Factors affecting milk urea nitrogen and its relationships with production traits in South African Holstein cattle

by

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Declaration

I declare that this thesis, which I hereby submit for the degree MSc (Agric) Animal Breeding and Genetics at the University of Pretoria, is my own work and has not previously been submitted by me for degree purposes at this or any other tertiary institution.

Signature…….. Date…….

Abstract

The efficiency of utilization of dietary nitrogen can be monitored using milk urea nitrogen (MUN). Overfeeding or underfeeding of protein can be identified through the observation of deviations from target MUN concentrations. This will assist in lowering feed costs of dairy farms, and improving nutrition management of herds. Higher efficiency of utilization of dietary nitrogen might result in a reduction in environmental pollution. Non-genetic factors affecting variation in MUN were herd-test-day (HTD), lactation stage and year of calving. The contribution of HTD was the highest, ranging from 58.56% to 63.18% in parity 1 to 3. Lactation stage had the second largest contribution to the MUN variation. Differences in least squares means for MUN in various years of calving were observed. The heritability estimate for MUN was 0.09±0.01 in the first parity, and remained constant at 0.11±0.01 in the second and third parity. Heritability estimates for milk, fat and protein yield ranged from 0.40±0.01 to 0.43±0.01, 0.21±0.01 to 0.26±0.01, and 0.32±0.01 to 0.38±0.01, respectively. These estimates were within acceptable ranges for South African Holstein cattle. Genetic correlations between MUN and milk production traits were low and positive, ranging from 0.01±0.003 to 0.10±0.004 across parities. Phenotypic correlations ranged from 0.02±0.11 to 0.16±0.07, being generally higher than the genetic correlations. The positive associations between MUN and milk production traits are undesirable as the dairy cows would be less efficient in utilizing dietary protein and may result in increased environmental pollution. The genetic trend for MUN was 0.44, 0.007 and 0.049 mg/dl in the first, second and third parity, respectively. Results of the current study indicate that MUN has potential as a management tool in South African Holstein dairy herds. It might be a good indicator of the efficiency of dietary protein utilization of dairy herds, and has practical advantage as it is currently collected by the national dairy herd recording and improvement scheme.

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Abbreviations

ANOVA	Analysis of variance
ARC	Agricultural research council
BLUP	Best linear unbiased prediction
BUN	Blood urea nitrogen
BW	Body weight
CP	Crude protein
DAFF	Department of agriculture forestry and fisheries
DHIA	Dairy herd improvement agency
DIM	Days in milk
DMI	Dry matter intake
EBV	Estimated breeding value
FY	Fat yield
F%	Fat percentage
GLM	Generalized linear model
HTD	Herd-test-day
Intergis	Integrated registration and genetic information system
IR	Infrared
LS	Least squares
MACE	Multiple across country evaluation
MME	Mixed model equations
MUN	Milk urea nitrogen
MPO	Milk producers' organization
MY	Milk yield
NMRIS	National milk recording and improvement scheme
PY	Protein yield
P%	Protein percentage
REML	Restricted maximum likelihood
RDP	Rumen degradable protein

RUP	Rumen undegradable protein
R^2	Coefficient of determination
r_g	Genetic correlation
r_p	Phenotypic correlation
SA	South Africa
SAS	Statistical analysis software
SCC	Somatic cell count
UUN	Urine urea nitrogen
US	United State
WC	Wet chemistry

Chapter 1: General introduction

The concentration of urea in milk, commonly known as Milk Urea Nitrogen (MUN), is an important tool in dairy herd management. It can be used to monitor the efficiency of utilization of dietary nitrogen (Jonker *et al.*, 2002a, b), thereby assisting dairy producers in nutrition management of their herds. Monitoring deviations from target MUN concentrations can be used to identify overfeeding or underfeeding of protein (Jonker *et al.*, 1998; Kohn *et al.*, 2002). High MUN values are also indicative of a deficiency of energy required for optimum utilization of protein in the diet (Nousiainen *et al.*, 2004; Calsamiglia *et al.*, 2010). Thus, MUN enables the efficient utilization of dietary protein, leading to lower feed costs. Feed costs make up to 80% of dairy farm costs (Agri review, 2007; Gourley *et al.*, 2012), hence the need for proper management of this valuable input.

Excessive intake or inefficient utilization of dietary nitrogen has been reported to increase the amount of nitrogen excreted in urine and faeces (Godden *et al.*, 2001b; Kebreab *et al.*, 2001; Hojman *et al.*, 2004). Most of the excess nitrogen is excreted via urine in the form of urea, which is easily volatilized to ammonia (Tamminga, 1992; Kebreab *et al.*, 2001), resulting in environmental pollution. Milk urea nitrogen is used to predict urinary nitrogen excretion (Burgos *et al.*, 2007). Thus, a reduction in environmental nitrogen pollution can be achieved through dietary manipulation and overall management adjustments (Schepers & Meijer, 1998, Jonker *et al.*, 2002a). Compared to other methods of measuring nitrogen excretion, MUN has practical advantages as it is determined through non-invasive methods and is routinely measured in dairy performance recording schemes.

Various non-genetic factors may contribute to the variation in MUN. Both nutritional and environmental factors have been reported to have an effect on MUN variation (Hojman *et al.*, 2004; Wattiaux & Karg, 2004; Burgos *et al.*, 2007). Positive associations have been reported between level of MUN and dry matter intake (DMI), MUN and rumen degradable protein (RDP), and MUN and crude protein (CP) (Godden *et al.*, 2001b; Hojman *et al.*, 2004; Burgos *et al.*, 2007). The interaction between CP and energy also has a notable impact on MUN variation (Nousiainen *et al.*, 2004; Rius *et al.*, 2010). Environmental factors such as herd-test-day, parity, lactation stage, and season of calving have been observed to have an effect on MUN (Wood *et al.*, 2003; Miglior *et al.*, 2006; Stoop *et al.*, 2007). In general, the herd-test-day interaction contributes the most to MUN variation, which may be mainly attributed to differences in herd management and nutrition practices (Jìlek *et al.*, 2006). For accurate interpretation of MUN data, knowledge of the environmental and nutritional factors influencing MUN is important. These factors should be taken into account when interpreting MUN results.

Recent studies (Castillo *et al.*, 2001; Frank & Swensson, 2002; Miglior *et al.*, 2007; Hossein-Zadeh & Ardala, 2010) have looked into the prospect of improving the efficiency of utilization of dietary nitrogen through selection on MUN. Variances and (co)variances for traits are population specific and are required for breeding value estimation. Heritability influences the accuracy of selection for a trait. Literature estimates of the heritability of MUN range from as low as 0.14 (Stoop *et al.*, 2007; Yazgan *et al.*, 2010) to as high as 0.59 (Wood *et al.*, 2003). These values indicate that there is scope for selection to reduce MUN, thus developing cows that utilize nitrogen more efficiently and contribute less to environmental pollution. There is consistency in the literature with regards to genetic and phenotypic correlations between MUN and production traits. Genetic and phenotypic correlations have been reported between MUN and milk yield (Wood *et al.*, 2003; Miglior *et al.*, 2007; Stoop *et al.*, 2007; König *et al.*, 2008), MUN and fat yield (Arunvipas *et al.*, 2003b; Miglior *et al.*, 2007; Stoop *et al.*, 2007) and MUN and protein yield (Arunvipas *et al.*, 2003b; Miglior *et al.*, 2007; Hossein-Zadeh & Ardala, 2010). Genetic correlations between MUN and milk yield, MUN and fat yield, and MUN and protein yield range from 0.11 to 0.79, -0.12 to 0.45, and -0.12 to 0.38, respectively (Wood *et al.*, 2003; Stoop *et al.*, 2007, Hossein-Zadeh & Ardalan, 2010).

These correlations indicate that MUN might be associated with milk production traits. Including MUN in breeding objectives of dairy cattle, taking into account the genetic correlations between MUN and production traits, may result in cows that are more efficient utilizers of dietary protein. There is limited literature on phenotypic correlations between MUN and production traits. High milk yield is generally associated with high MUN levels (Jonker *et al.*, 1999; Arunvipas *et al.*, 2003a; Hojman *et al.*, 2004). A phenotypic correlation estimate of 0.13 between MUN and milk yield was reported by Miglior *et al.* (2007) and König *et al*. (2008), indicating higher MUN levels as milk yield increases.

Milk urea nitrogen has been routinely measured in dairy herds participating in the National Milk Recording and Improvement Scheme in South Africa since 1994. There has, however, been limited research on factors influencing MUN and there are no available estimates of genetic parameters for MUN in South African dairy herds.

Aim of the study

The aim of the study was to identify and quantify factors influencing MUN and to estimate genetic and phenotypic parameters among MUN and production traits in South African Holstein cows. The specific objectives were to:

1. Identify and quantify non-genetic factors influencing MUN in South African Holstein herds. These factors need to be taken into account when using MUN data.
2. Estimate the heritability of MUN in South African Holstein cattle. Such an estimate gives an indication of the rate of genetic progress that can be achieved if dairy cows were selected on MUN.
3. Estimate genetic and phenotypic correlations between MUN and milk, fat and protein yield in South African Holstein cattle. These parameters may help to improve the accuracy of predictions for MUN.
4. Determine genetic trends for MUN in South African Holstein cattle. The genetic trends will help in assessing the impact genetic selection of milk production traits had on MUN within the South African Holstein breed.

Chapter 2: Literature review

2.1 Introduction

Milk urea nitrogen (MUN) is a practical manner of monitoring nutrition management of a dairy herd, as well as the efficiency of protein and energy utilization. It is less labor intensive compared to the collection of blood urea nitrogen (BUN) and urine urea nitrogen (UUN). The positive association between MUN and BUN (Kauffman & St-Pierre, 2001; Burgos *et al.*, 2007) suggests that MUN is a reliable predictor of the level of urea in the blood stream of animals and that excreted via urine. Milk urea nitrogen can be used to detect underfeeding and overfeeding of dietary protein. A decrease in the efficiency of nitrogen utilization has been reported by Castillo *et al.* (2001) when dietary protein intake was increased, and the efficiency increased with the reduction of dietary protein intake. These results were supported by Gourley *et al.* (2012), who observed a similar pattern with the effect of the level of dietary protein on efficiency of nitrogen utilization in dairy cows. Milk urea nitrogen might provide an accurate reflection of the amount of nitrogen absorbed by an animal that is not used for growth or milk protein synthesis.

Research on MUN has been limited in South Africa as the focus of research performed on dairy cattle has up to now been on nutrition, and conventional selection criteria such as milk, fat and protein. There is a need to perform research on MUN and its association with production traits. This is important as reliable estimates of genetic parameters for the specific population are necessary before the trait can be considered as a selection criterion and included in breeding strategies.

The aim of this chapter is to provide an overview on genetic and non-genetic factors influencing MUN, previously reported estimates of variance as well as various measuring methods used.

