ABSTRACT

Title of thesis: EFFECTS OF MILK UREA NITROGEN AND OTHER FACTORS ON PROBABILITY OF CONCEPTION OF DAIRY COWS

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Present study was to evaluate the relationships of milk urea nitrogen (**MUN**) and other factors with the probability of conception in dairy cows. A total of 713 dairy herds and 10,271 dairy cows were included in the study. Logistic regression was used to determine the effects of MUN, milk production, lactation number, and breeding season on the probability of conception for each of three services. Within herds, MUN displayed a slight negative association with probability of conception at first service. In among-herd regression analysis, there was no effect of MUN on probability of conception. These results suggest that MUN may be related to conditions affecting reproduction of individual cows within a herd. Diet formulation usually would affect MUN equally among all cows at a similar stage of lactation in a herd. Since there was no effect of MUN among herds, diet formulation to meet the protein requirements did not appear to affect conception rate.

EFFECTS OF MILK UREA NITROGEN AND OTHER FACTORS ON PROBABILITY OF CONCEPTION OF DAIRY COWS

by

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LIST OF ABBREVIATIONS

BUN	blood urea nitrogen
CP	crude protein
CR	conception rate
DHIA	Dairy Herd Improvement Association
DIM	days in milk
DM	dry matter
DMI	dry matter intake
LH	luteinizing hormone
MUN	milk urea nitrogen
NEB	negative energy balance
NRC	National Research Council
PUN	plasma urea nitrogen
RDP	rumen degradable protein
RUP	rumen undegradable protein

Chapter 1

Common Non-Nutritional and Nutritional Factors Affecting Dairy Cow Reproductive Performance: Literature Review

Common Non-Nutritional and Nutritional Factors Affecting Dairy Cow Reproductive Performance: Literature Review

Reproductive Performance

Reproductive performance is one of the most important factors affecting dairy farm profitability, because it directly or indirectly influences the amount of milk produced, reproductive culling rate, and the cost for breeding and calf sales (Plaizier et al., 1997; 1998). In the past 20-25 years, genetic selection for high production traits and advances in management practice have dramatically increased average milk yield per cow; however, a decline in reproductive performance has also puzzled animal scientists (Butler and Smith, 1989).

When animals are under adverse conditions, reproduction is one of the physiologic functions that is often most expendable to maintain critical body functions (Bauman and Currie, 1980). Within a dairy farm, herd reproductive performance is often affected by non-nutritional factors, such as the environment, managerial factors, and nutritional factors.

Non-nutritional factors

Non-nutritional factors refer to the factors such as environmental changes and managerial practices that could adversely affect dairy herd reproductive performance. It has been repeatedly reported that environmental changes such as extreme temperatures (heat or cold) and natural photoperiod can affect dairy cow reproductive performance.

Environmental factors

Among all the environmental factors that affect dairy cow reproduction, temperature is the most important factor. The upper critical temperature for lactating cows can be as low as 25-27°C (Berman et al., 1985). Therefore, heat-stress not only occurs in tropical areas, but sometimes can occur in the temperate zone in summer.

Summer temperature above the thermoneutral zone can significantly reduce CR in dairy cows (Cavestany et al., 1985). Thatcher (1974) and Ray et al. (1992) reported that cows first bred in cool weather could have up to three times better CR than cows bred hot weather. Rajala-Schultz et al. (2001) also reported that cows calving in summer were least likely to conceive. Cows calving in cool weather had fewer services per conception than cows that calved in hot weather (Monty and Wolf, 1974). Cool weather 12 days prior to breeding or 4-6 days after AI was beneficial for reproductive performance (Monty and Wolf, 1974).

Scientists proposed many theories in order to explain the heat-stress effect on fertility. First of all, heat-stressed cows tend to have declined duration of estrus (Abilay et al, 1985), which could lead to reduction in estrus detection. Heat-stress is also believed to be responsible for lowered CR by disrupting the establishment of pregnancy, altering normal embryonic development and causing fertilization failure (Putney et al., 1989; Ealy et al., 1993; Monty and Racowsky, 1987). Heat-stress can also cause pregnancy failure by reducing progesterone concentration and increasing prostaglandin F_2 alpha and estradiol secretion (Howell et al., 1994; Malayer et al, 1990).

Reproductive Management

Reproductive management factors usually affect reproductive performance at the herd level. Estrus detection is one of the most important factors in reproductive management. Early detection of estrus can increase the probability of conception and shorten the calving interval. Accurate and thorough detection of estrus is essential to improve dairy herd reproductive performance (Van Horn and Wilcox, 1999).

Some other factors can negatively affect dairy herd reproductive performance, such as inferior artificial insemination skill, improper ratio of age or parity in a herd (i.e. too many young or old animals in a herd), and long voluntary waiting period etc.

Reproductive health problem is another important managemental factor that can impair dairy cow reproductive performance. During early lactation, reproductive illnesses (such as endometritis, delayed placenta and metritis etc.) and metabolic diseases (such as hypocalcemia and ketosis etc.) are common due to the dramatic physiological changes and negative energy balance (**NEB**). Therefore, the prevention of reproductive disease is also related to nutritional management.

Nutritional factors

Bauman and Currie (1980) described the nutrient partitioning priority for nutrient utilization by lactating dairy cows. Within their system, reproduction is ranked below maintenance, milk production and growth. Therefore, when an animal is nutritionally stressed, available nutrients might primarily be conserved for critical body functions, and reproduction may be delayed until the nutritional status is improved.

Energy

Energy is one of the first limiting nutrients in diet formulation. Energy balance, (i.e. the difference between dietary energy intake and the requirements for milk production, maintenance etc.) is an important index of an animal's energy status.

Negative energy balance is common in lactating dairy cows during early lactation, because milk production increases much more rapidly than the increase in dry matter intake and the energy intake often does not meet the requirement for accelerating milk production. As a result, body fat and tissues will be mobilized to compensate for the energy deficits (Nebel et al, 1993).

