THE EFFECT OF BLOOD UREA NITROGEN ON REPRODUCTIVE PERFORMANCE OF BEEF HEIFERS ON DIFFERENT LEVELS OF NITROGEN SUPPLEMENTATION

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THE EFFECT OF BLOOD UREA NITROGEN ON REPRODUCTIVE PERFORMANCE OF BEEF HEIFERS ON DIFFERENT LEVELS OF NITROGEN SUPPLEMENTATION

BY

TAKULA TSHUMA

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Summary

Ruminants have a unique ability to acquire protein from non-protein nitrogen (NPN) sources, and to recycle nitrogen back into the rumen, instead of excreting all of it via the urine, faeces and milk. However, a high concentration of blood urea nitrogen (BUN) has a negative influence on conception. Additionally, a high dietary nitrogen intake poses a challenge to the environment in the form of ammonia emissions, eutrophication and bad odours. This calls for strategies to reduce the environmental impact of livestock production. Variation exists in the ability of cattle to recirculate nitrogen between as well as within cattle breeds. The purpose of this study was to investigate the effects of BUN concentration on reproductive performance in beef heifers under different management systems in South Africa. Serum samples from 369 Bonsmara heifers were taken in November and December 2010 to determine the BUN concentrations prior to the onset of the breeding season. Heifers were from five herds with different levels of protein supplementation during the weeks before the commencement of the breeding season. Body mass, age, body condition score (BCS) and reproductive tract score (RTS) were recorded at the same time as BUN concentration. Trans-rectal ultrasound and/or-palpation was performed four to eight weeks after the three-month breeding season to detect and estimate the stage of pregnancy. Days to pregnancy (DTP) was defined as the number of days from the start of the breeding season until a heifer was successfully mated. Logistic regression and Cox proportional hazards survival analysis were performed to estimate the effect of BUN concentration on subsequent pregnancy and DTP respectively, while stratifying by herd and adjusting for potential confounders. The correlations between BUN concentration, BCS and RTS were estimated using Spearman's rho. Pearson correlations were used for the normally distributed variables of age and body mass. BUN concentration was not a significant predictor of pregnancy status but was a significant (P = 0.007) and independent predictor of DTP in heavily and some moderately supplemented herds. As BUN concentration increased, DTP also increased [hazard ratio (HR) = 0.827; 95% CI: 0.721 – 0.949; P = 0.007], while the chance of becoming pregnant decreased, although this was not statistically significant [odds ratio (OR) = 0.882; 95% CI: 0.772 – 1.007; P = 0.063]. Bonsmara heifers with higher BUN

concentration, which suggests a better ability to recirculate nitrogen, might be at a disadvantage when the production system includes high levels of RDP supplementation because of this negative impact on reproductive performance. It is proposed that production systems be adapted to avoid selection against animals with an improved ability to recirculate nitrogen.

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List of acronyms and abbreviations

BCS	Body condition score
BUN	Blood urea nitrogen
CI	Confidence interval
CIDR	Controlled Internal Drug Release
CP	Crude protein
CRL	Crown rump length
DMI	Dry matter intake
DTC	Days to calving
DTP	Days to pregnancy
HR	Hazard ratio
LH	Luteinizing hormone
MUN	Milk urea nitrogen
NEB	Negative energy balance
NEFA	Non-esterified fatty acids
NPN	Non protein nitrogen
NSC	Non-structural carbohydrates
OR	Odds ratio
PE	Pregnancy examination

PER	Protein to energy ratio
PR	Pregnancy rate
RDP	Rumen degradable protein
RTS	Reproductive tract score
RUP	Rumen undegradable protein
SAWS	South African weather services
SC	Structural carbohydrates
SD	Standard deviation

1 Introduction

Ruminants are unique in that they are capable of recycling nitrogen back into the rumen, instead of excreting all of it in the urine (Erickson and Klopfenstein, 2010), faeces or milk (Dijkstra et al., 2011), thus supplying rumen microbes with their need for ammonia (Marini and van Amburgh, 2003). In light of the increasing public health and global warming concerns that have been focused on animal production systems as a source of environmental pollution (Marini and van Amburgh, 2005), there is a need for more research aimed at reducing nitrogen excretion into the environment. It is estimated that the proportion of dietary nitrogen that is retained in feedlot cattle is less than 20% (Bierman et al., 1999), implying that more than 80% of it is excreted. Most of this nitrogen (up to 97%) is excreted in the form of urea in urine and organic nitrogen in faeces (Varel et al., 1999; McCrory and Hobbs, 2001; Dijkstra et al., 2011). It is therefore logical to assume that even in less intensive beef production systems where the levels of dietary nitrogen supplementation are relatively lower, dietary nitrogen is still excreted into the environment.

Blood urea, which is synthesized in the liver in cattle, can be found in varying concentrations without causing any adverse effects to the animal. However if present at very high levels, it may be associated with reproductive problems (Larson et al., 1997; Kauffman and St-Pierre, 2001).

BUN concentration can be used to indicate the nitrogen recycling efficiency of cattle. The efficiency of ruminants in the utilisation of dietary nitrogen depends on the availability of dietary energy for the conversion of ammonia to microbial protein. In the presence of adequate amounts of energy, less ammonia is converted to urea for excretion (Ipharraguerre et al., 2005).

BUN concentration is known to vary with the dietary protein levels, hydration status of the animal, breed and time of blood sample collection (Godden et al., 2001). The dietary nitrogen content is the main determinant of BUN concentration and nitrogen excretion in cattle (Roseler et al., 1993; Kauffman and St-Pierre, 2001). Casper et al. (1994) suggested

that a balance in the protein to energy ratio (PER) is very critical in growing heifers because they have a limited dry matter intake (DMI) and fermentation capacity. Several studies have reported genetic variation in milk urea nitrogen (MUN) concentration between cows, suggesting that genetic differences in nitrogen recirculation efficiency do exist. The reported heritability estimates of MUN ranged between 0.14 and 0.44 (Mitchell et al., 2005; Stoop et al., 2007; Bouwman et al., 2010; Hossein-Zadeh and Ardalan, 2011). Selection of animals with the ability to optimally recirculate nitrogen could be useful to reduce environmental pollution from livestock production by reducing the need for dietary nitrogen supplementation.

2 Literature Review

2.1 Protein metabolism in the ruminant

Ruminants derive most of their energy and protein from microorganisms, which live symbiotically in the rumen. Hoover and Strokes (1991) identified carbohydrates and proteins as the major nutrients that are required to support microbial growth. It is logical to deduce that for ruminant diets to meet the requirements of the animal, they should first meet the microbial needs for growth and multiplication.

Ruminants use carbohydrates and fats for energy. During Negative energy balance (NEB), they will also utilise protein. Complex carbohydrates in the diet undergo microbial fermentation and enzyme breakdown in the rumen. The microbial fermentation process yields volatile fatty acids (VFA) which provide a large portion of the energy requirement in the ruminant (Demeyer, 1981; Fondevila and Dehority, 1994). In a study done by Leedle et al. (1986), it was shown that easily solubilized carbohydrates like sugars, starches, and pectins undergo the most rapid fermentation, while that of the less soluble polysaccharides (hemicellulose and cellulose) was slower.

The ruminant acquires its protein when the undegraded true protein (amino acids and peptides) fraction and the microbial protein, passes from the rumen to the abomasum and then to the small intestines, where it is digested and absorbed. The nitrogen for the process of microbial growth is obtained from protein nitrogen and non-protein nitrogen (NPN). The rumen degradable protein (RDP) fraction consists of NPN, soluble intake protein (SIP) and some more slowly degraded proteins. A proportion of the dietary true protein passes from the rumen into the abomasum and small intestine and this fraction is described as the rumen undegradable protein (RUP) (Schwab et al., 2003).

Bacteria acting on the structural carbohydrate (SC) fraction (cellulose and hemicellulose) of the diet require only ammonia for growth. Whereas bacteria acting on the non-structural carbohydrate (NSC) fraction (sugars, starches and pectins) derive about 65% of their

nitrogen from amino acids and peptides and the remainder from ammonia (Russell et al., 1992; McDonald et al., 1995).

Urea is quantitatively the most important end-product of nitrogen metabolism in ruminants, with at least 70% of dietary nitrogen passing through the urea pool of goats daily (Harmeyer and Martens, 1980). Urea is not only a waste product of nitrogen metabolism in ruminants, but it also serves the important functions of buffering the blood pH and providing an important precursor of protein biosynthesis (Harmeyer and Martens, 1980). The detoxification of ammonia into urea occurs in the liver and this is an energy dependant process, which may aggravate an existing energy shortage. A schematic summary of the fate of dietary crude protein (CP) in ruminants follows (Figure 2.1):

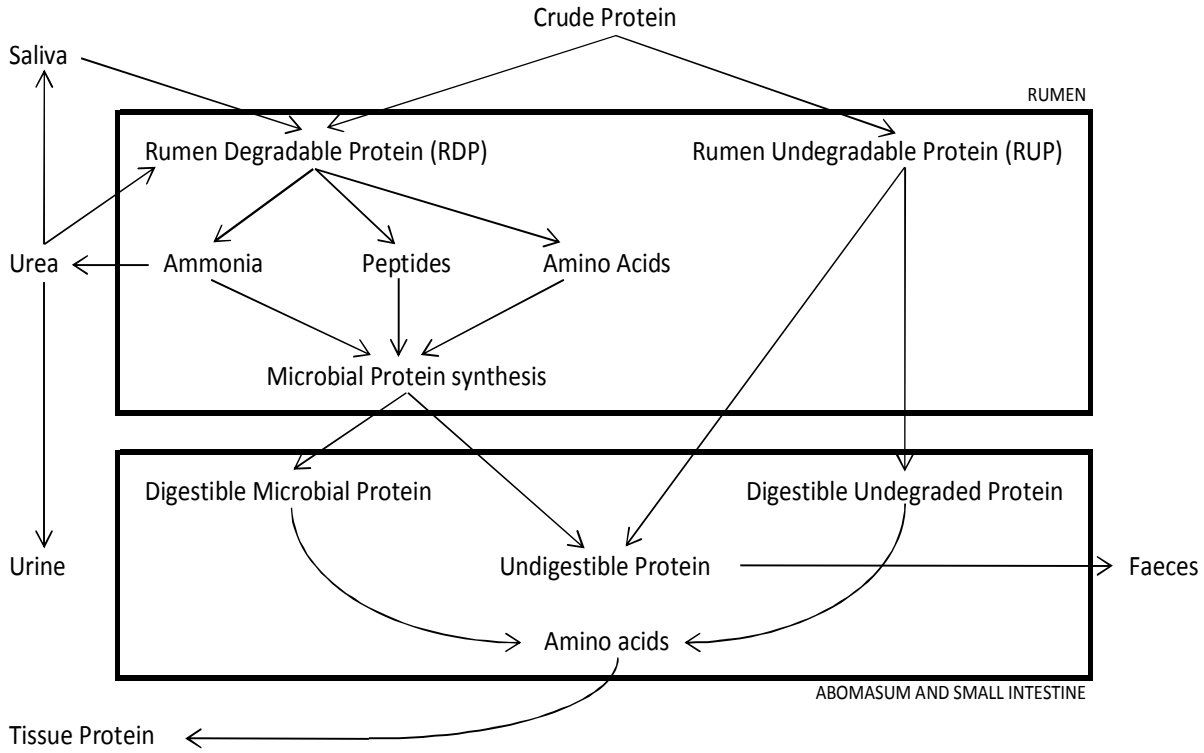

Figure 2.1: *Fate of dietary crude CP in the ruminant animal (Adapted from McDonald et al., 1995)*

The microbial degradation of the RDP fraction usually releases ammonia at a faster rate than its uptake by microorganisms. Excess ammonia gets absorbed through the rumen wall

into the portal vein and is transported to the liver where it is converted into urea (Roseler et al., 1993; Tamminga, 2006). Urea is a water-soluble molecule, which readily enters the blood circulation, and is distributed to all body fluids. Some of the urea recirculates back into the rumen via the saliva, or diffusion across the rumen wall. While most of the urea is excreted through urine and milk, the kidneys may also recirculate a fraction of the urea back into the blood. When high levels of ammonia exist in the rumen, the ruminal pH is elevated and this increases the rate of absorption through the rumen wall. This causes a rise in BUN concentration (Roseler et al., 1990; Elrod and Buetler, 1993).

Microbial protein is generally produced proportional to the amount of carbohydrate fermented in the rumen (Ørskov, 1994). Ruminal microbes utilise fermentable carbohydrates when metabolising dietary nutrients into microbial protein. It is therefore essential to ensure that the ruminant receives balanced proportions of fermentable carbohydrate and RDP; otherwise, most of the dietary RDP will be degraded into ammonia in the rumen (Chalupa and Sniffen, 1996; Schroeder and Titgemeyer, 2008).

2.2 Intraruminal nitrogen recycling

Recycling of nitrogen also occurs in the rumen. The outflow of nitrogen from the rumen is reduced by proteolytic bacteria and protozoa, which digest other rumen bacteria. Changing the microbial population of the rumen through antibiotics or some plant products including saponins and essential oils can have substantial effects on the anabolic nitrogen flow and hence the BUN concentration (Lapierre and Lobley, 2001).

2.3 The biosynthesis of urea nitrogen

Urea or carbamide is an organic chemical compound with the formula $CO(NH_2)_2$. It is mainly formed from the detoxification of ammonia in the liver, after which it equilibrates into the bloodstream and other body fluids (Harmeyer and Martens, 1980). The quantity of ammonia that is available for detoxification is a direct reflection of both dietary RDP and the availability of fermentable carbohydrates that support microbial growth and protein synthesis (Butler, 1998).

Other sources of urea in the body include the deamination of amino acids that occurs in the liver. Circulating amino acids that are not assimilated by the body are deaminated to produce urea and energy substrates (Butler, 1998). It has also been demonstrated in an in vitro study that ruminant gut tissues (ruminal epithelial and duodenal mucosal cells) have the capacity to produce urea in vitro (Oba et al., 2004). This production by gut tissues is thought to occur in vivo as well, thus contributing smaller amounts of urea to the circulating pool. Arginine catabolism in the mammary gland can also produce small amounts of urea that make up part of the MUN (Nousiainen et al., 2004).

The urea from the various sources circulates in the blood and equilibrates with other body tissues like milk and urine (Gustafsson and Palmquist, 1993). The urea is easily measured in plasma or serum by the nitrogen content (i.e., the urea nitrogen concentration) (Butler, 1998).

2.4 Sources of variation in BUN concentrations

Many studies in dairy cattle have shown that BUN concentration is directly related to the amount of CP in the diet, the proportions of RDP and RUP as well as the PER of the diet, especially the fermentable carbohydrates (Roseler et al., 1990; Roseler et al., 1993; Chalupa and Sniffen, 1996; Schroeder and Titgemeyer, 2008). The accepted target range for BUN concentrations in dairy cattle is between 8 and 18 mg/dL (Jonker et al., 1999), as obtained from directly converting the MUN range of between 10 and 16 mg/dL to BUN concentrations.

Previous studies have also demonstrated that several other factors in addition to feed intake and dietary composition are involved in the determination of BUN concentrations in cattle. These include factors like the time of sample collection, the mass of the animal, the method used to measure BUN concentration, parity, breed, the hydration status, the DMI and the method of analysis (Godden et al., 2001; Kauffman and St-Pierre, 2001; Rajala-Schultz and Saville, 2003; Hossein-Zadeh and Ardalan, 2011). Mitchell et al. (2005) clearly demonstrated that variation in BUN concentration is also genetically determined with a

moderate heritability. Most studies on nitrogen metabolism focus on urea because it is stable and easily measured (Butler et al., 1996).

BUN concentration is known to vary throughout the day in relation to the time of feeding in dairy cattle. Gustafsson and Palmquist (1993) determined that ruminal ammonia peaked one hour after feeding and returned to baseline levels after six hours. BUN concentrations peaked at 2.5 to 3 hours after feeding. This pattern is important to consider when collecting blood samples from a large group of animals as it may introduce an unintended variation among animals due to sampling order. It is yet to be determined how this variation interacts with other factors such as breed, dietary composition and the feeding schedule in determining the measured BUN concentration (Rodriguez et al., 1997).