2.2 South African dairy industry

The South African dairy industry is comprised of organizations that play different roles, and it is divided into the primary and secondary sectors. The primary sector represents milk producers, while the secondary sector consists of processors and producers who sell their own produce directly to consumers and retailers (MPO statistics, 2011). Dairy industry matters are coordinated by Milk South Africa, an organization financed by statutory contributions. The Milk Producers' Organization (MPO) negotiates with the government and other establishments on behalf of producers. This organization also makes statistics and management information available to producers, the dairy industry, and other authorities. The Agricultural Research Council (ARC) plays a major role in the Multiple Across-

Country Evaluation (MACE) for South African dairy breeds, and it manages the National Dairy Animal Recording and Improvement Scheme (SA yearbook, 2009/10; MPO statistics, 2011).

According to the SA Yearbook 2009/10, milk producers employ approximately 50 000 farm workers and 38 000 people are indirectly employed by the dairy industry. The gross value of milk produced in 2010 was estimated at R9 332 million, including milk for the producer and on farm consumption (DAFF, 2011). The dairy industry is therefore one of the most important industries in the South African agricultural sector as it is the fourth largest agricultural industry.

Dairy farming contributes to the supply of animal protein through production of milk and other dairy products such as cheese and yoghurt. In South Africa, the Western Cape, Eastern Cape, Kwa-Zulu Natal, Free State, North West, and Mpumalanga provinces contribute 26.6, 24.5, 23.6, 13.2, 4.8 and 3.8%, respectively, to the total milk production, with the remaining 3.5% being from the remaining three provinces (DAFF, 2011). Approximately 75% of all milk is produced in areas with predominantly pasture-based production systems, namely the Western Cape, Eastern Cape, and Kwa-Zulu Natal (Grobler, 2008). The number of milk producers decreased by 63% from January 2006 (4 184) to June 2011 (2 627) in South Africa (MPO statistics, 2011). This notable decline can be attributed to increased maize prices that resulted in inflated feed prices (Agri review, 2007). The high feed costs coupled with low producer prices might have been the cause of this reduction in milk producers nationwide.

The Holstein is one of the four major South African dairy breeds that undergo routine genetic evaluation by the Agricultural Research Council's Animal Production Institute (Mostert, 2007; SA Yearbook, 2009/10). The breed accounted for 57% of the cows participating in milk recording in South Africa (Mostert, 2007). In the 2004 test year, 39 093 registered and 33 824 commercial Holstein cows participated in performance testing in South Africa (Mostert, 2007). This accounted for 49 and 70% of the registered and commercial cows in the national herd, respectively. Tables 2.1 and 2.2 show productivity of registered and unregistered South African Holstein cows from the year 2001 to 2011, respectively.

Table 2.1 Productivity of registered South African Holstein cows

Period	N cows	Milk (kg)	Fat (kg)	Fat %	Protein (kg)	Protein %
2000 – 2001	37 463	8 219	286	3.47	257	3.12
2001 – 2002	34 603	8 388	292	3.48	265	3.15
2002 – 2003	35 399	8 388	312	3.74	266	3.18
2003 – 2004	39 093	8 676	329	3.79	277	3.19
2004 – 2005	31 350	8 877	332	3.74	285	3.21
2005 – 2006	32 748	9 285	349	3.76	300	3.23
2006 – 2007	30 734	9 308	351	3.78	296	3.18
2007 – 2008	29 091	9 331	356	3.81	297	3.18
2008 – 2009	33 654	9 508	359	3.78	303	3.19
2009 – 2010	29 004	9 567	359	3.78	305	3.20
2010 - 2011	28 260	9 830	369	3.76	316	3.22

*Adapted from National Dairy Animal Recoding and Improvement Scheme (2010)

More recent statistics shows that a decrease in the 2011 test year, where 28 260 registered and 24 350 commercial Holstein cows participated in milk recording in South Africa, accounted for 43 and 49%, respectively (National Animal Dairy Recording and Improvement Scheme, 2011). Despite the notable decrease in numbers, the Holstein breed still constitutes a high proportion (43%) of cows participating in performance testing in South Africa.

Table 2.2 Productivity of unregistered South African Holstein cows

Period	Number of cows	Milk (kg)	Fat (kg)	Fat %	Protein (kg)	Protein %
2000 – 2001	35 174	6 549	225	3.43	204	3.11
2001 – 2002	32 741	6 660	229	3.43	210	3.15
2002 – 2003	33 620	6 594	248	3.79	210	3.20
2003 – 2004	33 824	6 861	264	3.85	219	3.20
2004 – 2005	30 645	7 057	269	3.81	228	3.23
2005 – 2006	33 368	7 192	273	3.79	233	3.24
2006 – 2007	29 313	7 619	293	3.85	245	3.21
2007 – 2008	30 393	7 090	276	3.89	230	3.25
2008 – 2009	31 107	7 046	274	3.89	229	3.25
2009 – 2010	26 571	6 933	272	3.98	227	3.30
2010 – 2011	24 350	7 142	280	3.92	235	3.30

*Adapted from National Dairy Animal Recoding and Improvement Scheme (2010)

Test-day records are routinely measured in the National Dairy Animal Recording and Improvement Scheme, on a five weeks interval. On the test-day, milk yield of individual cows is

recorded at each milking. Parameters measured using milk samples for each cow are fat, protein, and lactose percentage, somatic cell count (SCC), and milk urea nitrogen (MUN). The test-day data, together with pedigree data, are captured in the Integrated Registration and Genetic Information System (Intergis). This data can be used by dairy producers for the identification and selection of productive dairy animals and farming enterprises (SA yearbook, 2009/10).

2.3 Metabolic pathway resulting in urea

During the process of protein digestion in the rumen some amino acids are further metabolized into ammonia, carbohydrates, and organic acids. The major end product of true protein and non-protein nitrogen metabolism is urea (Kauffman & St-Pierre, 2001). At times the degradation of protein proceeds more rapidly than the synthesis, resulting in excess nitrogen in the rumen. Excess nitrogen is transferred to the liver as amino acids alanine and citrulline as well as ammonia. In the liver, amino acids are deaminated and ammonium ions are converted to urea (Reece, 2004). Excess ammonia is converted into urea (because of the high toxicity of ammonia) in the liver, which is absorbed into the blood and returned to the rumen via saliva, or partly excreted in urine or milk (McDonald *et al.*, 2002). In the mammary gland, limited amounts of Milk Urea Nitrogen (MUN) can be derived from the catabolism of the amino acid arginine (Nousianen *et al.*, 2004). The concentration of urea in the blood is blood urea nitrogen (BUN), and that of urea in urine is referred to as urine area nitrogen (UUN) while MUN refers to the levels of urea nitrogen in milk (Jonker *et al.*, 1998; Nousiainen *et al.*, 2004; Wood *et al.*, 2003). The concentration of urea in bodily fluids can be used to identify nutritional deficiencies in cow herds.

The metabolic pathway of MUN has been well described by Arunvipas *et al.* (2008). Milk urea nitrogen is mainly derived from blood urea. Urea is a neutral molecule and it equilibrates with body water. It diffuses into and out of the mammary gland as milk is secreted in this gland. As a result, MUN is proportional to BUN (DePeterson & Ferguson, 1992; Roseler *et al.*, 1993; Jonker *et al.*, 1998). In studies by Ide *et al.* (1966), Roseler *et al.* (1993), Kauffman & St-Pierre (2001), and Burgos *et al.* (2007), a close association between MUN and BUN concentrations was observed. Milk urea nitrogen has also been reported to have a close association with urea nitrogen excretion (Burgos *et al.*, 2007; Zhai *et al.*, 2005). The correlation between MUN and UUN can be used to estimate nitrogen excretion, and as an indicator of nitrogen pollution by dairy herds. Compared to BUN and UUN excretion, MUN has a practical advantage as individual and bulk milk samples are routinely collected at dairy farms participating in national dairy improvement schemes. As a result, measuring MUN is more convenient. Measuring MUN is also non-invasive compared to collection of blood to measure BUN. Milk urea nitrogen is an excellent predictor of both BUN and UUN (Kohn, 1997). It can thus be applicable as a management tool to monitor efficiency of nitrogen utilization as well as to predict nitrogen excretion (Arunvipas *et al.*, 2008).

2.4 Methods used to measure milk urea nitrogen

There are two methods currently used to measure MUN, and those are the wet-chemistry (WC) determination method and infrared (IR) technology. These different methods of measurement differ in accuracy and precision, and some are more suitable for certain herds depending on management practices of the specific herd, affordability and convenience of using the specific measurement method.

The use of the wet-chemistry determination method, recommended by Jenkins *et al.* (2000), uses a biosensor and operates on-line in the milk parlor to measure MUN while the cows are being milked. The enzyme urease is added to the sample to convert urea to ammonia, the hydrolysis results in carbonate loss with the end products being ammonium and carbonate ions (Jenkins *et al.*, 1999). The change in pH of the sample is then measured, and used to estimate the amount of urea in milk (Jenkins *et al.*, 2000; Arunvipas *et al.*, 2003a). The WC method has been reported by Arunvipas *et al.* (2003a) as the most accurate method for detecting MUN. However, this method is highly labor intensive and costly (Godden *et al.*, 2000), making it impractical to use in larger dairy herds.

Infrared (IR) technology can also be used to quantify the concentration of urea in milk samples by measuring the amount of light absorbed at a wavelength that detects urea nitrogen, as recommended by Godden *et al.* (2000). Infrared measures of MUN are indirect measures of MUN (Hossein-Zadeh & Ardalan, 2010). Estimates of urea concentration are adjusted for concentrations of interfering substances (other milk components) using a computer algorithm. These interfering substances influence the accuracy of measurements as they also absorb some light at the urea wavelength (Arunvipas *et al.*, 2003a; Peterson *et al.*, 2004). This is a disadvantage as the IR method may produce different urea estimates for samples from different cows that have the same urea value (Godden *et al.*, 2000). The IR technology is however a fast and cost-effective method of measuring MUN. One of its main advantages is that multiple samples are not needed when other milk constituents must also be measured. The same instrument can be used (Godden *et al.*, 2000; Arunvipas *et al.*, 2003a) for measuring MUN and milk constituents in a single sample. In South Africa, the IR method is used for routine measurements performed in herds participating in the National Milk Recording Scheme of the country due to its practicality and cost effectiveness.

2.5 Non-genetic factors affecting milk urea nitrogen

Knowledge of factors affecting MUN is an important pre-requisite for proper use and accurate interpretation of MUN data. Several factors have been reported to influence MUN, both at an animal

and a herd level. Non-genetic factors include nutritional and environmental factors (Hojman *et al.*, 2004; Wattiaux & Karg, 2004; Burgos *et al.*, 2007), which will be discussed in more detail.