Negative energy balance can affect reproductive performance by increasing the period of postpartum anoestrus (Butler et al. 1981). It's important for cows to have multiple estruses prior to the breeding, because the CR of dairy cows was found positively associated with the number of estrus cycles pre-breeding (Butler and Smith, 1989). Therefore, prolonged interval to first ovulation could reduce length and number of ovulatory cycles, thus negatively affects CR at breeding.

The mechanisms of NEB effects on reproductive performance could be hormonal. Schillo (1992) indicated that NEB decreases luteinizing hormone (**LH**) pulse frequency by suppressing LH-releasing hormone and LH is one of the driving hormones for ovarian follicular development and ovulation. Evidence further indicates that about 10 days after NEB reaches its nadir (animal energy balance increasing afterwards) LH concentration and frequency increases and first ovulation can occur soon after in most of the cows (Canfield and Bulter, 1991). Spicer et al. (1990) indicated that NEB was associated with reduced insulin and insulin like growth factor (IGF-I), which are required for stimulating mitogenesis in bovine ovarian cells and normal follicle growth and ovulation (Spicer and Echternkamp, 1995). NEB may also affect fertility by reducing progesterone secretion. Decreased progesterone level may limit the luteal support for uterus during pregnancy, thus lower reproduction performance (Spicer et al, 1990; Villa-Godoy et al, 1988).

Dietary fat supplementation is a common practice to increase energy density in dairy rations. It has been considered beneficial to reproductive performance in dairy cows because cows can increase dietary energy intake without a large increase in DMI (Palmquist and Jenkins, 1980). However, the effect of dietary fat supplement on reproductive performance was mixed and depended on animal condition, the form of the fat added and the effects on milk production. Ferguson et al. (1990) reported that feeding prilled fat increased CR at first service at about 18% and slightly reduced days open without significant increase on milk production. Schneider et al. (1988) also observed an increase of CR in cows fed diets that supplemented with rumen inert fat. These studies indicated that supplementing dietary fat increased energy supply therefore improved dairy cow reproductive performance. On the other hand, fat supplementation may be accompanied by large increases in milk production, which diminished the beneficial effect of increased energy supply from diets and result in reduced CR and prolonged days open to first service (Lucy et al., 1992).

Protein

Protein metabolism can be directly related to the energy status of the cow. Because of the pre-gastric fermentation of ruminants, dietary protein can be divided into two fractions: rumen degradable protein (**RDP**) and rumen undegradable protein (**RUP**).

Rumen degradable protein provides a source of amino acids, ammonia and nucleic acid for rumen microbial protein synthesis. However, some of the ammonia can permeate through rumen wall and enter blood stream, and then is detoxified by conversion to urea in the liver. The efficiency of microbial protein synthesis (microbial protein synthesized divided by fermented energy) is determined by both dietary RDP and rumen carbohydrate availability. Deficient carbohydrate could increase the amount of ammonia that escapes into blood, thus increase blood urea and lower microbial protein synthesis. A small portion of RUP also joins the urea pool in the blood by catabolism in small intestine.

Currently most diet formulation for dairy cows is based on NRC (1989) model (Kohn et al., 1998), because current software has not been updated to the NRC 2001 model. Protein requirements are calculated according to animal body weight, milk production and physiological state. However, due to individual animal variation, accurate prediction of animal protein requirements is difficult. Therefore, in order to maintain high milk production, most of the high-producing cows are fed protein at greater level than recommended, thus resulting in elevated urea concentrations (Ferguson et al., 1993).

High protein diets containing 18% to 19% crude protein (**CP**) are recommended to support and stimulate high milk production in early lactation (NRC, 1989). Although high protein diets are important for high milk production, excessive protein has been reported to impair dairy-cow reproductive performance in many studies (Jordan and Swanson, 1979; Chalupa, 1989). Jordan and Swanson (1979) randomly assigned forty five high producing cows into three groups that fed three isocaloric diets with different level of CP (12.7, 16.3 or 19.3% DM). The results indicated that although high CP group (19.3%) displayed fewer days to first observed estrus, low to mid CP groups (12.7 or 16.3%) had fewer services per conception and fewer days open than the high CP group. Meta-analysis conducted by Ferguson and Chalupa (1989) indicated that excess protein intake may affect dairy cow reproduction through the toxic effect of ammonia and its metabolites on gametes and early embryos, and also by exacerbation of NEB.

Fertility has been reported to be affected by from both excessive RDP and RUP intake (Ferguson, 1989). Considering that RDP and RUP are digested and absorbed differently, the common intermediate in the metabolism is urea formation. Therefore, it is reasonable to speculate that blood or plasma urea and ammonia might be the common link for both RDP and RUP to be associated with dairy cow reproductive performance. Jordan et al. (1983) discovered that uterine urea concentration was positively associated with blood ammonia and urea concentration when animals were fed high CP diets. Studies also reported that excessive protein intake decreases uterine pH (Elrod et al., 1993; Elrod and Butler, 1993). These results may suggest that high dietary protein (including RDP and RUP) may affect fertility by altering the normal uterine environment and plasma progesterone concentration, which could be detrimental for embryo development. Jorritsma et al. (2003)'s review also suggested that exposure of oocytes in antral follicles to elevated ammonia or urea hampers cleavage and blastocyst formation, thus affects dairy cow reproduction.

The detrimental effect of excessive protein intake in early lactation could also be energy related. Negative energy balance, which is common in early lactation, was reported to suppress progesterone concentration (Spicer et al, 1990; Villa-Godoy et al, 1988). The NEB could be exacerbated by feeding excess protein because of the extra energy cost to detoxify and excrete urea, especially for RDP. Every gram of excess nitrogen from overfeeding CP can increase the energy requirements by 13.3 kcal of digestible energy (Butler, 1998; Tyrrell et al., 1970; NRC, 1989). Therefore, the negative effect of NEB on progesterone concentration may be worsened, which could reduce reproductive performance.

A living animal is a very complex system; factors affecting reproduction performance do not work in isolation. Nutritional and non-nutritional factors can interact with each other. For example, Carroll et al. (1988) and Barton et al. (1996) reported an interaction between high dietary protein intake and animal reproductive health problem. High CP diet tended to increase the days open when cows had major health problems, otherwise, it decreased days open.

