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Effects of milk urea nitrogen on reproduction parameters of dairy cows in South Africa

by

Elandri de Bruyn

BSc(Agric) Animal Science, (University of Pretoria)

Submitted in fulfilment of the requirements for the degree MScAgric Animal Science (Production Physiology and Product Quality)

Department Animal and Wildlife Sciences

Faculty of Natural and Agricultural Sciences

University of Pretoria

Pretoria

2018

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Ethics statement

I, Elandri de Bruyn, hereby declare that ethics approval was obtained for the research described in this dissertation. I further declare that I have observed the ethical standards required in terms of the University of Pretoria's Code of ethics for researchers and the Policy guidelines for responsible research.

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Declaration

I declare that this dissertation for the MScAgric Animal Science (Production Physiology and Product Quality) degree at the University of Pretoria has not been submitted by me for a degree at any other university.

Signed……

Elandri de Bruyn

Summary

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by
Elandri de Bruyn
Student number: 11006740
For the degree MScAgric Animal Science (Production Physiology and Product Quality)

Supervisor: Prof E.C. Webb

Department of Animal and Wildlife Sciences
Faculty of Natural and Agricultural Sciences
University of Pretoria

This study was based on measurements taken from the milk recording scheme of the Agricultural research Council (ARC). In this study the effects of milk urea nitrogen (MUN) on reproduction parameters of dairy cows in South Africa was investigated. The Analysis of Variance (ANOVA) procedure confirmed that MUN has a significant influence on reproduction performance in South African dairy cows. The correlation between MUN and intercalving period (ICP) demonstrated that cows are taking longer to fall pregnant as MUN increases. Other more complex reproduction parameters were also calculated as well as their correlations with MUN. The first is reproduction performance (RP) which was negatively correlated with MUN. The second parameter was reproductive index (RI) which was also negatively correlated with MUN. This means that overall reproduction in dairy cows decline as MUN concentrations are increased. The breeds used in this study were Holstein and Jersey dairy cows. Analysis of variance results confirmed breed differences for the effects of MUN on reproduction parameters. It was found that reproductive success declines with increasing MUN concentrations in Jersey cows regardless the season. Analyses of the interaction between breed and season, indicate that the most important interaction was between Jersey cows in summer. This implies a different urea threshold for Jersey cows between seasons, as well as between Jersey cows and Holstein cows.

Abstract

Milk urea nitrogen (MUN) is used as a tool to measure the inclusion rate of protein and non-protein nitrogen (NPN) in the diet of dairy cows. This study is based on data that was given from the Agricultural Research Councils (ARC) milk recording scheme that contains about 12000 observations. The data was recorded from 2006 to 2008 and contains measurements from both high and low producing dairy cows. In this study the interactions between the concentrations of MUN and reproduction parameters are investigated. The intercalving period (ICP) was calculated (415.5 ± 90.0 days) and investigated. It was found that MUN in South African dairy cows correlate significantly with ICP (r^2 = 0.02). These observations indicate that cows are taking longer to fall pregnant as MUN concentrations increases. Two more complex reproduction parameters were calculated and their correlation with MUN was further investigated. The first was reproduction performance (RP; 69.5 ± 12.49%) and the second is reproductive index (RI; 108.5 ± 2.95). Both correlated negatively with MUN (r^2 = -0.02 and r^2 = -0.10, respectively). Breed differences between Holstein and Jersey dairy cows were also investigated. Holstein cows had higher average concentrations of MUN than Jersey cows, 18.0 ± 4.72 mg/dL and 12.6 ± 5.2 mg/dL respectively. In each breed MUN was correlated with ICP, RP and RI but no significance was found in Holstein cows. In Jersey cows however both RP (70.8 ± 12.86%) and ICP (408.1 ± 91.79 days) correlated significantly with MUN, r^2 = -0.0001 and r^2 = 0.0007 respectively. This means that reproductive success declines as increasing MUN concentrations are measured in Jersey cows. Summer and winter was also included as factors in this study. In summer the MUN (14.1 ± 5.49 mg/dL) correlated with ICP (416.9 ± 88.43 days), RP (69.7 ± 12.36%) and RI (108.6 ± 2.93). Both RP and RI correlated negatively (r^2 = -0.02 and r^2 = -0.09, respectively) with MUN. While ICP correlated positively with MUN (r^2 = 0.02). In winter the MUN (15.1 ± 6.01 mg/dL) correlated with ICP (412.2 ± 94.18 days), RP (69.8 ± 12.68%) and RI (108.3 ± 2.99). RP and RI both correlated negatively with MUN, r^2 = -0.01 and r^2 = -0.12 respectively. MUN and ICP showed a positive correlation (r^2 = 0.008). These findings imply that the decline in reproduction as correlated with MUN is significant in both seasons for dairy cows in South Africa. Lastly the interaction between breed and season indicates that the most important interaction was that between Jersey cows in summer. The average MUN level for Jersey cows in summer was 12.5 ± 5.19 mg/dL. The present results suggest a different MUN threshold level for Jersey cows, compared to that currently recommended for Holstein cows.

Acknowledgements

I would firstly like to thank God for the incredible opportunity He has given me and for always being near me in life. I wish to express my sincere gratitude to my parents (Annie and Ferdie de Bruyn) and my sister (Cherene de Bruyn) for all their love, support and encouragement that never failed throughout my whole academic career. To Prof E.C. Webb who was my supervisor in this project, thank you for your time, advice, guidance and patience throughout the completion of this degree. I would also like to thank Mr Schalk Greyling for allowing me to use the data he collected to compile this dissertation. To Mr PH Duvenhage of SA Holstein and Ms Tessa Opperman of Jersey SA, thank you for allowing me to use the collected data in order to interpret the breed effects. To Ms Irene Joubert of the ARC – Soil, Climate and Water, thank you for allowing me to use temperature data in order to interpret the seasonal effects in my dissertation. Lastly, I would like to thank all my friends that supported and encouraged me throughout my studies over the years.

List of abbreviations

ADG	Average daily gain
AI	Artificial insemination
ANOVA	Analysis of variance
ARC	Agricultural Research Council
BCS	Body condition score
BHBA	β-hydroxybutyrate
BUN	Blood urea nitrogen
Butterfat %	Butterfat percentage
CP	Crude protein
CPS-I	Carbamyl phosphate synthetase I
DIM	Days in milk
DM	Dry matter
DO	Days open
DUP	Digestible undegradable protein
FME	Fermentable metabolizable energy
FSH	Follicle stimulating hormone
GLM	General linear model
GnRH	Gonadotropin-releasing hormone
ICP	Intercalving period
IGF-I	Insulin-like growth factor I
LDH	Lactate dehydrogenase
LH	Luteinizing hormone
ME	Metabolizable energy
MUN	Milk urea nitrogen
N	Nitrogen
NE	Net energy
NEBAL	Negative energy balance
NEFA	Non-esterified fatty acids
NPN	Non-protein nitrogen
NSC	Non-structural carbohydrates
$PGF_{2\alpha}$	Prostaglandin $F_{2\alpha}$
PUN	Plasma urea nitrogen
RDP	Rumen degradable protein
RI	Reproductive index
RP	Reproduction performance
RUP	Rumen undegradable protein

SUN	Serum urea nitrogen
THI	Temperature humidity index
TMR	Total mixed ration
Tn	Minimum temperature
Tx	Maximum temperature
UN	Urinary excretion of nitrogen

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Chapter 1: Introduction

1.1 Project title

Effects of milk urea nitrogen on reproduction parameters of dairy cows in South Africa.

1.2 Problem statement

The purpose of this study was to investigate the effects of milk urea nitrogen (MUN) on the reproduction of Jersey and Holstein dairy cows. It is suspected that dairy cattle are often overfed with protein and non-protein nitrogen (NPN) which may lead to reduced fertility, especially if protein and NPNP feeding is not synchronised with the level of milk production.

Much of the research to date has not clearly addressed whether the sensitivity of cows for blood urea nitrogen (BUN) or MUN on reproduction parameters are the result of breed differences or seasonal effects.

1.3 Project aims

The main objective of this study was to investigate how protein and NPN concentrations in the diet, and therefore MUN, affects reproduction parameters of dairy cows. A further aim of this study was to investigate the potential breed differences and seasonal effects on reproduction parameters between Jersey and Holstein dairy cows.

1.4 Motivation

In 2015 consumption of dairy products increased 0.6% from 2014 due to increased global population growth. It is estimated that the demand for dairy products will increase to 20 million tonnes per year of which 8 million tonnes is due to greater population growth and 12 million tonnes due to increased consumption per head. In South Africa the number of milk producers has decreased to 1593 in January 2017 from 3551 in January 2009. Even though the number of milk producers has decreased, the amount of milk produced still showed an increase in growth of 6.4% in 2015 from 2014 (Milk Producers' Organisation, 2017). Global warming is expected to not only increase overall temperatures but also lead to the country being drier, except in the Eastern Cape and central parts of the country where rainfall is expected to increase significantly (Williams *et al.*, 2016). The main factor concerning heat stress in dairy cattle is the negative effect it has on reproduction performance, fertility, physiology and milk production (De Rensis *et al.*, 2015). Most dairy cows that experience heat stress do not show oestrus, this in turn makes it difficult to perform artificial insemination (AI) and leads to fewer pregnancies (Takahashi, 2012).

Genetic selection for increased milk production directly lead to a higher need for nutrients from the diet as well as body reserves, therefore making health and reproduction disorders more extensive (Mulligan & Doherty, 2008). At calving a high producing lactating cow experiences an extremely high demand for nutrients that are used for maintenance, milk production and recommencing of the reproductive cycle. As a result the cows body stores are utilised (Tamminga, 2006). A major concern is that the cow does not receive enough energy from her diet to support her transition into lactation. This can be seen by a general drop in feed intake (Huzzey *et al.*, 2005) which leads to the cow experiencing an overall negative energy balance (NEBAL). Negative energy balance is associated with increased concentrations of triglycerides in the liver which negatively affects fertility and can lead to a prolonged interval to first ovulation (Rukkwamsuk *et al.*, 1999). Lactating cows experiencing NEBAL are more prone to reduced fertility when circulating urea or ammonia concentrations are high, than lactating cows in positive energy balance or dry cows (Sinclair *et al.*, 2000). Diets high in crude protein (CP) are given to cows to sustain milk production and are commonly fed during early lactation (Elrod & Butler, 1993; Butler, 2005a). Cows in NEBAL also have lowered circulating progesterone concentrations (Leroy *et al.*, 2008a). Reduced progesterone concentrations could possibly be as a result of increased metabolic demands from high milk production with increased dietary protein concentrations and NEBAL (Butler, 2000).

Blood urea nitrogen is a major end product of the urea cycle in ruminants and is used to determine the efficiency of nitrogen metabolism with high BUN concentrations indicating inefficient use of nitrogen in the body (Carlsson & Pehrson, 1993). According to Butler *et al.*, (1996) pregnancy rates decrease at BUN concentrations of more than 19mg/dl. Reduced fertility also occurs when BUN concentrations are below 7mg/dl due to deficient protein (Carlsson & Pehrson, 1993). Balancing protein to energy may lead to decreased nitrogen (N) losses to the environment and increased production efficiency (Hojman *et al.*, 2004). Excess rumen degradable protein (RDP) in the diet is degraded to ammonia by the rumen microbes and diffuses into the portal blood transporting it to the liver where it is converted to urea (Tamminga, 2006). When excess ammonia reaches the liver, there is increasing pressure to detoxify ammonia fast enough, which may lead to toxicity and reduced feed intake by the animal (Sinclair *et al.*, 2014). Urea is water soluble and is distributed across the body into the saliva, rumen fluid, blood serum, milk, follicular fluid, uterine fluid, urine and faeces. The follicular fluid of oocytes collected from heifers fed high protein had high concentrations of urea and cleavage rates were lowered (Butler, 2005a). Ammonia interferes with the blastocysts process to complete meiosis by reducing its cleavage rates and therefore disrupting its development, the exact interference of ammonia however is still not yet determined (Sinclair *et al.*, 2000). According to Butler, (2005) either the development of oocytes in ovarian follicles or embryo development and transport through oviduct is negatively affected by high concentrations of BUN. By decreasing BUN and increasing digestible undegradable protein (DUP) fertility may be improved in dairy cows (Sinclair *et al.*, 2014).

Some studies make use of MUN as an indicator of BUN, and as a measurement is non-invasive and easily obtained (Guo *et al.*, 2004). Both MUN and BUN gives an estimation on the level of N loss after absorption of ammonia from the rumen (Oltner *et al.*, 1985). The concentrations of MUN is dependent on the

CP, RDP and rumen undegradable protein (RUP) concentrations in the diet but is not affected by the amino acid balance of the diet (Baker *et al.*, 1995). The level of MUN recommended for optimal production is 8-12mg/dl (Ishler, 2016). If the ratio between protein and energy in the diet is correct then MUN is less likely to be significantly affected by different concentrations of protein inclusion in the diet (Oltner & Wiktorsson, 1983). During lactation there is an increased demand for protein per unit of energy to supply milk production. As such the protein to energy ratio at maintenance will increase as milk production increases. The nutritional status of a cow cannot be determined by a sole MUN estimation. However by determining the average MUN level in a herd a good estimate can be made on whether feeding is adequate (Oltner *et al.*, 1985). According to Godden *et al.*, (2001) MUN has limited use as a tool to measure fertility due to many conflicted reports of the association between MUN and reproduction performance. However, MUN may be used as a tool to measure N efficiency, decrease production costs and measuring N excretion into the environment.

1.5 Objectives

1. Milk urea nitrogen (MUN) influences reproduction parameters of dairy cattle.

2. The effects of MUN on reproduction parameters differ between Holstein and Jersey dairy cattle.

3. Season has a significant influence on the effects of MUN on reproduction parameters.

Chapter 2: Literature Review

2.1 Introduction

This chapter provides an overview of previous research conducted on the effects of urea on dairy reproduction characteristics. In this chapter literature focussing on factors that affect reproduction will be discussed. The purpose of this review is to consolidate current knowledge on milk urea nitrogen (MUN) in dairy cows, as well as to identify the gaps in current research on the topic.

In the Milk Producers' Organisation, (2017) edition of Lactodata, released by milkSA, it is stated that global milk production showed reduced growth in 2016 due to poor producer prices, unfavourable environmental factors and limitations created to slow down production. However, in 2015 consumption of dairy products increased 0.6% from 2014 due to increased global population growth. It is estimated that the demand for dairy will increase up to 20 million tonnes per year of which 8 million tonnes is due to greater population growth and 12 million tonnes due to increased consumption per head. In South Africa the number of milk producers has decreased to 1593 in January 2017 from 3551 in January 2009. Most producers are found in the Western Cape followed by the Eastern Cape, KwaZulu-Natal, Free State and North West. Gauteng, Mpumalanga, Limpopo. The Northern Cape has the least number of dairy producers. Even though the number of milk producers has decreased, the amount of milk produced still showed an increased growth of 6.4% in 2015 from 2014.

Global warming has been well documented worldwide and its effects are as severe as it is vast. The dairy industry (globally and locally) is not excluded from this event. As such, it is important that we include this factor into research aimed at improving the efficiency of the dairy industry. It is expected that global warming will lead to the overall increase in temperatures and a drier landscape in South Africa. However, in the Eastern Cape and central parts of the country rainfall is expected to increase significantly (figure 2.1.1 and figure 2.1.2) (Williams *et al.*, 2016).

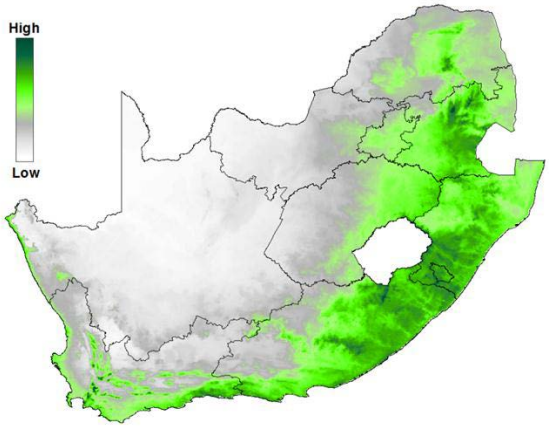

Figure 2.1.1 Map of the modelled suitability of geographical areas in South Africa for optimal milk production of Holstein dairy cattle on pasture (Williams *et al.*, 2016).

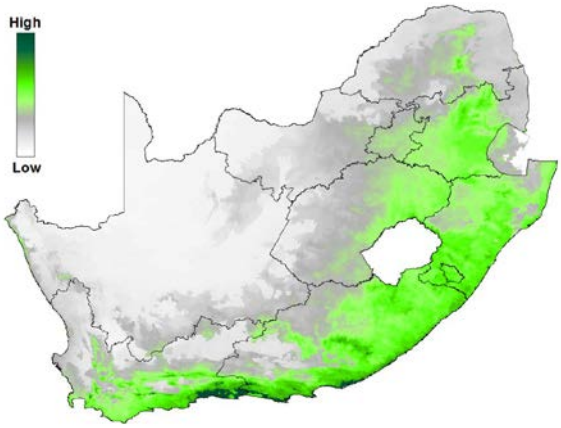

Figure 2.1.2 Map of modelled suitability of geographical areas in South Africa for optimal milk production with Holstein dairy cattle on pasture using projected climate-change data (2046-2065) (Williams *et al.*, 2016).

According to Kruger & Sekele, (2013) the highest annual average summer temperatures are recorded in parts of Limpopo, Gauteng, North West, Northern Cape and Western Cape. These regions also show the highest increases in temperatures over time, which include both maximum and minimum temperatures. This indicated that both summer and winter temperatures are steadily increasing in the Western and North Eastern parts of South Africa. The elevated temperature levels and drier climatic conditions will lead to increased intensity and length of heat stress experienced by dairy cows. This will affect reproduction, directly and indirectly, through nutritional and metabolic effects such as a drop in feed intake (De Rensis *et al.*, 2017). Sustainable strategies to reduce heat stress in dairy cows could be very important in improving reproduction in dairy cows.

2.2 Heat stress and reproduction

According to Gwazdauskas *et al.*, (1975) there are five important climatic factors that affect reproduction in dairy cows which are: 1) the maximum temperature the day after the cow was inseminated; 2) the level of rain on the day of inseminating the cow; 3) the lowest temperature on the day the cow is inseminated; 4) solar radiation on the day of inseminating the cow, and 5) the lowest temperature the day after the cow was inseminated. Heat stress is the condition in which external factors causes the body's temperature to be significantly increased to more than what it is when the animal is at rest. The main factor concerning heat stress in dairy cattle is the negative effect it has on reproduction performance, fertility and physiology as well as the extended effect on lactation (Lewis *et al.*, 1984; De Rensis *et al.*, 2015).

Heat stressed cows experience a less severe oestrous that lasts for shorter periods, than during lower environmental temperatures, which may lead to fewer cows being mounted by bulls in extensive systems (Goni

et al., 2015). Oestradiol is decreased in follicles 3 to 4 weeks after acute heat stress is experienced, which will then lead to less intensive and shorter periods of oestrus in cows (Thatcher *et al.*, 2010). Lowered luteal activity has been shown to occur during summer months which may be due to heat stress (De Rensis *et al.*, 2008). Reduced Luteinizing hormone (LH) secretion leads to a reduction in the size of the dominant follicle and may be due to the interactions between heat stress, milk production, lactation stage, energy balance and feed intake in the dairy cows (De Rensis & Scaramuzzi, 2003).

During summer when cows experience heat stress the secretion of Gonadotropin-releasing hormone (GnRH) is decreased which leads to repressed ovarian function (figure 2.2.1) (De Rensis *et al.*, 2017). It has been shown that 0°C to 16°C is the thermoneutral zone for bovine. Heat stress occurs at a relative humidity of 80% and when the temperature increases past 23.8°C (Du Preez *et al.*, 1991). Respiration rates of more than 60 breathes per minute as well as a rectal temperature of >39°C show that a cow is experiencing heat stress. At this level fertility and milk production could possibly be negatively affected (De Rensis *et al.*, 2017). A study by Cavestany *et al.*, (1985) showed rapidly decreasing conception rates at temperatures over 30°C. As temperatures increased above 35°C and reached a relative humidity between 65% to 70%, conception rates started nearing 0%. Most dairy cows that experience heat stress do not show oestrus, this makes it difficult to perform AI and leads to fewer pregnancies (Takahashi, 2012; De Rensis *et al.*, 2015).

It has been shown that the environmental temperatures on the day after AI may be more important to conception rates than the environmental temperature on the day of insemination (Gwazdauskas *et al.*, 1975). The number of zygotes that develop into blastocysts by day 8 after AI is higher during winter than summer (Al-Katanani *et al.*, 2002). According to De Rensis *et al.*, (2017) a decrease between 20% to 30% in conception and pregnancy rates, have been shown in various studies during summer. A reduction of 13% in pregnancy rates at 90 days after calving and the period of calving to fertilization increased by 13 days are also observed in a study by De Rensis *et al.*, (2002).

Heat stress experienced during pregnancy and the long-lasting effect thereafter on lactation may be due to a change in the endocrine status in the cow. These changes include reduced blood flow to the uterus, size of the corpus luteum after calving and ovarian volume as well as increased blood vessel mass and branching as well as production of Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (Lewis *et al.*, 1984). Cows experiencing heat stress also have higher concentrations of reactive oxygen species in their blood which can damage oocytes and embryos before implantation. By giving the cows antioxidants the oocytes and pre-implantation embryo may be protected (De Rensis *et al.*, 2017).

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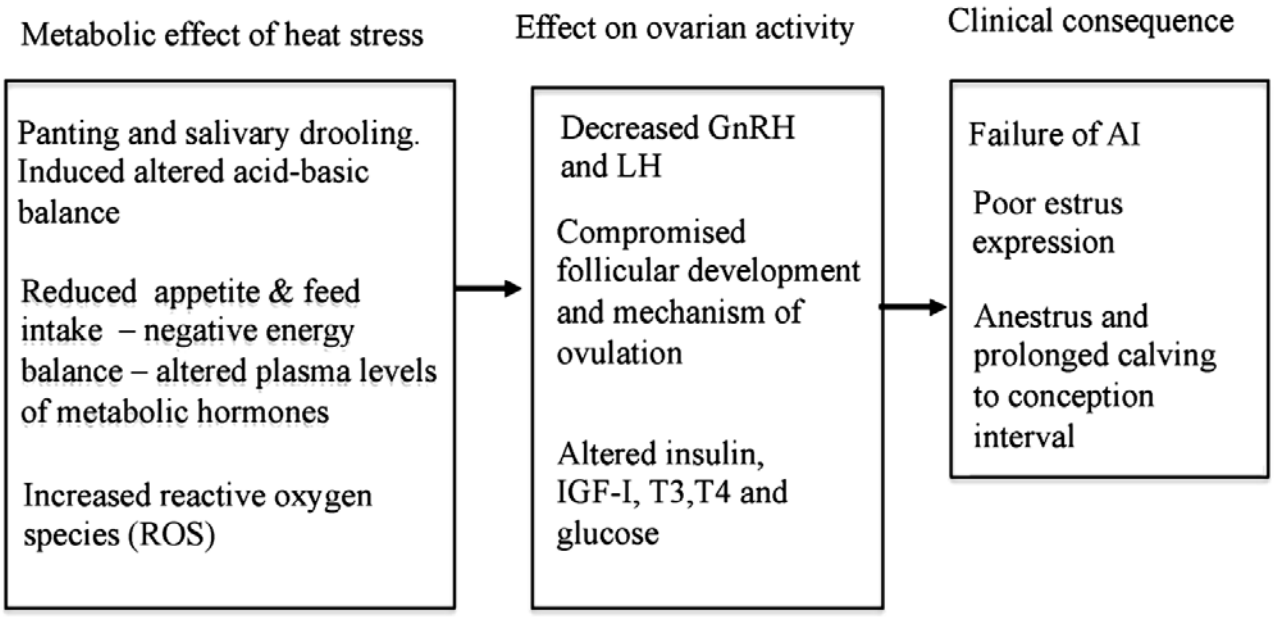

Figure 2.2.1 The main metabolic mechanisms that effect reproduction during periods of seasonal heat stress in dairy cows (De Rensis *et al.*, 2017).

The temperature humidity index (THI) is used to calculate heat stress in animals by making use of the wet bulb temperature and dry bulb temperature (Dash *et al.*, 2016). In this process a THI value past 70 indicate that cows are likely experiencing heat stress (Du Preez *et al.*, 1991). Cows are unable to maintain pregnancies due to increased body temperatures that leads to embryonic death (Thatcher *et al.*, 2010). Decreased fertility due to heat stress in the summer can carry over into autumn although dairy cows are no longer experiencing heat stress. Cows experiencing an increase in their THI during 21 to 30 days of pregnancy have an increased risk for abortion. The risk for abortion is 3.7 times more for cows expecting single calves and 5.4 times more for cows expecting twins up to day 90 of pregnancy (De Rensis *et al.*, 2017). An increase in pregnancy rates of 11% to 35% may occur if insemination takes place on a day with temperatures over 27°C, given that the temperature 3 days before to 3 days after insemination is lower than 27°C (Cavestany *et al.*, 1985)

Heat stress in dairy cows leads to a modified composition of hormones released as well as a decrease in follicular development. A hormone that may contribute to decreased fertility during summer, when cows experience heat stress is prolactin. Prolactin is a temperature sensitive hormone that increases in concentrations during summer. It may function in adaptation to heat by decreasing body heat through the evaporation of sweat. However, the mechanism by which this happens is not fully understood. Furthermore, prolactin also affects the growth of oocytes and can also lead to longer periods of anoestrous after calving. During summer the days are longer leading to a reduced release of melatonin which may also play a role in reduced reproduction in dairy cows (De Rensis *et al.*, 2017).

A decrease in inhibin leads to reduced dominance during follicular development. This could be responsible for changes in follicle stimulating hormone (FSH) secretion that escalates large follicle

development and therefore increases the incidence of twins in cows (Thatcher *et al.*, 2010). In a study conducted by Al-Katanani *et al.*, (2002) a group of cows were cooled in an effort to alleviate heat stress for 42 days before they were slaughtered and their oocytes collected. No improvement in oocyte quality was found compared to cows with no relieve from heat stress. The authors concluded that there are three possible reasons for this. Firstly, the method of cooling might have been insufficient to prevent heat stress from occurring. Secondly the oocyte quality might have already been reduced before the start of the cooling period. Or thirdly seasonal effects, other than heat stress, may be responsible for the decline in oocyte quality. The problem of heat stress leads to a snowball effect where fewer cows are successfully mated or inseminated. This leads to fewer births in winter and fewer cows that are ready for the next breeding season (Cavestany *et al.*, 1985).

As cows become adapted to summer temperatures, heat waves become a bigger concern not only at insemination but throughout pregnancy. It has been shown that cows exposed to extreme temperatures (≥38°C) for a short period of time (such as a day) might abort their calves (Fuquay, 1981). Several strategies to combat heat stress have been proposed and include breeding for adaptation to increased temperature as well as improving management and nutritional strategies (Williams *et al.*, 2016).

2.3 Factors that affect reproduction in dairy cattle

The reproductive success of high producing cows has been declining over the last 4 decades. This decline in reproduction has been shown to be associated with higher milk production (Dobson *et al.*, 2007). There are many factors affecting fertility such as, genetic factors, reproductive health management, calving environment, calf birth weight, sire selection, calf position during birth, retained placentas, nutritional imbalances, herd, year of calving, calving season, lactation number, days open, extended lactations, nutrient partitioning, heat detection, climatic conditions, general management practices, feeding routine, delayed first service after calving, number of services per conception, age of cow and breed (Muller *et al.*, 2014). Cows between lactation 1 and 4 shows lower pregnancy rates than heifers that have the best pregnancy rates. The lowest pregnancy rates are found in cows with a lactation number higher than 4 (Gwazdauskas *et al.*, 1975).

Genetically speaking fertility is considered as being moderately or lowly heritable. This is due to the success of pregnancy being affected by management practices and the environment, therefore making the genetic contribution difficult to estimate accurately (Perry, 2012). For reproduction to occur successfully there are certain phases that need to occur, with each phase being completed successfully leading to the birth of a calf. These phases start after birth and begin with involution of the uterus and the recommencement of the ovarian cycle by regression of the corpus luteum (Leroy *et al.*, 2008b; Ferguson & Skidmore, 2013). Oestrus finally occurs after the maturation of a normal follicle that will be ovulated and fertilized. After fertilization and the successful implantation of the embryo gestation takes place until the offspring is born. If any of these phases do not occur correctly reproduction failure will occur (Leroy *et al.*, 2008b) and the herd manager will have to wait for the next occurrence of oestrus in order to produce a calf. The time the herd manager waits

after calving before inseminating the cows is called the voluntary waiting period (VWP) and is usually a period of 30 to 50 days after calving. However a longer VWP has been shown to increase pregnancy rates (Ferguson & Skidmore, 2013). A decrease in fertility can be ascribed to higher rates of embryonic death during early gestation with lowered conception rates as well as to atypical oestrus cycles after calving (Leroy *et al.*, 2008b).

Another factor that influences fertility is the hormones circulating in the animals' body throughout the reproduction cycle. As the animal reaches puberty the negative feedback of oestradiol on GnRH decreases. This leads to pulsing LH concentrations that increases until it has reached the optimal level to induce first ovulation (Day & Anderson, 1998). Together with LH another hormone, namely FSH, is released which functions to stimulate the growth and development of the follicles in the ovary in preparation for ovulation of the dominant follicle (Butler, 2012). During summer FSH concentrations are amplified due to a decrease in the secretion of ovarian inhibin which changes the process of follicular dominance leading to more anovulatory follicles, ovarian cysts, double ovulations and giving birth to twins (De Rensis *et al.*, 2017). The frequency of LH pulses are important for the oestrus cycle to begin again after calving as well as for ovulation of the dominant follicle (Crowe, 2008). When the frequency of the LH pulse is insufficient, the dominant follicle fails to ovulate and is resorbed, therefore extending the period until first ovulation occurs after calving (Butler, 2012). Luteinizing hormone pulsing is repressed when a cow experiences heat stress which disrupts ovulation and prolongs the period between oestrus and ovulation leading to untimely inseminations (De Rensis *et al.*, 2017). Follicle stimulating hormone, LH, GnRH, oestrogen, progesterone and inhibin all work together to ensure that optimum follicle development occurs (Pryce *et al.*, 2004).

For an animal to reach puberty it also must be of the correct weight and size which differs among breeds. This means that leptin plays a small role in reproductive success as a lower limit in body condition is needed for puberty to occur (Perry, 2012). Differences in body condition, weight, body proportion and the amount of oestrus cycles a heifer had since the start of puberty have all been shown to have an effect on reproduction performance (Cavalieri *et al.*, 2005). Leptin is regulated by the current body condition of the animal and the availability of feed. It is produced in adipose tissue and plays a part in the hypothalamic-pituitary axis regulation (Williams *et al.*, 2002; Zieba *et al.*, 2005). The response of the hypothalamic-pituitary axis to leptin are decided by the metabolic state of the dairy cow (Barb & Kraeling, 2004). Leptin has been shown to be able to induce LH secretion in fasted cows that is mainly facilitated by GnRH acting on the pituitary gland to release LH (Williams *et al.*, 2002). The reproductive system also responds to Leptin as it has been shown that cow ovaries have a high sensitivity to Leptin and that embryos exposed to Leptin had a higher success rate of completing the blastocyst stage (Zieba *et al.*, 2005).

A new follicular wave can also be induced 1.6 days after injection of GnRH, as GnRH can cause a dominant follicle to ovulate earlier, but is influenced by the stage of oestrus (Perry, 2012). By inducing ovulation, reproduction performance of cows in anoestrous is increased. Submission rates to AI and timing of oestrus onset is also improved (Cavalieri *et al.*, 2005). In contrast Holness & Hurrell, (1977) stated that GnRH has been unsuccessful in inducing oestrus at a specific time as results have shown that conception rates after

treatment has been significantly low. Crowe, (2008) has shown that GnRH is only affective when a dominant follicle is present at the time of the first injection.

When a cow experiences heat stress a reduction in the concentrations of oestradiol in the blood occurs. This leads to follicular development being jeopardized, altered ovulation processes and the diminished quality of oocytes and embryos (De Rensis *et al.*, 2017). To force a new follicular wave oestradiol benzoate is injected. After which degeneration of the dominant follicle occurs between 4 to 5 days (Perry, 2012). Oestradiol benzoate is used to treat cows that have been synchronised with $PGF_{2\alpha}$, and have shown less variation in LH release time thereby increasing fertility (Holness & Hurrell, 1977). In a study by Cavalieri *et al.*,(2005) it was found that fertility is not significantly improved by the treatment of oestradiol benzoate and $PGF_{2\alpha}$ when compared to cows treated with $PGF_{2\alpha}$ alone. The study further found that the effect of oestradiol benzoate is greater in heifers, when first AI of heifers were to occur roughly 80 hours after the last $PGF_{2\alpha}$ treatment.

Prostaglandin $F_{2\alpha}$ decreases progesterone concentrations while increasing the concentrations of oestradiol (Perry, 2012) and is a relatively inexpensive method used to synchronize oestrous (Cavalieri *et al.*, 2005). Oxytocin binding to the endometrial oxytocin receptor stimulates the uterus to release $PGF_{2\alpha}$ (Perry, 2012). $PGF_{2\alpha}$ leads to the regression of the corpus luteum in a controlled manner and when given as a treatment at the right time induces a new follicular wave (Perry, 2012). On average oestrus occurs 48 to 96 hours after treatment with $PGF_{2\alpha}$ (Holness & Hurrell, 1977). Richardson *et al.*, (2002) found that heifers treated with $PGF_{2\alpha}$ and progesterone had higher conception rates than heifers that were only treated with $PGF_{2\alpha}$. First calving cows that have been treated with GnRH and $PGF_{2\alpha}$ and that have high body condition scores, have improved fertility during summer and autumn due to a better response to the GnRH treatment and consecutive follicular waves that were initiated (Friedman *et al.*, 2011).

Progesterone can help puberty to occur earlier and is therefore included in oestrous synchronization treatments by amplifying and changing the LH pulse frequency. Luteinizing hormone released from the pituitary gland is negatively affected by progesterone (Perry, 2012). A normal luteal phase and oestrus expression is associated with the level of progesterone in dairy cows (Crowe, 2008). Increased progesterone concentrations are related to improved conception rates which play an important role in reproduction efficiency (Aungier *et al.*, 2014). However, too high concentrations of progesterone compromises embryo survivability and can lead to reduced oestrus activity after embryo death (Richardson *et al.*, 2002). Embryo death can occur at the blastocyst stage that is directly after fertilization or later at the filamentous stage (Lucy, 2001).

There are other factors that affect reproduction in dairy cows that are not mentioned here. Discussing all factors that may influence reproduction parameters in dairy cows are beyond the scope of this study. The focus of this study is to investigate the effect of protein, NPN and their derivatives (specifically MUN) on reproduction in dairy cows,

2.4 Nutritional factors that influence reproduction in dairy cattle

Genetic selection for increased milk production directly lead to a higher need for nutrients from the diet and the body reserves, which makes health and reproduction disorders more extensive (Mulligan & Doherty, 2008). In contrast Leblanc, (2010) questions whether fertility has really declined with increasing milk production. In his study he claims that there has not been sufficient data presented to support this idea and also states that factors such as management choices have been neglected in the literature which could be a major contributing factor to longer conception rates than declining fertility.

Cows that are bred for increased fertility have greater body condition and milk yields during lactation compared to cows with decreased fertility (Cummins *et al.*, 2012). The high milk yield seen in dairy cows today are associated not only with increased genetic selection for higher milk production but also with diets that are specifically formulated for higher milk production to occur (Leroy *et al.*, 2008b). Energy balance before calving significantly affects metabolism, nutrient distribution as well as the reproductive axis postpartum (Kawashima *et al.*, 2016). At calving a high producing lactating cow experience an extremely high demand for nutrients that are used for maintenance, milk production and recommencing of the reproductive cycle. As a result body stores are utilised (Tamminga, 2006). This is due to nutrient prioritization in which early lactating animals prioritize milk production over fertility in order to ensure the survival of the new-born calf (Leroy *et al.*, 2008b). A major concern is that the cow does not receive enough energy from her diet to support her transition into lactation. This can be seen by a general drop in feed intake (Huzzey *et al.*, 2005) which leads to the cow experiencing an overall negative energy balance (NEBAL). Sever NEBAL is correlated with increased loss of body condition due to reduced feed (energy) intake. Cows that lose more than 0.5 of their body condition score (BCS) will have delayed first ovulation after calving, 30 to 40% of these cows may remain anovulatory. Cows that stay anovulatory after 50 days in milk (DIM) will be less likely to become pregnant, by 225 DIM non-pregnant cows are selected for culling when lactation finishes (Butler, 2012).

Hoedemaker *et al.*, (2004) suggests that insulin-like growth factor I (IGF-I), other IGFs and associated binding proteins are connected to the metabolic status of a cow as well as fertility due to their actions on the endocrine system. High concentrations of insulin in the first 2 weeks after calving are related to longer uterine involution intervals. NEBAL after calving leads to a decrease of IGF-I in the blood at a time when the uterus needs it to heal itself, as high concentrations of IGF-I is correlated with shorter time to uterine involution (Aungier *et al.*, 2014). During summer the level of insulin, IGF-I and glucose are significantly less than in winter (De Rensis *et al.*, 2017). Dairy cows experiencing NEBAL and lowered IGF-I concentrations also show lower rates of follicular growth, modified steroid manufacturing and less luteal activity (Hoedemaker *et al.*, 2004). This is because glucose and IGF-I are both used to stimulate follicular growth and implantation while glucose is also used as the main metabolic drive for the ovary and is also involved in the controlling of the LH pulsing in the hypothalamus (De Rensis *et al.*, 2017).

The nutritional status of the cow affects how many times a cow has to undergo AI before being confirmed pregnant. Poor nutritional status can lead to metabolic disorders, declining LH pulse frequency and less oestradiol production that could cause atresia of the dominant follicle instead of ovulation (Roche, 2006). After calving the normal increase in LH and FSH for resumption of ovarian cyclicity may be hindered when a cow experiences severe NEBAL which leads to the first ovulation to occur 3 or 4 weeks later (Butler, 2012). Using body stores of fat increases circulating non-esterified fatty acids (NEFA) and ketones like acetoacetate, acetone and β-hydroxybutyrate (BHBA) and is correlated with decreased circulating glucose, insulin and IGF-I concentrations (Tamminga, 2006). NEBAL is associated with increased concentrations of triglycerides in the liver which negatively affects fertility and can lead to a prolonged interval to first ovulation (Rukkwamsuk *et al.*, 1999). This increase in triglycerides in the liver is known as fatty liver which has been shown to deter metabolism in cows as well as slow down the immune reaction in the cow (Esposito *et al.*, 2014). A meta-analyses done by Rodney *et al.*, (2015) found that feeding fat during the transition period has a favourable effect on reproduction as well as milk yield. Cows in which oestrus are detected as well as cows at calving have been shown to have higher IGF-I concentrations in their blood compared to cows that experienced undetected heat or cows that fail to ovulate. Therefore follicular development that would lead to detectable oestrus and ovulation are correlated with immediate increased concentrations of IGF-I in the blood after calving (Obese *et al.*, 2011).

2.5 The influence of protein on reproduction

When protein requirements of the cow and rumen microbes are sufficiently met by the diet then protein feeding efficiency is maximized (Baker *et al.*, 1995). It has been shown that more than 70% of commercial dairy herds are inclined to overfeed protein (Jonker *et al.*, 2002a; Hristov *et al.*, 2018). High protein diets are given to dairy cows to increase their feed intake as well as milk production (Canfield *et al.*, 1990). Diets high in CP (17-19%) are recommended to sustain milk production and are commonly fed during early lactation (Elrod & Butler, 1993; NRC, 2001; Butler, 2005b) . However feeding cows diets with high CP content can lead to decreased feed intake, different amino acid configurations, therefore potentially leading to NEBAL (Westwood *et al.*, 1998). Protein supply is divided into RDP and RUP. It has been shown that high concentrations of RUP (where urea is produced in the liver by deamination of amino acids from RUP, body protein and microbial protein) is not as undermining to fertility as high concentrations of RDP is (Butler, 2005b; Tamminga, 2006). The reproduction of older cows (at fourth calving and older) are more significantly affected by CP in the diet than younger cows (Ferguson *et al.*, 1988).

In the rumen RDP is used by the rumen microbes to produce microbial protein with ammonia being produced as by-product (Butler, 2005b). Increasing concentrations of RDP have been shown to correlate with abnormal physiology of the ovaries and uterus which leads to embryo loss (Sinclair *et al.*, 2000). As RDP increases in the diet so does rumen production of ammonia, however this doesn't directly lead to this ammonia being utilized in the rumen (Kauffman & St-Pierre, 2001). The ammonia diffuses through the rumen wall into the portal circulation where it is transported to the liver and converted to urea (Butler, 2005b). It is unknown

exactly how a high protein diet is able to negatively affect fertility (Dawuda *et al.*, 2002). It is recommended to keep RDP concentrations at 10% and CP concentrations at 17% of dry matter (DM) in the diet for optimal production and fertility (Tamminga, 2006). A study by Canfield *et al.*, (1990) where cows were fed an isocaloric diet either containing 55.7% or 58.2% RDP to test the effect of feeding a high RDP diet to dairy cows found no significant effect, 14 days after calving or within the time of calving and first ovulation, between NEBAL and the diet containing high concentrations of RDP due to increased feed intake. A great supply of protein can potentially come from the microbial population in the rumen after digestion and absorption of the microbes in the small intestine because most of their cellular structures are made of protein (Tamminga, 2006). Diets low in RDP are able to spare energy for milk synthesis instead of using it for ammonia detoxification (Sinclair *et al.*, 2014).

The events that occur in order for conception to be successful may be interfered with by protein metabolism and lead to poor reproductive efficiency (Butler, 2005b). Reduced fertility can be due to LH pulse frequency, needed for ovarian follicle stimulation, being slowed down by high concentrations of NEFA and ketones as well as lowered blood glucose and insulin concentrations (Butler, 2005a). A high NEFA concentrations compromises liver function, thereby lowering the conversion rate of ammonia to urea leading to ammonia build-up in body fluids as well as follicular fluid (Tamminga, 2006). The intrafollicular condition should be balanced in order for oocyte growth and quality to be optimal (Takahashi, 2012). In a study by (Kurykin *et al.*, 2011) it was found that in 55.2% of repeat breeder cows fed high protein diets late postpartum had produced oocytes of poor quality. This was associated with high concentrations of lactate dehydrogenase (LDH) which is thought to increase when liver function is compromised, such as when cows experience NEBAL. The effect of NEBAL on fertility is worsened by excess RDP which delays first expression of ovulation and decreases conception rates at first AI thereby increasing calving interval, this is thought to be due to the increase in energy needed to convert the excess ammonia to urea (Tamminga, 2006). Lowered plasma progesterone concentrations due to negative effects of NEBAL increase the uterus sensitivity to urea (Butler, 2005b). In order to increase N efficiency through N recycling low protein diets are given to animals (Sinclair *et al.*, 2014).

A study by Barton *et al.*, (1996) found that cows fed diets with higher concentrations of CP (20%) were two times more likely to conceive in their next cycle and had a shorter interval of days open (DO) than cows fed a diet with low CP concentrations (13%). Diets with high CP responded negatively only when the health status of the cows was less than optimal and DO was increased, however the authors state that high CP diets may lead to a reduction in reproductive efficiency as cows may experience suppression in their immunity. Another study by Garcia-Bojalil *et al.*, (1998) on the effects of RDP and Calcium Salts of Long-Chain fatty acids in the diet of lactating dairy on reproduction found that high inclusion rates of RDP lead to the ovaries of early lactating cows having less luteal tissue. They describe this loss as being caused by the ovary that is opposite the foregoing expectant uterine horn being less active. By adding Calcium Salts of Long-Chain fatty acids the negative effects of high RDP inclusion were relatively mediated, showing that energy has some importance in preventing these effects.

The different stages in lactation have different nutrient requirements according to physiological status, milk yield and appetite which means that the level of protein included in the diet can potentially be adjusted according to production needs and optimal N efficiency (Sinclair *et al.*, 2014). Reproduction is improved if the CP, RDP and energy in the diet is properly balanced (Ferguson *et al.*, 1988). If amino acids are not properly balanced it leads to decreased milk production as well as milk protein production (Bahrami-yekdangi *et al.*, 2016). Methionine and Lysine are normally considered the first two limiting amino acids in terms of milk production and growth (Sinclair *et al.*, 2014). In a study by (Bahrami-yekdangi *et al.*, 2016) cows fed diets with low RDP had similar concentrations of milk production compared to cows fed diets with high RDP. The authors further concluded that there was also no significant difference found in milk composition between the two groups, therefore suggesting an RDP level of 9.3% DM in the diet to adequately support milk yield and milk composition.

In lactating dairy cows high concentrations of protein included in the diet may reduce concentrations of circulating progesterone but may not affect the concentrations of circulating progesterone in dry cows (Butler, 1998). Lower concentrations of circulating progesterone decreases fertility due to high protein concentrations impeding the activity of progesterone in the uterus and inhibiting embryo growth and development (Butler, 1999). Cows fed high concentrations of urea showed lowered oestrogen concentrations in the blood and decreased fertility (Erb *et al.*, 1976).

2.6 The influence of energy on reproduction

The most limiting factor to support high to moderate milk production of cows grazing pasture is metaboliable energy (ME) which is why non-structural carbohydrate (NSC) supplementation is needed (Brito *et al.*, 2017). If the energy demand of the rumen microbes are not met they struggle to efficiently synthesize microbial protein, this leads to the production of ammonia and therefore increases the conversion of ammonia to urea by the liver. High starch diets increases utilization of ammonia for microbialsyntesis and therefore decreases BUN (Useni *et al.*, 2018). Forage is usually high in RDP but lacking in NSC's that leads to unbalanced FME and ammonia supply to the rumen. During the day the fixation of carbon is at a greater rate than the rate of exporting of carbon out of cells and tissues, leading to NSC concentrations building up in grass and legume species (Antaya *et al.*, 2015). It was shown that supplementing NSC decreased concentrations of BUN and MUN but also did not indicate any negative effect on milk yield and milk composition (Brito *et al.*, 2017). When starch availability is increased in the rumen, the ruminal volatile fatty acide profile, percentage milk fat and rumen pH is changed (NRC, 2001). By increasing the rate of NSC digestion milk production is increased as well (Ferland *et al.*, 2018). It is important to balance RDP to fermentable metabolizable energy (FME) to increase the efficiency of microbial protein synthesis (by increasing the utilization of nutrients supplied) (Rezaii *et al.*, 2007). A study by Cabrita *et al.*, (2014) where cows were fed a total mixed ration (TMR) containing 40% corn silage, 5% coarsely chopped ryaegrass hay and 55% concentrate on a DM basis where the effective RDP to FME ratio varied between 6.7, 10.1 and 11.2 found that as the ratio of effective RDP to

FME increased so did dietary CP concentration. It was found that a ratio of 9.7 effective RDP to FME may lead to reduced DM intake and increased milk production (Rezaii *et al.*, 2007).

2.7 The distribution of urea through the body

Ammonia is produced from various sources such as amino acids and nucleic acids that are found in protein. It can also be produced from the metabolism, like in the kidneys from glutamine and as a by-product of bacterial digestive processes in the rumen and hindgut (Visek, 1984). High concentrations of ammonia that are produced in the rumen from RDP is detoxified and converted to urea in the liver (Miglior *et al.*, 2006). The main site of ammonia absorption in the rumen is via the ruminal and omasal epithelium where it enters the portal system to be transported to the liver (Davidovich *et al.*, 1977). In the rumen high concentrations of ammonia diffuses freely into the bloodstream, however ammonia may diffuse back into the digestive tract at the small intestine (Parker *et al.*, 1995). Ammonia is highly toxic to body tissues (except the liver), especially to the brain and is therefore converted to urea, which is a less toxic compound which stores N until it can be excreted. The concentrations of urea that is excreted is directly related to the level of urea in the milk and blood of a cow (Kohn *et al.*, 1997; Walker, 2009). Urea can also be produced through the deamination of amino acids in the liver of an animal (Miglior *et al.*, 2006; Leroy *et al.*, 2008a). This process is known as the urea cycle, also called the ornithine cycle, and uses five reactions to drive the cycle with each reaction having its own enzymes. These enzymes are carbamyl phosphate synthetase I (CPS-I) and ornithine carbamyltransferase which occur in the mitochondria. As well as argininosuccinate synthetase, argininosuccinate lyase and arginase which is located in the cytoplasm of the hepatocytes (Visek, 1984; Obitsu *et al.*, 2011). The production of urea requires the incorporation of two N atoms, one that originates from ammonia (via carbamyl phosphate synthesis) and the other from aspartate (via arginosuccinate synthesis) (Reynolds, 1992; Parker *et al.*, 1995). One of the aims of the urea cycle is therefore to detoxify ammonia to urea for excretion of N (Walker, 2009).

Urea that is produced by the liver, is excreted in the urine, with 40-60% into the intestinal lumen as exocrine secretions (e.g. saliva) or through diffusion of urea from the blood to the digestive tract epithelium (Reynolds, 1992; Obitsu *et al.*, 2011). The rate at which urea is dissipated from the blood is directly dependent on the permeability of urea as well as the blood flow rate (Obitsu *et al.*, 2011). Urea that enters the digestive tract can be recycled, through urease, to form ammonia which can be reabsorbed into the blood or be used by microbes for amino acid synthesis (Reynolds, 1992). Recycled urea N undergo hydrolysis by bacteria in the rumen after which it enters one of three pathways: 1) used for building protein in the body tissues via conversion to amino acids; 2) excreted in the faeces of the animal and; 3) it may also re-enter the urea cycle through the liver. (Obitsu *et al.*, 2011). Nonprotein N (NPN) are therefore constantly being cycled through the digestive tract and liver (Reynolds, 1992).When the amount of ammonia that enters the urea cycle is too high for the cycle to properly detoxify all of the ammonia after the first step in the cycle. The pyrimidine pathway is heightened to detoxify the excess ammonia to orotic acid for excretion via the urine. This can be measured to determine the level of ammonia in the animal (Visek, 1984).

