Genetic correlation between milk urea nitrogen and reproductive performance in seasonal grazing dairy cows

A thesis presented in partial fulfilment of the requirements for the degree of

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Abstract

Milk urea nitrogen (MUN) concentration is closely related to blood urea nitrogen concentration and is an indicator of the level of crude protein ingested by the cow. Studies with cows in indoor systems have reported an antagonistic phenotypic relationship between MUN and fertility traits, but no studies have reported estimates of genetic correlation between MUN and fertility traits in pasture-based systems. The objective of this study was to estimate genetic parameters for MUN concentration, fertility and production traits in New Zealand dairy grazing cows from two herds. Milk test records were collected from 637 cows from once-a-day and twice-a-day milking dairy herds from Massey University, Palmerston North, New Zealand, during the production seasons of 2016-17 and 2017-18. The average concentration of MUN ranged between 8.2 and 11.4 mg/dL with estimates of heritability and repeatability of 0.24 (0.09 SE) and 0.45 (0.05 SE). There were no significant heterosis nor breed effect for MUN. The estimates of genetic correlations between MUN and fertility traits (submission and pregnancy rate during the first 21 days after the start of mating) were moderate negative (-0.55 and -0.45), but the standard errors of the estimates were large due to the small data set. The phenotypic correlations were close to zero. The estimates of genetic parameters indicate that MUN concentration in milk can be reduced by genetic selection with a potential to increase the submission and pregnancy rate during the first 21 days after the start of mating, which are the two most important reproductive traits in grazing dairy cows. Further studies with a larger dataset would enable more accurate estimates of the genetic parameters.

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List of Abbreviations

AAs	Amino acids
AI	Artificial insemination
BUN	Blood urea nitrogen
CFS	Days from calving to first service
CI	Calving interval
CP	Crude protein
CPP	Crude protein percentage
CR42	Calving rate by day 42 after start of planned calving season
DDG	Dried distillers grains
DM	Dry matter
DMI	Dry matter intake
DO	Days open
F	Holstein-Friesian
FP	Fat percentage
FSCO	Days from first service to conception
FSH	Follicle stimulating hormone
FxJ	Holstein-Friesian x Jersey crossbred
FY	Fat yield
GH	Growth hormone
GL	Gestation length
GnRH	Gonadotropin-releasing hormone
IGF-1	Insulin-like growth factor 1
IR	Infra-red
J	Jersey
LH	Luteinizing hormone
LP	Lactose percentage
LW	Live weight
LY	Lactose yield
ME	Metabolizable energy
MP	Metabolizable protein
MU	Milk urea
MUN	Milk urea nitrogen
MY	Milk yield
N	Nitrogen
NDF	Neutral detergent fiber
NEB	Negative energy balance
NEFA	Nonesterified fatty acids
NEl	Net energy for lactation
NH3	Ammonia
NPN	Non proteic nitrogen
NR56	Non-return rate by day 56
NR90	Non-return rate by day 90

OAD	Once-a-day
PP	Protein percentage
PR21	Percentage of cows pregnant by day 21 after start of breeding
PR42	Percentage of cows pregnant by day 42 after start of breeding
PRFS	Pregnancy rate at first service
PUN	Plasma urea nitrogen
PxC	Plantain and chicory mix.
PxCxRC	Plantain, chicory and red clover mix.
PY	Protein yield
QDP	Quickly degradable protein
RDP	Rumen degradable protein
RDPB	Rumen degradable protein balance
r_G	Genetic correlation
r_P	Phenotypic correlation
RUP	Rumen undegradable protein
SB	Start of breeding season
SBCO	Days from start of breeding to conception
SBFS	Days from start of breeding to first service
SDP	Slowly degradable protein
SP	Interval from first to last insemination
SR21	Submission rate by 21 days after start of breeding season
TAD	Twice-a-day
TMR	Total mixed ration
WC	Wet chemistry

Chapter 1

General Introduction

Most farms in New Zealand are traditionally pasture-based twice-a-day milking systems, an average herd comprises of 435 cows, average milksolids production is 381 kg/cow and 1,081 kg/ha per season, and average lactation length is 271 days (New Zealand Dairy Statistics 2018-19). There is currently a growing interest in shifting from twice-a-day to once-a-day milking due to the lifestyle benefits involved (Bewsell et al. 2008). However, once-a-day milking systems have been reported to reduce milk solids production per cow by 29.4% for Holstein-Friesians and by 19.9% for Jerseys. Milk production per hectare also reduced by 17.7% and 9% for Holstein-Friesians and Jerseys, respectively. On the other hand, cows milked once-a-day were shown to have a better reproductive performance as they conceived 3 days earlier, had a 5 days shorter calving to conception, and needed 11% fewer controlled internal drug release devices than those milked twice-a-day (Clark et al. 2006).

In seasonal pasture-based systems, cows calve during a specific time of the year to align the seasonal pasture growth with cows feed demand throughout lactation. This requires a calving interval of 365 days. Therefore, fertility and reproductive performance are important for production efficiency and for the genetic progress of these systems. Having high submission and pregnancy rates in a short period of time is essential for achieving a concentrated calving pattern (Grosshans et al. 1997).

Milk urea nitrogen is an indicator of level of crude protein (CP) ingested by the cow. Crude protein measures the protein content of feed and is assumed to contain 16% of nitrogen (NRC 2001). New Zealand pastures can vary from 9 to 35% of CP, and dairy cows require 14-20% CP throughout lactation (Burke 2004). Studies have reported increased milk yield with increased CP, and some have reported increased dry matter intake (NRC 2001). However, there has been some controversy whether protein in excess of lactation requirements negatively affects cow fertility and production (Butler 1998). Urea and ammonia are the end products of nitrogen metabolism and are reported to have direct effect on oocyte, uterus, spermatozoa and indirect effects on the reproductive axis as a result of the energy cost of urea metabolism that lowers the nadir of negative energy balance (Dietz and Flipse 1969; Sinclair et al. 2000b; Tamminga 2006; Amundson et al. 2016; Ibtisham et al. 2018).

Previous studies have shown a negative phenotypic relationship between milk urea nitrogen and fertility in dairy cows fed total mixed ration (Butler et al. 1996; Larson et al. 1997; Melendez et al. 2000; König et al. 2008; Cutzal 2019). Whereas some have shown a positive (Mikkola et al. 2005) and others have shown a weak relationship between high CP/MU/MUN

and fertility of dairy cows in total mixed ration (Hossein-Zadeh and Ardalan 2011; Mucha and Strandberg 2011) and in grazing systems (Trevaskis and Fulkerson 1999; Kenny et al. 2001).

Excessive levels of nitrogen in farm systems is also the main cause of contamination of surface water and groundwater which is of environmental concern (Tamminga 1992). So, controlling the amount of nitrogen in the feed and controlling the use of N fertilizers is also a matter of sustainability of these systems.

The observed phenotypic relationship between two or more variables is a combination of the genetic and environment correlations (Falconer 1960). Some studies conducted overseas have estimated the heritability of milk urea (MU) or milk urea nitrogen (MUN) and described the genetic correlation with several reproductive traits and production traits to evaluate the applicability of MUN to support selection for fertility traits or production (Stoop et al. 2007; König et al. 2008; Hossein-Zadeh and Ardalan 2011; Mucha and Strandberg 2011; Rzewuska and Strabel 2014). However, the genetic correlation between MUN and fertility traits has not been described for New Zealand dairy systems, where dairy cows have been highly selected for production and fertility in grazing systems of low to very high levels of protein and nitrogen in pasture.

The objective of this thesis was to estimate the genetic parameters (heritabilities, repeatabilities, and genetic and phenotypic correlations) for MUN concentration and fertility and production traits in New Zealand dairy cows in pasture-based systems.

Chapter 2

Literature Review

New Zealand currently has 4.946 million cows, of which 48.5% are crossbred cows (Holstein-Friesian x Jersey), 33.1% are Holstein-Friesian, and 8.6% are Jersey (New Zealand Dairy Statistics 2018-19). The increasing use of crossbred cows is to exploit the benefits of heterosis, which has a positive impact on fertility and on production (Harris et al. 2000; Lopez-Villalobos and Garrick 2006). Most of dairy farms are seasonal pasture-based systems, as pasture is the cheapest source of feed which is needed at low milk prices (Penno et al. 1996). The activities to achieve this seasonal pattern are illustrated in Figure 2.1. Cows are managed to calve in early spring to match the period of maximum feed requirement during early lactation with the period of maximum grass growth, as 50-70% of pasture production occurs in spring (Holmes et al. 2002).

Cows are dried off in late summer or autumn, so that the reduced feed requirements of non-lactating cows coincide with winter when the growth of pasture is slow (Holmes et al. 2002). This calving concentration has been widely practiced around the country to reduce the costs of milk production, as it allows to maximise the use of the pasture grazed in situ and minimizes the need of purchasing supplementary feed (Macmillan et al. 1990). A result of this synchronisation is a shorter lactation length (220-240 days) compared with other non-seasonal dairy systems (Garcia and Holmes 1999).

A date is chosen to start the herd's seasonal breeding programme, usually in Spring between late September and early November. This decision will determine when the herd will start calving in relation to the predicted pasture supply and pasture growth (Macmillan et al. 1990; Grosshans et al. 1997). The aim is to have the entire herd calving over a short period of time (10 to 14 weeks) with a calving interval of 365 days (Verkerk 2003). Adjusting the time of the year at which calving occurs is one way of manipulating the lactation curve to supply milk to factories (Auldist et al. 2002). It is crucial to have high pregnancy rate in a short period of time after the start of the breeding season which will allow herds to have a concentrated calving pattern in the following season (Grosshans et al. 1997).

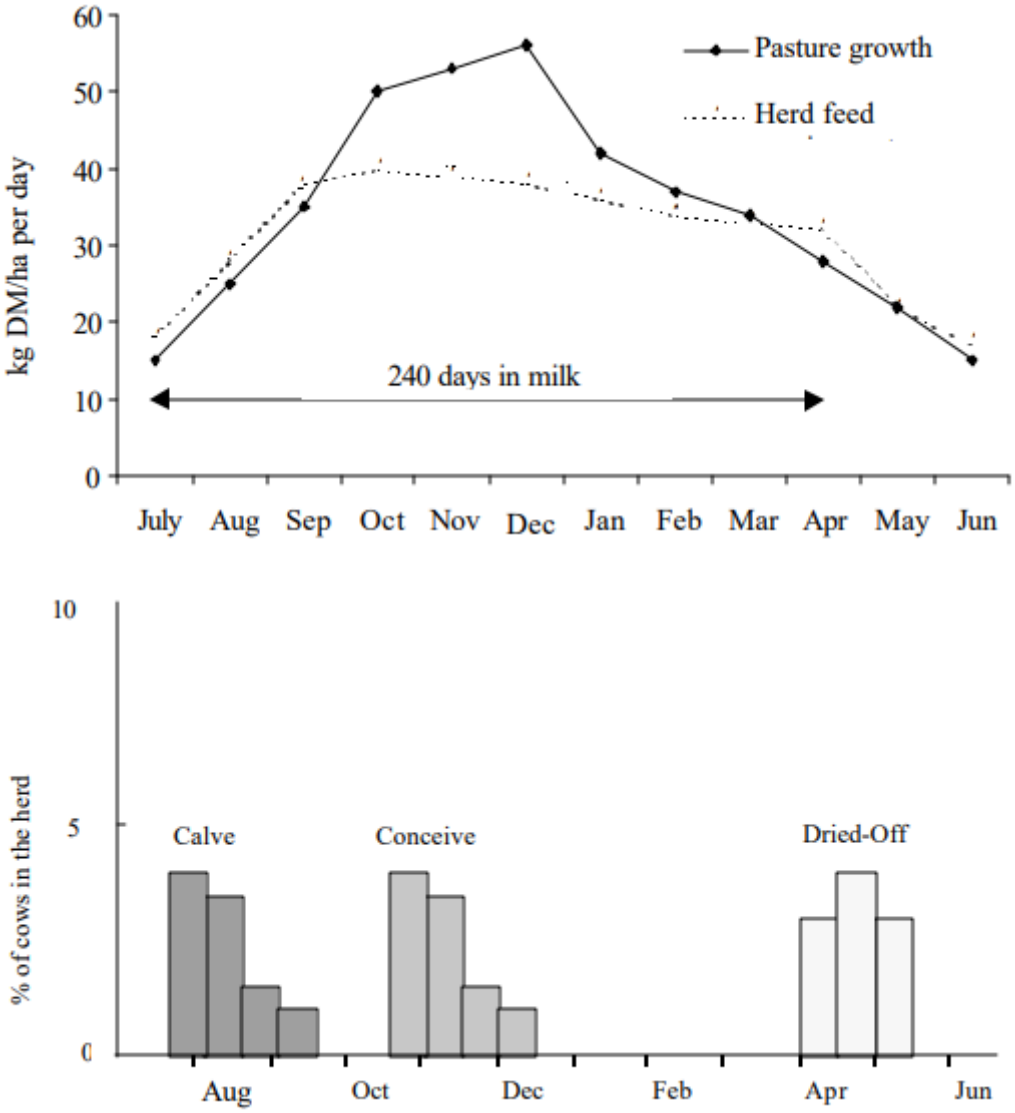

Figure 2.1. Illustration of the seasonal pattern of calving and drying-off, and the synchrony between feed requirements and pasture growth (Garrick et al. 2001).

2.1 Reproductive Performance

The aim of the reproductive system is to have submission rates to artificial insemination (AI) of 90-95% of the cows during the first 21 days after the start of the breeding season, pregnancy rate of 78% during the first 42 days, final conception rates to insemination of at least 60%, and empty rates of 12% or less (Smith et al. 2001; DairyNZ Ltd. 2017). Aiming for high submission rates in a short period of time will result in a compact calving in the following season which facilitates the management of the herd (McDougall 2006).

New Zealand dairy cows are considered to have good reproductive performance and the actual mean of 6-week in-calf rate of New Zealand dairy herd in the production season 2018-19 was 67.5%; mean 3-week submission rate was 80.7%, and mean conception rate was 54.1% which was higher than previous seasons (New Zealand Dairy Statistics 2018-19).

Prolonged postpartum anoestrus (interval from calving to first ovulation) is the main cause of poor reproduction in seasonal dairy herds and cows that have not ovulated 44 days after calving are considered to have prolonged anoestrus (Lamming and Darwash 1998). The main factors that extend the duration of anoestrus are poor body condition at calving and at post-calving, poor nutrition, parity, and periparturient diseases such as mastitis, lameness and ketosis (Rhodes et al. 2003).

2.2 Feed Requirements of Dairy Cows

Energy has been identified to be the main nutrient limiting milk production in pasture-based systems (Kolver and Muller 1998) and low energy intake can also affect reproductive performance due to the consequences of prolonged negative energy balance (NEB) (Butler 2003). However, protein also plays an important role as it can affect feed intake and can impair production when in deficiency (Edwards et al. 1980). On the other hand, excessive protein can impair reproduction and thus production because of the costs with detoxification of metabolites from protein degradation (Butler 1998).

2.2.1 Energy Requirements

Requirements of metabolizable energy (ME) for the cows increase as milk production increases and is at its highest at approximately six weeks after calving (Holmes et al. 2002). In general, a 400 kg dairy cow requires 160-180 MJME daily in early lactation and 110-130 MJME daily in late lactation (Burke 2004). The total ME requirement is estimated based on cow liveweight and on level of milksolids production and varies with the ME concentration of the diet (AFRC 1993).

$$ME_{total} = ME_m + ME_g + ME_c + ME_l + ME_a$$

ME_m = ME requirements for maintenance

ME_g = ME requirements for liveweight change (gain or loss)

ME_c = ME requirements of the conceptus (pregnancy)

ME_l = ME requirements for lactation

MEa = ME requirements for grazing and associated activity

2.2.2 Protein Requirements

Ruminants can live with low protein allowances due to their capacity of recycling urea used in saliva. On the other hand, ruminants can tolerate large amounts of protein due the liver ammonia detoxification system (Huntington and Archibeque 1999).

Protein needs for lactating cows are greatest in early lactation when milk yield production is at its highest (Huber and Limin Kung 1981). Overall, dairy cows require 18-20% of CP in early lactation, 16-18% in mid-lactation and 14-16% in late lactation (Burke 2004). Studies showing response to increased protein have generally shown higher energy intakes. An optimal level of 14-16% of crude protein was found to maintain or increase cows dry matter intake in late pregnancy (VandeHaar et al. 1999; Phillips et al. 2003).

Like the prediction of energy requirements, the protein requirement should account for maintenance and production. The protein requirements for maintenance, pregnancy, lactation and growth can be predicted as per NRC (2001). It is recommended that diets should have concentrations of rumen degradable protein (RDP) and rumen undegradable protein (RUP) and energy (NRC 2001) to reduce urea and ammonia concentrations in the ovary and uterus thus preventing negative impact on reproduction (Butler 2005). Requirements for rumen degradable protein (RDP), rumen undegradable protein (RUP), and total protein depend on animal size, diet energy, and dry matter intake (Table 2.1 and Table 2.2). In general, milk production between 18.8 and 44 kg/day requires 7.8 to 14.7% of RDP and 2.8 to 8.9 % RUP in the dry matter (NRC 2001).

Table 2.1. Daily nutrient requirements of small breed cows (LW= 454 kg) in early lactation (intake estimated at 11 days in milk). Values are appropriate for the diet with 78% Total Digestible Nutrient (adapted from NRC 2001).

Milk (kg)	Milk fat (kg)	Milk true protein (%)	DMI (kg)	LW change (kg)	NEl (Mcal)	RDP (%)	RUP (%)	CP (%)
15	4	3.0	9.4	-0.3	19.0	11.3	5.3	16.6
15	5	3.5	9.9	-0.5	20.8	11.2	6.2	17.4
30	4	3.0	12.9	-1.4	30.1	10.9	9.1	20
30	5	3.5	14.0	-1.8	33.7	10.8	10	20.8

DMI= dry matter intake.
LW= live weight.
NEl= net energy for lactation.
RDP= rumen degradable protein.
RUP= rumen undegradable protein.
CP= crude protein.

Table 2.2. Daily nutrient requirements of large breed cows (LW= 680 kg) in early lactation (intake estimated at 11 days in milk). Values are appropriate for the diet with 78% Total Digestible Nutrient (adapted from NRC 2001).

Milk (kg)	Milk fat (kg)	Milk true protein (%)	DMI (kg)	LW change (kg)	NEl (Mcal)	RDP (%)	RUP (%)	CP (%)
20	3	2.5	12.0	0	23.0	11.3	4.2	15.5
20	4	3.5	12.7	-0.4	26.0	11.3	6.5	17.8
40	4	2.5	17.4	-1.6	39.1	10.9	6.8	17.8
40	4	3.0	17.4	-1.8	40.2	10.9	8.9	19.8

DMI= dry matter intake.
LW= live weight.
NEl= net energy for lactation.
RDP= rumen degradable protein.
RUP= rumen undegradable protein.
CP= crude protein.

2.3 Nitrogen Metabolism

There are two sources of nitrogen: dietary or endogenous; and they can be proteic or non proteic (NPN). The dietary sources of nitrogen include nucleic acids, amino acids, proteins,

peptides, amines, amides, nitrates, nitrites, urea, and ammonia. Endogenous sources of nitrogen include sloughed epithelial cells and salivary urea (Huntington and Archibeque 1999).