The level of nitrogen in the diet also affects the efficiency of BUN recirculation in ruminants. Those animals that are fed low nitrogen diets tend to be more efficient at recycling nitrogen when compared to those that are fed high levels of nitrogen (Marini and van Amburgh, 2003; Marini et al., 2004). The mechanism that regulates the recycling of BUN back into the gastrointestinal tract (GIT) of ruminants remains unknown (Røjen et al., 2011). Some studies have shown that BUN concentration is related to the rate at which BUN is transferred back into the GIT of ruminants (Sunny et al., 2007; Kristensen et al., 2010). Kristensen et al. (2010) showed that the transport of urea nitrogen across gut epithelia is regulated by mass action and adaptive changes in their permeability in ruminants. Other studies have suggested that the transport system tends to adapt to dietary induced changes causing changes in the permeability of the gut to BUN and hence the rate of influx of BUN into the GIT (Calsamiglia et al., 2010). A factor named urea transporter B is expressed in the epithelial cells of the rumen (Stewart et al., 2005) confirming the involvement of transporters in the regulation of BUN recycling. However, in a recent study, Røjen et al. (2011) could not find a correlation between the expression of urea transporter B factor and changes in the arterial supply of nitrogen.

It is generally accepted that BUN concentration will be lower in heifers than in adult cows (Oltner et al., 1985; Canfield et al., 1990; Arunvipas et al., 2003), although other studies found no effect of age on BUN concentration (Eicher et al., 1999). Others even suggested

that BUN concentration is higher in younger cows and decreases with age (Doska et al., 2012). The proposed explanation for the increase in BUN concentration is that growing animals utilise amino acids more efficiently. This is thought to cause reduced deamination and urea formation in the liver, leading to lower BUN concentrations in younger animals (Oltner et al., 1985).

The influence of gender on BUN concentration in cattle has not received adequate attention in literature, but a study involving camels showed a significantly higher BUN concentration in females than in males (Patodkar et al., 2010).

Barton et al. (1996) demonstrated that breed significantly affects measured BUN concentrations in Holstein and Jersey cattle, but others reported that it has no effect (Miettinen and Juvonen, 1990).

2.5 Relationship between MUN and BUN concentrations

High dietary protein supplementation that is aimed at increasing production, leads to elevated concentrations of urea and ammonia, which impairs fertility in dairy cattle (Elrod and Butler, 1993). MUN concentration can be used to estimate BUN concentration (Ferguson et al., 1993) because of the strong linear correlation between the two (Roseler et al., 1993; Harris, 1996) in dairy cattle. In one study involving dairy cows, a strong correlation was observed between BUN and MUN concentrations (r^2 = 0.73; P < 0.001) (Gonda and Lindberg, 1994). This correlation is thought to be caused by rapid diffusion of urea from the blood compartment into the milk through the epithelium of the mammary gland after a bit of a time lag (Gustafsson and Palmquist, 1993). Broderick and Clayton et al. (1997) proposed the following equation indicating the relationship between MUN and BUN concentration:

$$y = 0.620x + 4.75\ (r^2 = 0.842),$$

where y = MUN concentration and x = BUN concentration.

The regression of MUN on BUN concentration they obtained in a study involving 2231 dairy cows (Figure 2.2):

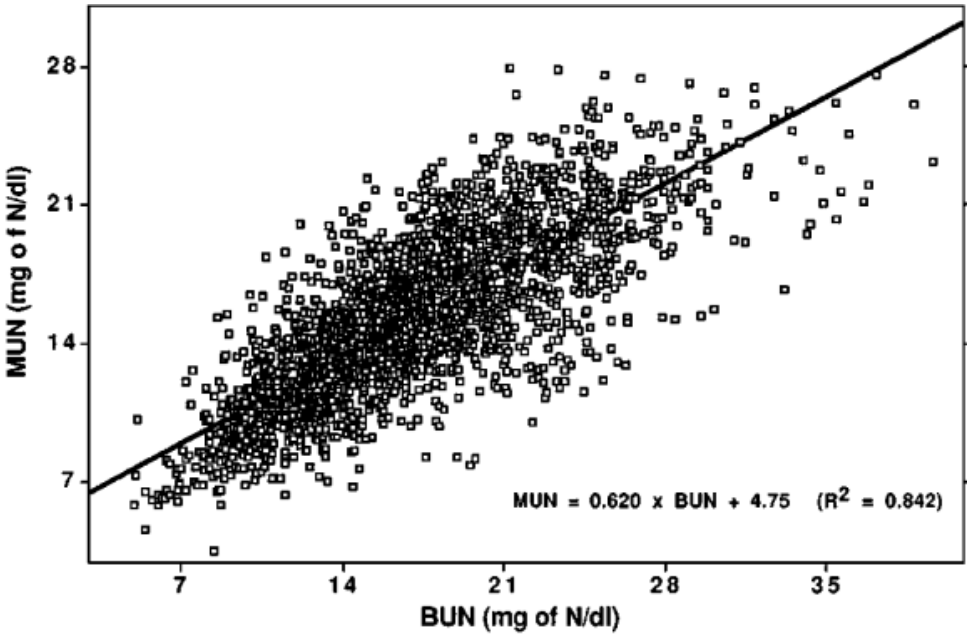

Figure 2.2: *Regression of MUN on BUN concentration (Adapted from Broderick and Clayton, 1997).*

2.6 Effect of BUN concentration on reproductive performance

The association between excess protein and fertility is controversial (Ward, 2000). Many studies have reported that high protein intakes or high BUN or MUN concentrations have negative effects on reproductive performance of dairy cattle (Butler et al., 1996; Larson et al., 1997; Rajala-Schultz et al., 2001; Arunvipas et al., 2007). Ward (2000) suggested that some of these negative effects might have been confused with those of a concurrent energy deficit. Several other studies found no association between protein intake or plasma urea levels at the time of service and reproductive performance in cattle (Whitaker et al., 1993; Kenny et al., 2002a; Kenny et al., 2002b).

Other studies have reported that reduced reproductive performance only occurs when MUN was either too low (<7 mg/dL) or too high (>17.6 mg/dL) (Pehrson et al., 1992; Carlsson and Pehrson, 1993). Butler et al. (1996) demonstrated that MUN concentrations in excess of 19 mg/dL have a negative effect on conception rates while Guo et al. (2004) reported that MUN concentrations had minimal effects on the rate of conception.

Many of the studies on the effects of protein and nitrogen supplementation on the reproductive performance were performed in dairy cattle (Elrod and Butler, 1993; Garcia-Bojalil et al., 1994; Barton et al., 1996; Smith et al., 2001; Kenny et al., 2002a; Babashahi et al., 2004; Rhoads et al., 2006) rather than beef heifers. Literature review failed to identify such studies within South Africa. Very few studies, to the knowledge of the researcher, have been published seeking to measure the association between genetic determinants of BUN or MUN concentrations and its reproductive performance. These studies identified that MUN concentrations are genetically determined with moderate heritability but the genetic correlations were too weak to justify inclusion of MUN concentration as an indicator trait for reproductive performance in a breeding program (Wood et al., 2003; Mitchell et al., 2005; König et al., 2008).

Although Schoeman (1989) reported on the existence of significant breed differences ($P < 0.01$) in BUN concentrations of Hereford, Bonsmara and Nguni breeds, Ndlovu et al. (2009) found no significant differences ($P > 0.05$) in a study involving the Nguni, Bonsmara and Angus breeds.

Several mechanisms by which high dietary CP may reduce cow fertility have been proposed. Degradation of protein in the rumen or its metabolism in the body for energy releases ammonia and urea (Tamminga, 2006). Ammonia is believed to play a negative role prior to ovulation, whereas urea mainly exerts its effects during cleavage and blastocyst formation of the embryo after fertilisation (Jorritsma et al., 2003). Elrod and Butler (1993) reported that high concentrations of BUN lowered the uterine pH. It is not known how urea causes this drop in uterine pH, but Zhu et al. (2000) suggested that ureagenesis lowers the pH by removing bicarbonate from the blood.

Other authors reported a direct effect of ammonia and BUN on reproductive performance. They suggested that urea acts directly on the oocyte and through altering the composition and pH of follicular, oviductal and uterine environments (Jordan and Swanson, 1979; Jordan et al., 1983; Ocon and Hansen, 2003). Sinclair et al. (2000) demonstrated a detrimental effect of ammonia on cleavage rates and blastocyst formation. However, another recent study demonstrated that the embryo survival rate is not affected by dietary urea

supplementation (Kenny et al., 2002a). The idea of disruptions to the oviductal environment as a cause of impaired fertility has been disputed by Kenny et al. (2002b).

Butler (1998) suggested that high levels of dietary RDP exert its effects through exacerbating the NEB and its negative effect on reproductive performance. The exacerbating effect is caused by the additional energy cost of detoxifying ammonia from the rumen and protein catabolism (Garcia-Bojalil et al., 1998). These authors also showed a negative effect of RDP on plasma progesterone, which could be rectified by dietary supplementation of fat (Garcia-Bojalil et al., 1998).

In an extensive review Leroy et al. (2008) suggested that NEB acts through a complex pathway involving the endocrine system causing a disruption in luteinizing hormone (LH) pulse frequency and amplitude, and this is responsible for compromising embryo survival. The same review reported that NEB also affects fertility by increasing the concentration of non-esterified fatty acids (NEFA), which have direct toxic effects on the developing oocyte. In the presence of NEB, protein catabolism causes elevated BUN concentrations. Leroy et al. (2008) further clarified that the detrimental effects of high blood urea and ammonia concentrations are at the level of both the embryo (especially through ammonia) and the oocyte (particularly through urea).

From current knowledge the interactions and potential confounding between the effects of NEB and increased BUN on reproductive performance has not been completely clarified.

2.7 Fate of nitrogen in the environment

Sixty-nine per cent of the nitrogen in urine of cows is in the form of urea (Bristow et al., 1992), and upon excretion, the urea is rapidly converted to ammonia by urease enzymes (Powell and Russelle, 2009). These enzymes are produced by bacteria that are present in faeces and the soil (Béline et al., 1998). In contrast, the degradation of organic nitrogen in faeces occurs more slowly and may require months or years to complete (Ndegwa et al., 2008).

Between 20 to 55% of nitrogen in the manure-urine mixture volatilises into the atmosphere, leading to air pollution (Varel et al., 1999; Powell and Russelle, 2009). Some of the nitrogen in the manure enters rivers and other surface water bodies and subsequently causes eutrophication. Over the past 15 years, strategies of reducing this environmental impact were aimed at manure management to mitigate runoff (Powell et al., 2008). Other strategies include reduction of protein in cattle diets (Wu and Satter, 2000), segregation of urine from faeces to reduce contact between urease and urine, use of urease inhibitors, lowering manure pH, use of chemical additives that bind to ammonia and biological agents that convert ammonium into non-volatile nitrogen such as nitrite, nitrate or gaseous nitrogen (Ndegwa et al., 2008).

2.8 Other factors affecting the reproductive performance of heifers

It is important to note that other factors such as age at puberty, body condition score (BCS), bull factors, farm management and environment have an effect on the reproductive performance of heifers. Heifers require a high plane of nutrition to attain puberty at an early age. Age at puberty can be defined as the age at which a heifer shows the first visual signs of oestrus (Pineda and Dooley, 2003).

Age at puberty is determined in individual heifers by a genetically determined body mass that has to be reached before the heifer will attain puberty. The age at which this mass will be reached is determined by the growth rate. Growth rate is partially determined by genetics but is mostly influenced by environmental factors, in particular nutrition (Figure 2.3) (Short and Bellows, 1971; Hall et al., 1997). Reproductive tract scoring (RTS) by transrectal palpation of the uterus and ovaries provides an indirect measure of age at puberty, and has been shown to have a good correlation with pregnancy proportion and days to calving (DTC) when applied before the first breeding season in heifers (Andersen et al., 1991; Holm et al., 2009).

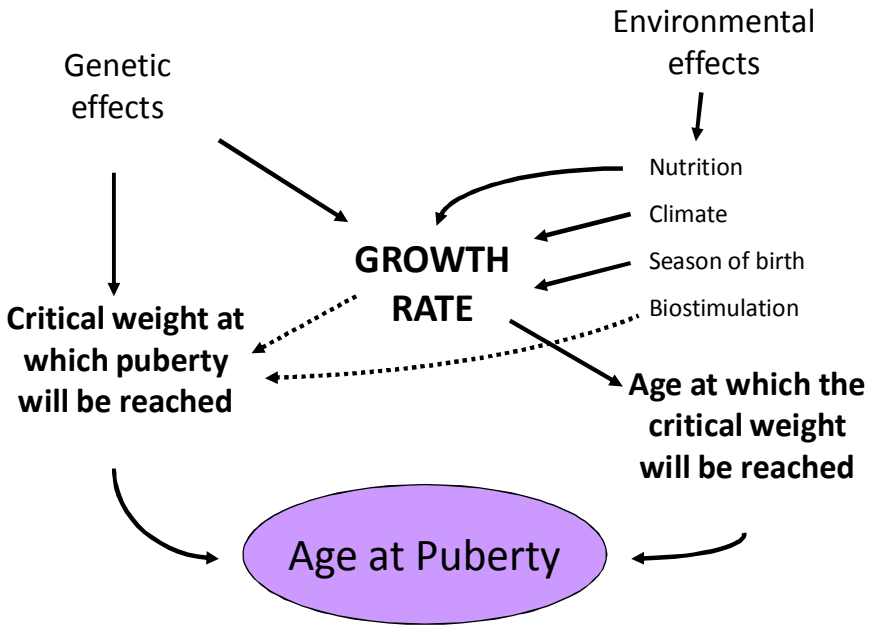

Figure 2.3: *Factors determining the age at puberty (Adapted from Holm et al., 2009)*

A balance in the PER of heifer rations is important because it determines how well the rumen microbes can synthesize microbial protein from dietary protein, thus affecting BUN concentrations (Ørskov, 1994). Fermentable carbohydrates and roughages play an important role in ensuring a healthy rumen environment (Chalupa and Sniffen, 1996; Schroeder and Titgemeyer, 2008).

It has been shown in previous studies that BCS, body mass and age at puberty of the heifers at the start of the breeding season are associated with reproductive performance (Buckley et al., 2003; Berry et al., 2003).

Reproductive performance in a production system that utilises natural service is a combination of heifer and bull fertility. The negative effects of an infertile bull can be exacerbated in single-sire herds where herd fertility is compromised by the bull's fertility. It is recommended that a bull breeding soundness examination be performed prior to the breeding season to reduce problem of infertile bulls (Irons et al., 2007; Alexander, 2008).

2.9 Measuring reproductive performance

Several methods for measuring and recording reproductive performance of beef heifers are available. These include, but are not limited to DTC, age at first calving, first insemination conception and pregnancy proportion (Cammack et al., 2009). As written by MacGregor and Casey (1999), the official National Beef Performance and Progeny Testing Scheme in South Africa uses age at first calving as the criteria for evaluating reproductive performance in beef herds. However, most of these methods may not be applicable to heifers that are bred in a restricted breeding season. Pregnancy proportion and DTC are considered the most useful criteria for beef heifers (MacGregor and Casey, 1999; Eler et al., 2002).

The DTC is estimated as the length of time from the onset of the breeding season to calving, and it is similar to measurements of the calving date (Meyer et al., 1990). It is easy to measure (Buddenberg et al., 1990), and is considered a strong and practical measure of reproductive performance in beef heifers because it is of great economic importance. Longer DTC will cause lighter weaning weights (Bourdon and Brinks, 1983), and the heifers that calve late in the season do not have adequate time to recuperate prior to the onset of the next breeding season (MacGregor and Casey, 1999).