2.8 Urea toxicity in dairy cows

Non-protein nitrogen that is included in the diets of dairy cows get broken down to ammonia or ammonium in the rumen. Under certain conditions excess ammonia can lead to acute ammonia toxicity, also known as urea toxicity (Hale & King, 1955; Visek, 1968). These conditions include 1) feeding NPN at concentrations suited for healthy adapted animals, to unhealthy or unaccustomed animals; 2) including palatable NPN sources at *ad lib* in the diet of animals, by allowing them free choice; 3) not ensuring that the ration is mixed thoroughly before giving it to the animals; 4) including high concentrations of NPN in a diet containing high concentrations of roughage and low concentrations of energy and protein and; 5) an animal that feeds aggressively may be predisposed to urea toxicity (Edjtehadi *et al.*, 1978; Kopcha, 1987). The pH of the rumen plays an important role in urea toxicity. A pH level that rises to 7.3 have been shown to induce toxicity in sheep. It was also shown that low concentrations of volatile fatty acids may make the rumen more sensitive to ammonia concentrations and therefore may result in an increased rumen pH (Coombe *et al.*, 1960). The rumen pH also determines how fast ammonia diffuses across the rumen wall into the portal system (Patra, 2015). When ammonia concentrations are high it may overstrain the livers ability to convert all the ammonia to urea, therefore leading to increased concentrations of ammonia in the blood and spinal fluid (Kopcha, 1987). Ammonia may diffuse into the brain from the blood as well as the spinal fluid and may also be produced in the brain (Singer, 2007).

At concentrations greater than 0.7mM ammonia in the blood may cause a disruption of the metabolism in the brain leading to tetany and death, since ammonia is highly toxic in tissues other than the liver (Kopcha, 1987; Parker *et al.*, 1995). The concentrations of ammonia in the brain is 1.5 to 3 times greater than that of blood under normal conditions (Walker, 2009). Ammonia travels into the brain cells via active transport across the blood-brain barrier into the astrocytes of the central nervous system therefore causing them to swell (Braissant *et al.*, 2013; Dasarathy *et al.*, 2017). Astrocytes have the ability to use ammonia to synthesize glutamine in order to maintain concentrations of ammonia at low concentrations to prevent toxicity (Singer, 2007). High concentrations of ammonia may change the blood-brain barrier permeability as a consequence of the of excess production of nitrous oxide stimulated by free radicals from ammonia (Dasarathy *et al.*, 2017). Once inside the cell ammonia alters the intracellular pH thereby interfering negatively with metabolism, ion transport and cell function (Dasarathy *et al.*, 2017). Cerebral oedema start to develop due to inadequate osmoregulation, which affects the whole brain leading to increased brain water content, inter cranial pressure, the swelling of astrocytes and herniation of the brain (Pratt, 1959; Singer, 2007; Walker, 2009; Braissant *et al.*, 2013). Astrocytes are particularly susceptible to ammonia toxicity as they are the only kind of brain cells capable of clearing ammonia and are therefore four times more likely to absorb ammonia than any other kind of brain cell (Dasarathy *et al.*, 2017).

Clinical signs of ammonia toxicity usually presents 10 to 60 minutes after the consumption of NPN feed (Kopcha, 1987). Distribution of ammonia across the body tissues and cells are influenced by pH and other factors, leading to concentrations of ammonia in some tissues being 10 to 50 times higher than in the blood

(Visek, 1984). Urea toxicity is indicated by irregular neurological conduct. This is thought to be caused by the build-up of α-ketoglutarate (a by-product of the Krebs cycle) in the spinal fluid as seen in human patients that have been diagnosed with hepatic encephalopathy. In horses it has been shown that concentrations of α-ketoglutarate declines within 1 to 2 hours after a toxic amount of urea is provided after which concentrations may increase slowly over time. This declining level of α-ketoglutarate may disrupt energy metabolism and ATP creation especially in the brain. (McKhann & Tower, 1961; Visek, 1984). Excess concentrations of ammonia in the brain may also lead to decreased utilisation of oxygen as seen in brain slices of cats (McKhann & Tower, 1961). Brain slices, specifically of the hippocampus, in rats exposed to high concentrations of ammonia before birth show decreased ability in memory and learning (Braissant *et al.*, 2013).

Elevated concentrations of ammonia in the blood may lead to lowered glucose tolerance, hyperglycaemia and reduced insulin concentrations in the blood (Visek, 1984). Another consequence of excess ammonia seen in human patients (due to cirrhosis) is declining protein synthesis as well as an increased rate of muscle resorption leading to lowered muscle mass and overall increased muscle weakness (Dasarathy *et al.*, 2017). In adult humans liver failure leads to excessive ammonia concentrations this causes hepatic encephalopathy (a neuropsychiatric disorder) to develop, leading to a changed mental state and patients may become comatose (Braissant *et al.*, 2013). In rats exposed to toxic concentrations of ammonia, tissue death of the kidneys are present. The damage occurred in both the cortex and the tubules of the kidney (Dasarathy *et al.*, 2017). Conditions that ensure that the urine has a lowered pH, leads to ammonia being trapped and therefore excreted, however when the pH of the urine is increased ammonia is more likely to escape and will not be excreted (Visek, 1968). It has been shown that pregnant cows (which have recovered from urea toxicity) performance is not affected by the exposure to toxicity and neither is their calves future performance (Word *et al.*, 1969). Studies have shown that cows experience NEBAL quickly after birth which could potentially lead to the development of fatty liver (Esposito *et al.*, 2014). This compromises the liver and could predispose cows to urea toxicity at lower concentrations of NPN inclusion in the diet.

Signs that show toxicity includes nervousness, skin tremors and muscle spasms, extreme salivation, urinating and defecating often, short quick breathes, incoordination, front limbs become rigid, weakness, tetany and death (Bartley *et al.*, 1976). Treatment for ammonia toxicity include administering acetic acid to animals before severe tetany starts to develop, or by emptying the ruminoreticulum when tetany develops thereby clearing ammonia rapidly (Bartley *et al.*, 1976). In 1914 three types of uremia conditions were defined in human patients. Uremia is the collective name for symptoms occurring when urine is prevented from being excreted. Three types have been described which are 1) Asthenic uremia which presents with symptoms such as lethargy, exhaustion, apathy, bodily weakness, NPN is not retained, heart failure and death; 2) Convulsive or epileptiform uremia that is marked with seizures, lack of sensation, hypertension, urinary secretion and NPN is retained and; 3) Psychotic uremia of which symptoms include mental decay, delusions, deep unconsciousness, muscle tremors, NPN concentrations that is not necessarily increased and hardening of the arteries in the brain. A combination of symptoms from the conditions may also be seen (Leiter, 1921). In a study where, various ammonia compounds were tested for toxicity it was found that organic compounds are

less toxic than inorganic compounds. The authors speculated that this may be due to the easiness with which organic compounds could be converted to urea in the liver (Underhill & Kapsinow, 1922).

2.9 The effect of blood urea nitrogen (BUN) on dairy cows

Blood urea nitrogen, RDP and CP have been shown to have a positive relationship (Tamminga, 2006). BUN refers to the urea circulating in the serum (SUN) or plasma (PUN) fractions of the blood and peaks around 4 to 6 hours after a meal (Butler, 2005a). As the degradability and solubility of the CP in the diet increases so does the level of ammonia in the rumen leading to higher concentrations of BUN (Hammond, 1997). Surplus N from the diet is always removed from the cow's body via the same pathway through the liver, whether from dietary RDP or RUP, which leads to an increased concentrations of BUN (Roseler *et al.*, 1993; Tamminga, 2006). When ammonia reaches a level where the liver struggles to detoxify ammonia fast enough it can lead to less feed intake by the animal (Sinclair *et al.*, 2014). The concentrations of BUN is found to be higher in lactating cows when compared to dry cows (Hwang *et al.*, 2001). Lactating cows experiencing NEBAL are more prone to reduced fertility when circulating urea or ammonia concentrations are high, than lactating cows in positive energy balance or dry cows (Sinclair *et al.*, 2000). Primiparous animals have lower milk production and should not experience NEBAL in the same severity as multiparous cows, thus higher concentrations of CP or BUN are not as troubling for these cows (Canfield *et al.*, 1990). Cows in NEBAL also have lowered circulating progesterone concentrations (Leroy *et al.*, 2008b). Reduced progesterone concentrations could possibly be as a result of the increased metabolic demands from high milk production with increased dietary protein concentrations and NEBAL (Butler, 2000).

Urea is a water soluble molecule and is distributed across the body into the saliva, rumen fluid, milk, blood serum, follicular fluid, uterine fluid, urine and faeces (Kauffman & St-Pierre, 2001; Butler, 2005b). According to Laven & Drew, (1999) it has been shown that dietary protein affects fertility in cows, however the mechanism that drivs this effect has not been successfully understood. The paper suggests that urea and ammonia are the likely causes that negatively affects fertility. Ammonia and urea both affect fertility at pre-ovulatory and the early stages of embryo development (Tamminga, 2006). In cows with high concentrations of BUN, glutamine serves as a carrier of ammonia therefore when the uterus uses glutamine it becomes a source of increased ammonium in the uterine fluid (Hammon *et al.*, 2005). The follicular fluid of oocytes collected from heifers who had been fed a high protein diet had high concentrations of urea and their cleavage rates were lowered (Butler, 2005b). Urea disrupts uterine pH and compromises the number of oocytes that develop due to ammonias' effect on cleavage rates and blastocyst formation as well as reduces the survivability of spermatozoa in the uterus (Westwood *et al.*, 1998; Tamminga, 2006). Oocytes that are exposed to high concentrations of urea are less likely to produce embryos that develop to the blastocyst stage due abnormal meiosis in oocytes (Kurykin *et al.*, 2011). This effect is most significant when the oocytes are of medium sized follicles (4-8 mm), this is because the ability of the oocytes to potentially develop to blastocysts occurs at this stage. This stage influences the blastocyst ability to perform *de novo* protein synthesis which is negatively affected by high concentrations of urea or ammonia. The affect occurs when meiosis activates from

metaphase II after fertilization has occurred. Ammonia interferes with the blastocysts process to complete meiosis by reducing its cleavage rates and therefore disrupting its development, the exact interference of ammonia however is still not yet determined (Sinclair *et al.*, 2000). The development of the embryo is also negatively affected by ammonia which converts α-ketoglutarate to glutamate. Thereby lowering its concentrations and flow through the Krebs cycle which decreases ATP concentrations in the embryonic cells (Hammon *et al.*, 2005).

According to Butler, (2005a) either the development of oocytes in ovarian follicles or embryo development and transport through the oviduct is negatively affected by high concentrations of BUN. No adverse effects were noted by (Dawuda *et al.*, 2002) on the pre-ovulatory follicle when they fed super ovulated dairy cows quickly degradable urea N for long periods of time. These animals were able to adapt to these toxic conditions due to the rumen microbes using more of the synthesised ammonia to produce methane, thereby reducing concentrations of ammonia. The urea in the blood or their liver function may have increased to metabolise the extra ammonia faster. The time at which high urea concentrations are fed was shown to have a significant impact on the toxic effect experienced by the embryo. By decreasing BUN and increasing DUP fertility may be improved in dairy cows (Sinclair *et al.*, 2014). According to Hammond, (1997) less degradable protein or DUP can be more efficiently used than non-protein N sources such as urea, which leads to decreased concentrations of BUN as well as better average daily gains (ADG).

High BUN concentrations prevents LH from attaching to ovarian receptors (Canfield *et al.*, 1990). During the luteal phase the uterine pH is altered by high protein diets leading to reduced fertility as uterine pH and BUN are negatively correlated (Butler, 2003). In cows with high concentrations of BUN the pH of the uterine fluid becomes lower than the pH of the blood which may cause ammonium to build up in the uterine fluid. This creates an ammonia gradient which traps ammonium in the uterine fluid. The concentrations of ammonium relative to ammonia increases thereby decreasing the pH of the uterus further (Hammon *et al.*, 2005). Bovine endometrial cell culture studies have demonstrated that urea does not only change uterine pH but also caused higher secretion of $PGF_{2\alpha}$ that is detrimental to embryo development (Butler, 1998, 2000). Furthermore ion flux across the endometrium is decreased, where phosphorous, magnesium and potassium concentrations declined and zinc increased during uterine flushing's in cows who were fed diets with high CP concentrations (Westwood *et al.*, 1998). The suboptimal uterine environment that is found leads to pregnancy failure and embryo implantation (Lucy, 2001). Embryo development is impeded when the conditions in the uterus is less than optimum due to the alteration of the uterine pH. This is because the normal effect of progesterone on the uterus is obstructed by high BUN concentrations (Butler, 2000). Specific proteins for growth and development are secreted by the endometrium through the occurrence of a sustainable embryo in the uterus. Therefore, any interference in the metabolic rate of protein could lead to the death of the embryo (Senosy *et al.*, 2012). Ammonia and urea both affect fertility at pre-ovulatory and the early stages of embryo development (Tamminga, 2006). It was found that embryo survivability was determined by the state of the donor cow more so than the condition of the recipient cow in embryo transfers. This implies that the ovarian and follicular

environment contributes more to the development of the embryo ensuring its longevity than the uterine environment (Leroy *et al.*, 2008a).

Blood urea nitrogen is a major end product of the urea cycle in ruminants and is used to determine the efficiency of N metabolism, with high BUN concentrations indicating inefficient use of N in the body (Carlsson & Pehrson, 1993). According to Butler *et al.*, (1996) reduced fertility occurs at BUN concentrations of more than 19mg/dl. Cows with BUN concentrations of over 20mg/dl are 3 times less likely to fall pregnant than cows with BUN concentrations of ≤20mg/dl. While a diet containing >20mg/dl BUN indicates excessive inclusion of protein in the diet and the diet should therefore be re-evaluated (Ferguson *et al.*, 1988). Hammond, (1997) states that for high producing dairy cows a urea level of <15mg/dl indicates a possible deficiency of protein in the diet. Reduced fertility also occurs when BUN concentrations are below 7mg/dl due to deficient proteins (Carlsson & Pehrson, 1993). A study by Senosy *et al.*, (2012) which aimed at finding factors after calving that lead to embryonic death in high-producing dairy cows, found that pregnant cows with low BUN concentrations had higher rates of embryonic death at week seven.

Energy concentrations interact with BUN and therefore plays an important role in determining whether cows become pregnant (Carlsson & Pehrson, 1993). Balancing protein to energy may lead to decreased N losses to the environment and increased production efficiency (Hojman *et al.*, 2004). A study by Elrod & Butler, (1993) showed that heifers who were fed a diet with high RDP concentrations as well as had a 70% recommended daily metabolizable energy (ME), had a first-service conception rate of 61% compared to heifers with a first-service conception rate of 82% who were fed normal concentrations of RDP. The lowered pregnancy rates were also shown to be correlated with elevated BUN concentrations. By holding protein intake constant with increasing energy included in the diet, BUN concentrations are likely to be lowered (Hammond, 1997). This is also shown in Hwang *et al.*, (2001) who found a negative correlation between BUN and energy where the BUN concentrations decreased as energy increased in diets containing similar concentrations of protein.

2.10 The effect of milk urea nitrogen (MUN) on dairy cows

As BUN cannot reliably and routinely be measured some studies make use of MUN as an indicator of BUN, and it is a measurement that is non-invasive and easily obtained. BUN and MUN is significantly correlated (r = 0.88) (Roseler *et al.*, 1993; Guo *et al.*, 2004; Nousiainen *et al.*, 2004). Both MUN and BUN gives an estimation on the level of N loss after absorption of ammonia from the rumen (Oltner *et al.*, 1985). The level of MUN and BUN are used to indicate the protein to energy ratio of the diet in healthy cows (Hammond, 1997; Hwang *et al.*, 2001). MUN can be used to determine whether a diet contains too much or too little protein (Baset *et al.*, 2010). Outflow rumen protein can be shown by aggregate samples of MUN (Hof *et al.*, 1997). The mammary gland is able to synthesize small amounts of urea, however the major contributor to MUN concentrations is the BUN concentrations (Gustafsson & Palmquist, 1993). As urea can easily diffuse from the blood into the milk of the cow, the concentrations of BUN directly affects the MUN concentrations (Kauffman

& St-Pierre, 2001). Since MUN and BUN show diurnal variation it can be interpreted incorrectly and lead to inaccurate protein inclusion in the diet (Gustafsson & Palmquist, 1993). Nitrogen not used for growth or milk protein synthesis is shown in the MUN value (Baset *et al.*, 2010). The level of MUN recommended for optimal production is 8-12mg/dl (Ishler, 2016). Oltner & Wiktorsson, (1983a) have shown that concentrations of MUN less than 14mg/dl could indicate a possible deficiency of CP relative to energy in the diet.

As the protein to energy ratio increases, so does MUN (Hof *et al.*, 1997). Non-protein N accounts for approximately 5% of milk protein, where urea makes up nearly half of this figure (Geerts *et al.*, 2004). The concentrations of MUN is dependent on the CP, RDP and RUP as well as the protein quality in the diet but is not affected by the amino acid balance of the diet (Baker *et al.*, 1995; Nousiainen *et al.*, 2004). It has been shown that the relationship between MUN and CP in the diet is similar to that of the ratio between CP and ME. Furthermore various studies have indicated that the energy balance are mixed up with CP concentrations in the diet (Nousiainen *et al.*, 2004). According to Geerts *et al.*, (2004) a possibility of error can occur when MUN is used as an index of energy and protein balance due to daily fluctuations of MUN concentrations in dairy cows. This can be avoided by collecting a 24 hour blended sample to use for determining MUN concentrations (Broderick & Clayton, 1997).

The conception rate of first service dairy cows are negatively associated with milk production and MUN (Guo *et al.*, 2004). A study done by Rajala-Schultz *et al.*, (2001) on 24 Holstein herds in Ohio (> 1200 cows) found that MUN values of low producing herds was less than MUN values of high producing herds, with the low producing herds also having greater variation. The level of MUN is not only dependent on the CP% in the diet but the protein to fermentable carbohydrate balance as well. Cows with >15.4mg/dl MUN were found to be less likely to become pregnant than cows with <10mg/dl MUN. Hojman *et al.*, (2004) reported similar results where MUN concentrations of <11.75 mg/dl was correlated with higher pregnancy rates in cows. A study in Finland by Shingfield *et al.*, (1999) that used data from the milk recording scheme of Finland found no affect on fertility when increasing MUN past the recommended concentration range of 200-300 mg/l to 200-350mg/l. It is mentioned that MUN concentrations greater than 386mg/l have been previously shown to negatively affect reproduction. The authors concluded that although they found no significant effects on reproduction by this change, they cannot say how individual herds may be affected. It is also worth mentioning that the changes they suggest is still below the given threshold and could therefore explain why no significant negative effects were observed with regards to reproductive traits.

At the time of AI an increase in the level of MUN to very high concentrations is a contributing factor to higher risk pregnancy failures (Albaaj *et al.*, 2017). If the ratio between protein and energy in the diet is correct then MUN is less likely to be significantly affected by different concentrations of protein inclusion in the diet (Oltner & Wiktorsson, 1983). For rumen microbes requirement to be reached a level of 11.7mg/dl MUN needs to be met (Nousiainen *et al.*, 2004). Another study at Cornell University (Larson *et al.*, 1997) found that low progesterone concentrations in the blood of nonpregnant lactating cows and high MUN concentrations were correlated. Furthermore, cows with increased MUN and lactation number had lower odds of falling pregnant.

Pregnant and nonpregnant lactating cows with high progesterone concentrations had similar concentrations of MUN that was lower than MUN concentrations in nonpregnant lactating cows with a low progesterone concentrations. Cows with MUN values of >21mg/dl were more likely to be found amongst the nonpregnant lactating cows with low progesterone concentrations group.

During lactation there is an increased demand for protein per unit of energy to supply milk production as such the protein to energy ratio at maintenance will increase as milk production increases (Oltner *et al.*, 1985). Hojman *et al.*, (2004) also found that MUN and milk production is positively correlated. The level of milk production determines how much N is required from the diet, this leads to the conclusion that the concentrations of MUN is highly dependent on milk production (Jonker *et al.*, 1999). MUN had significantly low effects on the conception rate but affected days open significantly greater, which suggests that urea does not affect early oocyte development but rather blastocyst formation and cleavage (Guo *et al.*, 2004).

According to Godden *et al.*, (2001) MUN has limited use as a tool to measure fertility due to many conflicted reports of the association between MUN and reproduction performance. However, it may be used as a tool to measure N efficiency, decrease production costs and measuring N excretion into the environment. The authors concluded that cows need at least 10 days to adapt to excess urea in the diet if the cows are started on the diet before breeding time to avoid urea's toxic effect. If no adaptation period is provided the intake of the animals will be modified in an effort to try and minimize the concentrations of ammonia that is produced within the rumen (Sinclair *et al.*, 2000) and thereby MUN. The nutritional status of a cow cannot be determined by a sole MUN estimation, however by determining the average MUN level in a herd a good estimate can be made on whether feeding is adequate (Oltner *et al.*, 1985; Schepers & Meijer, 1998). The level of MUN is reduced when N in the diet is used efficiently and is thus reflected in the milk in the form of true protein as a major source of N (Baker *et al.*, 1995).

Urea is excreted in the urine via the kidneys, as it is produced in the liver and carried to the kidneys through the blood (Miglior *et al.*, 2006). The excretion of urea is relative to BUN. Urinary excretion of N (UN) increases with animal size and could potentially have an effect on the level of MUN, such as with size differences within breeds (Jonker *et al.*, 1999). As a small neutral molecule urea continuously diffuses into the mammary gland and back out into circulation. Thereby establishing an equilibrium between the mammary gland and the blood. This makes MUN a good indicator of the level of N excreted in the urine (Jonker *et al.*, 1998). By measuring MUN milk production correlated with environmental N emission can be examined (Nousiainen *et al.*, 2004). Research showed that MUN and UN are linearly correlated as UN (g/d) = 12.54 * MUN (mg/dl) (Jonker *et al.*, 1998). However, Kauffman & St-Pierre, (2001) reported that more UN was excreted per unit of MUN. They also found that as CP increased, the efficiency of excreting N into the milk decreased while urinary excretion increased. The efficiency of excreting N into the milk decreased from 52.3% in diets containing 13% CP to 42.7% in diets containing 17% CP. There were no breed differences found as breed differences were accounted for by adjusting for difference in body weight. MUN increases in concentrations by 0.42 mg/dl with every 50 kg increase in body weight (Jonker *et al.*, 1998).

According to Rodriguez *et al.*, (1997) MUN concentrations is affected by breed as Holstein cows have a greater concentrations of MUN (as mg/dl) than Jersey cows. Another study found that test-day MUN concentrations was higher in Jersey cows than Holstein cows, but this was dependent on whether the measurement was taken from a single-breed herd or multiple breed herds (Wattiaux *et al.*, 2005). However, Kauffman & St-Pierre, (2001) have shown that breed effects were not significant for MUN and BUN concentrations, but were significant on other milk components (Volume, milk protein and milk fat). The concentrations of MUN is also affected by parity (Miglior *et al.*, 2006). Jonker *et al.*, (1998) found a 0.45 mg/dl difference in average MUN concentrations with primiparous cows having higher MUN concentrations than multiparous cows.

It has been suggested that seasonal effects may influence the interaction between MUN and reproduction. Cows who mated in winter with low MUN concentrations were more likely to fall pregnant than cows who mated in the summer with high MUN concentrations. Increased MUN concentrations may, directly or indirectly (by its interdependency on heat stress and its negative effects), cause a reduction in reproductive potential in dairy cows. Heat stress adversely affects the normal biological processes of the cow, thereby increasing the amount of energy needed to convert ammonia to urea (Melendez *et al.*, 2000). The concentrations of MUN is affected by the month of the year, with lower concentrations (11.8 mg/dl) seen in winter and higher concentrations (18.1 mg/dl) seen in summer and spring (Hojman *et al.*, 2004). Other researchers also found that MUN concentrations significantly differed within season and that MUN was higher in summer (Ferguson *et al.*, 1997; Godden *et al.*, 2001; Wattiaux *et al.*, 2005; Miglior *et al.*, 2006; Fatehi *et al.*, 2012),

A study by Jonker *et al.*, (2002) was done to test whether knowing the MUN concentrations in a herd could help farmers improve their feeding management and reduce costs. By replacing soybean meal with maize grain, a 11.05 g/day decrease of N intake per cow occurred thereby decreasing MUN by 0.52 mg/dl. An average replacement of 52.7kg maize per year per cow would then have led to an average saving of $0.113/kg (R1.19/kg) and $5.95/cow per year (R62.6/cow per year). The authors concluded that MUN analysis would cost $60/year (R631.2/year) while an average farmer could save $595/year (R6259.4/year) in feed cost.

The time that the cows are fed and the time MUN is sampled significantly influences the value of MUN that is obtained. It has been shown that BUN peaks around 2 to 4 hours after feeding, and MUN peaks 1.5 to 2 hours after BUN (Manston *et al.*, 1981; Gustafsson & Palmquist, 1993). The literature has not shown the best time for taking samples as various researchers have taken samples at 2, 3 or 4 hours after feeding. MUN peaks in the afternoon due to the feeding time between morning and afternoon milking (Hwang *et al.*, 2001). Other factors that affect MUN have been shown by Kgole *et al.*, (2012) these include stage of lactation, number of times cows are milked per day, herd-test-day, calving year, milk protein, milk fat and milk volume. The concentrations of MUN follows the same pattern throughout lactation as milk production in the lactation curve. The concentrations of MUN have been shown to be lower 30 DIM compared to the rest of the lactation cycle, due to the drop of feed intake seen immediately after parturition. The peak concentrations of MUN occurs

around 4 to 6 months after calving. Whereas milk production peaks at around 3 months, and then decreases gradually (at a slower rate than that of milk production), until the end of lactation (Fatehi *et al.*, 2012). In contrast to this a study by Wattiaux *et al.*, (2005) found that MUN concentrations peaks in the first month after calving. This indicates that MUN concentrations may not reveal sufficiency of intake but rather the balance between protein and energy in the diet. In a study on Canadian dairy breeds it has been shown that the concentrations of MUN increases with calving number (Miglior *et al.*, 2006). A study was done in the northeastern United States to determine the cause of fluctuating MUN concentrations that was obseverded by the Dairy Herd Improvement Agency (DHIA) over a 10-year period. It was concluded that the main reason for the fluctuations was the fluctuating feed price, in years when the cost of feed was too high alfalfa haylage would be included at higher rates in the diets, which lead to increasing MUN concentrations (Hristov *et al.*, 2018). This study did not however look at the affect of high MUN concentrations on any production or reproduction parameters in dairy cows.

2.11 Feeding systems of dairy cows

In South Africa it is generally assumed that Holstein dairy cows are kept on a total mixed ration (TMR) feeding system, while Jersey cows are kept on a pasture with concentrate feeding system. A TMR is formulated in such a way that with every bite a properly balanced diet is ingested. When any additional feeds such as hay or feed supplement is also given, the ration is no longer a TMR as it not properly balanced anymore. There are many advantages to feeding a TMR such as 1. The cow doesn't have a choice about what to eat as the feed is thoroughly mixed. 2. Protein, energy and fibre are balanced in the feed and reach the rumen microbes at the same time, therefore microbial growth and protein synthesis are supported more efficiently inside the rumen. 3. Because grain and roughage are added together in the feed and grain is ingested in smaller portions throughout the day, the cow experience less build-up of acid and her risk of acidosis is decreased. There is also one big disadvantage to feeding a TMR which is that the diet can only be formulated to feed a group of cows and can not meet the requirements of individual cows (De Ondarza, 2000).

Pasture feeding of dairy cows is a popular feeding system despite having hurdles that need to be overcome. These include pasture intake and variability of nutrients as well as soil fertility and fertilisation practices that influence the vegetation composition of the pasture. Fertilization may also increase the CP fraction while decreasing the non-structural carbohydrates (NSC) of the pasture leading to increased BUN (and therefore MUN) concentrations and decreased energy levels in cows. These difficulties are overcome by increasing the number of lower producing cows per hectare and getting high milk volume per area. Another method is to stock high producing animals that are well supplemented and managed (Dugmore, 2017).

These two systems are high input systems where dairy cows have high avergage production values for milk, fat and protein (Abin *et al.*, 2018). A study by Ferland *et al.*, (2018) found that component fed cows (such as pasture and concentrate) had higher concentrations of MUN and lower yields of milk, protein, lactose and fat than cows fed a TMR.

2.12 Conclusion

From the studies discussed in this chapter it can be concluded that extreme MUN concentrations and unbalanced protein diets could possibly have a negative effect on reproduction in dairy cows. To investigate this further literature discussing the metabolism of NPN sources such as urea as well as urea toxicity was reviewed. Furthermore, this section has also indicated that there is a lack of literature focussing on MUN and reproduction of dairy cows in South Africa. This lack of published studies emphasize the importance of undertaking a study focussing on the effect of MUN on reproduction in dairy cows in South Africa, which this current study aims to investigate (De Ondarza, 2000).

Chapter 3: Materials and methods

3.1 Introduction

In this study data was collected from high and low producing dairy cows from 2006 to 2008 for similar herds as part of the milk recording scheme of the Agricultural Research Council (ARC) (ca. 12 000 records). The data used in this study was representative of at least one complete lactation cycle where milk composition was measured on a monthly basis. Feed rations were specifically formulated and supplied by Epol. Epol also did the monitoring as well as analysis of the feed (on a dry matter basis). Wet chemistry analysis of the study was done by the UP Nutrilab. The dataset consisted of a total of about 12000 observations. The factors investigated in this study were:

- Breed (Jersey or Holstein)
- Season (summer or winter)
- Year (2006-2008)

The reproduction parameters were:

- Calving number
- Intercalving period (ICP) [days]
- Reproduction performance (RP) [%; Calculated parameter, see below]
- Reproductive index (RI) [Calculated parameter, see below]

The nutritional parameters (on dry matter basis) were:

- Rumen degradable protein (RDP) [%]
- Crude protein (CP) [%]
  - Holstein: 15%, 16% & 17%
  - Jersey: 16%, 17% & 19%
- Metabolizable energy (ME) [MJ/kg]

The dairy production parameters were:

- Total milk [kg/d]
  - Low producer: < 23.7kg/d
  - High producer: > 23.7kg/d
- Butterfat percentage (Butterfat %) [%]
- Milk protein [%]

- Milk lactose [%]
- Somatic cell count [cells/ml]
- Milk urea nitrogen (MUN) [mg/dl]
- Days in milk (DIM)

3.2 Preparation of data

The data received was captured on Microsoft excel. It was then checked and cleaned as part of the preparation for statistical analyses, which included standard summary statistics, frequency distributions and the identification of missing values and outliers. Data was analysed by means of repeated measures analyses since the data consisted of repeated recordings on the same animals (animals with incomplete data were omitted).

Reproduction performance (%RP) was calculated in an attempt to describe the reproduction success of the dairy cows. It was calculated as follows:

%RP = [278 / ICP] x 100,
where 278 is considered as the average gestation period of a cow (Everett & Magee, 1965; Foote, 1981).

Another variable that was calculated is Reproductive index (RI) as described by Webb *et al.*, (2017). It was calculated as:

RI = 200 – [Age(D) / [966 + [417 x [Calving number – 1]]] x 100],
where Age(D) is the age at last calving in days.

As Age(D) was not included in the dataset it had to be calculated. This was done by using the average number of calving's (also referred to as calving number in the data set obtained from the milk recording scheme) of the cow and correlating it to breed and thereby calculating the average age of the cow in months (Age(M)) as shown in Mostert *et al.*, (2001). Age in months was converted to average age in days (Age(D)) which was used to calculate RI as outlined in table 3.2.1.

Table 3.2.1 Calculation of average cow age (in days) according to breed and calving number, used in calculation of RI.

Breed	Calving number	Age(M) (Mostert *et al.*, 2001)	Age(D)
Jersey	1	28	852
	2	41	1247
	3	54	1643
	4	82	2494
Holstein (Holstein)	1	29	882
	2	42	1278
	3	58	1764
	4	90	2738

The data set was further split according to breed, season and breed x season interaction in separate Microsoft Excel documents for a more detailed investigation. The data was organised in different spreadsheets and labelled as :

- All cows included in the study
- Holstein cows and Jersey cows (to investigate within breed differences).
- Summer and winter (to investigate within season differences).
- Holstein x summer, Jersey x summer, Holstein x winter and Jersey x winter (to investigate breed x season interactions).

3.3 Statistical analyses

The data collected was analysed by means of the statistical program Statistical Analysis Software (SAS® 9.4, 2014). The univariate procedure was conducted to test for normal distribution of data. All the parameters used in this study had normal distributions.

Summary statistics (mean ± standard deviation) were calculated for all the cows in the study, including for breed, season and breed x season interaction. Analyses of variance (ANOVA) were done for all cows in the study, as well as within breeds and within seasons by using the general linear model (GLM) based on repeated measures. ANOVA was used to determine whether if there were significant differences between means of parameters. Differences between treatments were tested by means of Bonferroni's multiple range test, because the data was unbalanced (e.g. not equal number of observations between breeds or seasons). Intercalving period was used as the class characteristic for all data analysis. This was done because ICP was the only measured reproduction characteristic, therefore the most accurate predictor of reproduction in this study. The Pearson product moment correlation coefficient (r^2) was used to describe the linear relationship between parameters and indicate the strength and direction of the relationship. The relationship between MUN and the reproduction parameters was tested by means of correlation procedures, which also addressed the hypotheses of the study. If there were significance correlations between these parameters, it meant that the

hypotheses were not rejected and that MUN influenced reproduction. This procedure was done to analyse the data from all cows in the study, as well as those within breeds and within seasons. Scatterplots were used to illustrate the relationships between ICP vs MUN and RP & RI vs MUN. Plots were only created where MUN was shown to significantly interact with the reproduction parameters.

The GLM procedure was done within breeds, within seasons and breed x season interactions. This procedure was used to determine whether there were any significant differences within the parameters of breed, season and breed x season interaction. The results were included with the summary statistics to indicate which parameters showed significant differences. The latter was followed by ANOVA procedures to investigate the main effects on all parameters measured. Predictions about the effect of MUN on reproductive parameters were tested by means of the stepwise regression procedure. The Stepwise selection is a process in which every variable chosen out of the dataset is evaluated to fit the selected level of significance in building a model. Consideration is also given to parameters already included in the model, which get re-evaluated with every new variable being considered. These evaluations are based on the F-value of the variable. The results are then presented in a table form that indicates which of the chosen parameters are significant in the criteria given in order from most to least significant in the model. The process stops once all the parameters have been considered. Often parameters that were included in the model are removed later, as a result of the addition of other parameters in the model. The inclusion of the new parameters does not allow the previously included parameters to fit, as such they are excluded from the model. Stepwise selection was done for differences within breed, season and breed x season interactions.

The results are presented, explained and discussed in the next chapter (Chapter 4 - Results and Discussion). All results were presented in three categories (reproduction, nutritional and production parameters). Results are considered significant at $p \leq 0.05$.

Chapter 4: Results and Discussion

4.1 Effects of Milk urea nitrogen (MUN) on reproduction parameters of dairy cows

The results presented in this study were calculated from data collected from 2006 and 2008 as part of the milk recording scheme (ARC). In this section these results are used to investigate the effect of milk urea nitrogen (MUN) on the reproduction parameters for all the cows included in this study. The data was analysed, and specific reproduction parameters were calculated to describe reproduction efficiency in dairy cattle in South Africa. These calculations were based on established formulas and principles previously recorded in literature, as outlined in the previous chapter (Chapter 3 – Materials and Methods). An example of SAS output is given in Addendum D.

Summary statistics (mean ± SD) for reproduction parameters of dairy cows are presented in table 4.1.1. The average number of calving's (also referred to as the calving number) of all the cows was 2.1 ± 0.85, which is similar to the national average productive herd life of 2.3 lactations reported by Scholtz & Grobler, (2009). On average the overall intercalving period (ICP) was 415.5 ± 90.02 days, which is higher than the average calving interval of 396 days reported by Makgahlela *et al.*, (2007) for South African Holstein cattle. In Japan the average calving interval was about 431 days in 2008 and the average calving number was 2.7 (Dochi *et al.*, 2010), which are both higher than the values obtained in the present study. In the Netherlands the average calving interval for first parity cows was ca. 404 days in 2001 (Nauta *et al.*, 2006), which is lower than what is seen in this present study.

Table 4.1.1 Summary statistics (means ± SD) of reproduction parameters of all cows included in the study from 2006 to 2008.

Reproduction Parameters	**All**
Calving number	2.1 ± 0.85
Intercalving period (ICP) [days]	415.5 ± 90.02
Reproduction performance (RP) [%]	69.5 ± 12.49
Reproductive index (RI)	108.5 ± 2.95

Table 4.1.2 shows the mean and standard deviations of the nutritional parameters for all cows included in this study. The average crude protein (CP) was 17.19 ± 1.17%. This was higher than the 160 ± 19.1 g/kg recorded in the study by Nousiainen *et al.*, (2004), who also reported an average metabolizable energy (ME) value of 11.3 ± 0.47 MJ/kg. In their study the average ME-value was similar to that recorded in in the present study, which was 11.0 ± 0.59 MJ/kg.

Table 4.1.2 Summary statistics (mean ± SD) of the nutritional parameters of diets fed to cows included in the study from 2006 to 2008.

Nutritional Parameters (DM Basis)	**Average for all cows**
Rumen degradable protein (RDP) [%]	55.56 ± 3.68
Crude protein (CP) [%]	17.19 ± 1.17
Metabolizable energy (ME) [MJ/kg]	10.95 ± 0.59

The mean and standard deviations of the milk production parameters for all cows in the study are presented in table 4.1.3. Butterfat % and milk protein were on average 4.5 ± 1.04% and 3.5 ± 0.77%, respectively. These values are similar to that reported by Oltner & Wiktorsson, (1983), namely butterfat % was 4.52% and milk protein was 3.18%. There is a fair assumption about somatic cell count and udder health which was recently confirmed by Petzer *et al.*, (2017a). The average somatic cell count for this present study was 674.6 ± 1395.43 cells/ml, which was lower than the threshold set by Petzer *et al.*, (2017b) of 150 000 cells/ml in composite milk samples. Values exceeding 150 000 cells/ml indicate possible intramammary infection or mastitis, so cows included in the present study were relatively unaffected by intramammary infections or mastitis. As the data showed less somatic cell count than this threshold it means that these cows had good udder health. The average MUN for all cows included in this dataset was 14.4 ± 5.65 mg/dl which was higher than the MUN in the study of Rajala-Schultz *et al.*, (2001), who had an average MUN value of 12.6 ± 4.0 mg/dl across all herds where the high producing animals averaged at 13.6 mg/dl and low producing animals averaging at 11.1 mg/dl. The average days in milk (DIM) was 201.0 ± 133.67. This value was higher than the average DIM found in studies conducted by Nousiainen *et al.*, (2004) where the average was 109 ± 28.5 and Jonker *et al.*, (1998) who had an average of 108 ± 42 DIM.

Table 4.1.3 Summary statistics (mean ± SD) of the production parameters of all cows in this dataset from 2006 to 2008.

Production Parameters	**All**
Total milk [kg/d]	23.7 ± 8.47
Butterfat %	4.5 ± 1.04
Milk protein [%]	3.5 ± 0.77
Milk lactose [%]	4.5 ± 0.84
Somatic cell count [cells/ml]	674.6 ± 1395.43
Milk urea nitrogen (MUN) [mg/dl]	14.4 ± 5.65
Days in milk (DIM)	201.0 ± 133.67

All parameters (reproduction, nutritional, and production) were tested against ICP as a sensitive estimate of reproduction efficiency. table 4.1.4 shows that all the reproduction parameters were significantly affected ICP. Included in this table is %RP, whith ICP as a part of the calculation. By comparing these two parameters it is possible to determine the sensitivity of their relationship, eg the proportion of change and not the number of days per se, which may provide a more diserning measure of reproduction efficiency from a statistical analysis point of view.

Table 4.1.4 Influence of selected reproduction parameters on Intercalving period (ICP) (ANOVA results) of all cows included in the study from 2006 to 2008.

Reproduction Parameters	**All**	
	F	**p-value**
Calving number	6.40	<0.0001
Reproduction performance (RP) [%]	2901.44	<0.0001
Reproductive index (RI)	11.52	<0.0001

All the nutritional parameters were significant (table 4.1.5). This indicated that they interacted with the ICP. The nutritional parameters need to be formulated carefully in the ration of the animals for optimum reproduction to occur.

Table 4.1.5 Influence of selected nutritional parameters on Intercalving period (ICP) (ANOVA results) of all cows included in the study from 2006 to 2008.

Nutritional Parameters	**All**	
(DM Basis)	**F**	**p-value**
Rumen degradable protein (RDP) [%]	4.68	<0.0001
Crude protein (CP) [%]	4.54	<0.0001
Metabolizable energy (ME) [MJ/kg]	5.26	<0.0001

The interaction between the ICP and the production parameters were significant (table 4.1.6). As the focus of this study MUN was shown to be highly significant ($p<0.0001$) and therefore may have a great interaction with ICP. However, the total milk, butterfat %, milk protein and somatic cell count also showed the same p-value (<0.0001) and this could be as important to reproduction as MUN.

Table 4.1.6 Influence of selected production parameters on Intercalving period (ICP) (ANOVA results) of all cows included in the study from 2006 to 2008.

Production Parameters	All	
	F	p-value
Total milk [kg/d]	4.96	<0.0001
Butterfat %	1.73	<0.0001
Milk protein [%]	1.83	<0.0001
Milk lactose [%]	1.18	0.0232
Somatic cell count [cells/ml]	1.87	<0.0001
Milk urea nitrogen (MUN) [mg/dl]	3.41	<0.0001
Days in milk (DIM)	4.73	0.0088

The Pearson product moment correlation coefficient (r^2) is used to describe the linear relationship between parameters and indicates the strength and direction of the relationship. Both the reproduction performance (RP) and reproductive index (RI) indicated a negative relationship with MUN (table 4.1.7 and figure 4.1.1). This implies that an increase in MUN is associated with a decrease in both the RP and the RI, reproduction will therefore decline. The variation in these two reproductive measurements were explained by MUN at 2% and 10% respectively. Table 4.1.7 shows that MUN was not the only parameter that contributed to the variation of these two parameters. Total milk contributed 5% of the variation in RP and ICP contributed 93%. Total milk also contributed to the variation of RI at 14%, while rumen degradable protein (RDP) contributed 49% of the variation and calving number contributed 40%.

Milk urea nitrogen contributed 2% to the variation in ICP (table 4.1.7 and figure 4.1.2). There was a positive relationship between MUN and ICP. This indicates that when MUN increases, the ICP increases as well, i.e. it will take longer for a cow to deliver a calf when it is exposed to high concentrations of MUN. This significance is important as ICP was the only measured parameter to indicate the level of reproduction. Therefore, MUN needs to be explored further. Other parameters such as total milk which explained 4% of the variation and calving number contributed 6% of the variation in ICP.

Table 4.1.7 Pearson product moment correlation coefficient (r^2) and the probability associated with it of all Parameters of all cows included in the study from 2006 to 2008.

Parameters		Milk protein	MUN	RDP	CP	ME	ICP	RP	RI
Days in milk (DIM)	r^2	0.04	0.01	0.008	-0.10	-0.08	0.001	-0.002	-0.03
	p-value	<0.0001	<0.0001	0.0068	<0.0001	<0.0001	0.0152	0.0052	<0.0001
Total milk [kg/d]	r^2	-0.008	0.03	0.22	0.02	0.07	0.04	-0.05	-0.14
	p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Somatic cell count [cells/ml]	r^2	0.04	0.00002	-0.002	-0.008	-0.03	0.001	-0.002	-0.005
	p-value	<0.0001	0.6638	<0.0001	<0.0001	<0.0001	<0.0001	0.0014	<0.0001
Butterfat %	r^2	0.63	0.02	-0.04	0.003	-0.007	-0.005	0.008	0.03
	p-value	<0.0001	<0.0001	<0.0001	<0.0001	0.0043	<0.0001	<0.0001	<0.0001
Milk protein [%]	r^2	-	0.09	-0.02	-0.02	-0.04	-0.001	0.002	0.004
	p-value	-	<0.0001	<0.0001	<0.0001	<0.0001	0.0108	0.0062	<0.0001
Milk lactose [%]	r^2	0.49	0.24	0.003	-0.01	-0.004	0.0007	-0.002	-0.005
	p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0676	0.0090	<0.0001
Milk urea nitrogen (MUN) [mg/dl]	r^2	0.09	-	0.07	-0.10	-0.06	0.02	-0.02	-0.10
	p-value	<0.0001	-	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Rumen degradable protein (RDP) [%]	r^2	-0.02	0.07	-	0.0001	0.08	0.003	-0.006	-0.49
	p-value	<0.0001	<0.0001	-	0.3414	<0.0001	0.0006	<0.0001	<0.0001
Crude protein (CP) [%]	r^2	-0.02	-0.10	0.0001	-	0.35	-0.008	0.01	0.06
	p-value	<0.0001	<0.0001	0.3414	-	<0.0001	<0.0001	<0.0001	<0.0001
Metabolizable energy (ME) [MJ/kg]	r^2	-0.04	-0.06	0.08	0.35	-	-0.008	0.01	0.009
	p-value	<0.0001	<0.0001	<0.0001	<0.0001	-	<0.0001	<0.0001	<0.0001
Calving number	r^2	0.0007	0.0004	0.09	0.005	-0.0003	0.06	-0.06	-0.40
	p-value	<0.0001	0.0468	<0.0001	<0.0001	0.0479	<0.0001	<0.0001	<0.0001
Intercalving period (ICP) [days]	r^2	0.001	0.02	0.003	-0.008	-0.008	-	-0.93	-0.05
	p-value	0.0108	<0.0001	0.0006	<0.0001	<0.0001	-	<0.0001	<0.0001
Reproduction performance (RP) [%]	r^2	0.002	-0.02	-0.006	0.01	0.01	-0.93	-	0.07
	p-value	0.0062	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	-	<0.0001
Reproductive index (RI)	r^2	0.004	-0.10	-0.49	0.06	0.009	-0.05	0.07	-
	p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	-

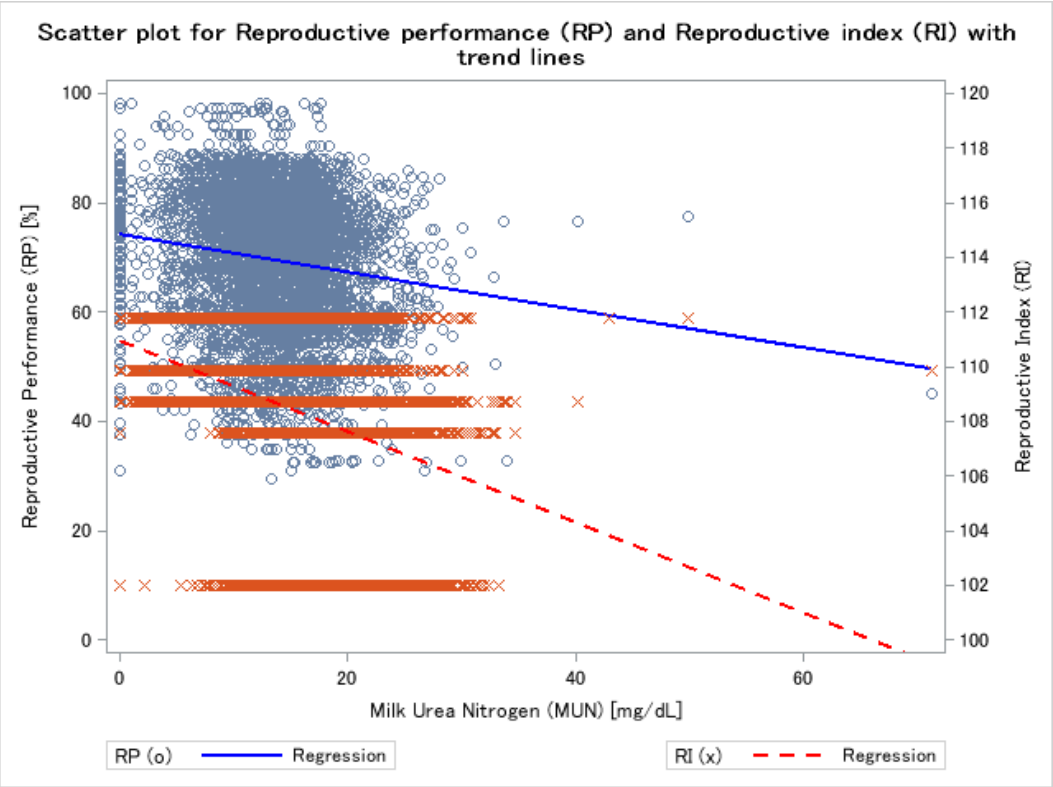

Figure 4.1.1 Scatterplot of Reproductive performance (RP) [%] and Reproductive index (RI) by Milk urea nitrogen (MUN) [mg/dl] overlaid with the fit line, for all cows included in the study from 2006 to 2008.

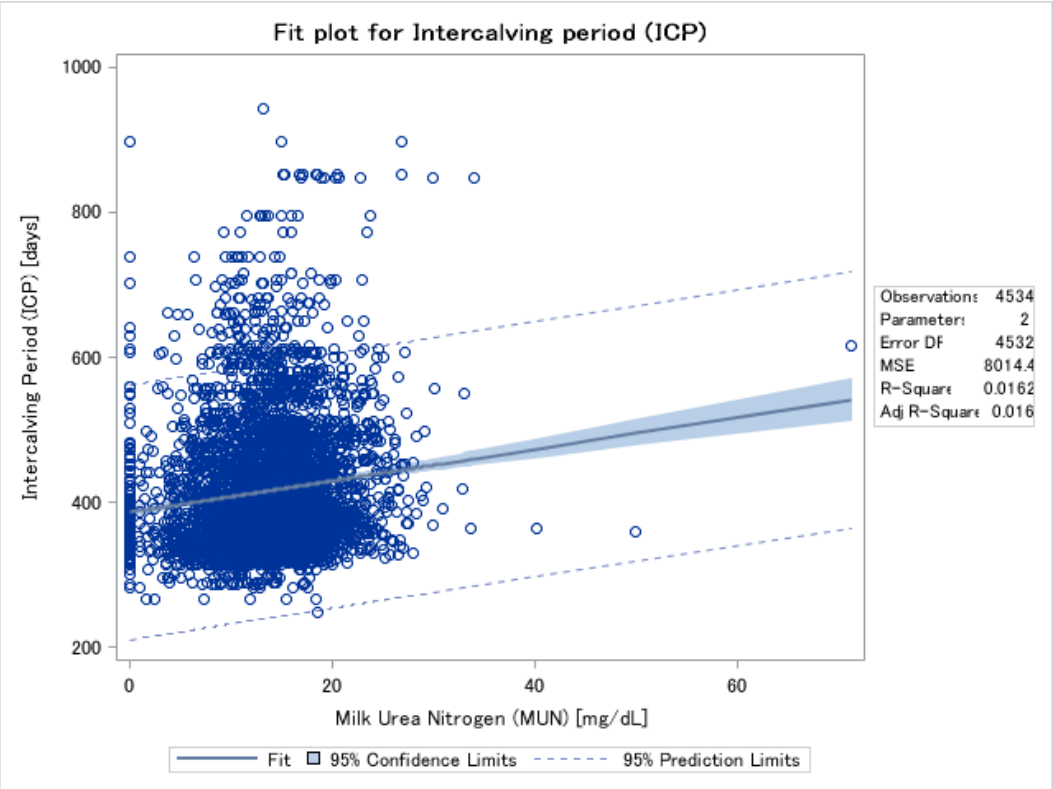

Figure 4.1.2 Scatterplot of Intercalving period (ICP) [days] by Milk urea nitrogen (MUN) [mg/dl] overlaid with the fit line, a 95% confidence interval and lower and upper 95% prediction limits, for all cows included in the study from 2006 to 2008.