In summary, the increase in milk production accompanied by a decline in fertility of dairy cows that has occurred over the past two decades may be caused by many factors. Further research is needed to better understand this subject and improve the reproductive performance of dairy cows.

Milk Urea Nitrogen (MUN)

Urea nitrogen is a byproduct of dairy cattle protein metabolism. Ammonia, which is toxic to the animal, is generated from dietary protein fermentation in the rumen and from body tissue protein catabolism, and is released into blood stream. The blood stream carries ammonia to the liver to be detoxified by converting it to urea. Urea is released back into blood stream and excreted proportionally from the body by the kidney through urine. Urea can easily diffuse from blood to milk; therefore MUN is highly correlated with blood urea nitrogen and plasma nitrogen (Jonker et al., 1998; Broderick and Clayton, 1997). Milk urea nitrogen is a normal component of milk and comprises about 20% to 75% of the milk non-protein nitrogen (DePeters and Ferguson, 1992).

Blood urea nitrogen (**BUN**) or MUN may vary under the influences of many different factors. Excessive protein intake is a common nutritional factor for elevated MUN (Jonker et al., 1998). Tyrrell et al. (1970) demonstrated the requirement of energy for the animal to excrete excess nitrogen as urea through urine. Therefore, if dietary intake is low in energy or high in protein to energy ratio, rumen bacteria will have reduced efficiency in utilizing free ammonia to synthesize protein, which can result in increased BUN or MUN (Broderick and Clayton, 1997; Hof et al. 1997; Rajala-Schultz and Saville, 2003). Similarly, cows under negative energy balance tend to have slightly higher urea concentration in milk, which could be associated with the increase of body protein mobilization (Schepers and Meijer, 1998).

Body weight or metabolic body weight have been reported to be negatively associated with MUN concentration by Oltner et al. (1985) and Jonker et al. (1998). The weight of blood of an animal is proportional to the animal’s body weight. Therefore, given same amount of urea in the blood, a large animal should have more diluted MUN concentration than a smaller animal (Oltner et al., 1985).

Blood urea nitrogen or plasma urea nitrogen may also be affected by diseases or medicines from various medical treatments (Vestweber et al., 1989). Any disease or body condition that reduces glomerular filtration such as dehydration, heart disease and renal disease or any condition that increases protein catabolism and/or reduces body fluid content can result in increased BUN level (Fraser, 1991).

Several DHIA laboratories provide MUN as a regular analysis while sampling milk. The results of MUN measurement can be a valuable indicator of nutritional status and health of a cow (Rajala-Schultz, 2001). MUN can also be used as a useful monitor of protein efficiency in dairy cows to help optimize dietary protein utilization efficiency (Baker et al., 1995; Hof et al, 1997; Schepers and Meijer, 1998).

Herd average MUN level can be useful to estimate nitrogen excreted from a group of cows. And in nitrogen balance experiments, MUN can be used to predict herd or animal urinary nitrogen excretion (Jonker et al, 1998). Milk urea nitrogen can also be used to estimate dry matter intake for high producing dairy cows under grazing conditions. Given MUN, milk yield, protein intake and pasture CP composition, DMI can be predicted (Jonker et al, 1998).

The current study used Bentley Chemspec Instrument to measure MUN. This laboratory method was modified from the Berthelot reaction, which was developed by Chaney and Marback (1962). Urea within a small amount of milk sample is split enzymatically by urease into ammonia and carbon dioxide. After sufficient incubation time, the solution is measured by a spectrophotometer for ammonia concentration, and MUN concentration can then be calculated.

In summary, MUN is a useful indicator of the animal nutritional (protein and energy specific) status. Monitoring dairy herd MUN level regularly can potentially improve animal nutritional management, reduce excessive protein intake and therefore reduce feed cost and reduce nitrogen load to the environment (Godden et al., 2000; Tamminga, 1992; Jonker et al., 1998, 2002).

MUN and Reproduction

In the past 10 to 15 years, scientists have done a lot of research on the relationship between MUN and reproductive performance in dairy cows. However, the studies have reported conflicting results. Many studies reported the negative relationship of blood urea nitrogen (BUN) or MUN with reproductive performance in dairy cows (Ferguson et al, 1989, 1993; Elrod and Bulter, 1993, Burke et al, 1997; Larson et al, 1997, Rajala-Schultz et al, 2001). Ferguson et al. (1993) indicated plasma urea nitrogen or serum urea nitrogen concentrations greater than a certain level were associated with decreased pregnancy rate in dairy cows. Rajala-Schultz et al (2001) analyzed the field data from 24 dairy herds using Cox proportional hazards model, and discovered that within-herd cows with MUN level less than 10 mg/dl were 2.4 times more likely, and cows with MUN level between 10 and 12.7 mg/dl were 1.4 times more likely, to be confirm pregnant than cows with MUN level over 15.4 mg/dl. Milk urea nitrogen level, which they discovered impaired dairy cow fertility was lower than in previous research.

However, the mechanism by which MUN affects reproductive performance is still unknown. Elrod and Butler (1993) examined uterine pH value of dairy cows feeding different levels of dietary protein. They found that high PUN concentration resulted in cows with high protein intake and also developed in lower uterine pH. The results suggested that high PUN, which may be associated with a decline in uterine pH, could render the environment within the uterus unsuitable for early embryo development. Larson et al (1997) found that non-pregnant cows with high MUN, which had long a inter-estrous interval, may be associated with a low-progesterone concentration in the blood. Melendez et al (2000) studied a total of 1073 cows from a commercial dairy farm located at north central Florida. They discovered that cows with high MUN (17-25 mg/dl) bred during summer were 18 times less likely to get pregnant than cows with low MUN (6-16 mg/dl) that were bred during winter.