2.3.1 Metabolizable Protein

Metabolizable protein (MP) is defined as the quantity of protein digested in the postruminal portion of the digestive tract of ruminants, in other words, it is the total amino acids (AAs) absorbed in the small intestine (Burroughs et al. 1975; Volden and Nielsen 2011). The total digestible protein (or AAs) available to the animal for metabolism comes from three sources: rumen degradable protein which is converted into microbial protein before leaving the rumen; dietary protein that escapes rumen degradation; and endogenous protein (Holmes et al. 2002; Volden and Nielsen 2011). MP requirement includes the requirement for maintenance and production which depends on the level of milk production, MP can be converted into milk protein with an average efficiency of 67% (NRC 2001).

Rumen degradable protein (RDP) is necessary for the symbiotic microorganisms in the rumen and if its intake is impaired, there is a reduced voluntary intake and digestibility. On the other hand, excessive RDP is absorbed as ammonia through the rumen wall, which must then be detoxified by the liver (Holmes et al. 2002).

Protein is degraded into peptides and amino acids by bacterial proteases and peptidases to form microbial protein, which is later absorbed in the small intestine (Leng and Nolan 1984). Bacterial protein provides essential amino acids which are not synthetized by the host such as methionine and lysine (Waghorn et al. 2007). 50 to 80% of bacterial N is derived from ammonia. The rate of flow of microbial N out from the rumen depends on bacteria concentrations in the ruminal fluid and attached to feed particles that move out of the rumen (Leng and Nolan 1984).

The rate and extent of protein degradation in the rumen depends on protein type (solubility and structure), ruminal dilution rate, ruminal pH, substrate being fermented, and predominant species of ruminal flora. The optimal pH of rumen proteolytic enzymes ranges from 5.5 to 7.0 and protein degradation reduces as pH decreases (Bach et al. 2005).

The degradation of slowly degradable protein (SDP) by ruminal microbes depends on the rate of passage through the rumen, and outflow rates are related to the level of feeding of the animal (Ørskov and McDonald 1979).

Quickly degradable protein (QDP) is released rapidly when the feed enters the rumen and its amount should be limited to up to 4% of the effective rumen degradable protein due to the risk of ammonia poisoning (AFRC 1993). However, approximately 80% of QDP can be utilised by the ruminal flora and a maximum of 20% is ammonia that is absorbed through the ruminal wall (Holmes et al. 2002). To avoid excessive ammonia production from intake of degradable protein, it is important to supply synchronous energy (carbohydrates) to increase microbial protein synthesis, which consequently increases the efficiency of N use (Sinclair et al. 2000b; Edwards et al. 2007).

Figure 2.2. Schematic representation of protein degradation and fate of end products in the rumen (Bach et al. 2005).

Another term that has been used is rumen degradable protein balance (RDPB), which is the fraction of RDP that is converted into ammonia and not to microbial protein in the rumen. RDPB % depends on the availability of degradable carbohydrates, level of CP, proportion of RDP and efficiency of microbial protein production. When the level of dietary RDP is lower than 10% the RDPB level is close to zero (Tamminga 2006).

Rumen undegradable protein (RUP) will pass the rumen degradation to be absorbed in the lower intestines (AFRC 1993) and will supply rest of metabolizable protein that was not

met from microbial protein sources. RUP digestibility depends on the type of feed and ranges from 70% to 80% for most pasture-based diets (Holmes et al. 2002).

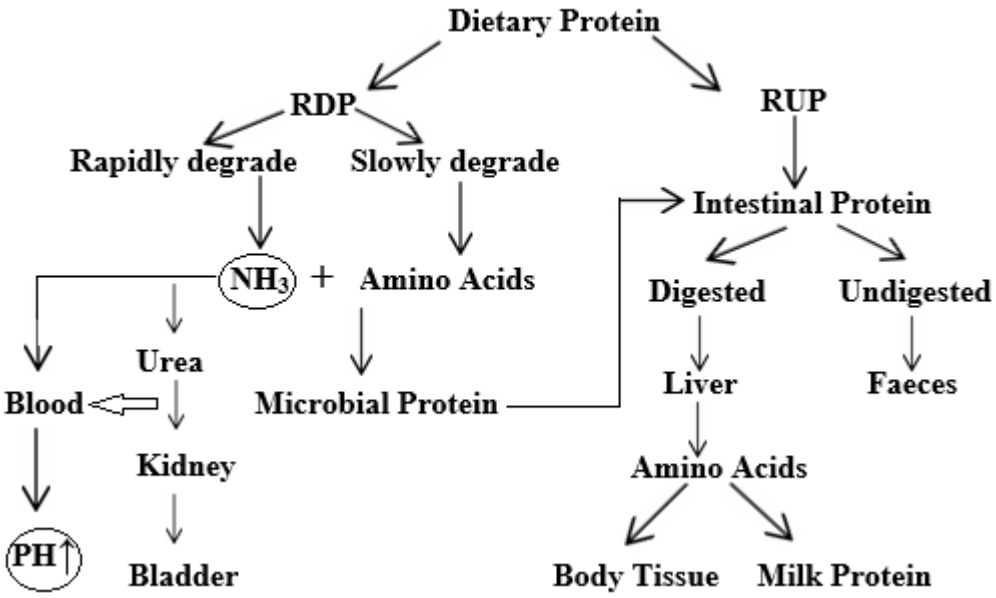

Figure 2.3. Schematic summary of protein metabolism and digestion in dairy animals (adapted from Ibtisham et al. 2018).

2.3.2 Non-Protein Nitrogen Sources

Sources of non-protein dietary nitrogen (NPN) include free amino acids, peptides, nucleic acids, free ammonia, ammonium salts, urea, biuret, nitrate, and other compounds (Huber and Limin Kung 1981). Urea enters the digestive tract and is hydrolysed into ammonia. This ammonia is then utilised by bacteria to synthesise cell constituents (and be subsequently absorbed mainly as amino acids) or is absorbed through the ruminal wall directly as ammonia (Lapierre and Lobley 2001).

Urea added to the feed is quickly degraded in the rumen, so it should be offered at a maximum of 30% of the dietary protein (Holmes et al. 2002). Amounts ranging from none to over 80% of ammonia from urea degradation are incorporated into bacterial N, and availability of energy is the major determinant of that (Huntington and Archibeque 1999).

The deamination and catabolism of amino acids from skeletal muscle and tissue protein are another source of nitrogen. Additionally, urea can be recycled in the form of salivary urea which can also be used by the ruminal flora. These are called endogenous sources of nitrogen (Huntington and Archibeque 1999).

2.3.3 Ammonia Removal

Ammonia is the main compound for protein synthesis in the rumen. It is produced from feed protein, dietary NPN, or blood urea recycled into the rumen through the saliva or rumen epithelia (Huber and Limin Kung 1981). The excess of ammonia in the blood is prejudicial due to its toxic effects and the liver is responsible for ammonia detoxification through the synthesis of urea (Huntington and Archibeque 1999).

Liver ammonia detoxification requires 13 to 19% of liver oxygen and is two-stage system where periportal and perivenous cells remove ammonia. Periportal cells synthesize urea from ammonia derived from hepatic portal blood. Whereas perivenous cells produce glutamine through glutamine synthetase. The urea that is produced is then excreted (through urine, milk) or re-enters the gut through saliva or through the gut wall (Emmanuel 1980).

Milk urea nitrogen is thus a convenient mean of monitoring protein metabolism, because it is close related to blood urea nitrogen (BUN) concentration, as urea molecules can easily diffuse through cell membranes, including the mammary gland (Butler et al. 1996; Broderick and Huhtanen 2007).

2.4 Protein Content in The Diet

New Zealand pastures are mainly composed by a mix of high-quality temperate forages: perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) (Verkerk 2003). These pastures usually have metabolizable energy (ME) concentrations above 11.5 MJME/kg DM (Dalley and Gardner 2012). In summer, these pastures can have 8-9 MJME/kg DM (Burke 2004).

Crude protein concentration can vary from 22-30% in DM of legumes (such as clover) to less than 15% in DM of grasses. Also, as grass matures, there is a reduction in CP due to a higher proportion of stem than leaf (Waghorn et al. 2007). Spring pastures can have 25-30% CP whilst in summer pastures can have less than 20% CP (Burke 2004). Overall, New Zealand pastures are high in CP and this can have negative effects on animal reproduction due to high blood ammonia/urea produced (Jordan et al. 1983). Also, nitrogen losses that occur mainly through NH3 volatilisation, biological denitrification, and nitrate (NO_3-) leaching, are of environmental and an economical concern to the dairy industry (Bolan et al. 2004; Dalley et al. 2017).

Table 2.3. Effect of season on composition of ryegrass-based pasture (DairyNZ 2017).

Season	Pasture composition			
	DM (%)	ME (MJ/kg)	CP (%)	NDF (%)
Spring	12.0 - 18.0	11.0- 12.5	18.0 - 35.0	35.0 -45.0
Summer	15.0 - 20.0	9.5 - 10.5	14.0 -22.0	42.0 - 52.0
Summer dry	20.0 - 30.0	8.0 - 9.5	9.0 - 14.0	52.0 - 65.0
Autumn/Winter	13.0 - 18.0	11.0 - 11.5	15.0 - 20.0	40.0 - 47.0

DM= dry matter.
ME= metabolizable energy.
CP= crude protein.
NDF= neutral detergent fibre.

2.5 Use of Supplementary Feed

The synchronisation of feed demand and feed supply in seasonal grazing systems is not perfect and there will periods of pasture deficit or surplus, and farmers need to use supplements (either purchased from off the farm, or conserved pasture in the form of hay or silage harvested on-farm) or use fertilizers to increase pasture growth, and conserve feed (in the form of silage or hay), respectively (Holmes 1999; Verkerk 2003).

2.5.2. Protein Supplementation in Summer

The main nutrient limiting production of milk in New Zealand grazing systems is metabolizable energy (Kolver and Muller 1998). However, during summer, pastures are low in CP (9-14%) and offering extra feed that is rich in protein can be beneficial if milk production is impaired by the poor protein intake (Penno 2002) and a level of at least 16-18% of CP has been recommended for cows producing greater than 18 L of milk (Macdonald et al. 1998; Fleming et al. 2018).

Legumes such as clover and lotus can be used during summer, as they contain more protein than ryegrass, and lotus is rich in condensed tannins which increases the efficiency of protein digestion (Holmes 1999). The use of urea (non-protein source of nitrogen) was not shown to increase milk solids yield and added a risk of urea toxicity to cattle. Thus, the source of nitrogen must be considered, as protein nitrogen sources are better utilized by the ruminal flora than non-protein nitrogen sources (Macdonald et al. 1998).

Turnips contain 12-18% CP and have been used as a summer crop in New Zealand, as it was previously shown to increase summer milksolids production, however it was only shown to be beneficial when up to 4kg DM/cow was offered (Harris et al. 1998). Protein

supplementation is also expensive and model simulations found that unless dietary crude protein drops below 12%, energy is still the limiting factor to milk production and there is no additional benefit in purchasing expensive protein supplements (Roche et al. 2011).

2.5.3. Use of Nitrogen Fertilizers

Nitrogen fertilizer is utilized to increase the rate of pasture growth (but reduces clover content) and has minor direct effects on pasture quality, mainly increasing the level of CP (Lambert et al. 2004). Responses to N fertilizer are highest when pasture growth rate is fastest and applications of up to 50 kg N/ha were considered to be most cost-effective, being the level of application dependent on the amount of feed-deficit to be covered. The first N application is usually given to cover feed deficit in winter/early spring and the second application is given to boost pasture growth for feed conservation (Figure 2.4, Roberts et al. 1992).

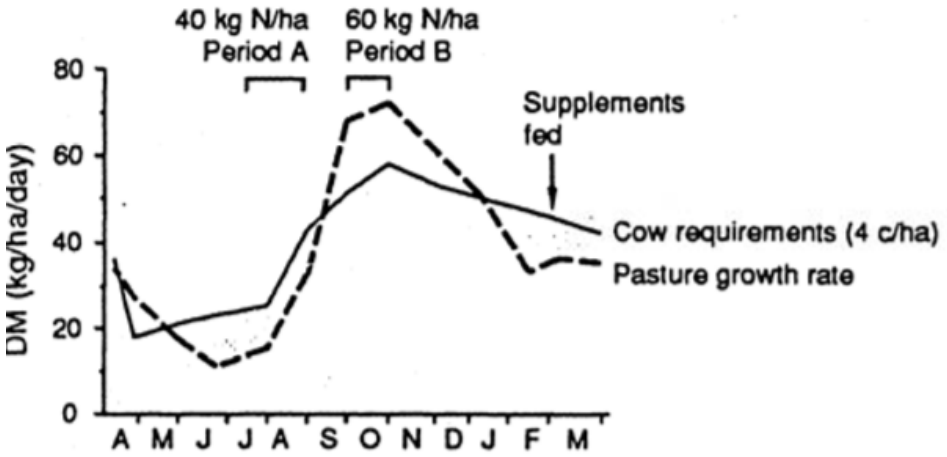

Figure 2.4. Pasture growth and dry matter (DM) requirements for the herd and strategic applications of nitrogen fertilizer (Roberts et al. 1992).

2.6 Milk Urea Nitrogen

Milk urea nitrogen (MUN) is highly correlated (0.69 to 0.99) to blood urea nitrogen (BUN) (Hof et al. 1997), this is a reflection of the easy diffusion of nitrogen from blood to milk (Gustafsson and Palmquist 1993) and is used to measure the nutritional protein status and health of the cow. It is a preferred method of measuring protein metabolism because it is non-invasive, economical and practical (Roy et al. 2011).

Milk urea nitrogen represents 2.5 to 3.0% of total milk nitrogen (Roy et al. 2011). Some studies found a negative association between MUN and milk protein percentage (Godden et al. 2001; Johnson and Young 2003), which suggested that low MUN is associated with nitrogen being used for milk protein synthesis (greater use of dietary CP), meaning a better nitrogen utilization efficiency. In housed dairy systems where cows are given total mixed rations, milk urea nitrogen levels throughout lactation have been reported to follow a similar pattern to that of milk yield, resembling a lactation curve, as can be seen on Figure 2.5 (Jonker et al. 1997; Johnson and Young 2003; Wood et al. 2003; Stoop et al. 2007).

Figure 2.5. Lactation curves for milk urea nitrogen (MUN) concentration and milk yield for Holstein-Friesian cows in housed systems (Johnson and Young 2003).

However, in New Zealand grazing conditions, where there is less control of the CP content in the diet, lactation curve for MU reflects the CP content of the diet and does not resemble the lactation curve for MY, as can be seen on Figure 2.6. The lower MU levels observed in early lactation is suggested to be due to the increased tissue mobilisation to deliver more nutrients in peak lactation. The increasing MU levels towards the end of lactation may be due to the reduced MY along with increasing dietary CP (Correa-Luna et al. 2018).

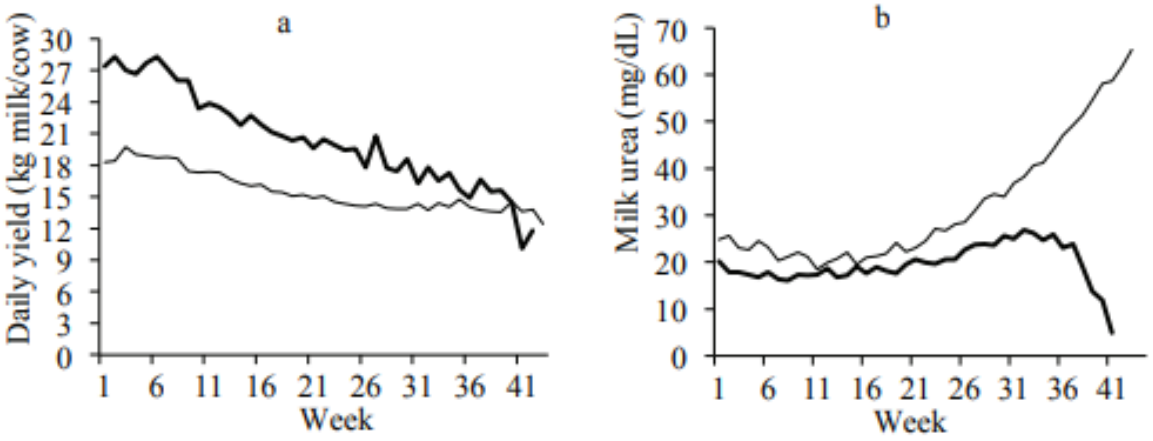

Figure 2.6. Lactation curves for milk yield (a) and milk urea (b) in a twice-a-day low supplementary higher CP (—) and once-a-day high supplementary lower CP (▬) dairy systems during season 2016-17 (Correa-Luna et al. 2018).

Since MUN has been negatively associated to fertility in dairy cows, the applicability of MUN as a useful tool of selection has been investigated (Larson et al. 1997; Miglior et al. 2007). MUN has been reported to be higher in high producing cows (Rzewuska and Strabel 2013), in heavier cows (Johnson and Young 2003) and with higher milking frequencies in total mixed ration system (Nilsen et al. 2005). In addition, MUN concentration was shown to be affected by season, parity, stage of lactation, so these variables should be controlled when investigating the relationship between MUN and other traits (Godden et al. 2001).

2.7 Fertility Traits

Fertility is the ability of the animal to conceive and maintain pregnancy if serviced at the appropriate time (Pryce et al. 2004). Successful conception and pregnancy involve a series of events that start from waves of follicular development, ovulation, oestrus detection, fertilization of the oocyte, embryo transport and development, maternal recognition, and implantation in the uterus (Butler 1998). In general, dairy cows ovulate 15 days after calving but oestrus is usually silent and 9 days later is followed by a detectable oestrus, from then, the average length of the cycle is 21 days and depends on the number of recruitment waves (Kojima 2003; Crowe 2008).

Early studies found an antagonistic genetic correlation between fertility traits and milk production (Philipson 1981; Grosshans et al. 1997; Dillon et al. 2006), suggesting that selection for high yield resulted in reduced fertility in dairy cows. Low fertility affects the profitability

of the farming system (Esslemont and Peeler 1993) and the rate of genetic progress (Congleton and King 1984). Although fertility traits are of low heritability (0.01 to 0.06) there is enough genetic variation to enable selection on fertility traits, which has enabled gains especially in seasonal dairy systems (Darwash et al. 1997; Grosshans et al. 1997). Fertility genetic evaluations in New Zealand currently use the binary traits SR21 (submission rate by 21 days after start of breeding season) and CR42 (calving rate by day 42 after start of planned calving season) (Bowley et al. 2015).

Fertility traits are categorised into interval traits; binary (binomial) traits; or count traits. Interval traits include interval from calving to first heat; interval from calving to first service; interval from calving to conception (days open); and interval from first service to conception. Binary traits are traits with two outcomes: pregnant or not pregnant; serviced or not serviced. They are the pregnancy rates and service rates. The count trait is usually the number of services (inseminations) needed until conception (Berry et al. 2014).

Traditionally, fertility traits are measured based on the season`s start of mating/breeding, which is the day when the first insemination is performed in the herd. Grosshans et al. (1997) investigated the applicability of using the fertility traits for cow selection in New Zealand. The traits SBCO (days from start of breeding to conception), PR21 (percentage of cows conceiving by day 21 after start of breeding) and PR42 (percentage of cows conceiving by day 42 after start of breeding) were most useful because they reflect not only the cow`s ability to show oestrus but also the ability to conceive to an insemination. PR21 was estimated using non-return within 21 days after start of breeding as an indication of conception. The high genetic correlation (r_G) between PR21 and SBCO (-1.00) and the high phenotypic correlation (r_P) between PR21 and DO (days open, -0.52) and CI (calving interval, -0.46) supported the use of PR21 as a selection criterion for fertility among dairy cows.