The pregnancy proportion in heifers is an indirect measure of sexual maturity at the onset of the breeding season. It is a binary measure (1 = pregnant; 0 = non-pregnant) defined as the probability of a heifer that was exposed to the bull at the onset of the breeding season becoming pregnant by the end of the breeding season and remaining pregnant to the time of examination for pregnancy (Evans et al., 1999; Eler et al., 2002).

2.10 Practical uses of BUN concentration data

BUN concentration data, when available can be useful for monitoring dietary CP and energy intake relative to the heifer's requirements (Rajala-Schultz and Saville, 2003). Monitoring BUN concentration in a beef herd can serve as an important management tool because excess dietary nitrogen increases the energy requirements of the animal and the producer has to spend money on feed to sustain the excess nitrogen in the diet. Besides, protein

supplementation is relatively expensive and BUN concentration data will help optimise protein supplementation. In addition to the negative effects of excess nitrogen on reproductive performance, excessive nitrogen excretion into the environment should be avoided (Broderick and Clayton, 1997; Frank and Swensson, 2002).

In Nguni cattle, it is believed that animals with high BUN concentrations are more capable of maintaining body condition (Schoeman, 1989), hence higher growth rates and should therefore have a lower age at puberty and better reproductive performance. BUN concentration data can be used as a management strategy to select for those animals that are better adapted for efficient utilisation of nitrogen resources.

3 Research Questions

1. Is BUN concentration associated with the reproductive performance of beef heifers, and if so, how is this influenced by nitrogen supplementation?
2. What is the correlation between BUN concentration, body mass, age, BCS and RTS in beef heifers?

4 Hypotheses

1. BUN concentration or its interaction with nitrogen supplementation has an effect on the reproductive performance of Bonsmara heifers.
2. A correlation exists between BUN concentration, body mass, age, BCS and RTS in Bonsmara heifers.

5 Objectives

1. To estimate the association between BUN concentration and reproductive performance of Bonsmara heifers at different levels of nitrogen supplementation.
2. To estimate the correlation between BUN concentration, body mass, age, BCS and RTS in Bonsmara heifers.

6 Materials and Methods

6.1 Model system and justification of the model

Three hundred and sixty-nine nulliparous Bonsmara heifers from five herds were used. All the herds were located between the latitudes 23˚ 21' 53" and 27˚29' 9" south. Herds were identified by convenience sampling and they all practised a restricted breeding for a period of 3 months, starting on 1 December. Herds were subsequently classified according to the level of dietary nitrogen supplementation that they practised during the month prior to sampling into none, low, moderate and high. Information on the weather elements were obtained from the South African Weather Services (SAWS). The SAWS stations nearest (less than 80 km) to the farms were used.

The first herd (Herd A) was a commercial Bonsmara herd in the sourish mixed bushveld of the Limpopo province. This herd was managed on natural rangeland (veldt) defined as a low input nutritional system. A commercial protein lick supplement with 45% CP was provided throughout the dormant season (winter) until approximately two months before the beginning of the breeding season when they were changed to a mineral lick, which lasted throughout the breeding season. Due to that, the herd was defined as having no supplementation of nitrogen for two months prior to the breeding season. In this herd, 106 heifers aged 22 to 26 months were bred by natural service in multisire groups of four bulls per 100 heifers. A breeding soundness examination was performed on all the bulls two months prior to the onset of the breeding season. The exact ages of the individual animals in this herd was not known. On the day of sampling, the heifers had access to drinking water whilst in the holding pens but feed was not available. The weather was cool with a minimum and maximum temperature of 17.8 ˚C and 31.4 ˚C respectively (SAWS). It rained a total of 2.8 mm during sampling and the humidity for the day was 55% (SAWS).

Herd B was a stud Bonsmara herd in the sweet mixed bushveld of the Limpopo province. The heifers in this herd were managed on veldt in a low input nutritional system. A commercial energy lick supplement (23% CP) was provided throughout the dormant season (winter and spring) until the beginning of the breeding season, when they were changed

onto a higher energy lick supplement (18% CP). Due to this, the herd was defined as having a low level of nitrogen supplementation prior to the breeding season. Thirty-three heifers aged 20 to 26 months were bred by natural service in a single sire group. The bull was examined for breeding soundness two months prior to the onset of the breeding season. On the day of sampling, these heifers had access to drinking water whilst they were in the holding pens. Sampling was done on a hot day with a humidity of 69%, minimum and maximum temperature of 18 ˚C and 35.5 ˚C respectively (SAWS).

Herd C was a Bonsmara herd in the sourish mixed bushveld of the Limpopo province. This herd was managed on irrigated oats pastures during the winter, defined as a medium input nutritional system. A commercial energy lick supplement (18% CP) was provided throughout the dormant season (winter and spring) until the beginning of the breeding season when they were moved to normal veldt with a mineral lick supplement, which lasted throughout the breeding season. Due to the high RDP content of the irrigated pasture, this herd was defined as having a moderate level of nitrogen supplementation. Thirty-four heifers aged 23 to 27 months were artificially inseminated after synchronisation with progesterone impregnated intravaginal devices (CIDR Easy Breed, Pfizer Animal Health), followed by natural mating with one bull. The bull was examined for breeding soundness two months prior to the onset of the breeding season. On the day of sampling, the heifers had no access to feed or water while in the holding pens. Sampling was done on a hot day with a humidity of 75%, minimum and maximum temperatures were 17.5 ˚C and 32.8 ˚C respectively (SAWS).

Herd D was a stud Bonsmara herd in the sourish mixed bushveld of Limpopo province. Heifers were managed on veldt in a low input nutritional system. A commercial protein lick supplement (45% CP) was provided during the dormant season until one month after the first significant spring rains have occurred, when they were changed onto a mineral lick for the duration of the rainy season (summer). Due to the high CP content in the protein lick supplement, this herd was defined as having a moderate level of nitrogen supplementation prior to the onset of the breeding season. These heifers were kept in two separate camps with differing grazing quality in a low input system. Twenty-two heifers aged 15 to 26

months were bred by natural service using a single sire group. The bull was examined for breeding soundness two months prior to the onset of the breeding season. On the day of sampling, the heifers had access to water, but not feed while in the holding pens. Sampling was done on a cool day with a humidity of 92%, minimum and maximum temperatures were 13.7 ˚C and 22.7 ˚C respectively (SAWS).

Herd E was a stud Bonsmara herd in the sour veldt of the Free State province. Heifers in this herd were managed on irrigated rye grass pastures, defined as a high input nutritional system. A commercial energy lick supplement (18% CP) was supplied for five months prior to the onset of the breeding season. On the first day of the breeding season, they were moved to natural pasture (over sown with *Themeda triandra*), and received a mineral lick for the duration of the breeding season. Due to the high RDP content of pasture and the energy lick supplement combined, this herd was defined as having a high level of nitrogen supplementation. One hundred and forty-three heifers aged 12 to 20 months were bred by natural mating in multisire groups of 25 to 30 heifers per bull. A breeding soundness examination of the bulls was performed two months prior to the onset of the breeding season. Sampling in this herd was done over two consecutive days. The weather on the first sampling day was cool, with intermittent rain and the sampling lasted the whole day until the evening. On the second day, sampling lasted until midday and it was a warm day. On both sampling days, the heifers did not have access to feed or water whilst in the holding pens. The heifers that were sampled on the second day spent the first day in the holding pen with the other group. It was cold and raining with a humidity of 76%, total daily rainfall of 1.4 mm, minimum and maximum temperatures were 16.1 ˚C and 26.7 ˚C respectively (SAWS) on the first day of sampling, but very hot on the second day with a humidity of 72 %, minimum and maximum temperatures were 14.4 ˚C and 28.4 ˚C respectively (SAWS).

Informed consent was obtained from all herd owners before commencement of the study.

6.2 Experimental design

The study was a prospective cohort study. The sample size was estimated at 438 heifers using the formula below, based on the normal approximation to the binomial assuming equal group sizes at a power of 80% and allowable alpha error of 5% (Fosgate, 2009):

$$\frac{\left(Z_{1-\alpha/2}\sqrt{2\bar{P}(1-\bar{P})}-Z_{\beta}\sqrt{P_1(1-P_1)+P_2(1-P_2)}\right)^2}{(P_2-P_1)^2},$$

where P_1 and P_2 are the expected proportions in each group, and $\bar{P}$ is the average of the expected proportions. Variables $Z_{1-\alpha/2}$ and Z_{β} are the standard normal Z values corresponding to the selected alpha (2-sided test) and beta, respectively.

All farmers were blinded to RTS, BCS and BUN concentration data.

6.3 Experimental procedures

The first visit to all the farms was performed within one week prior to the commencement of the breeding season. All heifers were driven into a holding pen, and from there they would enter the crush in batches averaging 20 animals and blood samples were collected by venepuncture from the coccygeal vein or artery into evacuated serum tubes. Immediately after sampling, the blood was centrifuged at 4000 rpm for 8 minutes. Serum was separated into labelled micro centrifuge tubes (2 ml) and then immediately frozen in a portable freezer at -18 °C. Delivery of the serum samples to the clinical pathology laboratory at the Faculty of Veterinary Science of the University of Pretoria was done on the first or second day after sampling, where the serum was frozen at -80 °C for a maximum of 30 days until analysed. Analysis for all samples was done using an auto analyser machine (Cobas Integra 400 plus, Roche, Switzerland).

After blood sampling, BCS and RTS were performed and recorded. BCS was assigned based on a 1 to 5 scale whereby score 1 represents emaciated animals and score 5 represents obese animals (Wildman et al., 1982) with scores further subdivided into halves. The

technique used for the scoring combined visual assessment of the whole animal and tactile assessment of the loin area and the ischiorectal fossa (Table 6.1).

Table 6.1: *BCS system* (Wildman et al., 1982).

Score	Description
1	Emaciated cow, distinct, sharp spinous processes, very prominent hooks, pins and tail head, line between hook & pin bone is V shaped, deep pigeon holes next to tail head and deep sunken area around hip joint (thurl area).
2	Less prominent spinous processes that feel rounded rather than sharp, half of the short rib covered with fat, hook and pin bones still prominent, line between hook and pin bone less V shaped, thurl area sunken and pigeon holes still deep.
3	Backbone forms straight line, individual processes still palpable, two thirds of short ribs covered with fat, hook and pin bones round and smooth, thurl area slightly depressed, pigeon holes have some fat and sacral ligaments less distinct.
4	Spinous processes of backbone not visible or palpable anymore, short ribs totally covered with fat, hook and pin bone rounded, span between backbone and hook and pin bones is flat and pigeon holes nearly filled with fat.
5	Over conditioned cow, bone structure of backbone, short ribs and hook and pin bones not visible, subcutaneous fat deposits very evident, whole back area can be compared with a rounded table top and tail head buried in fat.

The RTS score (Table 6.2) was determined using rectal palpation of the reproductive tract and ovarian structures and a score from 1 to 5 was assigned (Andersen et al., 1991; Holm et al., 2009).

Table 6.2: *RTS system (Adapted from Andersen et al., 1991; Holm et al., 2009).*

		Ovary			
RTS	**Uterine horn**	**Length (mm)**	**Height (mm)**	**Width (mm)**	**Ovarian structures**
1	Immature < 20 mm diameter, no tone	15	10	8	No palpable structures
2	20- to 25 mm diameter, no tone	18	12	10	8 mm follicles
3	25- to 30 mm diameter, slight tone	22	15	10	8 to 10 mm follicles
4	30 mm diameter, good tone	30	16	12	>10 mm follicles, corpus luteum possible
5	>30 mm diameter, good tone, erect	>32	20	15	>10 mm follicles, corpus luteum present

Four weeks after the breeding season, a combination of transrectal palpation and ultrasonography was performed to determine pregnancy status and stage. All examinations were performed by the researcher using a portable ultrasound machine and a 3.5 - 5 MHz linear transducer (CTS900V, Shantou Institute for Ultrasonic Instruments, China).

Rectal palpation was performed first, following the basic technique for pregnancy examination (PE) in cattle (Sheldon and Noakes, 2002; Youngquist, 2007). The stage of pregnancy was recorded in weeks. In cases where the pregnancy was judged to be more than 8 weeks, the stage of pregnancy was determined solely by rectal palpation (Table 6.3).

Table 6.3: *Determination of stage of pregnancy by rectal palpation (adapted from Sheldon and Noakes, 2002; Youngquist, 2007).*

	Pregnancy stage in weeks										
Palpable structures	4	6	8	10	12	14	16	18	20	22	24
CL ipsilateral to pregnant horn	■	■	■	■	■	■	■	■	■	■	■
Asymmetry and fluctuation of pregnant horn (Ipsilateral to CL)		■	■								
Amniotic vesicle		■	■								
Allantochorion			■	■	■	■	▒	▒	▒	▒	▒
Foetus				■	■	■	■	■			■
Placentomes				■	■	■	■	■	■	■	■
Fremitus on pregnant horn						■	■	■	■	■	■
Fremitus on non-pregnant horn									■	■	■

The green bars represent periods during which different structures can be clearly palpated while the light green bar delineates the period when structures can be palpated with some difficulty. Absence of a green bar indicates that the structure cannot be palpated during that period.

In cases where the pregnancy was determined to be less than 8 weeks on palpation, ultrasonography was performed to differentiate an early pregnancy from a non-gravid uterus. A presumptive diagnosis of an early pregnancy was made if nonechodense fluid was seen in the uterine lumen and a corpus luteum was present on the ipsilateral ovary. This was classified as a 4 week old pregnancy. Visualization of the allantochorion or embryo in the uterine lumen was also attempted, and where either was seen, it was used as a confirmation of pregnancy (Romano et al., 2006). For estimation of the age of the foetus, the crown rump length formula (CRL) was used (Riding et al., 2008):

$$y = -0.0009x^2 + 0.5509x + 29.184,$$

where x = CRL (mm) and y = estimated foetal age (days).

The farmers were requested to observe and record the days that the heifers were seen to be mated. This data, when available, was used to verify the accuracy of the estimated foetal age.

6.4 Observations

The exposure of interest was BUN concentration while the other covariates were BCS, RTS, age and body mass. The outcomes of interest were pregnancy status per three-month breeding season and DTP. DTP was calculated as the number of days from the onset of the breeding season to the fixed time of PE.

The units for BUN concentration in most literature is given in mg/dL, whilst in this study BUN concentration was measured in $mmolL^{-1}$. BUN concentration is converted from mg/dL to mmol/L by multiplying the value in mg/dL by 0.357 (Tresley and Sheean, 2008).

6.5 Data analysis

The normality assumption was assessed by plotting histograms, calculating descriptive statistics, and performing the Anderson-Darling Test. Data satisfying the normality assumption were presented as mean +/- standard deviation (SD) and non-normal data were presented as the median and interquartile range (IQR). Data were compared between days when herd sampling required multiple days to complete. Normally distributed data were compared using Student t tests and non-normal data using Mann-Whitney U tests.

Conditional logistic regression analysis was performed to measure the association between BUN concentration and subsequent pregnancy while adjusting for herd as the grouping factor and other potential confounders by including them as main effects in the models. Confounding was assessed by measuring the change in the odds ratio (OR) for BUN concentration in models with and without the covariate. Variables that caused 15% or greater change were considered important confounders.

Stratified Cox proportional hazards survival analysis was performed to investigate the effect of BUN concentration on the DTP. Herd was included as the stratifying factor and other potential confounders were evaluated as main effects. Sampling day was forced into all models in herds where sampling required two days. The odds ratio (OR) in all analyses was interpreted as the odds of becoming pregnant. The hazards is a rate defined as the number

of pregnant animals divided by the time that these animals were at risk of becoming pregnant. Therefore, the hazards ratio is an inverse of the average DTP. The hazards ratio (HR) is the estimate of effect analogous to the OR calculated from logistic regression. This means that when the HR is less than one, the relationship between the variable and the DTP is positive.

The ordinal scales of RTS and BCS were screened as ordinal variables in statistical models and dichotomized when significant associations ($P < 0.2$) with BUN were identified. Categorization was performed based on the relative frequencies within each category. Specifically, RTS was grouped as 1 to 3 versus 4 and 5. Results for the ordinal coding were reported when categorization did not suggest violation of the assumption of being linear in the natural logarithm on the odds or hazard scale. In addition to confounding variables, all variables with $P < 0.2$ were entered into all multivariable models and removed one by one in a backward elimination process based on Wald *P* values.