Nutrition has been reported to have an effect on the level of MUN. Positive associations between level of MUN and DMI (dry matter intake), CP (crude protein), and RDP (rumen degradable protein) were observed in studies by Godden *et al.* (2001b) and Hojman *et al.* (2004). To compensate for suboptimal intakes during early lactation, dairy farmers may increase nutrient density of dairy cow diets with the aim of sustaining milk production (Roy *et al.*, 2011). Zhai *et al.* (2006) observed an increase in MUN values when dietary CP was increased in Holstein cows. This is in agreement with results by Burgos *et al.* (2007) who reported an increase of 16.6 mg/dl (from 7.9 to 24.5ml/dl) in MUN concentration when CP was increased from 15.1 to 20.7% in the diet. A reduction in nitrogen efficiency when nitrogen intake is increased has been reported (Castillo *et al.*, 2001; Huhtanen *et al.*, 2008). A nitrogen efficiency of 32% was observed in high protein diets in the study by Frank & Swensson (2002). The efficiency increased to 42% in low protein diets. They concluded that MUN has a strong association with the protein content in the diet. An increase in nitrogen efficiency from 38.5% in cows fed low energy-low protein diets to 43% in cows fed high energy-low protein diets, was observed by Rius *et al.* (2010). These results are supported by those obtained by Gourley *et al.* (2012) where cows with the lowest level of feed nitrogen intake generally had the highest nitrogen efficiency. A positive relationship between MUN and RDP was observed in the study by Hojman *et al.* (2004), but no association between MUN and rumen undegradable protein (RUP) was found. An interaction between energy and CP was found to have a significant effect on MUN in study by Nousiainen *et al.* (2004). Results of these studies indicate that MUN can be used to monitor the efficiency of nitrogen utilization in dairy herds as there seems to be an association between nitrogen intake and MUN.

Environmental factors affecting MUN include herd, test-day, parity, lactation stage, season of calving, and interactions between some of these factors. In the study by Wood *et al.* (2003), effects of herd-test-day (HTD) were highly significant in lactations 1 to 3. These results are in agreement with those observed in the study by Stoop *et al.* (2007), where HTD accounted for 58% of the total variation. Significant effects of HTD may be due to the differences in herd management and nutrition practices (Jílek *et al.*, 2006). Parity also influences MUN concentrations, but results from various studies seem to be contradictory and effects are still debatable. An increase in MUN concentrations over parities was observed in studies by Hojman *et al.* (2005) and Miglior *et al.* (2006). A lower MUN concentration for primiparous cows of 12.41 mg/dl compared to the second (12.80 mg/dl) and third (12.74 mg/dl) lactations was also reported by Wood *et al.* (2003). This might be due to lean tissue growth and higher efficiency of amino acid utilization in primiparous cows that result in the reduction of amino acid deamination and subsequent urea formulation in the liver (Roy *et al.*, 2011).

Contrary to results obtained by Hojman *et al.* (2005) and Miglior *et al.* (2006), Abdouli *et al.* (2008) observed a negative relationship between MUN and parity, where MUN was high in the first parity but decreased in the second and third parities. However, Godden *et al.* (2001b) found no association between parity and herd mean MUN.

An association between MUN and lactation stage (DIM) was reported in studies by Godden *et al.* (2001a), Johnson & Young (2003), Wood *et al.* (2003), Jílek *et al.* (2006), and Abdouli *et al.* (2008). In general, the lowest MUN concentration was reported to be during the first 60 DIM (days in milk), increasing between 60 and 150 DIM, and decreasing again after about 150 DIM (Godden *et al.*, 2001a; Johnson & Young, 2003; Jílek *et al.*, 2006; Abdouli *et al*,. 2008; Cao *et al.*, 2010, Mucha & Strandberg, 2011). However, Godden *et al.* (2001b) and Rajala-Schultz & Saville (2003) found no association between MUN and DIM, as did Hojman *et al.* (2005). Results from these studies seem to contradict each other, but differences in MUN concentration over lactation stages may be attributed to physiological changes over the lactation period (Godden *et al.*, 2001b).

The effect of season on MUN concentration can be confounded by other factors such as stage of lactation and nutritional effects, making it difficult to describe their association (Godden *et al.*, 2001b). In the study by Godden *et al.* (2001a) the mean MUN concentration was highest during the late summer season (July – September). Lower MUN concentrations in winter and early summer, and higher values in spring, late summer, and fall (autumn) in Holstein cows were reported in the study by Miglior *et al.* (2006). Wattiaux *et al.* (2005) reported the lowest MUN values in autumn when cows were milked twice and in the spring when cows were milked three times per day. In the study by Abdouli *et al.* (2008) MUN was the lowest during the winter season (January – March), and highest during summer. Milk Urea Nitrogen concentration values that were 2.5 (winter), 1.8 (spring), and 2.8 (autumn) mg/dl lower than the summer concentrations in low producing herds were reported by Rajala-Schultz & Saville (2003). Contrary to this, Rajala-Schultz & Saville (2003) observed that, in the high producing herds, the MUN concentrations were lowest during summer with small differences among seasons. In the same study (Rajala-Schultz & Saville, 2003), season of calving was found to be more important in explaining MUN variations compared to test-day season. However, the association between MUN and test-day season was significant when accounting for DIM, parity, calving season and calving year in the model used.

Other factors reported to have an effect on MUN are body weight and breed of the cow. Body weight (BW) of lactating dairy cows was reported to have a negative correlation with MUN concentration in the studies by Jonker *et al.* (1998) and Hojman *et al.* (2005). In the former study a 100 kg change in BW produced a small change (a 100 kg increase in BW resulted in an increase of the mean MUN concentration 0.9 mg/dl) in the target MUN concentration. In the study by Johnson &

Young (2003) Jersey cows had a lower MUN mean value than Holstein cows. However, contrary to these results, Wattiaux *et al.* (2005) reported test-day MUN concentrations to be higher for the Jersey and Brown Swiss breeds compared to Holsteins, depending on whether a cow belonged to a single-breed or a multiple-breed herd. When compared to the Ayrshire breed in the study by Miglior *et al.* (2006), the MUN concentration of the Holsteins was lower than that of the Ayrshire breed.

Results of these studies indicate that MUN is affected by environmental, nutritional and other factors. These factors should be taken into account when interpreting MUN results. Most of the variation in MUN seems to be due to herd management factors or factors influencing MUN on the day of the test rather than the cow or animal factors (Wattiaux *et al.*, 2005). The effect of HTD was highly significant in studies by Wood *et al.* (2003) and Stoop *et al.* (2007), this environmental factor accounted for 58% of the total MUN variation in the latter study. Hence herd level MUN results may be difficult to interpret. As a result, the analysis of MUN might be more accurate when using individual cow level MUN measurements.

2.6 Genetic parameters for milk urea nitrogen and milk production traits

The heritability of a trait is important in selection as it influences selection accuracy and the rate of genetic progress. In Tables 2.3 and 2.4 lists of heritability estimates for MUN and production traits from various studies are given.

Table 2.3 MUN heritability estimates from various studies

Method	Parity 1	Parity 2	Parity 3	Across parities	Publication
Infrared	0.44±0.02	0.59±0.07	0.48±0.07		Wood *et al.* (2003)
technology	0.14±0.02	0.21±0.04	0.19±0.03		Hossein-Zadeh and Ardalan (2010)
	0.22±0.02	0.23±0.03		0.22±0.02	Mitchel *et al.* (2005)
	0.17±0.01				Mucha & Strandberg (2011)
Wet chemistry	0.14±0.02				Stoop *et al.*(2007)
	0.14±0.01	0.09±0.01		0.15±0.01	Mitchel *et al.* (2005)

Heritability estimates for MUN ranged from low (0.09±0.01 in the second parity; Mitchel *et al.* 2005) to high (0.59±0.07 in parity 2; Wood *et al.* 2003). The heritability estimates decreased lately, compared to the studies done earlier. This indicates that there might have been improvements in methods used for MUN determination and/or for analysis of MUN data.

Heritability estimates for yield traits were medium to high in literature and the estimates were fairly similar in the various studies. Yield traits have been included in breeding values of Holstein cattle worldwide and selection was applied for a long period of time, hence the similarity in heritability estimates was expected.

Table 2.4 Heritability estimates for production traits from various studies

Parity	Trait	Heritability	Publication
1	MY	0.20±0.01	Zink *et al.* (2012)
	FY	0.21±0.01	Yousefi-Golverd *et al.* (2012)
	PY	0.23±0.01	
	MY	0.22±0.09	
	FY	0.24±0.09	
	PY	0.28±0.08	
	MY	0.47±0.01	Mucha & Strandberg (2011)
	FY	0.36±0.01	
	PY	0.44±0.01	
	MY	0.33±0.04	Hossein-Zadeh & Ardalan (2010)
	F%	0.23±0.03	
	P%	0.27±0.04	
	MY	0.48±0.09	Wood *et al.* (2003)
	FY	0.38±0.08	
	PY	0.42±0.07	
2	MY	0.30±0.05	Hossein-Zadeh & Ardalan (2010)
	F%	0.22±0.04	
	P%	0.24±0.03	
	MY	0.45±0.10	Wood *et al.* (2003)
	FY	0.59±0.09	
	PY	0.47±0.09	
3	MY	0.28±0.05	Hossein-Zadeh & Ardalan (2010)
	F%	0.22±0.03	
	P%	0.25±0.05	
	MY	0.35±0.08	Wood *et al.* (2003)
	FY	0.50±0.09	
	PY	0.36±0.07	

MY=Milk yield; FY=Fat yield; PY=Protein yield; F%=Fat percentage; P%=Protein percentage

Before inclusion of a trait as a selection criterion in the breeding objective of a breed, reliable genetic parameters should be calculated. The high variation in heritability estimates for MUN in the above studies may be due to differences in certain factors such as design of the study and the number of animals used. This indicates the necessity of estimating breed-specific values for South African Holsteins. However, the estimated values indicate that MUN is heritable and selection can be applied based on MUN values. Heritability estimates reported in literature for production traits are generally moderate to high.

2.7 Correlations between milk urea nitrogen and production traits

Several studies have been performed to evaluate the association of MUN with production traits such as milk, fat and protein yield, as well as with fat and protein percentage. In the sections to follow, phenotypic and genetic correlations between MUN and production traits will be discussed.

Genetic correlations between MUN and production traits have been reported in several studies. In studies by Arunvipas *et al.* (2003b), Wood *et al.* (2003), and Miglior *et al.* (2007) genetic correlation values of 0.11±0.04, 0.17, and 0.22 (no standard errors provided), respectively, were estimated between MUN and milk yield. An estimated genetic correlation value of 0.24 was reported in the studies by Stoop *et al.* (2007) and Hossein-Zadeh & Ardalan (2010). However, the standard error in the study by Stoop *et al.* (2007) was too high (0.22) making the estimate unreliable, while Hossein-Zadeh & Ardalan (2010) did not report a standard error value. A higher value of 0.44±0.06 in German Holsteins was estimated by König *et al.* (2008), while Yazgan *et al.* (2010) estimated very high values of 0.67, 0.79, and 0.74 (no standard errors reported) for the first, second, and third lactations in Polish Holsteins. The positive genetic correlations between MUN and milk yield show that MUN increases as milk yield becomes higher. This unfavorable positive genetic correlation indicates that selecting for decreased MUN concentration in a herd might have a negative effect on milk yield.