Figure 2.7. Graphic representation of the fertility traits SBFS: interval from start of breeding (SB) to first service; SBCO: interval from SB to successful service (conception); CFS: interval from calving to first service; FSCO: interval from first to successful service; DO: interval from calving to successful service (days open); CI: interval between consecutive calvings; GL: gestation length (Grosshans et al. 1997).

2.8 Effect of Milking Frequency on Reproductive Performance

Studies have investigated the effect of milking frequency on the reproductive performance of dairy cows due to the growing interest of shifting from twice-a-day to once-a-day (Clark et al. 2006; Patton et al. 2006; McNamara et al. 2008). Patton et al. (2006) found that cows milked once-a-day had an interval to first ovulation 10 days shorter than that of cows milked thrice-a-day, this could be explained by the energy balance that tended to be less negative for the once-a-day cows during the first three weeks after calving. However, conception rate and final pregnancy rate did not differ due to milking frequency.

McNamara et al. (2008), on the contrary, found no significant difference in reproductive performance between the different milking frequency treatments. Although, cows milked once-a-day lost 0.25 BCS between calving and first service compared to 0.5 BCS for the cows milked twice- and thrice-a-day. In agreement with Patton et al. (2006), once-a-day cows were in less negative energy balance during the first week after calving (Figure 2.8). Cows milked once-a-day produced 500–600 kg less milk over a full lactation compared with cows milked twice-a-day.

Figure 2.8. Energy balance over the first 6 weeks of lactation for cows milked once- (▲) twice- (■) or thrice -(●) a- day (McNamara et al. 2008).

Clark et al. (2006) found that Jersey cows result in smaller production losses in once-a-day milking frequency compared with the higher yielding Holstein-Friesian cows, however there was no milking-frequency x breed interaction for reproductive traits other than use of controlled internal drug release device. It was concluded that once-a-day milking has the potential to improve energy balance and, therefore, may reduce interval to first ovulation. Once-a-day milking frequency does not necessarily improve pregnancy rates but will not impair reproductive performance.

2.9 Relationship Between Protein and Fertility

Although it has been found that prolonged protein deficiency can negatively affect reproductive efficiency of dairy cows (Gustafsson and Carlsson 1993; Ibtisham et al. 2018), the opposite is also true as excessive dietary protein has been associated with increased days open, more services per conception, and longer calving intervals in dairy cows in confinement and grazing conditions (Jordan and Swanson 1979; Elrod and Butler 1993; Larson et al. 1997; Sinclair et al. 2000a; Butler 2005; Lean et al. 2012).

There has been some controversy as to whether high CP impairs reproduction of dairy cows, but studies have found high blood urea and ammonia concentrations to produce adverse effects (directly or indirectly) on the oocyte, uterus and embryo (Jordan et al. 1983; Sinclair et al. 2000a; Ocon and Hansen 2003). Butler et al. (1996) stated that blood urea concentrations >19 mg/dL would result in a 20% decrease in pregnancy rate after insemination, which goes in

agreement with results from other studies (Ferguson et al. 1988; Ferguson et al. 1993; Westwood et al. 1998; Melendez et al. 2003).

Law et al. (2009), however, found no significant difference in fertility between cows fed high or low levels of CP, however, there was a tendency for animals on the low CP diet (114 g CP/kg DM) to have a higher 100 day in-calf rate (82.7%) compared with those on higher CP diets 144g (66.7%) and 173 g of CP/kg of DM (62.1%). Other studies also found no significant relationship between high CP or high MU/MUN and fertility of dairy cows in total mixed ration (Hossein-Zadeh and Ardalan 2011; Amundson et al. 2016) and grazing systems (Trevaskis and Fulkerson 1999; Kenny et al. 2001; Ordóñez et al. 2007).

Whereas some found the opposite effect of high CP on fertility. In Finland, Mikkola et al. (2005) found that a long-term moderate increase in dietary crude protein content from 14% to 18% was beneficial to the quality of embryos from Ayrshire heifers ($P=0.053$) despite the concomitant elevated blood urea concentrations ($P< 0.001$).

The discrepancies of results are likely to be due to differences in the energy status of animals, source of crude protein, physiological status of animals (heifers or multiparous), stage of lactation (early, mid or late lactation), duration of the feeding period (if under TMR) and feeding system (pasture-based vs TMR) (Amundson et al. 2016).

2.9.1 Effects on Ovarian Follicular Fluid and Oocytes

The maturation of oocytes during follicle development are vulnerable to the effect of various proteins and steroids that are present inside and surrounding the follicular environments. It was found that in vitro blastocyst production (10.9 vs 20.6%; $P=0.06$) and cleavage (47.4 vs 62.4%; $P= 0.02$) were negatively affected in oocytes from heifers with high ammonia concentrations in follicular fluid due to high CP diet (Sinclair et al. 2000).

It was suggested that ammonia would impair the following processes: meiotic maturity to metaphase II; resumption of meiosis following sperm activation; or cytoplasmic maturation, polymerization of tubulin into microtubules in meiosis and oocyte activation (Sinclair et al. 2000; De Wit et al. 2001). This goes in agreement with the findings of De Wit et al. (2001) where oocytes cultured in the presence of 6 mM urea decreased the subsequent percentage of fertilization, cleavage, and development on days 7 and 9. Accordingly, an in vitro study found ammonium chloride (NH_4Cl) to alter growth and metabolism of granulosa cells, affecting oocyte maturation (Rooke et al. 2004).

2.9.2 Effects on Circulating Progesterone Concentrations

Progesterone is crucial for maintenance of pregnancy (Larson et al. 1997). Plasma progesterone concentrations increase over the first three ovarian cycles during early lactation, and negative energy balance (NEB) moderates the rate of increase (Butler 1998). The association of NEB and excessive RDP in early lactation aggravates the NEB due to the energy cost of ammonia detoxification (Butler 1998). Also, ureagenesis has been reported to be less efficient in animals with fatty liver, further reducing the liver capacity to detoxify (Strang et al. 1998).

Some studies have associated high CP diets with low circulating progesterone levels in cows in early lactation. Jordan and Swanson (1979) found that cows fed low CP (12.7%) during the breeding period had higher serum progesterone concentrations than those fed 16.3 or 19.3% CP. Garcia-Bojalil et al. (1997) found that lactating cows fed diets with 15.7% of degradable protein had lower plasma progesterone accumulated over time (371 ng/ml), compared to cows fed diets with 11.1% degradable protein (848 ng/ml).

In a study conducted by Larson et al. (1997) MUN concentrations >21 mg/dl were associated with recycling in cows (oestrus detected at day 21 after breeding). There was a higher likelihood of cows that failed to become pregnant and had MUN concentrations >21 mg/dl to be included in the non-pregnant low progesterone category.

On the other hand, Law et al. (2009) found no significant effect of dietary protein concentration on progesterone measures, which goes in accordance with Jordan et al. (1983). However, only 0.54% of all blood samples had urea concentrations above 19 mg/dL (Law et al. 2009).

Figure 2.9. Regression of plasma progesterone concentrations throughout estrous cycle days for cows fed 12 or 23% CP diets standardized to a 21-day estrous cycle (Jordan et al. 1983).

Conversely, Sinclair et al. (2000) found that progesterone concentrations in medium-sized follicles harvested were significantly greater ($p < 0.05$) in animals offered the high- than in those offered low-ammonia generating diet suggesting that some follicles may have been cystic and that follicular-derived progesterone may have contributed significantly to the high levels of plasma progesterone observed in heifers offered the high-ammonia generating diet.

2.9.3 Effects on The Uterine Environment

Embryonic development requires adequate uterine environment to receive the blastocyst/embryo (Heap et al. 1979). An early study investigated the effects of dietary CP on minerals in uterine secretions, as mineral composition in uterine environment can affect cell metabolism of sperm, ova and zygotes (Hurley et al. 1976). It was found that cows fed 12% CP had greater concentration of P, K, Mg in uterine secretions than cows fed 23% CP, which can negatively affect the embryo (Jordan et al. 1983).

That study also found a relationship ($P<0.05$) between urea in uterine secretion and plasma urea and blood ammonia, suggesting that ammonia concentration in uterine secretion increased with high CP diets (23% CP) (Hurley et al. 1976). Ammonia was previously found to inhibit citric acid cycle of sperm cells (Dietz and Flipse 1969). In addition, urea nitrogen was found to inhibit the binding of chorionic gonadotropin to luteinizing hormone (LH) receptors in the corpus luteum in vitro (Haour and Saxena 1974), which decreases progesterone concentrations and consequently impairs fertility (Jordan and Swanson 1979).

Furthermore, studies have shown that increased level of blood urea nitrogen can decrease the uterine pH, which can affect embryo implantation (Law et al. 2009; Ibtisham et al. 2018). The normal uterine pH is around 6.8 at oestrus and increases to 7.1 on day 7 of the oestrous cycle, and diets with excessive RDP or RUP were shown to impede this increase to occur in heifers and in lactating cows (Butler 1998). Although, it is suggested that providing adequate levels of energy for excretion of excess ammonia may prevent these negative effects on reproduction (Ibtisham et al. 2018).

Figure 2.10. The time course of inverse changes in plasma urea nitrogen (PUN) and uterine luminal pH in a lactating cow. Feeding occurred at the times indicated by arrows during the 40-h period of study (Butler 1998).

The acidic pH from uterine flushings from cows fed high CP was also shown to affect the survival and motility of spermatozoids. There was a decreased percentage of motile spermatozoa with increasing concentrations of urea in vitro. Furthermore, higher deciliation, desquamation and ciliostasis of bovine oviductal tissue was found for tissue cultured in media that contained higher concentrations of ammonia (Westwood et al. 1993). On the other hand, Law et al. (2009) found that increases in the dietary protein concentration significantly ($P< 0.05$) decreased the proportion of animals diagnosed with metritis, indicating that a higher dietary protein concentration was associated with a better immune response of dairy cows.

2.9.4. Effects on The Embryo

Studies have evaluated the viability of embryos flushed from super-ovulated lactating cows fed different levels of protein. A study conducted by Rhoads et al. (2006) confirmed that the transfer of embryos from moderate PUN (15.5±0.7 mg/dl, 15.7% CP) donor cows resulted

in a higher pregnancy rate (35%; $P < 0.02$) than the transfer of embryos from high PUN (24.4±1.0, 21.9% CP) donor cows (11%) (Rhoads et al. 2006). The diet of recipient cows did not affect fertility, suggesting that the embryo or oocyte were affected rather than the uterine environment, which goes in agreement with a study conducted by Fahey et al. (2001) in ewes.

These results go in agreement with the findings from earlier studies (Blanchard et al. 1990; Sinclair et al. 2000). The exact mechanisms by which the embryo or oocyte is affected up to 7 days after insemination is unclear. However, a previous in-vitro study (Ocon and Hansen 2003) exposed oocytes to different urea concentrations, which was shown to interfere in the development of embryos to the blastocyst stage. It was noticed, however, oocyte resistance to very high levels of urea as 10 mM urea did not affect cleavage or subsequent development compared to 5 and 7.5 mM urea. Exposing embryos to urea did not affect its development, which suggests that the embryo itself is resistant to direct effects of urea. On the other hand, embryos were affected by low pH (15 and 20 mM dimethadione, equivalent to initial pH of 6.4 and 6.3, respectively) (Ocon and Hansen 2003).

2.9.5 Theory of Adaptation to High Crude Protein Diets

Previous studies suggested that ruminants can adapt their metabolism to high intakes of dietary protein and consequently high dietary protein may not affect fertility in the longer term. Some indicated that feeding urea and ammonium salts as the only source of nitrogen favours the growth of rumen bacterial strains that grow well with ammonia (Virtanen 1966). However, Sinclair et al. (2000b) found no significant metabolic adaptation to high-ammonia-generating diets during a 4-week experiment, the cows fed high ammonia-generating diets had altered their pattern of intake to prevent high levels of plasma ammonia. Plasma ammonia levels were significantly elevated in heifers offered diets that were asynchronous in nitrogen release.

Ordóñez et al. (2007) and Kenny et al. (2001), on the other hand, concluded that high pasture CP had no effect on the fertility of Friesian cows and beef heifers, respectively. CP intake of 25.4% and 21.6 % were compared (Ordóñez et al. 2007), and pasture CP content was increased from 12% to 23 % (Kenny et al. 2001). Both studies found a significant increase in plasma urea levels, but with no effect on fertility. Blood urea concentration of the high CP groups was significantly higher than 19 mg/dL (threshold), whereas the concentrations in the control groups were, significantly lower than the threshold.

It has been suggested that this tolerance would be due to an adaptation process that takes around 10 days, so insemination should be delayed for a minimum of 10 days after dietary changes. It is important to consider the timing of the increase in dietary nitrogen intake relative to fertilisation, and that larger negative energy balance increases the likelihood of an adverse effect of high dietary nitrogen on fertility (Dawuda et al. 2002).

2.9.6 Interaction Between High CP Diets and Negative Energy Balance

Studies indicate that the effect of high CP diets (or unbalanced RDP/RUP diets) on fertility is likely to be due to the effects on the energy balance and on the hypothalamic-pituitary-ovarian axis, and not due to the direct effects of urea/ammonia on follicles or uterus (Tamminga 2006; Amundson et al. 2016). Liver detoxification of excessive dietary protein is estimated to be 0.035 MJ per gram of excess N detoxified, which therefore aggravates and extends the NEB in the post-calving period (Westwood et al. 1998).

Most cows undergo a period of negative energy balance, when energy demand for maintenance and production exceeds energy intake, after calving, due to the high nutrient demand for milk production in early lactation. During NEB, nonesterified fatty acids (NEFA) are released from body fat reserves and are used by the liver as a source of energy; or transformed in ketone bodies and acetate and transported in the blood; or accumulated in the liver as tri-acyl glycerol, which can result in fatty liver (Wathes et al. 2007b). This excessive mobilisation of fat and accumulation in the liver can impair liver function, which further negatively affects the detoxification of ammonia into urea (Tamminga 2006).

In addition, high CP diets can alter plasma amino acid profiles and reduce feed intakes, further lowering energy balance (Bergen and Potter 1975) it was also shown that steers fed 40% CP reduced their feed intake by 56% compared with steers fed 10% CP (Fenderson and Bergen 1976). After calving, the first wave of follicular development occurs in 5-7 days in response to increased plasma FSH (Butler 2003), and two to three waves normally occur in the oestrus cycle (Beam and Butler 1999). The ovulation of the dominant follicle, however, depends on the re-establishment of pulsatile LH secretion and oestradiol production; and NEB affects the pattern of LH secretion and the ovarian responsiveness to LH (Butler 2003). During NEB, plasma concentration of growth hormone (GH) increases and insulin and IGF-1 decrease (Tamminga 2006). The lower levels of insulin are due to decreased level of circulating glucose; and insulin is important for follicle responsiveness to LH. Furthermore, it was shown that ammonia generated from high CP/RDP diets also supresses plasma insulin release following

consumption of food, which is another mean of affecting the reproductive axis (Sinclair et al. 2000b).

It was previously demonstrated in vitro that IGF-1 increases the number of LH-binding sites on the follicle and enhances production of progesterone and androstenedione. The number of IGF-1 receptors in granulosa cells is increased by oestradiol and FSH and may thus form a self-amplifying of IGF-1 stimulation in the dominant follicle (Beam and Butler 1999). Overall, since follicular development is primarily controlled by hypothalamic GnRH and pituitary gonadotrophins, and these are affected by nutrition and energy status, fertility can be compromised by high dietary protein diets (Garnsworthy and Webb 1999).

2.8. Genetic Parameters for Milk Urea Nitrogen, Production and Reproduction Traits

Estimates of heritability for MUN from previous studies ranged from 0.09 to 0.41 (Mitchell et al. 2005; Miglior et al. 2007; Stoop et al. 2007; König et al. 2008; Hossein-Zadeh and Ardalan 2011; Mucha and Strandberg 2011). Mitchell at al. (2005) estimated heritability for two different measuring methods of MUN: infrared (IR), which is an indirect measure; or wet chemistry (WC), which is a direct measure of urea nitrogen in milk samples. Heritability estimates were higher for IR MUN than for WC MUN, with IR MUN estimates of 0.22-0.23 and WC MUN estimates of 0.09-0.15. IR MUN values were of higher accuracy, so it was preferred in the analysis of metabolizable protein. Hossein-Zadeh and Ardalan (2011) also found low estimated heritabilities of IR MUN, which were 0.18, 0.20 and 0.22 for first, second and third lactation, respectively. Miglior et al. (2007) found higher heritabilities of MUN but that also increased with lactation number/parity, 0.39, 0.38 and 0.41 for first, second and third lactations, respectively.

The observed phenotypic correlation between characters (traits) can be explained by the genetics and by the environment. Genetic causes of correlation can be pleiotropy and linkage (when traits are inherited together). Pleiotropy is the property of a gene whereby it affects two or more traits. These genetic correlations between traits can be positive, where both traits increase; or they can be negative, where one trait enhances whilst the other trait reduces (Falconer 1960). Therefore, when two traits are highly genetically correlated, genetic improvement of one trait could cause similar parallel improvement in the other trait (Hossein-Zadeh and Ardalan 2011).

Strong negative genetic correlations between MUN and fertility traits such as DO, SBFS, SBCO, CFS, and FSCO would mean that an increase in MUN is favourable to fertility because these intervals are shorter. Strong negative correlations between MUN and fertility traits such as SR21, SR42, PR21, PR42 would mean that an increase in MUN is unfavourable to fertility because submission and pregnancy rates are lower. If the traits are of low heritability, then the phenotypic correlation is mainly determined by the environmental correlation (Falconer 1960).

2.8.1. Genetic Correlation Between MUN and Fertility Traits

A limited number of studies have described the genetic correlation (r_G) between milk MUN and fertility traits in dairy cows. These studies were conducted in the United States (Mitchell et al. 2005); Germany (König et al. 2008); Iran (Hossein-Zadeh and Ardalan 2011); Sweden (Mucha and Strandberg 2011); and Poland (Rzewuska and Strabel 2014).

Some found an overall antagonist genetic correlation between MUN and fertility (Mitchell et al. 2005; König et al. 2008; Hossein-Zadeh and Ardalan 2011), whereas others unexpectedly found a favourable genetic correlation between MUN and fertility (Mucha and Strandberg 2011; Rzewuska and Strabel 2014) indicating that animals with breeding values for increased MUN also had breeding values for improved fertility.

Mitchell et al. (2005) included in the analysis of reproductive traits cows in the first and second lactation that had a MUN value within ± 30 days of first service. Genetic correlations were low between MUN and CFS (-0.14) and pregnancy rate at first service (PRFS) (-0.06) in first lactation; MUN and CFS (0.18) and PRFS (0.01) in second lactation. Genetic correlation was higher between MUN and DO, with estimates of 0.21 in first and 0.41 in second lactation cows.

The study conducted by König et al. (2008) used average MUN obtained from measurements of the first 2 test days after calving. In agreement with Mitchell et al. (2005), genetic correlations were also low between MUN and reproductive traits. Genetic correlations with NR56 (non-return rate by day 56) and NR90 (non-return rate by day 90), were -0.13 and -0.12, respectively. Genetic correlation with CFS, however, was slightly higher (0.29).