Pearson's correlation coefficient was used to evaluate the relationship between normally distributed data and Spearman's rho was calculated for non-normal data. Results for variables that were not significantly correlated to any other variable in any herd were not presented. The trend in BUN concentrations over sampling order was described using scatter plots and simple linear regression. Data were analysed using commercially available software (IBM SPSS Statistics Version 20, International Business Machines Corp., Armonk, NY, USA; MINITAB Statistical Software, Release 13.32, Minitab Inc., State College, Pennsylvania, USA).

P-values less than 0.05 were defined as being significant, values between 0.05 and 0.1 as being close to significant and values greater than 0.1 as being not significant.

6.6 Experimental animals

All study animals were managed at their respective farms as described for the different herds (Section 6.1). On the day of sample collection and PE, all the heifers were moved into a holding pen and then into a crush in batches averaging 20 animals at a time. Farm

management was responsible for routine daily care of the animals and normal farming practices continued throughout the study. The researcher had no control over the loss of animals due to death, sales or loss of identification tags, which occurred between sample collection and the subsequent follow up at the time of PE.

7 Results

7.1 All herds combined

7.1.1 Descriptive statistics

Three hundred and sixty-nine heifers were sampled from five different farms at the start of the study and 338 were available at the time of examination for pregnancy (Table 7.1). Heifers that did not become pregnant were considered right censored, whilst those that were lost to follow up were excluded from the analysis. The mean breeding age ± the standard deviation (SD) for all herds was 19.03 months ± 4.54 (assuming that the heifers in herd A were born on the 15th of the month) and the overall pregnancy proportion was 63.1%. The mean BUN concentration for all herds was 5.27 mmolL^{-1} ± 1.80 at the onset of the breeding season. Heifers had a median BCS of 3.00 [Interquartile range (IQR): 3.00, 3.00] and RTS of 4.00 (IQR: 3.00, 4.00). The median BCS increased to 3.50 (IQR: 3.00, 3.50) at the time of PE. The earliest heifers became pregnant 4 days after the onset of the breeding season and the median DTP was 40 (IQR: 19, 54) days.

Table 7.1: *Descriptive statistics for all herds (n = 369)*

Variable	N	Mean	SE Mean	SD	Min	Q_1	Median	Q_3	Max
Age	333	19.03	0.25	4.54	11.93	14.03	18.77	22.57	30.57
Mass	221	283.00	3.24	48.19	190.00	244.00	271.00	305.00	440.00
BUN	368	5.27	0.09	1.80	1.20	4.30	5.20	6.50	9.70
BCS	367	3.00	0.02	0.30	2.00	3.00	3.00	3.00	4.00
RTS	367	3.78	0.04	0.71	1.00	3.00	4.00	4.00	5.00
DTP	233	38.00	1.43	21.75	4.00	19.00	40.00	54.00	120.00
BCS at PE	328	3.34	0.02	0.36	2.50	3.00	3.50	3.50	4.50

The mean breeding age ± SD for heifers that became pregnant was 20.53 months ± 4.23 whilst for those non-pregnant was 16.30 months ± 3.77 (Table 7.2). The mean body mass for pregnant heifers was 297 kg ± 45.12 whilst for those non-pregnant ones was 259 kg ± 44.88. The mean BUN concentration for heifers that became pregnant was 4.87 mmolL^{-1} ± 1.73 whilst that for those non-pregnant was 6.04 mmolL^{-1} ± 1.64. The median BCS at the onset of the breeding season was 3.00 (IQR: 3.00, 3.00) for pregnant and non-pregnant heifers. The

median RTS for pregnant heifers was 4.00 (IQR: 3.00 – 4.00) whilst for those non-pregnant was 4.00 (IQR: 3.50 – 4.00).

Table 7.2: *Descriptive statistics by pregnancy status for all herds, indicating pregnant (n = 233), non-pregnant (n = 102) and lost to follow up (n = 31) heifers*

Var	PE status	N	Mean	SE Mean	SD	Min	Q_1	Median	Q3	Max
Age	preg	217	20.53	0.29	4.23	12.17	18.77	19.77	24.53	30.57
	non-preg	95	16.30	0.39	3.77	11.93	13.43	14.03	18.77	29.57
	lost	21	16.07	0.76	3.47	12.53	13.30	13.67	18.77	24.03
Mass	preg	138	297.00	3.84	45.12	220.00	262.00	295.00	324.00	435.00
	non-preg	72	259.00	5.29	44.88	190.00	234.00	244.00	265.00	440.00
	lost	12	256.00	9.12	31.59	229.00	233.00	244.00	267.00	321.00
BUN	preg	233	4.87	0.11	1.73	1.20	3.70	5.00	6.10	9.50
	non-preg	102	6.04	0.16	1.64	2.20	4.90	5.80	7.20	9.70
	lost	30	5.67	0.35	1.93	2.50	4.50	5.30	6.85	9.70
BCS	preg	233	3.03	0.02	0.33	2.00	3.00	3.00	3.00	4.00
	non-preg	102	2.95	0.02	0.22	2.50	3.00	3.00	3.00	3.50
	lost	32	3.00	0.06	0.36	2.50	3.00	3.00	3.00	4.00
RTS	preg	233	3.76	0.05	0.78	1.00	3.00	4.00	4.00	5.00
	non-preg	102	3.83	0.06	0.58	2.00	3.50	4.00	4.00	5.00
	lost	29	3.72	0.08	0.45	3.00	3.00	4.00	4.00	4.00

Var = variable; preg = pregnant; lost = animals that were missing at the time of PE

7.1.2 Logistic regression analysis

7.1.2.1 Single variable logistic regression

When all herds were combined, and Herd E modelled as two separate herds based on sampling day, age (P = 0.010) and body mass (P = 0.037) were the only significant predictors of pregnancy outcome (Table 7.3). The older (OR = 1.082; 95% CI: 1.019 – 1.149) and heavier

(OR = 1.006; 95% CI: 1.000 – 1.012) heifers were more likely to become pregnant when compared to the lighter and younger ones. Herd was included as the grouping variable in the conditional logistic regression model.

Table 7.3: *Effects of BUN concentration, age, body mass and RTS on pregnancy status in all herds combined (single variable logistic regression)*

Variable	B	OR	95% CI of OR		*P* value
			Lower	Upper	
BUN	-0.126	0.882	0.772	1.007	0.063
Age	0.079	1.082	1.019	1.149	0.010
Mass	0.006	1.006	1.000	1.012	0.037
RTS	-0.102	0.903	0.746	1.093	0.297

B = beta; OR = odds ratio and CI = confidence interval

7.1.2.2 Multivariable logistic regression

When combined with potential confounding variables, BUN concentration was still not significant (*P* = 0.068; Table 7.4).

Table 7.4: *The combined effects of BUN concentration and age on pregnancy status in all herds (multivariable logistic regression*

Variable	B	OR	95% CI of OR		*P* value
			Lower	Upper	
BUN	-0.127	0.881	0.768	1.009	0.068
Age	0.079	1.082	1.019	1.149	0.011

B = beta; OR =odds ratio and CI = confidence interval

7.1.3 Proportional hazards survival analysis

7.1.3.1 Single variable survival analysis

BUN concentration ($P = 0.007$), age ($P < 0.001$), body mass ($P = 0.001$) and RTS ($P = 0.023$) were significant predictors of DTP (Table 7.5). Heifers with a higher BUN concentration (HR = 0.827; 95% CI: 0.721 – 0.949) and RTS (HR = 0.797; 95% CI: 0.656 – 0.969) had a higher number of DTP. The older (HR = 1.130; 95% CI: 1.061 – 1.202) and heavier (HR = 1.010; 95% CI: 1.004 – 1.016) heifers had a smaller number of DTP.

Table 7.5: *Effects of BUN concentration, age, body mass and RTS on DTP in all herds combined (single variable survival analysis)*

Variable	B	HR	95% CI of HR		*P* value
			Lower	Upper	
BUN	-0.190	0.827	0.721	0.949	0.007
Age	0.122	1.130	1.061	1.202	<0.001
Mass	0.010	1.010	1.004	1.016	0.001
RTS	-0.226	0.797	0.656	0.969	0.023

B = beta; HR = hazard ratio and CI = confidence interval

7.1.3.2 Multivariable survival analysis

BUN concentration was a significant predictor of DTP ($P = 0.012$) when adjusting for age (Table 7.6). Other evaluated covariates were not significant predictors and did not cause substantial confounding (< 15 %). Herd was included in the conditional logistic regression model as the grouping variable.

Table 7.6: *The combined effects of BUN concentration and age on DTP in all herds (multivariable survival analysis)*

Variable	B	HR	95% CI of HR		*P* value
			Lower	Upper	
BUN	-0.184	0.832	0.722	0.958	0.011
Age	0.122	1.130	1.061	1.203	<0.001

B = beta; HR = hazard ratio and CI = confidence interval

7.1.4 Correlation analysis

A significant positive correlation was estimated between RTS with age ($P = 0.004$) and BCS ($P < 0.001$; Figure 7.1). A significant negative correlation was estimated between BUN concentration with age ($P < 0.001$) and body mass ($P < 0.001$). Age was significantly correlated to body mass ($P < 0.001$) and BCS ($P = 0.021$). All the other variables were not significantly correlated (P >0.05).

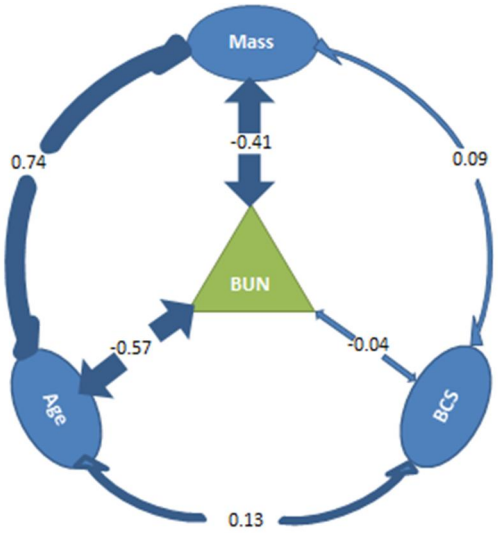

Figure 7.1: *Pearson's correlation for BUN concentration with age and body mass, and age with body mass, and Spearman's correlation for BCS to age, body mass and BUN concentration, in all herds combined.*

7.2 Herd A

7.2.1 Descriptive statistics

One hundred and fifteen heifers were sampled from this herd at the commencement of the study and 106 were available at the time of PE (Table 7.7). Heifers in this herd were bred at a mean age ± SD of 18.6 months ± 1.27 and had a pregnancy proportion of 71.3%. However, exact age was not known as only the month in which they were born was recorded. In this herd, it was assumed that all heifers were born on the 15th of the given month. Heifers that were sampled had a mean BUN concentration of 5.37 mmolL^{-1} ± 0.81 at the onset of the breeding season. Heifers were considered mature and ready for breeding based on their RTS. Median BCS was 3.00 (IQR: 3.00 – 3.00) before breeding and increased to 3.50 (IQR: 3.00 – 3.50) at the time of PE. The earliest heifer conceived 15 days after the onset of the breeding season and the median DTP was 43 (IQR: 29, 71) days. Body mass of the heifers were not available in this herd.

Table 7.7: *Descriptive statistics for Herd A (n = 115)*

Variable	n	Mean	SE Mean	SD	Min	Q1	Median	Q3	Max
Age	110	18.6	0.1	1.3	14	19	19	19	24
BUN	115	5.37	0.08	0.81	2.70	4.90	5.30	5.90	7.50
BCS	115	2.90	0.02	0.20	2.50	3.00	3.00	3.00	3.00
RTS	115	3.77	0.05	0.50	2.00	4.00	4.00	4.00	5.00
DTP	106	67.00	5.00	50.00	15.00	29.00	43.00	71.00	155.00
BCS at PE	106	3.40	0.03	0.28	2.50	3.00	3.50	3.50	4.00

There was no significant linear trend between BUN concentration and sampling order (P = 0.548; Figure 7.2). The least squares regression line for this herd was

$$y = 0.0004x + 5.3451,$$

where y is the BUN concentration and x is the sampling order.

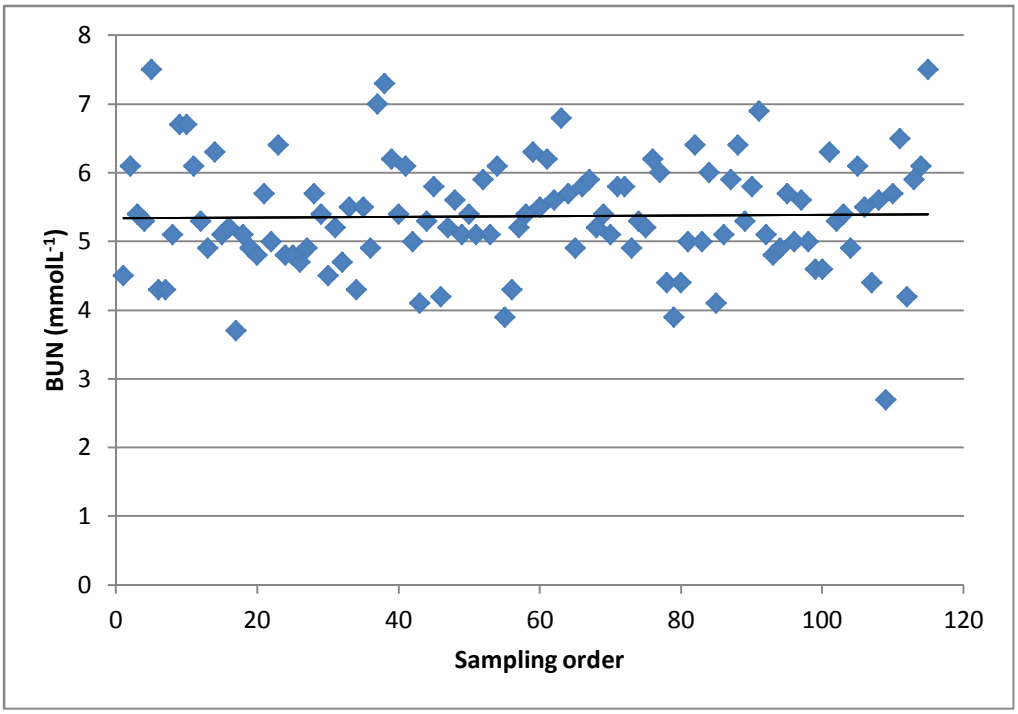

Figure 7.2: *BUN concentration in relation to sampling order in Herd A*

Mean BUN concentration ± SD for heifers that became pregnant was 5.37 mmolL^{-1} ± 0.77 whilst for those non-pregnant was 5.45 mmolL^{-1} ± 0.91 (Table 7.8). The median BCS at the

onset of the breeding season was 3.00 (IQR: 3.00 – 3.00 for both pregnant and non-pregnant heifers. The median RTS for the pregnant heifers was 4.00 (IQR: 3.00 – 4.00) whilst for those non-pregnant was 4.00 (IQR: 4.00 – 4.00).