The statistical analysis clearly indicated that MUN had an effect on reproduction in dairy cows. Even thugh the MUN only contributes 2% to the variation of ICP which seems biologically small. This value is still statistically significant and holds financial implications for the farmer. The cost for every extra day open (DO) in a cow with no available replacement is $0.10 (R0.66) - $1.60 (R10.48) for DO between 2 to 8 months. For a cow that can be replaced this range changes to $0 (R0) - $3 (R19.65). It is also said that this cost increases in lower producing anaimals, however various other factors also contribute to these calculations such as lactation stage (Groenendaal *et al.*, 2004). In South Africa the average number of cows in milk per producer is 332 cows (Milk Producers' Organisation, 2018). If this statistical effect of MUN on ICP is calculated in average milking herd size of 332 cows and multiplied by the average number of extra DO (average ICP 415 days in this study, in a system where one calf is produced every year the optimal ICP would be 90 days, therefore the extra DO in this study is 323 days), the financial loss up to R2 107 187.40 per herd, which is quite substantial. When adding the cost of overfeeding protein as illustrated by Jonker *et al.*, (2002b) a cumulative financial implication is observed which indicates that high MUN concentrations can adversely affect ICP. It is suggested that when ration formulation is calculated nutritionists should carefully consider the inclusion rate of protein, non-protein nitrogen (NPN) and fermentable metabolizable energy (FME) in order not to compromise reproduction. A similar outcome was also found by Guo *et al.*, (2004) who measured the conception rates in dairy cows. The results showed that dairy cows with low conception rates had correlating high concentrations of MUN. High concentrations of MUN have also been shown to negatively affect reproductive success, if high concentrations of urea were measured on the day of insemination (Albaaj *et al.*, 2017).

This study did not show the mechanism by which MUN affects reproduction success. This calls for more in-depth studies to be conducted. Understanding the interaction between MUN and reproduction could lead to preventative measures that will improve reproduction in dairy cows without compromising milk production. It is possible that the significance found for MUN in this present study may indicate that reproduction is affected at the ovaries and uterus of cows. As a water soluble molecule, urea, is distributed across the body into the saliva, rumen fluid, milk, blood, follicular fluid, uterine fluid, urine and faeces (Kauffman & St-Pierre, 2001; Butler, 2005b). Urea disrupts uterine pH and compromises the number of oocytes that develop due to compromised cleavage rates and blastocyst formation (Tamminga, 2006). Oocytes that are exposed to high concentrations of urea are less likely to produce embryos that develop to the blastocyst stage due to abnormal meiosis in oocytes (Kurykin *et al.*, 2011).

Decreasing the RDP fraction, so that rumen microbe survival is not negatively affected, could possibly lead to decreased concentrations of MUN and in turn lead to improved reproduction in cows. According to Hammond, (1997) less degradable protein or digestible undegradable protein (DUP) can be more efficiently used than NPN sources such as urea, which leads to decreased concentrations of BUN as well as better average daily gains (ADG).

4.2 Breed differences between dairy cattle

In this section the data was split into different breeds (Holstein and Jersey) before being analysed. These results are used to investigate the effect of MUN on reproduction parameters within breed.

Summary statistics (mean ± SDs) for reproduction parameters of dairy cows were presented in table 4.2.1 according to the breeds, Holstein and Jersey. The average ICP for Holstein dairy cows was 435.7 ± 82.49 days whereas it was 408.1 ± 91.79 days for Jersey cows. This was higher than what was observed in the study by Mostert *et al.*, (2010), where the ICP of Holstein cows averaged at around 395.67 days and the ICP of Jersey cows averaged at around 387.67 days. In the USA the calving interval in Holstein cows for 2006 was 422 (Norman *et al.*, 2009) which is lower than what is seen in this present study. The calving period for Jersey cows in 2006 was 410 (Norman *et al.*, 2009), which is higher than what is observed in this present study. The RP was higher in Jersey cows than in Holstein cows while Jersey cows had more variation. Holstein cows have a lower RI than Jersey cows, but with more variation. There were significant differences between breed for all reproduction parameters.

Table 4.2.1 Summary statistics (mean ± SD) of reproduction parameters in Holstein and Jersey cows.

Reproduction Parameters	**Holstein**	**Jersey**
Calving number	2.2 ± 0.82	2.1 ± 0.74
Intercalving period (ICP) [days]	435.7 ± 82.49	408.1 ± 91.79
Reproduction performance (RP) [%]	65.7 ± 10.63	70.8 ± 12.86
Reproductive index (RI)	105.5 ± 3.05	110.0 ± 1.33

Table 4.2.2 presents the mean and standard deviations of the nutritional parameters in Holstein and Jersey cows. In this study Holstein cows were fed higher amounts of RDP than Jersey cows. However, Jersey cows showed more variation. CP was fed in lower amounts to Holstein cows than Jersey cows. Greater variation was seen within Jersey cows. ME was fed relatively constant throughout both breeds. All the nutritional parameters had significant differences within the breeds.

Table 4.2.2 Summary statistics (mean ± SD) of nutritional parameters in Holstein and Jersey cows.

Nutritional Parameters (DM Basis)	**Holstein**	**Jersey**
Rumen degradable protein (RDP) [%]	64.42 ± 1.63	54.50 ± 2.60
Crude protein (CP) [%]	16.53 ± 0.86	17.51 ± 1.17
Metabolizable energy (ME) [MJ/kg]	10.87 ± 0.69	10.99 ± 0.54

The mean and standard deviations of the production parameters in Holstein and Jersey cows were also measured (table 4.2.3). Holstein cows had a total milk average of 28.6 ± 8.52 kg/d whereas Jerseys cows had an average of 21.4 ± 7.39 kg/d. In the study conducted by Kauffman & St-Pierre, (2001) Holstein cows had an average milk yield of 35.5 kg/d which was higher when compared to the results of this present study. Jersey cows on the other hand had an average total milk yield of 20.2 kg/d which was similar to the results found in this study. Total milk of Holstein cows was higher than Jersey cows, with greater variation within Holstein cows. In Holstein cows the butterfat % and milk protein was 4.0 ± 0.69% and 3.3 ± 0.53%. A slightly lower butterfat% of 3.58% and similar milk protein of 3.26% was recorded by Kauffman & St-Pierre, (2001). Similar concentrations of butterfat % and milk protein was observed by Barton *et al.*, (1996) at 3.69% and 3.05%, respectively. In Jersey cows the butterfat % and milk protein were 4.7 ± 1.12% and 3.6 ± 0.85%. This was similar to what was recorded in Kauffman & St-Pierre, (2001) which showed the butterfat % as 4.77% and milk protein as 3.91%. The same observations were also found in Barton *et al.*, (1996) study of Jersey cows where the butterfat % was 4.61% and the milk protein was 3.59%. In Holstein cows MUN was 18.0 ± 4.72 mg/dl which is higher than the level that was seen in Jersey cows at 12.6 ± 5.2 mg/dl. These results were much higher than those that were recorded by Kauffman & St-Pierre, (2001) who found that MUN in Holstein cows were 9.44 mg/dl and 9.47 mg/dl in Jersey cows. All production parameters showed significant differences within breed.

Table 4.2.3 Summary statistics (mean ± SD) of production parameters in Holstein and Jersey cows.

Production Parameters	**Holstein**	**Jersey**
Total milk [kg/d]	28.6 ± 8.52	21.4 ± 7.39
Butterfat %	4.0 ± 0.69	4.7 ± 1.12
Milk protein [%]	3.3 ± 0.53	3.6 ± 0.85
Milk lactose [%]	4.7 ± 0.5	4.5 ± 0.95
Somatic cell count [cells/ml]	610.0 ± 1362.72	705.9 ± 1409.99
Milk urea nitrogen (MUN) [mg/dl]	18.0 ± 4.72	12.6 ± 5.2
Days in milk (DIM)	228.0 ± 155.06	187.9 ± 119.84

In this study all parameters were also tested against ICP within both breeds, e.g. Holstein and Jersey. In both breeds the reproduction parameters influenced ICP, as summarised in table 4.2.2.

Table 4.2.4 Influence of selected reproduction parameters on Intercalving period (ICP) (ANOVA results) of Holstein and Jersey cows.

Reproduction Parameters	**Holstein**		**Jersey**	
	F	**p-value**	**F**	**p-value**
Calving number	7.83	<0.0001	6.18	<0.0001
Reproduction performance (RP) [%]	608.77	<0.0001	1090.53	<0.0001
Reproductive index (RI)	8.15	<0.0001	5.08	<0.0001

The interaction between ICP and RDP is only significant in Jersey cows (table 4.2.5). The interaction between both CP and ME with ICP was significant in both breeds. This indicated that CP and ME were important in both breeds for ICP, but RDP needs to be formulated more carefully in rations of Jersey cows in order for reproduction to occur optimally.

Table 4.2.5 Influence of selected nutritional parameters on Intercalving period (ICP) (ANOVA results) of Holstein and Jersey cows.

Nutritional Parameters	**Holstein**		**Jersey**	
(DM Basis)	**F**	**p-value**	**F**	**p-value**
Rumen degradable protein (RDP) [%]	1.11	0.2191	2.32	<0.0001
Crude protein (CP) [%]	5.70	<0.0001	3.56	<0.0001
Metabolizable energy (ME) [MJ/kg]	2.30	<0.0001	7.59	<0.0001

The interaction of the production parameters with ICP in Holstein cows was significant (table 4.2.6). There was no significant interaction between the ICP, butterfat %, milk lactose and DIM of Jersey cows. Milk urea nitrogen significantly interacted with ICP for both breeds. This means that the level of MUN found in the milk played a significant role in determining the length of the ICP of cows included in this study for both breeds. In Holstein cows the p-value of MUN is close to the level of significance ($p \leq 0.05$) considered for this study. This could indicate that although MUN is significant in Holstein cows, it may be less significant for reproduction to occur optimally. Therefore, when investigating reproduction in Holstein cow's other production parameters that may be more significant should be considered in conjunction with MUN.

Table 4.2.6 Influence of selected production parameters on Intercalving period (ICP) (ANOVA results) of Holstein and Jersey cows.

Production Parameters	Holstein		Jersey	
	F	p-value	F	p-value
Total milk [kg/d]	2.98	<0.0001	4.05	<0.0001
Butterfat %	2.10	<0.0001	0.96	0.6505
Milk protein [%]	2.04	<0.0001	1.47	<0.0001
Milk lactose [%]	1.41	0.0025	1.08	0.1928
Somatic cell count [cells/ml]	3.86	<0.0001	1.46	<0.0001
Milk urea nitrogen (MUN) [mg/dl]	1.24	0.0431	2.46	<0.0001
Days in milk (DIM)	3.04	0.0483	0.85	0.4286

The Pearson product moment correlation coefficient (r^2) is used to describe the linear relationship between parameters and indicates the strength and direction of the relationship. The relationship between MUN and RP as well as MUN and RI in Holstein cows was not significant as shown by the p-values 0.5075 and 0.1475, respectively (table 4.2.7). This indicated that MUN in Holstein cows did not significantly contribute to the variation of these two parameters. The table indicates that these parameters were affected by other parameters, such as total milk, CP, calving number, RI and ICP. The biggest contributors to the variation in RI were DIM, somatic cell count, milk protein, milk lactose, RDP, ME, calving number, and ICP.

The contribution of MUN to ICP was not significant. RP and RI together indicated that MUN was not significant in the reproduction of Holstein cows. The p-value of the ANOVA procedure for the ICP in Holstein cows was 0.0431. This is close to the level of significance considered for this study ($p \leq 0.05$) and indicated that MUN contributed less significantly to the ICP. The p-value with the Pearson product moment correlation coefficient for MUN and ICP was 0.3430 which is much higher than the level of significance considered for this study. Therefore, the significance indicated by the ANOVA showed that MUN had a slight influence on the ICP and therefore reproduction success in Holstein cows. This could mean that other parameters should be investigated to explain the declining fertility. The major parameters that contributed to the variation in ICP was CP (2%), calving number (9%) and RI (9%).

It was shown that in Holstein cows MUN may not be important for reproduction to occur successfully. This suggests that to improve fertility in Holstein cow's other factors, additional to MUN, should be looked at. Future studies that investigate the effect of MUN on reproduction in Holstein cows, should consider other parameters that may affect reproduction in combination with MUN.

Table 4.2.7 Pearson product moment correlation coefficient (r^2) and the probability associated with it of all Parameters of Holstein dairy cows.

Parameters		Milk protein	MUN	RDP	CP	ME	ICP	RP	RI
Days in milk (DIM)	r^2	0.20	0.003	0.25	-0.19	-0.20	0.02	-0.001	-0.02
	p-value	<0.0001	0.0011	<0.0001	<0.0001	<0.0001	0.1241	0.4758	<0.0001
Total milk [kg/d]	r^2	-0.17	0.0006	-0.18	0.25	0.22	0.009	-0.01	-0.006
	p-value	<.001	0.1588	<0.0001	<0.0001	<0.0001	0.0008	0.0099	<0.0001
Somatic cell count [cells/ml]	r^2	0.03	-0.005	0.01	-0.001	-0.006	0.006	-0.006	-0.04
	p-value	<0.0001	<0.0001	<0.0001	0.0549	<0.0001	0.0058	0.0757	<0.0001
Butterfat %	r^2	0.48	0.04	0.05	-0.05	-0.05	0.004	-0.003	-0.009
	p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0240	0.1884	<0.0001
Milk protein [%]	r^2	-	0.06	0.12	-0.13	-0.13	0.002	-0.0001	-0.01
	p-value	-	<0.0001	<0.0001	<0.0001	<0.0001	0.1677	0.7976	<0.0001
Milk lactose [%]	r^2	0.23	0.12	-0.01	* NS	0.001	* NS	0.0002	0.02
	p-value	<0.0001	<0.0001	<0.0001	0.9332	0.0330	0.8448	0.7097	<0.0001
Milk urea nitrogen (MUN) [mg/dl]	r^2	0.06	-	-0.001	-0.002	-0.01	0.0007	-0.001	0.0006
	p-value	<0.0001	-	0.1473	0.0079	<0.0001	0.3430	0.5075	0.1475
Rumen degradable protein (RDP) [%]	r^2	0.12	-0.001	-	-0.37	-0.50	-0.0004	-0.001	-0.08
	p-value	<0.0001	0.1473	-	<0.0001	<0.0001	0.5991	0.5060	<0.0001
Crude protein (CP) [%]	r^2	-0.13	-0.002	-0.37	-	0.55	-0.02	0.03	0.005
	p-value	<0.0001	0.0079	<0.0001	-	<0.0001	<0.0001	0.0001	<0.0001
Metabolizable protein (ME) [MJ/kg]	r^2	-0.13	-0.01	-0.50	0.55	-	-0.005	0.01	0.02
	p-value	<0.0001	<0.0001	<0.0001	<0.0001	-	0.0108	0.0196	<0.0001
Calving number	r^2	0.02	-0.001	0.15	-0.01	-0.04	0.09	-0.08	-0.89
	p-value	<0.0001	0.2000	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Intercalving period (ICP) [days]	r^2	0.002	0.0007	-0.0004	-0.02	-0.005	-	-0.94	-0.09
	p-value	0.1677	0.3430	0.5991	<0.0001	0.0108	-	<0.0001	<0.0001
Reproduction performance (RP) [%]	r^2	-0.0001	-0.001	-0.001	0.03	0.01	-0.94	-	0.07
	p-value	0.7976	0.5075	0.5060	0.0001	0.0196	<0.0001	-	<0.0001
Reproductive index (RI)	r^2	-0.01	0.0006	-0.08	0.005	0.02	-0.09	0.07	-
	p-value	<0.0001	0.1475	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	-

* NS: non-significant (value <0.00001)

In Holstein cows MUN did not contribute significantly to the variation of RI (table 4.2.8 and figure 4.2.1) Other parameters such as total milk, RDP, ICP, RP and calving number contributed the most to the variation of RI.

Some of the variation in RP was explained by MUN (0.01%). The relationship was shown as negative, therefore as MUN concentrations increased, the RP value decreased. This then indicates that reproduction declines with increasing concentrations of MUN. This small variation also indicated that there must be other parameters that contribute to the variation in RP. In this study, parameters were identified that contributed to the variation in RP, these included calving number, RI and ICP.

In this study as MUN increased so did the ICP, which indicates a positive relationship between MUN and ICP (table 4.2.8 and figure 4.2.2). This suggests that cows exposed to high concentrations of MUN take longer to reproduce. Milk urea nitrogen (0.7%) contributed to the variation in ICP. Because the contribution of MUN was small, it is recommended that other parameters should be considered to explain the variation in ICP. Considering this, total milk, calving number and RI in this study showed the greatest contribution to ICP.

In Jersey cows MUN was significant in reproduction. Although the effect of MUN in reproduction of Jersey cows was small, it needs to be considered for reproduction to be improved in a herd. However, it is suggested that other factors that could possibly work with MUN in declining fertility should be included in future studies.

Table 4.2.8 Pearson product moment correlation coefficient (r^2) and the probability associated with it of all Parameters of Jersey cows.

Parameters		Milk protein	MUN	RDP	CP	ME	ICP	RP	RI
Days in milk (DIM)	r^2	0.02	0.002	-0.03	-0.05	0.02	0.0006	-0.001	-0.009
	p-value	<0.0001	0.0006	<0.0001	<0.0001	<0.0001	0.1693	0.1719	<0.0001
Total Milk [kg/d]	r^2	-0.04	-0.0006	0.12	0.08	0.05	0.03	-0.01	-0.06
	p-value	<0.0001	0.0312	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Somatic cell count [cells/ml]	r^2	0.04	0.004	-0.001	-0.02	-0.05	0.0006	-0.003	-0.008
	p-value	<0.0001	<0.0001	0.040	<0.0001	<0.0001	0.1475	0.0321	<0.0001
Butterfat %	r^2	0.65	0.13	* NS	-0.001	0.00002	-0.003	0.0001	-0.003
	p-value	<0.0001	<0.0001	0.1147	0.0076	0.7150	0.0022	0.6872	<0.0001
Milk protein [%]	r^2	-	0.24	-0.001	-0.03	-0.03	* NS	-0.0007	-0.007
	p-value	-	<0.0001	0.0043	<0.0001	<0.0001	0.3793	0.2924	<0.0001
Milk lactose [%]	r^2	0.61	0.27	-0.001	-0.007	-0.007	0.0002	-0.003	0.0003
	p-value	<0.0001	<0.0001	0.0017	<0.0001	<0.0001	0.4379	0.0288	0.1730
Milk urea nitrogen (MUN) [mg/dl]	r^2	0.24	-	* NS	-0.04	-0.09	0.009	-0.01	* NS
	p-value	<0.0001	-	0.0364	<0.0001	<0.0001	<0.0001	<0.0001	0.2242
Rumen degradable protein (RDP) [%]	r^2	-0.001	-0.0006	-	0.06	0.04	0.0001	-0.003	-0.22
	p-value	0.0043	<0.0001	-	<0.0001	<0.0001	0.4999	0.0361	<0.0001
Crude protein (CP) [%]	r^2	-0.03	-0.04	0.06	-	0.32	-0.001	0.002	-0.03
	p-value	<0.0001	<0.0001	<0.0001	-	<0.0001	0.0324	0.0967	<0.0001
Metabolizable energy (ME) [MJ/kg]	r^2	-0.03	-0.09	0.04	0.32	-	-0.01	0.008	-0.01
	p-value	<0.0001	<0.0001	<0.0001	<0.0001	-	<0.0001	0.0003	<0.0001
Calving number	r^2	0.007	0.0003	0.20	0.03	0.009	0.04	-0.05	-0.98
	p-value	<0.0001	0.1428	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Intercalving period (ICP) [days]	r^2	* NS	0.009	0.0001	0.001	-0.01	-	-0.93	-0.05
	p-value	0.3793	<0.0001	0.4999	0.0324	<0.0001	-	<0.0001	<0.0001
Reproduction performance (RP) [%]	r^2	* NS	-0.01	-0.003	0.002	0.008	-0.93	-	0.06
	p-value	0.2924	<0.0001	0.0361	0.0967	0.0003	<0.0001	-	<0.0001
Reproductive index (RI)	r^2	-0.007	* NS	0.22	-0.03	-0.01	-0.05	0.06	-
	p-value	<0.0001	0.2242	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	-

* NS: non-significant (value <0.0001)

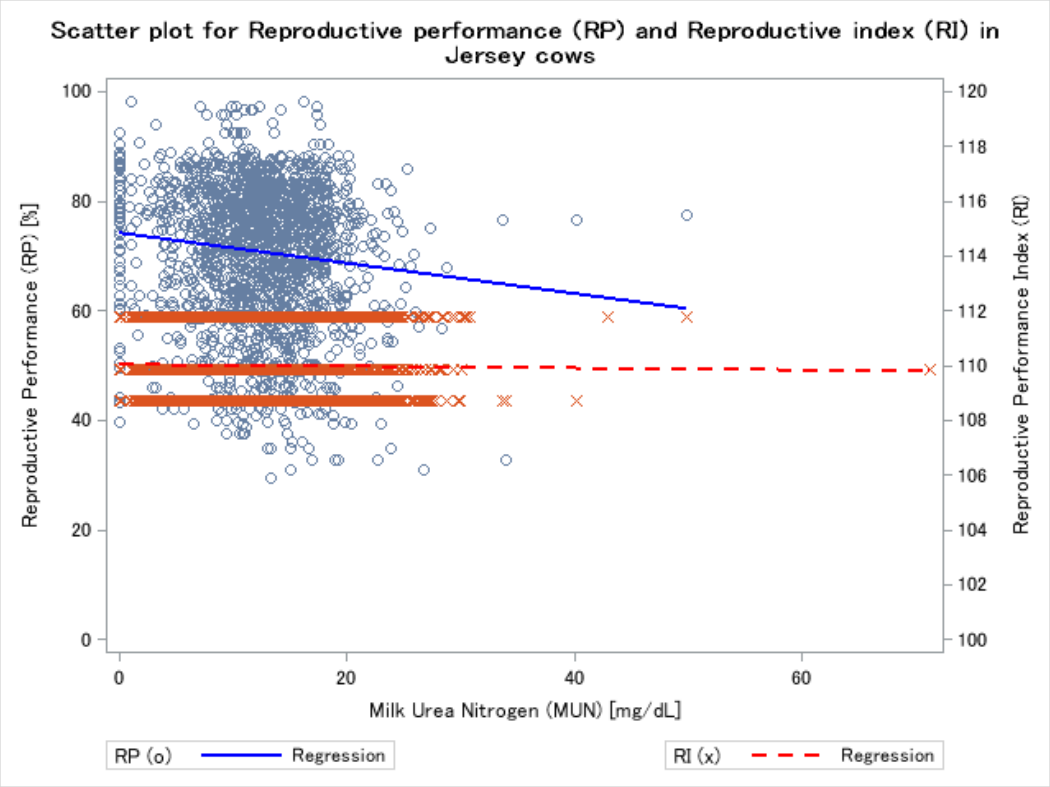

Figure 4.2.1 Scatterplot of Reproductive performance (RP) [%] and Reproductive index (RI) by Milk urea nitrogen (MUN) [mg/dl] overlaid with the fit line in Jersey cows.

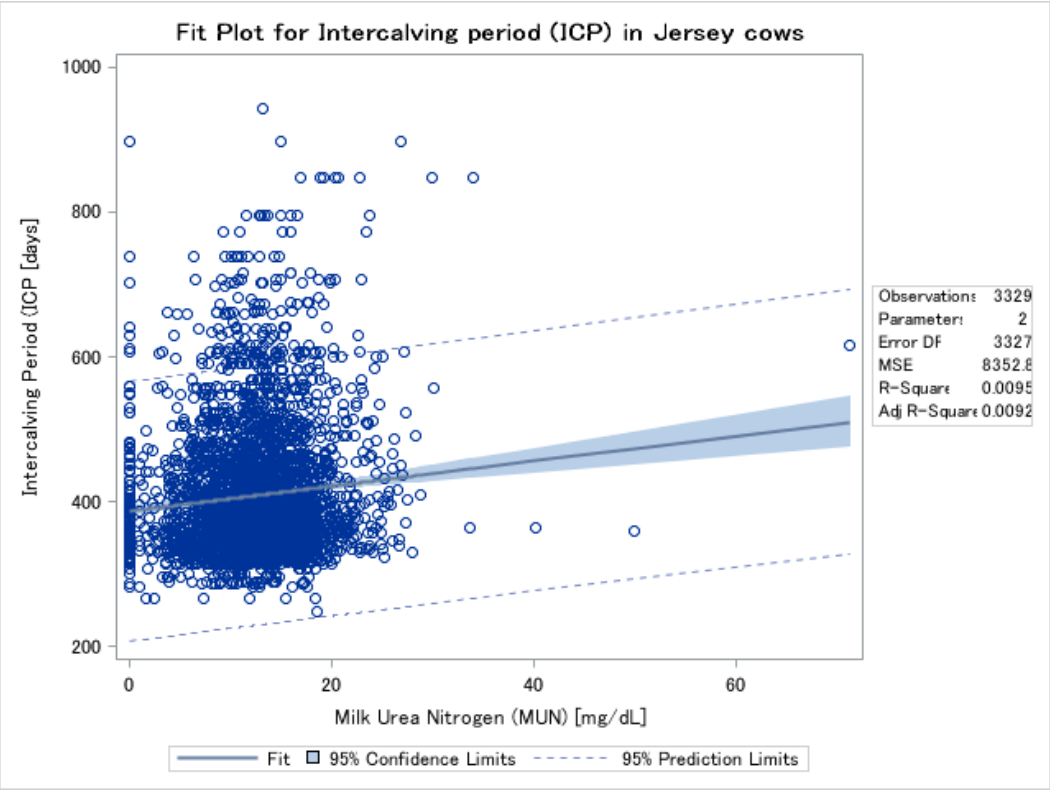

Figure 4.2.2 Scatterplot of Intercalving period (ICP) [days] by Milk urea nitrogen (MUN) [mg/dl] overlaid with the fit line in Jersey cows, a 95% confidence interval and lower and upper 95% prediction limits.

Stepwise selection is a statistical process in which every parameter chosen out of a dataset is evaluated to fit the selected level of significance in building a model. In this statistical analysis ICP was used as the model, as it is the only reproduction parameter that was measured to indicate the reproductive status of the cows. In terms of ICP there were two parameters that were significant and that fit the model in both breeds (table 4.2.9 and 4.2.10). Calving number was not significant enough to stay in the model and as such was removed from both breeds. The only reproduction parameter that had any significant impact on ICP, was RP since ICP was used in the calculation of RP.

Table 4.2.9 Stepwise selection of reproduction parameters on Intercalving period (ICP) as measured performance of reproduction in Holstein cows.

Reproduction Parameters		**Number**	**Score Chi-**	**Wald Chi-**	**p-**
Entered	**Removed**	**In**	**Square**	**Square**	**value**
Reproduction performance (RP)	-	1	554.66	-	<0.0001
Calving number	-	2	54.68	-	<0.0001
-	Calving number	1	-	0.0008	0.9770

entry: 0.05

stay: 0.01

Table 4.2.10 Stepwise selection of reproduction parameters on Intercalving period (ICP) as measured performance of reproduction in Jersey cows.

Reproduction Parameters		**Number**	**Score Chi-**	**Wald Chi-**	**p-value**
Entered	**Removed**	**In**	**Square**	**Square**	
Reproduction performance (RP)	-	1	1578.77	-	<0.0001
Calving number	-	2	100.34	-	<0.0001
-	Calving number	1	-	0.16	0.6874

entry: 0.05

stay: 0.01

The only nutritional parameter that was considered and included in the model was CP (table 4.2.11), but it was removed as it was not significant enough to stay in the model. It was found that there were not any nutritional parameters that were significant enough to build a model for Holstein cows out of these parameters. This indicated that the nutritional parameters were not significant in the reproduction success of Holstein cows, although it has previously shown to significantly affect ICP in Holstein cows in this study. This could mean that the significance was not great enough to affect ICP in a measurable way.

All the nutritional parameters were included in the model for Jersey cows (table 4.2.12). The most important parameters was ME, with CP being the least important. This indicated that ME needed more consideration than protein and NPN did in the diets of these cows. It also showed that reproduction were more

sensitive to ME. Although protein was not the most important nutritional factor on reproduction, it should still be a priority in Jersey cows when diets are formulated.

Table 4.2.11 Stepwise selection of nutritional parameters on Intercalving period (ICP) as measured performance of reproduction in Holstein cows.

Nutritional Parameters (DM Basis)		**Number In**	**Score Chi-Square**	**Wald Chi-Square**	**p-value**
Entered	**Removed**				
Crude protein (CP)	-	1	5.76	-	0.0164
-	Crude Protein (CP)	0	-	5.98	0.0145

entry: 0.05
stay: 0.01

Table 4.2.12 Stepwise selection of nutritional parameters on Intercalving period (ICP) as measured performance of reproduction in Jersey cows.

Nutritional Parameters (DM Basis)		**Number In**	**Score Chi-Square**	**Wald Chi-Square**	**p-value**
Entered	**Removed**				
Metabolizable energy (ME)	-	1	67.93	-	<0.0001
Rumen degradable protein (RDP)	-	2	17.67	-	<0.0001
Crude protein (CP)	-	3	19.33	-	<0.0001

entry: 0.05
stay: 0.01

The results presented in table 4.2.13 confirms previous findings in this study. Milk urea nitrogen was not included in the model as it was not an important consideration as a production parameter on the reproduction of Holstein cows. Milk protein was also not included in the model. This was similar to table 4.2.11 which found no nutritional parameters, specifically protein, significant to include in the model. Therefore, in this study protein inclusion was not found to be significant for reproduction success in Holstein cows.

Milk urea nitrogen was in the top three parameters included in the model (table 4.2.14), which confirms previous results that MUN was an important measurement to consider in the reproduction success of Jersey cows. Milk protein was included in the model which confirms together with MUN that greater consideration should be given to protein and NPN inclusion in Jersey cow rations in order to improve reproduction.

Table 4.2.13 Stepwise selection of production parameters on Intercalving period (ICP) as measured performance of reproduction in Holstein cows.

Production Parameters		Number In	Score Chi-Square	Wald Chi-Square	p-value
Entered	**Removed**				
Total milk	-	1	11.77	-	0.0006
Butterfat %	-	2	14.85	-	0.0001
Somatic cell count	-	3	11.47	-	0.0007
Days in milk (DIM)	-	4	11.88	-	0.0006
Milk lactose	-	5	8.90	-	0.0028

entry: 0.05

stay: 0.01

Table 4.2.14 Stepwise selection of production parameters on Intercalving period (ICP) as measured performance of reproduction in Jersey cows.

Production Parameters		Number In	Score Chi-Square	Wald Chi-Square	p-value
Entered	**Removed**				
Total milk	-	1	78.57	-	<0.0001
Days in milk (DIM)	-	2	43.35	-	<0.0001
Milk urea nitrogen (MUN)	-	3	43.69	-	<0.0001
Butterfat %	-	4	16.94	-	<0.0001
Somatic cell count	-	5	13.48	-	0.0002
Milk protein	-	6	9.03	-	0.0027

entry: 0.05

stay: 0.01

In a study by Rodriguez *et al.*, (1997) it was found that Holstein cows have higher MUN concentrations than Jersey cows. These results are similar to those obtained in this study. Different results such as that Jersey cows have higher concentrations of MUN was found by Wattiaux *et al.*, (2005), or that breed was not significant in the measured level of MUN (Kauffman & St-Pierre, 2001). MUN was not significant for reproduction in Holstein cows, possibly because the cows (18.0 ± 4.72 mg/dl) did not exceed the known threshold at which reproduction is compromised. The current recommendation for optimal MUN concentration is 8-12mg/dl (Ishler, 2016). However it was previously stated that when MUN exceeds 20 mg/dl production is compromised (Tamminga, 2006). Since the values in this study are less than the threshold mentioned by Tamminga it could mean that the level of MUN was not high enough to indicate significance or high enough to negatively affect reproduction. Therefore, although no significance was shown in this study, it does not necessarily indicate that MUN is not significant in Holstein cows. More studies are needed to further investigate this.

For ration formulation in Jersey cow's protein and NPN inclusion and the derivative, MUN, needs to be considered. Although Holstein cows had higher concentrations of MUN (18.0 ± 4.72 mg/dl) than Jersey cows (12.6 ± 5.2 mg/dl), the results of this study indicated that MUN is significant in terms of reproduction for Jersey cows but not for Holstein cows. When MUN is <14 mg/dl it could indicate that the protein inclusion in the diet was insufficient (Oltner & Wiktorsson, 1983). Since the MUN value in Jerseys cows are lower than the limit of <14 mg/dl and the average MUN value for Holstein cows in this study, it could mean that Jersey cows have a lower threshold for MUN in terms of negative implications on reproduction than Holstein cows. In a study on Israeli cows by Hojman *et al.*, (2004) the pregnancy rates of the cows increased when MUN concentrations were <11.75 mg/dl. Rajala-Schultz *et al.*, (2001) reported similar results in Holstein cow herds in Ohio with higher pregnancy rates recorded when MUN was <10 mg/dl, with lowered pregnancy rates at MUN concentrations >15.4 mg/dl. Jersey cows may have a lower threshold that is similar to what was found in these investigations. Further studies are needed to determine at which level MUN starts to negatively affect reproduction in Jersey cows, as well as other dairy breeds. As such studies should be conducted to understand why more emphasis should be placed on the protein and NPN balance of Jersey cows than on Holstein cows. Investigations should focus on how MUN affects reproduction and how this can be overcome without sacrificing growth and production, and thus profit for the farmer.

It was clearly indicated that when considering the effect of MUN on reproduction a difference existed within the breeds. Future studies should consider this breed effect, while also focusing on determining the concentrations at which this effect occurs within Holstein and Jersey cows as well as other breeds. This is important as this can affect the way in which rations are formulated and could further indicate that the formulation process need to be reconsidered to fit the needs of individual breeds.

4.3 The effect of season on reproduction parameters of dairy cows

In order to investigate the effect of MUN on reproduction parameters within season for all the cows included in the study, the data was split into season (summer and winter) before being analysed further. The results of this analysis will be discussed in this section.

Summary statistics (mean ± SDs) for reproduction parameters of dairy cows studied in this research was presented in table 4.3.1 according to season (summer and winter). The average calving number across both seasons was approximately 2, with some relatively high variation. Differences was seen within season, where the cows have a longer ICP in summer than in winter, with winter showing more variation. Season was relatively similar on average for RP and RI. Only calving number and RI had significant differences within season.

Table 4.3.1 Summary statistics (mean ± SD) of reproduction parameters in summer and winter.

Reproduction Parameters	**Summer**	**Winter**
Calving number [a]	2.1 ± 0.85	2.2 ± 0.83
Intercalving period (ICP) [days]	416.9 ± 88.43	412.2 ± 94.18
Reproduction performance (RP) [%]	69.7 ± 12.36	69.8 ± 12.68
Reproductive index (RI) [b]	108.6 ± 2.93	108.3 ± 2.99

a and b: Indicate reproduction parameters that showed significant mean differences within season.

Table 4.3.2 shows the mean and standard deviations of the nutritional parameters in summer and winter months. The level of RDP was similar in summer and winter, with summer being slightly lower than winter. For both seasons CP and ME were fed at similar concentrations. All nutritional parameters had significant differences within season.

Table 4.3.2 Summary statistics (mean ± SD) of nutritional parameters in summer and winter.

Nutritional Parameters (DM Basis)	**Summer**	**Winter**
Rumen degradable protein (RDP) [%] [a]	55.59 ± 3.73	56.63 ± 3.41
Crude protein (CP) [%] [b]	17.09 ± 1.10	17.46 ± 1.32
Metabolizable energy (ME) [MJ/kg] [c]	10.94 ± 0.59	10.99 ± 0.60

a, b and c: Indicate nutritional parameters that showed significant mean differences within season.

The mean and standard deviations of the production parameters in summer and winter were presented in table 4.3.3. Somatic cell count was higher in winter than summer, which could indicate compromised udder

health during winter. According to Petzer *et al.*, (2017b) a threshold of 150 000 cells/ml or more in composite milk samples indicate possible intramammary infection or mastitis. This indicates that even though the winter somatic cell count (704.7 ± 1421.08 cells/ml) was higher than in the summer month (663.9 ± 1386.12 cells/ml) no infection was indicated and the cows had good udder health. The average MUN over summer was at 14.1 ± 5.49 mg/dl and 15.1 ± 6.01 mg/dl in winter, showing that MUN in both seasons were relatively similar but winter was higher. A study by Hojman *et al.*, (2004) on dairy cows in Israel found that MUN concentrations were highest in June (Summer) at 18.1 mg/dl and lowest in November (Winter) at 11.8 mg/dl. Other researchers also found that MUN concentrations were higher in summer (Ferguson *et al.*, 1997; Godden *et al.*, 2001; Fatehi *et al.*, 2012). It should be noted that these studies were all performed outside of South Africa and mainly in the Northern hemisphere which could explain the difference in the results as climatic conditions for the same season differ between hemispheres. In summer DIM was longer than in winter. Only butterfat %, milk protein, MUN and DIM had significant mean differences within season. These differences occur due to seasonal changes in roughage quality.

Table 4.3.3 Summary statistics (mean ± SD) of production parameters in summer and winter.

Production Parameters	**Summer**	**Winter**
Total milk [kg/d]	23.7 ± 8.31	24.0 ± 8.91
Butterfat % [a]	4.4 ± 1.03	4.6 ± 1.05
Milk protein [%] [b]	3.4 ± 0.76	3.6 ± 0.80
Milk lactose [%]	4.6 ± 0.85	4.5 ± 0.81
Somatic cell count [cells/ml]	663.9 ± 1386.12	704.7 ± 1421.08
Milk urea nitrogen (MUN) [mg/dl] [c]	14.1 ± 5.49	15.1 ± 6.01
Days in milk (DIM) [d]	203.2 ± 132.45	194.8 ± 136.87

a, b, c and d: Indicate production parameters that showed significant mean differences within season.

In this study all parameters were also tested against ICP within both seasons. In both seasons the reproduction parameters influenced ICP, as summarised in table 4.3.4.

Table 4.3.4 Influence of selected reproduction parameters on Intercalving period (ICP) (ANOVA results) in summer and winter.

Reproduction Parameters	**Summer**		**Winter**	
	F	**p-value**	**F**	**p-value**
Calving number	5.44	<0.0001	2.36	<0.0001
Reproduction performance (RP) [%]	1089.00	<0.0001	568.46	<0.0001
Reproductive index (RI)	9.48	<0.0001	3.30	<0.0001

Intercalving period significantly interacted with all the nutritional parameters in both seasons (table 4.3.5). This indicated that the nutritional parameters should be carefully formulated in rations for optimum reproduction to occur in both seasons.

Table 4.3.5 Influence of selected nutritional parameters on Intercalving period (ICP) (ANOVA results) in summer and winter.

Nutritional Parameters	**Summer**		**Winter**	
(DM Basis)	**F**	**p-value**	**F**	**p-value**
Rumen degradable protein (RDP) [%]	3.90	<0.0001	2.09	<0.0001
Crude protein (CP) [%]	3.27	<0.0001	2.75	<0.0001
Metabolizable energy (ME) [MJ/kg]	4.24	<0.0001	2.43	<0.0001

The production parameters significantly interacted with ICP in summer (table 4.3.6). The only nutritional parameters that significantly interacted with ICP in the winter was total milk, butterfat %, somatic cell count and MUN. This indicated that these parameters need to be carefully managed during the respective seasons for optimal reproduction to occur. For the purpose of this study it was important to see that MUN interacted significantly with ICP during both seasons. However, the p-value for MUN in winter was higher, indicating that MUN may have less of an effect on ICP during winter than summer. A paper by Wattiaux & Sanjeewa, (2016) states that the difference in MUN concentrations between seasons may not be due to weather but could rather be explained by varying feedinding practice's within seasons.

Table 4.3.6 Influence of selected production parameters on Intercalving period (ICP) (ANOVA results) in summer and winter.

Production Parameters	**Summer**		**Winter**	
	F	**p-value**	**F**	**p-value**
Total milk [kg/d]	4.20	<0.0001	2.65	<0.0001
Butterfat %	1.64	<0.0001	1.24	0.0122
Milk protein [%]	2.08	<0.0001	1.14	0.0921
Milk lactose [%]	1.26	0.0044	0.93	0.7661
Somatic cell count [cells/ml]	1.85	<0.0001	1.22	0.0189
Milk urea nitrogen (MUN) [mg/dl]	3.41	<0.0001	1.21	0.0252
Days in milk (DIM)	5.74	0.0033	1.02	0.3624

In summer the contribution of MUN to RP and RI were shown to be significant (table 4.3.7 and figure 4.3.1). The relationship between MUN and these two parameters were also shown to be negative which means as MUN increases they decrease. Therefore, cows with declining reproduction also had higher concentrations of MUN in the milk. MUN explained 2% and 9% of the variation respectively in these two parameters during

summer. In RP other parameters that contributed to the variation was total milk, calving number, ICP and RI. Other major parameters that contributed significantly to RI were total milk, RDP, CP, ICP and calving number.

The variation in ICP was explained at 2% by MUN in the summer (table 4.3.7 and figure 4.3.2). This relationship was positive, which meant that as MUN increased so did the ICP. Even thugh the MUN only contributes 2% to the variation of ICP which seems biologically small. This value is still statistically significant and holds financial implications for the farmer, as previously explained. his suggested that during summer cows exposed to increasing concentrations of MUN will take longer to produce a calf and enter a new lactation cycle. Other parameters that contributed to ICP in summer were calving number and RI.

From these findings it can be concluded that during summer it is very important to ensure that cows were not overfed on protein and NPN. Too much protein and NPN or too little FME in the diet of cows can cause rising concentrations of MUN which have a negative effect on reproduction and fertility in a herd. Similar observations have been found by Fatehi *et al.*, (2012), where in their study higher MUN concentrations in the summer lead to reduced reproduction in Iranian dairy cows.

Table 4.3.7 Pearson product moment correlation coefficient (r^2) and the probability associated with it of all parameters in summer.

Parameters		Milk protein	MUN	RDP	CP	ME	ICP	RP	RI
Days in milk (DIM)	r^2	0.04	0.007	*	-0.07	-0.05	0.003	-0.003	-0.03
	p-value	<0.0001	<0.0001	0.7592	<0.0001	<0.0001	0.0028	0.9479	<0.0001
Total milk [kg/d]	r^2	-0.08	0.03	0.22	0.02	0.05	0.03	-0.04	-0.13
	p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Somatic cell count [cells/ml]	r^2	0.04	0.0003	-0.001	-0.005	-0.03	0.003	-0.005	-0.005
	p-value	<0.0001	0.1327	0.0047	<0.0001	<0.0001	0.0013	0.0038	<0.0001
Butterfat %	r^2	0.62	0.03	0.04	0.00003	-0.001	-0.003	0.0009	0.03
	p-value	<0.0001	<0.0001	<0.0001	0.6120	0.0190	0.0014	0.2291	<0.0001
Milk protein [%]	r^2	-	0.12	-0.02	-0.03	-0.04	-0.00001	* NS	0.003
	p-value	-	<0.0001	<0.0001	<0.0001	<0.0001	0.9015	0.9499	<0.0001
Milk lactose [%]	r^2	0.51	0.25	0.001	-0.02	-0.005	0.002	-0.003	-0.005
	p-value	<0.0001	<0.0001	0.0015	<0.0001	<0.0001	0.0268	0.0327	<0.0001
Milk urea nitrogen (MUN) [mg/dl]	r^2	0.12	-	0.06	-0.09	-0.07	0.02	-0.02	-0.09
	p-value	<0.0001	-	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Rumen degradable protein (RDP) [%]	r^2	-0.02	0.06	-	0.0001	0.06	0.002	-0.003	-0.49
	p-value	<0.0001	<0.0001	-	0.3681	<0.0001	0.0121	0.0594	<0.0001
Crude protein (CP) [%]	r^2	-0.03	-0.09	0.0001	-	0.27	-0.006	0.005	0.06
	p-value	<0.0001	<0.0001	0.3681	-	<0.0001	<0.0001	0.0065	<0.0001
Metabolizable energy (ME) [MJ/kg]	r^2	-0.04	-0.07	0.06	0.27	-	-0.01	0.01	0.01
	p-value	<0.0001	<0.0001	<0.0001	<0.0001	-	<0.0001	<0.0001	<0.0001
Calving number	r^2	0.007	0.0003	0.09	0.002	-0.002	0.07	-0.07	-0.41
	p-value	<0.0001	0.0969	<0.0001	0.0003	0.0004	<0.0001	<0.0001	<0.0001
Intercalving period (ICP) [days]	r^2	* NS	0.02	0.002	-0.006	-0.01	-	-0.93	-0.06
	p-value	0.9015	<0.0001	0.0121	<0.0001	<0.0001	-	<0.0001	<0.0001
Reproduction performance (RP) [%]	r^2	* NS	-0.02	-0.003	0.005	0.01	0.93	-	0.09
	p-value	0.9499	<0.0001	0.0594	0.0065	<0.0001	<0.0001	-	<0.0001
Reproductive index (RI)	r^2	0.003	-0.09	-0.49	0.06	0.01	-0.06	0.09	-
	p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	-

* NS: non-significant (value <0.00001)

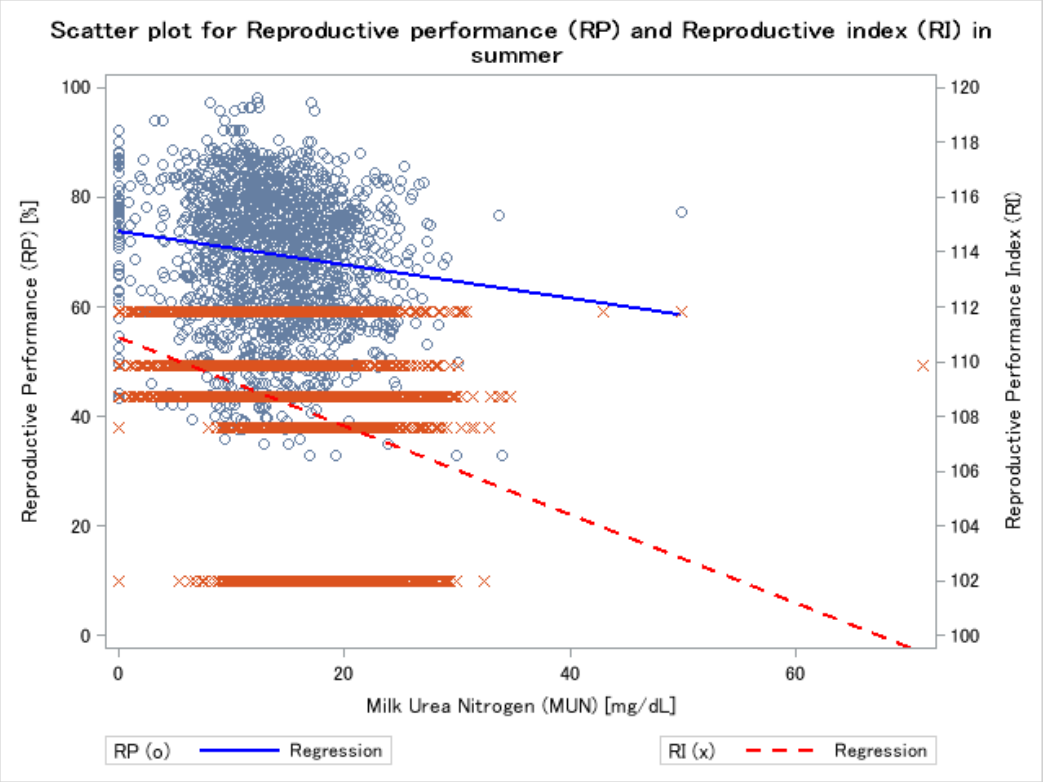

Figure 4.3.1 Scatterplot of Reproductive performance (RP) [%] and Reproductive index (RI) by Milk urea nitrogen (MUN) [mg/dl] overlaid with the fit line in the summer.

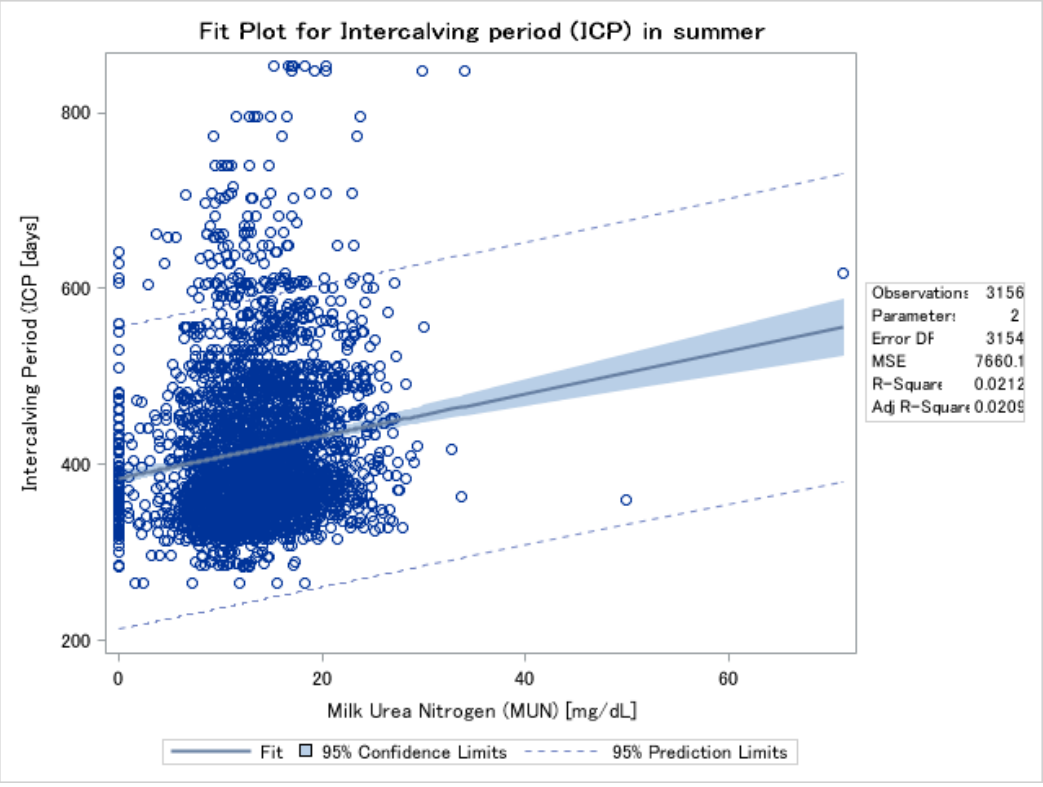

Figure 4.3.2 Scatterplot of Intercalving period (ICP) [days] by Milk urea nitrogen (MUN) [mg/dl] overlaid with the fit line in the summer, a 95% confidence interval and lower and upper 95% prediction limits.