Accordingly, Hossein-Zadeh and Ardalan (2011) found very low genetic correlations between MUN and several measures of reproductive performance (CFS, PRFS and FSCO); except for DO, which were 0.23 to 0.45, suggesting that higher MUN concentrations may be genetically associated with increased DO. The study required cows to have at least one MUN value (collected from routine test-day samples).

In the study conducted by Mucha and Strandberg (2011), genetic correlations between MUN and most fertility traits remained negative, except for CI that started positive (0.20) and turned negative after day 50. Genetic correlation between MUN and PRFS started negative (-0.10) turned positive after day 50, indicating that animals with breeding values for increased MUN after day 50 also had breeding values for improved pregnancy rates at first service. Rzewuska and Strabel (2014) found that r_G between MUN and SP (interval from first to last insemination), DO and NI (number of inseminations to conception) were negative in the first two months of lactation, also indicating high MUN to have favourable relationship with fertility.

Table 2.4. Estimates of genetic correlations between milk urea nitrogen and fertility traits reported in different studies.

Study	mean MUN	Genetic correlations between MUN and fertility traits						
		CFS	PRFS	DO	FSCO	CI	NR56	NR90
Mitchell et al. (2005)	12.92 and 14.30 mg/dL	-0.14 to 0.18	-0.06 to 0.01	0.21 to 0.41	-	-	-	-
König et al. (2008)	267.11 ppm (26.7 mg/dL)	0.29	-	-	-	-	-0.13	-0.12
Hossein-Zadeh and Ardalan (2011)	17.4 to 18.6 mg/dL	-0.12 to 0.19	-0.05 to 0.15	0.23 to 0.45	-0.09 to 0.16	-	-	-
Mucha and Strandberg (2011)	13.6 mg/dL	0* to -0.21	-0.10 to 0.28	-	-	0.20 to -0.22	-	-

MUN=milk urea nitrogen.
CFS=calving to first service, also named DCFS (days from calving to first service) or CFI (calving to first insemination).
PRFS=pregnancy rate at first service, also named PFI (pregnancy rate at first insemination) or FSCR (first service conception rate).
DO=days open.
FSCO=first service to conception, also named DFSC (days from first service to conception).
CI=calving interval.
NR56= non-return rate by day 56 after insemination.
NR90= non-return rate by day 90 after insemination.

*Genetic correlation was reported as close to zero.

Differences in the results reported in these studies may be due to differences in population genetics between the countries. Also, studies need to better define the production systems as they may differ in feed management and in general levels of CP and ME in pasture or ration, Iranian cows are generally fed rations of high CP content (Hossein-Zadeh and Ardalan 2011), cows in European countries are generally under high input systems (Van Arendonk and Liinamo 2003). Furthermore, each study used a different set of fertility traits in the analysis of correlation which hinders comparisons, some of them used test-day record traits and others used lactation records. These studies were also inconsistent with the time of sampling for MUN (some did not sample close to first service, nor predicted MUN at time of first service).

In countries where grazing dairy cows were highly selected for fertility on pastures of high CP content may have resulted in genetics for higher MUN, which is an undesirable effect due to the environmental consequences involved with nitrogen losses. This could be the case of New Zealand dairy cows. Therefore, an assessment of genetic correlations between MUN and fertility traits should be performed for each production system.

2.8.2. Genetic Correlation Between Milk Urea Nitrogen and Production Traits

Several studies have investigated the genetic correlation between milk urea and production traits such as yields of milk (MY), protein (PY), fat (FY), lactose (LY), and proportions of protein (PP), fat (FP) and lactose (LP) (Stoop et al. 2007; König et al. 2008; Hossein-Zadeh and Ardalan 2011; Mucha and Strandberg 2011; Rzewuska and Strabel 2013; Lopez-Villalobos et al. 2018). Positive correlations between MU/MUN and MY ranging from 0.22 to of 0.77 (Stoop et al. 2007; König et al. 2008; Lopez-Villalobos et al. 2018) have been reported, indicating that higher MU/MUN can be observed in high producing cows, and may due to energy deficiency in early lactation and higher tissue mobilization. Mucha and Strandberg (2011) found weak genetic correlations between MUN and MY, FY; these r_G were positive at the start and turned negative at the end of lactation. Miglior et al. (2007) found negative weak genetic correlation between MUN and MY (-0.094) in Canadian Holstein cows; MUN was genetically correlated with FP (0.425) and PP (0.200). In New Zealand, however, Lopez-Villalobos et al. (2018) found significant ($P<0.01$) negative genetic correlations between MU and FP (-0.80) and LP (-0.76). Lopez-Villalobos et al. (2018) and Rzewuska and Strabel (2013) found a negative r_G between MU and CPP (crude protein percentage) of -0.66 and -0.11 to -0.24, respectively, suggesting that cows producing milk with low MU may be partitioning nitrogen towards milk protein. The strong negative genetic correlations found between MU and production traits would support the applicability of selection for low MU.

2.9. Summary and Formulation of The Research Problem

Although several studies conducted overseas have found an antagonist phenotypic association between MUN and fertility traits, studies conducted overseas have found a poor genetic correlation between milk urea nitrogen and fertility traits, and others have found a positive genetic correlation during most of the lactation, indicating that animals with breeding values for increased MUN also had breeding values for improved fertility.

New Zealand dairy cows generally graze pastures of high CP content, whilst achieving high reproductive performance. Therefore, this thesis hypothesizes that milk urea nitrogen has a positive genetic correlation to reproductive traits in New Zealand dairy cows. It is also hypostatised that years of selection of cows for better reproductive performance in high CP pastures, whilst achieving good milk production could have changed MUN genetics in New Zealand dairy cows.

The objective of this study is to analyse the data collected from two experimental herds at Massey University during two seasons to estimate the phenotypic and genetic correlations between milk urea nitrogen concentration and reproductive performance traits of cows in once- and twice-a-day milking systems.

Chapter 3

Materials and Methods

3.1. Location

The data used in this experiment was obtained from 2016-17 and 2017-18 production seasons of two dairy herds from Massey University, Palmerston North, New Zealand. The two farms differed in their milking frequencies, being Dairy 1, a once-a-day milking system and Dairy 4, a twice-a-day milking system. Dairy 1 is 35 m above sea level, with 980 mm average annual rainfall, soils are alluvial free draining, of high natural fertility. Dairy 4 is 80 m above sea level, with 980 mm average annual rainfall, and soils are of moderate natural fertility and artificially drained.

The milk urea (MU) data set used for this experiment was obtained from a smaller set of animals in an earlier study in Dairy 1 and Dairy 4 (Correa-Luna et al. 2018). The fertility and production data set were obtained from a study that investigated the phenotypic relationship between milk urea nitrogen and fertility parameters in Dairy 1 and Dairy 4 (Cutzal 2019).

Each cow included in this study had six to ten records on milk yield (MY), fat yield (FY), protein yield (PY), fat percentage (FP), protein percentage (PP) and at least three records on milk urea (MU), lactose yield (LY) and lactose percentage (LP) throughout lactation, in each production season. Reproduction data and pedigree information for each cow were also available.

3.2. Animals

Animals were classified into farm, breed, and lactation number. Animals were also classified into 12 different contemporary groups defined as the group of cows that started milking production in the same farm (Dairy 1 or Dairy 4), production season (2016-17 or 2017-18) and lactation number (1, 2, or ≥ 3). The three different breed groups were defined: Jersey (J) with proportion of J ≥ 0.875, Holstein-Friesian (F) with proportion of F ≥ 0.875 and F×J crossbred with proportions of F and J <87.5 and >12.5. Numbers of cows per breed are shown in Table 3.1 and number of cows per lactation number are shown in Table 3.2. There was a total of 874 records obtained from 637 cows and 1160 animals were in the pedigree dataset (which included cows and respective sires and dams).

Table 3.1. Number of cows included in the analysis of milk urea nitrogen per season, farm and breed group.

	2016			2017		
Breed	Dairy 1	Dairy 4	Total	Dairy 1	Dairy 4	Total
Holstein-Friesian (F)	61	49	110	54	98	152
Jersey (J)	51	3	54	56	4	60
F×J crossbred	131	147	278	124	96	220
Total	243	199	442	234	198	432

Table 3.2. Number of cows included in the analysis of milk urea nitrogen per season, farm and lactation number.

	2016			2017		
Lactation Number	Dairy 1	Dairy 4	Total	Dairy 1	Dairy 4	Total
1	51	11	62	59	81	140
2	57	39	96	47	27	74
≥ 3	135	149	284	128	90	218
Total	243	199	442	234	198	432

3.3. Pedigree

Pedigree information was provided by Livestock Improvement (LIC, Hamilton, New Zealand). The breed composition of each cow was calculated as follows:

$$\alpha_i^P = \frac{(\alpha_i^s + \alpha_i^d)}{2},$$

where α_i^P is the proportion of genes from breed i in the cow, α_i^d and α_i^s are the proportions of breed i in the dam and sire, respectively, and i is for breed Friesian or Jersey.

Coefficient of heterosis for individual cows ($h_{F\times J}$) was obtained as follows (Dickerson 1973):

$$h_{F\times J} = \alpha_F^s\ \alpha_J^d + \alpha_J^s\ \alpha_F^d,$$

where α_F^s and α_J^s are the proportion of breeds Friesian and Jersey in the sire, and α_J^d and α_F^d are the proportion of breeds Jersey and Friesian in the dam, respectively.

3.4. Herd Management

Cows from Dairy 1 were milked once-a-day at 6.30 am and cows from Dairy 4 were milked twice-a-day at 5:30 am and 2:30 pm and had access to supplementary feed prior to and during milking (Correa-Luna et al. 2018).

Both herds had access to fresh ryegrass (*Lolium perenne*)/ white clover (*Trifolium repens*) pasture after each milking and were contained in their allocated forage area. However, cows at Dairy 1 were fed low levels of supplementary feed and cows at Dairy 4 were fed higher levels of non-pasture inputs (Correa-Luna et al. 2018).

During the first production season, Dairy 1 cows received pasture silage in August (early lactation). From December 2016 to February 2017 (mid-lactation) cows grazed a mixed herb crop comprising of chicory, red clover and plantain. In March and May, Lucerne was grazed directly from the paddock and pasture silage was given. In February 2017, cows were allowed turnips (Correa-Luna et al. 2018).

Dairy 4 cows received maize silage and grain-based concentrate before afternoon milking and in the parlour throughout lactation. In January (mid-lactation), pasture silage was fed in the paddock. In March, grain concentrate was fed during the morning milking, and cows were also allocated turnips crop (Correa-Luna et al. 2018).

During the second production season, Dairy 1 cows were supplemented with maize silage and pasture silage. During mid-lactation they were supplemented with pasture silage and a mix of plantain and chicory, dried distillers grains (DDG) and tapioca pellets. Dairy 4 cows in early lactation were fed pasture, maize silage, pasture silage, DDG and concentrate. The pasture silage was eliminated during mid-lactation, and tapioca pellets were added to the feed (Cutzal 2019).

Table 3.3. Dairy 1 and Dairy 4 feeding strategy during 2016 and 2017 production seasons (adapted from Correa-Luna et al. 2018; adapted from Cutzal 2019).

Season	Lactation stage	Dairy 1	Dairy 4
2016	Early lactation	100% [1]pasture	65% pasture
		69% pasture and 31% pasture silage in August	26% maize silage
			9% concentrate
	Mid-lactation	68% pasture.	44% pasture
		32% mix of chicory, plantain. and red clover	20% maize silage
			20% pasture silage
			16% concentrate
	Late lactation	48% pasture	51% pasture
		17% pasture silage	23% maize silage
		35% grazing Lucerne	11% concentrate
			11% turnips
			4% DDG
2017	Early lactation	86% pasture	45% pasture
		9.7% maize silage	18.3% maize silage
		4.3% pasture silage	24.5% pasture silage
			6.1% DDG
			6.1% tapioca pellets
	Mid-lactation	22.8% pasture	81.2% pasture
		41.6% plantain-chicory	4.7% maize silage
		17.8% pasture silage	4.7% DDG
		11.9% [2]DDG	4.7% concentrate
		5.9% tapioca pellets	4.7% tapioca pellets

[1]Pasture= ryegrass and white clover.
[2]DDG= dried distillers grains.

3.5. Pasture Analysis

Samples of fresh pastures and crops were taken for analysis the day before taking milk samples by hand-plucking, along with samples of silage and concentrate. These samples were freeze-dried and grounded (Wiley mill) and then analysed by the near infrared reflectance spectroscopy technique to evaluate metabolizable energy (ME), crude protein (CP) and neutral detergent fibre (NDF) content (Correa-Luna et al. 2018).

Table 3.4. Weighted averages of metabolizable energy (ME), crude protein (CP) and neutral detergent fibre (NDF) content in the feed offered at Dairy 1 and Dairy 4 during early and mid-lactation in the 2016 and 2017 production seasons (Cutzal 2019).

			Farm					
			Dairy 1			Dairy 4		
Season	Lactation stage	Feed	ME (MJ/kg)	CP (%)	NDF (%)	ME (MJ/kg)	CP (%)	NDF (%)
2016	Early lactation	[1]Pasture	11.4	21.0	46.0	11.4	17.0	41.0
		Maize silage	-	-	-	10.3	8.0	33.0
		Pasture silage	10.4	15.0	51.0	-	-	-
		Concentrate	-	-	-	12.5	15.0	19.0
		Average in August	11.0	19.0	47.5	-	-	-
		Average	11.4	21.0	46.0	11.2	14.0	37.0
	Mid lactation	Pasture	11.6	19.0	46.0	11.1	16.0	46.0
		[2]PxCxRC	12.5	22.0	27.0	-	-	-
		Maize silage	-	-	-	10.6	9.0	30.0
		Pasture silage	-	-	-	10.9	12.0	56.0
		Concentrate	-	-	-	11.4	22.0	29.0
		Average	11.9	20.0	40.0	11.0	15.0	42.0
2017	Early lactation	Pasture	12.1	19.0	37.0	11.0	22.0	48.0
		Maize silage	10.2	8.0	38.0	10.0	8.0	38.0
		Pasture silage	12.4	21.0	46.0	10.7	13.0	50.0
		[3]DDG	-	-	-	8.5	29.0	37.0
		Concentrate	-	-	-	12.1	10.0	24.0
		Average	11.9	18.0	37.0	10.6	17.0	45.0
	Mid lactation	Pasture	9.9	12.0	49.0	11.4	12.0	46.0
		[4]PxC	11.6	23.0	27.0	-	-	-
		Maize silage	-	-	-	10.3	8.0	33.0
		Pasture silage	12.7	15.0	42.0	-	-	-
		DDG	8.3	33.0	32.0	8.1	31.0	33.0
		Concentrate	-	-	-	11.6	12.0	27.0
		Tapioca	11.3	5.0	26.0	11.1	4.0	28.0
		Average	11.0	19.0	35.0	11.2	12.0	43.0

[1]Pasture= ryegrass and white clover.
[2]PxCxRC= Plantain, chicory and red clover mix.
[3]DDG= dried distillers grains.
[4]PxC= Plantain and chicory mix.

3.6. Reproductive Management

Calving dates were recorded for all cows from both farms (Dairy 1 and Dairy 4) for both seasons, so it was possible to establish calving patterns. The start of the mating season was recorded as the first insemination (first service) date and the end of the mating season was recorded as the last insemination date of each herd. The start of breeding was planned by the farmer according to the predicted availability of pasture for the following season, which fell in mid-October (spring).

Table 3.5. First and last insemination dates and length of the mating season (inseminations) at Dairy 1 and Dairy 4 in the 2016 and 2017 production seasons at Massey University, New Zealand.

Season	Farm	First insemination	Last insemination	Length of artificial insemination season
2016	Dairy 1	14/10/2016	23/12/2016	10 weeks
	Dairy 4	18/10/2016	28/11/2016	5 weeks and 6 days
2017	Dairy 1	20/10/2017	26/12/2017	9 weeks and 4 days
	Dairy 4	18/10/2017	24/12/2017	9 weeks and 4 days

Cows were inseminated when signs of oestrus were observed. Each service date was recorded, and each cow had a maximum of 5 services. The animals were tested for pregnancy by ultrasound scan to confirm conception date. If the cow was confirmed pregnant after the end of mating season, the conception date was recorded as the last service date the cow had.

Reproductive performance of the two herds was evaluated based on the measurement of the following fertility traits: interval from start of breeding (SB) to first service (SBFS); interval from SB to successful service/conception (SBCO); interval from calving to first service (CFS); interval from first to successful service (FSCO); interval from calving to successful service (days open, DO); percentage of cows pregnant in the first service (PRFS); percentage of cows in the herd pregnant by Day 21 after the start of breeding (PR21); and percentage of cows in the herd submitted to artificial insemination by Day 21 after the start of breeding (SR21).

Cows that did not have a conception date were the ones that were removed from the herd after the start of the breeding season. Removed cows were sold, culled or died and the reasons for removal included development of mastitis, low production, lameness, for example. For these cows with no conception dates, it was not possible to calculate the reproductive traits SBCO, PR21, DO, FSCO, PRFS. Although these variables were left in blank for these animals, these were not removed from study as they had their calving dates and service dates recorded.

3.7. Milk Samples

Records on milk, fat and protein yield (MY, FY, PY) and fat and protein percentages (FP, PP) were obtained from six to ten herd tests from all cows from both herds during the seasons of 2016-17 and 2017-18. Records on milk urea (MU), lactose yield (LY) and lactose percentage (LP) were obtained from additional milk samples (as per Table 3.1) at three time points: in early lactation (September/October), mid lactation (December/January) and late lactation (February/March), during both seasons. These samples were collected using herd-test milk meters provided by Livestock Improvement Corporation. These samples were analysed by MilkTestNZ (Hamilton, NZ) using the CombiFoss technique (infrared spectrophotometry) for MU (mg/dL) content. MU was converted into milk urea nitrogen (MUN) by multiplying it by 0.47 (MUN=MU X 0.47), as urea has 47% of nitrogen in its composition.

3.8. Closest Record to Date of First Insemination

Actual values from herd-tests that were obtained close to the date of first service were deemed as the best value to estimate genetic and phenotypic correlations between production and reproduction traits. The date difference between the closest herd-test day and first service day are shown on Tables 3.6 for MUN and 3.7 for MY.

Table 3.6. Difference in days between closest herd-test date (for MUN, LY and LP) and first service performed in Dairy 1 and Dairy 4 during 2016 and 2017 production seasons at Massey University, New Zealand.

	Farm									
	Dairy 1					Dairy 4				
Season	N	Mean	SD	Min	Max	N	Mean	SD	Min	Max
2016	243	34.6	6.3	24	46	199	6.5	4.4	0	32
2017	234	39.0	5.0	30	46	198	21.7	6.5	0	29

MUN= milk urea nitrogen; LY= lactose yield; LP= lactose percentage.

Table 3.7. Difference in days between closest herd-test date (for MY, FY, FP, PY, and PP) and first service performed in Dairy 1 and Dairy 4 during 2016 and 2017 production seasons at Massey University, New Zealand.

	Farm									
	Dairy 1					Dairy 4				
Season	N	Mean	SD	Min	Max	N	Mean	SD	Min	Max
2016	243	6.2	4.2	0	27	199	6.1	3.9	0	17
2017	234	8.4	3.9	0	23	198	4.6	2.8	0	11

MY= milk yield; FY= fat yield; FP= fat percentage; PY= protein yield; PP= protein percentage.

