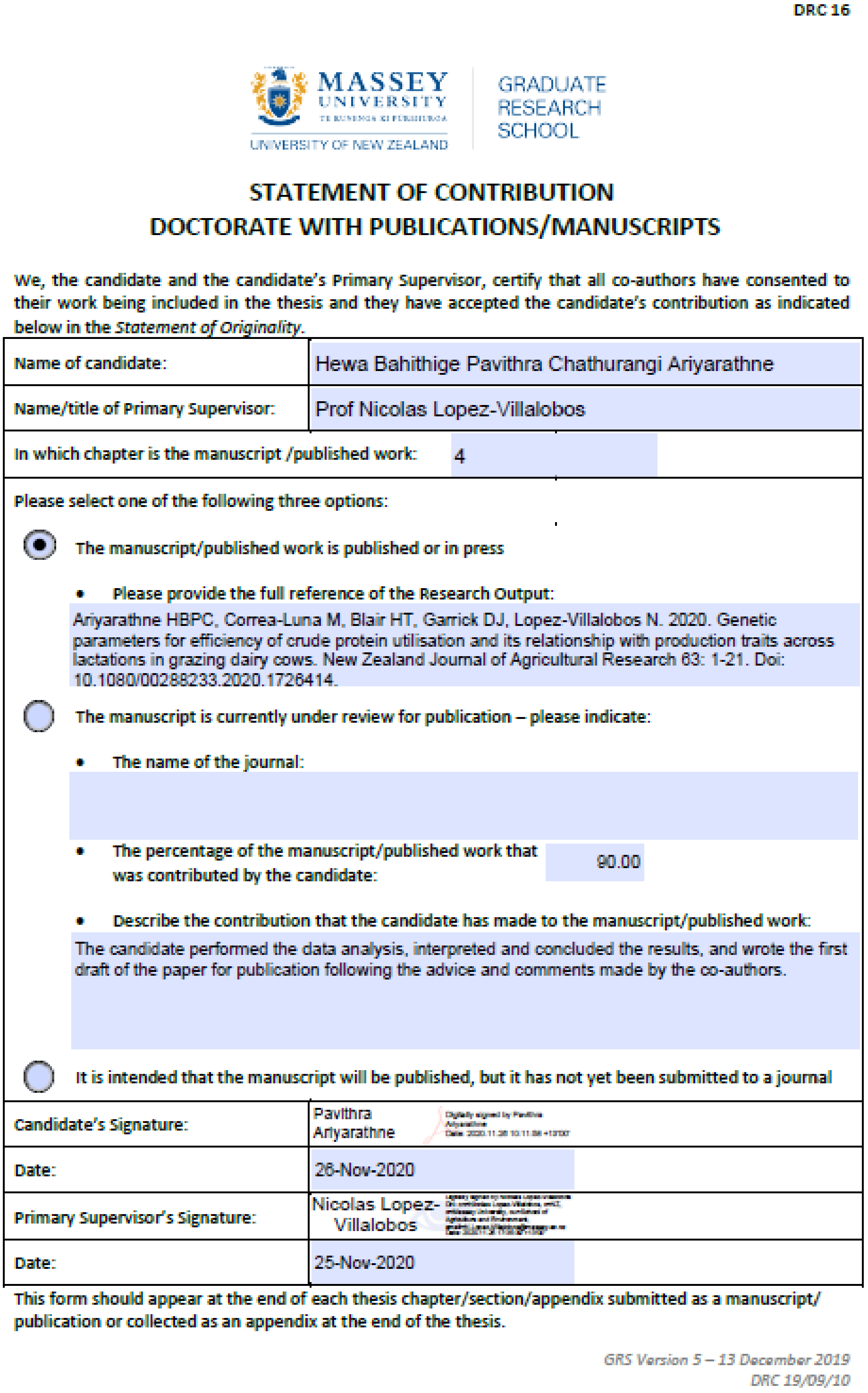
Appendix three

Statement of contribution to doctoral thesis containning publications for chapters 4, 5, and 6.



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Ariyarathne HBPC, Correa-Luna M, Blair HT, Garrick DJ, Lopez-Villalobos N. 2020. Genetic parameters for efficiency of crude protein utilisation and its relationship with production traits across lactations in grazing dairy cows. New Zealand Journal of Agricultural Research 63: 1-21. Doi: 10.1080/00288233.2020.1726414.