In winter the contribution of MUN to the variation of RP was significant and negative (table 4.3.8 and figure 4.3.3), as MUN increased the RP declined. This was mainly due to dairy cows being sensitive to the inclusion rate of protein and NPN in their diet for successful reproduction to occur during the winter. The contribution of MUN to the variation of RP was 1%, while total milk, RI and ICP were other parameters that contributed to the variation of RP during winter. This was a small biological effect but it was consistent and statistically significant.

The relationship between MUN and RI was negative. To improve RI, the level of MUN that was measured in cows needs to be lowered, as increasing concentrations of MUN lead to declining RI. The contribution of MUN to the variation of RI was 12%. This value is high and indicated that MUN may be important during the winter, as cows may be more sensitive to protein and NPN inclusion during winter. Other parameters that explain the variation in RI were total milk, RDP, CP and calving number.

The relationship between MUN and ICP was positive, because it was found that as MUN increased so did the ICP. Furthermore, MUN explained 0.8% of the variation in ICP (table 4.3.8 and figure 4.3.4). This suggested that cows with high measurements of MUN were more likely to take longer to produce a calf (although a biologically small effect, it was statictically significant as explained previously). There were other parameters that contributed to the variation in ICP, such as total milk. From the data we can infer that the processes leading to a successful reproduction event to occur, i.e. to ensure a calf was produced, was sensitive to the overfeeding of protein and NPN. Therefore, increasing concentrations of MUN may be harmful to fertility during the winter and the inclusion of protein and NPN in the diet of cows should be considered carefully.

These findings suggested that season (both summer and winter) was sensitive to the measurement of MUN in milk of dairy cows. For reproduction to occur successfully high concentrations of MUN should be avoided. In comparison RP was more sensitive in summer although the difference between summer and winter was 1%. RI was higher in winter, the difference between the two seasons being 3%. ICP was more sensitive in summer although the difference between summer and winter was 1.2%. Since these differences were not high, it suggested that cows were overfed protein and NPN in both seasons.

Table 4.3.8 Pearson product moment correlation coefficient (r^2) and the probability associated with it of all Parameters in winter.

Parameters		Milk protein	MUN	RDP	CP	ME	ICP	RP	RI
Days in milk (DIM)	r^2	0.04	0.03	0.02	-0.17	-0.19	* NS	-0.0003	-0.04
	p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.8269	0.5969	<0.0001
Total milk [kg/d]	r^2	-0.09	0.03	0.21	0.01	0.19	0.05	-0.05	-0.16
	p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Somatic cell count [cells/ml]	r^2	0.03	-0.0009	-0.47	-0.02	-0.04	-0.002	0.00001	-0.006
	p-value	<0.0001	0.1016	0.0008	<0.0001	<0.0001	0.5829	0.9098	<0.0001
Butterfat %	r^2	0.63	0.004	-0.05	0.01	-0.002	-0.01	0.004	0.04
	p-value	<0.0001	0.0007	<0.0001	<0.0001	0.0170	0.0001	0.0624	<0.0001
Milk protein [%]	r^2	-	0.04	-0.02	-0.003	-0.04	-0.01	0.005	0.01
	p-value	-	<0.0001	<0.0001	0.0038	<0.0001	<0.0001	0.0288	<0.0001
Milk lactose [%]	r^2	0.44	0.22	0.02	-0.01	-0.002	0.00001	-0.0002	-0.008
	p-value	<0.0001	<0.0001	<0.0001	<0.0001	0.0297	0.8955	0.6521	<0.0001
Milk urea nitrogen (MUN) [mg/dl]	r^2	0.04	-	0.10	-0.14	-0.04	0.008	-0.01	-0.12
	p-value	<0.0001	-	<0.0001	<0.0001	<0.0001	0.0012	0.0013	<0.0001
Rumen degradable protein (RDP) [%]	r^2	-0.02	0.10	-	-0.006	0.14	0.006	-0.01	-0.48
	p-value	<0.0001	<0.0001	-	0.0002	<0.0001	0.0069	0.0034	<0.0001
Crude protein (CP) [%]	r^2	-0.003	-0.14	-0.006	-	0.58	-0.01	0.01	0.09
	p-value	0.0038	<0.0001	0.0002	-	<0.0001	0.0002	0.0011	<0.0001
Metabolizable energy (ME) [MJ/kg]	r^2	-0.04	-0.04	0.14	0.58	-	-0.001	-0.0002	0.007
	p-value	<0.0001	<0.0001	<0.0001	<0.0001	-	0.2036	0.6691	<0.0001
Calving number	r^2	0.008	0.0002	0.08	0.02	0.001	0.03	-0.03	-0.38
	p-value	<0.0001	0.4883	<0.0001	<0.0001	0.0679	<0.0001	<0.0001	<0.0001
Intercalving period (ICP) [days]	r^2	-0.01	0.008	0.006	-0.01	-0.001	-	-0.92	-0.03
	p-value	<0.0001	0.0012	0.0069	0.0002	0.2036	-	<0.0001	<0.0001
Reproduction performance (RP)	r^2	0.005	-0.01	-0.01	0.01	* NS	-0.92	-	0.04
	p-value	0.0288	0.0013	0.0034	0.0011	0.6691	<0.0001	-	<0.0001
Reproductive index (RI)	r^2	0.01	-0.12	-0.48	0.09	0.007	-0.03	0.04	-
	p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	-

* NS: non-significant (value <0.00001)

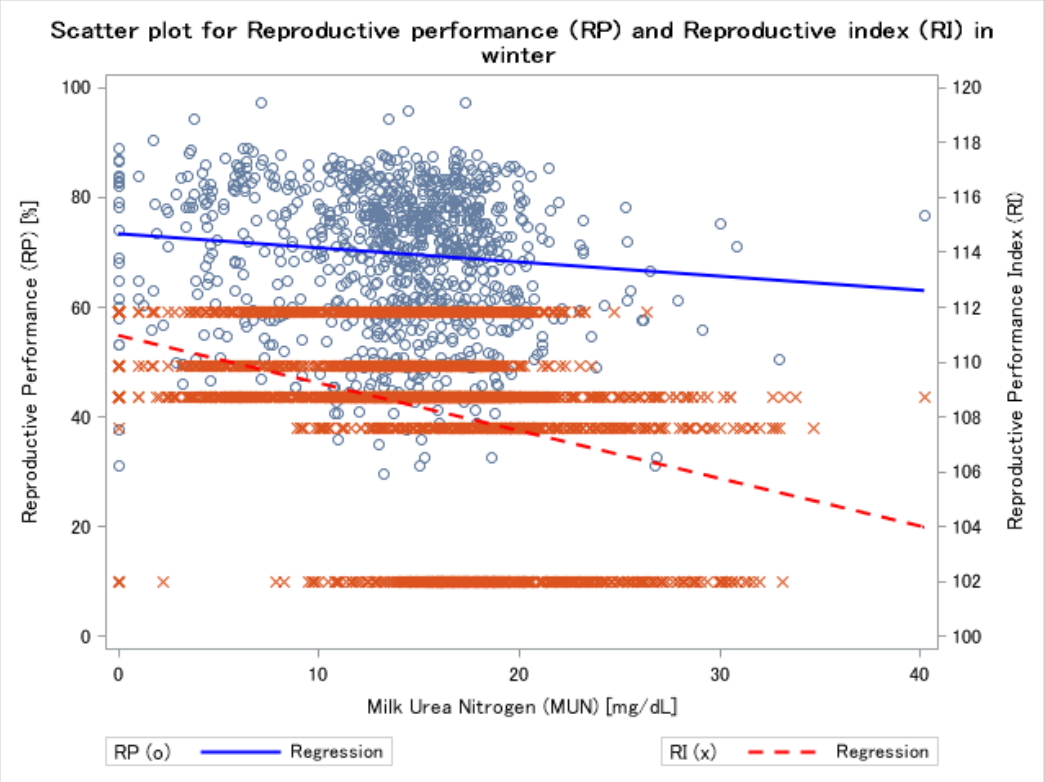

Figure 4.3.3 Scatterplot of Reproductive performance (RP) [%] and Reproductive index (RI) by Milk urea nitrogen (MUN) [mg/dl] overlaid with the fit line in the winter.

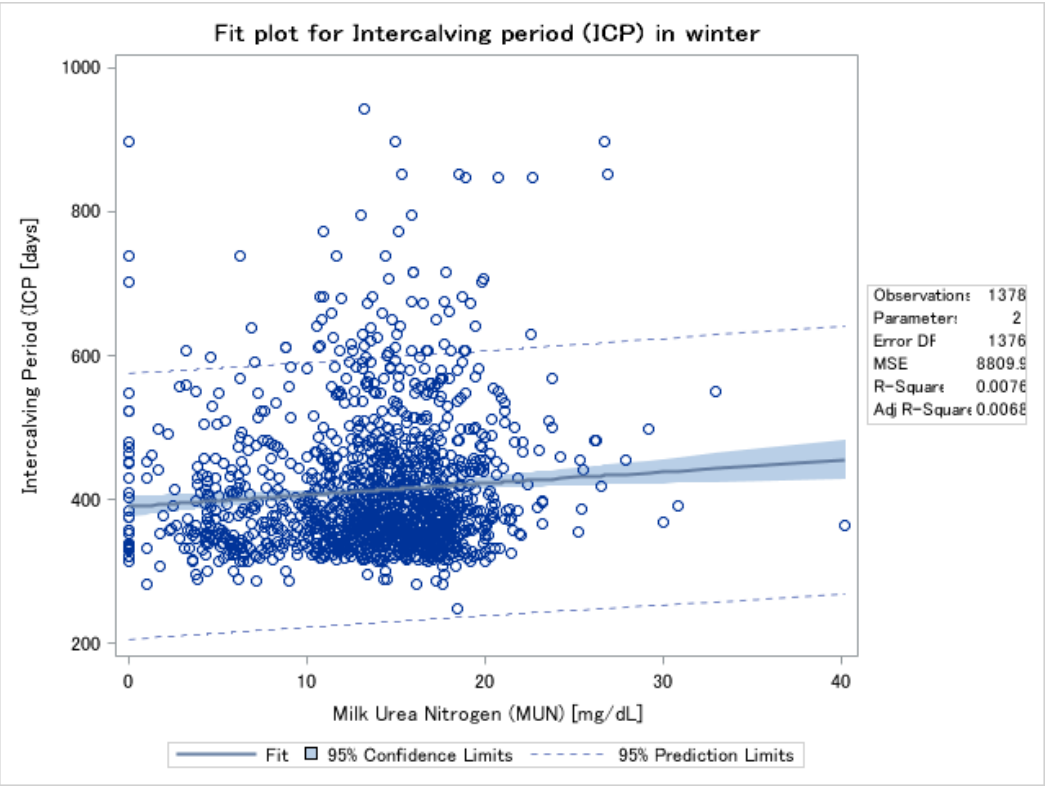

Figure 4.3.4 Scatterplot of Intercalving period (ICP) [days] by Milk urea nitrogen (MUN) [mg/dl] overlaid with the fit line in the winter, a 95% confidence interval and lower and upper 95% prediction limits**.**

Stepwise selection was conducted with ICP used as the model. In table 4.3.9 and 4.3.10 two parameters were included in the model. Only one parameter, RP, stayed in the model.

Table 4.3.9 Stepwise selection of reproduction parameters on Intercalving period (ICP) as measured performance of reproduction in summer.

Reproduction Parameters		Number	Score Chi-Square	Wald Chi-Square	p-value
Entered	**Removed**	**In**			
Reproduction performance (RP)	-	1	1565.16	-	<0.0001
Reproductive index (RI)	-	2	100.32	-	<0.0001
-	Reproductive index (RI)	1	-	0.0028	0.9575

entry: 0.05
stay: 0.01

Table 4.3.10 Stepwise selection of reproduction parameters on Intercalving period (ICP) as measured performance of reproduction in winter.

Reproduction Parameters		Number	Score Chi-Square	Wald Chi-Square	p-value
Entered	**Removed**	**In**			
Reproduction performance (RP)	-	1	1578.77	-	<0.0001
Reproductive index (RI)	-	2	100.34	-	<0.0001
-	Reproductive index (RI)	1	-	0.16	0.6874

entry: 0.05
stay: 0.01

In table 4.3.11 all the parameters were fitted in the model, with ME being the first parameter entered in the model and CP entered last. This indicated that when formulating rations during the summer ME needs to be emphasized more than protein and NPN. Protein has an effect on reproduction in summer and should be considered when formulating a ration.

Because CP was not significant enough it was removed from the model (table 4.3.12). Contrary to what was observed for summer, protein in the form of RDP needs to be emphasized more when formulating rations during winter. This suggest that during winter the reproduction may be more sensitive to RDP inclusion in the diet and it becomes more important to ensure that it was balanced in the diet.

Table 4.3.11 Stepwise selection of nutritional parameters on Intercalving period (ICP) as measured performance of reproduction in summer.

Nutritional Parameters (DM Basis)		**Number**	**Score Chi-**	**Wald Chi-**	**p-value**
Entered	**Removed**	**In**	**Square**	**Square**	
Metabolizable energy (ME)	-	1	36.50	-	<0.0001
Rumen degradable protein (RDP)	-	2	58.16	-	<0.0001
Crude protein (CP)	-	3	7.03	-	0.0080

entry: 0.05

stay: 0.01

Table 4.3.12 Stepwise selection of nutritional parameters on Intercalving period (ICP) as measured performance of reproduction in winter.

Nutritional Parameters (DM Basis)		**Number**	**Score Chi-**	**Wald Chi-**	**p-value**
Entered	**Removed**	**In**	**Square**	**Square**	
Rumen degradable protein (RDP)	-	1	24.76	-	<0.0001
Metabolizable energy (ME)	-	2	14.66	-	0.0001
Crude protein (CP)	-	3	4.31	-	0.0379
-	Crude protein (CP)	2	-	4.33	0.0375

entry: 0.05

stay: 0.01

Milk urea nitrogen was in top three parameters fitted in the model (table 4.3.13). This shows that during summer the level of MUN in the milk was important to successful reproduction. These results support previous findings in this study and indicated the significance of MUN in summer, because if MUN is carefully maintained reproduction can be expected to improve during the summer.

Total milk was the only parameter that stayed in the model (table 4.3.14), because in winter milk volume needs to be carefully managed in order to ensure that optimal reproduction occur. Milk urea nitrogen was not entered in the model because it was not significant during the winter. This means that it can be given less consideration. This was however not what was expected from the results in table 4.3.12, which showed RDP to be the most important nutritional parameter in winter. It is possible that RDP may affect reproduction but should be measured in another way than the pathway to MUN. In order to explain this, the pathway of RDP needs to be better understood.

Table 4.3.13 Stepwise selection of production parameters on Intercalving period (ICP) as measured performance of reproduction in summer.

Production Parameters		Number In	Score Chi-Square	Wald Chi-Square	p-value
Entered	**Removed**				
Total milk	-	1	11.77	-	0.0006
Days in milk (DIM)	-	2	14.85	-	0.0001
Milk urea nitrogen (MUN)	-	3	11.47	-	0.0007
Days in milk (DIM)	-	4	11.88	-	0.0006
Milk lactose	-	5	8.90	-	0.0028

entry: 0.05

stay: 0.01

-Table 4.3.14 Stepwise selection of production parameters on Intercalving period (ICP) as measured performance of reproduction in winter.

Production Parameters		Number In	Score Chi-Square	Wald Chi-Square	p-value
Entered	**Removed**				
Total milk	-	1	83.44	-	<0.0001
Days in milk (DIM)	-	2	6.32	-	0.0119
-	Days in milk (DIM)	1	-	6.49	0.0109

entry: 0.05

stay: 0.01

It has been shown that MUN concentrations in different seasons have an effect on reproduction, as MUN was shown to be more important in summer than winter. Similarly, Melendez *et al.*, (2000) found that cows that mated in winter, who also had low concentrations of MUN, were more likely to fall pregnant than cows who mated in summer with high concentrations of MUN. The authors indicated that during summer, heat stress and the level of MUN may interact in reducing reproductive success in dairy cows. Since heat stress may cause the animal to use energy needed to convert ammonia to urea, instead for thermoregulation. Heat stress occurs in cows when temperatures exceed 23.8°C (Du Preez *et al.*, 1991). In figure 4.3.5 the temperatures for 2005 to 2010 in the Pretoria area was shown. The lowest temperature during the summer months (Jan to Mar & Oct to Dec) was 14°C and the highest temperature was 32°C. These summer temperatures showed wide variations that were mostly above the thermoneutral zone for bovine, which was why dairy cows frequently experienced heat stress. According to Kruger & Sekele, (2013) the highest annual average summer temperatures are seen in parts of the Limpopo, Gauteng, North West, Northern Cape and Western Cape Provinces. These regions also show the highest increases in temperatures over time, which include both maximum and minimum temperatures. This indicated that both summer and winter temperatures are steadily increasing in the Western and North Eastern parts of South Africa. A decrease between 20% to 30% in conception and pregnancy rates, have been shown in various studies during summer. As well as a reduction of 13% in pregnancy rates at 90 days after calving and the period of calving to fertilization increased by 13 days (De Rensis *et al.*, 2017). Sustainable strategies to reduce heat stress in dairy cows could be very

important in improving reproduction in dairy cows. This can explain why in this study, ME can be considered a more important nutritional parameter than protein and NPN.

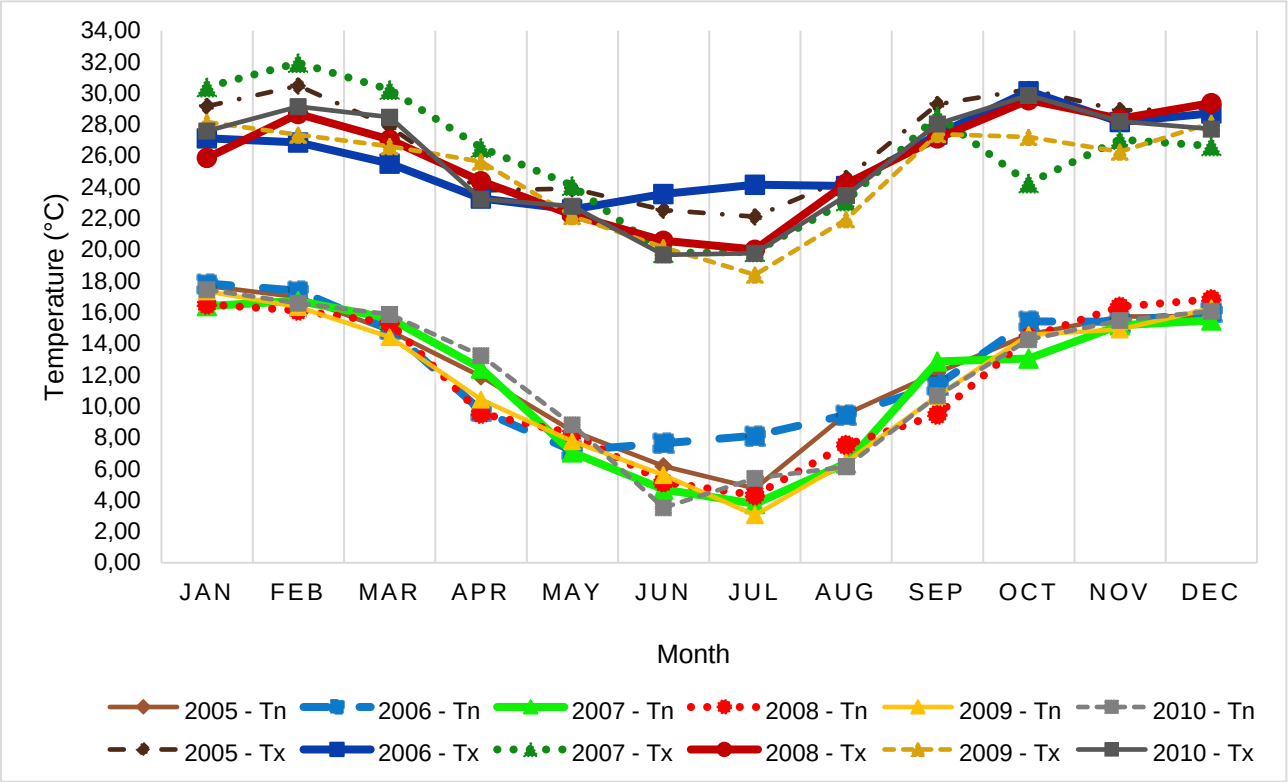

Figure 4.3.5 Monthly temperature data of the Pretoria area as provided by the ARC – Soil, Climate and Water department for the year 2005 to the year 2010 (Tn = Minimum Temperature, Tx = Maximum Temperature).

While ME was the most important nutritional parameter in summer, in winter RDP was the most important nutritional parameter. Rumen degradable protein is broken down to ammonia in the rumen and diffuses into the portal system where it is converted to urea by the liver and is known as blood urea nitrogen (BUN) (Butler, 2005b). Blood urea nitrogen has been shown to decrease uterine pH which can be detrimental to embryo survival (Butler, 1998, 2000, 2003). Urea is a water soluble molecule and easily diffuses into oocytes before ovulation, therefore compromising oocyte quality and cleavage rates of blastocysts after fertilization (Butler, 2005b; Tamminga, 2006). This can explain why MUN was not found to be significant in winter, but RDP was. Since BUN was not part of the measurements included in the data, its significance could not have been measured.

It was found that 12.54g of urinary nitrogen (UN) gets excreted per unit of MUN (Jonker *et al.*, 1998). However, Kauffman & St-Pierre, (2001) reported that more UN was excreted per unit of MUN than previously described. They also found that as CP increased, the efficiency of excreting N into the milk decreased while urinary excretion increased. The efficiency of excreting N into the milk decreased from 52.3% in diets containing 13% CP to 42.7% in diets containing 17% CP. In this study the average CP in the diet during winter was 17.4 ± 1.32%. Since UN was not measured in this study it is possible that urea originating from RDP was

excreted through the urinary tract instead of the mammary gland which would explain the absence of MUN included in the model for winter.

It was clearly indicated that a difference existed within season when considering the effect of MUN on reproduction. In summer it was found that ME needs to be emphazised more, which also indicated that dairy cows may need more energy in their diets during summer and that their diets contain too much protein and NPN which disturbs the balance between protein and energy requirements of the cows. However, since RDP was observed to be significant in the winter season, it may show that dairy cows are not fed enough protein and NPN during the winter months. Future studies should consider seasonal effects, to ensure that dairy cows are fed concentrations of protein, NPN and FME that would ensure optimal reproduction throughout the year.

4.4 Breed x season interaction effects on reproduction parameters

In this section the data was split into season within breed before being analysed further. These results are used to investigate the effect of MUN on reproduction parameters within the interaction between breed and season (Holstein x summer, Jersey x summer, Holstein x winter and Jersey x winter) for all the cows included in the study.

Summary statistics (mean ± SDs) for reproduction parameters of dairy cows studied in this research was presented in table 4.4.1 according to the interaction between breed and season. There was a slight difference in the average calving number between summer and winter within Holstein cows, however within Jersey cows the calving number was slightly higher in winter. In Holstein cows the ICP was relatively similar as was also seen in Jersey cows across both seasons. However, Holstein cows had a longer ICP in both seasons when compared to Jersey cows. Reproductive performance was similar across both seasons for Holstein cows and was also lower than what was seen in Jersey cows. In summer RP was slightly higher than winter in Jersey cows. Reproductive index was higher in Jersey cows and slightly higher in summer when compared to winter, however RI was relatively similar for both seasons in Holstein cows. The interaction between breed and season showed no significant differences in the reproduction parameters.

Table 4.4.1 Summary statistics (mean ± SD) of reproduction parameters of season within breed.

Reproduction Parameters	**Holstein**		**Jersey**	
	Summer	**Winter**	**Summer**	**Winter**
Calving number	2.1 ± 0.82	2.2 ± 0.81	2.1 ± 0.87	2.2 ± 0.85
Intercalving period (ICP) [days]	436.4 ± 80.55	433.1 ± 89.91	408.4 ± 90.37	407.7 ± 94.51
Reproduction performance (RP) [%]	65.9 ± 10.16	66.0 ± 11.88	71.3 ± 12.79	70.8 ± 12.70
Reproductive index (RI)	105.5 ± 3.05	105.4 ± 3.05	110.0 ± 1.34	109.9 ± 1.29

Table 4.4.2 show the mean and standard deviations of the nutritional parameters of season within breed. In both breeds RDP was fed at a similar level throughout summer and winter but at a higher level in Holstein cows than what Jersey cows were fed. Jersey cows were fed a higher level of CP than Holstein cows, however CP was fed at a similar level in summer and winter in both breeds. ME was fed at a similar level throughout breed and season, with Jersey cows and winter being slightly higher. There was no significant difference shown in ME within the interaction between breed and season.

Table 4.4.2 Summary statistics (mean ± SD) of nutritional parameters of season within breed.

Nutritional Parameters (DM Basis)	**Holstein**		**Jersey**	
	Summer	**Winter**	**Summer**	**Winter**
Rumen degradable protein (RDP) [%] [a]	61.46 ± 1.66	61.32 ± 1.55	54.24 ± 2.60	55.26 ± 2.45
Crude protein (CP) [%] [b]	16.52 ± 0.88	16.57 ± 0.82	17.36 ± 1.09	17.95 ± 1.29
Metabolizable energy (ME) [MJ/kg]	10.86 ± 0.67	10.89 ± 0.73	10.97 ± 0.55	11.05 ± 0.50

a and b: Indicate nutritional parameters that showed significant mean differences within the interaction between breed and season.

Table 4.4.3 shows the mean and standard deviations of the production parameters of season within breed. In both breeds winter concentrations of milk protein was relatively higher than that of summer. Jersey cows have relatively higher milk protein concentrations than Holstein cows. Jersey cows have higher concentrations of somatic cell count as compared to Holstein cows, that was especially high in winter. Holstein cows have similar somatic cell count concentrations across both seasons and showed slightly higher concentrations during the winter. In Jersey cows MUN was relatively similar across both seasons and was also lower than what was seen in Holstein cows. In winter MUN concentrations were greater than summer in Holstein cows. Furthermore, the data demonstrated that the interaction between breed and season was not significant for butterfat %, milk lactose and somatic cell count.

Table 4.4.3 Summary statistics (mean ± SD) of production parameters of season within breed.

Production Parameters	**Holstein**		**Jersey**	
	Summer	**Winter**	**Summer**	**Winter**
Total milk [kg/d] [a]	28.4 ± 8.26	29.0 ± 9.13	21.5 ± 7.36	21.2 ± 7.48
Butterfat %	4.0 ± 0.67	4.2 ± 0.73	4.6 ± 1.11	4.9 ± 1.11
Milk protein [%] [b]	3.3 ± 0.50	3.4 ± 0.57	3.5 ± 0.84	3.7 ± 0.88
Milk lactose [%]	4.7 ± 0.49	4.7 ± 0.51	4.5 ± 0.95	4.4 ± 0.92
Somatic cell count [cells/ml]	609.2 ± 1337.17	612.0 ± 1425.25	689.2 ± 1407.5	755.8 ± 1416.58
Milk urea nitrogen (MUN) [mg/dl] [c]	17.6 ± 4.44	19.1 ± 5.23	12.5 ± 5.19	12.9 ± 5.24
Days in milk (DIM) [d]	226.6 ± 151.86	231.5 ± 162.82	192.4 ± 120.98	174.6 ± 115.40

a, b, c and d: Indicate production parameters that showed significant mean differences within the interaction between breed and season.

Stepwise selection was done with ICP used as the model. In table 4.4.4 and 4.4.5 RP was the only parameter that was included in the model.

Table 4.4.4 Stepwise selection of reproduction parameters on Intercalving period (ICP) as measured performance of reproduction in Holstein cows and summer.

Reproduction Parameters		**Number In**	**Score Chi-Square**	**Wald Chi-Square**	**p-value**
Entered	**Removed**				
Reproduction performance (RP)	-	1	447.02	-	<0.0001
Reproductive index (RI)	-	2	41.20	-	<0.0001
-	Reproductive index (RI)	1	-	0.04	0.8370

entry: 0.05
stay: 0.01

Table 4.4.5 Stepwise selection of reproduction parameters on Intercalving period (ICP) as measured performance of reproduction in Jersey cows and summer.

Reproduction Parameters		**Number In**	**Score Chi-Square**	**Wald Chi-Square**	**p-value**
Entered	**Removed**				
Reproduction performance (RP)	-	1	1098.89	-	<0.0001
Calving number	-	2	64.93	-	<0.0001
-	Calving number	1	-	0.18	0.6760

entry: 0.05
stay: 0.01

In table 4.4.6 no parameters were kept in the model. This indicated that the nutritional parameters were not significant in Holstein cows during summer for reproduction. CP was entered in the model but then removed which could indicate that the CP inclusion in the diet needs to be looked at occasionally to ensure it is balanced.

All the nutritional parameters were included in the model with ME being the most important parameter (table 4.4.7). However, it was still important to ensure that the diets of Jersey cows do not include high concentrations of protein and NPN during summer as this could be damaging to reproduction.

Table 4.4.6 Stepwise selection of nutritional parameters on Intercalving period (ICP) as measured performance of reproduction in Holstein cows and summer.

Nutritional Parameters (DM Basis)		**Number In**	**Score Chi-Square**	**Wald Chi-Square**	**p-value**
Entered	**Removed**				
Crude protein (CP)	-	1	4.44	-	0.0351
-	Crude protein (CP)	0	-	4.72	0.0299

entry: 0.05
stay: 0.01

Table 4.4.7 Stepwise selection of nutritional parameters on Intercalving period (ICP) as measured performance of reproduction in Jersey cows and summer.

Nutritional Parameters (DM Basis)		**Number**	**Score Chi-**	**Wald Chi-**	**p-value**
Entered	**Removed**	**In**	**Square**	**Square**	
Metabolizable energy (ME)	-	1	64.59	-	<0.0001
Crude protein (CP)	-	2	6.66	-	0.0099
Rumen degradable protein (RDP)	-	3	7.37	-	0.0066

entry: 0.05

stay: 0.01

In table 4.4.8 MUN was not included in the model. This analysis found that although MUN was important during summer, as seen in table 4.3.13, it was still not significant in Holstein cows. As such these findings support the notion that when formulating diets for Holstein cows during the summer high emphasis does not need to be placed on the inclusion of protein and NPN in terms of reproduction.

As one of the top three parameters entered in the model (table 4.4.9) the presence of MUN was . indicated that not only was it important in Jersey cows but more so in the diets formulated during the summer for these cows. In figure 4.4.1 the relationship between MUN and ICP was shown for Jersey cows during the summer. Milk urea nitrogen explained 0.92% of the variation in ICP as shown by the Pearson product moment correlation coefficient. The positive relationship indicated that as MUN increases so did ICP. Even though the MUN only contributes 2% to the variation of ICP which seems biologically small. This value is still statistically significant and holds financial implications for the farmer. The cost for every extra day open (DO) in a cow with no available replacement is $0.10 - $1.60 for DO between 2 to 8 months. For a cow that can be replaced this range changes to $0 - $3. It is also said that this cost increases in lower producing anaimals, however various other factors also contribute to these calculations such as lactation stage (Groenendaal *et al.*, 2004). When adding the cost of overfeeding protein as illustrated by Jonker *et al.*, (2002b) a cumulative financial implication is observed which indicates that high MUN concentrations can adversely affect ICP. From these results it is clear that reproductive success could decline when Jersey cows have high measured concentrations of MUN during the summer and therefore take longer to produce a calf.

Table 4.4.8 Stepwise selection of production parameters on Intercalving period (ICP) as measured performance of reproduction in Holstein cows and summer.

Production Parameters		Number In	Score Chi-Square	Wald Chi-Square	p-value
Entered	**Removed**				
Total milk	-	1	8.98	-	0.0027
Butterfat %	-	2	11.45	-	0.0007
Milk lactose	-	3	9.56	-	0.0020
Milk protein	-	4	8.79	-	0.0030
-	Butterfat %	3	-	6.46	0.0110
Butterfat %	-	4	6.77	-	0.0093
-	Butterfat %	3	-	6.46	0.0110

entry: 0.05

stay: 0.01

Table 4.4.9 Stepwise selection of production parameters on Intercalving period (ICP) as measured performance of reproduction in Jersey cows and summer.

Production Parameters		Number In	Score Chi-Square	Wald Chi-Square	p-value
Entered	**Removed**				
Total milk	-	1	38.51	-	<0.0001
Days in milk	-	2	41.01	-	<0.0001
Milk urea nitrogen (MUN)	-	3	43.95	-	<0.0001
Butterfat %	-	4	13.05	-	0.0003
Milk protein	-	5	17.56	-	<0.0001
Milk lactose	-	6	8.63	-	0.0033

entry: 0.05

stay: 0.01

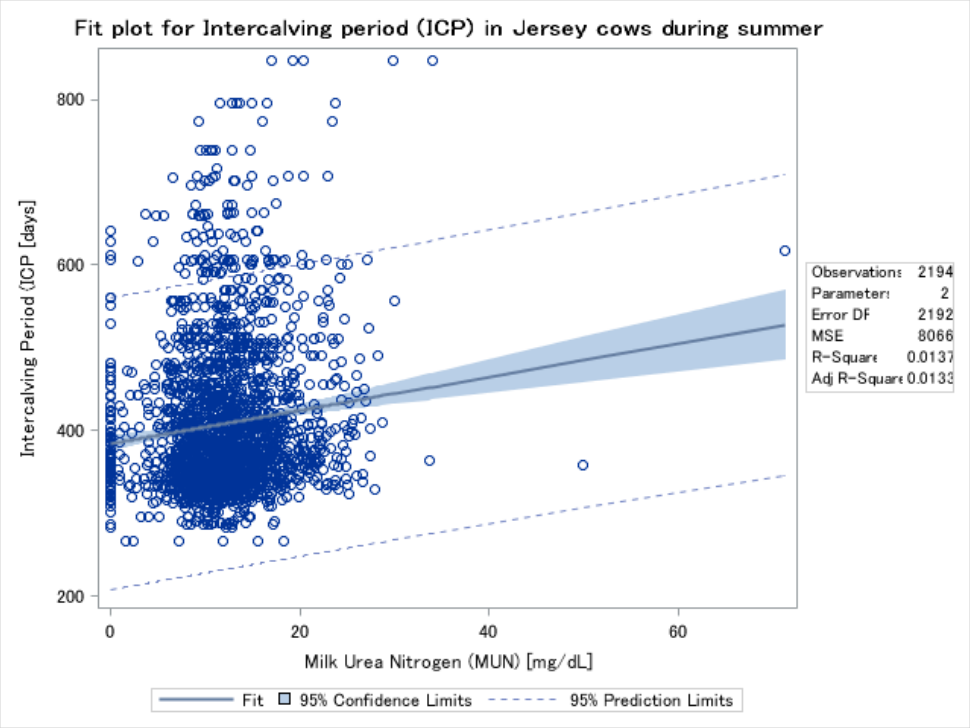

Figure 4.4.1 Scatterplot of Intercalving period (ICP) [days] by Milk urea nitrogen (MUN) [mg/dl] overlaid with the fit line in Jersey cows during summer, a 95% confidence interval and lower and upper 95% prediction limits**.**

In table 4.4.10 and 4.4.11 RP was the only parameter that was included in the model.

Table 4.4.10 Stepwise selection of reproduction parameters on Intercalving period (ICP) as measured performance of reproduction in Holstein cows and winter.

Reproduction Parameters		**Number In**	**Score Chi-Square**	**Wald Chi-Square**	**p-value**
Entered	**Removed**				
Reproduction performance (RP)	-	1	184.10	-	<0.0001
Calving number	-	2	12.50	-	0.0004
-	Calving number	1	-	0.12	0.7282

entry: 0.05

stay: 0.01

Table 4.4.11 Stepwise selection of reproduction parameters on Intercalving period (ICP) as measured performance of reproduction in Jersey cows and winter.

Reproduction Parameters		**Number In**	**Score Chi-Square**	**Wald Chi-Square**	**p-value**
Entered	**Removed**				
Reproduction performance (RP)	-	1	656.19	-	<0.0001
Calving interval	-	2	37.03	-	<0.0001
-	Calving interval	1	-	0.07	0.7856

entry: 0.05

stay: 0.01

In table 4.4.12 all parameters were considered and entered the model, however, none fit the model completely. The analysis provides evidence to indicate that these parameters could be important in the formulation of the diets of Holstein cows during the winter. But they were not significant enough to warrant specific emphasis. If these parameters were balanced in the diet, then it should not be expected that they would harm the reproduction success of a herd.

In table 4.4.13 all the nutritional parameters were included in the model. Just as was seen in table 4.3.12, RDP was the most significant parameter in the model. This indicated that when formulating the diet of Jersey cows during the winter the protein inclusion rate, specifically RDP, was important in order to ensure optimal reproduction occur in the herd.

Table 4.4.12 Stepwise selection of nutritional parameters on Intercalving period (ICP) as measured performance of reproduction in Holstein cows and winter.

Nutritional Parameters (DM Basis) **(Eligible for Entry)**	**Score Chi-Square**	**p-value**
Rumen degradable protein (RDP)	0.0005	0.9819
Crude protein (CP)	1.00	0.3181
Metabolizable energy (ME)	0.01	0.9216

entry: 0.05

stay: 0.01

Table 4.4.13 Stepwise selection of nutritional parameters on Intercalving period (ICP) as measured performance of reproduction in Jersey cows and winter.

Nutritional Parameters (DM Basis)		**Number**	**Score Chi-**	**Wald Chi-**	**p-value**
Entered	**Removed**	**In**	**Square**	**Square**	
Rumen degradable protein (RDP)	-	1	13.40	-	0.0003
Metabolizable energy (ME)	-	2	15.71	-	<0.0001
Crude protein (CP)	-	3	9.91	-	0.0016

entry: 0.05

stay: 0.01

In table 4.4.14 the only parameter entered in the model was total milk, however because it was not statistically significant to stay it was left out of the model. This indicated that none of the production parameters significantly affected reproduction in Holstein cows during winter. Although total milk needs to be occasionally checked to ensure that it is at an optimal level for reproduction success to occur successfully.

In table 4.4.15 total milk was the only parameter that was considered significant and included in the model. Milk urea nitrogen was not considered to be significant enough to enter the model. This indicates that during winter the level of MUN measured in Jersey cows do not significantly contribute to the success of reproduction.

Table 4.4.14 Stepwise selection of production parameters on Intercalving period (ICP) as measured performance of reproduction in Holstein cows and winter.

Production Parameters		**Number In**	**Score Chi-Square**	**Wald Chi-Square**	**p-value**
Entered	**Removed**				
Total milk	-	1	6.36	-	0.0117
-	Total milk	0	-	5.98	0.0145

entry: 0.05

stay: 0.01

Table 4.4.15 Stepwise selection of production parameters on Intercalving period (ICP) as measured performance of reproduction in Jersey cows and winter.

Production Parameters		**Number**	**Score Chi-Square**	**Wald Chi-Square**	**p-value**
Entered	**Removed**	**In**			
Total milk	-	1	44.77	-	<0.0001
Days in milk (DIM)	-	2	5.15	-	0.0232
-	Days in milk (DIM)	1	-	5.50	0.0190

entry: 0.05

stay: 0.01

In this section MUN concentration of Holstein cows did not significantly affect reproduction during either season. The results demonstrated that the inclusion of protein and NPN needs to be considered for the ration formulation in Jersey cows. Jersey cows had 12.5 ± 5.19 mg/dl MUN on average in summer, but 12.9 ± 5.24 mg/dl MUN on average for winter. On average the difference between summer and winter MUN concentrations in Jersey cows are relatively similar, however, only summer indicated significance for MUN. This could mean that Jersey cows have a lower threshold for MUN in terms of reproduction in summer than winter. Future research should therefore concentrate on investigating at which level MUN starts to negatively affect reproduction in Jersey cows, as well as other dairy breeds within season. As such future research needs to examine more closely the protein and NPN balance of Jersey cows, especially in summer. Another possible area of future research would be to investigate how MUN affects reproduction of cows and how this can be overcome without sacrificing growth and production, while also allowing the farmer to make a profit.

Another important finding was that in Jersey cows during the winter RDP was significant, even though MUN was not. Rumen degradable protein is broken down to ammonia in the rumen and is converted to urea in the liver (BUN) (Butler, 2005b). Blood urea nitrogen decreases uterine pH which reduces embryo survival (Butler, 1998, 2000, 2003). As a water soluble molecule urea easily diffuses into oocytes before ovulation, therefore compromising oocyte quality and cleavage rates of blastocysts after fertilization (Butler, 2005b; Tamminga, 2006). This distribution of urea into oocytes and the uterus would not show in MUN, this could explain why MUN was not found to be significant in winter, but RDP was. Since BUN was not part of the measurements included in the data, its significance could not have been measured.

Kauffman & St-Pierre, (2001) reported that more UN was excreted per unit of MUN, than was expected as indicated by Jonker *et al.*, (1998) (UN [g/d] = 12.54 * MUN [mg/dl]) . They also found that as CP increased, the efficiency of excreting N into the milk decreased while urinary excretion increased. The efficiency of excreting N into the milk decreased from 52.3% in diets containing 13% CP to 42.7% in diets containing 17% CP. In this study the average CP in the diet for Jerseys during winter was 18.0 ± 1.29%. Since UN was not measured in this study it is possible that urea originating from RDP was excreted through the urinary tract instead of the mammary gland which would explain the absence of MUN included in the model for winter.

This indicated that the Jersey cow's reproductive process was sensitive to protein and NPN inclusion in the diet. High concentrations of protein and NPN could be detrimental to reproduction success during both seasons although it was only expressed through MUN in the summer. One of the limitations of this study is the lack of studies and research that have been conducted that focusses on the effect of breed and season interaction and how this interaction together with MUN may affect reproductive success in Jersey cows and other dairy cows.

Chapter 5: Conclusion and critical evaluation

5.1 Conclusion

This study set out to investigate the concentrations of milk urea nitrogen (MUN) in South African dairy cows with the purpose of determining the effects of MUN on reproduction. The results from this study indicates that MUN significantly affects reproduction in dairy cows in South Africa, and a positive relationship exists between MUN and intercalving period (ICP) which is undesirable from a reproduction perspective. Reproduction performance (RP) and reproductive index (RI) have also been plotted against MUN to illustrate the negative relationships. Calving number shares a positive (but not significant) relationship with MUN and a negative relationship with RI. Increasing concentrations of MUN lead to an increase in RI which indicated poorer reproduction. Therefore, it was concluded that MUN significantly effects reproduction. The study suggests that a better understanding of the interaction between MUN and reproduction could lead to preventative measures to improve reproduction in dairy cows without compromising milk production.

The second major finding was that the effects of MUN was less significant in Holstein cows compared to Jersey cows. This may be due to the intense scrutinization of Holstein cow diets seen on farm as the most popular dairy breed. This intense management could have made the effect of MUN on reproduction less significant and lead to the results found in this study. However, this does not mean that more protein and NPN could be included in the diet than there already was. If too much dietary protein and NPN are included it could lead to a decrease in reproduction, due to the adverse effects of excess MUN on the gonadotrophic axes and hence reproduction. Holstein cows had an average MUN value that is less than the threshold proposed by Tamminga, (2006). Another reason for this is that Holstein dairy cows are mainly on TMR systems where each mouthful of feed is a properly balanced diet. This could indicate that the level of MUN was not high enough to indicate significance as it was not high enough to negatively affect reproduction. Therefore, although no significance was observed in this study for MUN in Holstein cows, it does not mean that nutritionist should not be cautious about excess MUN in Holstein cows. Future research should assess the impact of MUN in Holstein cows fed diets with higher rates of protein and NPN inclusion, so that it can be determined at what level MUN starts to negatively impact reproduction of Holstein cows in South African conditions.

The results of this investigation show that MUN significantly affected reproduction parameters in Jersey cows and needs to be well managed in order to improve reproduction. Therefore, nutritionists should re-evaluate the inclusion rate of protein and NPN in the diets of Jersey cows in order to improve reproduction. Jersey cows are primarily on pasture based systems where each mouthful of pasture is not a balanced diet which may lead to overfeeding protein and underfeeding energy. Jersey cows are generally smaller and have a much lower genetic capacity for milk production than Holstein cows, which makes them more vulnerable because their diets are more variable due to higher roughage intake and more variable concentrate supplementation. Although Holstein cows had higher concentrations of MUN than Jersey cows on average, MUN was not significant in Holstein cows. From the results of this study it appears that Jersey cows may have

a lower threshold for MUN in terms of reproduction than Holstein cows. Further studies are needed to determine at what level MUN starts to negatively affect reproduction in Jersey cows, as well as other dairy breeds. Investigations should focus on how MUN affects reproduction and how this can be overcome without sacrificing growth and production, and thus profit for the farmer.

During summer dairy cows have been shown to frequently experience heat stress which may lead to metabolic disorders and make dairy cows more susceptible to imbalanced diets that quickly affect reproduction. It has been shown that MUN and protein, together with metabolizable energy (ME) significantly affect reproduction parameters. The evidence from this study suggests that the balance between protein and ME may be just as important as the individual characteristics. Dairy cows can be said to be sensitive for protein and NPN inclusion in terms of reproduction in both seasons, but during summer months they need enough ME to be included in the diet as well. In South Africa summer temperatures were constantly above the thermoneutral zone for bovine, which was why dairy cows frequently experience heat stress. Heat stress ensures that dairy cows use energy for temperature regulation in an attempt to maintain homeostasis, instead of production. This could be why during summer ME was indicated as important, dietary ME intake in summer is higher because more energy dense diets are fed due to the adverse effects of heat stress on dry matter intake. Milk urea nitrogen was also indicated as significant during summer. This suggests that the level of MUN is important and may affect reproduction during summer. The effect of MUN on reproduction may be worsened when considering heat stress, making it very difficult for a cow to produce a calf and enter a new lactation cycle. Both summer and winter temperatures are steadily increasing in parts of South Africa. Therefore, sustainable strategies to reduce heat stress and MUN concentrations in dairy cows are very important in improving reproduction in dairy cows. It was also shown that the effects of the level of MUN on reproduction parameters measured during summer was more significant than during winter. This could be because during winter dairy cows were closer to their thermoneutral zone in which climatic stress was reduced, thereby improving their reproductive ability. However, RDP has a small but significant effect during winter which indicates that overfeeding of RDP may have detrimental effects on reproduction success.

In Holstein cows MUN was not significant in either season. The reason for this may be that Holstein cows were intensively managed and that MUN concentrations were not high enough to negatively impact reproduction in this study. Protein should not be included at concentrations higher than what is recommended in order to ensure that reproduction is not negatively affected. In Jersey cows MUN was significant only during summer. Milk urea nitrogen concentrations were similar in both seasons in Jerseys. The significance in summer may indicate that the threshold for summer is lower than in winter. This should be included in future studies to determine at what level the breed and season interaction together with MUN negatively affects reproduction. It was shown that ME was still important in summer and could indicate that together with protein and NPN should not exceed optimal concentrations for reproduction to occur successfully. During winter protein in the form of RDP was significant although MUN was not significant. It was previously suggested that urea was distributed to the oocytes and uterus of cows instead of the milk in cows, whith adverse effects on reproduction of Jersey cows, if protein and NPN concentrations exceed that which was recommended.

The findings of this study have a number of important implications for future practice. It was shown that MUN did not only influence reproduction, but that its affects differed between breed and season. There are still many questions that this study was unable to answer due to a lack of more comprehensive data. It is conceded that there are still a number of remaining questions, but the present study makes several noteworthy contributions to understanding how MUN affects reproduction and more specifically breed and seasonal effects and interactions. It is clear that more research is needed to understand how the environment interacts with nutrition to affect reproduction. This study has demonstrated that it is important for farmers to know the MUN concentrations in their herds (herd baseline MUN value) as it can lead to financial savings on feed according to Jonker *et al.*, (2002b).

5.2 Critical evaluation

This study focused on the effects of MUN as well as the related effects of breed, season and breed x season interactions on MUN and reproduction in dairy cows. Literature on the effects of MUN on reproduction parameters in dairy cattle is limited, especially for feeding systems in subtropical regions such as South Africa.

The data used for this study was a large dataset collected by the Milk Recording Scheme from a number of productive dairy herds over several years and seasons and consisted of repeated measures. This dataset was limited as a result of some parameters that were not always being well recorded, and missing values that may have affected the observed statistical effects. However, due to the great number of observations still included in the study, it was still possible for relevant trends to be projected and analysed. The evidence from this study suggests that the balance between protein and ME may be just as important as the individual characteristics. It was conceded that net energy (NE) values were not available for this data set, but ME values were available and included in the analyses. More research is required to address the relation between BUN, MUN and reproduction parameters in dairy cattle, and to confirm some of the observed effects of MUN on reproduction parameters observed in the present study. Although expensive, the specific effects and interaction of MUN and BUN on reproduction parameters may be better addressed by means of a completely randomised control study.

A limitation of the present study was the unavailability of more accurate estimates of cow age (in days), which would have made the calculation of reproductive index more accurate. The definition of reproduction could be expanded by including more parameters in future studies such as, the conception rate and number of times a cow was inseminated before conception. The data set used was recorded about ten years ago and future studies need to include more recent data which is based on current feeding practices and dairy cow genetics. Nevertheless, the present study provides very useful information about the effects and interactions between MUN and reproductive parameters of dairy cattle as affected by breed, season, year and cow age.

When the study was started it was expected that MUN would have an effect on reproduction in both breeds, just at different concentrations of significance. However, MUN was not significant in Holstein cows because they are fed properly balanced TMR's. It is unclear whether this is due to the measured MUN

concentrations being too low to have a negative effect on reproduction, or if that is an accurate representation of the interaction between MUN and reproduction in Holstein cows. Further studies with more balanced datasets need to be carried out to clear up this question.

As one of the first studies to be performed in South Africa on this topic it is clear that more research is needed to better evaluate these effects. Future studies could possibly include other variables with MUN such as energy measurements that better explain the use of ammonia for microbial synthesis (i.e. FME, starch or non-fiber carbohydrates). In this way other considerations such protein and energy balance can also be investigated for reproduction.

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Addendum A

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Natural and Agricultural Sciences
Ethics Committee

E-mail: ethics.nas@up.ac.za

Date: 9 March2018

ETHICS SUBMISSION: LETTER OF APPROVAL

Name of Applicant	Prof E C Webb (student Ms E de Bruyn)
Department	Animal and Wildlife Sciences
Reference number	EC171101-156
Title	Effects of milk urea nitrogen (MUN) on fertility of dairy cows

Dear Prof Webb

The submission conforms to the requirements of the NAS EC. Any amendments must be submitted to the NAS EC on a relevant application form as used for the original application quoting the reference number and detailing the required amendment. An amendment would be for example differentiating within the research target population.

You are required to submit a progress report no later than two months after the anniversary of this application as indicated by the reference number. The progress report document is accessible of the NAS faculty's website: Research/Ethics Committee.