3.9. Statistical Analysis

Descriptive statistics (mean, standard deviation, minimum and maximum values) were obtained in SAS version 9.4 software (SAS Institute Inc., Cary, NC, USA) per farm, per season and per lactation number for all dependent variables.

Analysis of variances were performed in SAS using the MIXED procedure for continuous variables and using the GLIMMIX procedure with a logit transformation for binomial variables. The model included as fixed effects farm, season, interaction of farm and season, and lactation number; as covariates the proportion of Friesian, heterosis and deviation from median calving date, and cow and residual error as random effects.

Estimates of variances and covariances required for estimation of genetic parameters were obtained using the ASReml 4.0 software package (VSN International Ltd.) with single and bi-variate repeatability animal models. In matrix notation, the bivariate model was represented as follows:

$$\begin{bmatrix}\mathbf{y_1}\\ \mathbf{y_2}\end{bmatrix} = \begin{bmatrix}\mathbf{X_1} & \mathbf{0}\\ \mathbf{0} & \mathbf{X_2}\end{bmatrix}\begin{bmatrix}\mathbf{b_1}\\ \mathbf{b_2}\end{bmatrix} + \begin{bmatrix}\mathbf{Z_1} & \mathbf{0}\\ \mathbf{0} & \mathbf{Z_2}\end{bmatrix}\begin{bmatrix}\mathbf{a_1}\\ \mathbf{a_2}\end{bmatrix} + \begin{bmatrix}\mathbf{W_1} & \mathbf{0}\\ \mathbf{0} & \mathbf{W_2}\end{bmatrix}\begin{bmatrix}\mathbf{c_1}\\ \mathbf{c_2}\end{bmatrix} + \begin{bmatrix}\mathbf{e_1}\\ \mathbf{e_2}\end{bmatrix},$$

where $\mathbf{X_1}$, $\mathbf{X_2}$, $\mathbf{Z_1}$, $\mathbf{Z_2}$, $\mathbf{W_1}$, and $\mathbf{W_2}$ are design matrices relating the fixed, additive genetic, and cow permanent effects to the phenotypes; $\mathbf{b_1}$ and $\mathbf{b_2}$ are the vectors of fixed effects of contemporary group, the proportion of Friesian (breed effect), heterosis Holstein-Friesian × Jersey and deviation from median calving date (calving date minus the median calving date of the herd); $\mathbf{a_1}$ and $\mathbf{a_2}$ are the vectors of random effects of animal for each trait; $\mathbf{c_1}$ and $\mathbf{c_2}$ are the vectors of random effects of cow permanent environment for each trait; and $\mathbf{e_1}$ and $\mathbf{e_2}$ are vectors of residual errors. The distributional properties of the model with E and Var indicating the expectation and variance were as follows:

$$E\begin{bmatrix}\mathbf{y_1}\\ \mathbf{y_2}\end{bmatrix} = \begin{bmatrix}\mathbf{X_1} & \mathbf{0}\\ \mathbf{0} & \mathbf{X_2}\end{bmatrix}\begin{bmatrix}\mathbf{b_1}\\ \mathbf{b_2}\end{bmatrix} \text{ and}$$

$$\text{Var}\begin{bmatrix}\mathbf{a_1}\\ \mathbf{a_2}\\ \mathbf{c_1}\\ \mathbf{c_2}\\ \mathbf{e_1}\\ \mathbf{e_2}\end{bmatrix} = \begin{bmatrix}\mathbf{A\sigma^2_{a1}} & \mathbf{A\sigma_{a1a2}} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0}\\ \mathbf{A\sigma_{a1a2}} & \mathbf{A\sigma^2_{a2}} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0}\\ \mathbf{0} & \mathbf{0} & \mathbf{I_1\sigma^2_{c1}} & \mathbf{I_1\sigma_{c1c2}} & \mathbf{0} & \mathbf{0}\\ \mathbf{0} & \mathbf{0} & \mathbf{I_1\sigma_{c1c2}} & \mathbf{I_1\sigma^2_{c2}} & \mathbf{0} & \mathbf{0}\\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{I_2\,\sigma^2_{e1}} & \mathbf{I_2\sigma_{e1e2}}\\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{I_2\sigma_{e1e2}} & \mathbf{I_2\,\sigma^2_{e2}}\end{bmatrix},$$

where $\mathbf{A}$ is the numerator relationship matrix of order equal to the total number of animals in the pedigree file; $\mathbf{I_1}$ is an identity matrix of order equal to the number of cows with lactation records; $\mathbf{I_2}$ is an identity matrix of order equal to the number of records.

Heritability (h^2) and repeatability (t) of each trait were calculated as follows (Falconer and Mackay 1996):

$$h^2 = \frac{\sigma^2_a}{\sigma^2_p} \text{ and } t = \frac{\sigma^2_a + \sigma^2_c}{\sigma^2_p},$$

where σ^2_a is the additive genetic (animal) variance, σ^2_p is the phenotypic variance for any trait, calculated as follows:

$$\sigma^2_p = \sigma^2_a + \sigma^2_c + \sigma^2_e$$

where σ^2_c is the cow permanent variance, and σ^2_e is the residual variance of the trait. Repeatability was considered high when it was >0.40; medium when it was between 0.10 and 0.40; and low when it was <0.10.

Genetic (r_G) and phenotypic (r_P) correlations were calculated as follows (Falconer and Mackay 1996):

$$r_G = \frac{\sigma_{a1a2}}{\sigma_{a1} \times \sigma_{a2}} \text{ and } r_P = \frac{\sigma_{p1p2}}{\sigma_{p1} \times \sigma_{p2}},$$

where σ_{a1a2} is the animal (genotypic) covariance between traits 1 and 2; σ_{p1p2} is the phenotypic covariance between traits 1 and 2; σ_{a1} and σ_{p1} are the additive genetic and phenotypic standard deviations for trait 1, respectively; and σ_{a2} and σ_{p2} are the additive genetic and phenotypic standard deviations for trait 2.

Chapter 4

Results

4.1. Productive and Reproductive Performance

Descriptive statistics for milk urea nitrogen (MUN), yield and percentages of production traits, and reproductive traits for both farms in seasons 2016 and 2017 are presented in Table A.1 and Table A.2, respectively, of the appendix.

Least-squares means and standard errors for productive and reproductive performance traits and MUN for both farms are presented in Table 4.1 (season 2016) and Table 4.2 (season 2017). MUN was higher (P<0.001) for Dairy 1 than for Dairy 4 in both seasons. Cows from Dairy 1 farm produced 2.37 mg/dL and 2.31 mg/dL more MUN than cows from Dairy 4 farm in seasons 2016 and 2017, respectively. The highest MUN value (22.7 mg/dL) was recorded from Dairy 1 in 2017 (Table A.2).

Table 4.1. Least-squares means, standard errors (SE) and probability values of farm effects on milk urea nitrogen, productive and reproductive performance of cows in Massey University Dairy 1 and Dairy 4 farms during the 2016 production season. Milk urea nitrogen and productive traits were the obtained at the start of the breeding season (closest date to first service).

	Farm				
	Dairy 1		Dairy 4		
Trait[1]	Mean	SE	Mean	SE	P-value
MUN, mg/dL	10.49	0.152	8.12	0.170	<0.001
MY, kg/day	17.85	0.219	22.54	0.245	<0.001
FY, kg/day	0.85	0.011	0.90	0.012	0.0009
PY, kg/day	0.68	0.008	0.80	0.009	<0.001
LY, kg/day	1.01	0.013	1.16	0.014	<0.001
FP, %	4.84	0.040	4.14	0.044	<.0001
PP, %	3.83	0.015	3.60	0.017	<.0001
LP, %	5.05	0.010	5.17	0.011	<.0001
SBFS, days	11	0.447	10	0.516	0.081
SBCO, days	20	1.075	16	1.261	0.0214
CFS, days	78	0.447	84	0.516	<.0001
FSCO, days	9	1.003	6	1.187	0.0619
DO, days	88	1.075	91	1.261	0.0487
SR21, %	95.9	1.4	94.7	1.9	0.5835
PR21, %	64.2	3.3	59.8	3.9	0.3737
PRFS, %	64.4	3.2	59.6	3.9	0.3198

[1] MUN = milk urea nitrogen, MY = milk yield, FY = fat yield, PY = protein yield, LY = lactose yield, FP = fat percentage, PP = protein percentage, LP = lactose percentage, SBFS = interval from start of breeding to first service, SBCO = interval from start of breeding to conception, CFS = interval from calving to first service, FSCO = interval from first service to conception, DO = days open or interval from calving to conception, SR21 = submission rate in 21 days

after start of breeding, PR21 = pregnancy rate in 21 days after start of breeding, PRFS = pregnancy rate at first service.

Table 4.2. Least-squares means, standard errors (SE) and probability values of farm effects on milk urea nitrogen, productive and reproductive performance of cows in Massey University Dairy 1 and Dairy 4 farms during the 2017 production season. Milk urea nitrogen and productive traits were the obtained at the start of the breeding season (closest date to first service).

	Farm				
	Dairy 1		Dairy 4		
Trait[1]	Mean	SE	Mean	SE	P-value
MUN, mg/dL	11.42	0.159	9.11	0.171	<.0001
MY, kg/day	18.81	0.230	23.15	0.246	<.0001
FY, kg/day	0.94	0.011	0.99	0.012	0.0048
PY, kg/day	0.73	0.008	0.87	0.009	<.0001
LY, kg/day	1.02	0.013	1.15	0.014	<.0001
FP, %	5.04	0.042	4.30	0.045	<.0001
PP, %	3.86	0.016	3.75	0.017	<.0001
LP, %	5.01	0.010	5.21	0.011	<.0001
SBFS, days	9	0.462	7	0.502	0.0016
SBCO, days	18	1.112	21	1.215	0.0668
CFS, days	83	0.462	80	0.502	<.0001
FSCO, days	8	1.033	14	1.131	0.0006
DO, days	91	1.112	93	1.215	0.2183
SR21, %	97.9	1.0	96.4	1.3	0.3653
PR21, %	68.7	3.3	47.7	3.8	<.0001
PRFS, %	66.6	3.3	45.3	3.8	<.0001

[1] MUN = milk urea nitrogen, MY = milk yield, FY = fat yield, PY = protein yield, LY = lactose yield, FP = fat percentage, PP = protein percentage, LP = lactose percentage, SBFS = interval from start of breeding to first service, SBCO = interval from start of breeding to conception, CFS = interval from calving to first service, FSCO = interval from first service to conception, DO = days open or interval from calving to conception, SR21 = submission rate in 21 days after start of breeding, PR21 = pregnancy rate in 21 days after start of breeding, PRFS = pregnancy rate at first service.

Milk yield (MY), protein yield (PY) and lactose yield (LY) were greater (P<0.001) for Dairy 4 cows in both seasons. Cows from Dairy 4 farm produced 4.7 kg/day and 4.3 kg/day more milk yield than cows from Dairy 1 farm in seasons 2016 and 2017, respectively. Fat yield was also higher for cows in Dairy 4 in seasons 2016 (P=0.0009) and 2017 (P=0.0048), respectively.

Percentages of milk fat (FP) and milk protein (PP) were higher (P<0.0001) for Dairy 1 in both seasons, but lactose percentage (LP) was higher (P<0.0001) for Dairy 4 in both seasons. Cows from Dairy 1 farm produced 0.70% more FP, 0.23% more PP, and 0.12% less LP than Dairy 4 cows in 2016. In 2017, cows from Dairy 1 farm produced 0.74% more FP, 0.11% more PP, and 0.20% less LP than Dairy 4 cows.

In 2016, cows from Dairy 4 farm conceived 4 days earlier (P=0.0214) after the start of the breeding season (SBCO), but had the first service after calving (CFS) 6 days later (P<0.0001), and took 3 days more (P=0.0487) to conceive after calving (DO), when compared with cows from Dairy 1 farm. However, cows from Dairy 1 and Dairy 4 took a similar amount of days to be serviced after the start of breeding (SBFS) and to become pregnant after first service (FSCO), which indicates that the chosen date of start of breeding in Dairy 4 was relatively later or calving was relatively earlier. Dairy 1 and Dairy 4 farms had similar pregnancy (PR21 and PRFS) and submission rates (SR21) in season 2016.

In 2017, cows from Dairy 4 farm were serviced 2 days earlier (P=0.0016) after the start of the breeding season (SBFS), and were serviced 3 days earlier (P<0.0001) after calving (CFS), but took 6 days longer (P=0.0006) to conceive after the first service (FSCO), when compared with cows from Dairy 1 farm. This indicates that cows from Dairy 4 farm had oestrus detected earlier but were not able to get pregnant until later and thus were likely to require more services. Dairy 1 farm had higher (P<0.0001) pregnancy rates (PR21 and PRFS) than Dairy 4 farm in season 2017. Dairy 1 and Dairy 4 farms had similar submission rates (SR21) in season 2017.

4.2. Estimates of Genetic Parameters

Estimates of heritability (h^2), repeatability (t), heterosis (FxJ), breed (F-J) and deviation from median calving date (DMCD) effects are presented in Table 4.3. MUN heritability was 0.24 and repeatability was 0.45. PP and FP had the highest heritability estimates (0.77 and 0.55, respectively) and highest repeatability estimates (0.78 and 0.57). LP had a moderate h^2 of 0.30 and repeatability of 0.54. Heritability of yield traits (MY, FY, PY, LY) were moderate, ranging from 0.27 to 0.35, with moderate to high repeatabilities ranging from 0.37 to 0.56. Reproductive traits had low heritabilities, ranging from 0.0 to 0.13, PRFS had a heritability of 0.0. Repeatabilities for reproductive traits were low to moderate ranging from 0.08 to 0.30, but repeatatiliby for SR21 was high (0.46). The standard errors of heritabilities and repeatabilities of reproductive traits were high.

Table 4.3. Estimates of heritability (h^2), repeatability (t), variance components, and effects of heterosis (FxJ), breed (F-J), and deviation from median calving date (DMCD) for milk and fertility traits from Dairy 1 and Dairy 4 during the 2016 and 2017 production seasons.

Trait[1]	h^2	SE	t	SE	Variance components[2]				Heterosis			Breed effect			DMCD		
					σ^2_a	σ^2_c	σ^2_e	σ^2_{Total}	FxJ	SE	P	F-J	SE	P	DMCD	SE	P
MUN	0.24	0.09	0.45	0.05	1.29	1.10	2.98	5.37	-0.3529	0.3054	0.1241	0.4090	0.3213	0.1017	-0.0096	0.0052	0.0338
MY	0.35	0.09	0.51	0.04	3.97	1.84	5.62	11.44	1.0580	0.4523	0.0098	5.4940	0.4860	0.0000	0.0798	0.0074	0.0000
FY	0.32	0.10	0.45	0.05	0.01	0.00	0.01	0.03	0.0392	0.0212	0.0324	0.0206	0.0227	0.1822	0.0029	0.0004	0.0000
PY	0.27	0.09	0.37	0.05	0.00	0.00	0.01	0.01	0.0437	0.0152	0.0021	0.1175	0.0161	0.0000	0.0016	0.0003	0.0000
LY	0.28	0.10	0.56	0.04	0.01	0.01	0.02	0.04	0.0897	0.0262	0.0003	0.2645	0.0277	0.0000	0.0024	0.0004	0.0000
FP	0.55	0.09	0.57	0.04	0.21	0.01	0.16	0.38	-0.1575	0.0827	0.0286	-1.1420	0.0923	0.0000	-0.0031	0.0013	0.0076
PP	0.77	0.08	0.78	0.02	0.05	0.00	0.01	0.06	-0.0486	0.0338	0.0754	-0.4583	0.0392	0.0000	-0.0065	0.0004	0.0000
LP	0.30	0.10	0.54	0.04	0.01	0.01	0.01	0.02	0.0213	0.0202	0.1460	-0.1083	0.0215	0.0000	-0.0016	0.0003	0.0000
SBFS	0.02	0.07	0.11	0.06	0.76	4.35	39.39	44.50	-0.7866	0.7907	0.1601	0.8995	0.7907	0.1278	0.0167	0.0156	0.1432
SBCO	0.03	0.08	0.30	0.07	6.56	64.52	166.34	237.42	-2.6240	2.0470	0.1001	0.3452	2.0650	0.4336	0.0303	0.0382	0.2138
CFS	0.02	0.07	0.11	0.06	0.76	4.35	39.40	44.51	-0.7866	0.7906	0.1600	0.8994	0.7907	0.1278	-0.9833	0.0156	0.0000
FSCO	0.05	0.08	0.20	0.07	10.23	30.30	164.28	204.81	-1.5480	1.8640	0.2032	0.1336	1.8930	0.4719	0.0419	0.0357	0.1207
DO	0.03	0.08	0.30	0.07	6.56	64.54	166.16	237.26	-2.6240	2.0470	0.1001	0.3463	2.0660	0.4335	-0.9699	0.0382	0.0000
SR21	0.13	0.10	0.46	0.06	0.01	0.01	0.02	0.04	0.0216	0.0264	0.2067	-0.0349	0.0271	0.0991	-0.0001	0.0005	0.4457
PR21	0.03	0.07	0.12	0.07	0.01	0.02	0.20	0.23	0.0815	0.0574	0.0780	0.0683	0.0577	0.1184	-0.0024	0.0011	0.0154
PRFS	0.00	0.00	0.08	0.07	0.00	0.02	0.22	0.23	0.0473	0.0564	0.2011	0.0867	0.0561	0.0612	-0.0023	0.0011	0.0642

[1] MUN = milk urea nitrogen (mg/dL), MY = milk yield (kg/day), FY = fat yield (kg/day), PY = protein yield (kg/day), LY = lactose yield (kg/day), FP = fat percentage (%), PP = protein percentage (%), LP = lactose percentage (%), SBFS = interval from start of breeding to first service (days), SBCO = interval from start of breeding to conception (days), CFS = interval from calving to first service (days), FSCO = interval from first service to conception (days), DO = days open or interval from calving to conception (days), SR21 = submission rate in 21 days after start of breeding (days), PR21 = pregnancy rate in 21 days after start of breeding (days), PRFS = pregnancy rate at first service (days).[2] σ^2_a = genetic variance, σ^2_c = permanent environmental variance, σ^2_e = residual error variance, σ^2_{Total} = sum of all variances, FxJ = heterosis effect of Holstein-Friesian Jersey crossbred, F-J = breed effect expressed as the difference between Holstein-Friesian and Jersey, DMCD = deviation from median calving date effect.

Heterosis effects (FxJ) were positive and significant (P<0.05) for the yield traits (MY, FY, PY and LY), meaning that crossbred cows produced significantly more yield/day than the average of the purebred cows. Heterosis resulted in 1.06 kg/day more MY (P=0.0098); 0.04 kg/day more FY (P=0.0324); 0.04 kg/day more PY (P=0.0021); 0.09 kg/day more LY (P=0.0003). Heterosis effect was negative and significant (P=0.0286) for fat percentage (FP), meaning that crossbred cows had 0.16 % less fat in the milk, compared to the purebred cows. Heterosis effect for MUN was not significant.