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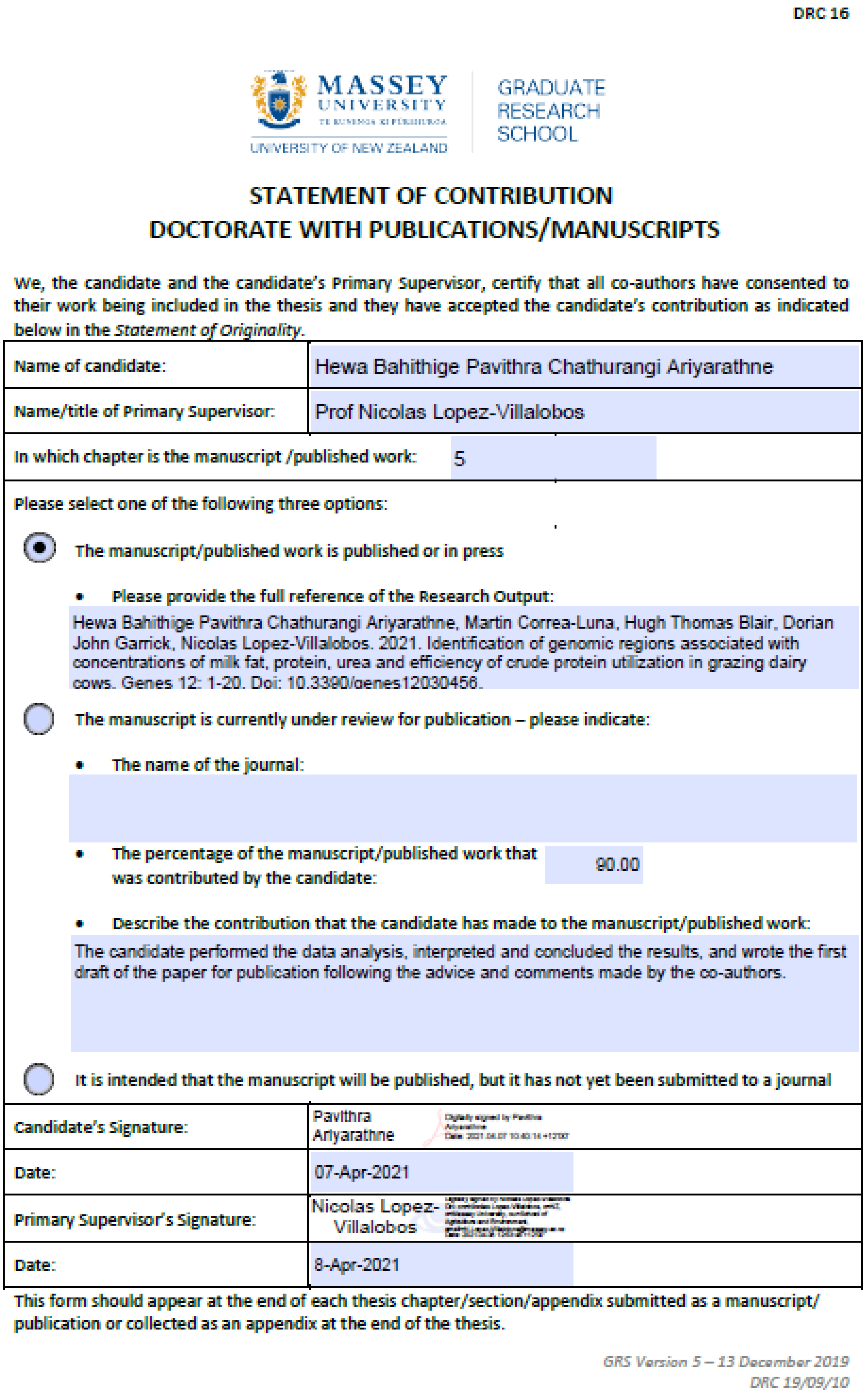

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Hewa Bahithige Pavithra Chathurangi Ariyarathne, Martin Correa-Luna, Hugh Thomas Blair, Dorian John Garrick, Nicolas Lopez-Villalobos. 2021. Identification of genomic regions associated with concentrations of milk fat, protein, urea and efficiency of crude protein utilization in grazing dairy cows. Genes 12: 1-20. Doi: 10.3390/genes12030456.

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Hewa Bahithige Pavithra Chathurangi Ariyarathne, Martin Correa-Luna, Hugh Blair, Dorian Garrick, Nicolas Lopez-Villalobos. 2021. Can nitrogen excretion of dairy cows be reduced by genetic selection for low milk urea nitrogen concentration? Animals 12: 1-20. Doi: 10.3390/ani11030737.

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