You are required to notify the NAS EC upon the completion or ending of the project using the form Project Completed. Completion will be when the data has been analysed and documented in a postgraduate student's thesis or dissertation, or in a paper or a report for publication.

The digital archiving of data is a requirement of the University of Pretoria. The data should be accessible in the event of an enquiry or further analysis of the data.

The NAS EC wishes you well with your research project.

Yours sincerely,

p/p **Chairperson**
NAS Ethics Committee

Addendum B

SA HOLSTEIN – THE GLOBAL BREED

141 President Reitz Avenue / Laan • Hydropark 2 • Westdene
Box / Posbus 544 • Bloemfontein • South Africa / Suid Afrika • 9300

Tel: 051 4479123 • Fax / Faks: 051 4304224
email / epos: info@saholstein.co.za • website / webadres:www.saholstein.co.za

To Whom It May Concern. 2 March 2018

I hereby give consent on behalf of S A Holstein to Elandri de Bruyn to use the Holstein data concerning for her study of the effects of MUN on physiological parameters to better understand dairy production in typical South African conditions.

I wish you all the best.

Regards.

PH DUVENAGE
RASDIREKTEUR/BREED DIRECTOR

For profitability . . .
milk Jerseys
Vir winsgewendheid . . .
melk Jerseys
Tel: 051 430 8020 Fax: 051 430 7719
Website: www.jerseysa.co.za PO Box 100893 BRANDHOF 9324

JERSEY SA

2018-03-06

Elandri de Bruyn

RE: DATA REQUEST

Permission is hereby given for the use of Jersey SA data, as specified in your request letter to the society, towards the completion of your MSc project under the supervision of Prof E.C. Webb at the University of Pretoria.

Jersey SA would like to request a copy of the final report on your research.

Wishing you the best for your endeavour.

Tessa Opperman
Chief Admin Officer

Addendum C

Gmail **Elandri de Bruyn <elan3db@gmail.com>**

Fw: Fw: Aanvaarding: Studentesimposium

1 message

Pretorius, Rudi <Pretorw@unisa.ac.za> Fri, Oct 5, 2018 at 12:54 AM
To: Rudi Pretorius <pretorius_rudi@yahoo.com>

Geagte deelnemer

Dit is vir my aangenaam om jou in kennis te stel dat jou voorlegging om aan die jaarlikse simposium vir nagraadse studente in die natuurwetenskappe deel te neem, in orde is. Jy kan dus met jou voorbereidings voortgaan en jou aanbieding regkry.

Indien nog nie gedoen nie, versoek ons jou aandag aan die volgende drie sake (indien reeds gedoen, ignoreer asb):
1) Betaling van registrasiefooi - Laat weet ons as jy 'n faktuur benodig en onthou om die betaalbewys vir ons aan te stuur.
2) Motivering deur studieleier - 'n bevestiging dat die studieleier weet dat jy gaan deelneem, dit goedkeur en jou ondersteun.
3) Lang Afrikaanse en kort Engelse opsomming – indien nog nie gelaai, moet dit asseblief teen middel Oktober gedoen wees.

Indien verstellings aan jou opsommings nodig is of nog inligting benodig word, sal ons jou daaroor kontak.

Ons fokus tans om die kort Afrikaanse opsommings vir die programboek reg te kry. Jou kort opsomming is nou reeds deur ons afgelaai. Verdere veranderings daaraan is nie meer moontlik nie, tensy absoluut noodsaaklik, en dan moet jy ons direk daarvan per e-pos laat weet.

Ons sien baie daarna uit om jou by die simposium te ontvang!

Vriendelike groete

Prof Rudi Pretorius
Pretorius_rudi@yahoo.com
0847275022

Rudi Pretorius
Department of Geography, Unisa
Room 035, Calabash, Science Campus
Office Tel: 011-471-3680
Cell: 084 7275022
Email: pretorw@unisa.ac.za

Invloed van melk ureum stikstof op reproduksie parameters van melkkoeie in Suid-Afrika

E de Bruyn en EC Webb

Departement van Vee- en Wildkunde, Universiteit van Pretoria

elan3db@gmail.com

Gevorderd

MScAgric (Produksie Fisiologie en Produk Kwaliteit)

Melk ureum stikstof (MUS) word as ‘n maatstaaf gebruik om die proteïen vlak in die voer van melkkoeie te meet. Die studie is gebaseer op data wat van die Landbou Navorsing Raad (LNR) ontvang is en dit is gebaseer op sowat 12000 waarnemings. Die data is aangeteken vanaf 2006 tot 2008 en bevat metings van hoë en lae produksie melkkoeie. In hierdie studie is die interaksies tussen die konsentrasie van MUS met reproduksie eienskappe van melkkoeie ondersoek. Interkalf periode (415.5±90.0 dae) is bereken en bestudeer. Daar is bevind dat MUS in Suid-Afrikaanse melkkoeie betekenisvol korreleer met hul interkalf periode (r^2=0.02). Waarnemings dui daarop dat koeie toenemend langer neem om dragtig te raak met toenemende konsentrasies van MUS. Twee meer komplekse repdrokusie eienskappe is bereken en hulle korrelasies met MUS is bepaal. Die eerste is reproduksie prestasie (69.5±12.49%) en die tweede is reproduksie indeks (108.5±2.95). Beide korreleer negatief met MUS (r^2=-0.02 en r^2=-0.10, onderskeidelik).

Addendum D

The SAS System

The MEANS Procedure

Analysis Variable : DIM

N	Mean	Std Dev	Minimum	Maximum
10956	200.9728003	133.6683737	2.0000000	1323.00

The SAS System

The MEANS Procedure

Analysis Variable : DIM

N	Mean	Variance	Std Dev	Std Error
10956	200.9728003	17867.23	133.6683737	1.2770346

The SAS System

The MEANS Procedure

Analysis Variable : T_MILK T#MILK

N	Mean	Std Dev	Minimum	Maximum
10953	23.7332694	8.4716317	0.4000000	70.7000000

The SAS System

The MEANS Procedure

Analysis Variable : T_MILK T#MILK

N	Mean	Variance	Std Dev	Std Error
10953	23.7332694	71.7685442	8.4716317	0.0809470

The SAS System

The MEANS Procedure

Analysis Variable : FAT FAT

N	Mean	Std Dev	Minimum	Maximum
10956	4.4542789	1.0419034	0.2900000	12.9000000

The SAS System

The MEANS Procedure

Analysis Variable : FAT FAT

N	Mean	Variance	Std Dev	Std Error
10956	4.4542789	1.0855628	1.0419034	0.0099541

The SAS System

The MEANS Procedure

Analysis Variable : PROT PROT

N	Mean	Std Dev	Minimum	Maximum
10955	3.4813939	0.7725546	0	13.6000000

The SAS System

The MEANS Procedure

Analysis Variable : PROT PROT

N	Mean	Variance	Std Dev	Std Error
10955	3.4813939	0.5968406	0.7725546	0.0073811

The SAS System

The MEANS Procedure

Analysis Variable : LACT LACT

N	Mean	Std Dev	Minimum	Maximum
10954	4.5444924	0.8360430	0	5.5300000

The SAS System

The MEANS Procedure

Analysis Variable : LACT LACT

N	Mean	Variance	Std Dev	Std Error
10954	4.5444924	0.6989679	0.8360430	0.0079881

The SAS System

The MEANS Procedure

Analysis Variable : CELLS CELLS

N	Mean	Std Dev	Minimum	Maximum
10956	674.6341731	1395.43	0	24995.00

The SAS System

The MEANS Procedure

Analysis Variable : CELLS CELLS

N	Mean	Variance	Std Dev	Std Error
10956	674.6341731	1947214.30	1395.43	13.3315567

The SAS System

The MEANS Procedure

Analysis Variable : UREA UREA

N	Mean	Std Dev	Minimum	Maximum
10928	14.3913580	5.6485727	0	71.2300000

The SAS System

The MEANS Procedure

Analysis Variable : UREA UREA

N	Mean	Variance	Std Dev	Std Error
10928	14.3913580	31.9063738	5.6485727	0.0540342

The SAS System

The MEANS Procedure

Analysis Variable : RDP__ RDP %%

N	Mean	Std Dev	Minimum	Maximum
9193	55.8580518	3.6756285	50.1600000	64.3300000

The SAS System

The MEANS Procedure

Analysis Variable : RDP__ RDP %%

N	Mean	Variance	Std Dev	Std Error
9193	55.8580518	13.5102451	3.6756285	0.0383357

The SAS System

The MEANS Procedure

Analysis Variable : CP__ CP %%

N	Mean	Std Dev	Minimum	Maximum
10956	17.1891064	1.1712546	14.8000000	19.4600000

The SAS System

The MEANS Procedure

Analysis Variable : CP__ CP %%

N	Mean	Variance	Std Dev	Std Error
10956	17.1891064	1.3718374	1.1712546	0.0111899

The SAS System

The MEANS Procedure

Analysis Variable : ME__MJ ME# MJ

N	Mean	Std Dev	Minimum	Maximum
10956	10.9508744	0.5944726	9.6000000	11.7400000

The SAS System

The MEANS Procedure

Analysis Variable : ME__MJ ME# MJ

N	Mean	Variance	Std Dev	Std Error
10956	10.9508744	0.3533977	0.5944726	0.0056794

The SAS System

The MEANS Procedure

Analysis Variable : ICP ICP

N	Mean	Std Dev	Minimum	Maximum
4538	415.4493169	90.2336336	248.0000000	943.0000000

The SAS System

The MEANS Procedure

Analysis Variable : ICP ICP

N	Mean	Variance	Std Dev	Std Error
4538	415.4493169	8142.11	90.2336336	1.3394799

The SAS System

The MEANS Procedure

Analysis Variable : _RP %%RP

N	Mean	Std Dev	Minimum	Maximum
4538	69.5284928	12.4921143	29.4803818	112.0967742

The SAS System

The MEANS Procedure

Analysis Variable : _RP %%RP

N	Mean	Variance	Std Dev	Std Error
4538	69.5284928	156.0529205	12.4921143	0.1854401

The SAS System

The MEANS Procedure

Analysis Variable : RI__ RI %%

N	Mean	Std Dev	Minimum	Maximum
10956	108.5302706	2.9462854	102.0000000	111.8012422

The SAS System

The MEANS Procedure

Analysis Variable : RI__ RI %%

N	Mean	Variance	Std Dev	Std Error
10956	108.5302706	8.6805978	2.9462854	0.0281481

The SAS System

The MEANS Procedure

Analysis Variable : C_ C#

N	Mean	Std Dev	Minimum	Maximum
10956	2.1213034	0.8482154	1.0000000	3.0000000

The SAS System

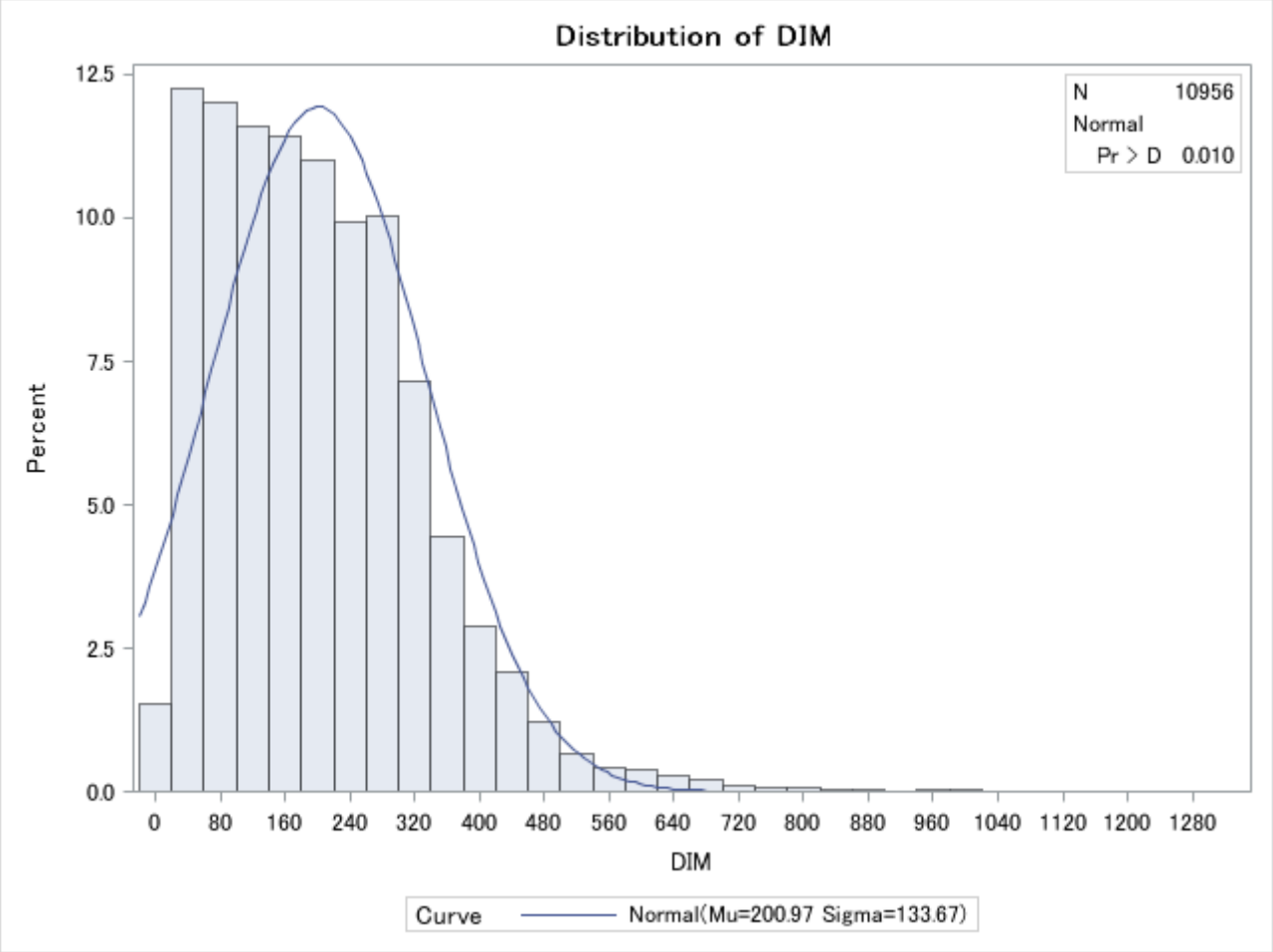
The MEANS Procedure

Analysis Variable : C_ C#

N	Mean	Variance	Std Dev	Std Error
10956	2.1213034	0.7194694	0.8482154	0.0081036

The SAS System

The UNIVARIATE Procedure



The SAS System

The UNIVARIATE Procedure
Fitted Normal Distribution for DIM (DIM)

Parameters for Normal Distribution

Parameter	Symbol	Estimate
Mean	Mu	200.9728
Std Dev	Sigma	133.6684

Goodness-of-Fit Tests for Normal Distribution

Test		Statistic		p Value
Kolmogorov-Smirnov	**D**	0.074353	**Pr > D**	<0.010
Cramer-von Mises	**W-Sq**	15.782159	**Pr > W-Sq**	<0.005
Anderson-Darling	**A-Sq**	118.376235	**Pr > A-Sq**	<0.005

Histogram Bin Percents for Normal Distribution

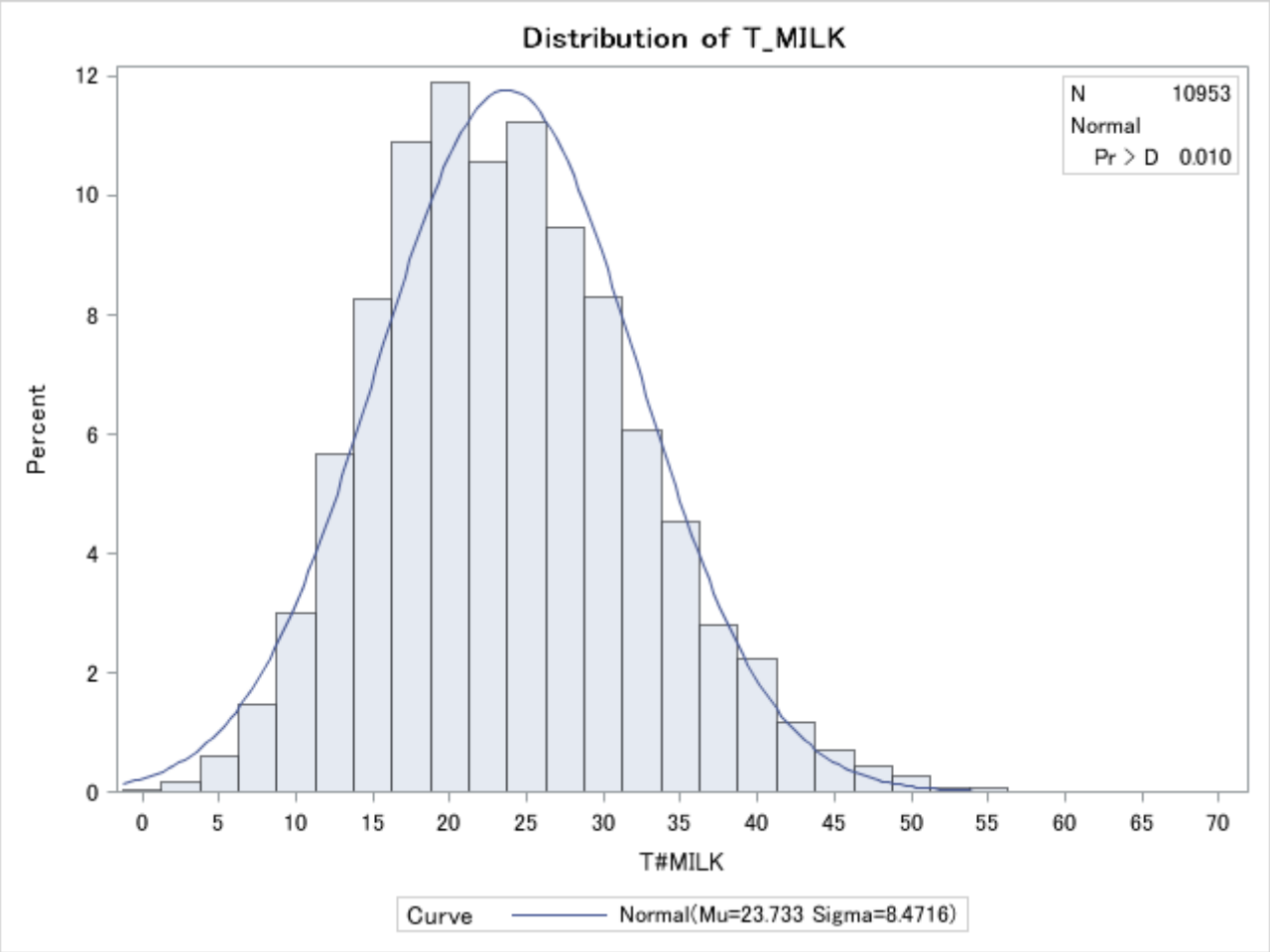
Bin Midpoint	Percent	
	Observed	Estimated
0	1.515	3.873
40	12.258	5.791
80	12.003	7.921
120	11.592	9.914
160	11.409	11.352
200	10.989	11.894
240	9.940	11.401
280	10.049	10.000
320	7.165	8.025
360	4.454	5.892
400	2.875	3.958
440	2.072	2.433
480	1.205	1.368
520	0.657	0.704
560	0.429	0.331
600	0.402	0.143
640	0.274	0.056
680	0.201	0.020
720	0.110	0.007
760	0.082	0.002
800	0.073	0.001
840	0.027	0.000

Histogram Bin Percents for Normal Distribution

Bin Midpoint	Percent	
	Observed	Estimated
880	0.055	0.000
920	0.018	0.000
960	0.027	0.000
1000	0.027	0.000
1040	0.018	0.000
1080	0.018	0.000
1120	0.009	0.000
1160	0.009	0.000
1200	0.009	0.000
1240	0.009	0.000
1280	0.009	0.000
1320	0.009	0.000

Quantiles for Normal Distribution

Percent	Quantile	
	Observed	Estimated
20.0	80.0000	88.4747
40.0	148.0000	167.1083
60.0	220.0000	234.8373
80.0	301.0000	313.4709



The SAS System

The UNIVARIATE Procedure
Fitted Normal Distribution for T_MILK (T#MILK)

Parameters for Normal Distribution

Parameter	Symbol	Estimate
Mean	Mu	23.73327
Std Dev	Sigma	8.471632

Goodness-of-Fit Tests for Normal Distribution

Test		Statistic		p Value
Kolmogorov-Smirnov	**D**	0.0389008	**Pr > D**	<0.010
Cramer-von Mises	**W-Sq**	4.0328728	**Pr > W-Sq**	<0.005
Anderson-Darling	**A-Sq**	25.2350654	**Pr > A-Sq**	<0.005

Histogram Bin Percents for Normal Distribution

Bin Midpoint	Percent	
	Observed	Estimated
0.0	0.027	0.238
2.5	0.164	0.519
5.0	0.603	1.036
7.5	1.461	1.896
10.0	3.013	3.183
12.5	5.670	4.901
15.0	8.253	6.922
17.5	10.901	8.966
20.0	11.905	10.652
22.5	10.554	11.608
25.0	11.230	11.601
27.5	9.477	10.634
30.0	8.308	8.940
32.5	6.062	6.894
35.0	4.538	4.875
37.5	2.812	3.162
40.0	2.228	1.881
42.5	1.187	1.027
45.0	0.703	0.514
47.5	0.429	0.236
50.0	0.274	0.099
52.5	0.082	0.038
55.0	0.082	0.014
57.5	0.009	0.004
60.0	0.018	0.001
62.5	0.000	0.000
65.0	0.000	0.000
67.5	0.000	0.000

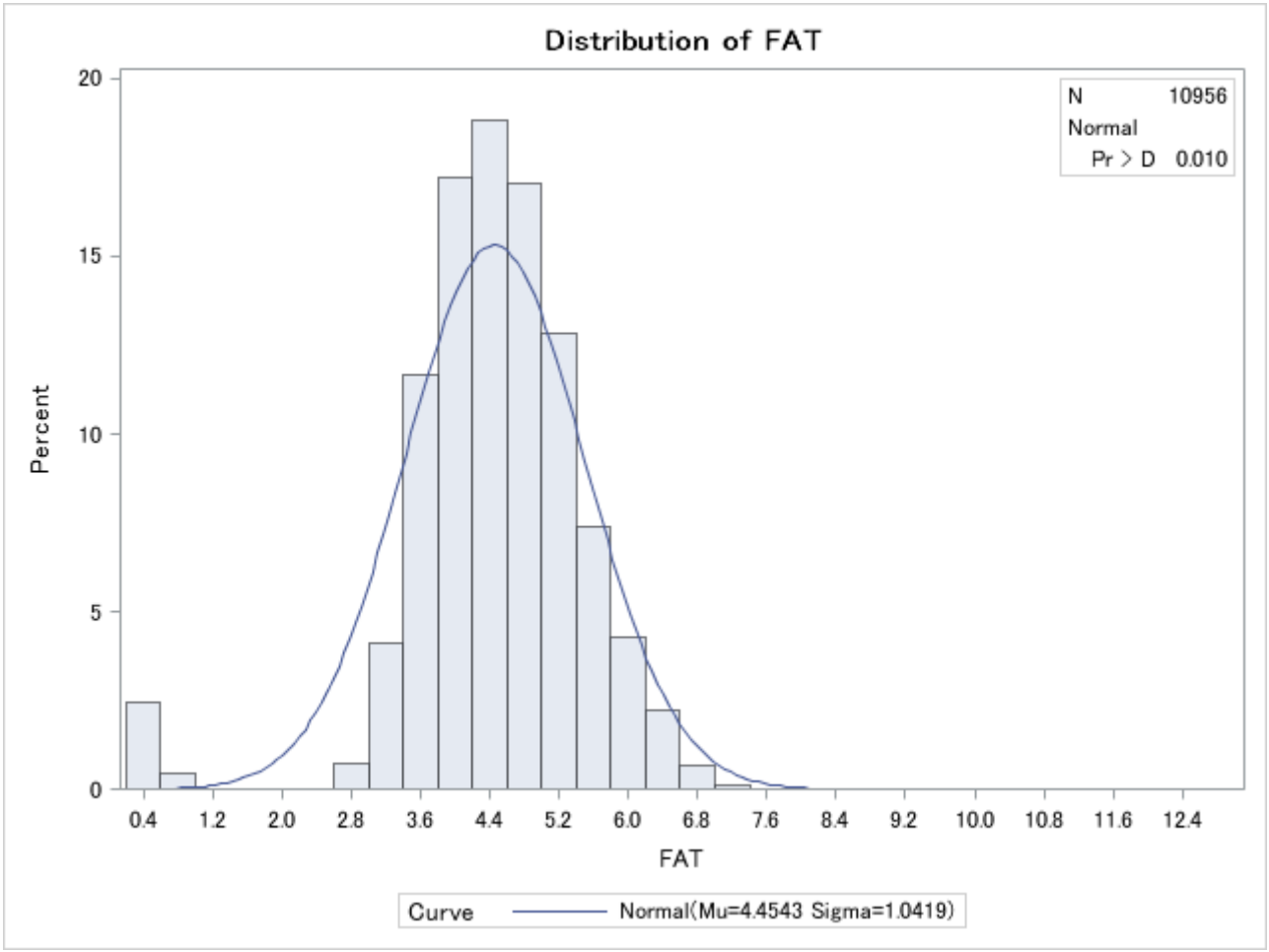
Histogram Bin Percents for Normal Distribution

Bin Midpoint	Percent	
	Observed	Estimated
70.0	0.009	0.000

Quantiles for Normal Distribution

Percent	Quantile	
	Observed	Estimated
20.0	16.5000	16.6034
40.0	20.8000	21.5870
60.0	25.4000	25.8795
80.0	30.7000	30.8632

The UNIVARIATE Procedure



The UNIVARIATE Procedure
Fitted Normal Distribution for FAT (FAT)

Parameters for Normal Distribution

Parameter	Symbol	Estimate
Mean	Mu	4.454279
Std Dev	Sigma	1.041903

Goodness-of-Fit Tests for Normal Distribution

Test		Statistic		p Value
Kolmogorov-Smirnov	**D**	0.084201	**Pr > D**	<0.010
Cramer-von Mises	**W-Sq**	19.581732	**Pr > W-Sq**	<0.005
Anderson-Darling	**A-Sq**	172.392169	**Pr > A-Sq**	<0.005

Histogram Bin Percents for Normal Distribution

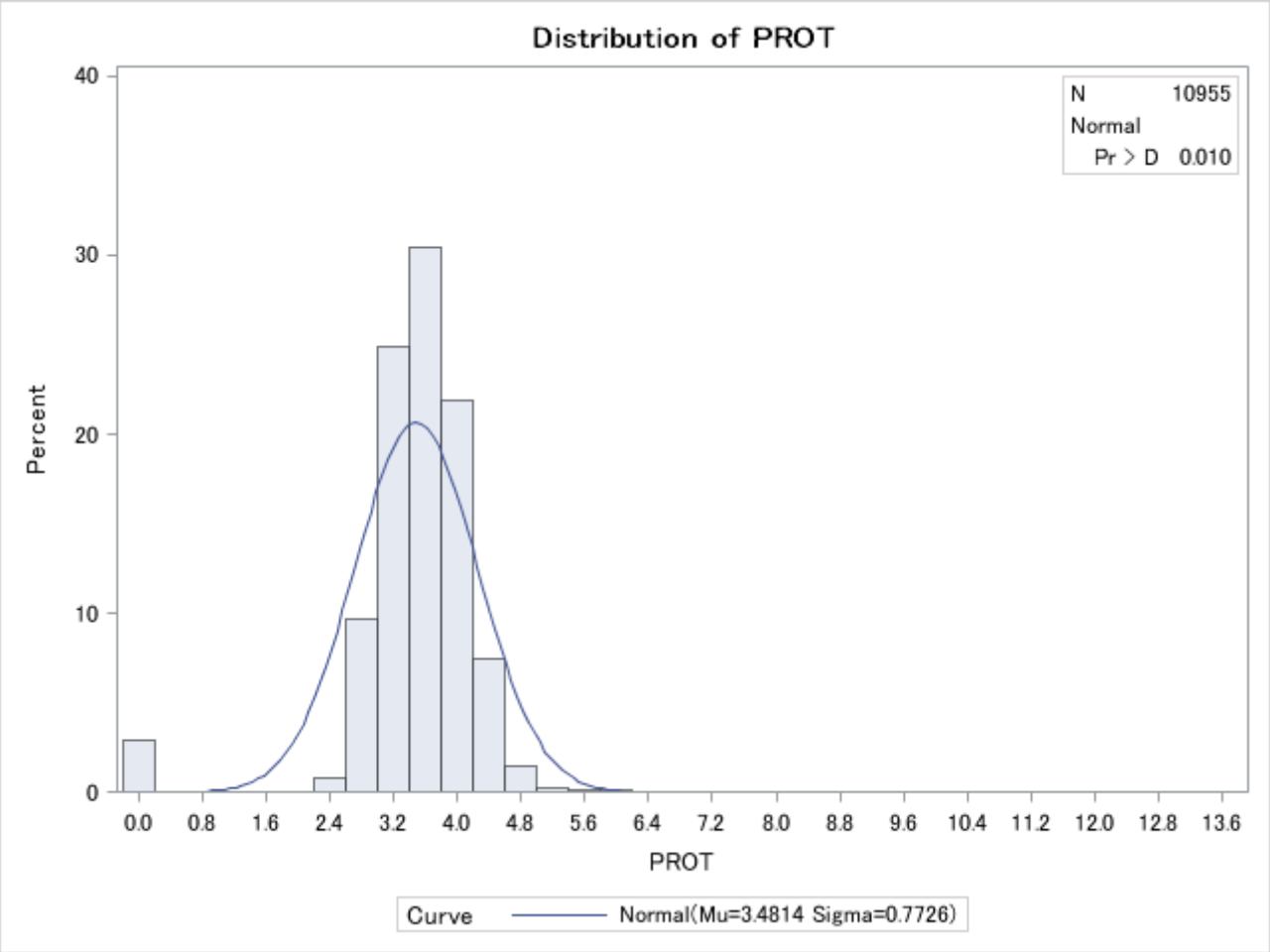
Bin Midpoint	Percent	
	Observed	Estimated
0.4	2.428	0.009
0.8	0.447	0.035
1.2	0.000	0.123
1.6	0.000	0.374
2.0	0.000	0.982
2.4	0.027	2.232
2.8	0.721	4.383
3.2	4.116	7.441
3.6	11.665	10.922
4.0	17.223	13.858
4.4	18.812	15.202
4.8	17.032	14.417
5.2	12.824	11.820
5.6	7.375	8.377
6.0	4.263	5.133

Histogram Bin Percents for Normal Distribution

Bin Midpoint	Percent	
	Observed	Estimated
6.4	2.227	2.719
6.8	0.666	1.245
7.2	0.110	0.493
7.6	0.009	0.169
8.0	0.009	0.050
8.4	0.009	0.013
8.8	0.000	0.003
9.2	0.018	0.001
9.6	0.000	0.000
10.0	0.000	0.000
10.4	0.009	0.000
10.8	0.000	0.000
11.2	0.000	0.000
11.6	0.000	0.000
12.0	0.000	0.000
12.4	0.000	0.000
12.8	0.009	0.000

Quantiles for Normal Distribution

Percent	Quantile	
	Observed	Estimated
20.0	3.81000	3.57739
40.0	4.26000	4.19032
60.0	4.70000	4.71824
80.0	5.21000	5.33117



The SAS System

The UNIVARIATE Procedure
Fitted Normal Distribution for PROT (PROT)

Parameters for Normal Distribution

Parameter	Symbol	Estimate
Mean	Mu	3.481394
Std Dev	Sigma	0.772555

Goodness-of-Fit Tests for Normal Distribution

Test		Statistic		p Value
Kolmogorov-Smirnov	**D**	0.132632	**Pr > D**	<0.010
Cramer-von Mises	**W-Sq**	68.407043	**Pr > W-Sq**	<0.005
Anderson-Darling	**A-Sq**	490.842330	**Pr > A-Sq**	<0.005

Histogram Bin Percents for Normal Distribution

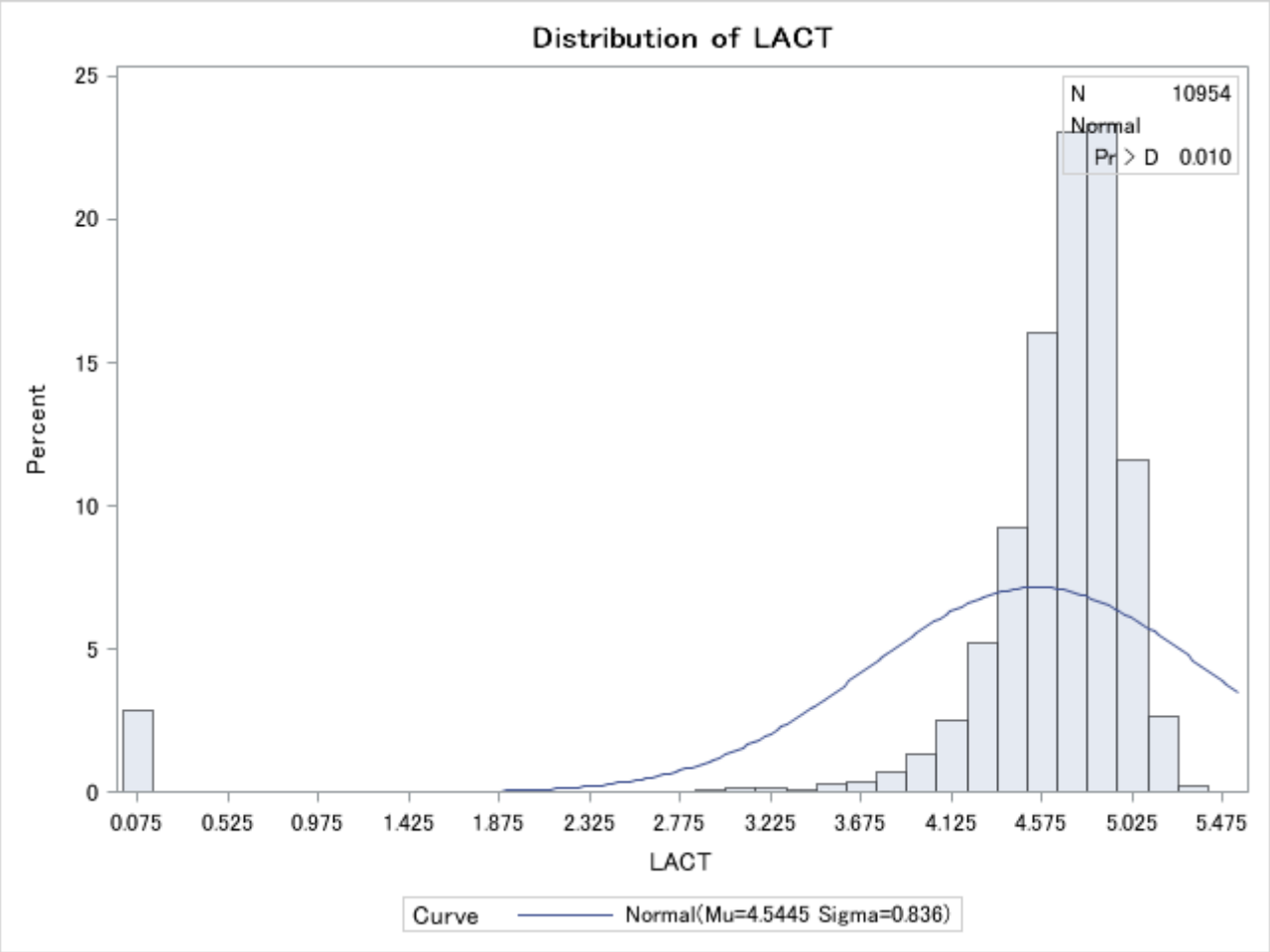
Bin Midpoint	Percent	
	Observed	Estimated
0.0	2.875	0.001
0.4	0.000	0.009
0.8	0.000	0.056
1.2	0.000	0.287
1.6	0.000	1.123
2.0	0.027	3.383
2.4	0.776	7.837
2.8	9.731	13.964
3.2	24.893	19.144
3.6	30.397	20.193
4.0	21.890	16.388
4.4	7.494	10.232
4.8	1.424	4.915
5.2	0.219	1.816
5.6	0.100	0.516
6.0	0.082	0.113
6.4	0.018	0.019
6.8	0.037	0.002
7.2	0.009	0.000
7.6	0.000	0.000
8.0	0.009	0.000
8.4	0.000	0.000
8.8	0.000	0.000
9.2	0.009	0.000
9.6	0.000	0.000
10.0	0.000	0.000
10.4	0.000	0.000
10.8	0.000	0.000

Histogram Bin Percents for Normal Distribution

Bin Midpoint	Percent	
	Observed	Estimated
11.2	0.000	0.000
11.6	0.000	0.000
12.0	0.000	0.000
12.4	0.000	0.000
12.8	0.000	0.000
13.2	0.000	0.000
13.6	0.009	0.000

Quantiles for Normal Distribution

Percent	Quantile	
	Observed	Estimated
20.0	3.12000	2.83120
40.0	3.42000	3.28567
60.0	3.68000	3.67712
80.0	3.97000	4.13159



The SAS System

The UNIVARIATE Procedure
Fitted Normal Distribution for LACT (LACT)

Parameters for Normal Distribution

Parameter	Symbol	Estimate
Mean	Mu	4.544492
Std Dev	Sigma	0.836043

Goodness-of-Fit Tests for Normal Distribution

Test		Statistic		p Value
Kolmogorov-Smirnov	**D**	0.27054	**Pr > D**	<0.010
Cramer-von Mises	**W-Sq**	320.54405	**Pr > W-Sq**	<0.005
Anderson-Darling	**A-Sq**	1757.48266	**Pr > A-Sq**	<0.005

Histogram Bin Percents for Normal Distribution

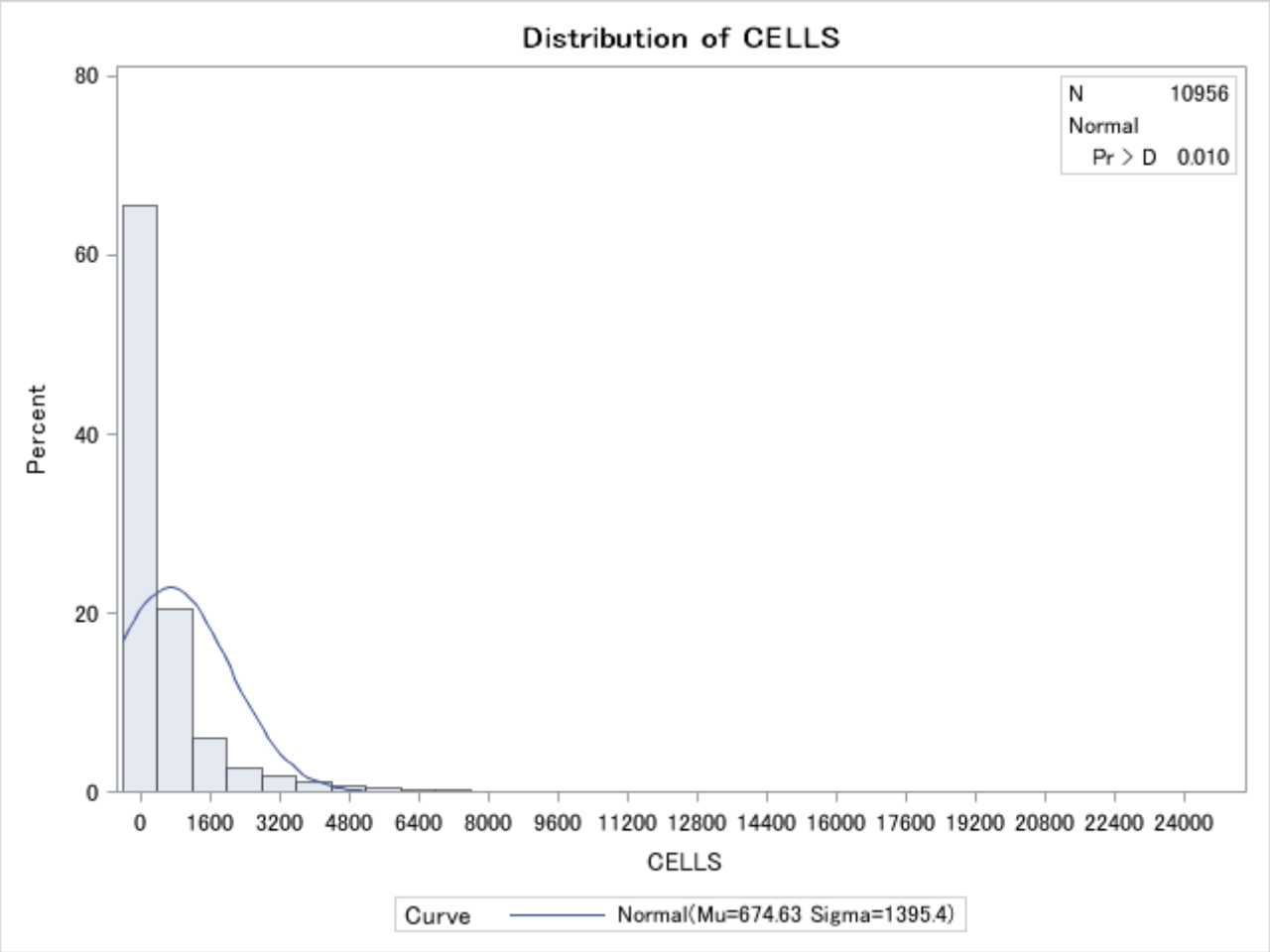
Bin Midpoint	Percent	
	Observed	Estimated
0.075	2.876	0.000
0.225	0.000	0.000
0.375	0.000	0.000
0.525	0.000	0.000
0.675	0.000	0.000
0.825	0.000	0.000
0.975	0.000	0.001
1.125	0.000	0.002
1.275	0.000	0.003
1.425	0.000	0.007
1.575	0.000	0.013
1.725	0.000	0.025
1.875	0.009	0.044
2.025	0.009	0.077
2.175	0.000	0.130
2.325	0.018	0.213
2.475	0.018	0.337
2.625	0.009	0.516
2.775	0.009	0.766
2.925	0.073	1.100
3.075	0.128	1.532
3.225	0.164	2.064
3.375	0.100	2.694
3.525	0.301	3.405
3.675	0.374	4.168
3.825	0.676	4.941
3.975	1.360	5.672
4.125	2.492	6.305

Histogram Bin Percents for Normal Distribution

Bin Midpoint	Percent	
	Observed	Estimated
4.275	5.240	6.787
4.425	9.230	7.076
4.575	16.022	7.143
4.725	23.051	6.984
4.875	23.343	6.612
5.025	11.603	6.063
5.175	2.620	5.383
5.325	0.237	4.629
5.475	0.037	3.854

Quantiles for Normal Distribution

Percent	Quantile	
	Observed	Estimated
20.0	4.45000	3.84086
40.0	4.65000	4.33268
60.0	4.78000	4.75630
80.0	4.91000	5.24812



The SAS System

The UNIVARIATE Procedure
Fitted Normal Distribution for CELLS (CELLS)

Parameters for Normal Distribution

Parameter	Symbol	Estimate
Mean	Mu	674.6342
Std Dev	Sigma	1395.426

Goodness-of-Fit Tests for Normal Distribution

Test		Statistic		p Value
Kolmogorov-Smirnov	**D**	0.31438	**Pr > D**	<0.010
Cramer-von Mises	**W-Sq**	354.88084	**Pr > W-Sq**	<0.005
Anderson-Darling	**A-Sq**	1783.31973	**Pr > A-Sq**	<0.005

Histogram Bin Percents for Normal Distribution

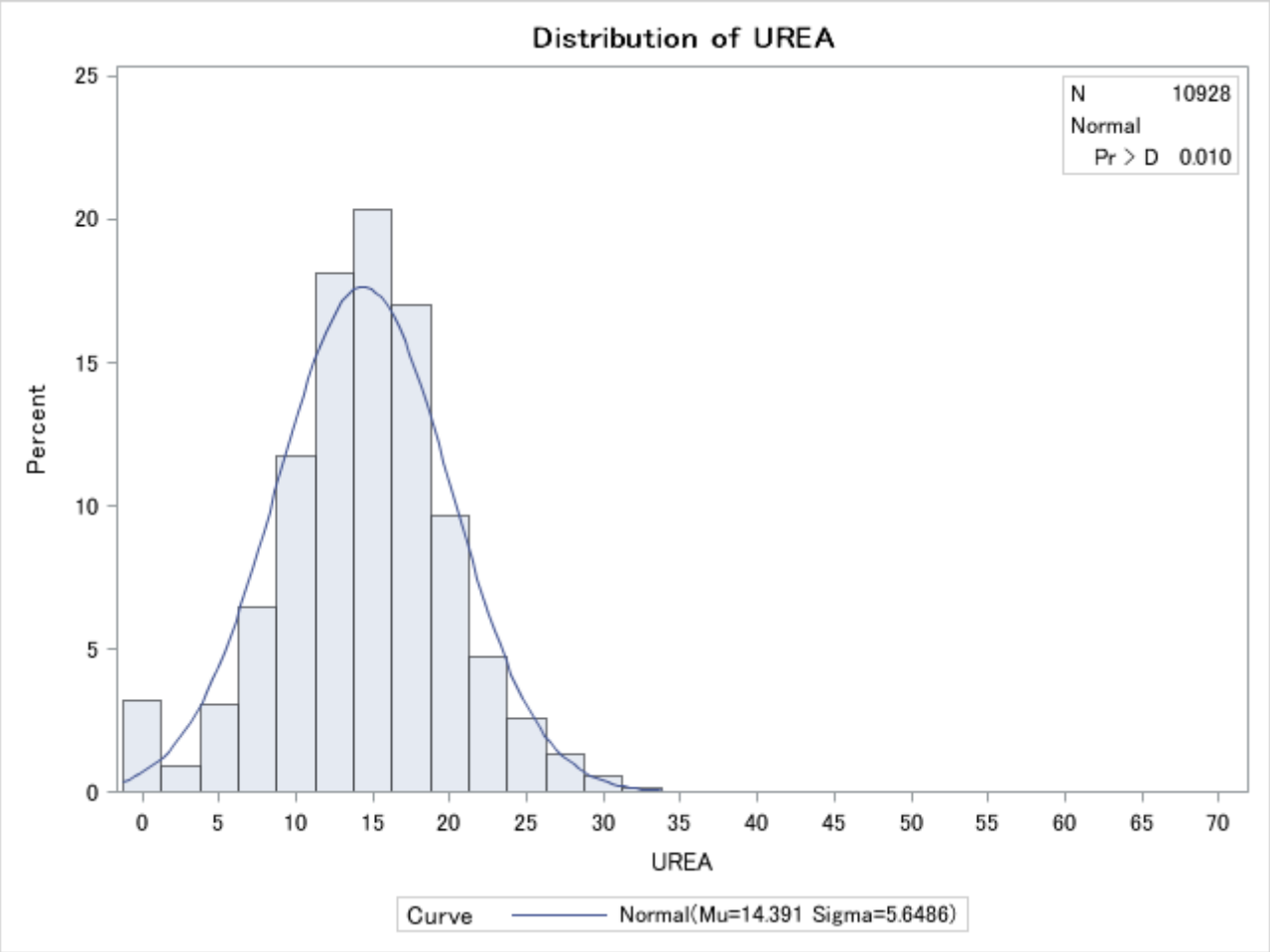
Bin Midpoint	Percent	
	Observed	Estimated
0	65.462	20.137
800	20.528	22.474
1600	6.033	18.217
2400	2.601	10.724
3200	1.707	4.584
4000	1.068	1.423
4800	0.748	0.320
5600	0.475	0.052
6400	0.301	0.006
7200	0.338	0.001
8000	0.137	0.000
8800	0.137	0.000
9600	0.064	0.000
10400	0.119	0.000
11200	0.091	0.000
12000	0.027	0.000
12800	0.009	0.000
13600	0.037	0.000
14400	0.027	0.000
15200	0.018	0.000
16000	0.009	0.000
16800	0.009	0.000
17600	0.000	0.000
18400	0.000	0.000
19200	0.027	0.000
20000	0.000	0.000
20800	0.000	0.000
21600	0.000	0.000

Histogram Bin Percents for Normal Distribution

Bin Midpoint	Percent	
	Observed	Estimated
22400	0.000	0.000
23200	0.009	0.000
24000	0.009	0.000
24800	0.009	0.000

Quantiles for Normal Distribution

Percent	Quantile	
	Observed	Estimated
20.0	71.0000	-499.786
40.0	153.0000	321.107
60.0	315.0000	1028.161
80.0	822.0000	1849.054



The SAS System

The UNIVARIATE Procedure
Fitted Normal Distribution for UREA (UREA)

Parameters for Normal Distribution

Parameter	Symbol	Estimate
Mean	Mu	14.39136
Std Dev	Sigma	5.648573

Goodness-of-Fit Tests for Normal Distribution

Test		Statistic		p Value
Kolmogorov-Smirnov	**D**	0.0350846	**Pr > D**	<0.010
Cramer-von Mises	**W-Sq**	4.9664529	**Pr > W-Sq**	<0.005
Anderson-Darling	**A-Sq**	35.4229269	**Pr > A-Sq**	<0.005

Histogram Bin Percents for Normal Distribution

Bin Midpoint	Percent	
	Observed	Estimated
0.0	3.184	0.719
2.5	0.933	1.979
5.0	3.084	4.496
7.5	6.451	8.422
10.0	11.768	13.010
12.5	18.119	16.574
15.0	20.315	17.414
17.5	17.030	15.090
20.0	9.672	10.783
22.5	4.704	6.355
25.0	2.562	3.089
27.5	1.354	1.238
30.0	0.567	0.409
32.5	0.183	0.111
35.0	0.037	0.025
37.5	0.000	0.005
40.0	0.009	0.001
42.5	0.009	0.000
45.0	0.000	0.000
47.5	0.000	0.000
50.0	0.009	0.000
52.5	0.000	0.000
55.0	0.000	0.000
57.5	0.000	0.000
60.0	0.000	0.000
62.5	0.000	0.000
65.0	0.000	0.000
67.5	0.000	0.000