The genetic correlation between MUN and fat yield was estimated at 0.01 (no standard error provided) in the study by Wood *et al.* (2003). However, they concluded that the genetic correlation between these traits was inconsistent. Stoop *et al.* (2007) estimated a high genetic correlation value of 0.41±0.19 for MUN and fat yield. In the study by Miglior *et al.* (2007) a value of 0.45 was estimated for the genetic correlation between MUN and fat percentage, while Hossein-Zadeh & Ardala (2010) estimated a lower value of 0.21 (no standard error provided). On the contrary, a negative value of -0.12 (no standard error provided) was reported in the study by Arunvipas *et al.* (2003b). The contradiction between the various studies necessitates the estimation of genetic correlation values in the South African Holstein population.

An estimate of 0.38±0.20 for the correlation between MUN and protein yield was obtained by Wood *et al.* (2003), while Stoop *et al.* (2007) reported a value that was much lower (0.04; no standard error provided). In the study by Miglior *et al.* (2007), a genetic correlation of 0.20 (no standard error provided) between MUN and protein percentage was estimated, while Hossein-Zadeh & Ardalan (2010) estimated a similar value of 0.30 (no standard error provided). However, Arunvipas *et al.* (2003b) estimated a negative genetic correlation of -0.117 (no standard error provided) between MUN and protein percentage. Milk urea nitrogen is genetically and phenotypically correlated with

production traits. Thus, these correlations should not be ignored when MUN is included as a selection criterion in breeding objectives of dairy herds.

Phenotypic correlations between MUN and milk yield were estimated in a number of studies. In studies by Jonker *et al.* (1999) and Hojman *et al.* (2004) high milk yields were correlated with high MUN concentrations on a herd level. In agreement with these results, Jílek *et al.* (2006) reported a positive quadratic (milk squared) relationship between MUN and milk yield in commercial Holstein herds. A non-linear relationship between the cow level MUN and milk yield was reported by Godden *et al.* (2001a) and Cao *et al.* (2010). The phenotypic correlation between MUN and milk yield was found to be low (0.13; no standard error reported) in the studies by Miglior *et al*, (2007) and König *et al.*, (2008). An increase of 0.05 mg/dl of MUN concentration was observed when milk production increased by 1 kg in herds that contained Holstein, Ayrshire, Guernsey, Jersey, and milking Shorthorn breeds (Arunvipas *et al.*, 2003b). This is in agreement with the 0.044 mg/dl MUN concentration increase as the fat corrected milk yield increased by 1 kg per day in the study by Cao *et al.* (2010) using Chinese Holsteins. In the study by Rajala-Schultz & Saville (2003) the test-day milk yield showed a positive phenotypic correlation with MUN in the high production group, where cows with a milk yield exceeding 41.2kg/day (highest producers) had MUN values that were on average 0.8mg/dl higher than the cows the lowest production group. Contrary to the estimates in the above studies, Stoop *et al*. (2007) observed a phenotypic correlation of -0.031 (no standard error reported) between MUN and milk yield. In general, high MUN values are associated with high milk yield. However, quadratic relationships should also be taken into consideration as one cannot assume only a linear relationship exists between MUN and milk yield.

Genetic and phenotypic correlations between MUN and production traits have been estimated for several Holstein populations. A wide range of estimates were reported in literature. However, there are some contradictions between the different studies with regards to the estimated values as well as the significance of the correlations. These differences in estimates could be due to the different populations and methods used for the analysis. It is in this light that it can be postulated that these genetic and phenotypic correlation parameters should be estimated for the South African Holstein population.

2.8 Environmental concerns regarding excess nitrogen excretion

Environmental pollution is a global concern, and one of the main pollutants is nitrogen (Tamminga, 1992). Excess nitrogen is excreted by livestock in feces and urine as ammonia, nitrous oxide or nitrogen oxides. Ammonia is produced when urea, excreted via feces and urine, is broken down by the enzyme urease (found in feces and soil) resulting in ammonia gas and carbamine. Further decomposition releases another molecule of ammonia gas and carbon dioxide. Ammonia is then

volatilized at a rate that is dependent on various factors, with urinary urea concentration and temperature being primary factors (Monteny, 2000; Lupis *et al.*, 2010). Ammonia emissions cause environmental acidification and eutrophication, which may result in poisoning and death of organisms living in rivers and dams (De Boer *et al.*, 2002; Di & Cameron, 2002; Van Duinkerken *et al.*, 2005). There are also concerns with regards to the effect of ammonia on human health. Ammonia has been reported by Becker & Graves (2004), Samet & Krewski (2007) and Lupis *et al.* (2010) to increase incidence of cardiorespiratory morbidity and mortality in humans, and also to cause eye irritation. Reports by various studies on the negative impact that ammonia has on the environment and human health indicate that measures should be taken to reduce ammonia air emissions.

Milk urea nitrogen can be used as a tool to monitor ammonia air emissions. A positive correlation between MUN and UUN has been reported by Jonker *et al.* (1998) and Burgos *et al.* (2007). Dietary nitrogen intake has a positive correlation with both MUN and UUN, hence the need to improve the efficiency of nitrogen utilization to reduce ammonia emissions (Kebreab *et al.*, 2001; Rotz, 2004). In the study by Van Duinkerken *et al.* (2011) the emission of ammonia was strongly influenced by diet and temperature. Due to the positive association between MUN and dietary nitrogen, and MUN and efficiency of nitrogen utilization (Godden *et al.*, 2001b; Zhai *et al.*, 2006; Hughtanen *et al.*, 2008) MUN can be used to monitor and control ammonia emissions. Results of the study by Van Duinkerken *et al.* (2011) showed an exponential increase of ammonia emission with increasing MUN concentration. The use of MUN to monitor ammonia emission is more practical and cost effective as MUN data is collected with routine measurements in South African dairy herds.

Dairy farmers need new approaches that would help in the improvement of nitrogen management in the various dairy farming systems, as the prices of feed continue to increase (Gourley *et al.* (2012). Overfeeding of protein results in more nitrogen being wasted as the portion not utilized by the animal is excreted. Protein is an expensive portion of animal rations and the farmer will incur higher feed costs. The use of MUN results to create awareness of the linkages between excess CP in dairy rations and increases in MUN concentration, UUN excretion and ammonia emissions from dairy farms was recommended by Powell *et al.* (2011).

2.9 Conclusion

Selection criteria that are currently included in selection indices of Holstein cattle in various countries, as well as South Africa include fitness, production, and welfare traits (Miglior *et al.*, 2005; Nielsen *et al.*, 2005). Milk urea nitrogen has not been routinely included in these selection indices and estimates of its economic value have not been reported in literature. Other prerequisites for a trait to be included in selection indices include information on the trait's variances, heritability, and

correlations with other traits (du Plessis & Roux, 1999). These have been estimated in various studies in other countries, but the inclusion of MUN in selection indices has to date not been established.

Estimates of genetic and phenotypic parameters, (co)variances, and economic values are important constituents for breeding value estimation (Mostert *et al.*, 2006a) and they are population specific. There is a need to determine MUN's variance components, heritability, and correlations with other traits. Upon estimation of the above mentioned parameters, MUN can then be considered for inclusion in breeding objectives of dairy herds. Milk urea nitrogen might also be used to monitor ammonia air emissions with the aim of reducing environmental pollution due to excess nitrogen.

In the current study, non-genetic factors influencing MUN will be determined. Factors with significant contributions to the variation in MUN will be taken into account when estimating genetic and phenotypic parameters, as well as (co)variance components for MUN.

Chapter 3: Materials and methods

3.1 Introduction

This chapter describes the data used in the current study. The procedures used for data preparation and editing, as well as statistical analysis, are presented subsequently.

3.2 Materials

Data were obtained from the Integrated Registration and Genetic Information System (Intergis). Test-day records and pedigree data of Holstein cows participating in the South African National Milk Recording and Improvement Scheme during the period from 1 January 2007 to 31 July 2012 were used. Cows participating in the National Milk Recording and Improvement Scheme (NMRIS) are tested every 5 weeks. On the test-day, each individual cow's milk is weighed and recorded at each milking. A milk sample is also collected from each cow and sent for laboratory testing. For each sample, MUN, fat, protein and lactose percentage, and somatic cell count (SCC) are determined using a System 4000 Infrared Analyzer (Foss Electric, Hillerod, Denmark) at the Lacto Lab (Pty) Ltd in Irene. Table 3.1 shows an example of test-day records for a few South African Holstein cows.

Table 3.1 An example of test-day records

Comp no.	Herd no.	Test date	Calving date	Parity	Milk yield	Fat percent	Protein percent	Lactose percent	SCC	MUN
2001585	42865	25/07/2010	05/06/2010	1	45.3	4.75	5.67	3.54	678	18.6
2006842	37564	03/12/2009	10/11/2009	1	39.7	4.40	5.13	3.25	752	20.1
2003285	44085	25/07/2010	07/06/2010	2	44.7	3.98	4.97	3.68	699	19.4
2164889	25786	04/11/2011	15/09/2011	3	55.4	4.45	5.87	3.43	712	22.4
1349852	19425	05/04/2007	19/02/2007	2	45.8	4.15	5.04	3.75	807	27.2
1752498	34895	18/02/2004	05/01/2004	3	44.7	4.08	4.18	3.48	674	21.8

The unedited data set consisted of 2 059 494 test-day records of 139 178 Holstein cows, from 571 herds.

3.3 Methods

3.3.1 Data preparation and editing

In order to work with a smaller and less computationally demanding data set, only data from the first three parities were used. Restrictions on age at calving were imposed to ensure reasonable calving ages in a specific lactation. Age at calving was required to be in the ranges 20 – 42, 30 – 54,

and 40 – 66 months for the first, second, and third lactation, respectively, following Mostert *et al.* (2006a). Table 3.2 gives a description of how days in milk (DIM) were ordered into lactation stages. The lactation was divided into 30-day intervals each, except for the last stage which was a 35-day interval, resulting in ten lactation stages (Ojango & Pollot, 2001).

Table 3.2 Classification of days in milk into lactation stages

Lactation stage	Days in milk range
1	1 – 30
2	31 – 60
3	61 – 90
4	91 – 120
5	121 – 150
6	151 – 180
7	181 – 210
8	211 – 240
9	241 – 270
10	271 – 305

Seasons of calving were defined as summer (November – January), autumn (February – April), winter (May – July) and spring (August – October). Herd-test-day (HTD) was used to define contemporary groups.

Records with missing results for MUN and/or milk, fat and protein percent were discarded. Cow tests that were recorded less than 5 DIM or after 305 DIM were excluded. Colostrum contains approximately 23% total solids compared to 12 – 13% in milk; it is also thicker and higher in protein, energy, minerals and vitamins. The milk becomes normal after about 4 to 5 days after calving (Harris & Schmidt, 2009). Cows are generally dried 2 months prior to calving to prepare them for calving and the next lactation. Hence the 305 day lactation period is normally considered as the standard lactation period. Outliers (i.e. observations outside 3 standard deviations from the mean) for MUN and the three milk production traits were discarded. Table 3.3 gives the acceptable range for each trait.