On the other hand, some scientists did not find such negative effects of MUN on fertility of dairy cows (Godden et al, 2001). Howard et al. (1987) and Carroll et al., (1988) both compared the influences of two diets with different dietary protein concentrations (15% vs. 20% and 13% vs. 20%) on dairy cow reproductive performances. Cows in the high protein (20% CP) group had higher MUN; however no differences were found on days open, service per conception and CR as compared to the low protein group. Godden et al. (2001) found that the odds for pregnancy were the highest when the milk urea on the test day preceding the insemination was either below 4.5 mmol/l or greater than 6.49 mmol/L, compared with a concentration between 4.5 and 6.49 mmol/l for first, second and third service.

Therefore, studying the relationship between MUN and reproduction could help us further understand the complicated interactions among dietary protein, energy and health condition on dairy cow reproductive performance.

The present study, analyzed a large dataset (a total of 713 dairy herds, 10,271 cows) from Lancaster Dairy Herd Improvement Association, which enabled us to examine the within-herd and among-herd effects of MUN and some other factors, such as milk production, season and parity on reproductive performance of Pennsylvania dairy cows. It provided valuable new information by comparing MUN effects between dairy herds and individual cows.

Chapter 2

Effects of Milk Urea Nitrogen and Other Factors on Probability of Conception of Dairy Cows*

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INTRODUCTION

Milk urea nitrogen (**MUN**) is a byproduct of dairy cattle protein metabolism and a reflection of urinary nitrogen excretion (Jonker et al., 1998). It is highly correlated with blood urea nitrogen and plasma urea nitrogen. Excessive feeding of protein can lead to increased MUN concentration (DePeters and Ferguson, 1992; Broderick and Clayton, 1997). Milk urea nitrogen measurement is convenient and non-invasive, and several Dairy Herd Improvement Association (**DHIA**) laboratories provide MUN as a regular analysis while sampling milk.

Reproductive performance has a substantial impact on economic profitability of dairy farms (Mourits et al., 1997). Jorritsma et al. (2003) have reviewed the influences of metabolic changes during early lactation on reproductive performance. Nutrition management may be an important means to improve dairy cow reproductive performance (Ferguson and Chalupa, 1989). Several studies reported the negative effects of blood urea nitrogen or MUN on reproductive performance in dairy cows, and suggested that overfeeding CP caused reproductive stress (Ferguson et al., 1993; Rajala-Schultz et al., 2001). However, others did not find such negative effects of high MUN on fertility of cows (Carroll et al., 1988; Godden et al., 2001).

The previous studies were conducted with a small number of animals (<200 to 300) and herds (< 30), therefore our objective was to evaluate the associations of MUN and other factors on probability of conception of dairy cows based on data from a large number of herds.

MATERIALS AND METHODS

Data Collection

Data were retrieved from the Lancaster DHIA (Manheim, PA) for herds in Pennsylvania. Cows that were first bred between June 1, 2000 and May 31, 2001 were included in the study. A total of 10,271 cows from 713 herds were selected (from a total of 44,090 cows and 1066 herds) where data on milk production, MUN, pregnancy status and breeding date were available. Milk urea nitrogen values were measured using Bentley Chemspec Instrument, which is based on a modified Berthelot reaction (Chaney and Marback, 1962) to detect ammonia after urea hydrolysis (Bentley Instruments, Chaska, MN).

The average days open to first service was 91 days, the interval between first and second service was 60 days, and the interval between second and third service was 49 days (Table 1). Therefore we used MUN and milk production data from 60 to 90 days post calving for regression against the probability of conception at first service. By the same token, we used MUN and milk production from 120 to 150 days, and 170 to 200 days, after calving for regression against probability of conception at second service and third service respectively. We chose not to use the MUN value at breeding because typically MUN value increases and then declines during lactation (Jonker et al., 1998). Thus, higher MUN at breeding may be confounded with fewer days in milk at breeding, and therefore reduced probability on conception.

The time range for the study was divided into four seasons: winter (December, January, and February), spring (March April and May), summer (June, July, and August), and fall (September, October, and November).

Statistical Analysis

Within-herd Logistic Regression Analyses. The probability of conception at each service was analyzed separately using Logistic Regression of PROC GENMOD of SAS (2000). The full model is listed as follows:

$$Ln\left(\frac{p}{1-p}\right) = I + H + L + S + N + M + LS + LN + LM + SN + SM + NM$$
$$+ LSN + LSM + LMN + SMN + LSMN + N^2 + M^2 + LN^2 + LM^2$$

where, p is the probability of conception for a cow.

I = intercept of the model;

H = random effect of herd;

L = fixed effect of the lactation number;

S = fixed effect of season;

N = MUN at 60-90, 120-150 and 170-200 days post calving for first service, second and third service respectively;

M = daily milk production at 60-90, 120-150 and 170-200 days post calving for first service, second and third service respectively;

A GEE analysis in PROC GENMOD was used to account for correlation among cows within a herd. All quantitative variables were centered to reduce multicollinearity (SAS 2000). Insignificant (P>0.05) terms were removed by stepwise elimination. Logistic regression fits the logit of the probability of conception to a linear model of factors.

Among-herds analyses. In order to analyze the among-herd effects, means for each herd were computed for conception rate (**CR**), MUN (60-90 days, 120-150 days and 170-

200 days), milk production (60-90 days, 120-150 days and 170-200 days) and lactation number at first, second and third services. Medians were used for days open because they were not normally distributed and sometimes cows were culled prior to a next service. Herds with data from fewer than three cows were dropped. A total of 506 herds comprised of 9810 cows were included in the analysis.

Conception rate and days open were analyzed using multiple regression model of JMP (2000) according to the following model:

$$Y = I + L + N + N^2 + M + M^2 + LN + LM + NM + LN^2 + LM^2 + LNM + e$$

where,

Y= CR or median Days open to first, second or third service,

I = intercept of the model;

L = fixed effect of the lactation number;

N = fixed effect MUN at 60-90, 120-150 and 170-200 days post calving for first service, second and third service respectively;

M = fixed effect of daily milk production at 60-90, 120-150 and 170-200 days post calving for first service, second and third service respectively;

e = error term.

Partial F tests were used to select the variables that were significantly related to conception rate and days open at first, second and third service respectively.

RESULTS

Among all the cows included in the study, a mean of 2.8 services were required for pregnancy. The overall CR's were 31%, 33% and 35% for first, second and third services respectively (Table 2.1).