Breed effects were significant (P<0.05) for all milk production traits, except for FY. Friesian cows produced 5.49 kg/day more milk than Jersey cows (P<0.001). Friesian cows also produced 0.12 kg/day and 0.27 kg/day more (P<0.001) protein and lactose yield than Jersey cows, respectively. However, Jersey cows produced milk with 1.14% more fat percentage, 0.46% more protein percentage, and 0.11% more lactose percentage, compared with Friesian cows (P<0.001). Breed effect for MUN was not significant.

Effects of deviation from median calving date were significant (P<0.05) for most traits, except for the reproductive traits SBFS, SBCO, FSCO, SR21, and PRFS. Deviation from median calving date (days) was a positive value for later calving cows and negative for early calving cows. Cows that calved 1 day later than the median calving date of the herd produced 0.01 mg/dL less MUN (P=0.0338), 0.08 kg/day more MY, 0.003 kg/day more FY, 0.002 kg/day more PY, and 0.002 kg/day more LY (P<0.001); but produced 0.003% less fat (P=0.0076), 0.007% less protein and 0.002% less lactose (P<0.001). Cows that calved later than the median calving date had a shorter time from calving to first service and a shorter calving to conception (P<0.001), but lower (P=0.0154) probability of becoming pregnant up to 21 days after the start of the breeding season.

4.3. Genetic and Phenotypic Correlations

Genetic (r_G) and phenotypic (r_P) correlations are presented in Table 4.4. The genetic correlation between MUN and reproductive traits were strong negative, but the phenotypic correlations were close to zero. The r_G between MUN and SR21 was -0.50, and between MUN and PR21 was -0.45, but the standard errors were large (>0.44).

Overall, MUN had an antagonistic genetic correlation with production traits. The r_G and r_P between MUN and MY were close to zero. MUN had a moderate negative genetic correlation with FY, FP and LP. The r_G between MUN and the other milk production traits (PY, LY, PP)

were weak negative. The r_P between MUN and milk production traits were weak, ranging from -0.03 to 0.15 (0.04 SE).

Milk yield had a moderate positive genetic correlation with the reproductive traits SR21 and PR21, but the standard errors of these estimates were large. The corresponding r_P were close to zero. The estimates of r_G between MY and other yield traits (FY, PY, LY) were strong positive, ranging from 0.41 to 0.99 (0.07 to 0.19 SE). The r_G between MY and milk percentage traits (FP, PP, LP) were moderate negative, ranging from -0.38 to -0.61 (0.11 to 0.21 SE).

The other milk yield traits (FY, PY, LY) had a positive moderate r_G with PR21. Protein percentage had a moderate positive r_G with PR21, but the standard error was large. The estimates of r_P between milk and reproductive traits were close to zero.

The estimate of r_G between SR21 and PR21 was strong positive (0.96), but the r_P between these two traits was much lower (0.25).

Table 4.4. Estimated genetic (below diagonal) and phenotypic (above diagonal) correlations with standard errors (SE) among milk urea nitrogen, milk yields, milk percentages and reproductive traits from Dairy 1 and Dairy 4 herds in 2016 and 2017 production seasons.

	MUN		MY		FY		PY		LY		FP		PP		LP		SR21		PR21		PRFS	
		SE		SE		SE		SE		SE		SE		SE		SE		SE		SE		SE
MUN			0.07	0.04	0.04	0.04	0.07	0.04	0.15	0.04	-0.03	0.04	-0.03	0.04	0.01	0.04	-0.07	0.04	-0.01	0.03	0.03	0.03
MY	-0.01	0.24			0.63	0.02	0.86	0.01	0.68	0.02	-0.35	0.03	-0.40	0.03	-0.06	0.04	-0.01	0.04	0.03	0.03	‡	‡
FY	-0.37	0.28	0.41	0.17			0.66	0.02	0.40	0.03	0.42	0.03	-0.04	0.04	0.01	0.04	-0.02	0.04	0.05	0.04	‡	‡
PY	-0.09	0.26	0.80	0.07	0.64	0.13			0.57	0.02	-0.19	0.04	0.03	0.04	0.00	0.04	-0.01	0.04	0.06	0.03	-0.02	0.06
LY	-0.04	0.27	0.99	0.09	0.55	0.18	0.73	0.15			-0.29	0.04	-0.29	0.04	0.16	0.04	-0.04	0.04	0.03	0.04	0.02	0.04
FP	-0.18	0.21	-0.54	0.14	0.50	0.14	-0.16	0.18	-0.42	0.18			0.41	0.03	0.12	0.04	‡	‡	‡	‡	‡	‡
PP	-0.05	0.18	-0.61	0.11	0.05	0.16	-0.02	0.16	-0.47	0.15	0.63	0.09			0.14	0.04	‡	‡	0.08	0.04	‡	‡
LP	-0.31	0.28	-0.38	0.21	-0.03	0.24	-0.34	0.24	-0.08	0.26	0.30	0.18	0.15	0.16			-0.01	0.04	‡	‡	‡	‡
SR21	-0.50	0.44	0.14	0.32	0.36	0.42	0.29	0.35	0.10	0.36	‡	‡	‡	‡	0.10	0.36			0.25	0.03	‡	‡
PR21	-0.45	0.70	0.38	0.75	0.03	0.59	0.61	0.92	0.49	0.75	‡	‡	0.38	0.40	‡	‡	0.96	0.80			‡	‡
PRFS	0.00	0.00	‡	‡	‡	‡	-0.16	0.36	0.39	0.39	‡	‡	‡	‡	‡	‡	‡	‡	‡	‡		

MUN = milk urea nitrogen (mg/dL), MY = milk yield (kg/day), FY = fat yield (kg/day), PY = protein yield (kg/day), LY = lactose yield (kg/day), FP = fat percentage (%), PP = protein percentage (%), LP = lactose percentage (%), SR21 = submission rate in 21 days after start of breeding (days), PR21 = pregnancy rate in 21 days after start of breeding (days), PRFS = pregnancy rate at first service (days).
‡Genetic and phenotypic correlations were not possible to be estimated due to the small dataset.

Chapter 5

Discussion

5.1. Means

Cows from Dairy 1 had higher MUN than cows from Dairy 4. This could be due to Dairy 1 having higher total crude protein in the diet, compared with Dairy 4, during both production seasons (Table 3.4).

The maximum concentration of MUN reported in the current study was above 19 mg/dL for Dairy 1 during both production seasons, which is above the threshold reported to affect fertility of dairy cows (Butler et al. 1996). Cutzal (2019), however, reported that MUN did not exceed the threshold levels in both Dairy 1 and Dairy 4 during the same production seasons. The difference in the results may be due to the MUN values used in that study being predicted for the time of first insemination, whilst this study used actual MUN values from milk-test day that was performed close to the date of first service.

The average concentration of MUN found in this study was below 19 mg/dL for both farms and both production seasons (between 8.2 and 11.4 mg/dL), and was similar to the previous findings in those farms (Lopez-Villalobos et al. 2018) and in Brazil (Oliveira et al. 2012), but lower than those reported by Beatson et al. (2019) from farms located throughout New Zealand (14.0 mg/dL). Average MUN was also lower than those reported by other studies conducted overseas (Mitchell et al. 2005; König et al. 2008; Hossein-Zadeh and Ardalan 2010; Mucha and Strandberg 2011) and similar to that reported by Miglior et al. (2006).

Milk urea nitrogen concentrations are routinely predicted using mid-infrared spectroscopy (Oliveira et al. 2012; Gengler et al. 2019). Oliveira et al. (2012) reported that the CombiScope FTIR equipment, which is similar to the instrument used in this study, was a reliable method for analysis of MUN content in raw milk (r=0.89). However, Mitchell et al. (2005) reported different estimated of genetic correlations between reproduction traits and MUN measured either by direct wet-chemistry or predicted using mid-infrared spectroscopy. This highlight a need to review the calibration equations to predict MUN using mid-infrared spectroscopy.

The average milk yield produced in Dairy 4 was higher than the average milk yield produced in Dairy 1, during both production seasons, which is expected as Dairy 1 cows were milked OAD whereas Dairy 4 cows were milked TAD during the entire season. Milk protein and fat percentages were significantly higher for Dairy 1 than for Dairy 4 farm, and lactose percentage was higher for twice-a-day farm (Dairy 4) in both seasons. Concentrations of fat

and protein have been reported to be significantly greater ($P<0.05$) for cows milked OAD when compared to cows milked TAD (Clark et al. 2006); and lactose concentration has been reported to decrease with OAD milking frequency (Davis et al. 1999). The changes in milk composition are likely to be caused by changes in permeability of tight junctions between the secretory epithelial cells resulting in increased exchange of milk and interstitial fluid (Stelwagen et al. 1994).

The reproductive performance of Dairy 1 and Dairy 4 cows was better than those reported by Grosshans et al. (1997) and Brownlie et al. (2014) in New Zealand dairy herds. In this study, both farms achieved high submission rates (above 90%) during the first 21 days after the start of breeding season and high pregnancy rates 21 days after the start of the breeding season. Dairy 1 had significantly higher pregnancy rates (PR21 and PRFS) than Dairy 4 in season 2017, this better reproductive performance has been expected from once-a-day milking system when compared to twice-a-day milking system and is likely to be due to the less negative energy balance for the once-a-day cows during the first three weeks after calving, reducing interval to first ovulation (Patton et al. 2006).

5.2. Genetic Parameters

Heritability for MUN found in this study (0.24) was similar to that reported by Beatson et al. (2019) (0.22) and equal to that reported by Lopez-Villalobos et al. (2018) (0.24), both studies using New Zealand dairy cows. Also, heritability for milk urea nitrogen was similar to those reported overseas by Mitchell et al. (2005) (0.22) and Hossein-Zadeh and Ardalan (2010) (0.18 to 0.22) but higher than those reported by Stoop et al. (2007) (0.14) and Mucha and Strandberg (2011) (0.16) and König et al. 2008 (0.13 and 0.15). The moderate heritability for MUN found in this study indicates that MUN concentration could be modified by selective breeding.

The genetic parameters for yield traits (MY, FY, PY, LY) reported in this study are higher than the range of 0.12 to 0.20 previously reported by Lopez-Villalobos et al. (2018) in Dairy 1 and Dairy 4, and by Sneddon et al. (2015) in New Zealand dairy herds. Pryce and Harris (2006) reported h^2 for MY similar to that reported in the current study (0.36). Lembeye et al. (2016) also reported similar h^2 estimates for MY (0.33) and PY (0.22). Other studies have reported higher h^2 estimates for FY and PY, 0.36–0.45 and 0.32–0.40, respectively (Berry et al. 2003, Miglior et al. 2007).

The h^2 estimates for percentage traits (FP, PP and LP) were also higher than those reported by Sneddon et al. (2015) and Lopez-Villalobos et al. (2018). Heritability estimates for percentage traits are usually at least twice as high as yield traits and range from 0.50 to 0.60 (Lopez-Villalobos 2012). However, Lembeye et al. (2016) also reported higher heritabilities for FP (0.62) and PP (0.67) in cows milked OAD in New Zealand. Heritability for LP found in this study was similar to that found by Rzewuska and Strabel (2013) (0.26 to 0.34), but it was the highest h^2 value reported by them. Stoop et al. (2007) and Miglior et al. (2007) estimated h^2 values for LP higher than the one reported in this study, 0.64 and 0.48 to 0.51, respectively.

In general, h^2 estimates for fertility traits are low (less than 0.13) indicating that these traits are largely influenced by management and environmental factors (Berglund, 2008). The h^2 estimates for reproduction traits estimated in this study were low, which are in agreement with the estimated values reported by previous studies (Darwash 1997; Grosshans et al. 1997; Berry et al. 2014). However, the h^2 for SR21 estimated in this study (0.13) was higher than the value reported by Amer et al. (2016), which ranged from 0.031 to 0.058 across different parities in New Zealand dairy cows. Bowley et al. (2015) also reported low values (0.035) from New Zealand cows. Grosshans et al. (1997) found higher h^2 estimate for SBFS of 0.06, and similar h^2 for SBCO and PR21. The h^2 estimate for FSCO (0.05) was higher than that reported by Grosshans et al. (1997) (0.01). Grosshans et al. (1997) found low h^2 estimates for CFS and DO for first lactation (0.03 and 0.02) and even less for second lactation, which goes in agreement with the low values reported in this study. The h^2 for PRFS estimated in the current study (0.00) was similar to that reported by Berry et al. (2003) (0.01) in Ireland.

Repeatabilities estimated for MUN and production traits found in this study were high, agreeing with Sneddon et al. (2015), Lembeye et al. (2016), and Lopez-Villalobos et al. (2018). Repeatabilities estimated for fertility traits were low to moderate. Repeatability expresses the proportion of variance in multiple measurements of a trait that are due to permanent (genetic and environmental) differences among individuals (Falconer 1960), whereas heritability expresses the proportion of variance of a trait that are due to genetic variation only. High repeatability indicates that individuals perform consistently throughout time in the same environment and there is little gain in accuracy from repeated measures (Boake 1989). In the case of reproductive traits, repeatability was much higher than heritability and indicates that environmental variation is high. High repeatability and high heritability of production traits FP and PP indicates environmental variation is low and most of the genetic variation is additive.

Tenghe et al. (2015) also reported low repeatability estimates for classical fertility traits (calving interval and calving to first service), but moderate repeatability estimates for endocrine fertility traits (obtained from milk progesterone records), indicating that these are more influenced by the cow itself and could be a better measure of cow physiology than the classical fertility measures that are mainly controlled by farm management decisions and by oestrus detection.

Breed effect was significant for milk, protein and lactose yields; Holstein-Friesian cows had higher MY, PY and LY than Jersey cows, but Jersey cows produced milk with higher values of FP, PP and LP than Holstein-Friesian cows, in agreeing with Lopez-Villalobos et al. (2018). Breed effect was not significant for MUN. Kauffman and St-Pierre (2001) also reported no significant difference for MUN between milk from Holstein-Friesian and Jersey cows. There was no significant difference in fertility between the two breeds although Grosshans et al. (1997) showed significant differences in reproductive performance, with Jersey cows being superior to Friesian cows in New Zealand (SBFS intervals were 3.5 and 5.4 days shorter for Jersey than for Friesian cows). However, Coffey et al. (2016) also reported no breed effect on reproductive traits in Irish dairy herds, as these traits are highly under the influence of the environment. The very good reproductive performance in both Dairy 1 and Dairy 4 herds in may be the reason why no significant differences between farms and breeds were detected in season 2016.

Heterosis is defined as the differential performance of crossbred animals compared with the average of both purebred parental breeds (Sørensen et al. 2008). In this study, heterosis effect was significant for all yield traits, also confirming what has been reported by Lopez-Villalobos et al. (2018), crossbred cows performed better than the average of the purebreds for milk yield traits. Heterosis effect was not significant for most milk composition traits, except for FP. There was no significant heterosis effect for any reproductive variable, this may be due to the good reproductive performance achieved in the two herds and production seasons. Although, crossbreeding is known to benefit traits such as reproduction, health and survival (Lopez-Villalobos and Garrick 2002), Coffey et al. (2016) also found no heterosis effect for calving to first service (CFS) nor for submission rate (SR).

Cows that calved later than the median calving date of the herd had a higher value of deviation from median calving date (DMCD). DMCD effect was significant for MUN, for all production traits and for some of the reproductive traits (CFS, DO and PR21). Late calving cows had lower levels of content traits MUN, FP, PP and LP, but higher yield traits MY, FY, PY LY. Early calving may result in an underfeeding of the cows because the calving period is planned to be synchronized with the supply of feed and, consequently, these cows may produce lower daily milk yields during the first weeks of lactation (Garcia and Holmes 1999). Late calving cows had a shorter calving to first service, shorter days open and lower pregnancy rate at 21 days after start of breeding season, later calving cows have low probability to become pregnant because they have shorter recovery time from calving to the start of mating.

5.3. Correlations

Concentration of MUN had a moderate negative genetic correlation with the two most important reproductive traits of seasonal grazing systems (SR21 and PR21). These estimates are in agreement with estimates of genetic correlations between MUN and 56- and 90-day nonreturn rates in German dairy cattle, -0.19 and -0.23, respectively (König et al. 2008). The results from König et al. (2008) and our study suggest that selection for low MUN would result in better reproductive performance, however, König et al. (2008) demonstrated by selection index calculations that the genetic correlations between MUN and nonreturn rates were too weak to justify the use of MUN as an additional trait in genetic selection for fertility. Moreover, in our study the standard error for r_G between MUN and PR21 was large and therefore these estimates should be considered with caution.

The estimates of r_P between MUN and fertility traits SR21 and PR21 were close to zero. These results agree with estimates of r_P between MUN and fertility traits in first-lactation Swedish Holstein cows as reported by Mucha et al. (2011). The low phenotypic correlation between MUN and fertility supports the theory that high MUN is not necessarily associated with low fertility in cows as they may adapt to high CP diets whilst producing high MUN, and that the low negative energy balance at post-calving is more likely to affect fertility of cows.

The genetic and phenotypic correlations between MUN and continuous fertility traits SBFS, SBCO, CFS, FSCO and DO were not reported in this study because of the high standard errors encountered due to the nature of these variables. The traits related to the start of breeding and to the services performed (SBFS, SBCO, CFS and FSCO) are related to farmer's decision when to start the breeding period rather than the fertility of the cow. These interval traits are not the best indicators of cow fertility in seasonal dairy systems where animals that calve early are withheld from being bred until the seasonal breeding season starts, further resulting in longer CFS (Grosshans et al. 1997; Bowley et al. 2015).

In this study, more than 90% of first inseminations were performed during 21 days after the start of the breeding season, so there is no genetic difference in SBFS among these cows, being SBFS a poor predictor of fertility. Also, SBFS only measures the ability of a cow to show oestrus after the start of the breeding season and does not measure the ability of a cow to conceive. However, the traits related to conception (SBCO, FSCO and DO) were obtained using only cows that became pregnant, calves that failed to conceive were left in blank and therefore ended up not being analyzed by the model.

The estimates of r_G and r_P between milk urea nitrogen and milk yield were close to zero agreeing with Miglior et al. (2007), although moderate positive r_G ranging from 0.22 to of 0.77 have been previously reported (Stoop et al. 2007; König et al. 2008; Lopez-Villalobos et al. 2018) and indicated that higher MUN could be observed in high producing cows, which was suggested to be due to energy deficiency and higher tissue mobilization in early lactation.

The r_G between MUN and other production traits were also close to zero, except between MUN and FY, FP and LP, which were moderate negative (-0.37, -0.18 and -0.31, respectively). Lopez-Villalobos et al. (2018) found stronger negative genetic correlations between MU and FP (-0.80) and LP (-0.76). The weak negative genetic correlation between MUN and PP found in the current study (-0.05) was weaker than the estimate (-0.66) reported by Lopez-Villalobos et al. (2018). The difference between these estimates is caused that in the current study only herd-test close to the mating season were used, whereas in the study by Lopez-Villalobos the estimates of r_G were obtained using all records during the lactation.

Milk yield measured close to the start of breeding had a favorable genetic correlation with fertility, which can be a consequence of the combined selection for high MY, culling of empty cows, use of fertility in breeding objectives (Pryce et al. 2014) and crossbreeding to explore heterosis effect (Berglund 2008). Whilst earlier studies from overseas (Abdallah and McDaniel 2000; Pryce et al. 2001; Berry et al. 2003) and from New Zealand (Grosshans et al. 1997) reported moderate to high unfavorable genetic correlations between milk production traits and fertility, which shows New Zealand dairy cows have been more recently selected for improved fertility. There was a weak phenotypic correlation between MY and fertility which agrees with the findings of Grosshans et al. (1997) and Muir et al. (2004) and suggests that fertility is more under the influence of environmental effects and of reproductive management rather than genetic factors.