Table 3.3 Acceptable ranges for MUN and milk production traits

Trait	Acceptable range
MUN (mg/dl)	0.82 – 28.9
Milk yield (kg)	2 – 90
Fat percentage	2 – 9%
Protein percentage	2 – 6%

The acceptable ranges for milk yield, fat and protein percentages are those currently used by the NMRIS for Holstein cows. Yields of milk components (fat and protein) were calculated using the following equation:

Component yield (kg) = (component percent ÷ 100%) * milk yield (kg) (DHIA, 2011) [1]

The edited data set consisted of 1 240 562 test-day records on 137 088 cows from 571 herds. The data set was then separated according to parity. Observations from different parities, for a particular trait, were considered to be separate traits; hence a distinct analysis was carried out for each parity.

Descriptive statistics for MUN and the milk component yields were calculated from a sample of data from 40 randomly selected herds. This was done in order to work with a smaller and more manageable data set. A summary of the data by parity is shown in Table 3.4. This data set was eventually used for the analysis of variance (ANOVA) to test for fixed effects.

Table 3.4 Data used to calculate descriptive statistics and ANOVA

Parity	No. records	No. cows	No. HTD
1	110 672	12 402	2 433
2	107 324	10 373	1 841
3	100 151	9 214	2 520

A second data set (Table 3.5) was created from the edited data set described above, for the subsequent estimation of (co)variance components. This data set comprised only cows that calved from 2009; including earlier years made the data set too large and therefore computationally challenging.

3.3.2 Pedigree file preparation

Animals with unknown birth dates were excluded from the pedigree file. The pedigree was prepared with consideration of three generations of the studied animals. Only cows with known sires and dams were retained. Sires without daughters in at least three contemporary groups were not included in the analysis. Contemporary groups with less than three sires were deleted, together with contemporary groups that had less than five records. This was done to reduce the prediction error variance and to enhance the accuracy of the estimation of breeding values. The editing resulted in the data set given in Table 3.5.

Table 3.5 A summary of the data used for the estimation of (co) variance components

Parity	No. Records	No. HTD	No. Cows	No. Sires	No. Dams
1	135 703	3 144	22 995	1 002	19 595
2	112 782	3 196	20 497	989	17 785
3	73 667	2 916	13 559	901	12 223

This data set was used to estimate (co)variance components for MUN and milk production traits, as well as genetic trends for MUN.

3.3.3 Data analysis

3.3.3.1 Non-genetic factors influencing milk urea nitrogen

Descriptive statistics for MUN and milk, fat and protein yield were computed using the *Proc Means* procedure of the Statistical Analysis System (SAS 9.2, 2009). To determine non-genetic factors affecting MUN, an Analysis of Variance (ANOVA) was performed using the General Linear Models (GLM) procedure of SAS (SAS 9.2, 2009). The following model was used for the analysis:

$$y = \mu + Xb + e \qquad [2]$$

Where:

y = vector of observations for MUN;

μ = vector of the mean for MUN observations;

b = vector of unknown fixed effects. Fixed effects that were tested for were herd-test-day (HTD), season and year of calving, year of test, age at calving, lactation stage, as well as interactions among these factors;

X = incidence matrix relating fixed effects to MUN observations;

e = vector of random residual errors.

It was assumed that residual errors were independent and identically normally distributed with mean 0 and varianceσ_e^2, i.e.:

$e \overset{iid}{\sim} N(0, \sigma_e^2)$;

3.3.3.2 Estimation of genetic parameters

Variance and covariance components were estimated by the Restricted Maximum Likelihood procedure (REML) using the ASReml software (Gilmour *et al.*, 2002). This software is optimized for

working with genetics data. It handles large data sets efficiently, and it is faster compared to other genetic analysis software. Single-trait analyses for MUN, milk yield, fat yield and protein yield were used to derive starting values for the subsequent analyses. The general model was as follows:

$$y = Xb + Zu + Wpe + e \quad [3]$$

Where:

y = vector of test-day observations;

X = incidence matrix relating fixed effects to observations;

b = a vector of fixed effects;

Fixed effects were HTD, lactation stage, year of calving and age at calving for MUN. For milk, fat and protein yield the fixed effects were HTD, lactation stage, year-season of calving and age at calving;

Z = incidence matrix relating random animal additive genetic effects to observations;

u = a vector of animal additive genetic effects;

W = incidence matrix relating random permanent environmental effects to observations;

pe = a vector of permanent environmental effects;

e = vector of random residual effects;

Random animal additive genetic effects (a) were assumed to have the distribution N ~ (0, Aσ_a^2), where A is the additive genetic relationship matrix and σ_a^2 is the animal additive genetic variance. Residual effects (e) were assumed to be distributed with N ~ (0,$I\sigma_e^2$), where I is an identity matrix, σ_e^2 is the residual variance and cov (a, e) = 0. Permanent environmental effects were assumed to be distributed with N ~ (0,$I\sigma_{pe}^2$), where I is an identity matrix, σ_{pe}^2 is the variance due to permanent environmental effects and cov (a, pe) = 0.

Bivariate analyses were subsequently performed to estimate (co) variance components using the following general equation:

$$\begin{bmatrix} y_1 \\ y_2 \end{bmatrix} = \begin{bmatrix} X_1 & 0 \\ 0 & X_2 \end{bmatrix} \begin{bmatrix} b_1 \\ b_2 \end{bmatrix} + \begin{bmatrix} Z_1 & 0 \\ 0 & Z_2 \end{bmatrix} \begin{bmatrix} u_1 \\ u_2 \end{bmatrix} + \begin{bmatrix} W_1 & 0 \\ 0 & W_2 \end{bmatrix} \begin{bmatrix} pe_1 \\ pe_2 \end{bmatrix} + \begin{bmatrix} e_1 \\ e_2 \end{bmatrix} \quad [4]$$

Where:

y, b, X, u, Z, pe, W and e are the same as in equation 3, and superscript i refers to the i^{th} trait.

The (co) variance matrix for random effects in the model is given by:

$$\text{Var} \begin{bmatrix} a \\ pe \\ e \end{bmatrix} = \begin{bmatrix} A\sigma_a^2 & 0 & 0 \\ 0 & I\sigma_{pe}^2 & 0 \\ 0 & 0 & I\sigma_e^2 \end{bmatrix} \quad [5]$$

Heritability (h^2) was calculated as the ratio of animal additive genetic variance to phenotypic variance as follows:

$$h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_{pe}^2 + \sigma_e^2} \quad [6]$$

Repeatability (r) was calculated as:

$$r = \frac{\sigma_a^2 + \sigma_{pe}^2}{\sigma_a^2 + \sigma_{pe}^2 + \sigma_e^2} \quad [7]$$

Phenotypic (r_p) and genetic (r_g) correlations were estimated using equations 8 and 9 below:

$$r_p = \frac{COV_{pxy}}{\sigma_{px}\,\sigma_{py}} \quad [8]$$

Where:

r_p = phenotypic correlation between traits x and y;

COV_{pxy} = phenotypic covariance between traits x and y;

σ_{px} = phenotypic standard deviation for trait x;

σ_{py} = phenotypic standard deviation for trait y.

$$r_g = \frac{COV_{gxy}}{\sigma_{gx}\,\sigma_{gy}} \quad [9]$$

Where:

r_g = genetic correlation between traits x and y;

COV_{gxy} = genetic covariance between traits x and y;

σ_{gx} = genetic standard deviation for trait x;

σ_{gy} = genetic standard deviation for trait y.

3.3.3.3 Estimation of breeding values and determination of genetic trends

Estimated breeding values (EBVs) for MUN, for each of the three parities, were calculated by solving Best Linear Unbiased Prediction (BLUP) mixed model equations (Henderson, 1984) using the ASReml software (Gilmour *et al.*, 2002). The following mixed model equations (MME) were used:

$$\begin{bmatrix} X'R^{-1}X & X'R^{-1}Z & X'R^{-1}W \\ Z'R^{-1}X & Z'R^{-1}Z + A^{-1}\,{}^{1}\!/_{\sigma_a^2} & Z'R^{-1}W \\ W'R^{-1}X & W'R^{-1}Z & W'R^{-1}W + I\,{}^{1}\!/_{\sigma_{pe}^2} \end{bmatrix} \begin{bmatrix} \hat{b} \\ \hat{u} \\ \hat{pe} \end{bmatrix} = \begin{bmatrix} X'R^{-1}Y \\ Z'R^{-1}Y \\ W'R^{-1}Y \end{bmatrix} \quad [10]$$

Because R^{-1} is an identity matrix, it can be factored out from both sides of the equation, resulting in:

$$\begin{bmatrix} X'X & X'R^{-1}Z & X'W \\ Z'X & Z'Z + A^{-1}\alpha_1 & Z'W \\ W'X & W'R^{-1}Z & W'W + IW\alpha_2 \end{bmatrix} \begin{bmatrix} \hat{b} \\ \hat{u} \\ p\hat{e} \end{bmatrix} = \begin{bmatrix} X'Y \\ Z'Y \\ W'Y \end{bmatrix} \quad [11]$$

Where:

$\alpha_1 = \sigma_e^2 / \sigma_a^2$

$\alpha_2 = \sigma_e^2 / \sigma_{pe}^2$

$\hat{b}$,û and pê are estimates of b, u and pe, respectively, in equation 3. σ_a^2 ,σ_e^2, σ_{pe}^2, W, X, and Z are the same as in equation 3. W', X' and Z' are transposes of W, X and Z, respectively.

The EBVs were used to determine genetic trends by calculating the mean EBVs per year of birth. Genetic trends for MUN were determined by calculating mean EBVs by year of birth, using the SAS software (SAS 9.2, 2009). Genetic trends show the change in average genetic merit for the population over successive years.

Chapter 4: Results

4.1 Introduction

This chapter presents a description of the results obtained from the analyses described in chapter 3. Test-day data for Holstein cows participating in the South African National Milk Recording and Improvement Scheme was used to determine non-genetic factors affecting milk urea nitrogen (MUN) and yield traits. Heritability, genetic and phenotypic correlations between MUN and the yield traits, as well as genetic trends were estimated.

4.2 Descriptive statistics

Means and standard deviations for MUN and the three production traits, per parity, are shown in Table 4.1. The data used consisted of 110 672, 107 324 and 100 151 records from the first, second and third parity, respectively, from 12 202, 10 373 and 9 214 cows. The overall means for MUN and milk, fat and protein yields were 14.86 mg/dl, 27.83 kg, 1.05 kg and 0.89 kg, respectively.

Table 4.1 Means and standard deviations (SD) for MUN and yield traits for South African Holstein cows in parities 1 to 3 from 1 January 2007 to 31 July 2012

Parity	Trait	Min.	Max.	Mean	SD
1	MUN (mg/dl)	5.66	26.10	14.47	3.26
	Milk yield (kg)	7.40	45.20	26.43	8.25
	Fat yield (kg)	0.26	1.78	0.97	0.34
	Protein yield (kg)	0.27	1.54	0.84	0.25
2	MUN (mg/dl)	1.00	28.88	15.25	3.28
	Milk yield (kg)	2.60	79.80	29.83	10.00
	Fat yield (kg)	0.09	5.75	1.17	0.47
	Protein yield (kg)	0.09	2.78	0.96	0.30
3	MUN (mg/dl)	1.00	28.90	14.86	3.23
	Milk yield (kg)	2.70	88.50	27.24	10.21
	Fat yield (kg)	0.06	5.41	1.01	0.42
	Protein yield (kg)	0.09	3.31	0.88	0.36

Mean MUN was lowest in the first parity. There was a slight increase in the second parity, and then a decrease in parity 3. Milk, fat and protein yields followed a similar trend, with their means being lowest in parity 1, increasing in parity 2, and slightly decreasing in parity 3.