Within-herd Analysis

Among cows within herds, the effects on probability of conception at first to third service are shown in tables 2 to 4 respectively. There was a negative association of MUN with CR at first service (Fig. 2.1) but not in subsequent services. For example, a change in MUN from 9 to 18 mg/dl resulted in a 2.2 or 4.4 percentage unit change in conception rate at first service for low-producing cows bred in spring and high-producing cows bred in fall respectively (Fig. 2.1). Within herds, there was a negative association of milk production with probability of conception at all three services (Table 2.2 to 2.4 and Fig. 2.2). Seasonal effects were significant for all three services with higher CR in the spring (Table 2.2 to 2.4 and Fig. 2.1). There was an interaction of lactation number by season by milk production for first service (Fig 2.3.)

Among-herd Analyses

Among herds, the main linear effects on probability of conception at first service were not significant (P>0.05); but there was a positive quadratic effect of milk production. Lactation number by milk production and lactation number by MUN interactions were also significant (Table 2.5). For the second service, only milk production had a negative impact on CR (Table 2.6). There was a quadratic effect of milk production on CR at third service (Table 2.7).

In regression analysis on days open to first service (Table 2.8), lactation number and MUN had no effect (P>0.05), however, milk production had a negative linear effect on days open and a positive quadratic effect. There were lactation number by MUN and milk production by MUN interactions (Fig. 2.4). For the second service, milk production had a positive quadratic effect on days open (Table 2.9). No significant effects were found at third service.

DISCUSSION

MUN Effect

Jorritsma et al. (2003) have reviewed the influences of urea and ammonia on reproduction during early lactation. Larson et al (1997) found that non-pregnant cows with low-progesterone postbreeding were often associated with high MUN. Elrod and Butler (1993) suggested that high MUN may be associated with a decrease in uterine pH, which could make the environment within the uterus unsuitable for early embryo development. Previous research has also shown that cows within herds with high MUN were associated with reduced probability of conception at first service, but not subsequent services (Ferguson et al., 1993). In this study, we saw a negative effect of MUN on conception rate at first service among cows within herds, but no such effects were found at second and third service. In among herd analyses, MUN had minimal effect on conception rate, but was associated with greater days open among high-producing herds. These results agree with the hypothesis that urea affects cleavage and blastocyst formation and not necessarily early oocyte development (Jorritsma et al, 2003).

High MUN may be caused by many factors. Excessive protein intake is a common nutritional factor (Jonker et al., 1998). Blood urea nitrogen or plasma urea nitrogen which is the origin of MUN, may also be affected by diseases or medicines from treatments (Vestweber et al., 1989). Any disease or body condition that reduces glomerular filtration such as dehydration, heart disease and renal disease or any condition that increases protein catabolism can result in increased blood urea nitrogen level (Fraser, 1991). In this geographic region, most herds (75%) are fed a single diet (Jonker et al., 2002), and certainly most cows within a herd are fed the same diet during the same stage of lactation (e.g. 60-90 days post partum). Therefore, ration formulation is likely to affect MUN equally among all cows in the herd at a similar stage of lactation. On the other hand, any number of factors including health or energy balance can affect MUN among individual cows within a herd (Collard et al., 2000; Stockham and Scott, 2002).

In the present study, we detected a negative association of MUN with CR at first service among cows within herds. This implies that the within-herd negative association of MUN with probability of conception during early lactation could relate to the status or condition of individual cows. Negative energy balance and illnesses are common during 60 to 90 days post calving (Waltner et al., 1993; Collard et al., 2000). Some illnesses may result in higher MUN or BUN as well as reproductive problems (Finco et al. 1997; Stockham and Scott, 2002). When cows are at second or third service, increased energy supply may reduce the stress from milk production. As body condition improves, the incidence of illness may be less likely and the relationship between MUN and CR would disappear.

Diet formulation is usually similar for all animals in a similar stage of lactation within a herd. Therefore, among-herd effects are likely to reflect diet differences. Among herds, CR was largely unaffected by MUN, although there was a significant negative interaction of MUN and lactation number for CR at first service. The magnitude of this effect was negligible. Thus, diet formulation appeared to have a minimal effect on CR.

Although diet formulation did not appear to affect CR, it may have been associated with days open at first service. Figure 4 shows that herds with high milk production (>40 kg) from 60 to 90 d postpartum had increased days open at first service, especially when those herds also had high MUN. These high-producing herds may have delayed estrus due to negative energy balance caused by high milk production. The high MUN associated with this effect may have resulted from high-protein diets exacerbating the negative energy balance due to the energy required to excrete nitrogen (Tyrrell et al., 1970). This leaves open the possibility that feeding high protein diets can affect reproduction by increasing the days open at first service.

Milk Production Effect

The negative effect of milk production on conception rate has long been recognized (Spalding et al., 1975). In the present study, high milk production of individual cows within herds was associated with reduced probability of conception at all three services (Fig. 2.2, Table 2.2 to 2.4). During early lactation, dietary energy intake does not meet energy requirements for increasing milk production. As a result, body fat is mobilized.

High producing dairy cows have more severe negative energy balance, which was shown to reduce progesterone secretion and the luteal support for the uterus during pregnancy, and thereby lower the CR (Spicer et al., 1990; Villa-Godoy et al., 1988).

Herds that are well managed can maintain reproduction even in the face of high milk production. Among herds, the positive quadratic association of milk production with CR at first and third service may be due to the fact that herds with effective reproduction programs can have higher culling rates and shorter calving intervals. The contrasting negative association of milk production with CR at second service may be due to the reduced CR from the stress of higher milk production.

This interplay of milk production and reproduction among herds is also apparent for days open. In this study, number of days open was positively associated with milk production among herds with cows averaging more than 45 kg per day during first and second service. Negative energy balance that results from high milk production can delay the estrous cycle therefore prolonging days open (Butler and Smith, 1989). Producers with high-producing cows may choose to delay breeding compared with other producers. However, at lower levels of milk production, number of days open was negatively associated with milk production at first and second service. This latter observation is consistent with Laben et al. (1982) who indicated that on average the highest yielding herds had 21 fewer days open than the low-producing herds. Better reproduction enables greater culling rates and thus higher milk production.