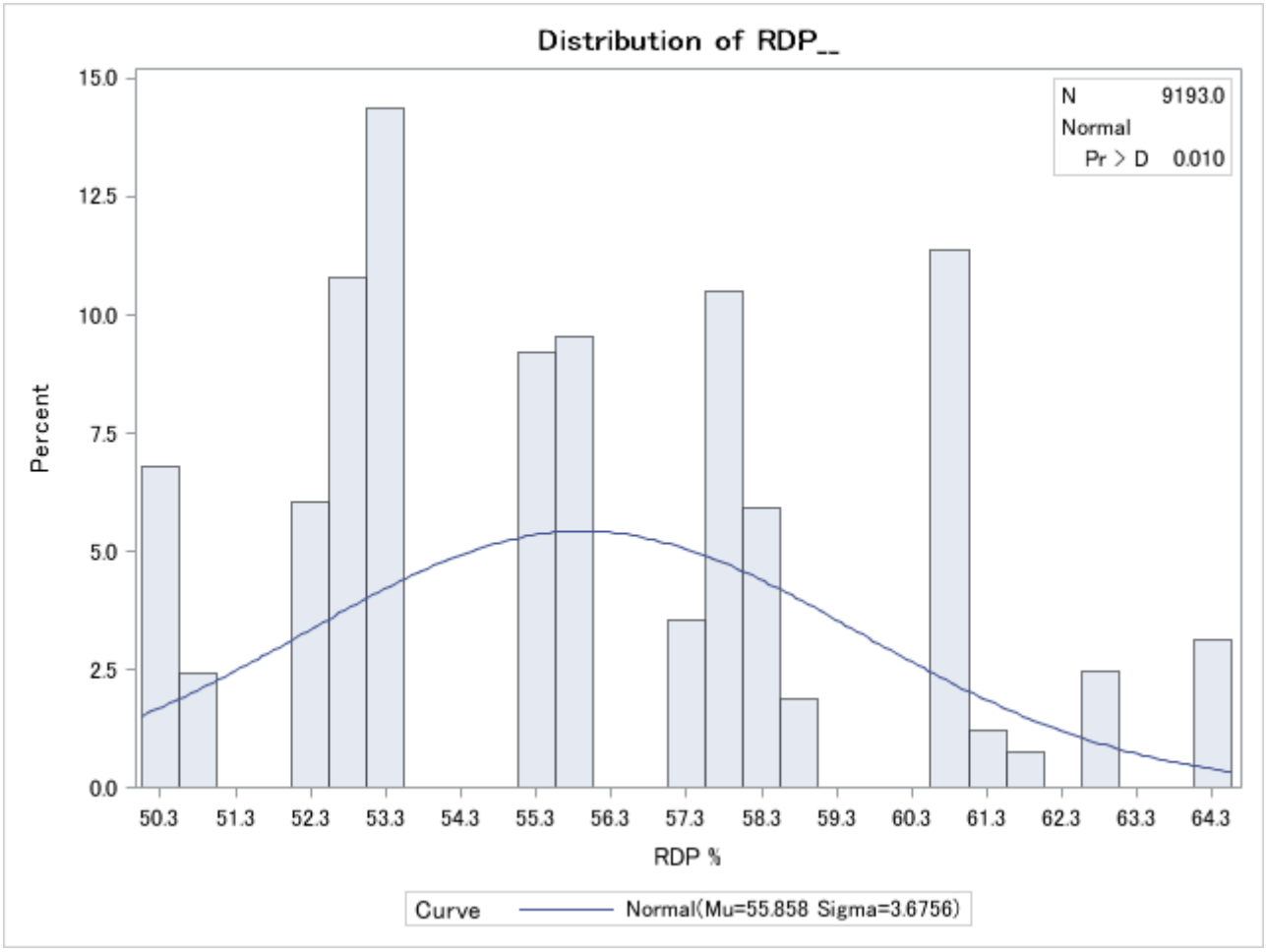
Histogram Bin Percents for Normal Distribution

Bin Midpoint	Percent	
	Observed	Estimated
70.0	0.009	0.000

Quantiles for Normal Distribution

Percent	Quantile	
	Observed	Estimated
20.0	10.2600	9.63740
40.0	13.2900	12.96031
60.0	15.7600	15.82241
80.0	18.5900	19.14532

The UNIVARIATE Procedure



The UNIVARIATE Procedure
Fitted Normal Distribution for RDP_ (RDP %)

Parameters for Normal Distribution

Parameter	Symbol	Estimate
Mean	Mu	55.85805
Std Dev	Sigma	3.675629

Goodness-of-Fit Tests for Normal Distribution

Test		Statistic		p Value
Kolmogorov-Smirnov	**D**	0.152609	**Pr > D**	<0.010
Cramer-von Mises	**W-Sq**	27.785039	**Pr > W-Sq**	<0.005
Anderson-Darling	**A-Sq**	168.431990	**Pr > A-Sq**	<0.005

Histogram Bin Percents for Normal Distribution

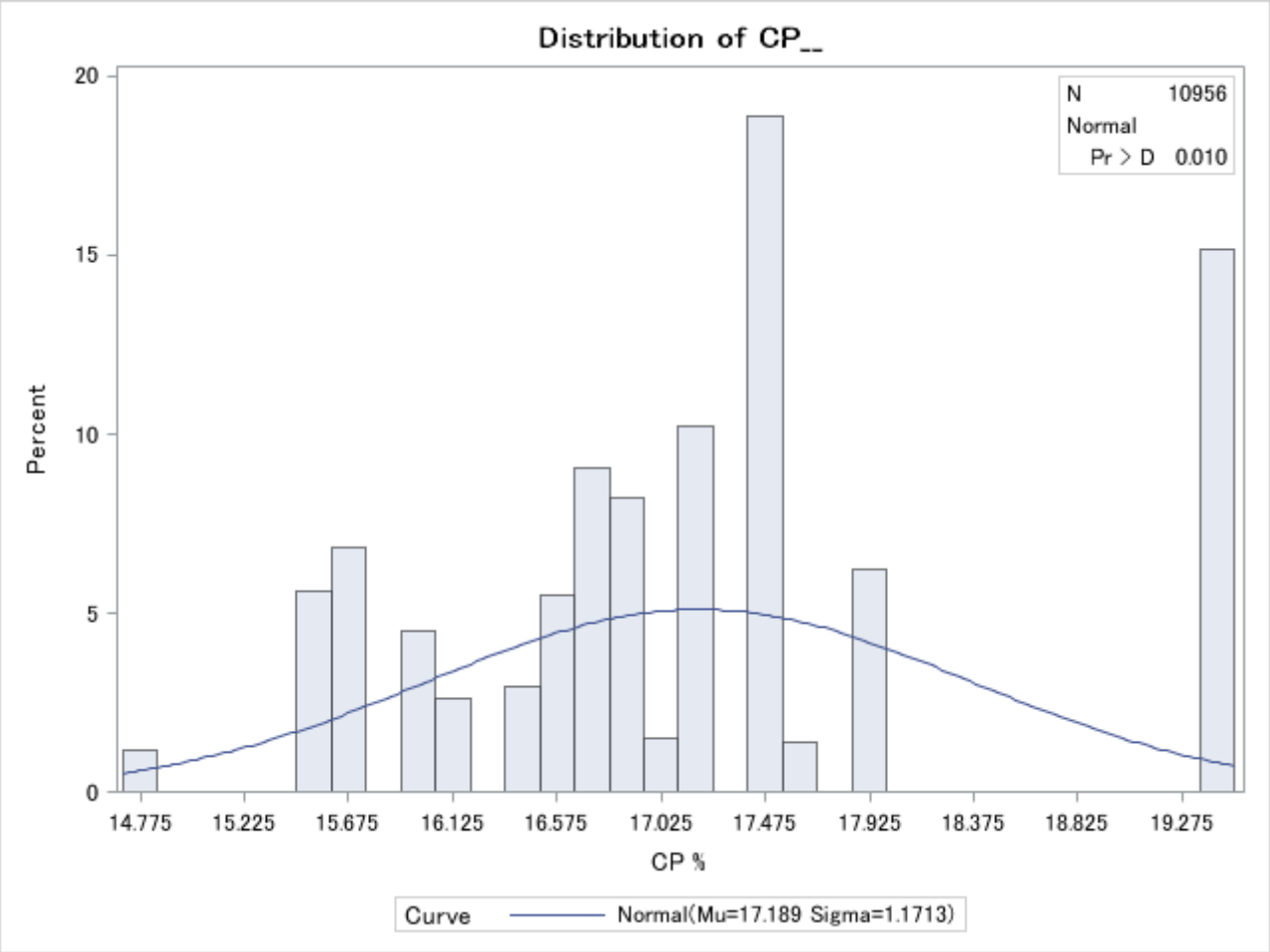
Bin Midpoint	Percent	
	Observed	Estimated
50.25	6.788	1.696
50.75	2.437	2.068
51.25	0.000	2.474
51.75	0.000	2.907
52.25	6.037	3.352
52.75	10.791	3.795
53.25	14.391	4.218
53.75	0.000	4.601
54.25	0.000	4.929
54.75	0.000	5.182
55.25	9.214	5.349
55.75	9.529	5.420
56.25	0.000	5.392
56.75	0.000	5.266
57.25	3.535	5.048

Histogram Bin Percents for Normal Distribution

Bin Midpoint	Percent	
	Observed	Estimated
57.75	10.508	4.751
58.25	5.928	4.389
58.75	1.893	3.981
59.25	0.000	3.545
59.75	0.000	3.098
60.25	0.000	2.659
60.75	11.378	2.240
61.25	1.197	1.852
61.75	0.772	1.504
62.25	0.000	1.198
62.75	2.469	0.937
63.25	0.000	0.720
63.75	0.000	0.543
64.25	3.133	0.402

Quantiles for Normal Distribution

Percent	Quantile	
	Observed	Estimated
20.0	52.5900	52.7646
40.0	53.4000	54.9268
60.0	57.1600	56.7893
80.0	58.5900	58.9515



The SAS System

The UNIVARIATE Procedure
Fitted Normal Distribution for CP_ (CP %)

Parameters for Normal Distribution

Parameter	Symbol	Estimate
Mean	Mu	17.18911
Std Dev	Sigma	1.171255

Goodness-of-Fit Tests for Normal Distribution

Test		Statistic		p Value
Kolmogorov-Smirnov	**D**	0.164141	**Pr > D**	<0.010
Cramer-von Mises	**W-Sq**	39.524429	**Pr > W-Sq**	<0.005
Anderson-Darling	**A-Sq**	313.415343	**Pr > A-Sq**	<0.005

Histogram Bin Percents for Normal Distribution

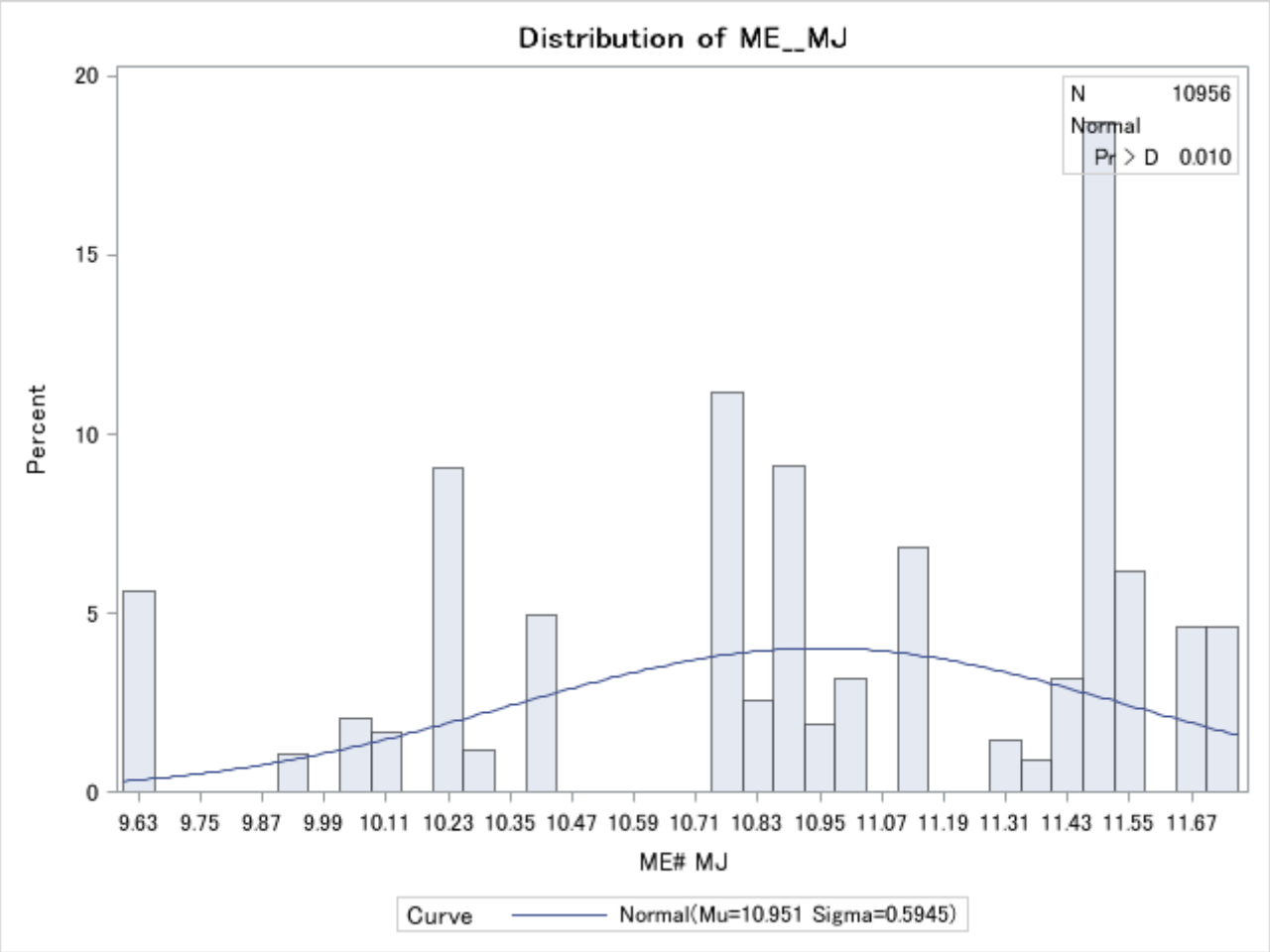
Bin Midpoint	Percent	
	Observed	Estimated
14.775	1.159	0.612
14.925	0.000	0.790
15.075	0.000	1.004
15.225	0.000	1.254
15.375	0.000	1.541
15.525	5.622	1.863
15.675	6.855	2.216
15.825	0.000	2.594
15.975	4.518	2.986
16.125	2.638	3.381
16.275	0.000	3.767
16.425	2.966	4.128
16.575	5.531	4.451
16.725	9.054	4.721
16.875	8.233	4.926
17.025	1.497	5.056
17.175	10.241	5.105
17.325	0.000	5.071
17.475	18.894	4.956
17.625	1.378	4.765
17.775	0.000	4.506
17.925	6.234	4.192
18.075	0.000	3.837
18.225	0.000	3.455
18.375	0.000	3.060
18.525	0.000	2.667
18.675	0.000	2.286
18.825	0.000	1.928

Histogram Bin Percents for Normal Distribution

Bin Midpoint	Percent	
	Observed	Estimated
18.975	0.000	1.599
19.125	0.000	1.305
19.275	0.000	1.048
19.425	15.179	0.828

Quantiles for Normal Distribution

Percent	Quantile	
	Observed	Estimated
20.0	16.0900	16.2034
40.0	16.8800	16.8924
60.0	17.4700	17.4858
80.0	17.8500	18.1749



The SAS System

The UNIVARIATE Procedure
Fitted Normal Distribution for ME_MJ (ME# MJ)

Parameters for Normal Distribution

Parameter	Symbol	Estimate
Mean	Mu	10.95087
Std Dev	Sigma	0.594473

Goodness-of-Fit Tests for Normal Distribution

Test		Statistic		p Value
Kolmogorov-Smirnov	**D**	0.151932	**Pr > D**	<0.010
Cramer-von Mises	**W-Sq**	44.556935	**Pr > W-Sq**	<0.005
Anderson-Darling	**A-Sq**	293.355919	**Pr > A-Sq**	<0.005

Histogram Bin Percents for Normal Distribution

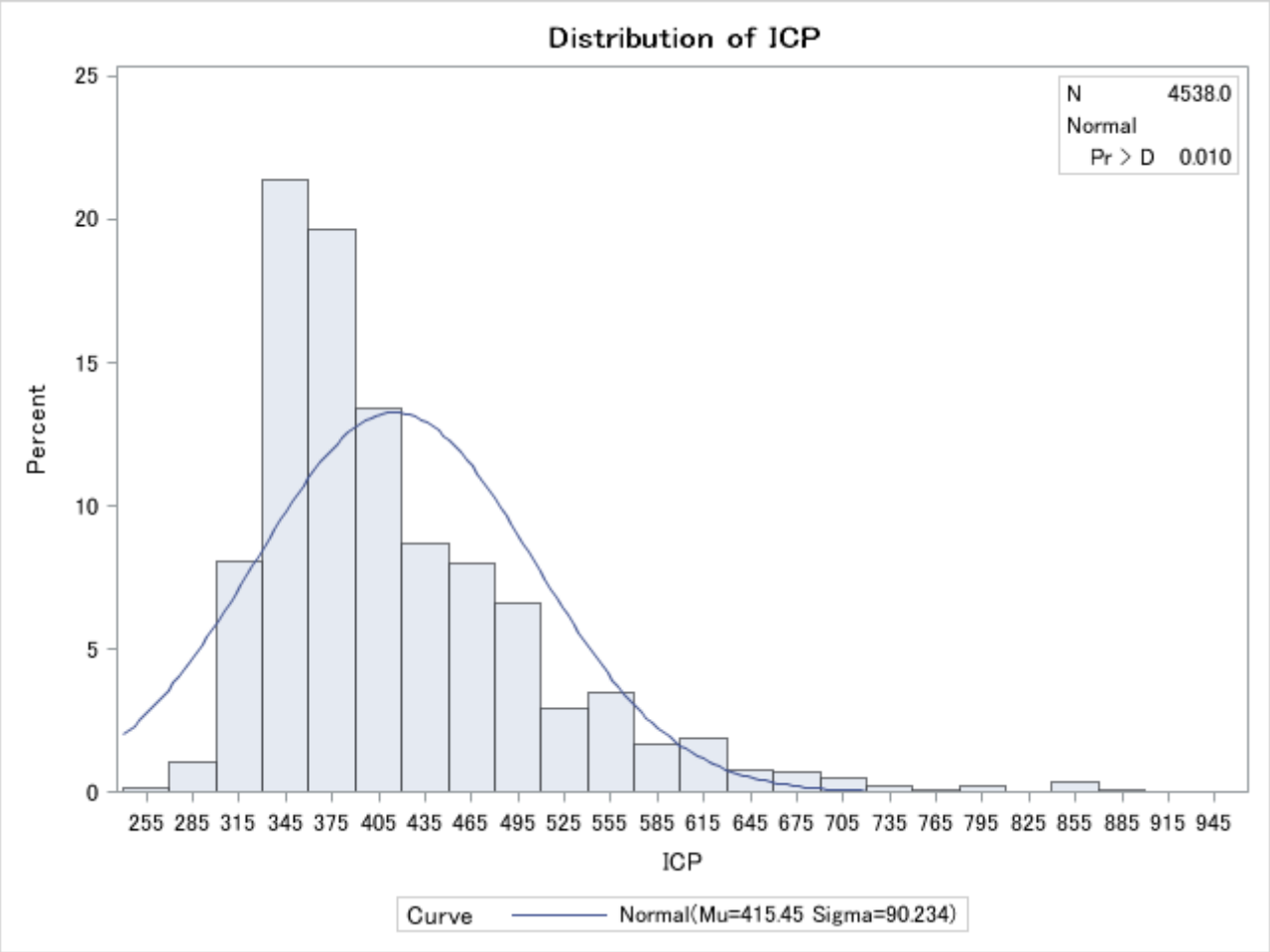
Bin Midpoint	Percent	
	Observed	Estimated
9.63	5.622	0.342
9.69	0.000	0.425
9.75	0.000	0.524
9.81	0.000	0.639
9.87	0.000	0.772
9.93	1.068	0.922
9.99	0.000	1.091
10.05	2.045	1.278
10.11	1.698	1.481
10.17	0.000	1.700
10.23	9.054	1.931
10.29	1.159	2.171
10.35	0.000	2.416
10.41	4.974	2.662
10.47	0.000	2.903
10.53	0.000	3.133
10.59	0.000	3.348
10.65	0.000	3.541
10.71	0.000	3.708
10.77	11.163	3.843
10.83	2.556	3.943
10.89	9.109	4.004
10.95	1.880	4.025
11.01	3.158	4.005
11.07	0.000	3.945
11.13	6.855	3.846
11.19	0.000	3.712
11.25	0.000	3.547

Histogram Bin Percents for Normal Distribution

Bin Midpoint	Percent	
	Observed	Estimated
11.31	1.460	3.354
11.37	0.913	3.140
11.43	3.167	2.909
11.49	18.702	2.669
11.55	6.179	2.423
11.61	0.000	2.178
11.67	4.628	1.938
11.73	4.609	1.706

Quantiles for Normal Distribution

Percent	Quantile	
	Observed	Estimated
20.0	10.3000	10.4506
40.0	10.8800	10.8003
60.0	11.1300	11.1015
80.0	11.5000	11.4512



The SAS System

The UNIVARIATE Procedure
Fitted Normal Distribution for ICP (ICP)

Parameters for Normal Distribution

Parameter	Symbol	Estimate
Mean	Mu	415.4493
Std Dev	Sigma	90.23363

Goodness-of-Fit Tests for Normal Distribution

Test		Statistic		p Value
Kolmogorov-Smirnov	**D**	0.128534	**Pr > D**	<0.010
Cramer-von Mises	**W-Sq**	26.540274	**Pr > W-Sq**	<0.005
Anderson-Darling	**A-Sq**	154.621510	**Pr > A-Sq**	<0.005

Histogram Bin Percents for Normal Distribution

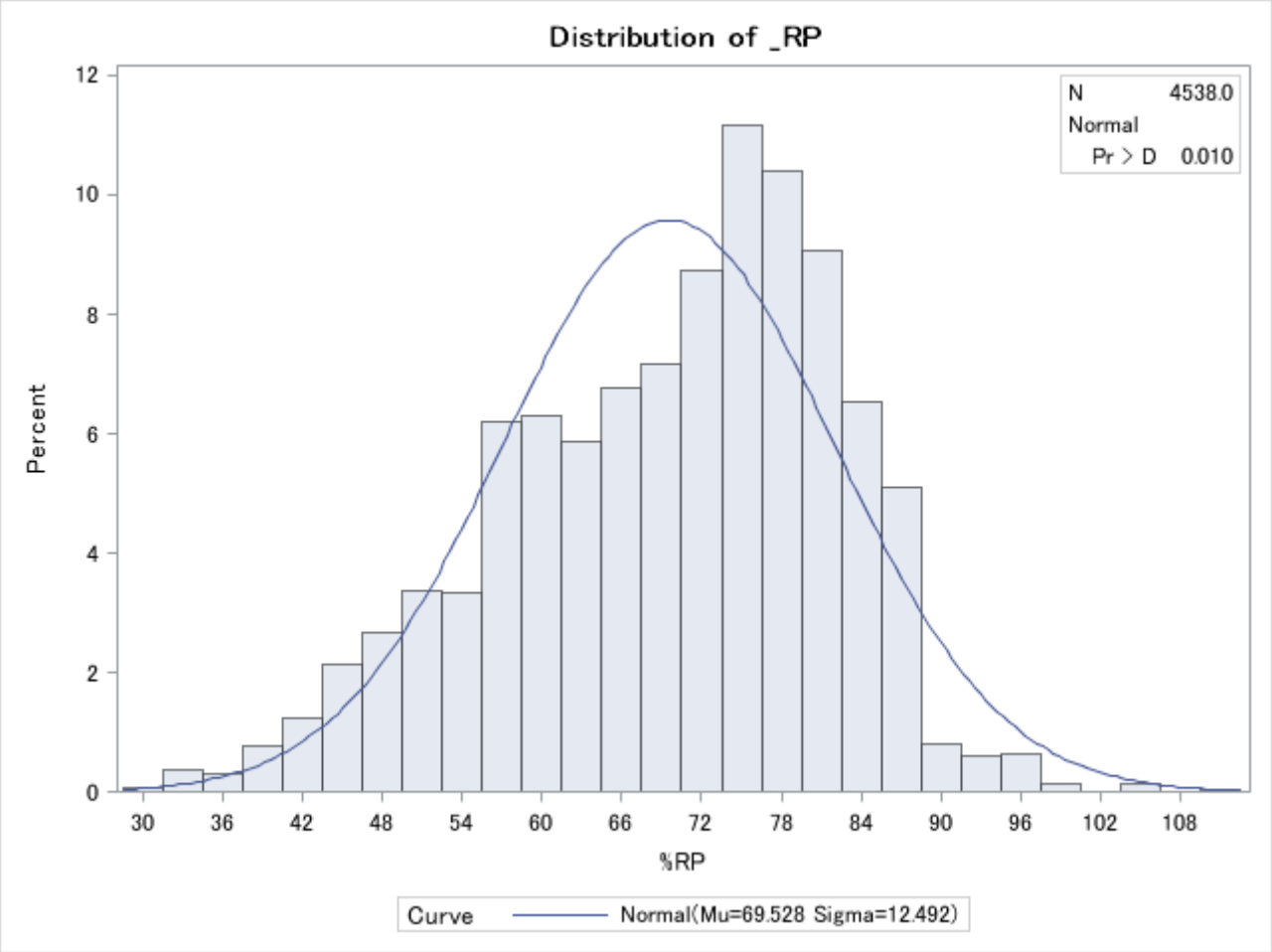
Bin Midpoint	Percent	
	Observed	Estimated
255	0.154	2.757
285	1.036	4.688
315	8.065	7.146
345	21.375	9.761
375	19.656	11.952
405	13.376	13.115
435	8.682	12.899
465	8.021	11.371
495	6.633	8.983
525	2.931	6.361
555	3.460	4.037
585	1.697	2.296
615	1.917	1.170
645	0.771	0.535
675	0.683	0.219
705	0.529	0.080
735	0.242	0.026
765	0.110	0.008
795	0.198	0.002
825	0.000	0.000
855	0.375	0.000
885	0.066	0.000
915	0.000	0.000
945	0.022	0.000

Quantiles for Normal Distribution

Percent	Quantile	
	Observed	Estimated
20.0	345.000	339.507
40.0	374.000	392.589
60.0	409.000	438.310
80.0	479.000	491.392

The SAS System

The UNIVARIATE Procedure



The SAS System

The UNIVARIATE Procedure
Fitted Normal Distribution for _RP (%RP)

Parameters for Normal Distribution

Parameter	Symbol	Estimate
Mean	Mu	69.52849
Std Dev	Sigma	12.49211

Goodness-of-Fit Tests for Normal Distribution

Test		Statistic		p Value
Kolmogorov-Smirnov	**D**	0.0723241	**Pr > D**	<0.010
Cramer-von Mises	**W-Sq**	5.5400764	**Pr > W-Sq**	<0.005
Anderson-Darling	**A-Sq**	32.4524714	**Pr > A-Sq**	<0.005

Histogram Bin Percents for Normal Distribution

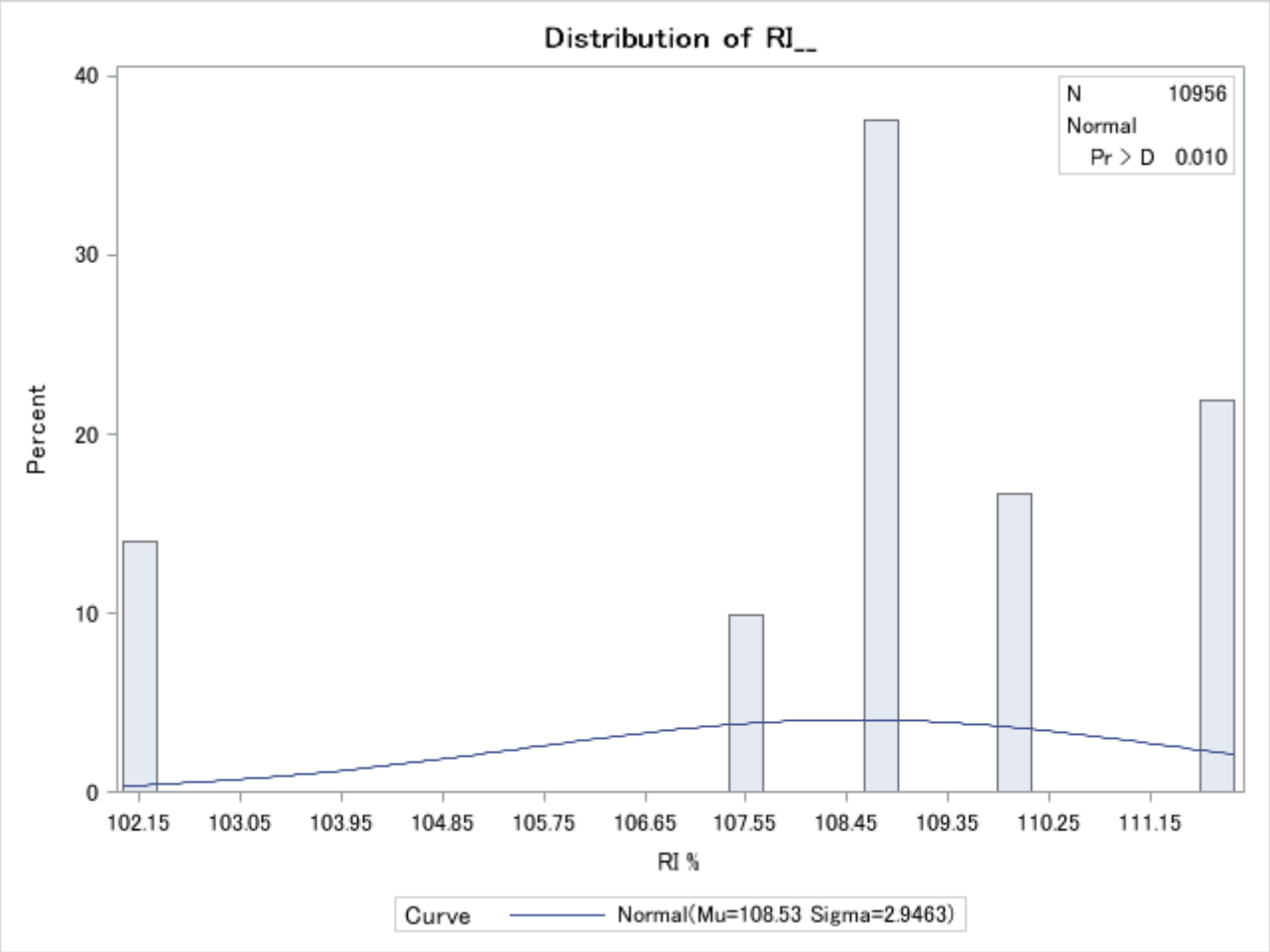
Bin Midpoint	Percent	
	Observed	Estimated
30	0.088	0.066
33	0.375	0.136
36	0.309	0.265
39	0.771	0.489
42	1.234	0.853
45	2.138	1.403
48	2.666	2.180
51	3.372	3.198
54	3.349	4.430
57	6.214	5.794
60	6.302	7.155
63	5.884	8.343
66	6.765	9.186
69	7.162	9.549
72	8.748	9.373
75	11.150	8.687
78	10.401	7.603
81	9.079	6.282

Histogram Bin Percents for Normal Distribution

Bin Midpoint	Percent	
	Observed	Estimated
84	6.545	4.902
87	5.112	3.611
90	0.815	2.512
93	0.595	1.650
96	0.639	1.023
99	0.132	0.599
102	0.000	0.331
105	0.132	0.173
108	0.000	0.085
111	0.022	0.040

Quantiles for Normal Distribution

Percent	Quantile	
	Observed	Estimated
20.0	58.0376	59.0149
40.0	67.9707	66.3637
60.0	74.3316	72.6933
80.0	80.5797	80.0421



The SAS System

The UNIVARIATE Procedure
Fitted Normal Distribution for RI_ (RI %)

Parameters for Normal Distribution

Parameter	Symbol	Estimate
Mean	Mu	108.5303
Std Dev	Sigma	2.946285

Goodness-of-Fit Tests for Normal Distribution

Test		Statistic		p Value
Kolmogorov-Smirnov	**D**	0.283882	**Pr > D**	<0.010
Cramer-von Mises	**W-Sq**	149.857272	**Pr > W-Sq**	<0.005
Anderson-Darling	**A-Sq**	922.063990	**Pr > A-Sq**	<0.005

Histogram Bin Percents for Normal Distribution

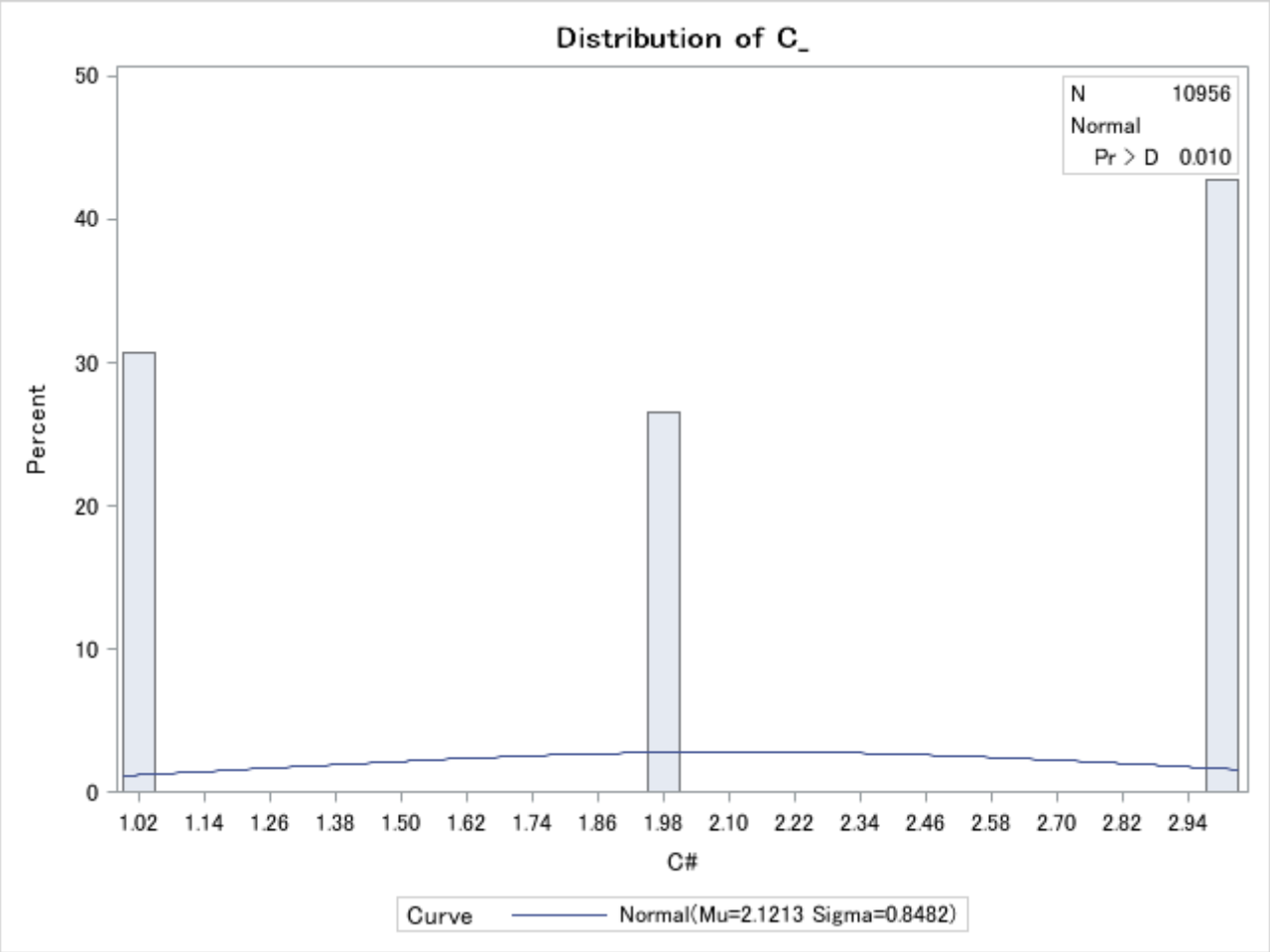
Bin Midpoint	Percent	
	Observed	Estimated
102.15	13.956	0.390
102.45	0.000	0.484
102.75	0.000	0.594
103.05	0.000	0.721
103.35	0.000	0.867
103.65	0.000	1.031
103.95	0.000	1.214
104.25	0.000	1.415
104.55	0.000	1.632
104.85	0.000	1.862
105.15	0.000	2.104
105.45	0.000	2.352
105.75	0.000	2.602
106.05	0.000	2.850
106.35	0.000	3.089
106.65	0.000	3.313
106.95	0.000	3.517
107.25	0.000	3.695
107.55	9.894	3.842
107.85	0.000	3.954
108.15	0.000	4.027
108.45	0.000	4.059
108.75	37.541	4.049
109.05	0.000	3.998
109.35	0.000	3.906
109.65	0.000	3.778
109.95	16.694	3.616
110.25	0.000	3.425

Histogram Bin Percents for Normal Distribution

Bin Midpoint	Percent	
	Observed	Estimated
110.55	0.000	3.211
110.85	0.000	2.979
111.15	0.000	2.736
111.45	0.000	2.486
111.75	21.915	2.236

Quantiles for Normal Distribution

Percent	Quantile	
	Observed	Estimated
20.0	107.592	106.051
40.0	108.722	107.784
60.0	108.722	109.277
80.0	111.801	111.010



The SAS System

The UNIVARIATE Procedure
Fitted Normal Distribution for C_ (C#)

Parameters for Normal Distribution

Parameter	Symbol	Estimate
Mean	Mu	2.121303
Std Dev	Sigma	0.848215

Goodness-of-Fit Tests for Normal Distribution

Test		Statistic		p Value
Kolmogorov-Smirnov	**D**	0.27759	**Pr > D**	<0.010
Cramer-von Mises	**W-Sq**	146.07216	**Pr > W-Sq**	<0.005
Anderson-Darling	**A-Sq**	1036.27683	**Pr > A-Sq**	<0.005

Histogram Bin Percents for Normal Distribution

Bin Midpoint	Percent	
	Observed	Estimated
1.02	30.641	1.215
1.08	0.000	1.328
1.14	0.000	1.445
1.20	0.000	1.565
1.26	0.000	1.685
1.32	0.000	1.806
1.38	0.000	1.926
1.44	0.000	2.044
1.50	0.000	2.158
1.56	0.000	2.267
1.62	0.000	2.369
1.68	0.000	2.464
1.74	0.000	2.550
1.80	0.000	2.626
1.86	0.000	2.691
1.92	0.000	2.743
1.98	26.588	2.783
2.04	0.000	2.808
2.10	0.000	2.821
2.16	0.000	2.818
2.22	0.000	2.802
2.28	0.000	2.772
2.34	0.000	2.729
2.40	0.000	2.673
2.46	0.000	2.605
2.52	0.000	2.526
2.58	0.000	2.438
2.64	0.000	2.340

Histogram Bin Percents for Normal Distribution

Bin Midpoint	Percent	
	Observed	Estimated
2.70	0.000	2.236
2.76	0.000	2.125
2.82	0.000	2.010
2.88	0.000	1.891
2.94	0.000	1.771
3.00	42.771	1.650

Quantiles for Normal Distribution

Percent	Quantile	
	Observed	Estimated
20.0	1.00000	1.40743
40.0	2.00000	1.90641
60.0	3.00000	2.33620
80.0	3.00000	2.83518

The GLM Procedure

Class Level Information

Class	Levels	Values
SEASON	2	SUMMER WINTER
BREED	2	FRIES JERSEY

Number of Observations Read 10962

Number of Observations Used 10956

The GLM Procedure

Dependent Variable: DIM DIM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	4321953.5	1440651.2	82.43	<.0001
Error	10952	191413596.4	17477.5		
Corrected Total	10955	195735549.9			

R-Square	Coeff Var	Root MSE	DIM Mean
0.022081	65.78129	132.2025	200.9728

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SEASON	1	149746.669	149746.669	8.57	0.0034
BREED	1	3926206.184	3926206.184	224.64	<.0001
SEASON*BREED	1	246000.660	246000.660	14.08	0.0002

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SEASON	1	80072.239	80072.239	4.58	0.0323
BREED	1	3965873.655	3965873.655	226.91	<.0001
SEASON*BREED	1	246000.660	246000.660	14.08	0.0002

The SAS System

The GLM Procedure
Least Squares Means

SEASON	BREED	DIM LSMEAN
SUMMER	**FRIES**	226.597882
SUMMER	**JERSEY**	192.389050
WINTER	**FRIES**	231.470588
WINTER	**JERSEY**	174.570426

The SAS System

The GLM Procedure

Class Level Information

Class	Levels	Values
SEASON	2	SUMMER WINTER
BREED	2	FRIES JERSEY

Number of Observations Read	10962
Number of Observations Used	10953

The SAS System

The GLM Procedure

Dependent Variable: T_MILK T#MILK

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	123968.4790	41322.8263	683.41	<.0001
Error	10949	662040.6176	60.4659		
Corrected Total	10952	786009.0966			

R-Square	Coeff Var	Root MSE	T_MILK Mean
0.157719	32.76405	7.775979	23.73327

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SEASON	1	201.3265	201.3265	3.33	0.0681
BREED	1	123457.1926	123457.1926	2041.77	<.0001
SEASON*BREED	1	309.9599	309.9599	5.13	0.0236

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SEASON	1	42.5717	42.5717	0.70	0.4014
BREED	1	103253.5004	103253.5004	1707.63	<.0001
SEASON*BREED	1	309.9599	309.9599	5.13	0.0236

The SAS System

The GLM Procedure
Least Squares Means

SEASON	BREED	T_MILK LSMEAN
SUMMER	**FRIES**	28.4094584
SUMMER	**JERSEY**	21.4613702
WINTER	**FRIES**	28.9614706
WINTER	**JERSEY**	21.2078791

The SAS System

The GLM Procedure

Class Level Information

Class	Levels	Values
SEASON	2	SUMMER WINTER
BREED	2	FRIES JERSEY

Number of Observations Read	10962
Number of Observations Used	10956

The SAS System

The GLM Procedure

Dependent Variable: FAT FAT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	1098.64652	366.21551	371.59	<.0001
Error	10952	10793.69348	0.98555		
Corrected Total	10955	11892.34000			

R-Square	Coeff Var	Root MSE	FAT Mean
0.092383	22.28748	0.992746	4.454279

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SEASON	1	98.1641130	98.1641130	99.60	<.0001
BREED	1	996.8381501	996.8381501	1011.46	<.0001
SEASON*BREED	1	3.6442573	3.6442573	3.70	0.0545

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SEASON	1	98.6034209	98.6034209	100.05	<.0001
BREED	1	842.1446287	842.1446287	854.50	<.0001
SEASON*BREED	1	3.6442573	3.6442573	3.70	0.0545

The SAS System

The GLM Procedure
Least Squares Means

SEASON	BREED	FAT LSMEAN
SUMMER	**FRIES**	3.97325618

SEASON	BREED	FAT LSMEAN
SUMMER	JERSEY	4.59341525
WINTER	FRIES	4.15673529
WINTER	JERSEY	4.86423098

The SAS System

The GLM Procedure

Class Level Information

Class	Levels	Values
SEASON	2	SUMMER WINTER
BREED	2	FRIES JERSEY

Number of Observations Read	10962
Number of Observations Used	10955

The SAS System

The GLM Procedure

Dependent Variable: PROT PROT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	238.432124	79.477375	138.17	<.0001
Error	10951	6299.360291	0.575232		
Corrected Total	10954	6537.792415			

R-Square	Coeff Var	Root MSE	PROT Mean
0.036470	21.78553	0.758440	3.481394

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SEASON	1	51.9709075	51.9709075	90.35	<.0001
BREED	1	181.3223833	181.3223833	315.22	<.0001
SEASON*BREED	1	5.1388332	5.1388332	8.93	0.0028

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SEASON	1	43.7561290	43.7561290	76.07	<.0001
BREED	1	169.8895068	169.8895068	295.34	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SEASON*BREED	1	5.1388332	5.1388332	8.93	0.0028

The SAS System

The GLM Procedure
Least Squares Means

SEASON	BREED	PROT LSMEAN
SUMMER	FRIES	3.27170655
SUMMER	JERSEY	3.51801012
WINTER	FRIES	3.37116667
WINTER	JERSEY	3.72118187

The SAS System

The GLM Procedure

Class Level Information

Class	Levels	Values
SEASON	2	SUMMER WINTER
BREED	2	FRIES JERSEY

Number of Observations Read	10962
Number of Observations Used	10954

The SAS System

The GLM Procedure

Dependent Variable: LACT LACT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	180.970632	60.323544	88.37	<.0001
Error	10950	7474.824596	0.682632		
Corrected Total	10953	7655.795228			

R-Square	Coeff Var	Root MSE	LACT Mean
0.023638	18.18059	0.826216	4.544492

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SEASON	1	0.4626981	0.4626981	0.68	0.4104
BREED	1	180.2902191	180.2902191	264.11	<.0001
SEASON*BREED	1	0.2177146	0.2177146	0.32	0.5723

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SEASON	1	0.9539333	0.9539333	1.40	0.2372
BREED	1	148.4722526	148.4722526	217.50	<.0001
SEASON*BREED	1	0.2177146	0.2177146	0.32	0.5723

The GLM Procedure
Least Squares Means

SEASON	BREED	LACT LSMEAN
SUMMER	FRIES	4.73189486
SUMMER	JERSEY	4.46381710
WINTER	FRIES	4.72022549
WINTER	JERSEY	4.43079914

The GLM Procedure

Class Level Information

Class	Levels	Values
SEASON	2	SUMMER WINTER
BREED	2	FRIES JERSEY

Number of Observations Read	10962
Number of Observations Used	10956

The GLM Procedure

Dependent Variable: CELLS CELLS

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	28272086	9424029	4.84	0.0023

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Error	10952	21303460588	1945166		
Corrected Total	10955	21331732674			

R-Square	Coeff Var	Root MSE	CELLS Mean
0.001325	206.7331	1394.692	674.6342

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SEASON	1	3522996.77	3522996.77	1.81	0.1784
BREED	1	22800790.65	22800790.65	11.72	0.0006
SEASON*BREED	1	1948298.45	1948298.45	1.00	0.3169

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SEASON	1	2298760.35	2298760.35	1.18	0.2770
BREED	1	23910437.96	23910437.96	12.29	0.0005
SEASON*BREED	1	1948298.45	1948298.45	1.00	0.3169

The GLM Procedure
Least Squares Means

SEASON	BREED	CELLS LSMEAN
SUMMER	FRIES	609.222440
SUMMER	JERSEY	689.148175
WINTER	FRIES	611.975490
WINTER	JERSEY	755.759849

The GLM Procedure

Class Level Information

Class	Levels	Values
SEASON	2	SUMMER WINTER
BREED	2	FRIES JERSEY

Number of Observations Read 10962

Number of Observations Used 10928

The SAS System

The GLM Procedure

Dependent Variable: UREA UREA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	71727.4109	23909.1370	943.19	<.0001
Error	10924	276913.5352	25.3491		
Corrected Total	10927	348640.9460			

R-Square	Coeff Var	Root MSE	UREA Mean
0.205734	34.98481	5.034789	14.39136

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SEASON	1	1887.17536	1887.17536	74.45	<.0001
BREED	1	69245.85555	69245.85555	2731.69	<.0001
SEASON*BREED	1	594.37996	594.37996	23.45	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SEASON	1	1582.96532	1582.96532	62.45	<.0001
BREED	1	60402.52455	60402.52455	2382.83	<.0001
SEASON*BREED	1	594.37996	594.37996	23.45	<.0001

The SAS System

The GLM Procedure
Least Squares Means

SEASON	BREED	UREA LSMEAN
SUMMER	**FRIES**	17.6056336
SUMMER	**JERSEY**	12.5402325
WINTER	**FRIES**	19.0737647
WINTER	**JERSEY**	12.8927361

The SAS System

The GLM Procedure

Class Level Information

Class	Levels	Values
SEASON	2	SUMMER WINTER
BREED	2	FRIES JERSEY

Number of Observations Read	10962
Number of Observations Used	9193

The SAS System

The GLM Procedure

Dependent Variable: RDP_ RDP %

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	70949.7787	23649.9262	4082.15	<.0001
Error	9189	53236.3945	5.7935		
Corrected Total	9192	124186.1732			

R-Square	Coeff Var	Root MSE	RDP__ Mean
0.571318	4.309079	2.406967	55.85805

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SEASON	1	1910.72019	1910.72019	329.80	<.0001
BREED	1	68643.68915	68643.68915	11848.4	<.0001
SEASON*BREED	1	395.36938	395.36938	68.24	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SEASON	1	230.74868	230.74868	39.83	<.0001
BREED	1	52407.04740	52407.04740	9045.85	<.0001
SEASON*BREED	1	395.36938	395.36938	68.24	<.0001

The SAS System

The GLM Procedure
Least Squares Means

SEASON	BREED	RDP__ LSMEAN
SUMMER	**FRIES**	61.4597474

SEASON	BREED	RDP__ LSMEAN
SUMMER	JERSEY	54.2432779
WINTER	FRIES	61.3236178
WINTER	JERSEY	55.2605720

The SAS System

The GLM Procedure

Class Level Information

Class	Levels	Values
SEASON	2	SUMMER WINTER
BREED	2	FRIES JERSEY

Number of Observations Read	10962
Number of Observations Used	10956

The SAS System

The GLM Procedure

Dependent Variable: CP_ CP %

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	2773.36417	924.45472	826.16	<.0001
Error	10952	12255.11458	1.11898		
Corrected Total	10955	15028.47875			

R-Square	Coeff Var	Root MSE	CP__ Mean
0.184541	6.154017	1.057820	17.18911

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SEASON	1	284.366361	284.366361	254.13	<.0001
BREED	1	2349.970277	2349.970277	2100.09	<.0001
SEASON*BREED	1	139.027534	139.027534	124.24	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SEASON	1	197.473323	197.473323	176.48	<.0001
BREED	1	2358.603096	2358.603096	2107.81	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SEASON*BREED	1	139.027534	139.027534	124.24	<.0001

The SAS System

The GLM Procedure
Least Squares Means

SEASON	BREED	CP__ LSMEAN
SUMMER	FRIES	16.5171204
SUMMER	JERSEY	17.3583375
WINTER	FRIES	16.5688529
WINTER	JERSEY	17.9495089

The SAS System

The GLM Procedure

Class Level Information

Class	Levels	Values
SEASON	2	SUMMER WINTER
BREED	2	FRIES JERSEY

Number of Observations Read	10962
Number of Observations Used	10956

The SAS System

The GLM Procedure

Dependent Variable: ME_MJ ME# MJ

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	46.121071	15.373690	44.01	<.0001
Error	10952	3825.350753	0.349283		
Corrected Total	10955	3871.471823			