4.3 Non-genetic factors influencing milk urea nitrogen and milk production traits

Non-genetic factors influencing MUN and milk production traits where determined with generalized linear models (GLM) of the Statistical Analysis Software (SAS) (SAS9.2, 2009). The contributions to variation in MUN by non-genetic factors significantly influencing MUN ($P < 0.05$) across parities are shown in Table 4.2. These factors were herd-test-day (HTD), stage of lactation, year of calving (year) and age at calving. The coefficient of variation (R^2) ranged from 62 to 66%, indicating that the model explained 62 to 66% of the variation in MUN.

The herd-test-day (HTD) contemporary group had the highest contribution (ranged from 58.56% to 63.18%) to the variation in MUN, across parities. It was followed by lactation stage, age at calving and then year of calving respectively. The trend was similar in all three parities. Lactation stage had the highest contribution in the first lactation, decreasing in the second and third lactation. Year of calving effect had a minimal contribution in the three parities with negligible variation between parities. The contribution of age at calving increased with increase in parity.

Table 4.2 Contribution to MUN variation by non-genetic factors

Factor	Parity 1 ($R^2 = 0.64$)	Parity 2 ($R^2 = 0.62$)	Parity 3 ($R^2 = 0.66$)
Herd-test-day	59.94%	58.56%	63.18%
Lactation stage	1.43%	1.08%	0.84%
Year of calving	0.003%	0.002%	0.004%
Age at calving	0.04%	0.07%	0.10%

Milk, fat and protein yields were affected by the above mentioned non-genetic factors, as well as the year-season of calving interaction. Non-genetic factors influencing milk production traits and their level of contribution are given in Table 4.3 to 4.5.

Table 4.3 Contribution to milk yield variation by non-genetic factors ($P < 0.05$)

Factor	Parity 1 ($R^2 = 0.66$)	Parity 2 ($R^2 = 0.64$)	Parity 3 ($R^2 = 0.65$)
Herd-test-day	56.92%	46.72%	44.53%
Lactation stage	2.34%	5.79%	6.26%
Age at calving	0.64%	0.88%	0.37%
Year-season of calving	0.04%	0.03%	0.04%

In all three parities, HTD showed the highest contribution, followed by lactation stage, age at calving, and year-season of calving. The contribution by HTD was high in the first parity, decreasing in the second and third parity. Lactation stage followed a different trend, having the lowest influence in the first parity and then increasing in the second and third parity. Age at calving had the highest

contribution in the second parity compared to the first and third parity. The contribution by year-season of calving was similar for all parities but it was very low compared to the other non-genetic factors.

Table 4.4 Contribution to fat yield variation by non-genetic factors (P < 0.05)

Factor	Parity 1 (R^2 = 0.52)	Parity 2 (R^2 = 0.54)	Parity 3 (R^2 = 0.54)
Herd-test-day	47.46%	43.67%	41.34%
Lactation stage	0.28%	2.64%	3.21%
Age at calving	0.68%	0.69%	0.45%
Year-season of calving	0.05%	0.05%	0.08%

Herd-test-day had the highest contribution to the variation in fat yield. The contribution was lowest in the third parity compared to the first two parities. Lactation stage contributed less in the first parity; there was a notable increase in the second and third parity. The contribution by age at calving was similar in the first two parities, and it decreased in the third parity. Year-season of calving followed a trend similar to that of age at calving; however the contribution was extremely low.

Table 4.5 Contribution to protein yield variation by non-genetic factors (P < 0.05)

Factor	Parity 1 (R^2 = 0.65)	Parity 2 (R^2 = 0.62)	Parity 3 (R^2 = 0.63)
Herd-test-day	58.15%	51.08%	48.93%
Lactation stage	1.12%	2.65%	3.01%
Age at calving	0.64%	0.66%	0.26%
Year-season of calving	0.03%	0.03%	0.05%

For protein yield, the contribution by HTD was highest in the first parity; it decreased in the second and third parity. The effect of lactation stage was low in parity 1, increasing in the second and third parity. Age at calving contributed more to the protein yield variation in the second parity compared to the first and third parities. This contribution decreased notably in parity 3. Year-season of calving had a low contribution in all three parities, with the contribution in the third parity being slightly higher compared to that of parity 1 and 2.

Least squares means (LS) are adjusted for multiple factors, either categorical and/or continuous covariates, thereby minimizing the residual variance. Least squares (LS) means by stage of lactation for parities 1 to 3 are given in Figure 4.1.

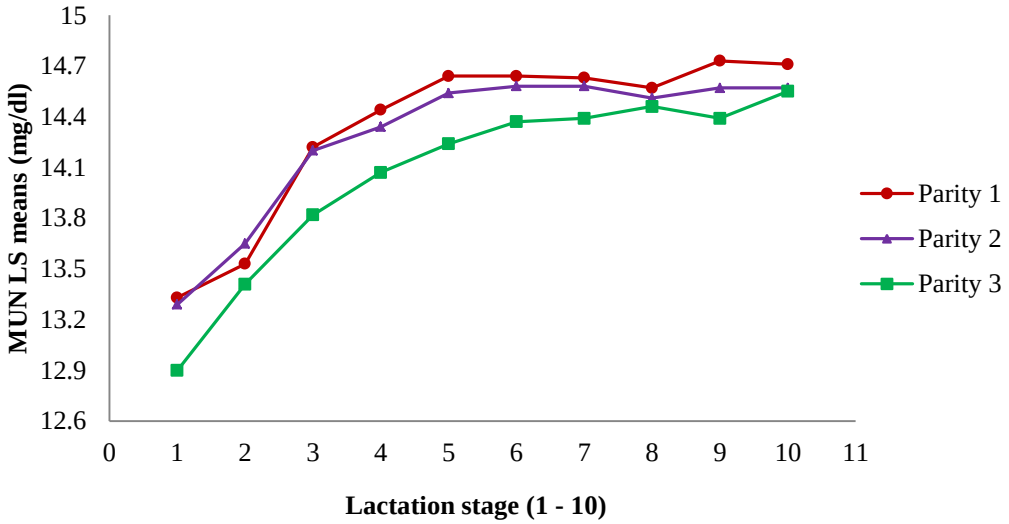

Figure 4.1 Trends in MUN LS means for lactation stages over parities 1 to 3

The MUN LS means for parity 3 were slightly lower compared to those of the first two parities. For all three parities, MUN was lowest in the first lactation stage and increased over the lactation period. In parity 1, a peak was reached in lactation stage 9. A peak was reached in lactation stage 6 in the second parity, and in lactation stage 10 in the third parity. Trends in MUN LS means over lactation stages were similar for all three parities.

4.4 Genetic parameters

4.4.1 Heritability estimates

Variance components and heritability estimates for MUN and milk, fat and protein yield for parities one to three are given in Table 4.6. Heritability was low for MUN across parities (0.09-0.11), moderate for both fat yield (FY) and protein yield (PY) (0.21 – 0.256) and high for MY (0.40-0.43). Estimates tended to be higher in the second and third parities.

Table 4.6 Estimates of variance components and heritability ±standard error for MUN and milk (MY), fat (FY) and protein (PY) yield

Parity	Trait	σ_a^2	σ_{pe}^2	σ_e^2	σ_p^2	r ± SE	h^2 ± SE
1	MUN	0.608	5.779	0.537	6.924	0.924±0.007	0.088±0.006
	MY	4.315	12.497	14.456	31.268	0.862±0.013	0.400±0.012
	FY	0.006	0.016	0.057	0.079	0.928±0.008	0.207±0.007
	PY	0.004	0.010	0.018	0.032	0.870±0.012	0.317±0.011
2	MUN	0.845	6.517	0.423	7.784	0.946±0.007	0.109±0.006
	MY	6.513	23.151	23.654	53.320	0.878±0.014	0.434±0.012
	FY	0.008	0.032	0.092	0.133	0.937±0.008	0.244±.008
	PY	0.005	0.019	0.028	0.052	0.902±0.010	0.371±0.011
3	MUN	0.808	6.432	0.423	7.663	0.945±0.008	0.105±0.007
	MY	7.569	24.853	27.85	49.430	0.873±0.014	0.418±0.016
	FY	0.011	0.039	0.103	0.153	0.930±0.011	0.256±0.011
	PY	0.005	0.022	0.031	0.058	0.916±0.012	0.379±0.013

h2 = heritability; r = repeatability; SE = standard error; σ_a^2 = additive variance; σ_{pe}^2 = variance due to permanent environmental effects; σ_e^2 = variance due to residual effects; σ_p^2 = phenotypic variance

The heritability estimate for MUN was lowest in parity 1, it increased slightly in the second parity, and it remained constant in the third parity. Heritability estimates for fat and protein yield followed a similar trend, being lowest in the first parity and then increasing slightly in the second and third parity. For milk yield, the heritability estimate increased in second parity then decreased in third parity.

The permanent environmental effects accounted for a large proportion of the phenotypic variation for both MUN, as shown by a high repeatability, which should be expected in a repeatability model. The repeatability for production traits remained fairly similar across parities. The residual variance for MUN was higher in the first parity compared to the second and third parities, which may indicating that there might be factors affecting MUN variation in the first parity that were not accounted for by the model used. The residual variance for production traits was lowest in the first parity, increasing in the second and third parities.

4.4.2 Genetic and phenotypic correlations

Genetic and phenotypic correlations between MUN and milk production traits for parity 1 to 3 are shown in Table 4.7. The correlations indicate the degree of association between MUN and milk production traits, as well as among the yield traits in the South African Holstein cattle population.

Table 4.7 Estimates of genetic (above diagonal) and phenotypic (below diagonal) correlations between MUN and milk production traits in parities1 to 3

Parity	Trait	MUN	MY	FY	PY
1	MUN		0.05±0.003	0.01±0.003	0.05±0.004
	MY	0.16±0.07		0.62±0.002	0.89±0.001
	FY	0.15±0.07	0.72±0.04		0.61±0.002
	PY	0.12±0.07	0.87±0.02	0.75±0.03	
2	MUN		0.10±0.004	0.03±0.004	0.06±0.004
	MY	0.09±0.08		0.66±0.04	0.91±0.001
	FY	0.06±0.09	0.72±0.04		0.66±0.002
	PY	0.03±0.09	0.89±0.02	0.79±0.03	
3	MUN		0.08±0.05	0.04±0.004	0.07±0.005
	MY	0.11±0.10		0.67±0.003	0.91±0.001
	FY	0.15±0.10	0.71±0.05		0.67±0.003
	PY	0.02±0.11	0.86±0.02	0.78±0.04	

Genetic correlations between MUN and production traits were positive and low across all parities. The genetic correlation (r_g) between MUN and fat yield was weaker in all parities compared to the correlation between MUN and both milk and protein yield. Genetic correlations between MUN and production traits were lowest in the first parity and increased across parities, with the exception of MUN with MY which decreased slightly in the third parity. A range in genetic correlations of 0.61±0.002 (between fat yield and protein yield, parity 1) to 0.91±0.001 (between milk and protein yield, parities 2 and 3) was observed among milk production traits, with the highest genetic correlations being between milk and protein yield in all parities.