Lactation Number Effect

Gwazdauskas et al. (1975) indicated that the reproductive performance (CR) decreased as cows grew older. Ray et al (1992) found that first and sixth lactation cows had highest number of services per conception and lactation 2 to 5 had better reproductive performance. In the current study, lactation number had a near significant positive effect and strong interaction with breeding season and milk production on CR among cows within herds at first service. Conception rate was lowest among first lactation cows, and increased as cows were more mature at second lactation. However, after second lactation, the effect of lactation number varied greatly under the influences of milk production and breeding season (Fig. 2.3). Similar interaction with milk production can also be found in within-herd analysis at second service and among-herd analyses. This implies that lactation number, as an indicator of maturity might not be the primary factor affecting reproductive performance of dairy cows; however it might be important to consider while analyzing other factors, such as milk production and breading season.

Seasonal Effect

The reproductive performance of dairy cows fluctuated throughout the year. Logistic regression indicated that cows first bred in winter and spring had much higher CR than cows bred in summer and fall. Previous research (Thatcher, 1974; Ray et al., 1992) reported similar results. Rajala-Schultz et al. (2001) also reported that cows calving in summer were least likely to conceive.

Climatic temperature change is associated with fertility (Thatcher, 1974). High temperature in the summer above the thermoneutral zone could significantly reduce CR in dairy cows (Cavestany et al., 1985). Monty and Wolf (1974) indicated that cows calving in cool weather had fewer services per conception than cows that calved in hot weather. Cool weather 12 days prior to breeding or 4-6 days after AI was beneficial for reproductive performance (Monty and Wolf, 1974).

The results of this study agree with previous studies (Thatcher, 1974; Cavestany et al., 1985) that in early lactation (first service), cows bred in relatively hot weather (summer and fall) had lower CR's than those bred in cooler weather (winter and spring). However, cows at second and third service during summer and fall did not show the same negative effects on CR as at first service. At second or third service, cows may have been less influenced by the stress of negative energy balance as they gained sufficient energy to cope with the environmental changes. Therefore, high temperature in summer or fall did not have the same negative effect on probability of conception.

CONCLUSION

Within herd, milk production and MUN (60-90 days after calving) had negative effects on conception rate of dairy cows at first service. Among herds, MUN interacted with milk production and lactation number on days open to first service. The results suggest that the negative effect of MUN on reproduction relates to the status or condition of individual cows. High MUN among herds, which might result from diet formulation, was not associated with reduced conception rates, but was associated with a slight increase in days open to first service in high producing herds.

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TABLE 2.1**.** Distributions of variables

Variables	N	Mean	SD
Cows per herd	713	14.4	
Milk production at 60-90 days (kg/d)	10,271	38.1	10.5
MUN at 60-90 days (mg/dl)	10,271	13.4	3.9
Lactation Number	10,271	2.41	1.5
Service number of current pregnancy	10,271	2.77	2.0
Days to first service (d)	10,271	90.6	53.7
Interval between first & second service (d)	6581	59.9	48.2
Interval between second & third service (d)	4138	49.0	34.3
Calving interval (d)	9174	451.3	99.9
Days open (d)	10,271	177	103.1
Average conception rate at first service	10,271	31%	
Average conception rate at second service	6581	33%	
Average conception rate at third service	4138	35%	
305-ME Milk (kg)	10,271	23,785	6654

1. Milk production & MUN values were test-day measurements at 60-90 days after calving (within 30 days before the first service).

TABLE 2.2. Within herd logistic regression for probability of conception at first service in dairy cows (713 herds, 10,271 cows).

Factor	Estimates	SE	*P*<
Intercept	-1.028	0.08	0.0001
Lactation #	0.053	0.03	0.06
MUN[1]	-0.020	0.008	0.01
Milk production (Milk)[1]	-0.021	0.005	0.0001
Season			
Winter	0	0	
Spring	0.66	0.06	0.0001
Summer	-0.28	0.1	0.02
Fall	-0.36	0.1	0.001
Lactation # * Season			
Lactation # * Winter	0	0	
Lactation # * Spring	-0.056	0.03	0.05
Lactation # * Summer	0.018	0.07	0.1
Lactation # * Fall	-0.091	0.05	0.8
Lactation # * Milk	-0.0002	0.003	0.9
Season * Milk			
Winter * Milk production	0	0	
Spring * Milk production	0.012	0.001	0.03
Summer * Milk production	0.016	0.01	0.2
Fall * Milk production	-0.010	0.01	0.3
Lactation # * Season * Milk			
Lactation # * Winter * Milk	0	0	
Lactation # * Spring * Milk	-0.002	0.004	0.6
Lactation # * Summer * Milk	-0.028	0.006	0.0001
Lactation # * Fall * Milk	-0.0008	0.005	0.2

1. Milk production and MUN were the test-day measurements at 60-90 days after calving.
2. Seasonal effects were estimated by using winter as the baseline.

Table 2.3. Within herd logistic regression for probability of conception at second service in dairy cows (496 herds, 6358 cows).

Factor	Estimates	SE	*P*<
Intercept	-1.002	0.07	0.0001
Lactation #	-0.007	0.03	0.8
Milk production (Milk)[1]	-0.022	0.008	0.005
Milk*Milk	0.003	0.0002	0.3
Season			
Winter	0	0	
Spring	0.668	0.08	0.0001
Summer	0.434	0.09	0.0001
Fall	-0.040	0.1	0.7
Milk * Season			
Milk * Winter	0	0	
Milk * Spring	0.013	0.009	0.1
Milk * Summer	0.005	0.01	0.6
Milk * Fall	0.029	0.01	0.02
Lactation # * Milk	0.0002	0.002	0.9
Lactation # * Milk*Milk	-0.0003	0.0002	0.04

1. Milk production was the test-day measurements at 120-150 days after calving.
2. Seasonal effects were estimated by using winter as the baseline.