The current study found moderate positive r_G and weak positive r_P between milk protein percentage (PP) and pregnancy rate at 21 days after start of breeding (PR21), which implies that cows producing milk with high protein percentage have higher probability to become pregnant during the 21 days after the start of the mating period. This result agrees with results published by Morton et al. (2001) and Yang et al. (2010). Cows producing low PP have been reported to undergo a more severe and prolonged NEB compared to cows producing high PP (Fulkerson et al. 2001); low PP would be a result of the shortage of glucose, which is used in the synthesis of milk protein in the udder (de Vries & Veerkamp 2000). Also, studies have suggested that nitrogen being partitioned towards milk protein synthesis (PP) implies that less nitrogen is being excreted in the form of MUN which therefore means there is less circulating urea to affect fertility (Godden et al. 2001; Johnson and Young 2003).

5.4. Conclusions

Cows from both OAD and TAD dairy farms from Massey University had an average MUN concentration ranging from 8.2 to 11.4 mg/dL, which is lower than the threshold reported to affect fertility of dairy cows. Milk urea nitrogen had a moderate heritability of 0.24 meaning that a reduction in MUN would be expected if this trait is included in a selection index. The estimates of genetic correlations between MUN and fertility traits (SR21 and PR21) were moderate negative (-0.55 and 0.45), but the standard errors of the estimates were large due to the small data set. The estimates of genetic parameters indicate that MUN concentration may be reduced by genetic selection with a potential to increase the submission and pregnancy rate during the first 21 days after the start of mating, which are the two most important reproductive traits in grazing dairy cows. The phenotypic correlations, however, were close to zero indicating that high MUN is not necessarily associated with impaired fertility of dairy cows and that other factors may be contribute more to lower fertility, like low negative energy balance or a sudden change in CP content of the diet.

The satisfactory reproductive performance achieved in both farms (mainly reflected as high submission and pregnancy rates) is mainly a result of the good reproductive management and is less attributable to genetics. The results indicate that selection for low MUN should not have detrimental effects on reproduction performance and should be able to occur concurrently to selection for reproduction in New Zealand dairy farms. Further studies with a larger dataset would enable more accurate estimates of the genetic parameters for MUN, productive and reproductive traits.

References

Abdallah JM, McDaniel BT. 2000. Genetic parameters and trends of milk, fat, days open, and body weight after calving in North Caroline experimental herds. Journal of Dairy Science 83: 1364-1370.

AFRC. 1993. Energy and protein requirements of ruminants. An advisory manual prepared by the AFRC Technical Committee on Responses to Nutrients. CAB International, Wallington, UK.

Amer PR, Stachowicz K, Jenkins GM, Meier S. 2016. Short communication: Estimated of genetic parameters for dairy fertility in New Zealand. Journal of Dairy Science 99: 8227-8230.

Amundson OL, Larimore EL, McNeel AK, Chase CC, Cushman RA, Freetly HC, Perry GA. 2016. Uterine environment and pregnancy rate of heifers with elevated plasma urea nitrogen. Animal Reproduction Science 173: 56–62.

Auldist MJ, MacDonald KA, Thomson NA, Coulter M, Lancaster JAS, Penno JW. 2002. Effect of time of calving on dairy production. Proceedings of New Zealand Society of Animal Production 62: 42- 43.

Bach A, Calsamiglia S, Stern MD. 2005. Nitrogen metabolism in the rumen. Journal of Dairy Science 88: 9-21.

Beam S, Butler W. 1999. Effects of energy balance on follicular development and first ovulation in postpartum dairy cows. Journal of Reproduction and Fertility-Supplement: 411-424.

Beatson PR, Meier S, Cullen NG, Eding H. 2019. Genetic variation in milk urea nitrogen concentration of dairy cattle and its implications for reducing urinary nitrogen excretion. Animal 13: 2164 –2171.

Bergen WG, Potter EL. 1975. Effect of dietary protein level on plasma and tissue free amino acid concentrations in nursing lambs. Journal of Animal Science 40: 789- 794.

Berglund B. 2008. Genetic improvement of dairy cow reproductive performance. Reproduction in Domestic Animals 43: 89-95.

Berry DP, Buckley F, Dillon P, Evans RD, Rath M, Veerkamp RF. 2003. Genetic parameters for body condition score, body weight, milk yield, and fertility estimated using random regression models. Journal of Dairy Science 86: 3704- 3717.

Berry DP, Wall E, Pryce JE. 2014. Genetics and genomics of reproductive performance in dairy and beef cattle. Animal 8: 105-121.

Bewsell D, Clark DA, Dalley DE. 2008. Understanding motivations to adopt once-a-day milking amongst New Zealand dairy farmers. The Journal of Agricultural Education and Extension 14: 69-80.

Blanchard T, Ferguson JD, Love L, Takeda T, Henderson B, Hasler J, Chalupa W. 1990. Effect of dietary crude-protein type on fertilization and embryo quality in dairy cattle. American Journal of Veterinary Research 51: 905-908.

Boake CRB. 1989. Repeatability: Its role in evolutionary studies of mating behavior. Evolutionary Ecology 3: 173–182.

Bolan NS, Saggar S, Luo J, Bhandral R, Singh J. 2004. Gaseous emission of nitrogen from grazed pasture: processes, measurements and modelling, environmental implications, and mitigation. Advances in Agronomy 84: 37-120.

Bowley FE, Green RE, Amer PR, Meier S. 2015. Novel approaches to genetic analysis of fertility traits in New Zealand dairy cattle. Journal of Dairy Science 98: 2005-2012.

Broderick GA and Huhtanen P. 2007. Application of milk urea nitrogen levels. Proceedings of the Cornell Nutrition Conference: 185-193.

Brownlie T, Morton J, Heuer C, Hunnam J, McDougall S. 2014. Reproductive performance of seasonal-calving, pasture-based dairy herds in four regions of New Zealand. New Zealand Veterinary Journal 62: 77-86.

Burke JL. 2004. Determination of digestion parameters to develop and evaluate forage mixtures for pasture-fed ruminants. Doctor of Philosophy in Animal Science, Massey University, Palmerston North, New Zealand.

Burroughs W, Nelson DK, Mertens DR. 1975. Evaluation of protein nutrition. Journal of Dairy Science 58: 611-619.

Butler WR. 1998. Review: effect of protein nutrition on ovarian and uterine physiology in dairy cattle. Journal of Dairy Science 8: 2533-2539.

Butler WR. 2003. Energy balance relationships with follicular development, ovulation and fertility in postpartum dairy cows. Livestock Production Science 83: 211-218.

Butler WR. 2005. Relationships of dietary protein and fertility. Advances in Dairy Technology 17: 159-168.

Butler WR, Calaman J, Beam S. 1996. Plasma and milk urea nitrogen in relation to pregnancy rate in lactating dairy cattle. Journal of animal science 74: 858-865.

Clark DA, Phyn CVC, Tong MJ, Collis SJ, Dalley DE. 2006. A systems comparison of once- versus twice-daily milking of pastured dairy cows. Journal of Dairy Science 89: 1854-1862.

Coffey EL, Horan B, Evans RD, Berry DP. 2016. Milk production and fertility performance of Holstein, Friesian, and Jersey purebred cows and their respective crosses in seasonal-calving commercial farms. Journal of Dairy Science 99: 5681-5689.

Congleton WR, King LW. 1984. Profitability of Dairy Cow Herd Life. Journal of Dairy Science 67: 661-674.

Correa-Luna M, Lopez-Villalobos N, Almeida Jr GA, Donaghy DJ, Kemp PD. 2018. Phenotypic correlations of milk urea and the efficiency of crude protein utilization with milk yield traits and cow performance in two contrasting dairy systems in New Zealand. New Zealand Journal of Animal Science and Production 78: 76-81.

Crowe MA. 2008. Resumption of ovarian cyclicity in post-partum beef and dairy cows. Reproduction in Domestic Animals 43: 20-28.

Cutzal LXR. 2019. Relationship between milk urea nitrogen and fertility parameters in dairy cows under New Zealand grazing conditions. Massey University, Palmerston North, NZ.

DairyNZ Ltd. 2017. The InCalf Book for New Zealand dairy farmers 2nd Edition. Hamilton, NZ.

Dalley DE, Gardner G. 2012. Pasture growth and quality on West Coast dairy farms. Proceedings of the New Zealand Grasslands Association 74: 231-236.

Dalley DE, Malcolm BJ, Chakwizira E, de Ruiter JM. 2017. Range of quality characteristics of New Zealand forages and implications for reducing the nitrogen leaching risk from grazing dairy cows. New Zealand Journal of Agricultural Research 60: 319-332.

Darwash AO, Lamming GE, Woolliams JA. 1997. Estimation of genetic variation in the interval from calving to postpartum ovulation of dairy cows. Journal of Dairy Science 80: 1227-1234.

Davis SR, Farr VC, Stelwagen K. 1999. Regulation of yield loss and milk composition during once-daily milking: A review. Livestock Production Science 59: 77-94.

Dawuda PM, Scaramuzzi RJ, Leese HJ, Hall CJ, Peters AR, Drew SB, Wathes DC. 2002. Effect of timing or urea feeding on the yield and quality of embryos in lactating dairy cows. Theriogenology 58: 1443-1455.

de Vries MJ, Veerkamp RF. 2000. Energy balance of dairy cattle in relation to milk production variables and fertility. Journal of Dairy Science 83: 62-69.

De Wit AAC, Cesar MLF, Kruip TAM. 2001: Effect of urea during in vitro maturation on nuclear maturation and embryo development of bovine cumulus-oocyte-complexes. Journal of Dairy Science 84: 1800-1804.

Dickerson GE. 1973. Inbreeding and heterosis in animals. Journal of Animal Science: 54-77.

Dietz RW, Flipse RJ. 1969. Metabolism of bovine semen. Role of ammonia in interactions between citric acid and urea cycle. Biology of Reproduction 1: 200-206.

Dillon P, Berry DP, Evans RD, Buckley F, Horan B. 2006. Consequences of genetic selection for increased milk production in European seasonal pasture-based systems of milk production. Livestock Science 99: 141-158.

Dohm MR. 2002. Repeatability estimates do not always set an upper limit to heritability. Functional Ecology 16: 273-280.

Edwards GR, Parsons AJ, Rasmussen S, Bryant RH. 2007. High sugar ryegrasses for dairy systems. Proceedings of the New Zealand Grassland Association 69: 161-171.

Edwards JS, Bartley EE, Dayton AD. 1980. Effects of dietary protein concentration on lactating cows. Journal of Dairy Science 63: 243-248

Elrod CC, Butler WR. 1993. Reduction of fertility and alternation of uterine pH in heifers fed excess ruminally degradable protein. Journal of Animal Science 71: 694-701.

Emmanuel B. 1980. Urea cycle enzymes in tissues (liver, rumen epithelium, heart, kidney, lung and spleen) of sheep (ovis aries). Comparative Biochemistry and Physiology 65: 693-697.

Esslemont RJ, Peeler EJ. 1993. The scope for raising margins in dairy herds by improving fertility and health. British Veterinary Journal 149: 537-547.

Fahey J, Boland MP, and O'Callaghan D. 2001.The effects of dietary urea on embryo development in superovulated donor ewes and on early embryo survival and development in recipient ewes. Animal Science 72: 395-400.

Falconer DS, Mackay M. 1996. Introduction To Quantitative Genetics. Longman Group, Essex, UK.

Falconer DS. 1960. Introduction To Quantitative Genetics. The Ronald Press Company, New York, USA.

Fenderson CL, Bergen WG. 1976. Effects of excess dietary protein on feed intake and nitrogen metabolism in steers. Journal of Animal Science 42: 1323-1330.

Ferguson JD, Blanchard T, Galligan DT, Hoshall DC, Chalupa W. 1988. Infertility in dairy cattle fed a high percentage of protein degradable in the rumen. Journal of the American Veterinary Medical Association 192: 659-662.

Ferguson JD, Galligan DT, Blanchard T, Reeves M. 1993. Serum urea nitrogen and conception rate: the usefulness of test information. Journal of Dairy Science 76: 3742-3746.

Fleming AE, Edwards GR, Bryant RH, Dalley D, Gregorini P. 2018. Milk production and milk fatty acid composition of grazing dairy cows supplemented with fodder beet. New Zealand Journal of Animal Science and Production 78: 6-10.

Fulkerson WJ, Wilkins J, Dobos RC, Hough GM, Goddard ME, Davidson T. 2001. Reproductive performance in Holstein-Friesian cows in relation to genetic merit and level of feeding when grazing pasture. Animal Science 73: 397-406.

Garcia SC, Holmes CW. 1999. Effects of time of calving on the productivity of pasture-based dairy systems: a review. New Zealand Journal of Agricultural Research 42: 347-362.

Garcia SC, Holmes CW. 1999. Effects of time of calving on the productivity of pasture-based system: A review. New Zealand Journal of Agricultural Research 42: 347-362.

Garcia-Bojalil CM, Staples CR, Risco CA, Savio JD, Thatcher WW. 1997. Protein degradability and calcium salts of long-chain fatty acids in the diets of lactating dairy cows: reproductive responses. Journal of Dairy Science 81: 1385-1395.

Garnsworthy PC, Webb R. 1999. The influence of nutrition on fertility in dairy cows. Recent Advances in Animal Nutrition, Nottingham University Press, Nottingham, UK: 39- 57.

Garrick DJ, Lopez-Villalobos N, Holmes CW. 2001. Recent pre- and post-farm gate developments in the New Zealand dairy industry. Advances in Dairy Technology 13: 369-378.

Gengler N, Soyeurt H, Dehareng F, Bastin C, Colinet F, Hammami H, Vanrobays ML, Lainé A, Vanderick S, Grelet C, Vanlierde A, Froidmont E, Dardenne P. 2016. Capitalizing on fine milk composition for breeding and management of dairy cows. Journal of Dairy Science 99: 4071–4079.

Godden SM, Lissemore KG, Kelton DF, Leslie KE, Walton JS, Lumsden JH. 2001. Factors associated with milk urea concentrations in Ontario dairy cows. Journal of Dairy Science 84: 107-114.

Grosshans T, Xu ZZ, Burton LJ, Johnson DL, Macmillan KL. 1997. Performance and genetic parameters for fertility of seasonal dairy cows in New Zealand. Livestock Production Science 51: 41-51.

Guliński P, Salamończyk E, Młynek K. 2016. Improving nitrogen use efficiency of dairy cows in relation to urea in milk – a review. Animal Science Papers and Reports 34: 5-24.

Gustafsson AH, Carlsson J. 1993. Effects of silage quality, protein evaluation systems and milk urea content on milk yield and reproduction in dairy cows. Livestock Production Science 37: 91-105.

Gustafsson AH, Palmquist DC. 1993. Diurnal variation of rumen ammonia, serum urea, and milk urea in dairy cows at high and low yields. Journal of Dairy Science 76: 475-484.

Haour R, Saxena BB. 1974. Characterizatioon and solubilization of gonadotropin receptor of bovine corpus luteum. Journal of Biological Chemistry 249: 2195-2205.

Harris BL, Winkelman AM, Burton JL. 2000. Comparisons of fertility measures in strains of Holstein Friesian cows, Jersey cows and their crosses. Dairy farming Annual, Massey University 52: 71-78.

Harris SL, Clark DA, Waugh CD, Copeman PJA, Napper AR. 1998. Use of Barkant turnips or supercow sorghum to increase summer-autumn milk production. Proceedings of the New Zealand Society of Animal Production 58: 121-124.

Heap RB, Flint APF, Gadsby JE, Rice C. 1979. Hormones, the early embryo and the uterine environment. Journal of Reproduction and Fertility 55: 267-275.

Hof G, Vervoorn MD, Lenaers PJ, Tamminga S. 1997. Milk urea nitrogen as tool to monitor protein nutrition of dairy cows. Journal of Dairy Science 80: 3333-3340.

Holmes CW. 1999. Responses To Extra Feeds In Pastoral Dairy Production Systems. AGMARDT Massey University, Palmerston North, NZ.

Holmes CW, Brookes IM, Garrick DJ, Mackenzia DDS, Parkinson TJ, Wilson GF. 2002. Milk Production From Pasture. Massey University, Palmerston North, NZ.

Hossein-Zadeh NG, Ardalan M. 2011. Genetic relationship between milk urea nitrogen and reproductive performance in Holstein dairy cows. Animal 5: 26-32.

Hossein-Zadeh NG, Ardalan M. 2011 Estimation of genetic parameters for milk urea nitrogen and its relationship with milk constituents in Iranian Holsteins. Livestock Science 135: 274-281.

Huber JT, Limin Kung Jr. 1981. Protein and nonprotein nitrogen utilization in dairy cattle. Journal of Dairy Science 64: 1170-1195.

Huntington GB, Archibeque SL. 1999. Practical aspects of urea and ammonia metabolism in ruminants. Proceedings of the American Society of Animal Science: 1-11.

Hurley LS, Cosens G, Theriault LL. 1976. Teratogenic effects of magnesium deficiency in rats. Journal of Nutrition 106: 1254-1260.

Ibtisham F, Nawab A, Li Guanghui, Xiao M, An L, Naseer G. 2018. Effect of nutrition on reproductive efficiency of dairy animals. Medycyna Weterynaryjna 74: 356-361.

Janson L. 1980. Studies on fertility traits in swedish dairy cattle. Acta Agriculturae Scandinavica 30: 427-436.

Johnson RG, Young AJ. 2003. The association between milk urea nitrogen and DHI production variables in western commercial herds. Journal of Dairy Science 89: 3008-3015.

Jonker JS, Kohn RA, Erdman RA, Andrew SM. 1997. Estimation and evaluation of nutritional parameters from milk urea nitrogen. Journal of Dairy Science 80: 85.

Jordan ER, Chapman TE, Holtan DW, Swanson LV. 1983. Relationship of dietary crude protein to composition of uterine secretions and blood in high-producing postpartum dairy cows. Journal of Dairy Science 66: 1854-1862.

Jordan ER, Swanson LV. 1979. Serum progesterone and luteinizing hormone in dairy cattle fed varying levels of crude protein. Journal of Animal Science 48: 1154-1158.

Kauffman AJ, St-Pierre NR. 2001. The relationship of milk urea nitrogen to urine nitrogen excretion in Holstein and Jersey cows. Journal of Dairy Science 84: 2284-2294.

Kenny DA, Boland MP, Diskin MG, Sreenan JM. 2001. Effect of pasture crude protein and fermentable energy supplementation on blood metabolite and progesterone concentrations and on embryo survival in heifers. Animal Science 73: 501-511.

Kojima FN. 2003. The estrous cycle in cattle: physiology, endocrinology, and follicular waves. The Professional Animal Scientist 19: 83-95.

Kolver ES, Muller LD. 1998. Performance and nutrient intake of high producing Holstein cows consuming pasture or a total mixed ration. Journal of Dairy Science 81: 1403–1411.

König S, Chang YM, Borstel UUv, Gianola D, Simianer H. 2008. Genetic and phenotypic relationships among milk urea nitrogen, fertility and milk yield in Holstein cows. Journal of Dairy Science 91: 4372-4382.

Lambert MG, Clark DA, Litherland AJ. 2004. Advances in pasture management for animal productivity and health. New Zealand Veterinary Journal 52: 311-319.