R-Square	Coeff Var	Root MSE	ME__MJ Mean
0.011913	5.396847	0.591002	10.95087

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SEASON	1	5.98885329	5.98885329	17.15	<.0001
BREED	1	38.98636674	38.98636674	111.62	<.0001
SEASON*BREED	1	1.14585063	1.14585063	3.28	0.0701

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SEASON	1	4.88859161	4.88859161	14.00	0.0002
BREED	1	36.63321285	36.63321285	104.88	<.0001
SEASON*BREED	1	1.14585063	1.14585063	3.28	0.0701

The GLM Procedure
Least Squares Means

SEASON	BREED	ME__MJ LSMEAN
SUMMER	FRIES	10.8589094
SUMMER	JERSEY	10.9728750
WINTER	FRIES	10.8850000
WINTER	JERSEY	11.0479385

The GLM Procedure

Class Level Information

Class	Levels	Values
SEASON	2	SUMMER WINTER
BREED	2	FRIES JERSEY

Number of Observations Read	10962
Number of Observations Used	10956

The GLM Procedure

Dependent Variable: C_ C#

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	15.915996	5.305332	7.39	<.0001

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Error	10952	7865.871791	0.718213		
Corrected Total	10955	7881.787788			

R-Square	Coeff Var	Root MSE	C_ Mean
0.002019	39.95066	0.847475	2.121303

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SEASON	1	7.07918056	7.07918056	9.86	0.0017
BREED	1	7.60755045	7.60755045	10.59	0.0011
SEASON*BREED	1	1.22926536	1.22926536	1.71	0.1908

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SEASON	1	4.33995308	4.33995308	6.04	0.0140
BREED	1	3.83388343	3.83388343	5.34	0.0209
SEASON*BREED	1	1.22926536	1.22926536	1.71	0.1908

The GLM Procedure
Least Squares Means

SEASON	BREED	C_ LSMEAN
SUMMER	FRIES	2.15417811
SUMMER	JERSEY	2.08402602
WINTER	FRIES	2.17647059
WINTER	JERSEY	2.15704263

The GLM Procedure

Class Level Information

Class	Levels	Values
SEASON	2	SUMMER WINTER
BREED	2	FRIES JERSEY

Number of Observations Read 10962

Number of Observations Used 4538

The SAS System

The GLM Procedure

Dependent Variable: ICP ICP

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	677391.48	225797.16	28.23	<.0001
Error	4534	36263355.37	7998.09		
Corrected Total	4537	36940746.84			

R-Square	Coeff Var	Root MSE	ICP Mean
0.018337	21.52659	89.43206	415.4493

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SEASON	1	21481.7793	21481.7793	2.69	0.1013
BREED	1	654906.1370	654906.1370	81.88	<.0001
SEASON*BREED	1	1003.5611	1003.5611	0.13	0.7232

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SEASON	1	2383.2787	2383.2787	0.30	0.5852
BREED	1	441282.5434	441282.5434	55.17	<.0001
SEASON*BREED	1	1003.5611	1003.5611	0.13	0.7232

The SAS System

The GLM Procedure

Least Squares Means

SEASON	BREED	ICP LSMEAN
SUMMER	**FRIES**	436.386694
SUMMER	**JERSEY**	408.351228
WINTER	**FRIES**	433.144033
WINTER	**JERSEY**	407.660793

The SAS System

The GLM Procedure

Class Level Information

Class	Levels	Values
SEASON	2	SUMMER WINTER
BREED	2	FRIES JERSEY

Number of Observations Read	10962
Number of Observations Used	4538

The SAS System

The GLM Procedure

Dependent Variable: _RP %RP

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	23231.1269	7743.7090	51.27	<.0001
Error	4534	684780.9735	151.0324		
Corrected Total	4537	708012.1004			

R-Square	Coeff Var	Root MSE	_RP Mean
0.032812	17.67552	12.28952	69.52849

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SEASON	1	955.38836	955.38836	6.33	0.0119
BREED	1	22220.86404	22220.86404	147.13	<.0001
SEASON*BREED	1	54.87449	54.87449	0.36	0.5467

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SEASON	1	170.36489	170.36489	1.13	0.2883
BREED	1	14765.75633	14765.75633	97.77	<.0001
SEASON*BREED	1	54.87449	54.87449	0.36	0.5467

The SAS System

The GLM Procedure
Least Squares Means

SEASON	BREED	_RP LSMEAN
SUMMER	**FRIES**	65.6131821

SEASON	BREED	_RP LSMEAN
SUMMER	JERSEY	70.8064988
WINTER	FRIES	66.4373685
WINTER	JERSEY	71.0338802

The SAS System

The GLM Procedure

Class Level Information

Class	Levels	Values
SEASON	2	SUMMER WINTER
BREED	2	FRIES JERSEY

Number of Observations Read	10962
Number of Observations Used	10956

The SAS System

The GLM Procedure

Dependent Variable: RI_ RI %

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	48886.49522	16295.49841	3862.16	<.0001
Error	10952	46209.45368	4.21927		
Corrected Total	10955	95095.94890			

R-Square	Coeff Var	Root MSE	RI__ Mean
0.514075	1.892639	2.054086	108.5303

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SEASON	1	167.15234	167.15234	39.62	<.0001
BREED	1	48718.03485	48718.03485	11546.6	<.0001
SEASON*BREED	1	1.30804	1.30804	0.31	0.5777

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SEASON	1	16.89296	16.89296	4.00	0.0454
BREED	1	38540.69801	38540.69801	9134.45	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SEASON*BREED	1	1.30804	1.30804	0.31	0.5777

The SAS System

The GLM Procedure
Least Squares Means

SEASON	BREED	RI__ LSMEAN
SUMMER	**FRIES**	105.511402
SUMMER	**JERSEY**	110.028342
WINTER	**FRIES**	105.443545
WINTER	**JERSEY**	109.908161

The SAS System

The CORR Procedure

13 With Variables: DIM T_MILK FAT PROT LACT CELLS RDP__ CP__ ME__MJ C_ ICP _RP RI__

1 Variables: UREA

Sums of Squares and Crossproducts
SSCP / Row Var SS / Col Var SS

	UREA
DIM	32471672.4
DIM	637267271
	2611951.970
T_MILK	3821935.8
T#MILK	6940128
	2611711.812
FAT	710284.4
FAT	228782
	2611951.970
PROT	562109.4
PROT	138957
	2611385.530

Sums of Squares and Crossproducts
SSCP / Row Var SS / Col Var SS

	UREA
LACT	739747.6
LACT	233357
	2611178.458
CELLS	106572573.4
CELLS	26303555763
	2611951.970
RDP__	7033422.5
RDP %	28725436
	1970923.752
CP__	2680466.3
CP %	3243573
	2611951.970
ME__MJ	1713065.8
ME# MJ	1314289
	2611951.970
C_	334831.7
C#	57095
	2611951.970
ICP	25825071.1
ICP	819684874
	960164.480
_RP	4231147.9
%RP	22620219
	960164.480
RI__	17009611.9
RI %	128799676

Sums of Squares and Crossproducts
SSCP / Row Var SS / Col Var SS

	UREA
	2611951.970

Variances and Covariances
Covariance / Row Var Variance / Col Var Variance / DF

	UREA
DIM	77.35584042
DIM	17876.389
	31.90637376
	10927
T_MILK	8.18004092
T#MILK	71.811
	31.89397242
	10924
FAT	0.87506989
FAT	1.083
	31.90637376
	10927
PROT	1.33679656
PROT	0.594
	31.90119123
	10926
LACT	2.29981222
LACT	0.700
	31.90411125
	10925
CELLS	32.81338007
CELLS	1951046.055

Variances and Covariances
Covariance / Row Var Variance / Col Var Variance / DF

	UREA
	31.90637376
	10927
RDP__	5.32169930
RDP %	13.526
	28.94247989
	9164
CP__	-2.07996754
CP %	1.373
	31.90637376
	10927
ME__MJ	-0.83378518
ME# MJ	0.354
	31.90637376
	10927
C_	0.09109353
C#	0.719
	31.90637376
	10927
ICP	60.62903555
ICP	8144.684
	27.83562553
	4533
_RP	-9.65737995
%RP	156.054
	27.83562553
	4533

Variances and Covariances
Covariance / Row Var Variance / Col Var Variance / DF

	UREA
RI__	-5.29607719
RI %	8.685
	31.90637376
	10927

Simple Statistics

Variable	N	Mean	Std Dev	Sum	Minimum	Maximum	Label
DIM	10956	200.97280	133.66837	2201858	2.00000	1323	DIM
T_MILK	10953	23.73327	8.47163	259951	0.40000	70.70000	T#MILK
FAT	10956	4.45428	1.04190	48801	0.29000	12.90000	FAT
PROT	10955	3.48139	0.77255	38139	0	13.60000	PROT
LACT	10954	4.54449	0.83604	49780	0	5.53000	LACT
CELLS	10956	674.63417	1395	7391292	0	24995	CELLS
RDP__	9193	55.85805	3.67563	513503	50.16000	64.33000	RDP %
CP__	10956	17.18911	1.17125	188324	14.80000	19.46000	CP %
ME__MJ	10956	10.95087	0.59447	119978	9.60000	11.74000	ME# MJ
C_	10956	2.12130	0.84822	23241	1.00000	3.00000	C#
ICP	4538	415.44932	90.23363	1885309	248.00000	943.00000	ICP
_RP	4538	69.52849	12.49211	315520	29.48038	112.09677	%RP
RI__	10956	108.53027	2.94629	1189058	102.00000	111.80124	RI %
UREA	10928	14.39136	5.64857	157269	0	71.23000	UREA

Pearson Correlation Coefficients
Prob > |r| under H0: Rho=0
Number of Observations

	UREA
DIM	0.10243
DIM	<.0001
	10928

Pearson Correlation Coefficients
Prob > |r| under H0: Rho=0
Number of Observations

	UREA
T_MILK	0.17093
T#MILK	<.0001
	10925
FAT	0.14884
FAT	<.0001
	10928
PROT	0.30719
PROT	<.0001
	10927
LACT	0.48649
LACT	<.0001
	10926
CELLS	0.00416
CELLS	0.6638
	10928
RDP__	0.26896
RDP %	<.0001
	9165
CP__	-0.31425
CP %	<.0001
	10928
ME__MJ	-0.24811
ME# MJ	<.0001
	10928
C_	0.01902

Pearson Correlation Coefficients
Prob > |r| under H0: Rho=0
Number of Observations

	UREA
C#	0.0468
	10928
ICP	0.12733
ICP	<.0001
	4534
_RP	-0.14653
%RP	<.0001
	4534
RI__	-0.31816
RI %	<.0001
	10928

The CORR Procedure

13 With Variables:	DIM T_MILK FAT LACT CELLS UREA RDP__ CP__ ME__MJ C_ ICP _RP RI__
1 Variables:	PROT

Sums of Squares and Crossproducts
SSCP / Row Var SS / Col Var SS

	PROT
DIM	7878968.72
DIM	638248794
	139313.5249
T_MILK	884681.93
T#MILK	6955424
	139292.0472

Sums of Squares and Crossproducts
SSCP / Row Var SS / Col Var SS

	PROT
FAT	176816.65
FAT	229100
	139313.5249
LACT	178154.36
LACT	233882
	139128.5649
CELLS	27956805.92
CELLS	26317712596
	139313.5249
UREA	562109.42
UREA	2611386
	138957.0027
RDP__	1792014.88
RDP %	28804200
	118372.2808
CP__	654345.78
CP %	3251880
	139313.5249
ME__MJ	416639.43
ME# MJ	1317610
	139313.5249
C_	81525.39
C#	57179
	139313.5249
ICP	6626488.19
ICP	820076162

Sums of Squares and Crossproducts
SSCP / Row Var SS / Col Var SS

	PROT
	59061.4872
_RP	1112738.56
%RP	22638938
	59061.4872
RI__	4140832.21
RI %	129131780
	139313.5249

Variances and Covariances
Covariance / Row Var Variance / Col Var Variance / DF

	PROT
DIM	19.4902693
DIM	17865.809
	0.5968406441
	10954
T_MILK	-1.8604163
T#MILK	71.752
	0.5958870679
	10951
FAT	0.6359123
FAT	1.079
	0.5968406441
	10954
LACT	0.4469344
LACT	0.699
	0.5875465059
	10953

Variances and Covariances
Covariance / Row Var Variance / Col Var Variance / DF

	PROT
CELLS	203.3149387
CELLS	1947392.050
	0.5968406441
	10954
UREA	1.3367966
UREA	31.901
	0.5936137330
	10926
RDP__	-0.4073897
RDP %	13.512
	0.6455577999
	9191
CP__	-0.1119956
CP %	1.372
	0.5968406441
	10954
ME__MJ	-0.0923686
ME# MJ	0.353
	0.5968406441
	10954
C_	0.0567170
C#	0.720
	0.5968406441
	10954
ICP	-2.6758537
ICP	8142.615
	0.6142827327

Variances and Covariances
Covariance / Row Var Variance / Col Var Variance / DF

	PROT
	4536
_RP	0.3978822
%RP	156.053
	0.6142827327
	4536
RI__	0.1494040
RI %	8.681
	0.5968406441
	10954

Simple Statistics

Variable	N	Mean	Std Dev	Sum	Minimum	Maximum	Label
DIM	10956	200.97280	133.66837	2201858	2.00000	1323	DIM
T_MILK	10953	23.73327	8.47163	259951	0.40000	70.70000	T#MILK
FAT	10956	4.45428	1.04190	48801	0.29000	12.90000	FAT
LACT	10954	4.54449	0.83604	49780	0	5.53000	LACT
CELLS	10956	674.63417	1395	7391292	0	24995	CELLS
UREA	10928	14.39136	5.64857	157269	0	71.23000	UREA
RDP__	9193	55.85805	3.67563	513503	50.16000	64.33000	RDP %
CP__	10956	17.18911	1.17125	188324	14.80000	19.46000	CP %
ME__MJ	10956	10.95087	0.59447	119978	9.60000	11.74000	ME# MJ
C_	10956	2.12130	0.84822	23241	1.00000	3.00000	C#
ICP	4538	415.44932	90.23363	1885309	248.00000	943.00000	ICP
_RP	4538	69.52849	12.49211	315520	29.48038	112.09677	%RP
RI__	10956	108.53027	2.94629	1189058	102.00000	111.80124	RI %
PROT	10955	3.48139	0.77255	38139	0	13.60000	PROT

Pearson Correlation Coefficients
Prob > |r| under H0: Rho=0
Number of Observations

	PROT
DIM	0.18875
DIM	<.0001
	10955
T_MILK	-0.28452
T#MILK	<.0001
	10952
FAT	0.79237
FAT	<.0001
	10955
LACT	0.69742
LACT	<.0001
	10954
CELLS	0.18859
CELLS	<.0001
	10955
UREA	0.30719
UREA	<.0001
	10927
RDP__	-0.13794
RDP %	<.0001
	9192
CP__	-0.12377
CP %	<.0001
	10955
ME__MJ	-0.20112

Pearson Correlation Coefficients
Prob > |r| under H0: Rho=0
Number of Observations

	PROT
ME# MJ	<.0001
	10955
C_	0.08655
C#	<.0001
	10955
ICP	-0.03784
ICP	0.0108
	4537
_RP	0.04064
%RP	0.0062
	4537
RI__	0.06564
RI %	<.0001
	10955

The SAS System

The CORR Procedure

13 With Variables: DIM T_MILK FAT PROT LACT CELLS UREA CP__ ME__MJ C_ ICP _RP RI__

1 Variables: RDP__

Sums of Squares and Crossproducts
SSCP / Row Var SS / Col Var SS

	RDP__
DIM	99491550.9
DIM	490229801
	28807467.25

Sums of Squares and Crossproducts
SSCP / Row Var SS / Col Var SS

	RDP__
T_MILK	12067980.9
T#MILK	5622143
	28797013.49
FAT	2314659.4
FAT	198581
	28807467.25
PROT	1792014.9
PROT	118372
	28804199.98
LACT	2314604.2
LACT	193497
	28801113.07
CELLS	346683872.5
CELLS	21404969489
	28807467.25
UREA	7033422.5
UREA	1970924
	28725436.19
CP__	8949794.0
CP %	2803520
	28807467.25
ME__MJ	5681943.3
ME# MJ	1126044
	28807467.25
C_	1093613.6
C#	47777

Sums of Squares and Crossproducts
SSCP / Row Var SS / Col Var SS

	RDP__
	28807467.25
ICP	93826852.1
ICP	712714344
	12974338.82
_RP	16021604.8
%RP	20507141
	12974338.82
RI__	55965359.8
RI %	109492376
	28807467.25

Variances and Covariances
Covariance / Row Var Variance / Col Var Variance / DF

	RDP__
DIM	13.0814176
DIM	15879.522
	13.51024513
	9192
T_MILK	14.4547214
T#MILK	71.184
	13.50661391
	9189
FAT	-0.7778231
FAT	1.157
	13.51024513
	9192
PROT	-0.4073897

Variances and Covariances
Covariance / Row Var Variance / Col Var Variance / DF

	RDP__
PROT	0.646
	13.51153062
	9191
LACT	0.1892383
LACT	0.757
	13.51299121
	9190
CELLS	-205.9999684
CELLS	1867801.952
	13.51024513
	9192
UREA	5.3216993
UREA	28.942
	13.52645387
	9164
CP__	0.0403084
CP %	1.221
	13.51024513
	9192
ME__MJ	0.5185652
ME# MJ	0.259
	13.51024513
	9192
C_	0.9567468
C#	0.734
	13.51024513
	9192

Variances and Covariances
Covariance / Row Var Variance / Col Var Variance / DF

	RDP__
ICP	15.8497028
ICP	8170.275
	10.57284839
	4024
_RP	-3.1169950
%RP	155.661
	10.57284839
	4024
RI__	-6.5608295
RI %	6.544
	13.51024513
	9192

Simple Statistics

Variable	N	Mean	Std Dev	Sum	Minimum	Maximum	Label
DIM	10956	200.97280	133.66837	2201858	2.00000	1323	DIM
T_MILK	10953	23.73327	8.47163	259951	0.40000	70.70000	T#MILK
FAT	10956	4.45428	1.04190	48801	0.29000	12.90000	FAT
PROT	10955	3.48139	0.77255	38139	0	13.60000	PROT
LACT	10954	4.54449	0.83604	49780	0	5.53000	LACT
CELLS	10956	674.63417	1395	7391292	0	24995	CELLS
UREA	10928	14.39136	5.64857	157269	0	71.23000	UREA
CP__	10956	17.18911	1.17125	188324	14.80000	19.46000	CP %
ME__MJ	10956	10.95087	0.59447	119978	9.60000	11.74000	ME# MJ
C_	10956	2.12130	0.84822	23241	1.00000	3.00000	C#
ICP	4538	415.44932	90.23363	1885309	248.00000	943.00000	ICP
_RP	4538	69.52849	12.49211	315520	29.48038	112.09677	%RP
RI__	10956	108.53027	2.94629	1189058	102.00000	111.80124	RI %

Simple Statistics

Variable	N	Mean	Std Dev	Sum	Minimum	Maximum	Label
RDP__	9193	55.85805	3.67563	513503	50.16000	64.33000	RDP %

Pearson Correlation Coefficients
Prob > |r| under H0: Rho=0
Number of Observations

	RDP__
DIM	0.02824
DIM	0.0068
	9193
T_MILK	0.46617
T#MILK	<.0001
	9190
FAT	-0.19670
FAT	<.0001
	9193
PROT	-0.13794
PROT	<.0001
	9192
LACT	0.05916
LACT	<.0001
	9191
CELLS	-0.04101
CELLS	<.0001
	9193
UREA	0.26896
UREA	<.0001
	9165
CP__	0.00992

Pearson Correlation Coefficients
Prob > |r| under H0: Rho=0
Number of Observations

	RDP__
CP %	0.3414
	9193
ME__MJ	0.27721
ME# MJ	<.0001
	9193
C_	0.30379
C#	<.0001
	9193
ICP	0.05393
ICP	0.0006
	4025
_RP	-0.07683
%RP	<.0001
	4025
RI__	-0.69779
RI %	<.0001
	9193

The CORR Procedure

13 With Variables: DIM T_MILK FAT PROT LACT CELLS UREA RDP__ ME__MJ C_ ICP _RP RI__

1 Variables: CP__

Sums of Squares and Crossproducts
SSCP / Row Var SS / Col Var SS

	CP__
DIM	37308657.7
DIM	638249118
	3252147.179
T_MILK	4482322.5
T#MILK	6955484
	3251318.535
FAT	839544.7
FAT	229266
	3252147.179
PROT	654345.8
PROT	139314
	3251879.856
LACT	854377.7
LACT	233882
	3251501.165
CELLS	125495570.1
CELLS	26318150840
	3252147.179
UREA	2680466.3
UREA	2611952
	3243573.139
RDP__	8949794.0
RDP %	28807467
	2803519.599
ME__MJ	2066809.1
ME# MJ	1317733

Sums of Squares and Crossproducts
SSCP / Row Var SS / Col Var SS

	CP__
	3252147.179
C_	400234.0
C#	57183
	3252147.179
ICP	32908824.8
ICP	820191083
	1395118.940
_RP	5524338.7
%RP	22645663
	1395118.940
RI__	20448071.1
RI %	129143844
	3252147.179

Variances and Covariances
Covariance / Row Var Variance / Col Var Variance / DF

	CP__
DIM	-49.2299221
DIM	17867.234
	1.371837403
	10955
T_MILK	1.2750360
T#MILK	71.769
	1.372001525
	10952
FAT	0.0636915
FAT	1.086

Variances and Covariances
Covariance / Row Var Variance / Col Var Variance / DF

	CP__
	1.371837403
	10955
PROT	-0.1119956
PROT	0.597
	1.371898356
	10954
LACT	-0.1183146
LACT	0.699
	1.371552772
	10953
CELLS	-141.8653338
CELLS	1947214.302
	1.371837403
	10955
UREA	-2.0799675
UREA	31.906
	1.373021495
	10927
RDP__	0.0403084
RDP %	13.510
	1.221135674
	9192
ME__MJ	0.4106121
ME# MJ	0.353
	1.371837403
	10955

Variances and Covariances
Covariance / Row Var Variance / Col Var Variance / DF

	CP__
C_	0.0677287
C#	0.719
	1.371837403
	10955
ICP	-10.8994677
ICP	8142.109
	1.823343252
	4537
_RP	1.8799488
%RP	156.053
	1.823343252
	4537
RI__	0.8427842
RI %	8.681
	1.371837403
	10955

Simple Statistics

Variable	N	Mean	Std Dev	Sum	Minimum	Maximum	Label
DIM	10956	200.97280	133.66837	2201858	2.00000	1323	DIM
T_MILK	10953	23.73327	8.47163	259951	0.40000	70.70000	T#MILK
FAT	10956	4.45428	1.04190	48801	0.29000	12.90000	FAT
PROT	10955	3.48139	0.77255	38139	0	13.60000	PROT
LACT	10954	4.54449	0.83604	49780	0	5.53000	LACT
CELLS	10956	674.63417	1395	7391292	0	24995	CELLS
UREA	10928	14.39136	5.64857	157269	0	71.23000	UREA
RDP__	9193	55.85805	3.67563	513503	50.16000	64.33000	RDP %
ME__MJ	10956	10.95087	0.59447	119978	9.60000	11.74000	ME# MJ

Simple Statistics

Variable	N	Mean	Std Dev	Sum	Minimum	Maximum	Label
C_	10956	2.12130	0.84822	23241	1.00000	3.00000	C#
ICP	4538	415.44932	90.23363	1885309	248.00000	943.00000	ICP
_RP	4538	69.52849	12.49211	315520	29.48038	112.09677	%RP
RI__	10956	108.53027	2.94629	1189058	102.00000	111.80124	RI %
CP__	10956	17.18911	1.17125	188324	14.80000	19.46000	CP %

Pearson Correlation Coefficients
Prob > |r| under H0: Rho=0
Number of Observations

	CP__
DIM	-0.31445
DIM	<.0001
	10956
T_MILK	0.12849
T#MILK	<.0001
	10953
FAT	0.05219
FAT	<.0001
	10956
PROT	-0.12377
PROT	<.0001
	10955
LACT	-0.12084
LACT	<.0001
	10954
CELLS	-0.08680
CELLS	<.0001
	10956

Pearson Correlation Coefficients
Prob > |r| under H0: Rho=0
Number of Observations

	CP__
UREA	-0.31425
UREA	<.0001
	10928
RDP__	0.00992
RDP %	0.3414
	9193
ME__MJ	0.58972
ME# MJ	<.0001
	10956
C_	0.06817
C#	<.0001
	10956
ICP	-0.08945
ICP	<.0001
	4538
_RP	0.11145
%RP	<.0001
	4538
RI__	0.24423
RI %	<.0001
	10956

The CORR Procedure

13 With Variables:	DIM T_MILK FAT PROT LACT CELLS UREA RDP__ CP__ C_ ICP _RP RI__
1 Variables:	ME__MJ

Sums of Squares and Crossproducts
SSCP / Row Var SS / Col Var SS

	ME__MJ
DIM	23865210.80
DIM	638249118
	1317733.072
T_MILK	2861121.14
T#MILK	6955484
	1317367.389
FAT	534229.42
FAT	229266
	1317733.072
PROT	416639.43
PROT	139314
	1317609.640
LACT	544789.26
LACT	233882
	1317477.390
CELLS	79416931.81
CELLS	26318150840
	1317733.072
UREA	1713065.77
UREA	2611952
	1314289.345
RDP__	5681943.31
RDP %	28807467
	1126043.592
CP__	2066809.08

Sums of Squares and Crossproducts
SSCP / Row Var SS / Col Var SS

	ME__MJ
CP %	3252147
	1317733.072
C_	254404.88
C#	57183
	1317733.072
ICP	20816016.30
ICP	820191083
	555792.305
_RP	3490906.53
%RP	22645663
	555792.305
RI__	13023023.41
RI %	129143844
	1317733.072

Variances and Covariances
Covariance / Row Var Variance / Col Var Variance / DF

	ME__MJ
DIM	-22.5522245
DIM	17867.234
	0.3533977018
	10955
T_MILK	1.3186825
T#MILK	71.769
	0.3534888045
	10952
FAT	-0.0168942

Variances and Covariances
Covariance / Row Var Variance / Col Var Variance / DF

	ME__MJ
FAT	1.086
	0.3533977018
	10955
PROT	-0.0923686
PROT	0.597
	0.3534276520
	10954
LACT	-0.0315986
LACT	0.699
	0.3534323854
	10953
CELLS	-139.1308613
CELLS	1947214.302
	0.3533977018
	10955
UREA	-0.8337852
UREA	31.906
	0.3539473791
	10927
RDP__	0.5185652
RDP %	13.510
	0.2590110575
	9192
CP__	0.4106121
CP %	1.372
	0.3533977018
	10955

Variances and Covariances
Covariance / Row Var Variance / Col Var Variance / DF

	ME__MJ
C_	-0.0095292
C#	0.719
	0.3533977018
	10955
ICP	-4.6794260
ICP	8142.109
	0.3190146993
	4537
_RP	0.8023615
%RP	156.053
	0.3190146993
	4537
RI__	0.1645348
RI %	8.681
	0.3533977018
	10955

Simple Statistics

Variable	N	Mean	Std Dev	Sum	Minimum	Maximum	Label
DIM	10956	200.97280	133.66837	2201858	2.00000	1323	DIM
T_MILK	10953	23.73327	8.47163	259951	0.40000	70.70000	T#MILK
FAT	10956	4.45428	1.04190	48801	0.29000	12.90000	FAT
PROT	10955	3.48139	0.77255	38139	0	13.60000	PROT
LACT	10954	4.54449	0.83604	49780	0	5.53000	LACT
CELLS	10956	674.63417	1395	7391292	0	24995	CELLS
UREA	10928	14.39136	5.64857	157269	0	71.23000	UREA
RDP__	9193	55.85805	3.67563	513503	50.16000	64.33000	RDP %
CP__	10956	17.18911	1.17125	188324	14.80000	19.46000	CP %

Simple Statistics

Variable	N	Mean	Std Dev	Sum	Minimum	Maximum	Label
C_	10956	2.12130	0.84822	23241	1.00000	3.00000	C#
ICP	4538	415.44932	90.23363	1885309	248.00000	943.00000	ICP
_RP	4538	69.52849	12.49211	315520	29.48038	112.09677	%RP
RI__	10956	108.53027	2.94629	1189058	102.00000	111.80124	RI %
ME__MJ	10956	10.95087	0.59447	119978	9.60000	11.74000	ME# MJ

Pearson Correlation Coefficients
Prob > |r| under H0: Rho=0
Number of Observations

	ME__MJ
DIM	-0.28381
DIM	<.0001
	10956
T_MILK	0.26181
T#MILK	<.0001
	10953
FAT	-0.02728
FAT	0.0043
	10956
PROT	-0.20112
PROT	<.0001
	10955
LACT	-0.06358
LACT	<.0001
	10954
CELLS	-0.16772
CELLS	<.0001
	10956

Pearson Correlation Coefficients
Prob > |r| under H0: Rho=0
Number of Observations

	ME__MJ
UREA	-0.24811
UREA	<.0001
	10928
RDP__	0.27721
RDP %	<.0001
	9193
CP__	0.58972
CP %	<.0001
	10956
C_	-0.01890
C#	0.0479
	10956
ICP	-0.09182
ICP	<.0001
	4538
_RP	0.11372
%RP	<.0001
	4538
RI__	0.09394
RI %	<.0001
	10956

The CORR Procedure

13 With Variables: DIM T_MILK FAT PROT LACT CELLS UREA RDP__ CP__ ME__MJ C_ _RP RI__

1 Variables: ICP

Sums of Squares and Crossproducts
SSCP / Row Var SS / Col Var SS

	ICP
DIM	288032337
DIM	147344318
	820191083.0
T_MILK	49112044
T#MILK	3373954
	819817270.0
FAT	8412362
FAT	96071
	820191083.0
PROT	6626488
PROT	59061
	820076162.0
LACT	8533970
LACT	95769
	819891262.0
CELLS	1487464567
CELLS	12566090830
	820191083.0
UREA	25825071
UREA	960164
	819684874.0
RDP__	93826852
RDP %	12974339
	712714344.0
CP__	32908825

Sums of Squares and Crossproducts
SSCP / Row Var SS / Col Var SS

	ICP
CP %	1395119
	820191083.0
ME__MJ	20816016
ME# MJ	555792
	820191083.0
C_	4833372
C#	30695
	820191083.0
_RP	126156400
%RP	22645663
	820191083.0
RI__	203257196
RI %	52917014
	820191083.0

Variances and Covariances
Covariance / Row Var Variance / Col Var Variance / DF

	ICP
DIM	314.557563
DIM	9360.898
	8142.108627
	4537
T_MILK	160.623170
T#MILK	84.643
	8144.183816
	4534
FAT	-7.006019

Variances and Covariances
Covariance / Row Var Variance / Col Var Variance / DF

	ICP
FAT	1.110
	8142.108627
	4537
PROT	-2.675854
PROT	0.614
	8142.614871
	4536
LACT	1.974158
LACT	0.650
	8144.363788
	4535
CELLS	5078.199657
CELLS	2166208.990
	8142.108627
	4537
UREA	60.629036
UREA	27.836
	8144.684191
	4533
RDP__	15.849703
RDP %	10.573
	8170.275150
	4024
CP__	-10.899468
CP %	1.823
	8142.108627
	4537

Variances and Covariances
Covariance / Row Var Variance / Col Var Variance / DF

	ICP
ME__MJ	-4.679426
ME# MJ	0.319
	8142.108627
	4537
C_	12.552613
C#	0.345
	8142.108627
	4537
_RP	-1085.804099
%RP	156.053
	8142.108627
	4537
RI__	-57.613541
RI %	7.725
	8142.108627
	4537

Simple Statistics

Variable	**N**	**Mean**	**Std Dev**	**Sum**	**Minimum**	**Maximum**	**Label**
DIM	10956	200.97280	133.66837	2201858	2.00000	1323	DIM
T_MILK	10953	23.73327	8.47163	259951	0.40000	70.70000	T#MILK
FAT	10956	4.45428	1.04190	48801	0.29000	12.90000	FAT
PROT	10955	3.48139	0.77255	38139	0	13.60000	PROT
LACT	10954	4.54449	0.83604	49780	0	5.53000	LACT
CELLS	10956	674.63417	1395	7391292	0	24995	CELLS
UREA	10928	14.39136	5.64857	157269	0	71.23000	UREA
RDP__	9193	55.85805	3.67563	513503	50.16000	64.33000	RDP %
CP__	10956	17.18911	1.17125	188324	14.80000	19.46000	CP %

Simple Statistics

Variable	N	Mean	Std Dev	Sum	Minimum	Maximum	Label
ME__MJ	10956	10.95087	0.59447	119978	9.60000	11.74000	ME# MJ
C_	10956	2.12130	0.84822	23241	1.00000	3.00000	C#
_RP	4538	69.52849	12.49211	315520	29.48038	112.09677	%RP
RI__	10956	108.53027	2.94629	1189058	102.00000	111.80124	RI %
ICP	4538	415.44932	90.23363	1885309	248.00000	943.00000	ICP

Pearson Correlation Coefficients
Prob > |r| under H0: Rho=0
Number of Observations

	ICP
DIM	0.03603
DIM	0.0152
	4538
T_MILK	0.19346
T#MILK	<.0001
	4535
FAT	-0.07370
FAT	<.0001
	4538
PROT	-0.03784
PROT	0.0108
	4537
LACT	0.02714
LACT	0.0676
	4536
CELLS	0.03824
CELLS	0.0100
	4538

Pearson Correlation Coefficients
Prob > |r| under H0: Rho=0
Number of Observations

	ICP
UREA	0.12733
UREA	<.0001
	4534
RDP__	0.05393
RDP %	0.0006
	4025
CP__	-0.08945
CP %	<.0001
	4538
ME__MJ	-0.09182
ME# MJ	<.0001
	4538
C_	0.23668
C#	<.0001
	4538
_RP	-0.96327
%RP	<.0001
	4538
RI__	-0.22972
RI %	<.0001
	4538

The CORR Procedure

13 With Variables:	DIM T_MILK FAT PROT LACT CELLS UREA RDP__ CP__ ME__MJ C_ ICP RI__
1 Variables:	_RP

Sums of Squares and Crossproducts
SSCP / Row Var SS / Col Var SS

	_RP
DIM	47737820.0
DIM	147344318
	22645663.02
T_MILK	7984232.8
T#MILK	3373954
	22626504.40
FAT	1418443.7
FAT	96071
	22645663.02
PROT	1112738.6
PROT	59061
	22638938.05
LACT	1424871.2
LACT	95769
	22634758.28
CELLS	241121909.0
CELLS	12566090830
	22645663.02
UREA	4231147.9
UREA	960164
	22620218.81
RDP__	16021604.8
RDP %	12974339
	20507140.85
CP__	5524338.7

Sums of Squares and Crossproducts
SSCP / Row Var SS / Col Var SS

	_RP
CP %	1395119
	22645663.02
ME__MJ	3490906.5
ME# MJ	555792
	22645663.02
C_	791246.1
C#	30695
	22645663.02
ICP	126156400.0
ICP	820191083
	22645663.02
RI__	34102560.9
RI %	52917014
	22645663.02

Variances and Covariances
Covariance / Row Var Variance / Col Var Variance / DF

	_RP
DIM	-50.179040
DIM	9360.898
	156.0529205
	4537
T_MILK	-24.602350
T#MILK	84.643
	156.0589506
	4534
FAT	1.157921

Variances and Covariances
Covariance / Row Var Variance / Col Var Variance / DF

	_RP
FAT	1.110
	156.0529205
	4537
PROT	0.397882
PROT	0.614
	156.0529939
	4536
LACT	-0.390689
LACT	0.650
	156.0821640
	4535
CELLS	-872.895463
CELLS	2166208.990
	156.0529205
	4537
UREA	-9.657380
UREA	27.836
	156.0537208
	4533
RDP__	-3.116995
RDP %	10.573
	155.6611741
	4024
CP__	1.879949
CP %	1.823
	156.0529205
	4537

Variances and Covariances
Covariance / Row Var Variance / Col Var Variance / DF

	_RP
ME__MJ	0.802362
ME# MJ	0.319
	156.0529205
	4537
C_	-1.790375
C#	0.345
	156.0529205
	4537
ICP	-1085.804099
ICP	8142.109
	156.0529205
	4537
RI__	9.308194
RI %	7.725
	156.0529205
	4537

Simple Statistics

Variable	N	Mean	Std Dev	Sum	Minimum	Maximum	Label
DIM	10956	200.97280	133.66837	2201858	2.00000	1323	DIM
T_MILK	10953	23.73327	8.47163	259951	0.40000	70.70000	T#MILK
FAT	10956	4.45428	1.04190	48801	0.29000	12.90000	FAT
PROT	10955	3.48139	0.77255	38139	0	13.60000	PROT
LACT	10954	4.54449	0.83604	49780	0	5.53000	LACT
CELLS	10956	674.63417	1395	7391292	0	24995	CELLS
UREA	10928	14.39136	5.64857	157269	0	71.23000	UREA
RDP__	9193	55.85805	3.67563	513503	50.16000	64.33000	RDP %
CP__	10956	17.18911	1.17125	188324	14.80000	19.46000	CP %

Simple Statistics

Variable	N	Mean	Std Dev	Sum	Minimum	Maximum	Label
ME__MJ	10956	10.95087	0.59447	119978	9.60000	11.74000	ME# MJ
C_	10956	2.12130	0.84822	23241	1.00000	3.00000	C#
ICP	4538	415.44932	90.23363	1885309	248.00000	943.00000	ICP
RI__	10956	108.53027	2.94629	1189058	102.00000	111.80124	RI %
_RP	4538	69.52849	12.49211	315520	29.48038	112.09677	%RP

Pearson Correlation Coefficients
Prob > |r| under H0: Rho=0
Number of Observations

	_RP
DIM	-0.04152
DIM	0.0052
	4538
T_MILK	-0.21406
T#MILK	<.0001
	4535
FAT	0.08799
FAT	<.0001
	4538
PROT	0.04064
PROT	0.0062
	4537
LACT	-0.03880
LACT	0.0090
	4536
CELLS	-0.04748
CELLS	0.0014
	4538

Pearson Correlation Coefficients
Prob > |r| under H0: Rho=0
Number of Observations

	_RP
UREA	-0.14653
UREA	<.0001
	4534
RDP__	-0.07683
RDP %	<.0001
	4025
CP__	0.11145
CP %	<.0001
	4538
ME__MJ	0.11372
ME# MJ	<.0001
	4538
C_	-0.24384
C#	<.0001
	4538
ICP	-0.96327
ICP	<.0001
	4538
RI__	0.26808
RI %	<.0001
	4538

The SAS System

The CORR Procedure

13 With Variables:	DIM T_MILK FAT PROT LACT CELLS UREA RDP__ CP__ ME__MJ C_ ICP _RP
1 Variables:	RI__

Sums of Squares and Crossproducts
SSCP / Row Var SS / Col Var SS

	RI__
DIM	238176706.8
DIM	638249118
	129143843.8
T_MILK	28110101.0
T#MILK	6955484
	129108383.8
FAT	5301974.8
FAT	229266
	129143843.8
PROT	4140832.2
PROT	139314
	129131780.4
LACT	5400685.7
LACT	233882
	129119959.9
CELLS	798870576.4
CELLS	26318150840
	129143843.8
UREA	17009611.9
UREA	2611952
	128799675.6
RDP__	55965359.8
RDP %	28807467
	109492376.1
CP__	20448071.1

Sums of Squares and Crossproducts
SSCP / Row Var SS / Col Var SS

	RI__
CP %	3252147
	129143843.8
ME__MJ	13023023.4
ME# MJ	1317733
	129143843.8
C_	2504961.5
C#	57183
	129143843.8
ICP	203257195.6
ICP	820191083
	52917014.1
_RP	34102560.9
%RP	22645663
	52917014.1

Variances and Covariances
Covariance / Row Var Variance / Col Var Variance / DF

	RI__
DIM	-72.2535567
DIM	17867.234
	8.680597800
	10955
T_MILK	-9.3484157
T#MILK	71.769
	8.682736771
	10952
FAT	0.5093874

Variances and Covariances
Covariance / Row Var Variance / Col Var Variance / DF

	RI__
FAT	1.086
	8.680597800
	10955
PROT	0.1494040
PROT	0.597
	8.681235150
	10954
LACT	-0.1811901
LACT	0.699
	8.682024371
	10953
CELLS	-301.9940049
CELLS	1947214.302
	8.680597800
	10955
UREA	-5.2960772
UREA	31.906
	8.684538854
	10927
RDP__	-6.5608295
RDP %	13.510
	6.543509945
	9192
CP__	0.8427842
CP %	1.372
	8.680597800
	10955

Variances and Covariances
Covariance / Row Var Variance / Col Var Variance / DF

	RI__
ME__MJ	0.1645348
ME# MJ	0.353
	8.680597800
	10955
C_	-1.5874487
C#	0.719
	8.680597800
	10955
ICP	-57.6135411
ICP	8142.109
	7.725491833
	4537
_RP	9.3081936
%RP	156.053
	7.725491833
	4537

Simple Statistics

Variable	N	Mean	Std Dev	Sum	Minimum	Maximum	Label
DIM	10956	200.97280	133.66837	2201858	2.00000	1323	DIM
T_MILK	10953	23.73327	8.47163	259951	0.40000	70.70000	T#MILK
FAT	10956	4.45428	1.04190	48801	0.29000	12.90000	FAT
PROT	10955	3.48139	0.77255	38139	0	13.60000	PROT
LACT	10954	4.54449	0.83604	49780	0	5.53000	LACT
CELLS	10956	674.63417	1395	7391292	0	24995	CELLS
UREA	10928	14.39136	5.64857	157269	0	71.23000	UREA
RDP__	9193	55.85805	3.67563	513503	50.16000	64.33000	RDP %
CP__	10956	17.18911	1.17125	188324	14.80000	19.46000	CP %

Simple Statistics

Variable	N	Mean	Std Dev	Sum	Minimum	Maximum	Label
ME__MJ	10956	10.95087	0.59447	119978	9.60000	11.74000	ME# MJ
C_	10956	2.12130	0.84822	23241	1.00000	3.00000	C#
ICP	4538	415.44932	90.23363	1885309	248.00000	943.00000	ICP
_RP	4538	69.52849	12.49211	315520	29.48038	112.09677	%RP
RI__	10956	108.53027	2.94629	1189058	102.00000	111.80124	RI %

Pearson Correlation Coefficients
Prob > |r| under H0: Rho=0
Number of Observations

	RI__
DIM	-0.18347
DIM	<.0001
	10956
T_MILK	-0.37449
T#MILK	<.0001
	10953
FAT	0.16594
FAT	<.0001
	10956
PROT	0.06564
PROT	<.0001
	10955
LACT	-0.07355
LACT	<.0001
	10954
CELLS	-0.07345
CELLS	<.0001
	10956

Pearson Correlation Coefficients
Prob > |r| under H0: Rho=0
Number of Observations

	RI__
UREA	-0.31816
UREA	<.0001
	10928
RDP__	-0.69779
RDP %	<.0001
	9193
CP__	0.24423
CP %	<.0001
	10956
ME__MJ	0.09394
ME# MJ	<.0001
	10956
C_	-0.63521
C#	<.0001
	10956
ICP	-0.22972
ICP	<.0001
	4538
_RP	0.26808
%RP	<.0001
	4538

The PHREG Procedure

Model Information

Data Set	WORK.TEST_DATA

Model Information

Dependent Variable	UREA	UREA
Ties Handling	BRESLOW	

Number of Observations Read	10962
Number of Observations Used	4016

Summary of the Number of Event and Censored Values

Total	Event	Censored	Percent Censored
4016	4016	0	0.00

Analysis of Effects Eligible for Entry

Effect	DF	Score Chi-Square	Pr > ChiSq	Effect Label
DIM	1	2.4529	0.1173	DIM
T_MILK	1	54.6423	<.0001	T#MILK
FAT	1	4.5821	0.0323	FAT
PROT	1	111.8466	<.0001	PROT
LACT	1	1360.7625	<.0001	LACT
CELLS	1	0.2575	0.6118	CELLS
RDP__	1	209.2424	<.0001	RDP %
CP__	1	288.1717	<.0001	CP %
ME__MJ	1	129.6555	<.0001	ME# MJ
C_	1	15.5497	<.0001	C#
ICP	1	51.8762	<.0001	ICP
_RP	1	71.1268	<.0001	%RP
RI__	1	253.5971	<.0001	RI %

Residual Chi-Square Test

Chi-Square	DF	Pr > ChiSq
2538.1731	13	<.0001

Step 1. Effect LACT is entered. The model contains the following effects:

LACT

Convergence Status

Convergence criterion (GCONV=1E-8) satisfied.

Model Fit Statistics

Criterion	Without Covariates	With Covariates
-2 LOG L	58638.291	57845.232
AIC	58638.291	57847.232
SBC	58638.291	57853.530

Testing Global Null Hypothesis: BETA=0

Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	793.0584	1	<.0001
Score	1360.7625	1	<.0001
Wald	921.9405	1	<.0001

Analysis of Maximum Likelihood Estimates

Parameter	DF	Parameter Estimate	Standard Error	Chi-Square	Pr > ChiSq	Hazard Ratio	Label
LACT	1	-0.92799	0.03056	921.9405	<.0001	0.395	LACT

Analysis of Effects Eligible for Entry

Effect	DF	Score Chi-Square	Pr > ChiSq	Effect Label
DIM	1	6.2982	0.0121	DIM
T_MILK	1	9.7144	0.0018	T#MILK
FAT	1	16.5645	<.0001	FAT
PROT	1	25.9595	<.0001	PROT
CELLS	1	59.4073	<.0001	CELLS
RDP__	1	189.8825	<.0001	RDP %
CP__	1	193.3226	<.0001	CP %

Analysis of Effects Eligible for Entry

Effect	DF	Score Chi-Square	Pr > ChiSq	Effect Label
ME__MJ	1	87.4028	<.0001	ME# MJ
C_	1	9.8188	0.0017	C#
ICP	1	52.2209	<.0001	ICP
_RP	1	65.1271	<.0001	%RP
RI__	1	213.7355	<.0001	RI %

Residual Chi-Square Test

Chi-Square	DF	Pr > ChiSq
782.9497	12	<.0001

Step 2. Effect RI_ is entered. The model contains the following effects:

LACT RI_

Convergence Status

Convergence criterion (GCONV=1E-8) satisfied.

Model Fit Statistics

Criterion	Without Covariates	With Covariates
-2 LOG L	58638.291	57606.083
AIC	58638.291	57610.083
SBC	58638.291	57622.679

Testing Global Null Hypothesis: BETA=0

Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	1032.2076	2	<.0001
Score	1600.6288	2	<.0001
Wald	1176.2230	2	<.0001

Analysis of Maximum Likelihood Estimates

Parameter	DF	Parameter Estimate	Standard Error	Chi-Square	Pr > ChiSq	Hazard Ratio	Label
LACT	1	-0.88474	0.02987	877.0815	<.0001	0.413	LACT
RI__	1	0.09750	0.00675	208.7907	<.0001	1.102	RI %

Analysis of Effects Eligible for Entry

Effect	DF	Score Chi-Square	Pr > ChiSq	Effect Label
DIM	1	0.2863	0.5926	DIM
T_MILK	1	15.2956	<.0001	T#MILK
FAT	1	3.0517	0.0807	FAT
PROT	1	143.3201	<.0001	PROT
CELLS	1	56.1969	<.0001	CELLS
RDP__	1	27.4871	<.0001	RDP %
CP__	1	153.1060	<.0001	CP %
ME__MJ	1	204.7720	<.0001	ME# MJ
C_	1	31.6273	<.0001	C#
ICP	1	27.2422	<.0001	ICP
_RP	1	30.1416	<.0001	%RP

Residual Chi-Square Test

Chi-Square	DF	Pr > ChiSq
558.6727	11	<.0001

Step 3. Effect ME_MJ is entered. The model contains the following effects:

LACT ME_MJ RI_

Convergence Status

Convergence criterion (GCONV=1E-8) satisfied.

Model Fit Statistics

Criterion	Without Covariates	With Covariates
-2 LOG L	58638.291	57397.723
AIC	58638.291	57403.723
SBC	58638.291	57422.617

Testing Global Null Hypothesis: BETA=0

Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	1240.5680	3	<.0001
Score	1770.8883	3	<.0001
Wald	1402.2218	3	<.0001

Analysis of Maximum Likelihood Estimates

Parameter	DF	Parameter Estimate	Standard Error	Chi-Square	Pr > ChiSq	Hazard Ratio	Label
LACT	1	-0.83558	0.02988	782.0233	<.0001	0.434	LACT
ME__MJ	1	0.44453	0.03127	202.0363	<.0001	1.560	ME# MJ
RI__	1	0.12301	0.00703	306.2889	<.0001	1.131	RI %

Analysis of Effects Eligible for Entry

Effect	DF	Score Chi-Square	Pr > ChiSq	Effect Label
DIM	1	3.1683	0.0751	DIM
T_MILK	1	0.3873	0.5337	T#MILK
FAT	1	2.6128	0.1060	FAT
PROT	1	60.8528	<.0001	PROT
CELLS	1	24.5446	<.0001	CELLS
RDP__	1	135.3253	<.0001	RDP %
CP__	1	4.5181	0.0335	CP %
C_	1	65.2217	<.0001	C#
ICP	1	16.9652	<.0001	ICP
_RP	1	14.7288	0.0001	%RP

Residual Chi-Square Test

Chi-Square	DF	Pr > ChiSq
350.7341	10	<.0001

Step 4. Effect RDP_ is entered. The model contains the following effects:

LACT RDP_ ME_MJ RI_

Convergence Status

Convergence criterion (GCONV=1E-8) satisfied.

Model Fit Statistics

Criterion	Without Covariates	With Covariates
-2 LOG L	58638.291	57262.422
AIC	58638.291	57270.422
SBC	58638.291	57295.614

Testing Global Null Hypothesis: BETA=0

Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	1375.8689	4	<.0001
Score	1929.6628	4	<.0001
Wald	1589.8585	4	<.0001

Analysis of Maximum Likelihood Estimates

Parameter	DF	Parameter Estimate	Standard Error	Chi-Square	Pr > ChiSq	Hazard Ratio	Label
LACT	1	-0.81270	0.02957	755.2169	<.0001	0.444	LACT
RDP__	1	-0.07314	0.00630	134.8994	<.0001	0.929	RDP %
ME__MJ	1	0.58060	0.03282	312.8763	<.0001	1.787	ME# MJ
RI__	1	0.06934	0.00833	69.3600	<.0001	1.072	RI %

Analysis of Effects Eligible for Entry

Effect	DF	Score Chi-Square	Pr > ChiSq	Effect Label
DIM	1	4.9327	0.0264	DIM
T_MILK	1	1.0953	0.2953	T#MILK
FAT	1	12.0355	0.0005	FAT
PROT	1	75.3390	<.0001	PROT
CELLS	1	23.6725	<.0001	CELLS
CP__	1	1.6975	0.1926	CP %
C_	1	34.9020	<.0001	C#
ICP	1	21.6842	<.0001	ICP
_RP	1	16.1315	<.0001	%RP

Residual Chi-Square Test

Chi-Square	DF	Pr > ChiSq
210.4387	9	<.0001

Step 5. Effect PROT is entered. The model contains the following effects:

PROT LACT RDP_ ME_MJ RI_

Convergence Status

Convergence criterion (GCONV=1E-8) satisfied.