Table 2.4. Within herd logistic regression for probability of conception at third service in dairy cows (460 herds, 4138 cows).

Factor	Estimates	SE	*P*<
Intercept	-0.927	0.08	0.0001
Milk Production[1]	-0.017	0.004	0.001
Season			
Winter	0	0	
Spring	0.559	0.09	0.001
Summer	0.334	0.1	0.001
Fall	0.653	0.1	0.001

1. Milk production was the test-day measurements at 170-200 days after calving.
2. Seasonal effects were estimated by using winter as the baseline.

Table 2.5. Among herd regression on conception rate at first service (506 herds, 9810 cows).

Term	Estimate	Std Error	*P*<
Intercept	0.40	0.01	0.0001
Milk production (Milk) [1]	-0.001	0.002	0.5
MUN[1]	0.005	0.005	0.3
Lactation #	0.02	0.02	0.3
Milk*Milk	0.0005	0.0001	0.004
Lactation # * Milk	0.007	0.003	0.006
Lactation # * MUN	-0.013	0.005	0.02

1. Milk production and MUN was the test-day measurements at 60-90 days after calving.

Table 2.6. Among herd regression on conception rate at second service (305 herds, 5737 cows).

Term	Estimate	Std Error	*P*<
Intercept	0.504	0.01	0.0001
Milk production [1]	-0.005	0.002	0.02

1. Milk production was the test-day measurements at 120-150 days after calving.

Table 2.7. Among herd regression on conception rate at third service (207 herds, 2840 cows)

Term	Estimate	Std Error	*P*<
Intercept	0.577	0.02	0.0001
Milk production (Milk) [1]	-0.004	0.003	0.1
Milk*Milk	0.0006	0.0003	0.03

1. Milk production was the test-day measurements at 170-200 days after calving

Table 2.8. Among herd regression on days open to the first service (506 herds, 9810 cows)

Term	Estimate	Std Error	P<
Intercept	84.0	1.4	0.0001
Milk production (Milk) [1]	-0.410	0.2	0.05
MUN[1]	0.218	0.5	0.7
Lactation #	-0.145	0.7	0.9
Milk*Milk	0.057	0.02	0.003
Milk * MUN	0.153	0.07	0.02
Lactation # * MUN	-1.191	0.5	0.03

1. Milk production and MUN were the test-day measurements at 60-90 days after calving

Table 2.9. Among herd regression on days open to the second service (305 herds, 5737 cows).

Term	Estimate	Std Error	P<
Intercept	135.2	2.6	0.0001
Milk production (Milk)[1]	-0.369	0.4	0.3
Milk*Milk	0.087	0.03	0.01

1. Milk production was the test-day measurements at 120-150 days after calving

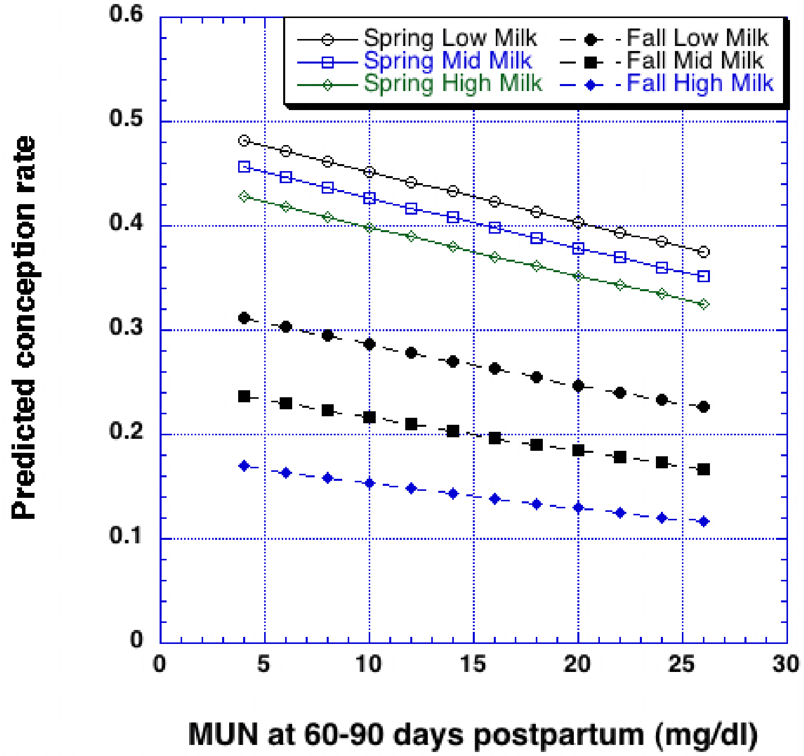

Figure 2.1. The within-herd MUN effect and interaction of milk production and season (spring and fall) on conception rate at first service. (Lactation = 2, Low Milk = 25.5 kg, Mid Milk = 37.7 kg, High Milk = 51.4 kg; Low, Mid and High milk are the milk production of lower 10%, median and upper 10% of all the observations respectively; Probability of conception was calculated from the logistic regression model; non-parallel lines do not necessarily represent interactions.)