Phenotypic correlations (r_p) between MUN and production traits were weaker in parity two compared to other parities, except for protein which was weakly correlated to MUN in both the second and third parities. The phenotypic correlations were low and positive, ranging from 0.02±0.11 (between MUN and protein yield, parity 3) to 0.16±0.07 (between MUN and milk yield in the first parity). The r_p between MUN and protein yield was the lowest compared to those between MUN and milk yield, and MUN and fat yield, in all three parities. Phenotypic correlations among milk production traits were much higher compared to those between MUN and the milk production traits. They ranged from 0.71±0.05 (between milk and fat yield in the third parity) to 0.89±0.02 (between milk and protein yield in parity 2).

4.4.3 Genetic trends for MUN

Figures 4.2 to 4.5 show the genetic trends for MUN and milk production traits for Holstein cows that were born from 1995 to 2010. Estimated breeding values were averaged per year of birth.

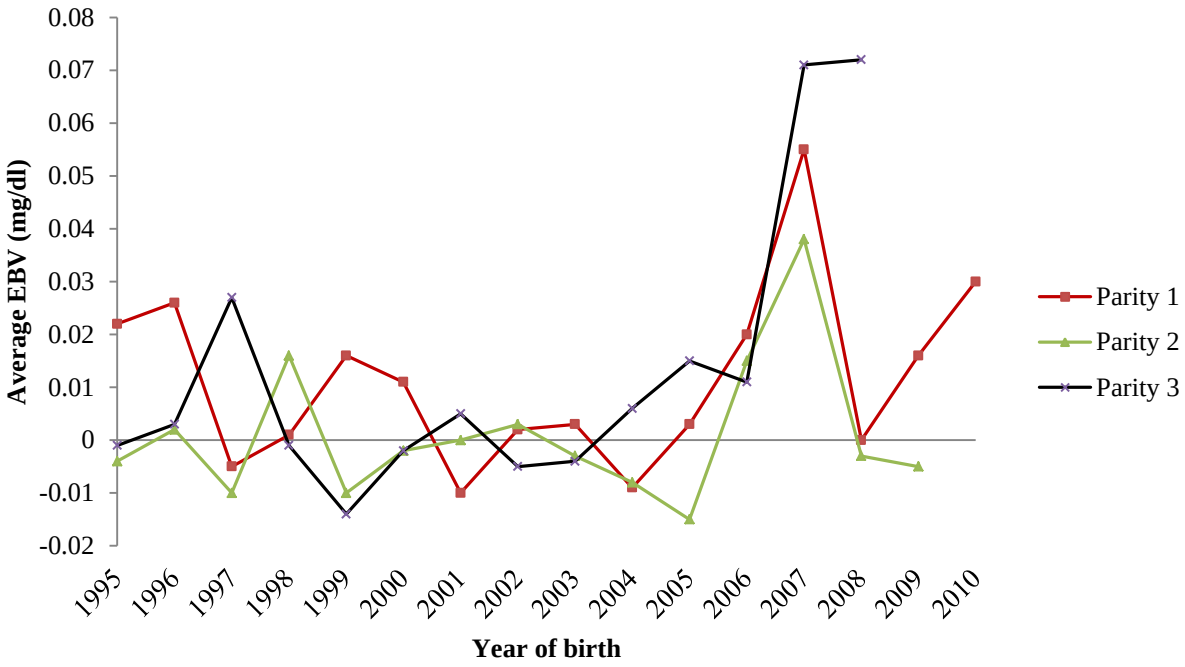

Figure 4.2 The genetic trend for MUN in parity 1 to 3

For parity 1, no distinct genetic trend in MUN was observed. There was a genetic increase in MUN of 0.044 mg/dl over the 15 year period, at 0.0029 mg/dl per year. The genetic trend in the second parity was 0.007 mg/dl over a 14 year period, and it was a bit higher than in the first parity. In parity 3, there was a decrease in MUN in cows born in 1999 and a peak in those born in 2007. The genetic trend was 0.049 mg/dl over a thirteen year period. A peak in cows born in 2007 was observed in all three parities.

Chapter 5: Discussion

5.1 Introduction

Test-day records and pedigree data of Holstein cows participating in the South African National Milk Recording and Improvement Scheme during the period 2007 to 2012 were obtained from the Integrated Registration and Genetic Information System (Intergis). These data were analyzed to determine environmental factors influencing MUN and subsequently estimate genetic and phenotypic parameters among MUN and yield traits. Estimated breeding values and genetic trends were obtained for MUN.

Traits currently included in selection objectives of Holstein cattle in South Africa include cow fertility, production, and udder health traits (Banga, 2009). Milk urea nitrogen may be used as a predictor of some economically important traits; however the utility of the trait in this regard has not been reported in literature. The focus of this study was to estimate variance components for MUN and its correlations with milk production traits in the South African Holstein population. These parameters may assist in determining the value of MUN in predicting traits in the breeding objectives.

The main findings of the study are discussed in this chapter. Results obtained are compared to those reported in literature and their practical application discussed.

5.2 Descriptive statistics

The overall mean across parities for milk urea nitrogen (MUN) was 14.86 mg/dl, which was lower than the 24.1±1.43 mg/dl previously observed in South African Holstein-Friesian cows on grazing (Van der Merwe *et al.*, 2001). This mean is comparable to those reported by Hojman *et al.* (2004) in Israeli dairy herds (14.4 mg/dl) and Ouda (2008) in Holstein and Czech Spotted dairy cattle (14.8 mg/dl). Abdouli *et al.* (2008) and Hossein-Zadeh & Ardalan (2010) observed higher means of 30.4 and 17.97 mg/dl in Tunisian and Iranian Holstein cows, respectively. Infrared technology was used in most of these studies, while some (Van der Merwe *et al.*, 2001; Ouda, 2008) did not specify the method used to measure MUN. The differences in the overall means may be due to environmental and nutritional, as well as breed effects. These factors have been reported to have an effect on the variation in MUN in studies such as those by Johnson & Young (2003), Hojman *et al.* (2004) and Burgos *et al.* (2007).

Mean MUN was lowest in parity 1 (14.47±3.26 mg/dl), increasing in the second parity (15.25±3.28 mg/dl), and then slightly decreasing in the third parity (14.86±3.23 mg/dl). This trend is similar to that obtained by Wood *et al.* (2003) where primiparous cows had the lowest mean MUN of 12.41 mg/dl, with an increase in second parity (12.80 mg/dl) and a slight decrease in parity 3 (12.74 mg/dl). Means of 14.3, 14.7, and 14.5 mg/dl for parities 1, 2 and 3, respectively, were reported by Hojman *et al.* (2004). Standard deviations were not specified in the study by Wood *et al.* (2003) and Hojman *et al.* (2004). The low mean MUN for the first parity, observed in the current study and that of Wood *et al.* (2003) and Hojman *et al.* (2004), may be attributable to what Roy *et al.* (2011) reported; tissue growth and higher efficiency of amino acid utilization in primiparous cows result in the reduction of amino acid deamination and subsequent urea synthesis in the liver. The effects of parity on MUN concentration are still debatable, and results from various studies seem to be contradictory. For example, the mean MUN was highest in the first parity in the study by Abdouli *et al.* (2008), it decreased in the second parity and furthermore in the third parity. This trend is completely different from that of the above mentioned studies.

Mean milk yield was higher than the 22.82±8.50 (across parity 1 to 3) obtained for South African Holstein cattle that calved from 1982 to 2004 (Mostert *et al.*, 2006a). There might have been an increase in the mean milk of the South African Holstein cattle over the years. Mean milk yield was 26.43±8.25, 29.83±10.00, and 27.24±10.21 kg in the first, second and third parity, respectively. These means were generally lower than those reported in the literature (Wood *et al.* 2003; Hojman *et al.* 2004; Miglior *et al.* 2007). The decrease of the mean in parity 3 was unexpected as older cows, up to the 4^{th} or 5^{th} parity, are normally higher producers compared to primiparous cows. The decrease in mean milk yield was contradictory to results obtained by Wood *et al.* (2003), Hojman *et al.* (2004) and Miglior *et al.* (2007); the mean increased from 33.42±9.25 to 35.40±9.97, 35.5±9.30 to 37.3±10.5, and 31.70±9.1 to 33.5±9.7 from the second to the third parity, respectively. Means for fat yield were 0.97±0.34, 1.17±0.47, and 1.01±0.42 kg for parities 1, 2, and 3, respectively. This was higher than the mean reported by Mostert *et al.* (2006) in South African Holsteins that calved from 1982 to 2004. Protein yield followed a similar trend, with means of 0.84±0.25, 0.96±0.30, and 0.88±0.36 in parity 1, 2, and 3, respectively. These estimates were also higher than the 0.73±0.26 mean in South African Holsteins (Mostert *et al.*, 2006). The differences in the means of yield traits might be due to an increase in these traits over the years of calving of the South African Holstein cows, as a result of genetic selection.

5.3 Non-genetic factors influencing milk urea nitrogen

Non-genetic factors that had an effect on variation in MUN were herd-test-day (HTD), lactation stage and year of calving. Herd-test-day (HTD) had the highest contribution (58.56% to 63.18%) to the variation in MUN in all three parities. This is in agreement with Stoop *et al.* (2007), who reported that HTD accounted for 58% of the total MUN variation. Effects of HTD were also highly significant in parities 1 to 3 in a study by Wood *et al.* (2003). The high contribution of HTD indicates that this environmental effect should be accounted for when MUN data is analysed. The reason for the high contribution may be because HTD includes effects of the herd management and the season of the test.

Lactation stage had the second largest contribution to variation in MUN. It contributed 1.43, 1.08 and 0.84% in the first, second and third parities, respectively. There was a notable decrease in the percentage contributed by lactation stage from parity 1 to 3; this factor became less important in the third parity. This indicates that physiological changes occurring throughout the lactation period might have less impact in older cows compared to primiparous cows. Lactation stage was also reported to have an effect on MUN variation in a number of other studies (Godden *et al.*, 2001a; Johnson & Young, 2003; Jílek *et al.*, 2006; Abdouli *et al.*, 2008). The decrease in MUN after 150 days in milk (DIM), equivalent to the fifth lactation stage, was however not observed in these other studies. The least squares (LS) mean for MUN stayed fairly constant after the fifth lactation stage in all three parities. A peak in MUN LS means was reached in lactation stage 9 (14.73mg/dl), 6 (14.58 mg/dl) and 10 (14.55 mg/dl) in the first, second and third parities, respectively. These results differ from those obtained by Mucha & Strandberg (2011), where the MUN maximum value (14 mg/dl) was reached at 75 DIM (equivalent to the third lactation stage of the current study) in first parity Swedish Holstein cows. Results of the current study contradict those obtained by Godden *et al.* (2001b), Rajala-Schultz & Saville (2003) and Hojman *et al.* (2005) that found no association between MUN and lactation stage. The differences in MUN concentration over lactation stages may be a result of physiological changes over the lactation period (Godden *et al.*, 2001b). The increase in MUN after the peak of lactation might be due to a decrease in metabolic demands of lactation and the lower milk production (Hossein-Zadeh & Ardalan, 2010).