Table 7.8: *Descriptive statistics by pregnancy status for Herd A, indicating pregnant (n = 82), non-pregnant (n = 24) and lost to follow up (n = 9) heifers*

Var	PE status	n	Mean	SE Mean	SD	Min	Q1	Median	Q3	Max
Age	preg	78	18.80	0.12	1.03	14.67	18.77	18.77	18.77	23.8
	non-preg	23	18.37	0.27	1.27	14.67	18.77	18.77	18.77	19.77
	lost	9	17.53	0.73	2.20	13.67	15.70	18.77	18.77	18.77
BUN	preg	82	5.37	0.09	0.77	2.70	4.90	5.35	5.80	7.50
	non-preg	24	5.45	0.19	0.91	3.90	4.90	5.35	6.20	7.50
	lost	9	5.18	0.33	0.98	4.20	4.35	5.10	5.90	7.00
BCS	preg	82	2.92	0.02	0.19	2.50	3.00	3.00	3.00	3.00
	non-preg	24	2.90	0.04	0.21	2.50	3.00	3.00	3.00	3.00
	lost	9	2.83	0.08	0.25	2.50	2.50	3.00	3.00	3.00
RTS	preg	82	3.70	0.06	0.51	2.00	3.00	4.00	4.00	4.00
	non-preg	24	4.04	0.07	0.36	3.00	4.00	4.00	4.00	5.00
	lost	9	3.67	0.17	0.50	3.00	3.00	4.00	4.00	4.00

Var = variable; preg = pregnant; lost = animals that were missing at the time of PE

7.2.2 Logistic regression analysis

7.2.2.1 Single variable logistic regression

RTS was the only significant predictor of pregnancy status in this herd (*P* = 0.017; Table 7.9). Those heifers which had a higher RTS had lower chances of becoming pregnant (OR = 0.083; 95% CI: 0.011 – 0.638). When using the dichotomised RTS data, RTS was a significant predictor of pregnancy status (B = -2.149, OR = 0.268 and *P* = 0.041).

Table 7.9: *Effects of BUN concentration, age, body mass and RTS on pregnancy status in Herd A (single variable logistic regression)*

Variable	B	OR	95% CI of OR		*P* value
			Lower	Upper	
BUN	-0.182	0.833	0.460	1.511	0.548
Age	0.331	1.392	0.923	2.099	0.115
Mass	-	-	-	-	-
RTS	-2.494	0.083	0.011	0.638	0.017

B = beta; OR = odds ratio and CI = confidence interval

7.2.2.2 Multivariable logistic regression

Multivariable analysis did not identify a model that was an improvement over models with only a single predictor.

7.2.3 Proportional hazards survival analysis

7.2.3.1 Single variable survival analysis

RTS was the only significant predictor of DTP (*P* = 0.023) but age was close to the significance threshold (*P* = 0.051; Table 7.10). Heifers with a higher RTS took longer to become pregnant than those with a lower RTS (HR = 0.636; 95% CI: 0.431 - 0.939). Using the dichotomised RTS data, RTS was close to significance in predicting DTP (B = -0.487, HR = 0.614 and *P* = 0.053).

Table 7.10: *Effects of BUN concentration, age, body mass and RTS on DTP in Herd A (single variable survival analysis)*

Variable	B	HR	95% CI of HR		*P* value
			Lower	Upper	
BUN	-0.031	0.969	0.737	1.276	0.824
Age	0.266	1.305	0.999	1.704	0.051
Mass	-	-	-	-	-
RTS	-0.453	0.636	0.431	0.939	0.023

B = beta; HR = hazard ratio and CI = confidence interval

7.2.3.2 Multivariable survival analysis

Multivariable survival analysis did not identify a model that was an improvement over models with only a single predictor.

7.2.4 Correlation analysis

RTS and age were positively correlated (P = 0.043; Figure 7.3). No other significant correlations were identified (P > 0.05). Correlations between body mass and other variables could not be computed since the body masses were not available.

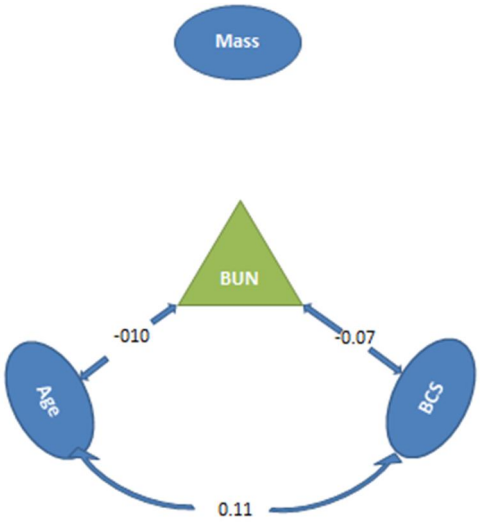

Figure 7.3: *Pearson's correlation for BUN with age, and Spearman's correlation for BCS with age and BUN concentration, in Herd A*

7.3 Herd B

7.3.1 Descriptive statistics

Thirty-three heifers were sampled at the commencement of the study and all of them were pregnant at the time of PE. Heifers were bred at a mean age ± SD of 24.87 months ± 1.50 and had a 100% pregnancy proportion (Table 7.11). Sampled heifers had a mean mass of 318 kg ± 32, and a mean BUN concentration of 2.20 $mmolL^{-1}$ ± 0.67. Median BCS and RTS were 3.00 (IQR: 2.50 – 3.00) and 3.00 (IQR: 2.75 – 4.00) respectively. The earliest heifer became pregnant 10 days after the start of the breeding season and the median DTP was 24 (IQR: 17, 31) days.

Table 7.11: *Descriptive statistics for Herd B (n = 33)*

Variable	n	Mean	SE Mean	SD	Min	Q1	Median	Q3	Max
Age	33	24.87	0.27	1.50	20.43	24.13	25.23	26.13	26.37
Mass	33	318.00	6.00	32.00	242.00	290.00	323.00	342.00	371.00
BUN	33	2.20	0.11	0.67	1.20	1.70	2.00	2.80	4.40
BCS	33	2.80	0.05	0.27	2.00	2.50	3.00	3.00	3.00
RTS	33	3.27	0.18	1.02	1.00	2.75	3.00	4.00	5.00
DTP	33	26.00	2.00	14.00	10.00	17.00	24.00	31.00	59.00
BCS at PE	33	3.92	0.04	0.25	3.50	4.00	4.00	4.00	4.50

There was no significant linear trend between BUN concentration and sampling order (P = 0.205; Figure 7.4). The least squares regression line for this herd was:

$$y = -0.0158x + 2.4875,$$

where y is the BUN concentration and x is the sampling order.

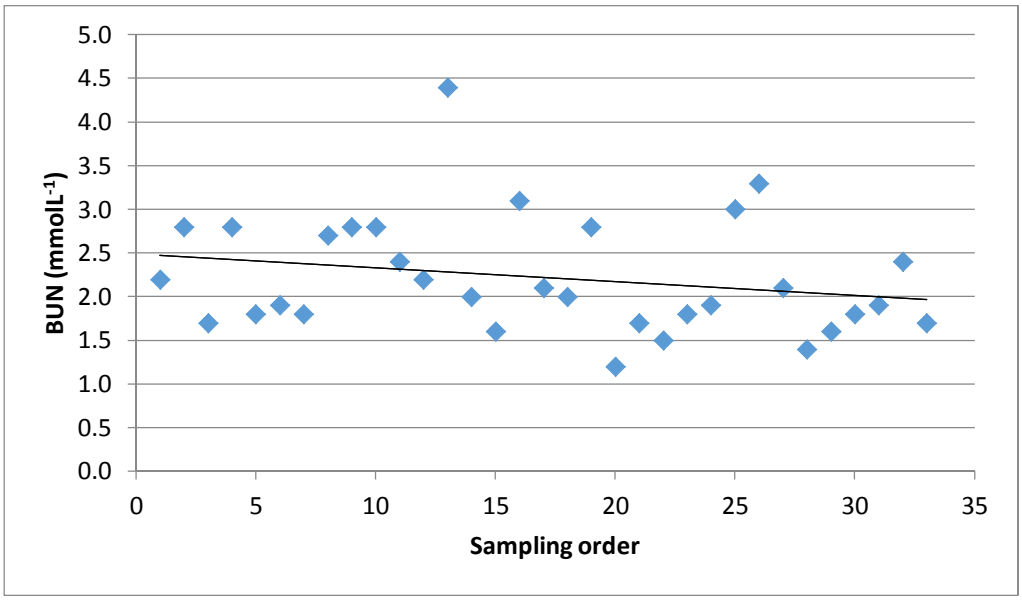

Figure 7.4: *BUN concentration in relation to sampling order in Herd B*

7.3.2 Logistic regression analysis

Logistic regression was not possible because the pregnancy proportion was 100%.

7.3.3 Proportional hazards survival analysis

7.3.3.1 Single variable survival analysis

In this herd, none of the evaluated variables significantly predicted DTP (P > 0.05; Table 7.12).

Table 7.12: *Effects of BUN concentration, age, body mass and RTS on DTP in Herd B (single variable survival analysis)*

Variable	B	HR	95% CI of HR		*P* value
			Lower	Upper	
BUN	0.107	1.113	0.644	1.926	0.701
Age	-0.139	0.870	0.663	1.142	0.317
Mass	0.002	1.002	0.990	1.013	0.793
RTS	-0.051	0.951	0.671	1.347	0.776

B = beta; HR = hazard ratio and CI = confidence interval

7.3.3.2 Multivariable survival analysis

Multivariable survival analysis was not possible because none of the variables were significant predictors of DTP.

7.3.4 Correlation analysis

There was a positive correlation between age and body mass (*P* = 0.002) and BCS and body mass (*P* = 0.009; Figure 7.5). No other variables were significantly correlated (*P* > 0.05).

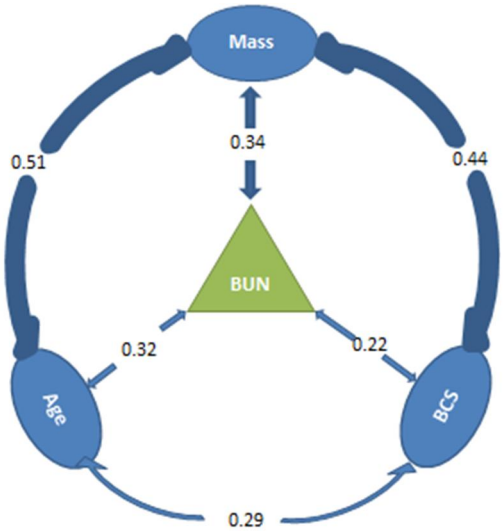

Figure 7.5: *Pearson's correlation for BUN concentration with age and body mass, and age with body mass, and Spearman's correlation for BCS to age, body mass and BUN concentration, in Herd B*

7.4 Herd C

7.4.1 Descriptive statistics

Thirty-four heifers were sampled at the beginning of the study, and all were pregnant at the time of PE (Table 7.13). Mean age ± SD at breeding was 25.73 months ± 0.87 and the pregnancy proportion was 100%. Sampled heifers had a mean mass of 297 kg ± 29 and a mean BUN concentration of 4.14 mmolL^{-1} ± 0.92. Their median BCS and RTS was 3.50 (IQR: 3.50 – 3.50) and 5.00 (4.00 – 5.00) respectively. The earliest heifer became pregnant 4 days after the onset of the breeding season and the median DTP was 4 (IQR: 4, 25).

Table 7.13: *Descriptive statistics for Herd C (n=34)*

Variable	n	Mean	SE Mean	SD	Min	Q1	Median	Q3	Max
Age	31	25.73	0.17	0.87	23.87	24.83	26.13	26.47	27.17
Mass	31	297.00	5.00	29.00	220.00	287.00	300.00	304.00	361.00
BUN	34	4.14	0.15	0.92	2.60	3.45	4.20	4.68	6.90
BCS	34	3.60	0.04	0.23	3.00	3.50	3.50	3.50	4.00
RTS	34	4.69	0.09	0.52	3.00	4.00	5.00	5.00	5.00
DTP	34	18.00	4.00	24.00	4.00	4.00	4.00	25.00	81.00
BCS at PE	26	3.38	0.05	0.26	3.00	3.00	3.50	3.50	4.00

There was no significant linear trend between BUN concentration and sampling order (P = 0.438; Figure 7.6). The least squares regression line for this herd was:

$$y = 0.0105x + 3.9775,$$

where y is the BUN concentration and x is the sampling order.

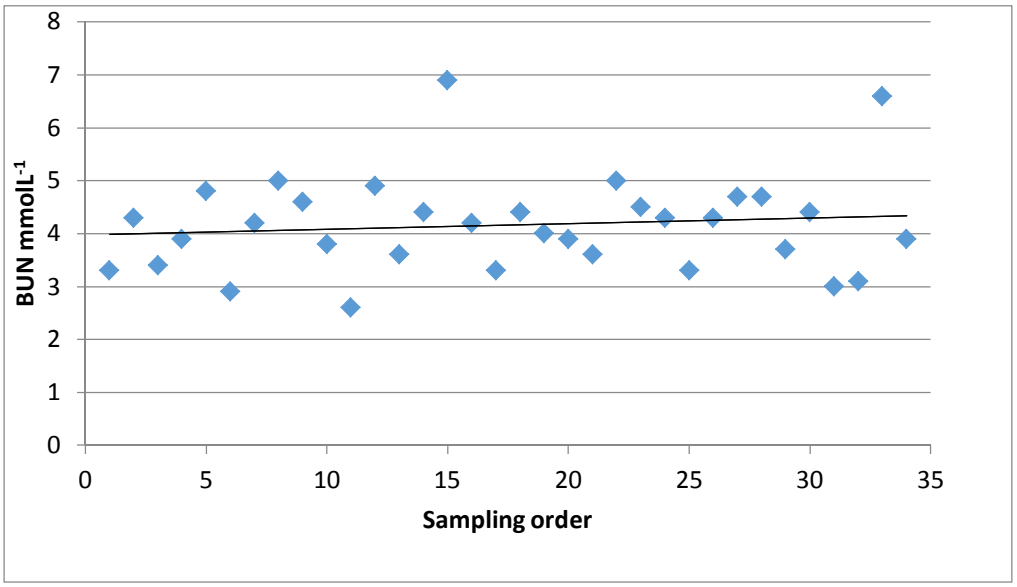

Figure 7.6: *BUN concentration in relation to sampling order in Herd C*

7.4.2 Logistic regression analysis

Logistic regression was not possible in this herd because the pregnancy proportion was 100%.

7.4.3 Proportional hazards survival analysis

7.4.3.1 Single variable survival analysis

In this herd, none of the evaluated variables significantly predicted DTP (P > 0.05; Table 7.14).

Table 7.14: *Effects of BUN concentration, age, body mass and RTS on DTP in Herd C (single variable survival analysis)*

Variable	B	HR	95% CI of HR		*P* value
			Lower	Upper	
BUN	-0.003	0.997	0.647	1.535	0.989
Age	-0.157	0.855	0.580	1.259	0.427
Mass	0.002	1.002	0.989	1.014	0.796
RTS	-0.244	0.784	0.407	1.510	0.466

B = beta; HR = hazard ratio and CI = confidence interval

7.4.3.2 Multivariable logistic regression

Multivariable logistic regression was not possible because none of the evaluated variables were significant.

7.4.4 Correlation analysis

Age and body mass were positively correlated ($P < 0.001$; Figure 7.7). No other variables were significantly correlated ($P > 0.05$).

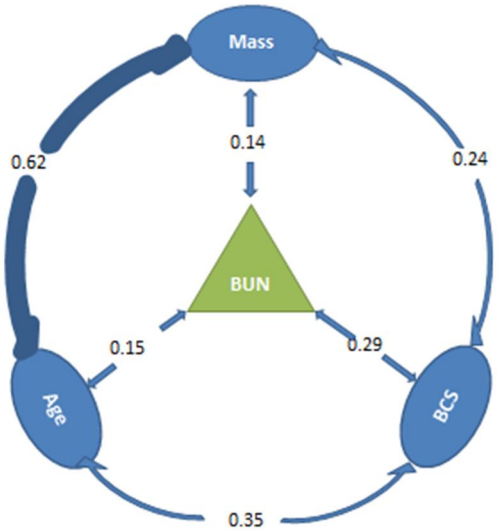

Figure 7.7: *Pearson's correlation for BUN concentration with age and body mass, and age with body mass, and Spearman's correlation for BCS to age, body mass and BUN concentration, in Herd C*

7.5 Herd D

7.5.1 Descriptive statistics

Twenty-nine heifers were sampled at the beginning of the study, and 22 were later examined at the time of PE (Table 7.15). Heifers were bred at a mean age of 24.7 months ± 2.27 and their pregnancy proportion was 68.2%. Sampled heifers had a mean mass of 380 kg ± 38 and a mean BUN concentration of 4.50 mmolL^{-1} ± 1.80. The median BCS and RTS were 3.00 (IQR: 3.00 – 3.00) and 4.00 (IQR: 3.00 – 4.00) respectively. At the time of PE, the median BCS had increased to 3.50 (IQR: 3.50 – 3.50). The earliest heifer became pregnant 12 days after the onset of the breeding season and the median DTP was 47 (IQR: 40, 61) days.