Model Fit Statistics

Criterion	Without Covariates	With Covariates
-2 LOG L	58638.291	57183.432
AIC	58638.291	57193.432
SBC	58638.291	57224.922

Testing Global Null Hypothesis: BETA=0

Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	1454.8592	5	<.0001

Testing Global Null Hypothesis: BETA=0

Test	Chi-Square	DF	Pr > ChiSq
Score	2225.8361	5	<.0001
Wald	1563.2710	5	<.0001

Analysis of Maximum Likelihood Estimates

Parameter	DF	Parameter Estimate	Standard Error	Chi-Square	Pr > ChiSq	Hazard Ratio	Label
PROT	1	-0.27037	0.03117	75.2600	<.0001	0.763	PROT
LACT	1	-0.73668	0.03294	500.1184	<.0001	0.479	LACT
RDP__	1	-0.07785	0.00636	150.0050	<.0001	0.925	RDP %
ME__MJ	1	0.50156	0.03435	213.2409	<.0001	1.651	ME# MJ
RI__	1	0.08599	0.00854	101.4451	<.0001	1.090	RI %

Analysis of Effects Eligible for Entry

Effect	DF	Score Chi-Square	Pr > ChiSq	Effect Label
DIM	1	27.7499	<.0001	DIM
T_MILK	1	12.1495	0.0005	T#MILK
FAT	1	9.3984	0.0022	FAT
CELLS	1	11.6887	0.0006	CELLS
CP__	1	0.9168	0.3383	CP %
C_	1	58.4668	<.0001	C#
ICP	1	27.3016	<.0001	ICP
_RP	1	22.8088	<.0001	%RP

Residual Chi-Square Test

Chi-Square	DF	Pr > ChiSq
145.2012	8	<.0001

Step 6. Effect C_ is entered. The model contains the following effects:

PROT LACT RDP_ ME_MJ C_ RI_

Convergence Status

Convergence criterion (GCONV=1E-8) satisfied.

Model Fit Statistics

Criterion	Without Covariates	With Covariates
-2 LOG L	58638.291	57124.393
AIC	58638.291	57136.393
SBC	58638.291	57174.181

Testing Global Null Hypothesis: BETA=0

Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	1513.8976	6	<.0001
Score	2282.3611	6	<.0001
Wald	1528.5611	6	<.0001

Analysis of Maximum Likelihood Estimates

Parameter	DF	Parameter Estimate	Standard Error	Chi-Square	Pr > ChiSq	Hazard Ratio	Label
PROT	1	-0.32202	0.03285	96.0985	<.0001	0.725	PROT
LACT	1	-0.72987	0.03409	458.5226	<.0001	0.482	LACT
RDP__	1	-0.06876	0.00649	112.1310	<.0001	0.934	RDP %
ME__MJ	1	0.50371	0.03424	216.4046	<.0001	1.655	ME# MJ
C_	1	0.24420	0.03201	58.1890	<.0001	1.277	C#
RI__	1	0.13027	0.01050	153.9706	<.0001	1.139	RI %

Analysis of Effects Eligible for Entry

Effect	DF	Score Chi-Square	Pr > ChiSq	Effect Label
DIM	1	22.3471	<.0001	DIM
T_MILK	1	14.8022	0.0001	T#MILK
FAT	1	6.6330	0.0100	FAT
CELLS	1	10.7314	0.0011	CELLS
CP__	1	6.0903	0.0136	CP %

Analysis of Effects Eligible for Entry

Effect	DF	Score Chi-Square	Pr > ChiSq	Effect Label
ICP	1	44.7844	<.0001	ICP
_RP	1	36.9817	<.0001	%RP

Residual Chi-Square Test

Chi-Square	DF	Pr > ChiSq
84.0092	7	<.0001

Step 7. Effect ICP is entered. The model contains the following effects:

PROT LACT RDP_ ME_MJ C_ ICP RI_

Convergence Status

Convergence criterion (GCONV=1E-8) satisfied.

Model Fit Statistics

Criterion	Without Covariates	With Covariates
-2 LOG L	58638.291	57076.535
AIC	58638.291	57090.535
SBC	58638.291	57134.621

Testing Global Null Hypothesis: BETA=0

Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	1561.7560	7	<.0001
Score	2338.2398	7	<.0001
Wald	1542.5808	7	<.0001

Analysis of Maximum Likelihood Estimates

Parameter	DF	Parameter Estimate	Standard Error	Chi-Square	Pr > ChiSq	Hazard Ratio	Label
PROT	1	-0.34354	0.03304	108.1101	<.0001	0.709	PROT
LACT	1	-0.73156	0.03440	452.2119	<.0001	0.481	LACT

Analysis of Maximum Likelihood Estimates

Parameter	DF	Parameter Estimate	Standard Error	Chi-Square	Pr > ChiSq	Hazard Ratio	Label
RDP__	1	-0.07006	0.00650	116.0872	<.0001	0.932	RDP %
ME__MJ	1	0.49321	0.03439	205.6832	<.0001	1.638	ME# MJ
C_	1	0.28379	0.03235	76.9727	<.0001	1.328	C#
ICP	1	-0.00116	0.0001737	44.6279	<.0001	0.999	ICP
RI__	1	0.12978	0.01050	152.7580	<.0001	1.139	RI %

Analysis of Effects Eligible for Entry

Effect	DF	Score Chi-Square	Pr > ChiSq	Effect Label
DIM	1	24.0720	<.0001	DIM
T_MILK	1	8.9814	0.0027	T#MILK
FAT	1	4.0415	0.0444	FAT
CELLS	1	7.8623	0.0050	CELLS
CP__	1	4.8862	0.0271	CP %
_RP	1	2.3989	0.1214	%RP

Residual Chi-Square Test

Chi-Square	DF	Pr > ChiSq
41.3465	6	<.0001

Step 8. Effect DIM is entered. The model contains the following effects:

DIM PROT LACT RDP_ ME_MJ C_ ICP RI_

Convergence Status

Convergence criterion (GCONV=1E-8) satisfied.

Model Fit Statistics

Criterion	Without Covariates	With Covariates
-2 LOG L	58638.291	57052.678

Model Fit Statistics

Criterion	Without Covariates	With Covariates
AIC	58638.291	57068.678
SBC	58638.291	57119.062

Testing Global Null Hypothesis: BETA=0

Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	1585.6129	8	<.0001
Score	2407.4775	8	<.0001
Wald	1557.1233	8	<.0001

Analysis of Maximum Likelihood Estimates

Parameter	DF	Parameter Estimate	Standard Error	Chi-Square	Pr > ChiSq	Hazard Ratio	Label
DIM	1	0.0008620	0.0001758	24.0270	<.0001	1.001	DIM
PROT	1	-0.39851	0.03550	126.0347	<.0001	0.671	PROT
LACT	1	-0.70534	0.03530	399.3485	<.0001	0.494	LACT
RDP__	1	-0.07142	0.00644	123.0544	<.0001	0.931	RDP %
ME__MJ	1	0.49057	0.03433	204.2074	<.0001	1.633	ME# MJ
C_	1	0.27610	0.03262	71.6492	<.0001	1.318	C#
ICP	1	-0.00118	0.0001742	46.1636	<.0001	0.999	ICP
RI__	1	0.13719	0.01066	165.5016	<.0001	1.147	RI %

Analysis of Effects Eligible for Entry

Effect	DF	Score Chi-Square	Pr > ChiSq	Effect Label
T_MILK	1	2.3188	0.1278	T#MILK
FAT	1	4.3087	0.0379	FAT
CELLS	1	6.1033	0.0135	CELLS
CP__	1	1.0566	0.3040	CP %
_RP	1	2.2451	0.1340	%RP

Residual Chi-Square Test

Chi-Square	DF	Pr > ChiSq
17.2556	5	0.0040

Step 9. Effect CELLS is entered. The model contains the following effects:

DIM PROT LACT CELLS RDP_ ME_MJ C_ ICP RI_

Convergence Status

Convergence criterion (GCONV=1E-8) satisfied.

Model Fit Statistics

Criterion	Without Covariates	With Covariates
-2 LOG L	58638.291	57046.119
AIC	58638.291	57064.119
SBC	58638.291	57120.802

Testing Global Null Hypothesis: BETA=0

Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	1592.1714	9	<.0001
Score	2513.3653	9	<.0001
Wald	1521.0776	9	<.0001

Analysis of Maximum Likelihood Estimates

Parameter	DF	Parameter Estimate	Standard Error	Chi-Square	Pr > ChiSq	Hazard Ratio	Label
DIM	1	0.0008278	0.0001763	22.0612	<.0001	1.001	DIM
PROT	1	-0.38101	0.03624	110.5291	<.0001	0.683	PROT
LACT	1	-0.74360	0.03875	368.2503	<.0001	0.475	LACT
CELLS	1	-0.0000306	0.0000124	6.1091	0.0134	1.000	CELLS
RDP__	1	-0.07109	0.00645	121.4802	<.0001	0.931	RDP %
ME__MJ	1	0.47788	0.03477	188.8777	<.0001	1.613	ME# MJ
C_	1	0.27351	0.03263	70.2459	<.0001	1.315	C#

Analysis of Maximum Likelihood Estimates

Parameter	DF	Parameter Estimate	Standard Error	Chi-Square	Pr > ChiSq	Hazard Ratio	Label
ICP	1	-0.00115	0.0001745	43.6038	<.0001	0.999	ICP
RI__	1	0.13460	0.01071	158.0012	<.0001	1.144	RI %

Analysis of Effects Eligible for Entry

Effect	DF	Score Chi-Square	Pr > ChiSq	Effect Label
T_MILK	1	2.0461	0.1526	T#MILK
FAT	1	4.5767	0.0324	FAT
CP__	1	1.3590	0.2437	CP %
_RP	1	2.5260	0.1120	%RP

Residual Chi-Square Test

Chi-Square	DF	Pr > ChiSq
11.1860	4	0.0246

Step 10. Effect FAT is entered. The model contains the following effects:

DIM FAT PROT LACT CELLS RDP_ ME_MJ C_ ICP RI_

Convergence Status

Convergence criterion (GCONV=1E-8) satisfied.

Model Fit Statistics

Criterion	Without Covariates	With Covariates
-2 LOG L	58638.291	57041.567
AIC	58638.291	57061.567
SBC	58638.291	57124.548

Testing Global Null Hypothesis: BETA=0

Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	1596.7235	10	<.0001

Testing Global Null Hypothesis: BETA=0

Test	Chi-Square	DF	Pr > ChiSq
Score	2517.1165	10	<.0001
Wald	1532.7605	10	<.0001

Analysis of Maximum Likelihood Estimates

Parameter	DF	Parameter Estimate	Standard Error	Chi-Square	Pr > ChiSq	Hazard Ratio	Label
DIM	1	0.0008331	0.0001764	22.2930	<.0001	1.001	DIM
FAT	1	0.05563	0.02600	4.5769	0.0324	1.057	FAT
PROT	1	-0.43397	0.04385	97.9617	<.0001	0.648	PROT
LACT	1	-0.74605	0.03858	373.8652	<.0001	0.474	LACT
CELLS	1	-0.0000312	0.0000124	6.3493	0.0117	1.000	CELLS
RDP__	1	-0.06939	0.00651	113.7345	<.0001	0.933	RDP %
ME__MJ	1	0.45670	0.03619	159.2793	<.0001	1.579	ME# MJ
C_	1	0.26756	0.03273	66.8212	<.0001	1.307	C#
ICP	1	-0.00112	0.0001750	41.1874	<.0001	0.999	ICP
RI__	1	0.13215	0.01076	150.9590	<.0001	1.141	RI %

Analysis of Effects Eligible for Entry

Effect	DF	Score Chi-Square	Pr > ChiSq	Effect Label
T_MILK	1	1.6200	0.2031	T#MILK
CP__	1	1.5650	0.2109	CP %
_RP	1	2.7457	0.0975	%RP

Residual Chi-Square Test

Chi-Square	DF	Pr > ChiSq
6.5960	3	0.0860

Step 11. Effect _RP is entered. The model contains the following effects:

DIM FAT PROT LACT CELLS RDP_ ME_MJ C_ ICP _RP RI_

Convergence Status

Convergence criterion (GCONV=1E-8) satisfied.

Model Fit Statistics

Criterion	Without Covariates	With Covariates
-2 LOG L	58638.291	57038.771
AIC	58638.291	57060.771
SBC	58638.291	57130.049

Testing Global Null Hypothesis: BETA=0

Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	1599.5201	11	<.0001
Score	2521.7793	11	<.0001
Wald	1540.5316	11	<.0001

Analysis of Maximum Likelihood Estimates

Parameter	DF	Parameter Estimate	Standard Error	Chi-Square	Pr > ChiSq	Hazard Ratio	Label
DIM	1	0.0008301	0.0001763	22.1745	<.0001	1.001	DIM
FAT	1	0.05697	0.02599	4.8050	0.0284	1.059	FAT
PROT	1	-0.43095	0.04383	96.6601	<.0001	0.650	PROT
LACT	1	-0.74778	0.03852	376.9347	<.0001	0.473	LACT
CELLS	1	-0.0000319	0.0000124	6.6361	0.0100	1.000	CELLS
RDP__	1	-0.07017	0.00652	115.8761	<.0001	0.932	RDP %
ME__MJ	1	0.46651	0.03664	162.1187	<.0001	1.594	ME# MJ
C_	1	0.27046	0.03280	67.9811	<.0001	1.311	C#
ICP	1	-0.00205	0.0005906	12.0495	0.0005	0.998	ICP
_RP	1	-0.00734	0.00443	2.7440	0.0976	0.993	%RP
RI__	1	0.13348	0.01079	153.1515	<.0001	1.143	RI %

Step 12. Effect _RP is removed. The model contains the following effects:

DIM FAT PROT LACT CELLS RDP_ ME_MJ C_ ICP RI_

Convergence Status

Convergence criterion (GCONV=1E-8) satisfied.

Model Fit Statistics

Criterion	Without Covariates	With Covariates
-2 LOG L	58638.291	57041.567
AIC	58638.291	57061.567
SBC	58638.291	57124.548

Testing Global Null Hypothesis: BETA=0

Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	1596.7235	10	<.0001
Score	2517.1165	10	<.0001
Wald	1532.7605	10	<.0001

Analysis of Maximum Likelihood Estimates

Parameter	DF	Parameter Estimate	Standard Error	Chi-Square	Pr > ChiSq	Hazard Ratio	Label
DIM	1	0.0008331	0.0001764	22.2930	<.0001	1.001	DIM
FAT	1	0.05563	0.02600	4.5769	0.0324	1.057	FAT
PROT	1	-0.43397	0.04385	97.9617	<.0001	0.648	PROT
LACT	1	-0.74605	0.03858	373.8652	<.0001	0.474	LACT
CELLS	1	-0.0000312	0.0000124	6.3493	0.0117	1.000	CELLS
RDP__	1	-0.06939	0.00651	113.7345	<.0001	0.933	RDP %
ME__MJ	1	0.45670	0.03619	159.2793	<.0001	1.579	ME# MJ
C_	1	0.26756	0.03273	66.8212	<.0001	1.307	C#
ICP	1	-0.00112	0.0001750	41.1874	<.0001	0.999	ICP
RI__	1	0.13215	0.01076	150.9590	<.0001	1.141	RI %

Analysis of Effects Eligible for Entry

Effect	DF	Score Chi-Square	Pr > ChiSq	Effect Label
T_MILK	1	1.6200	0.2031	T#MILK

Analysis of Effects Eligible for Entry

Effect	DF	Score Chi-Square	Pr > ChiSq	Effect Label
CP__	1	1.5650	0.2109	CP %
_RP	1	2.7457	0.0975	%RP

Residual Chi-Square Test

Chi-Square	DF	Pr > ChiSq
6.5960	3	0.0860

Note: Model building terminates because the effect to be entered is the effect that was removed in the last step.

Summary of Stepwise Selection

Step	Effect Entered	Effect Removed	DF	Number In	Score Chi-Square	Wald Chi-Square	Pr > ChiSq	Effect Label
1	LACT		1	1	1360.7625		<.0001	LACT
2	RI__		1	2	213.7355		<.0001	RI %
3	ME__MJ		1	3	204.7720		<.0001	ME# MJ
4	RDP__		1	4	135.3253		<.0001	RDP %
5	PROT		1	5	75.3390		<.0001	PROT
6	C_		1	6	58.4668		<.0001	C#
7	ICP		1	7	44.7844		<.0001	ICP
8	DIM		1	8	24.0720		<.0001	DIM
9	CELLS		1	9	6.1033		0.0135	CELLS
10	FAT		1	10	4.5767		0.0324	FAT
11	_RP		1	11	2.7457		0.0975	%RP
12		_RP	1	10		2.7440	0.0976	%RP

The REG Procedure
Model: MODEL1
Dependent Variable: ICP Intercalving Period (ICP) [days]

Number of Observations Read	10962
Number of Observations Used	4534
Number of Observations with Missing Values	6428

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	598613	598613	74.69	<.0001
Error	4532	36321241	8014.39553		
Corrected Total	4533	36919853			

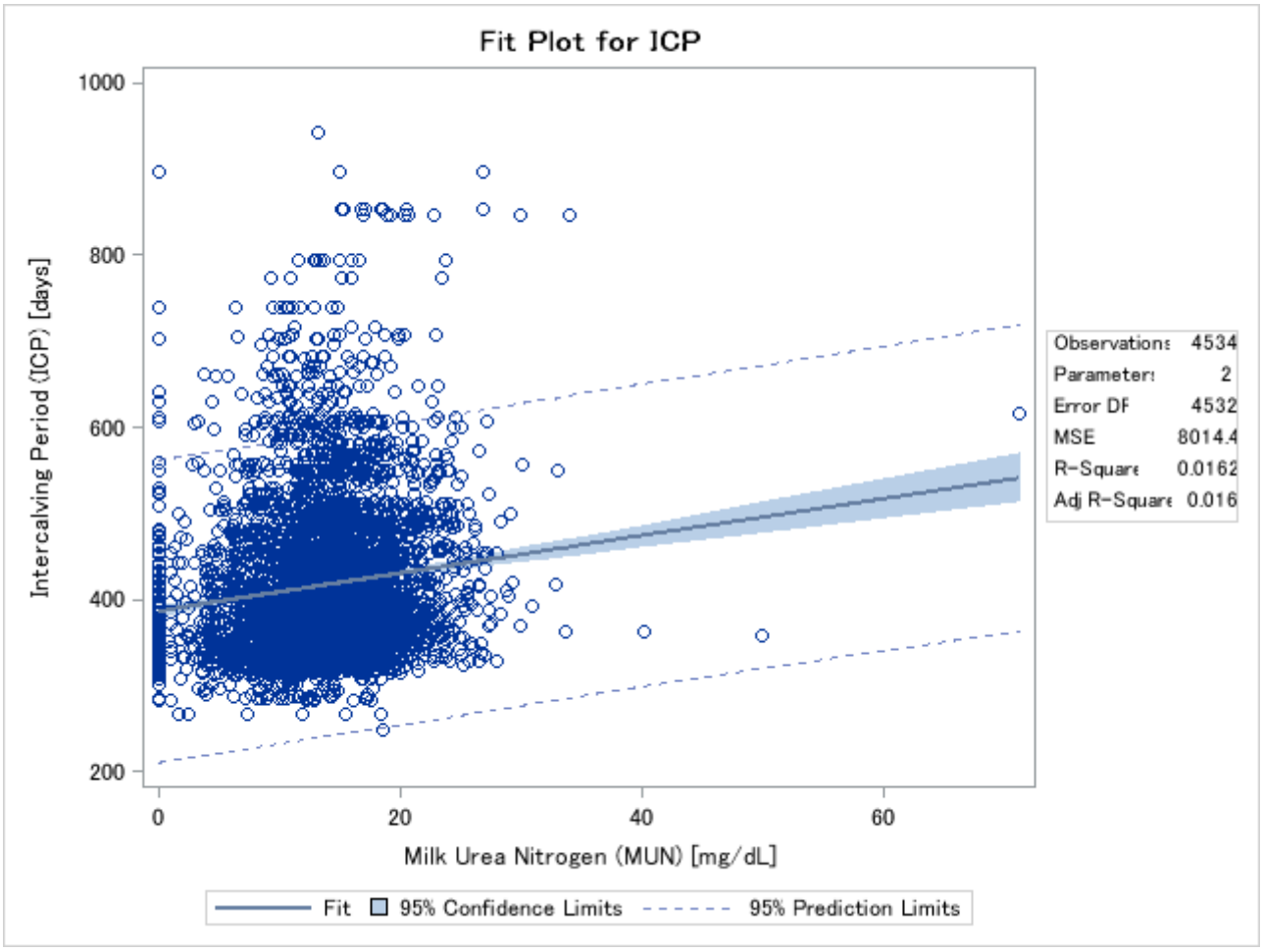
Root MSE	89.52316	**R-Square**	0.0162
Dependent Mean	415.50375	**Adj R-Sq**	0.0160
Coeff Var	21.54569		

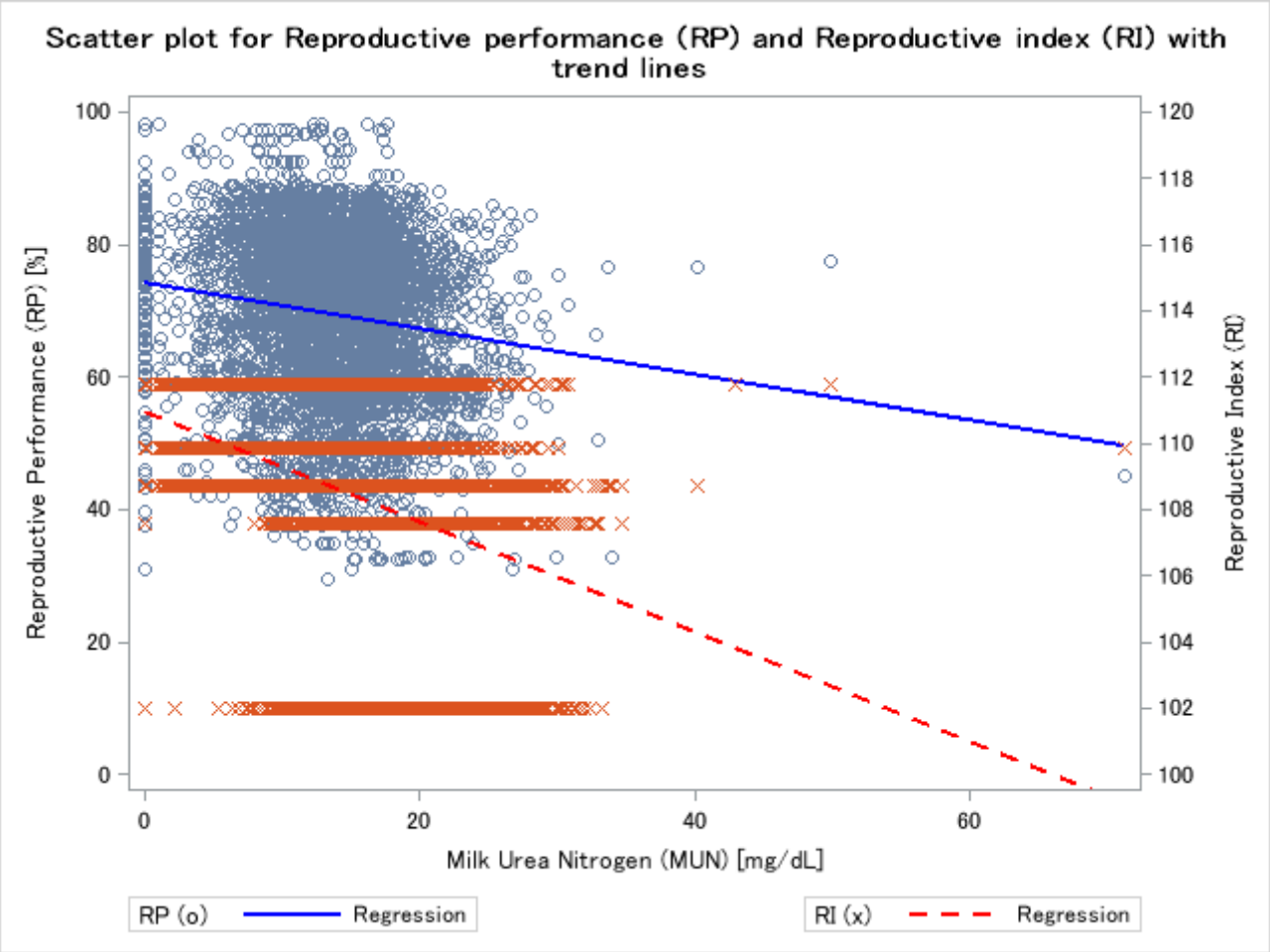
Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	Intercept	**1**	385.96323	3.66753	105.24	<.0001
UREA	Milk urea nitrogen (MUN) [mg/dL]	**1**	2.17811	0.25202	8.64	<.0001

The SAS System

The REG Procedure
Model: MODEL1
Dependent Variable: ICP Intercalving Period (ICP) [days]


Fit Plot for ICP
Intercalving Period (ICP) [days]
1000
800
600
400
200
0
20
40
60
Milk Urea Nitrogen (MUN) [mg/dL]
Observations 4534
Parameters 2
Error DF 4532
MSE 8014.4
R-Square 0.0162
Adj R-Square 0.016
Fit
95% Confidence Limits
95% Prediction Limits



Scatter plot for Reproduction performance (RP) and Reproductive index (RI) with trend lines

The ANOVA Procedure

Class Level Information

Class	Levels	Values
DIM	3	200 to 370・Less than 200・Over 370

Number of Observations Read	10962
Number of Observations Used	4538

Scatter plot for Reproduction performance (RP) and Reproductive index (RI) with trend lines

The ANOVA Procedure

Dependent Variable: ICP

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	76939.11	38469.56	4.73	0.0088

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Error	4535	36863807.73	8128.73		
Corrected Total	4537	36940746.84			

R-Square	Coeff Var	Root MSE	ICP Mean
0.002083	21.70168	90.15949	415.4493

Source	DF	Anova SS	Mean Square	F Value	Pr > F
DIM	2	76939.11098	38469.55549	4.73	0.0088

Scatter plot for Reproduction performance (RP) and Reproductive index (RI) with trend lines

The ANOVA Procedure

Class Level Information

Class	Levels	Values
ICP	284	248 266 283 286 288 290 295 296 301 307 308 311 313 314 315 316 317 318 319 320 321 322 323 324 325 326 327 328 329 330 331 332 333 334 335 336 337 338 339 340 341 342 343 344 345 346 347 348 349 350 351 352 353 354 355 356 357 358 359 360 361 362 363 364 365 366 367 368 369 370 371 372 373 374 375 376 377 378 379 380 381 382 383 384 385 386 387 388 389 390 391 392 393 394 395 396 397 398 399 400 401 402 403 404 405 406 407 408 409 410 411 412 413 414 415 416 417 418 419 420 421 422 423 424 425 426 427 428 429 430 431 432 433 434 435 436 438 439 440 441 442 443 444 445 446 447 449 450 451 452 453 454 455 456 457 458 459 460 461 462 463 464 465 466 467 468 469 470 471 472 473 474 475 476 477 478 479 481 482 483 484 485 486 487 488 489 490 491 493 497 498 499 500 501 502 504 505 506 508 509 510 511 512 513 514 517 522 523 524 525 530 531 534 535 536 537 539 542 543 544 545 547 549 550 551 554 556 557 560 561 562 563 564 566 568 569 571 572 573 579 582 584 585 590 592 594 597 601 605 606 607 608 611 612 613 617 620 626 629 634 638 642 647 649 659 662 663 672 675 680 682 683 697 702 706 708 716 739 773 795 847 853 898 943

Number of Observations Read	10962
Number of Observations Used	4538

Scatter plot for Reproduction performance (RP) and Reproductive index (RI) with trend lines

The ANOVA Procedure

Dependent Variable: C_ C#

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	283	468.086074	1.654014	6.40	<.0001

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Error	4254	1099.322697	0.258421		
Corrected Total	4537	1567.408770			

R-Square	Coeff Var	Root MSE	C_ Mean
0.298637	20.06521	0.508351	2.533495

Source	DF	Anova SS	Mean Square	F Value	Pr > F
ICP	283	468.0860738	1.6540144	6.40	<.0001

Scatter plot for Reproduction performance (RP) and Reproductive index (RI) with trend lines

The ANOVA Procedure

Class Level Information

Class	Levels	Values
ICP	284	248 266 283 286 288 290 295 296 301 307 308 311 313 314 315 316 317 318 319 320 321 322 323 324 325 326 327 328 329 330 331 332 333 334 335 336 337 338 339 340 341 342 343 344 345 346 347 348 349 350 351 352 353 354 355 356 357 358 359 360 361 362 363 364 365 366 367 368 369 370 371 372 373 374 375 376 377 378 379 380 381 382 383 384 385 386 387 388 389 390 391 392 393 394 395 396 397 398 399 400 401 402 403 404 405 406 407 408 409 410 411 412 413 414 415 416 417 418 419 420 421 422 423 424 425 426 427 428 429 430 431 432 433 434 435 436 438 439 440 441 442 443 444 445 446 447 449 450 451 452 453 454 455 456 457 458 459 460 461 462 463 464 465 466 467 468 469 470 471 472 473 474 475 476 477 478 479 481 482 483 484 485 486 487 488 489 490 491 493 497 498 499 500 501 502 504 505 506 508 509 510 511 512 513 514 517 522 523 524 525 530 531 534 535 536 537 539 542 543 544 545 547 549 550 551 554 556 557 560 561 562 563 564 566 568 569 571 572 573 579 582 584 585 590 592 594 597 601 605 606 607 608 611 612 613 617 620 626 629 634 638 642 647 649 659 662 663 672 675 680 682 683 697 702 706 708 716 739 773 795 847 853 898 943

Number of Observations Read	10962
Number of Observations Used	4535

Scatter plot for Reproduction performance (RP) and Reproductive index (RI) with trend lines

The ANOVA Procedure

Dependent Variable: T_MILK T#MILK

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	283	95201.4673	336.4009	4.96	<.0001

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Error	4251	288568.5476	67.8825		
Corrected Total	4534	383770.0149			

R-Square	Coeff Var	Root MSE	T_MILK Mean
0.248069	32.08622	8.239084	25.67795

Source	DF	Anova SS	Mean Square	F Value	Pr > F
ICP	283	95201.46734	336.40094	4.96	<.0001

Scatter plot for Reproduction performance (RP) and Reproductive index (RI) with trend lines

The ANOVA Procedure

Class Level Information

Class	Levels	Values
ICP	284	248 266 283 286 288 290 295 296 301 307 308 311 313 314 315 316 317 318 319 320 321 322 323 324 325 326 327 328 329 330 331 332 333 334 335 336 337 338 339 340 341 342 343 344 345 346 347 348 349 350 351 352 353 354 355 356 357 358 359 360 361 362 363 364 365 366 367 368 369 370 371 372 373 374 375 376 377 378 379 380 381 382 383 384 385 386 387 388 389 390 391 392 393 394 395 396 397 398 399 400 401 402 403 404 405 406 407 408 409 410 411 412 413 414 415 416 417 418 419 420 421 422 423 424 425 426 427 428 429 430 431 432 433 434 435 436 438 439 440 441 442 443 444 445 446 447 449 450 451 452 453 454 455 456 457 458 459 460 461 462 463 464 465 466 467 468 469 470 471 472 473 474 475 476 477 478 479 481 482 483 484 485 486 487 488 489 490 491 493 497 498 499 500 501 502 504 505 506 508 509 510 511 512 513 514 517 522 523 524 525 530 531 534 535 536 537 539 542 543 544 545 547 549 550 551 554 556 557 560 561 562 563 564 566 568 569 571 572 573 579 582 584 585 590 592 594 597 601 605 606 607 608 611 612 613 617 620 626 629 634 638 642 647 649 659 662 663 672 675 680 682 683 697 702 706 708 716 739 773 795 847 853 898 943

Number of Observations Read	10962
Number of Observations Used	4538

Scatter plot for Reproduction performance (RP) and Reproductive index (RI) with trend lines

The ANOVA Procedure

Dependent Variable: FAT FAT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	283	519.918248	1.837167	1.73	<.0001

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Error	4254	4515.106661	1.061379		
Corrected Total	4537	5035.024909			

R-Square	Coeff Var	Root MSE	FAT Mean
0.103260	23.00181	1.030233	4.478920

Source	DF	Anova SS	Mean Square	F Value	Pr > F
ICP	283	519.9182481	1.8371670	1.73	<.0001

Scatter plot for Reproduction performance (RP) and Reproductive index (RI) with trend lines

The ANOVA Procedure

Class Level Information

Class	Levels	Values
ICP	284	248 266 283 286 288 290 295 296 301 307 308 311 313 314 315 316 317 318 319 320 321 322 323 324 325 326 327 328 329 330 331 332 333 334 335 336 337 338 339 340 341 342 343 344 345 346 347 348 349 350 351 352 353 354 355 356 357 358 359 360 361 362 363 364 365 366 367 368 369 370 371 372 373 374 375 376 377 378 379 380 381 382 383 384 385 386 387 388 389 390 391 392 393 394 395 396 397 398 399 400 401 402 403 404 405 406 407 408 409 410 411 412 413 414 415 416 417 418 419 420 421 422 423 424 425 426 427 428 429 430 431 432 433 434 435 436 438 439 440 441 442 443 444 445 446 447 449 450 451 452 453 454 455 456 457 458 459 460 461 462 463 464 465 466 467 468 469 470 471 472 473 474 475 476 477 478 479 481 482 483 484 485 486 487 488 489 490 491 493 497 498 499 500 501 502 504 505 506 508 509 510 511 512 513 514 517 522 523 524 525 530 531 534 535 536 537 539 542 543 544 545 547 549 550 551 554 556 557 560 561 562 563 564 566 568 569 571 572 573 579 582 584 585 590 592 594 597 601 605 606 607 608 611 612 613 617 620 626 629 634 638 642 647 649 659 662 663 672 675 680 682 683 697 702 706 708 716 739 773 795 847 853 898 943

Number of Observations Read	10962
Number of Observations Used	4537

Scatter plot for Reproduction performance (RP) and Reproductive index (RI) with trend lines

The ANOVA Procedure

Dependent Variable: PROT PROT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	283	303.189323	1.071340	1.83	<.0001

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Error	4253	2483.197153	0.583870		
Corrected Total	4536	2786.386475			

R-Square	Coeff Var	Root MSE	PROT Mean
0.108811	21.69622	0.764114	3.521873

Source	DF	Anova SS	Mean Square	F Value	Pr > F
ICP	283	303.1893228	1.0713404	1.83	<.0001

Scatter plot for Reproduction performance (RP) and Reproductive index (RI) with trend lines

The ANOVA Procedure

Class Level Information

Class	Levels	Values
ICP	284	248 266 283 286 288 290 295 296 301 307 308 311 313 314 315 316 317 318 319 320 321 322 323 324 325 326 327 328 329 330 331 332 333 334 335 336 337 338 339 340 341 342 343 344 345 346 347 348 349 350 351 352 353 354 355 356 357 358 359 360 361 362 363 364 365 366 367 368 369 370 371 372 373 374 375 376 377 378 379 380 381 382 383 384 385 386 387 388 389 390 391 392 393 394 395 396 397 398 399 400 401 402 403 404 405 406 407 408 409 410 411 412 413 414 415 416 417 418 419 420 421 422 423 424 425 426 427 428 429 430 431 432 433 434 435 436 438 439 440 441 442 443 444 445 446 447 449 450 451 452 453 454 455 456 457 458 459 460 461 462 463 464 465 466 467 468 469 470 471 472 473 474 475 476 477 478 479 481 482 483 484 485 486 487 488 489 490 491 493 497 498 499 500 501 502 504 505 506 508 509 510 511 512 513 514 517 522 523 524 525 530 531 534 535 536 537 539 542 543 544 545 547 549 550 551 554 556 557 560 561 562 563 564 566 568 569 571 572 573 579 582 584 585 590 592 594 597 601 605 606 607 608 611 612 613 617 620 626 629 634 638 642 647 649 659 662 663 672 675 680 682 683 697 702 706 708 716 739 773 795 847 853 898 943

Number of Observations Read	10962
Number of Observations Used	4536

Scatter plot for Reproduction performance (RP) and Reproductive index (RI) with trend lines

The ANOVA Procedure

Dependent Variable: LACT LACT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	283	214.790589	0.758977	1.18	0.0232

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Error	4252	2731.697061	0.642450		
Corrected Total	4535	2946.487650			

R-Square	Coeff Var	Root MSE	LACT Mean
0.072897	17.71861	0.801530	4.523660

Source	DF	Anova SS	Mean Square	F Value	Pr > F
ICP	283	214.7905893	0.7589773	1.18	0.0232

Scatter plot for Reproduction performance (RP) and Reproductive index (RI) with trend lines

The ANOVA Procedure

Class Level Information

Class	Levels	Values
ICP	284	248 266 283 286 288 290 295 296 301 307 308 311 313 314 315 316 317 318 319 320 321 322 323 324 325 326 327 328 329 330 331 332 333 334 335 336 337 338 339 340 341 342 343 344 345 346 347 348 349 350 351 352 353 354 355 356 357 358 359 360 361 362 363 364 365 366 367 368 369 370 371 372 373 374 375 376 377 378 379 380 381 382 383 384 385 386 387 388 389 390 391 392 393 394 395 396 397 398 399 400 401 402 403 404 405 406 407 408 409 410 411 412 413 414 415 416 417 418 419 420 421 422 423 424 425 426 427 428 429 430 431 432 433 434 435 436 438 439 440 441 442 443 444 445 446 447 449 450 451 452 453 454 455 456 457 458 459 460 461 462 463 464 465 466 467 468 469 470 471 472 473 474 475 476 477 478 479 481 482 483 484 485 486 487 488 489 490 491 493 497 498 499 500 501 502 504 505 506 508 509 510 511 512 513 514 517 522 523 524 525 530 531 534 535 536 537 539 542 543 544 545 547 549 550 551 554 556 557 560 561 562 563 564 566 568 569 571 572 573 579 582 584 585 590 592 594 597 601 605 606 607 608 611 612 613 617 620 626 629 634 638 642 647 649 659 662 663 672 675 680 682 683 697 702 706 708 716 739 773 795 847 853 898 943

Number of Observations Read	10962
Number of Observations Used	4538

Scatter plot for Reproduction performance (RP) and Reproductive index (RI) with trend lines

The ANOVA Procedure

Dependent Variable: CELLS CELLS

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	283	1088883911	3847646	1.87	<.0001

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Error	4254	8739206278	2054350		
Corrected Total	4537	9828090189			

R-Square	Coeff Var	Root MSE	CELLS Mean
0.110793	184.5239	1433.301	776.7558

Source	DF	Anova SS	Mean Square	F Value	Pr > F
ICP	283	1088883911	3847646	1.87	<.0001

Scatter plot for Reproduction performance (RP) and Reproductive index (RI) with trend lines

The ANOVA Procedure

Class Level Information

Class	Levels	Values
ICP	284	248 266 283 286 288 290 295 296 301 307 308 311 313 314 315 316 317 318 319 320 321 322 323 324 325 326 327 328 329 330 331 332 333 334 335 336 337 338 339 340 341 342 343 344 345 346 347 348 349 350 351 352 353 354 355 356 357 358 359 360 361 362 363 364 365 366 367 368 369 370 371 372 373 374 375 376 377 378 379 380 381 382 383 384 385 386 387 388 389 390 391 392 393 394 395 396 397 398 399 400 401 402 403 404 405 406 407 408 409 410 411 412 413 414 415 416 417 418 419 420 421 422 423 424 425 426 427 428 429 430 431 432 433 434 435 436 438 439 440 441 442 443 444 445 446 447 449 450 451 452 453 454 455 456 457 458 459 460 461 462 463 464 465 466 467 468 469 470 471 472 473 474 475 476 477 478 479 481 482 483 484 485 486 487 488 489 490 491 493 497 498 499 500 501 502 504 505 506 508 509 510 511 512 513 514 517 522 523 524 525 530 531 534 535 536 537 539 542 543 544 545 547 549 550 551 554 556 557 560 561 562 563 564 566 568 569 571 572 573 579 582 584 585 590 592 594 597 601 605 606 607 608 611 612 613 617 620 626 629 634 638 642 647 649 659 662 663 672 675 680 682 683 697 702 706 708 716 739 773 795 847 853 898 943

Number of Observations Read	10962
Number of Observations Used	4534

Scatter plot for Reproduction performance (RP) and Reproductive index (RI) with trend lines

The ANOVA Procedure

Dependent Variable: UREA UREA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	283	23322.5276	82.4118	3.41	<.0001

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Error	4250	102856.3630	24.2015		
Corrected Total	4533	126178.8905			

R-Square	Coeff Var	Root MSE	UREA Mean
0.184837	36.27293	4.919502	13.56246

Source	DF	Anova SS	Mean Square	F Value	Pr > F
ICP	283	23322.52756	82.41176	3.41	<.0001

Scatter plot for Reproduction performance (RP) and Reproductive index (RI) with trend lines

The ANOVA Procedure

Class Level Information

Class	Levels	Values
ICP	284	248 266 283 286 288 290 295 296 301 307 308 311 313 314 315 316 317 318 319 320 321 322 323 324 325 326 327 328 329 330 331 332 333 334 335 336 337 338 339 340 341 342 343 344 345 346 347 348 349 350 351 352 353 354 355 356 357 358 359 360 361 362 363 364 365 366 367 368 369 370 371 372 373 374 375 376 377 378 379 380 381 382 383 384 385 386 387 388 389 390 391 392 393 394 395 396 397 398 399 400 401 402 403 404 405 406 407 408 409 410 411 412 413 414 415 416 417 418 419 420 421 422 423 424 425 426 427 428 429 430 431 432 433 434 435 436 438 439 440 441 442 443 444 445 446 447 449 450 451 452 453 454 455 456 457 458 459 460 461 462 463 464 465 466 467 468 469 470 471 472 473 474 475 476 477 478 479 481 482 483 484 485 486 487 488 489 490 491 493 497 498 499 500 501 502 504 505 506 508 509 510 511 512 513 514 517 522 523 524 525 530 531 534 535 536 537 539 542 543 544 545 547 549 550 551 554 556 557 560 561 562 563 564 566 568 569 571 572 573 579 582 584 585 590 592 594 597 601 605 606 607 608 611 612 613 617 620 626 629 634 638 642 647 649 659 662 663 672 675 680 682 683 697 702 706 708 716 739 773 795 847 853 898 943

Number of Observations Read	10962
Number of Observations Used	4025

Scatter plot for Reproduction performance (RP) and Reproductive index (RI) with trend lines

The ANOVA Procedure

Dependent Variable: RDP_ RDP %

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	280	11031.80353	39.39930	4.68	<.0001

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Error	3744	31513.33838	8.41702		
Corrected Total	4024	42545.14191			

R-Square	Coeff Var	Root MSE	RDP__ Mean
0.259296	5.118384	2.901211	56.68217

Source	DF	Anova SS	Mean Square	F Value	Pr > F
ICP	280	11031.80353	39.39930	4.68	<.0001

Scatter plot for Reproduction performance (RP) and Reproductive index (RI) with trend lines

The ANOVA Procedure

Class Level Information

Class	Levels	Values
ICP	284	248 266 283 286 288 290 295 296 301 307 308 311 313 314 315 316 317 318 319 320 321 322 323 324 325 326 327 328 329 330 331 332 333 334 335 336 337 338 339 340 341 342 343 344 345 346 347 348 349 350 351 352 353 354 355 356 357 358 359 360 361 362 363 364 365 366 367 368 369 370 371 372 373 374 375 376 377 378 379 380 381 382 383 384 385 386 387 388 389 390 391 392 393 394 395 396 397 398 399 400 401 402 403 404 405 406 407 408 409 410 411 412 413 414 415 416 417 418 419 420 421 422 423 424 425 426 427 428 429 430 431 432 433 434 435 436 438 439 440 441 442 443 444 445 446 447 449 450 451 452 453 454 455 456 457 458 459 460 461 462 463 464 465 466 467 468 469 470 471 472 473 474 475 476 477 478 479 481 482 483 484 485 486 487 488 489 490 491 493 497 498 499 500 501 502 504 505 506 508 509 510 511 512 513 514 517 522 523 524 525 530 531 534 535 536 537 539 542 543 544 545 547 549 550 551 554 556 557 560 561 562 563 564 566 568 569 571 572 573 579 582 584 585 590 592 594 597 601 605 606 607 608 611 612 613 617 620 626 629 634 638 642 647 649 659 662 663 672 675 680 682 683 697 702 706 708 716 739 773 795 847 853 898 943

Number of Observations Read 10962

Number of Observations Used 4538

Scatter plot for Reproduction performance (RP) and Reproductive index (RI) with trend lines

The ANOVA Procedure

Dependent Variable: CP_ CP %

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	283	1920.228256	6.785259	4.54	<.0001

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Error	4254	6352.280077	1.493249		
Corrected Total	4537	8272.508333			

R-Square	Coeff Var	Root MSE	CP__ Mean
0.232122	6.990112	1.221986	17.48163

Source	DF	Anova SS	Mean Square	F Value	Pr > F
ICP	283	1920.228256	6.785259	4.54	<.0001

Scatter plot for Reproduction performance (RP) and Reproductive index (RI) with trend lines

The ANOVA Procedure

Class Level Information

Class	Levels	Values
ICP	284	248 266 283 286 288 290 295 296 301 307 308 311 313 314 315 316 317 318 319 320 321 322 323 324 325 326 327 328 329 330 331 332 333 334 335 336 337 338 339 340 341 342 343 344 345 346 347 348 349 350 351 352 353 354 355 356 357 358 359 360 361 362 363 364 365 366 367 368 369 370 371 372 373 374 375 376 377 378 379 380 381 382 383 384 385 386 387 388 389 390 391 392 393 394 395 396 397 398 399 400 401 402 403 404 405 406 407 408 409 410 411 412 413 414 415 416 417 418 419 420 421 422 423 424 425 426 427 428 429 430 431 432 433 434 435 436 438 439 440 441 442 443 444 445 446 447 449 450 451 452 453 454 455 456 457 458 459 460 461 462 463 464 465 466 467 468 469 470 471 472 473 474 475 476 477 478 479 481 482 483 484 485 486 487 488 489 490 491 493 497 498 499 500 501 502 504 505 506 508 509 510 511 512 513 514 517 522 523 524 525 530 531 534 535 536 537 539 542 543 544 545 547 549 550 551 554 556 557 560 561 562 563 564 566 568 569 571 572 573 579 582 584 585 590 592 594 597 601 605 606 607 608 611 612 613 617 620 626 629 634 638 642 647 649 659 662 663 672 675 680 682 683 697 702 706 708 716 739 773 795 847 853 898 943

Number of Observations Read	10962
Number of Observations Used	4538

Scatter plot for Reproduction performance (RP) and Reproductive index (RI) with trend lines

The ANOVA Procedure

Dependent Variable: ME_MJ ME# MJ

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	283	375.440480	1.326645	5.26	<.0001

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Error	4254	1071.929211	0.251981		
Corrected Total	4537	1447.369691			

R-Square	Coeff Var	Root MSE	ME__MJ Mean
0.259395	4.541784	0.501978	11.05243

Source	DF	Anova SS	Mean Square	F Value	Pr > F
ICP	283	375.4404796	1.3266448	5.26	<.0001

Scatter plot for Reproduction performance (RP) and Reproductive index (RI) with trend lines

The ANOVA Procedure

Class Level Information

Class	Levels	Values
_RP	3	70 to 90・ Less than 70・ Over 90

Number of Observations Read	10962
Number of Observations Used	4538

Scatter plot for Reproduction performance (RP) and Reproductive index (RI) with trend lines

The ANOVA Procedure

Dependent Variable: ICP ICP

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	20735649.25	10367824.62	2901.44	<.0001
Error	4535	16205097.60	3573.34		
Corrected Total	4537	36940746.84			

R-Square	Coeff Var	Root MSE	ICP Mean
0.561322	14.38862	59.77742	415.4493

Source	DF	Anova SS	Mean Square	F Value	Pr > F
_RP	2	20735649.25	10367824.62	2901.44	<.0001

Scatter plot for Reproduction performance (RP) and Reproductive index (RI) with trend lines

The ANOVA Procedure

Class Level Information

Class	Levels	Values
ICP	284	248 266 283 286 288 290 295 296 301 307 308 311 313 314 315 316 317 318 319 320 321 322 323 324 325 326 327 328 329 330 331 332 333 334 335 336 337 338 339 340 341 342 343 344 345 346 347 348 349 350 351 352 353 354 355 356 357 358 359 360 361 362 363 364 365 366 367 368 369 370 371 372 373 374 375 376 377 378 379 380 381 382 383 384 385 386 387 388 389 390 391 392 393 394 395 396 397 398 399 400 401 402 403 404 405 406 407 408 409 410 411 412 413 414 415 416 417 418 419 420 421 422 423 424 425 426 427 428 429 430 431 432 433 434 435 436 438 439 440 441 442 443 444 445 446 447 449 450 451 452 453 454 455 456 457 458 459 460 461 462 463 464 465 466 467 468 469 470 471 472 473 474 475 476 477 478 479 481 482 483 484 485 486 487 488 489 490 491 493 497 498 499 500 501 502 504 505 506 508 509 510 511 512 513 514 517 522 523 524 525 530 531 534 535 536 537 539 542 543 544 545 547 549 550 551 554 556 557 560 561 562 563 564 566 568 569 571 572 573 579 582 584 585 590 592 594 597 601 605 606 607 608 611 612 613 617 620 626 629 634 638 642 647 649 659 662 663 672 675 680 682 683 697 702 706 708 716 739 773 795 847 853 898 943

Number of Observations Read	10962
Number of Observations Used	4538

Scatter plot for Reproduction performance (RP) and Reproductive index (RI) with trend lines

The ANOVA Procedure

Dependent Variable: RI_ RI %

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	283	15205.45408	53.72952	11.52	<.0001
Error	4254	19845.10237	4.66505		
Corrected Total	4537	35050.55645			

R-Square	Coeff Var	Root MSE	RI__ Mean
0.433815	2.000812	2.159872	107.9497

Source	DF	Anova SS	Mean Square	F Value	Pr > F
ICP	283	15205.45408	53.72952	11.52	<.0001

Addendum E

Original dairy cow data from the milk recording scheme and temperature data from the ARC – Soil, Climate and Water are available on request.