The LS mean for MUN fluctuated across years of calving; this might be due to annual variation in nutrition. Nutrition has been reported to have an effect on MUN concentration, with positive associations between level of MUN and dry matter intake (DMI), crude protein (CP) and

rumen degradable protein (RDP) being observed by Hojman *et al.* (2004) and Zhai *et al.* (2006). An interaction between energy and CP was also found to have a significant effect on MUN by Nousiainen *et al.* (2004). Changes in levels of any of these nutrients in dairy rations might result in a decrease or increase of the mean MUN concentration.

Herd-test-day, stage of lactation and year of calving need to be taken into account when analysing MUN data. Neglecting these factors may increase errors in the estimation of genetic and phenotypic parameters for MUN.

5.4 Genetic parameters

5.4.1 Heritability estimates

The heritability of MUN was lower in the first parity (0.09±0.01) than in parities 2 and 3 (0.11±0.01). Much higher estimates, ranging from 0.44±0.02 to 0.48±0.07 in the first three parities were obtained by Wood *et al.* (2003) in Canadian Holstein cattle. Mitchel *et al.* (2005) reported significantly higher estimates of 0.22±0.02 and 0.23±0.03 for parity 1 and 2, respectively, in Danish Holstein cows. Recent studies (Hossein-Zadeh & Ardalan 2010; Mucha & Strandberd, 2011) also observed larger estimates (0.14±0.02 to 0.21±0.04) in Iranian Holsteins and in Swedish Holsteins. The low heritability estimates indicate that the rate of genetic progress would be very slow if South African Holstein cattle were selected on MUN.

Heritability estimates for milk yield were 0.40±0.01, 0.43±0.01 and 0.42±0.02 for parities 1, 2 and 3, respectively. These estimates were slightly lower than those reported by Wood *et al.* (2003), in Canadian Holstein cattle for first (0.48±0.09) and second (0.45±0.10) parities, but higher for parity 3 (0.35±0.08). Previously reported heritability estimates of South African Holsteins that calved from 1980 to 2005 had lower estimates of 0.33±0.02, 0.25±0.02 and 0.25±0.03 for parities 1, 2 and 3, respectively (Makgahlela *et al.*, 2007). The differences in heritability estimates for South African Holsteins may be due to the different models used for analysis. Hossein-Zadeh & Ardalan (2010) also observed lower heritability estimates, ranging from 0.30±0.04 in the second parity, to 0.35±0.08 in parity 3 in Iranian Holstein cattle. Selection on milk yield has been a success and the medium to high heritability in the current study and the literature shows why the genetic progress has been significant in South Africa and globally.

Heritability estimates for fat yield in the current study were 0.21±0.01, 0.24±0.01 and 0.26±0.01 for the first, second and third parity, respectively. Estimates were similar to those observed by Makgahlela *et al.* (2007) in the first (0.24±0.02) and third parity (0.22±0.03); however heritability was slightly lower in parity 2 (0.19±0.02), in the current study. Similar estimates of 0.24±0.01 and 0.21±0.01 were observed in the first parity of Iranian (Yousefi-Golverdi *et al.*, 2012) and Czech (Zink *et al.*, 2012) Holstein cattle, respectively. Higher estimates were obtained in an earlier study by Wood *et al.* (2003) for parity 1 (0.38±0.08), 2 (0.59±0.09) and 3 (0.50±0.09). Mucha & Strandberg (2011) also reported a higher heritability estimate of 0.36±0.01 in the first parity of Swedish Holstein cattle.

Protein yield heritability estimates slightly increased across parities being 0.32±0.01, 0.37±0.01 and 0.38±0.01 in the first, second and third parities, respectively. Higher estimates for the first (0.42±0.07) and second (0.47±0.09) parities and a fairly similar estimate for the third parity (0.36±0.07) were reported for Canadian Holstein cattle by Wood *et al.* (2003). Lower estimates were observed by Makgahlela *et al.* (2007) in South African Holsteins, being 0.28±0.02, 0.24±0.02 and 0.26±0.03 in the first, second and third parities, respectively. Mucha & Strandberg (2011) obtained a higher heritability estimate of 0.44±0.01 in the first parity of Swedish Holstein cattle. Lower estimates of 0.28±0.08 and 0.23±0.01 were observed by Yousefi-Golverd *et al.* (2012) and Zink *et al.* (2012) in Iranian and Czech Holstein cattle, respectively.

Differences in heritability estimates between the current study and the literature may be because of the different populations studied, as genetic parameters are population specific. Estimates from more recent studies are lower compared to those obtained in earlier years, which may be a result of a reduction in genetic variation due to selection. Methods used for analysis might have had an effect on the heritability estimates. For example, random regression models were used by Wood *et al.*, 2003 and Hossein-Zadeh, 2010, repeated records animal model by Miglior *et al.*, 2005 and a random regression sire model by Mucha & Strandberg, 2011. The repeated records animal model was used in the current study.

5.4.2 Genetic and phenotypic correlations between milk urea nitrogen and milk production traits

Genetic correlation estimates observed in the current study were much lower compared to those reported in the literature. There are no estimates currently available for comparison of the genetic and phenotypic correlations between MUN and production traits in South Africa. An estimate

of 0.24 (no standard errors reported) was obtained by both Stoop *et al*. (2007) and Hossein-Zadeh & Ardalan (2010) for the genetic correlation between MUN and milk yield. Much higher values were reported by Yazgan *et al.* (2010) in Polish Holsteins for the first (0.67), second (0.79) and third (0.74) parity (no standard errors reported). The positive genetic correlations between MUN and milk yield are unfavourable as they indicate that MUN increases with increase in milk yield. Selection for higher milk yield is likely to result in decreased genetic merit for MUN.

The genetic correlation between MUN and fat yield was extremely low in all three parities, ranging from 0.01±0.003 in the first parity to 0.04±0.004 in parity 3. This might be an indication that selection for fat yield in unlikely to result in a correlated change in MUN. The estimate of 0.01±0.003 was similar to that reported by Wood *et al.* (2003) in Canadian Holsteins. A much higher estimate of 0.41±0.19 was observed by Stoop *et al*. (2007). The genetic correlation increased from the first to the third parity in the current study, indicating that selecting for higher fat yield may have more effect on the genetic merit for MUN in the third parity compared to parity 1.

The genetic correlation between MUN and protein yield remained fairly constant in parities 1 (0.05±0.005), 2 (0.06±0.004) and 3 (0.07±0.005). A similar estimate for the genetic correlation between MUN and protein yield (0.04±0.04) was observed by Wood *et al.* (2003) in the first parity. The standard error is, however, very high; indicating that the estimate is inconsistent / unreliable. The same can be said about the genetic correlation estimate of 0.06±0.15 in the third parity of the same study (Wood *et al.*, 2003). In the second parity (Wood *et al.*, 2003) the genetic correlation between MUN and protein yield was much higher (0.22±0.12) than that of the current study. Stoop *et al*. (2007) also reported a higher estimate of 0.38±0.20, which had a high standard error, making it unreliable. Results of the current study indicate that the genetic correlation between MUN and protein yield is extremely weak; selection applied on protein yield is unlikely to affect genetic merit for MUN.

Phenotypic correlations between MUN and milk yield were 0.16±0.07, 0.09±0.08 and 0.11±0.10 in the first, second and third parities, respectively. These correlations are comparable to the correlation of 0.13 (standard error not reported) reported by Miglior *et al.* (2007) and König *et al.* (2008) in Canadian and German Holsteins, respectively. Though phenotypic correlation estimates between MUN and milk yield were not reported by Cao *et al.* (2010), they observed a linear

relationship between these two traits. These phenotypic correlations show a possible increase in MUN concentration if milk yield was increased.

There were no estimates for the phenotypic correlation between MUN and fat yield, and MUN and protein yield to compare with in literature. The phenotypic correlation between MUN and fat yield was 0.15±0.07 in the first and third parities, and 0.06±0.09 in the second parity. These phenotypic correlations show that an increase in fat yield may have a resultant increase in MUN, more so in the first and third parities.

A phenotypic correlation of 0.12±0.07 was obtained between MUN and protein yield in the first parity. There was a notable decrease in the second (0.03±0.09) and third (0.02±0.11) parities. Older Holstein cows may have higher efficiency of utilization of dietary nitrogen, hence the extremely weak phenotypic correlation between MUN and protein yield correlation.

The genetic and phenotypic correlations between MUN and milk production traits are positive and very weak. This shows that increases in milk production traits may result in Holstein cows with slightly higher MUN levels. Increased MUN levels are undesirable as they are not only an indication of low dietary protein utilization efficiency, but also show that more urea might be excreted resulting in environmental pollution.

5.4.3 Genetic trends for milk urea nitrogen

Genetic trends observed in the current study showed a very low but positive increase in MUN in all three parities. There was an increase of 0.044mg/dl in the first parity, which decreased to 0.007mg/dl in the second parity and an increased to 0.049mg/dl in parity 3 over a 15, 14 and 13 year period for the first, second and third parity, respectively. There is currently no genetic trend estimates reported in literature for comparison. Results obtained in this study show that there has been an increase in MUN levels in South African Holstein cattle over the past 15 years.

Milk urea nitrogen is currently not included in the breeding objective of South African Holstein cattle. The genetic trend indicates that there was an increase in MUN in the past 15 years.

Although the increase is very low, this is a call for concern as it may imply that Holstein dairy cows are less efficient in utilizing dietary protein.

5.5 Concluding remarks

Variation in MUN levels of South African Holstein cattle is influenced by non-genetic factors such as the herd-test-day, lactation stage and year of calving. These factors should be accounted for when using MUN data. The low heritability observed indicates that the rate of genetic progress would be limited if selection is applied on MUN in the South African Holstein cattle population. However, due to the correlations between MUN and milk, fat and protein yield, which have medium to high heritabilities, the accuracy of prediction for MUN may be improved. Genetic trends for MUN were extremely low and positive, indicating a slight increase in MUN concentration over the past 15 years. Results of this study necessitate further research on MUN; this can help in the prediction of economically important traits that are correlated with MUN. This has a practical advantage as its data is currently being collected in cows participating the national dairy animal improvement scheme.

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Addendum

1. The following presentations were made at South African Society for Animal Science congresses;

- Environmental factors affecting milk urea nitrogen in South African Holstein cattle.
  - At the 44th SASAS congress held at the Stellenbosch University in July 2011.
- Genetic parameter estimates for milk urea nitrogen and its relationships with yield traits in South African Holstein cattle
  - At the 46th SASAS congress held at the University of Free State in June 2013

2. A paper emanating from this study was published;

- Environmental factors influencing milk urea nitrogen in South African Holstein cattle. 2012. SA J. Anim. Sci. 42 (Issue 5, Supplement 1)