Table 7.15: *Descriptive statistics for Herd D (n = 29)*

Variable	n	Mean	SE Mean	SD	Min	Q1	Median	Q3	Max
Age	23	24.70	0.47	2.27	21.73	23.00	24.13	24.90	30.57
Mass	20	380.00	8.00	38.00	320.00	343.00	385.00	410.00	440.00
BUN	29	4.50	0.33	1.80	1.50	2.75	4.70	6.00	7.00
BCS	29	3.00	0.05	0.27	2.50	3.00	3.00	3.00	3.50
RTS	29	3.62	0.17	0.90	1.00	3.00	4.00	4.00	5.00
DTP	15	46.00	5.00	18.00	12.00	40.00	47.00	61.00	75.00
BCS at PE	22	3.52	0.06	0.29	3.00	3.50	3.50	3.50	4.00

There was a significant linear trend between BUN concentration and sampling order ($P < 0.001$; Figure 7.8). The least squares regression line for this herd was

$$y = 0.1721x + 1.9155,$$

where y is the BUN concentration and x is the sampling order.

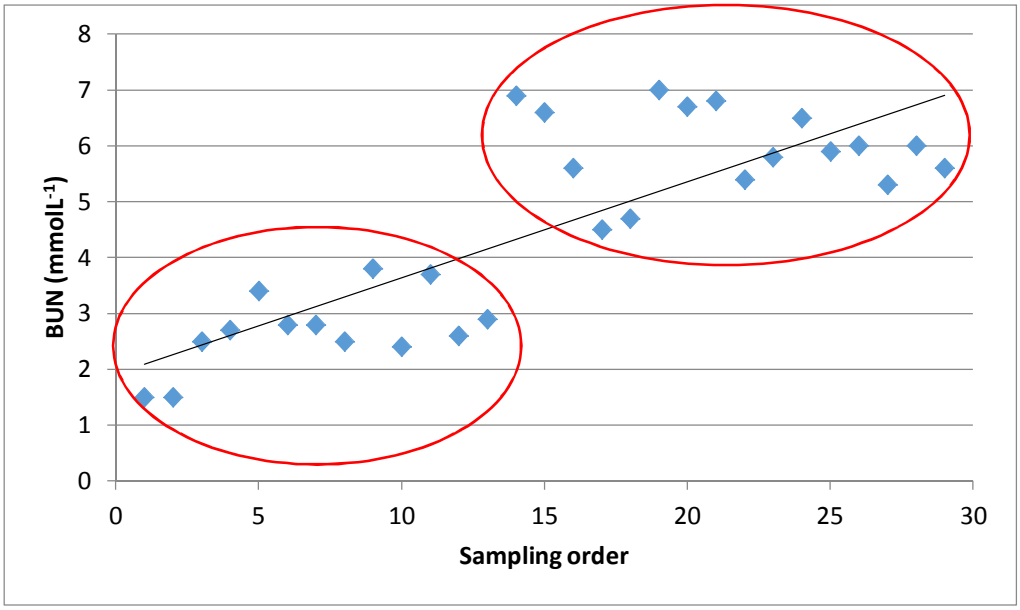

Figure 7.8: *BUN concentration in relation to sampling order in Herd D*

The mean body mass ± SD for heifers that became pregnant was 382 kg ± 37 whilst that for those non-pregnant was 375 kg ± 40 (Table 7.16). The mean BUN concentration for the pregnant heifers was 3.98 $mmolL^{-1}$ ± 1.77 whilst that for those non-pregnant was 5.84 $mmolL^{-1}$ ± 1.43. Median BCS for pregnant heifers was 3.00 (IQR: 3.00 – 3.00) whilst that for

those non-pregnant was 3.00 (IQR: 3.00 – 3.00). The median RTS for pregnant heifers was 3.00 (IQR: 3.00 – 4.00) whilst that for those non-pregnant was and 4.00 (IQR: 3.00 – 5.00).

Table 7.16: *Descriptive statistics by pregnancy status for Herd D, indicating pregnant (n = 15), non-pregnant (n = 7) and lost to follow up (n = 7) heifers*

Var	PE status	n	Mean	SEMean	SD	Min	Q1	Median	Q3	Max
Age	preg	15	24.73	0.57	2.20	22.77	23.73	24.13	24.70	30.57
	non-preg	7	24.80	1.03	2.73	21.73	22.40	24.67	26.57	29.57
	lost	1	24.03	-	-	24.03	-	24.03	-	24.03
Mass	preg	13	382.00	11.00	37.00	320.00	343.00	390.00	413.00	435.00
	non-preg	7	375.00	15.00	40.00	325.00	340.00	375.00	400.00	440.00
	lost	0	-	-	-	-	-	-	-	-
BUN	preg	15	3.98	0.46	1.77	1.50	2.50	3.70	5.90	7.00
	non-preg	7	5.84	0.54	1.43	2.80	5.60	6.50	6.70	6.90
	lost	7	4.29	0.64	1.70	2.50	2.60	4.70	5.40	6.80
BCS	preg	15	3.00	0.05	0.19	2.50	3.00	3.00	3.00	3.50
	non-preg	7	3.00	0.11	0.29	2.50	3.00	3.00	3.00	3.50
	lost	7	3.00	0.15	0.41	2.50	2.50	3.00	3.50	3.50
RTS	preg	15	3.33	0.25	0.98	1.00	3.00	3.00	4.00	5.00
	non-preg	7	4.14	0.34	0.90	3.00	3.00	4.00	5.00	5.00
	lost	7	3.71	0.18	0.49	3.00	3.00	4.00	4.00	4.00

Var = variable; preg = pregnant; lost = animals that were missing at the time of PE

7.5.2 Logistic regression analysis

7.5.2.1 Single variable logistic regression

BUN concentration was the only significant predictor of pregnancy status (*P* = 0.046; Table 7.17). Heifers which had a higher BUN concentration had reduced chances of becoming pregnant (OR = 0.478; 95% CI: 0.232 – 0.987). When using the dichotomised RTS data, RTS

was still not a significant predictor of pregnancy status (B = -1.050, OR = 0.350 and *P* = 0.286).

Table 7.17: *Effects of BUN concentration, age, body mass and RTS on pregnancy status in Herd D (single variable logistic regression)*

Variable	B	OR	95% CI of OR		*P* value
			Lower	Upper	
BUN	-0.737	0.478	0.232	0.987	0.046
Age	-0.014	0.986	0.665	1.460	0.943
Mass	0.005	1.005	0.980	1.031	0.689
RTS	-1.079	0.340	0.097	1.187	0.091

B = beta; OR = hazard ratio and CI = confidence interval

7.5.2.2 Multivariable logistic regression

A multivariable model was not identified that improved the prediction of pregnancy status over the models with only single variables.

7.5.3 Proportional hazards survival analysis

7.5.3.1 Single variable survival analysis

BUN concentration (*P* = 0.033) and RTS (*P* = 0.039) were significant predictors of DTP, while age and mass were not (*P* > 0.05; Table 7.18). Heifers with a higher BUN concentration took longer to become pregnant (HR = 0.719; 95% CI: 0.531 – 0.974). In addition, heifers that had a higher RTS took longer to become pregnant (HR = 0.545; 95% CI: 0.306 – 0.970). When using the dichotomised RTS data, RTS was still not a significant predictor of DTP (B = -0.516, HR = 0.597 and *P* = 0.323).

Table 7.18: *Effects of BUN concentration, age, body mass and RTS on DTP in Herd D (single variable survival analysis)*

Variable	B	HR	95% CI of HR		*P* value
			Lower	Upper	
BUN	-0.330	0.719	0.531	0.974	0.033
Age	0.009	1.009	0.813	1.252	0.934
Mass	0.002	1.002	0.986	1.017	0.844
RTS	-0.607	0.545	0.306	0.970	0.039

B = beta; HR = hazard ratio and CI = confidence interval

7.5.3.2 Multivariable survival analysis

Neither BUN concentration (*P* = 0.207) nor RTS (*P* = 0.282) were significant predictors of DTP when combined together in a multivariable model (Table 7.19).

Table 7.19: *The combined effects of BUN concentration and RTS on DTP in Herd D (multivariable survival analysis)*

Variable	B	HR	95% CI of HR		*P* value
			Lower	Upper	
BUN	-0.225	0.799	0.564	1.132	0.207
RTS	-0.373	0.689	0.349	1.358	0.282

B = beta; HR = hazard ratio and CI = confidence interval

7.5.4 Correlation analysis

Age and body mass (*P* = 0.024), and BUN concentration and RTS (*P* = 0.001) were positively correlated (Figure 7.9). No other variables were significantly correlated (*P* > 0.05).

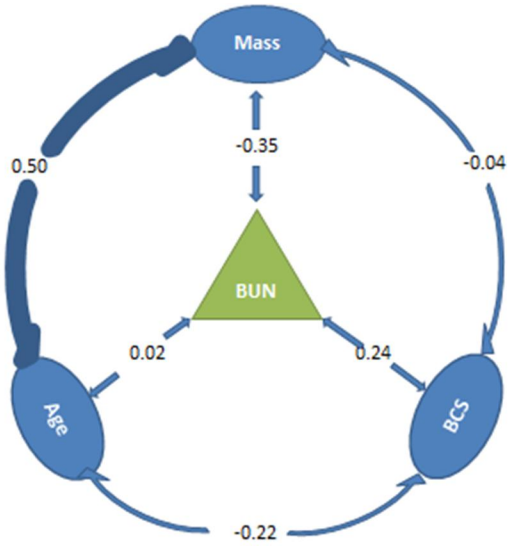

Figure 7.9: *Pearson's correlation for BUN concentration with age and body mass, and age with body mass, and Spearman's correlation for BCS to age, body mass and BUN concentration, in Herd D*

7.6 Herd E

7.6.1 Descriptive statistics

One hundred and fifty-eight heifers were sampled at the commencement of the study and 143 were available at the time of PE (Table 7.20 and Table 7.21). Sampled heifers were bred at a mean age ± SD of 15.6 months ± 3.37 and had a pregnancy proportion of 47.5%. The mean body mass was 256.50 kg ± 27.00. The mean BUN concentration for the first and second day of sampling was 7.40 $mmolL^{-1}$ ± 1.14 and 4.77 $mmolL^{-1}$ ± 1.16 respectively. The median BCS and RTS for these heifers were 3.00 (IQR: 3.00 – 3.00) and 4.00 (IQR: 3.00 – 4.00) respectively. In this herd, the earliest heifer became pregnant 12 days after the onset of the breeding season while the median DTP was 47 (IQR: 33, 61) days.

Table 7.20: *Descriptive statistics for Herd E on day 1 of sampling (n = 88)*

Variable	n	Mean	SE Mean	SD	Min	Q1	Median	Q3	Max
Age	79	15.16	0.37	3.27	12.13	13.17	13.60	14.70	22.50
Mass	79	256.00	2.92	26.00	199.00	239.00	250.00	270.00	335.00
BUN	87	7.40	0.12	1.14	4.60	6.60	7.30	8.20	9.70
BCS	87	2.97	0.03	0.24	2.50	3.00	3.00	3.00	3.50
RTS	87	3.71	0.06	0.57	3.00	3.00	4.00	4.00	5.00
DTP	43	46.84	3.19	20.92	12.00	33.00	40.00	68.00	89.00
BCS	80	3.11	0.03	0.27	2.50	3.00	3.00	3.50	3.50

Table 7.21: *Descriptive statistics for Herd E on day 2 of sampling (n =70)*

Variable	n	Mean	SE Mean	SD	Min	Q1	Median	Q3	Max
Age	58	16.03	0.47	3.47	11.93	13.53	14.10	20.67	21.50
Mass	58	257.00	4.00	28.00	190.00	238.00	251.00	275.00	340.00
BUN	70	4.77	0.14	1.16	2.20	4.00	4.65	5.45	8.90
BCS	69	2.98	0.03	0.25	2.50	3.00	3.00	3.00	3.50
RTS	69	3.74	0.07	0.61	1.00	3.50	4.00	4.00	5.00
DTP	26	46.00	4.00	20.00	12.00	31.00	47.00	56.00	89.00
BCS at PE	63	3.17	0.03	0.25	3.00	3.00	3.00	3.50	4.00

There was no significant liner trend between BUN concentration and sampling order on the first day of sampling (P = 0.099; r^2 = 0.032; Figure 7.10). The least squares regression line for this group was

$$y = -0.008x + 7.7478,$$

where y is the BUN concentration and x is the sampling order.

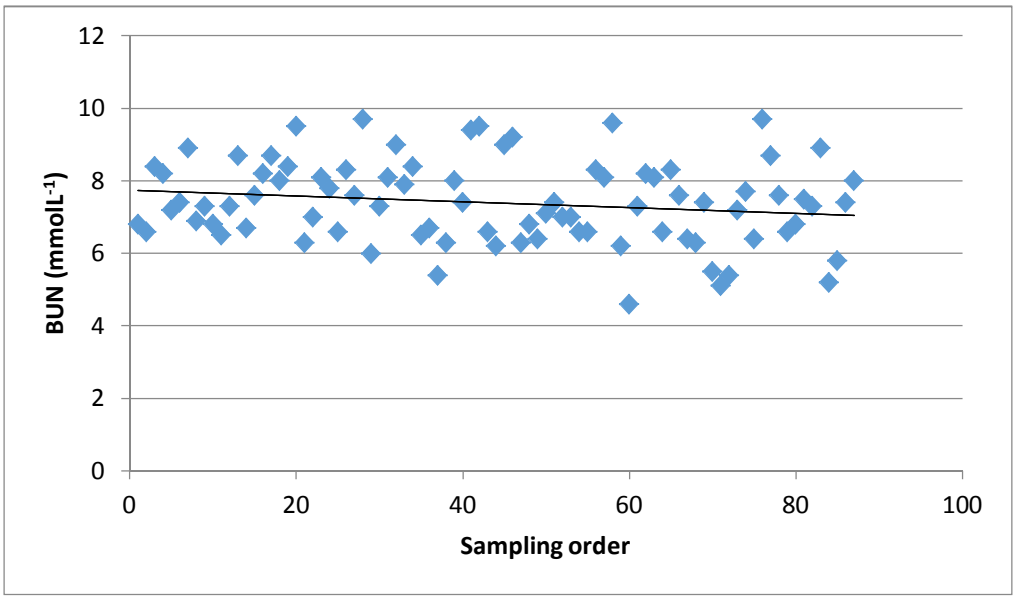

Figure 7.10: *BUN concentration in relation to sampling order in Herd E on day 1 of sampling*

On the second day of sampling, there was no significant linear trend between BUN concentration and sampling order (P = 0.957; Figure 7.11). The least squares regression line for this group was

$$y = 9E - 06x + 4.7725.$$

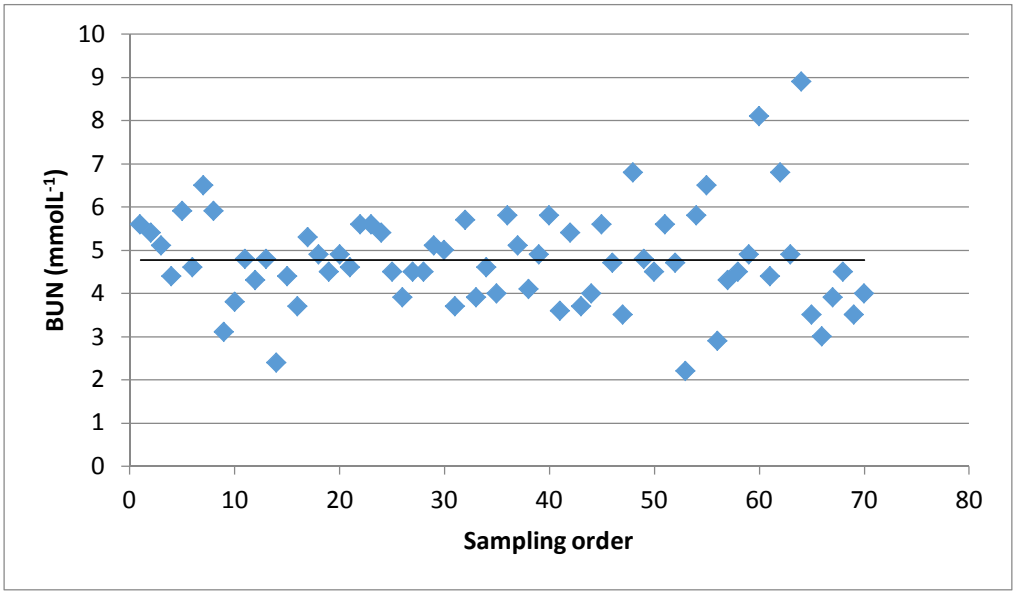

Figure 7.11: *BUN concentration in relation to sampling order in Herd E on day 2 of sampling*

Mean breeding age ± SD for heifers that became pregnant was 16.99 months ± 3.64 whilst those not pregnant had a mean breeding age of 14.65 months ± 2.57 (Table 7.22 and 7.23). The mean mass for the heifers that became pregnant was 268.00 kg ± 27.00 whilst those not pregnant had a mean breeding mass of 246.50 kg ± 21.00. On the first day of sampling, the mean BUN concentration for heifers that became pregnant was 7.07 $mmolL^{-1}$ ± 1.02 whilst for those non-pregnant was 7.65 $mmolL^{-1}$ ± 1.12. On the second day of sampling, the mean BUN concentration for heifers that became pregnant was 4.52 $mmolL^{-1}$ ± 1.06 whilst for those non-pregnant was 4.86 $mmolL^{-1}$ ± 1.15. Median BCS and RTS were 3.00 (IQR: 3.00, 3.00) and 4.00 (IQR: 3.00, 4.00) respectively for both pregnant and non-pregnant heifers.