Lamming GE, Darwash AO. 1998. The use of milk progesterone profiles to characterise components of subfertility in milked dairy cows. Animal Reproduction Science 52: 175-190.

Lapierre H, Lobley GE. 2001. Nitrogen recycling in the ruminant: a review. Journal of Dairy Science 84: 223-236

Larson SF, Butler WR, Currie WB. 1997. Reduced fertility associated with low progesterone post breeding and increased milk urea nitrogen in lactating cows. Journal of Dairy Science 80: 1288-1295.

Law RA, Young FJ, Patterson DC, Kilpatrick DJ, Wylie ARG, Mayne CS. 2009. Effect of dietary protein content on the fertility of dairy cows during early and mid-lactation. Journal of Dairy Science 92: 2737-2746.

Lean IJ, Celi P, Raadsma H, McNamara J, Rabiee AR. 2012. Effects of dietary crude protein on fertility: meta-analysis and meta-regression. Animal Feed Science and Technology 171: 31-42.

Lembeye F, Lopez-Villalobos N, Burke JL, Davis SR. 2016. Estimation of genetic parameters for milk traits in cows milked once- or twice-daily in New Zealand. Livestock Science 185: 142-147.

Leng RA and Nolan JV. 1984. Nitrogen metabolism in the rumen. Journal of Dairy Science 67: 1072-1089.

Lopez-Villalobos N. 2012. Analysing the genetic basis of milk production traits. Animal Science Reviews, CAB International: 65-82.

Lopez-Villalobos N, Correa-Luna M, Burke JL, Sneddon NW, Schutz MM, Donaghy DJ, Kemp PD. 2018. Genetic parameters for milk urea concentration and milk traits in New Zealand grazing dairy cattle. New Zealand Journal of Animal Science and Production 78: 56-61.

Lopez-Villalobos N, Garrick DJ. 2002. Economic heterosis and breed complementarity for dairy cattle in New Zealand. Proceedings of the 7th World Congress on Genetics Applied to Livestock Production: 19-23.

Lopez-Villalobos N, Garrick DJ. 2006. Crossbreeding systems for dairy production in New Zealand. Proceedings of the 8th World Congress on Genetics Applied to Livestock: 32-07.

Lopez-Villalobos N, Garrick DJ, Holmes CW, Blair HT and Spelman RJ. 2000. Profitabilities of some mating systems for dairy herds in New Zealand. Journal of Dairy Science 83: 144-153.

Macdonald KA, Penno JW, Kolver ES, Carter WA, Lancaster JA. 1998. Increasing protein concentration in diets of summer pasture and maize silage by including urea, soybeans, or fishmeal. Proceedings of the New Zealand Society of Animal Production 58: 102-105.

Macmillan KL, Henry RI, Taufa VK, Phillips P. 1990. Calving patterns in seasonal dairy herds. New Zealand Veterinary Journal 38: 151-155.

McDougall S. 2006. Reproduction performance and management of dairy cattle. Journal of Reproduction and Development 52: 185-194.

McNamara S, Murphy JJ, O'Mara FP, Rath M, Mee JF. 2008. Effect of milking frequency in early lactation on energy metabolism, milk production and reproductive performance of dairy cows. Livestock Science 117: 70-78.

Melendez P, Donovan A, Hernandez J. 2000. Milk urea nitrogen and infertility in Florida Holstein cows. Journal of Dairy Science 83: 459-463.

Melendez P, Donovan A, Hernandez J, Bartolome J, Risco CA, Staples C, Thatcher WW. 2003. Milk, plasma, and blood urea nitrogen concentrations, dietary protein, and fertility in dairy cattle. Journal of the American Veterinary Medical Association 223: 628-634.

Miglior F, Sewalem A, Jamrozik J, Bohmanova J, Lefebvre DM, Moore RK. 2007. Genetic analysis of milk urea nitrogen and lactose and their relationships with other production traits in Canadian Holstein cattle. Journal of Dairy Science 90: 2468-2479.

Mikkola M, Mäntysaari P, Tammiranta N, Peippo J, Taponen J. 2005. Effect of dietary protein on embryo recovery rate and quality in superovulated heifers. Animal Reproduction Science 87: 193-202.

Mitchell RG, Rogers GW, Dechow CD, Vallimont JE, Cooper JB, Sander-Nielsen U, Clay JS. 2005. Milk urea nitrogen concentration: heritability and genetic correlations with reproductive performance and disease. Journal of Dairy Science 88: 4434-4440.

Morton JM. 2001. High genetic merit and high-producing dairy cows in commercial Australian herds don't have substantially worse reproductive performance. British Society of Animal Science 26: 305-311.

Mucha S, Strandberg E. 2011. Genetic analysis of milk urea nitrogen and relationships with yield and fertility across lactation. Journal of Dairy Science 94: 5665-5672.

Muir BL, Fatehi J, Schaeffer LR. 2004. Genetic relationships between persistency and reproductive performance in first-lactation Canadian Holsteins. Journal of Dairy Science 87: 3029-3037.

Anonymous. 2019. New Zealand Dairy Statistics 2018-19. Livestock Improvement Corporation Limited & DairyNZ Limited, Hamilton, NZ.

Nielsen NI, Larsen T, Bjerring M, Ingvartsen KL. 2005. Quarter health, milking interval, and sampling time during milking affect the concentration of milk constituents. Journal of Dairy Science 88: 3186-3200.

NRC. 2001. Nutrient Requirements of Dairy Cattle. National Academy Press 7th Edition, Washington, D.C., USA.

Ocon OM, Hansen PJ. 2003. Disruption of bovine oocytes and preimplantation embryos by urea and acidic pH. Journal of Dairy Science 86: 1194-1200.

Oliveira MCPP, Silva NMA, Bastos LPF, Fonseca LM, MMOP Cerqueira, Leite MO, Conrrado RS. 2012. Fourier Transform Infrared Spectroscopy (FTIR) for MUN analysis in normal and adulterated Milk. Arquivo Brasileiro de Medicina Veterinaria e Zootecnia 64: 1360-1366.

Ordóñez A, Parkinson T, Holmes CW, Matthew C, Miller RD, Lopez-Villalobos N, Burke JL, Brookes I. 2007. Effects of application in spring of urea fertiliser on aspects of reproductive performance of pasture-fed dairy cows New Zealand Veterinary Journal 55: 69-76.

Ørskov ER, McDonald I. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. The Journal of Agricultural Science 92: 499-503.

Patton J, Kenny DA, Mee JF, O'Mara FP, Wathes DC, Cook M, Murphy JJ. 2006. Effect of milking frequency and diet on milk production, energy balance, and reproduction in dairy cows. Journal of Dairy Science 89: 1478-1487.

Penno JW. 2002. The response by grazing dairy cows to supplementary feeds. Doctor of Philosophy in Animal Science, Massey University, Palmerston North, New Zealand.

Penno JW, Macdonald KA, Bryant AM. 1996. The economics of No. 2 Dairy systems. Ruakura Farmers Conference: 11-19.

Philipsson J. 1981. Genetic aspects of female fertility in dairy cattle. Livestock Production Science 8: 307-319.

Phillips GJ, Citron TL, Sage JS, Cummins KA, Cecava MJ, McNmara JP. 2003. Adaptations in body muscle and fat in transition dairy cattle fed differing amounts of protein and methionine hydroxy analog. Journal of Dairy Science 86: 3634-3647.

Pryce JE, Coffey MP, Simm G. 2001. The relationship between body condition score and reproductive performance. Journal of Dairy Science 84: 1508-1515.

Pryce JE, Harris BL. 2006. Genetics of body condition score in New Zealand dairy cows. Journal of Dairy Science 89: 4424: 4432.

Pryce JE, Royal MD, Garnsworthy PC, Mao IL. 2004. Fertility in the high-producing dairy cow. Livestock Production Science 86: 125-135.

Rhoads ML, Rhoads RP, Gilbert RO, Toole R, Butler WR. 2006. Detrimental effects of high plasma urea nitrogen levels on viability of embryos from lactating dairy cows. Animal Reproduction Science 91: 1-10.

Rhodes FM, McDougall S, Burke CR, Verkerk GA, Macmillan KL. 2003. Invited review: treatment of cows with an extended postpartum anestrous interval. Journal of Dairy Science 86: 1876-1894.

Roberts AHC, Ledgard SF, O'Connor MB, Thomson NZ. 1992. Effective use of nitrogen. Ruakura Farmers Conference: 77-83.

Roche J, Kay J, Hedley P. 2011. Is protein supplementation needed during summer? Dairy New Zealand Technical Series 6: 6-10.

Rooke JA, Ewen M, Mackie K, Staines ME, McEvoy TG, Sinclair KD. 2004: Effect of ammonium chloride on the growth and metabolism of bovine ovarian granulosa cells and the development of ovine oocytes matured in the presence of bovine granulosa cells previously exposed to ammonium chloride. Animal Reproduction Science 84: 53-71.

Roy B, Brahma B, Ghosh S, Pankaj PK. 2011. Evaluation of milk urea concentration as useful indicator for dairy herd management: a review. Asian Journal of Animal and Veterinary Advances 6: 1-19.

Rzewuska K, Strabel T. 2013. Effects of some non-genetic factors on concentration of urea in milk in Polish Holstein-Fresian cows. Journal of Animal and Feed Sciences 22: 197- 203.

Rzewuska K, Strabel T. 2013. Genetic parameters for milk urea concentration and milk traits in Polish Holstein-Friesian cows. Journal of Applied Genetics 54: 473- 482.

Rzewuska K, Strabel T. 2014. The genetic relationship between reproduction traits and milk urea concentration. Animal Science Papers and Reports 32: 243- 256.

Silvestre A, Martins A, Santos V, Ginja M, Colaço J. 2009. Lactation curves for milk, fat and protein in dairy cows: a full approach. Livestock Science 122: 308-313.

Sinclair KD, Kuran M, Gebbie FE, Webb R, McEvoy TG. 2000a. Nitrogen metabolism and fertility in cattle: II. Development of oocytes recovered from heifers offered diets differing in their rate of nitrogen release in the rumen. Journal of Animal Science 78: 2670-2680.

Sinclair KD, Sinclair LA, Robinson JJ. 2000b. Nitrogen metabolism and fertility in cattle: I. Adaptive changes in intake and metabolism to diets differing in their rate of energy and nitrogen release in the rumen. Journal of Animal Science 78: 2659- 2669.

Smith JF, Verkerk GA, Clark BA, McKay BJ, Duganzich DM. 2001. Relationships between milk production, nutrition and reproduction in “benchmark” herds. Proceedings of the New Zealand Society of Animal Production 61: 195-198.

Sneddon NW, Lopez-Villalobos N, Davis SR, Hickson RE, Shalloo L. 2015. Genetic parameters for milk components including lactose from test day records in the New Zealand dairy herd. New Zealand Journal of Agricultural Research 58: 97- 107.

Sørensen MK, Norberg E, Pedersen J, Christensen LG. 2008. Invited review: Crossbreeding in dairy cattle: A Danish perspective. Journal of Dairy Science 91: 4116-4128.

Stelwagen K, Davis SR, Farr VC, Eichler SJ, and Politis I. 1994. Effects of once-daily milking and concurrent somatotropin on mammary tight junction permeability and yield of cows. Journal of Dairy Science 77: 2994-3001.

Stoop WM, Bovenhuis, H, van Arendonk JAM. 2007. Genetic parameters for milk urea nitrogen in relation to milk production traits. Journal of Dairy Science 90: 1981-1986.

Strang BD, Bertics SJ, Grummer RR, Armentano LE. 1998. Effect of long-chain fatty acids on triglyceride accumulation, gluconeogenesis, ureagenesis in bovine hepatocytes. Journal of Dairy Science 81: 728-739.

Tamminga S. 1992. Nutrition management of dairy cows as a contribution to pollution control. Journal of Dairy Science 75: 345-357.

Tamminga S. 2006. The effect of the supply of rumen degradable protein and metabolisable protein on negative energy balance and fertility in dairy cows. Animal Reproduction Science 96: 227-239.

Tenghe AMM, Bouwman AC, Berglund B, Strandberg E, Blom JY, Veerkamp RF. 2015. Estimating genetic parameters for fertility in dairy cows from in-line milk progesterone profiles. Journal of Dairy Science 98: 5763-5773.

Trevaskis LM, Fulkerson WJ 1999. The relationship between various animal and management factors and milk urea, and its association with reproductive performance of dairy cows grazing pasture. Livestock Production Science 57: 255-265.

Van Arendonk JA, Liinamo AE. 2003. Dairy cattle production in Europe. Theriogenology 59: 563-569.

VandeHaar MJ, Yousif G, Sharma BK, Herdt TH, Emery RS, Allen MS, Liesman JS. 1999. Effect of energy and protein density of prepartum diets on fat and protein metabolism of dairy cattle in the periparturient period. Journal of Dairy Science 82: 1282-1295.

Verkerk G. 2003. Pasture-based dairying: challenges and rewards for New Zealand producers. Theriogenology 59: 553-561.

Virtanen AI. 1966. Milk production of cows on protein-free feed. Science 153: 1603-1614.

Volden H, Nielsen NI. 2011. Energy and metabolizable protein supply. The Nordic Feed Evaluation System: 81-84.

Waghorn CG, Burke JL, Kolver ES. 2007. Principles of feeding value. Pasture and Supplements for Grazing Animals. New Zealand Society of Animal Production Occasional Publication 14.

Wathes DC, Bourne N, Cheng Z, Taylor VJ, Mann GE, Coffey MP. 2007a. Multiple correlation analyses of metabolic and endocrine profiles with fertility in primiparous and multiparous cows. Journal of Dairy Science 90: 1310-1325.

Wathes DC, Fenwick M, Cheng Z, Bourne N, Llewellyn S, Morris DG, Kenny D, Murphy J, Fitzpatrick R. 2007b. Influence of negative energy balance on cyclicity and fertility in the high producing dairy cow. Theriogenology 68: 232-241.

Westwood CT, Lean IJ, Kellaway RC. 1998. Indications and implications for testing of milk urea in dairy cattle: a quantitative review. Part 2: effect of dietary protein on reproductive performance. New Zealand Veterinary Journal 46: 123-130.

Wood GM, Boettcher PJ, Jamrozik J, Jansen GB, Kelton DF. 2003. Estimation of genetic parameters for concentrations of milk urea nitrogen. Journal of Dairy Science 86: 2462-2469.

Yang L, Lopez-Villalobos N, Berry DP, Parkinson T. 2010. Phenotypic relationships between milk protein percentage and reproductive performance in three strains of Holstein Friesian cows in Ireland. Proceedings of the New Zealand Society of Animal Production 70: 29-32.

Appendix

Table A.1. Mean, standard deviation (SD), minimum (Min) and maximum (Max) values of milk urea nitrogen, milk yields and milk percentages, and reproductive traits of cows from Dairy 1 and Dairy 4 during the 2016 production season. Milk urea nitrogen and productive traits were the obtained at the start of the breeding season (closest date to first service).

	Farm									
	Dairy 1					Dairy 4				
Trait[1]	N	Mean	SD	Min	Max	N	Mean	SD	Min	Max
MUN, mg/dL	243	10.6	2.6	4.3	20.9	199	8.2	1.9	2.9	13.3
MY, kg/day	243	18.7	4.9	8.6	35.3	199	25.6	4.8	12.8	37.7
FY, kg/day	243	0.9	0.2	0.5	1.3	199	1.0	0.2	0.5	1.5
PY, kg/day	243	0.7	0.2	0.3	1.2	199	0.9	0.1	0.5	1.2
LY, kg/day	243	1.1	0.3	0.5	1.9	199	1.3	0.2	0.7	1.9
FP, %	243	4.93	0.77	3.14	7.14	199	4.03	0.68	2.46	6.57
PP, %	243	3.86	0.34	2.99	4.79	199	3.55	0.28	2.92	4.43
LP, %	243	5.05	0.15	4.52	5.36	199	5.14	0.14	4.74	5.50
SBFS, days	243	11	7	0	35	199	10	7	0	39
SBCO, days	217	20	17	0	69	168	16	11	0	41
CFS, days	243	79	16	30	116	199	83	17	25	116
FSCO, days	217	9	16	0	59	168	6	10	0	40
DO, days	217	88	20	30	152	168	89	19	33	133
SR21, %	243	95.9	19.9	-	-	199	94.5	22.9	-	-
PR21, %	243	63.4	48.3	-	-	199	59.8	49.2	-	-
PRFS, %	243	63.8	48.2	-	-	199	59.8	49.2	-	-

[1] MUN = milk urea nitrogen, MY = milk yield, FY = fat yield, PY = protein yield, LY = lactose yield, FP = fat percentage, PP = protein percentage, LP = lactose percentage, SBFS = interval from start of breeding to first service, SBCO = interval from start of breeding to conception, CFS = interval from calving to first service, FSCO = interval from first service to conception, DO = days open or interval from calving to conception, SR21 = submission rate in 21 days after start of breeding, PR21 = pregnancy rate in 21 days after start of breeding, PRFS = pregnancy rate at first service.

Table A.2. Mean, standard deviation (SD), minimum (Min) and maximum (Max) values of MUN, milk yields and milk percentages, and reproductive traits of cows from Dairy 1 and Dairy 4 during the 2017 production season. Milk urea nitrogen and productive traits were the obtained at the start of the breeding season (closest date to first service).

	Farm									
	Dairy 1					Dairy 4				
Trait[1]	N	Mean	SD	Min	Max	N	Mean	SD	Min	Max
MUN, mg/dL	234	11.4	2.5	4.3	22.7	198	9.1	2.3	1.6	16.0
MY, kg/day	234	19.4	5.3	5.8	36.5	198	23.8	5.5	6.5	40.4
FY, kg/day	234	1.0	0.2	0.3	1.7	198	1.0	0.2	0.2	1.6
PY, kg/day	234	0.8	0.2	0.2	1.2	198	0.9	0.2	0.3	1.4
LY, kg/day	234	1.0	0.3	0.5	1.8	198	1.2	0.3	0.3	2.0
FP, %	234	5.15	0.86	3.46	9.17	198	4.15	0.60	2.75	6.24
PP, %	234	3.90	0.33	3.03	4.77	198	3.68	0.28	3.01	4.55
LP, %	234	5.01	0.16	4.48	5.36	198	5.20	0.19	4.27	5.53
SBFS, days	234	9	6	0	31	198	8	7	0	37
SBCO, days	214	17	15	0	66	176	21	17	0	67
CFS, days	234	83	16	34	143	198	80	14	43	110
FSCO, days	214	8	14	0	63	176	14	16	0	65
DO, days	214	91	20	38	151	176	94	21	46	149
SR21, %	234	97.9	14.5	-	-	198	95.5	20.9	-	-
PR21, %	234	67.1	47.1	-	-	198	48.0	50.1	-	-
PRFS, %	234	65.4	47.8	-	-	198	46.5	50.0	-	-

[1] MUN = milk urea nitrogen, MY = milk yield, FY = fat yield, PY = protein yield, LY = lactose yield, FP = fat percentage, PP = protein percentage, LP = lactose percentage, SBFS = interval from start of breeding to first service, SBCO = interval from start of breeding to conception, CFS = interval from calving to first service, FSCO = interval from first service to conception, DO = days open or interval from calving to conception, SR21 = submission rate in 21 days after start of breeding, PR21 = pregnancy rate in 21 days after start of breeding, PRFS = pregnancy rate at first service.