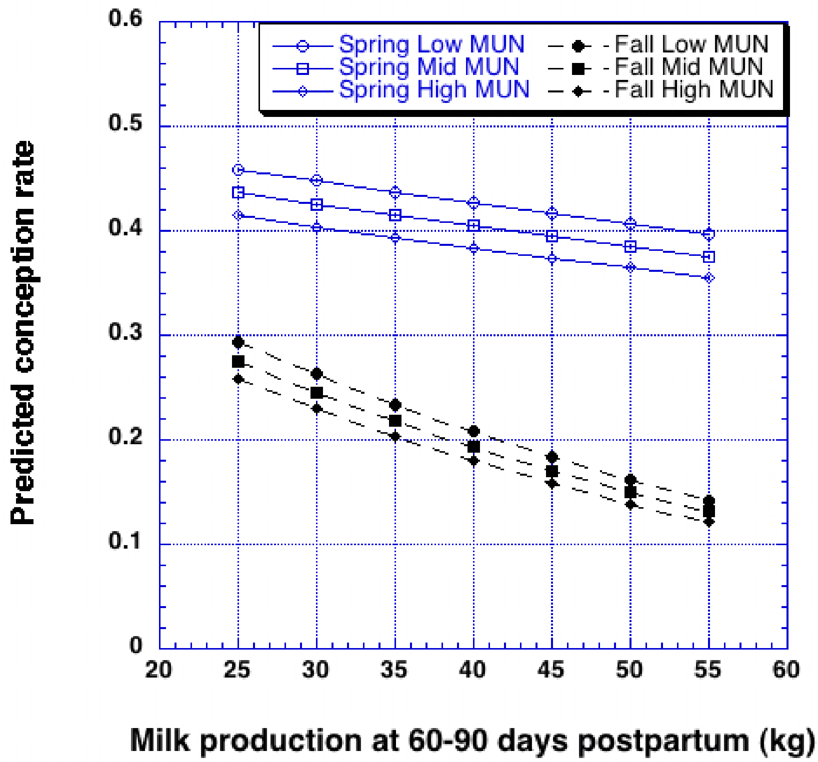

Figure 2.2 The within-herd MUN effect and the interaction milk production and season (spring and fall) on conception rate at first service. (Lactation = 2, Low MUN = 9 mg/dl, Mid MUN = 13.5 mg/dl, High MUN = 18 mg/dl; Low, Mid and High MUN are MUN of lower 10%, median and upper 10% of all the observations respectively.)

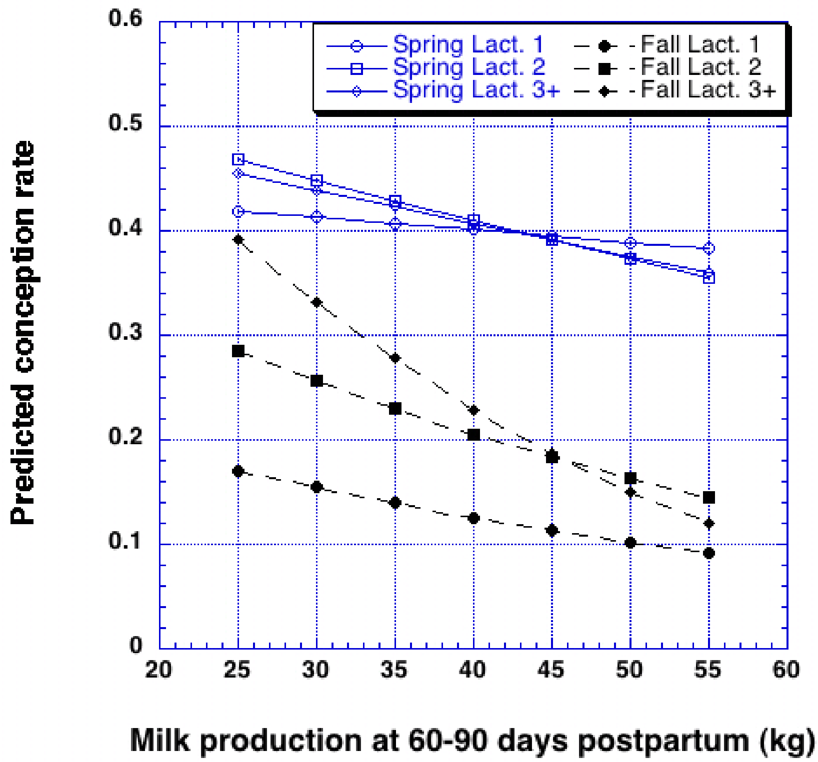

Figure 2.3. The within-herd milk production by lactation number by season (spring and fall) interaction on conception rate at first service.

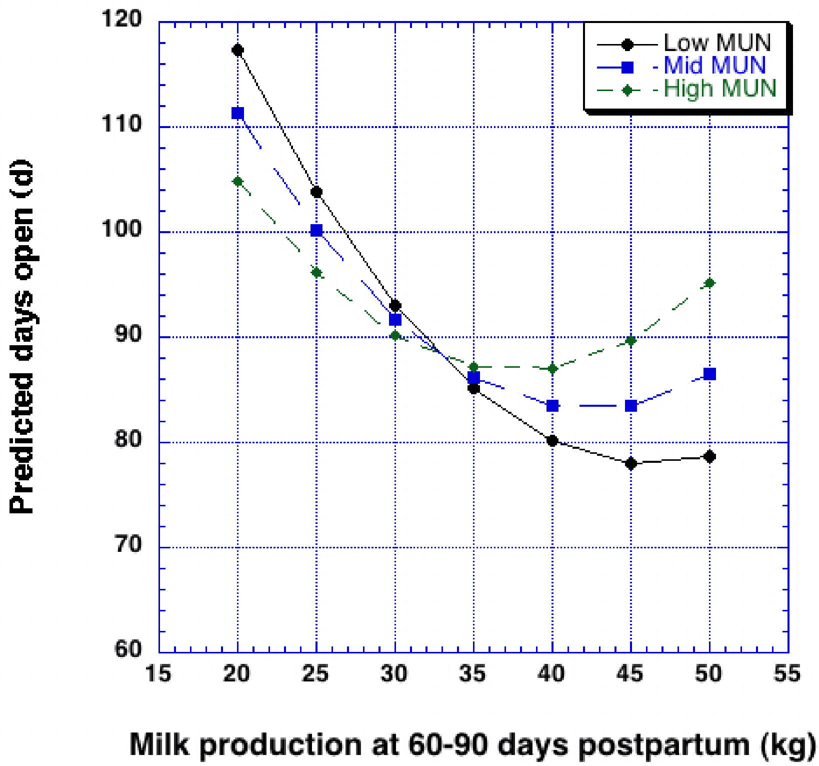

Figure 2.4. Among-herd interactions of milk production and MUN on days open at first service. (Low MUN = 10.7 mg/dl, Mid MUN = 13.7 mg/dl, High MUN = 17 mg/dl; Low, Mid and High MUN are MUN of lower 10%, median and upper 10% of all observations respectively.)

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