Table 7.22: *Descriptive statistics by pregnancy status for Herd E day 1, indicating pregnant (n = 43), non-pregnant (n = 37) and lost to follow up (n = 8) heifers*

Var	PE Status	n	Mean	SEMean	SD	Min	Q1	Median	Q3	Max
Age	preg	40	16.27	0.60	3.77	12.13	13.27	13.90	20.97	21.93
	non-preg	33	13.90	0.33	1.83	12.30	13.10	13.47	13.90	21.27
	lost	6	14.93	1.53	3.73	12.87	12.93	13.47	16.30	22.50
Mass	preg	40	266.00	4.00	27.00	220.00	244.00	258.00	289.00	335.00
	non-preg	33	245.00	3.00	18.00	199.00	234.00	242.00	258.00	288.00
	lost	6	256.00	13.00	32.00	230.00	231.00	247.00	278.00	315.00
BUN	preg	43	7.07	0.16	1.02	4.60	6.40	7.00	7.60	9.50
	non-preg	37	7.65	0.18	1.12	5.40	6.60	7.80	8.40	9.70
	lost	7	8.09	0.54	1.42	5.80	6.60	8.20	9.20	9.70
BCS	preg	43	2.95	0.04	0.26	2.50	3.00	3.00	3.00	3.50
	non-preg	37	2.97	0.03	0.20	2.50	3.00	3.00	3.00	3.50
	lost	7	3.00	0.11	0.29	2.50	3.00	3.00	3.00	3.50
RTS	preg	43	3.63	0.09	0.58	3.00	3.00	4.00	4.00	5.00
	non-preg	37	3.76	0.10	0.60	3.00	3.00	4.00	4.00	5.00
	lost	7	4.00	0	0	4.00	4.00	4.00	4.00	4.00

Var = variable; preg = pregnant; lost = animals that were missing at the time of PE

Table 7.23: *Descriptive statistics by pregnancy status for Herd E day 2, indicating pregnant (n = 26), non-pregnant (n = 37) and lost to follow up (n = 7) heifers*

Var	PE status	n	Mean	SEMean	SD	Min	Q1	Median	Q3	Max
Age	preg	21	17.70	0.76	3.50	13.17	14.23	20.03	21.03	21.43
	non-preg	32	15.40	0.57	3.30	11.93	13.43	13.83	18.70	21.50
	lost	5	13.20	0.20	0.43	12.53	12.87	13.20	13.57	13.63
Mass	preg	21	270.00	6.00	27.00	220.00	250.00	265.00	284.00	340.00
	non-preg	32	248.00	4.00	24.00	190.00	233.00	244.00	263.00	310.00
	lost	5	260.00	17.00	37.00	229.00	233.00	246.00	295.00	321.00
BUN	preg	26	4.52	0.21	1.06	2.40	3.70	4.40	5.18	6.80
	non-preg	37	4.86	0.19	1.15	2.20	4.20	4.80	5.50	8.90
	lost	7	5.26	0.56	1.47	3.50	4.50	4.80	5.80	8.10
BCS	preg	26	3.00	0.06	0.28	2.50	3.00	3.00	3.00	3.50
	non-preg	37	2.95	0.04	0.23	2.50	3.00	3.00	3.00	3.50
	lost	6	3.08	0.08	0.20	3.00	3.00	3.00	3.13	3.50
RTS	preg	26	3.85	0.13	0.68	1.00	4.00	4.00	4.00	5.00
	non-preg	37	3.70	0.09	0.57	2.00	3.00	4.00	4.00	5.00
	lost	6	3.50	0.22	0.55	3.00	3.00	3.50	4.00	4.00

Var = variable; preg = pregnant; lost = animals that were missing at the time of PE

7.6.2 Logistic regression analysis

Sampling day was forced into all logistic regression models to account for potential confounding.

7.6.2.1 Single variable logistic regression

BUN concentration ($P = 0.012$), age ($P < 0.001$) and body mass ($P < 0.001$) were significant predictors of pregnancy status (

Table **7.24**). Animals with a higher BUN concentration were less likely to become pregnant (OR = 0.656; 95% CI: 0.472 – 0.912). Older (OR = 1.264; 95% CI: 1.117 – 1.431) and heavier (OR = 1.041; 95% CI: 1.021 – 1.061) animals were more likely to become pregnant.

Table 7.24: *Effects of BUN concentration, age, body mass and RTS on pregnancy status in Herd E (single variable survival analysis)*

Variable	B	OR	95% CI of OR		*P* value
			Lower	Upper	
BUN	-0.421	0.656	0.472	0.912	0.012
Age	0.235	1.264	1.117	1.431	<0.001
Mass	0.040	1.041	1.021	1.061	<0.001
RTS	-0.030	0.971	0.557	1.691	0.916
Sample day	0.503	1.654	0.849	3.222	0.139

B = beta; OR = hazard ratio and CI = confidence interval

7.6.2.2 Multivariable logistic regression

BUN concentration (*P* = 0.017) was a significant predictor of pregnancy status when adjusting for body mass (Table 7.25). Other evaluated covariates were not significant predictors and did not cause substantial confounding (< 15 %).

Table 7.25: The combined e*ffects of BUN concentration and body mass on pregnancy status in Herd E (multivariable logistic regression)*

Variable	B	OR	95% CI of OR		*P* value
			Lower	Upper	
BUN	-0.482	0.617	0.422	0.903	0.013
Mass	0.041	1.042	1.022	1.062	<0.001
Sample day	1.969	7.163	1.983	25.877	0.003

B = beta; OR = hazard ratio and CI = confidence interval

7.6.3 Proportional hazards survival analysis

All models were stratified by sampling day to account for potential confounding.

7.6.3.1 *Single variable survival analysis*

BUN concentration (P = 0.008), age (P < 0.001) and mass (P < 0.001) were significant predictors of DTP (Table 7.26). Heifers with a higher BUN concentration took a longer time to conceive (HR = 0.736; 95% CI: 0.588 – 0.922). Older (HR = 0.1.159; 95% CI: 1.081 – 1.242) and heavier (HR = 1.024; 95% CI: 1.015 – 1.033) heifers had shorter DTP.

Table 7.26: *Effects of BUN concentration, age, body mass and RTS on DTP in Herd E (single variable survival analysis)*

Variable	B	HR	95% CI of HR		*P* value
			Lower	Upper	
BUN	-0.306	0.736	0.588	0.922	0.008
Age	0.148	1.159	1.081	1.242	<0.001
Mass	0.024	1.024	1.015	1.033	<0.001
RTS	-0.052	0.950	0.641	1.406	0.797
Sample day	-0.341	0.711	0.437	1.157	0.170

B = beta; HR = hazard ratio and CI = confidence interval

7.6.3.2 *Multivariable survival analysis*

BUN concentration (P = 0.017) was a significant predictor of DTP when adjusting for mass (Table 7.27). Other evaluated covariates were not significant predictors and did not cause substantial confounding (<15%).

Table 7.27: *The combined effects of BUN concentration and body mass on DTP in Herd E (multivariable survival analysis)*

Variable	B	HR	95% CI of HR		*P* value
			Lower	Upper	
BUN	-0.290	0.748	0.589	0.950	0.017
Mass	0.023	1.023	1.014	1.032	<0.001
Sample day	1.221	3.390	1.542	7.452	0.002

B = beta; HR = hazard ratio and CI = confidence interval

7.6.4 Correlation analysis

Age and body mass were positively correlated ($P < 0.001$) in the groups that were sampled on both days (Figure 7.12 and Figure 7.13). Age and BCS were negatively correlated in the group of heifers that were sampled on the first ($P = 0.015$) and second days ($P = 0.012$). No other variables were significantly correlated ($P > 0.05$).

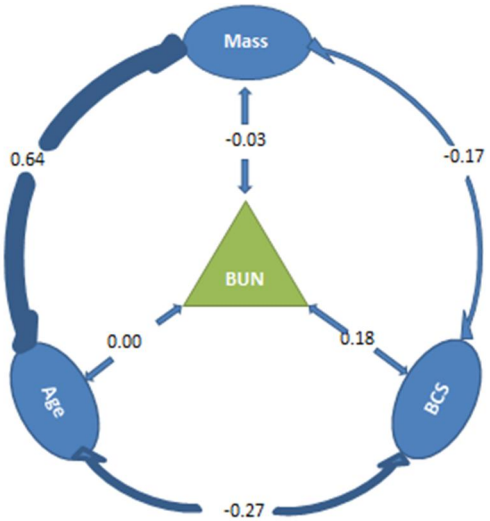

Figure 7.12: *Pearson's correlation for BUN concentration with age and body mass, and age with body mass, and Spearman's correlation for BCS to age, body mass and BUN concentration, in Herd E day 1*

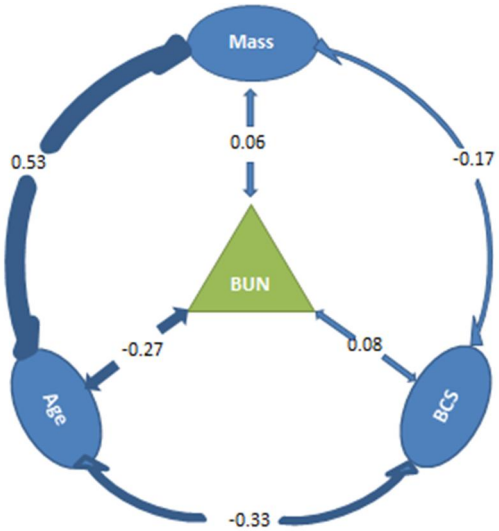

Figure 7.13: *Pearson's correlation for BUN concentration with age and body mass, and age with body mass, and Spearman's correlation for BCS to age, body mass and BUN concentration, in Herd E day 2*

8 Discussion

8.1 Introduction

The hypothesis of the study was that BUN concentration or its interaction with nitrogen supplementation would have a significant effect on the reproductive performance of Bonsmara heifers. The correlations between BUN concentration, body mass, age, BCS and RTS in these heifers were also estimated. Potential classification bias was controlled by blinding farmers to RTS, BCS, and BUN concentration data, and adjusting for herd and evaluating other potential confounders in the statistical models.

As a scientific study, this prospective cohort study offered the advantage of being able to demonstrate an appropriate temporal sequence between BUN concentration and reproductive outcome. Heifers were raised under typical South African commercial beef cow-calf enterprise conditions. The researcher had no control over farming practices including the sale of animals, provision of dietary supplements and the use of reproductive technologies such as synchronisation and artificial insemination that were potential sources of bias. Despite this limitation, the results from this study have an important practical application for the evaluation of the hypothesis because they were obtained from real, commercially viable operations.

Comparison of the descriptive statistics of different herds revealed that herd origin was an important determinant of the measured variables as well as the reproductive performance (Table 7.7; Table 7.8; Table 7.11; Table 7.13; Table 7.15; Table 7.16; Table 7.20; Table 7.21; Table 7.22; Table 7.23). The observed between-herd differences in these parameters clearly indicate that differences in environmental factors, including herd management, had an important role to play in reproductive performance. A large number of factors, both known and unknown could have caused the differences in reproductive performance between herds (Section 2.8, page 12); hence, the statistical significance of the observed differences between herds was not tested.

Sampling from different herds could have contributed towards the observed between-herd differences in BUN concentration because sampling was performed on different days, at different times of the day, and under different weather conditions. The effects of these conditions on BUN concentration were reviewed (Section 2.8, page 12). Stratifying data by herd and sampling day was performed to control for the variation due to different herds in this study.

Most authors have advocated the use of pregnancy proportion and DTC as the most appropriate measure of reproductive performance (Meyer et al., 1990; MacGregor and Casey, 1999; Eler et al., 2002). In this study, DTP was used instead of DTC in order to exclude abortions and variations in gestational length (Andersen and Plum, 1965; Foote, 1981; Norman et al., 2009), which are not related to BUN concentration. Using DTP also reduced the necessary follow up for the animals. The longer follow up period that is associated with DTC would have inevitably increased the loss of study animals, considering that 31 animals were lost within the first five months of the study period.

In a restricted breeding season, DTP is a better measure of reproductive performance than pregnancy proportion because if the breeding season had been long enough, most heifers would eventually become pregnant and pregnancy proportion alone would not differentiate between animals with good and poor reproductive performance.

Assuming that cycling occurs randomly in heifers, except in herds where oestrous synchronisation is practised, it follows that heifers were at random stages of their oestrus cycles when the breeding season commenced. Due to this, those heifers that were at oestrus were likely to become pregnant sooner (hence a shorter DTP) than those that were at dioestrous. The major drawback of using DTP as a parameter for measuring reproductive performance is that it makes those heifers that became pregnant earlier, to appear as if they were more fertile than those that became pregnant later during the first 21-day period. However, because of the large sample size, it was assumed that this random bias did not affect the usefulness of DTP.

The mean BUN concentration ± SD of 5.27 mmolL^{-1} ± 1.80 (14.76 mg/dl ± 5.04) obtained in this study was higher and more variable than the 3.17 mmolL^{-1} ± 0.11 in 8-month old Bonsmara steers raised on sweetveld, reported by Ndlovu et al. (2009). The difference in reported BUN concentration was most likely because steers in Ndlovu et al.'s (2009) study were raised on pasture and received no nitrogen supplementation. Previous studies have demonstrated a direct relationship between urea nitrogen concentration and the amount of dietary CP (Roseler et al., 1993; Chalupa and Sniffen, 1996; Schroeder and Titgemeyer, 2008).

BUN concentration showed a significant negative correlation with age ($P < 0.001$) and body mass ($P < 0.001$) in the combined data set, although the correlation was not present within all herds. The correlation with age is in agreement with findings of recent work, which showed that BUN concentration decreases with age (Doska et al., 2012) in cows but is contrary to findings from earlier work (Oltner et al., 1985). It is logical to assume that the correlation between BUN concentration and age is a weak one hence the conflicting results reported both in literature and in this study. BUN concentration did not show any significant correlations with BCS in the beef heifers of this study. In a study of lactating dairy cows, animals in a lower BCS were likely to have higher BUN concentrations (Ward, 2000; Guo et al., 2004; Tamminga, 2006), most likely because mature cows calving in a poor body condition have less adipose tissue available for milk production, leading to protein mobilisation to support gluconeogenesis.

It was expected that those heifers that were sampled on hot days with no access to drinking water would get dehydrated causing an increase in BUN concentration with increasing sampling order (Weeth and Lesperance, 1965; Burgos et al., 2001). However, this study found no association between BUN concentration and the sampling order except in Herd D where the heifers were managed as two separate groups. It is likely that the apparent linear trend between BUN concentration and sampling order was caused by differences in the way the two groups were managed (Figure 7.8).

8.2 Herd A

The mean BUN concentration ± SD of 5.37 $mmolL^{-1}$ ± 0.81 obtained from this herd was higher than the 2.20 $mmolL^{-1}$ ± 0.67 obtained in Herd B, which practised low levels of nitrogen supplementation. The reason for the high concentration cannot be explained by the level of dietary nitrogen supplementation at the time of sampling alone, because this herd was classified as having no supplementation of nitrogen (Section 6.1, page 19). Other farm specific factors likely caused the high BUN concentration. It is also thought that the energy lick that was supplemented in Herd B probably helped to reduce BUN concentrations, as reviewed in the literature section (Section 2.1, page 3).

The pregnancy proportion (71.3%) in this herd was lower than the benchmark for beef cow - calf operations (> 90% pregnancy rate) with a 62-day breeding season (Chenoweth and Sanderson, 2001). The reason for the lower pregnancy proportion in this herd is not clear, but is thought to be due to some heifers being immature at the onset of the breeding season and probably not cycling, considering that the age range at breeding was 14 to 24 months with a median breeding age of 18.60 months. The high number of DTP in this herd further strengthens the suspicion of immaturity at the onset of the breeding season.

This would also explain why RTS was the only significant predictor of pregnancy status and DTP, whilst age was close to significance in predicting DTP. The positive correlation between age and RTS also indicates that maturity of the reproductive tract was the main determinant of reproductive performance. This is in agreement with Holm et al. (2009), who observed that RTS was associated more strongly with age than with the body weight of the heifer.

However, it is important to note that only the month of birth was known and not the exact birth dates of the heifers. Using the 15th of the month as the birth date led to either overestimating or underestimating the age of heifers.

8.3 Herd B

The mean BUN concentration ± SD of 2.20 $mmolL^{-1}$ ± 0.67 obtained in this herd was lower than that of the first herd. This low BUN concentration could be due to heifer management. It is logical to assume that the production lick supplement that was provided in this herd resulted in lower BUN concentration by supplying the rumen microbes with fermentable carbohydrates leading to less ammonia and urea production (Ørskov, 1994; Chalupa and Sniffen, 1996; Schroeder and Titgemeyer, 2008).

The 100% pregnancy proportion obtained in this herd was probably because all the heifers were mature and cycling before the onset of the breeding season. The suspicion that most heifers were already cycling before the onset of the breeding season is strengthened by the low median DTP of 24 days. This suggests that most heifers became pregnant within the first oestrus cycle after the onset of the breeding season.

Since all the heifers became pregnant, it was not possible to perform logistic regression. Multivariable survival analysis was also not possible because none of the measured variables was a significant predictor of DTP. In this herd, as anticipated, there was a significant positive correlation between body mass and age because healthy growing heifers are expected to gain weight.

8.4 Herd C

The mean BUN concentration ± SD of 4.14 $mmolL^{-1}$ ± 0.92 obtained from this herd is higher than the 2.20 $mmolL^{-1}$ ± 0.67 seen in Herd B. This may be partly due to the moderate level of dietary nitrogen supplementation practised in Herd C. This finding is in agreement with that of other workers who found that increasing the levels of dietary CP leads to higher BUN concentration (Roseler et al., 1993; Chalupa and Sniffen, 1996; Schroeder and Titgemeyer, 2008).

Similar to Herd B, this herd had a 100% pregnancy proportion. It is believed that this was because all the heifers were mature and cycling before the onset of the breeding season.

This was confirmed by the median RTS of 5 obtained in this herd. It is likely that the heifers with a RTS of 3 were actually above 3 but they were scored down because they were not distinguishable from a score of 4 because their corpora lutea were not discernible by palpation. The suspicion that most heifers were already cycling before the onset of breeding is further strengthened by the fact that 22 out of 34 (64.7%) heifers became pregnant after the first synchronised artificial insemination. However, it is important to note that oestrus synchronisation and artificial insemination is known to affect both the pregnancy proportion and DTP (Xu and Burton, 1999). Although it seemed proper to exclude this herd from the study, because of its different management style, it was decided to retain it in order for the study to be relevant to commercial operations where the choice of management practices is driven by economics. Herd effects were controlled by adjusting for herd as the stratifying factor in the statistical models and thus prevented confounding.

Similar to Herd B, it was not possible to perform logistic regression because all heifers became pregnant. Multivariable survival analysis was also not possible because none of the measured variables was a significant predictor of DTP. As anticipated, there was a significant positive correlation between body mass and age because healthy growing heifers are expected to gain weight. BCS did not correlate with any other variable because there was no variation in BCS data in this herd.

8.5 Herd D

The mean BUN concentration ± SD of 4.50 $mmolL^{-1}$ ± 1.80 obtained from this herd was higher than that of Herd B. This is thought to be at least partly due to the moderate level of dietary nitrogen supplementation practised in this herd (Section 8.4, page 63). The two subgroups in this herd seem to be different (Figure 7.8), and this caused the overall mean not to be a good representation of the entire herd. It was anticipated that the larger group with a higher mean would determine the overall effect of BUN concentration on reproductive performance.

The pregnancy proportion (68.2%) was lower than the industry benchmark of more than 90% (Chenoweth Radostis 2001). It is thought that the high BUN concentration played a

significant role in determining the pregnancy proportion considering that the heifers in this herd were mature, as indicated by their age and RTS (Table 7.15). However, the BCS of these heifers were low compared to other herds, suggesting an energy deficiency. Other factors that might have played important roles in determining the overall pregnancy proportion were the sale of some study animals and the subgrouping that occurred in this herd. It is assumed that the animals in good condition (most likely to be pregnant) are the ones that were sold. This herd could have been excluded from the study because of the unexpected changes in the management of the heifers but the researcher decided to retain the herd and adjust for herd in the statistical models.

BUN concentration was the only predictor of pregnancy status and DTP in this herd. It is assumed that this was because all the heifers were mature, and the other measured variables ceased to be important in determining the reproductive outcome.

8.6 Herd E

In this herd, the mean BUN concentration ± SD for the heifers that were sampled on the second day was significantly lower ($P < 0.001$) than for those sampled on the first day (Table 7.20; Table 7.21). The reason for this is not clear but it is thought that this was due to reduced DMI of the group that was sampled on the second day after spending the previous day in the holding pen. Sampling day was forced into all regression models to account for potential confounding. The mean BUN concentration of 6.23 $mmolL^{-1}$ ± 1.74 obtained from this herd was higher than that obtained in Herd B. This is thought to be due to the high level of dietary nitrogen supplementation practised in this herd, as discussed for Herd C (Section 8.4, page 63).

This herd had a pregnancy proportion of 47.5% and median DTP of 44 days. BUN concentration, age and body mass were significant predictors of both pregnancy status and DTP. Age and body mass are thought to have been significant in this herd because some of the heifers in this herd were young with low body mass (Table 7.20; Table 7.21). This suggests that some had not attained puberty. Older heifers, and thus more mature, had a higher chance of becoming pregnant and at an increased rate. This is in agreement with the

work done by other researchers that have reported that heifers that mature and undergo a few complete oestrous cycles before the breeding season tend to have better reproductive performance (Yelich et al., 1996; Berry et al., 2003; Buckley et al., 2003; Whittier et al., 2008).

The significant positive correlation between mass and age was anticipated. The negative correlation that was seen on the second day of sampling between BUN concentration and age is in agreement with Doska et al. (2012), who suggested that BUN concentration is higher in young cows and tends to decrease with age. RTS was significantly correlated with age ($P = 0.004$) and BCS ($P < 0.001$). This is consistent with the view that age, BCS and frame size of the heifer has an influence on the RTS that is assigned (Rosenkrans and Hardin, 2003).

Although other studies (Patterson et al., 2000; Rosenkrans and Hardin, 2003; Holm et al., 2009) have shown that increasing RTS predicts reproductive performance, the reason for the failure of RTS to predict reproductive performance in this herd was likely due to a lack of variability in RTS data in this herd. Regarding RTS as categorical data through dichotomisation did not lead to different conclusions in the significance of its prediction of reproductive performance.

8.7 Effect of BUN concentration on reproductive performance

BUN concentration was a significant independent predictor of DTP (Table 7.6) but was not significant in predicting pregnancy status in all herds combined (Table 7.4). This suggests that high BUN concentration before the onset of the breeding season negatively affects the chances of becoming pregnant only for a short period. Another possibility is that some animals adapted to the increased BUN concentration as suggested by Calsamiglia et al. (2010), or their BUN levels decreased during the course of the breeding season. The latter option is highly possible because RDP supplementation was stopped in all herds at the beginning of the breeding season. The results of this study are in agreement with those of Guo et al. (2004), who showed that in among-herd analyses, MUN concentration had minimal effect on conception rate but was associated with greater days open. Ferguson et

al. (1993) also showed that within dairy herds with mean MUN concentrations above 20 mg/dL, cows with higher MUN levels were associated with poorer conception rates at first service, but not at subsequent services. The assumption that BUN concentration reduced the chances of becoming pregnant for a short period after the onset of the breeding season is consistent with the hypothesis that urea affects cleavage and blastocyst formation but not necessarily the early development of the oocyte (Jorritsma et al., 2003).

This is in contrast with the theory, which states that the negative effect of high BUN concentration is exerted through the exacerbation of an underlying NEB by the energy costs of detoxifying large quantities of ammonia in post-partal dairy cattle (Staples et al., 1990; Garcia-Bojalil et al., 1998; Overton et al., 1999). It is logical to assume that beef heifers will not suffer severely from NEB like lactating dairy cattle. In this study, BCS was used as an indicator of the animal's energy reserves. This is widely supported in literature for both beef and dairy cattle (Wildman et al., 1982; Edmonson et al., 1989; Houghton et al., 1990). However, BCS has been shown to have lower accuracy in young growing cows because growing animals tend to have less fat deposits (Nicholson and Butterworth, 1986). The heifers in this study were in a good BCS with little variation in the data. The lack of variation made it impossible to estimate the effect of BCS on reproductive performance. As recommended by Ndlovu et al. (2007) in their review, measuring of NEFAs would have been a more reliable way to assess the energy status of the heifers.

BUN concentration was only determined once prior to the onset of the breeding season. However, it is likely that BUN concentration in the heifers continued to vary during the breeding season because of dietary and environmental changes that occurred (Rodriguez et al., 1997; Godden et al., 2001; Schroeder and Titgemeyer, 2008). Nevertheless, since the environmental changes are likely to affect all the heifers in a herd in a similar way, and that urea concentration is genetically determined (Mitchell et al., 2005; Hossein-Zadeh and Ardalan, 2011), it is assumed that those heifers with a higher BUN concentration would remain high relative to the rest of the herd throughout the breeding season.

BUN concentration was a significant independent predictor of both DTP and pregnancy status only in Herds D and E (Table 8.1). These herds practised moderate to high levels of nitrogen supplementation in order to achieve early breeding.

Table 8.1: *Summary of the effect of BUN concentration on pregnancy status and DTP in the different herds*

						BUN as predictor of pregnancy		**BUN as predictor of DTP**
Herd	**n**	**Age Range (months)**	**Nitrogen supplement level**	**BUN ± SD**	PP (%)	Odds Ratio	DTP	Hazard Ratio
All	338	12 - 31		5.27 ± 1.80	63.1	0.882*	40	0.827**
A	106	14 - 24	None	5.37 ± 0.81	71.3	0.833	43	0.969
B	33	20 - 26	Low	2.20 ± 0.67	100.0	N.A.	24	1.113
C	34	24 - 27	Moderate	4.14 ± 0.92	100.0	N.A.	4	0.997
D	22	22 - 31	Moderate	4.50 ± 1.80	68.2	0.478**	47	0.719**
E	143	12 - 23	High	6.23 ± 1.74	47.5	0.656**	44	0.736**

* = *P* - value < 0.1
** = *P* - value < 0.05
DTP = median DTP
PP = pregnancy proportion
BUN = mean BUN concentration

In this study, those heifers that were fed high levels of nitrogen supplementation had the highest BUN concentrations and the lowest pregnancy proportion. Although the current study was not designed to investigate the causal relationship between nitrogen supplementation and BUN concentration, this finding is in agreement with the findings of other workers who demonstrated that high dietary NPN levels lead to high BUN concentration (Canfield et al., 1990; Kenny et al., 2002a). This is known to lead to a decrease in reproductive performance of cattle (Rhoads et al., 2006).

Variation in the genetic ability to recirculate nitrogen has been proposed, with animals with improved nitrogen recirculating ability having higher BUN levels (Schoeman, 1989). Feeding high levels of RDP is known to down regulate the efficiency of nitrogen recirculation (Marini and van Amburgh, 2003; Marini et al., 2004), leading to higher BUN concentration and renal loss. This suggests that those heifers with better abilities to recirculate nitrogen within herds that were over supplied with RDP probably lost their advantage in the presence of an oversupply of RDP. These heifers might have suffered more negative consequences from the effects of high BUN concentrations. Since BUN concentration affected reproductive performance only in herds where the mean BUN concentration was high (similar to findings by Ferguson et al. (1993)), one could reason that the potential exists that heifers with an improved ability to recirculate nitrogen within herds heavily supplemented with RDP are at risk of being culled for poor fertility in a restricted breeding system.

8.8 Potential weaknesses of the study

Due to unavailability of herds that met the study criteria, only 369 heifers were enrolled for the study instead of the calculated minimum sample size of 438. Of these, only 338 heifers finished the study.

The hydration status of the heifers in the study was not recorded. It is well known that the hydration status of ruminants has an influence on measured BUN concentration (Weeth and Lesperance, 1965; Utley et al., 1970; Mousa et al., 1983; Aganga et al., 1989; Maloiy et al., 2000). It was not practical to assess the hydration status of the heifers in this study as that would have increased the time spent on data collection, leading to more variation in the BUN concentration data (Gustafsson and Palmquist, 1993).

The CRL formula was accurate in estimating the stage of pregnancy when checked against the 20 recorded mating dates. Although this was a small number, the accuracy of the formula satisfied the researcher that it is useful for estimating the age of early pregnancies. According to Riding et al. (2008), this formula is known to be most accurate between day 36 and 103 of gestation. In the current study, some animals fell outside this range because the breeding season was longer than 67 days.

Time spent collecting additional data during BUN sampling potentially exacerbated the effect of sampling order in this study. There is evidence in the literature that MUN concentration in dairy cows is associated with the time of sampling in relation to feeding (Gustafsson and Palmquist, 1993), the hydration status and the DMI of the animal (Godden et al., 2001). Since BUN concentration is also known to vary with season and diurnal patterns, one should attempt to collect blood for BUN concentration analysis at the same time of the day and make all known factors as similar as possible (Wattiaux et al., 2005).

9 Conclusion

In this study, blood urea nitrogen (BUN) concentration at the start of breeding was independently associated with reproductive performance of Bonsmara heifers, especially in those herds where management included heavy supplementation of dietary protein to achieve early breeding.

It is recommended that production systems designed to achieve early breeding in beef heifers investigate whether oversupplying rumen degradable protein (RDP) selects against animals with an improved ability to recirculate nitrogen.

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