Association between Plasma and Milk Urea on the Insemination Day and Pregnancy Rate in Early Lactation Dairy Cows

Research Article

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ABSTRACT

Lactating dairy cows (n=177) fed with grass and corn silage *ad libitum* kept in pasture, were randomly assigned to evaluate how urea nitrogen in plasma and milk can be related to their pregnancy rate. Blood and milk samples were collected on the artificial insemination (AI) day to evaluate plasma urea nitrogen (PUN) and milk urea nitrogen (MUN) as well as progesterone levels, excluding cows with progesterone higher than 0.5 ng/mL. Cows were considered pregnant if six weeks after artificial insemination did not return to estrus. Concentrations of PUN or MUN greater than the average (16 mg/dL) were associated with decreased pregnancy rates (13% and 14%, respectively) (P<0.05) compared with the cows with urea levels less than this value on the insemination day. As PUN and MUN increased to greater than 16 mg/dL, the likelihood ratio for pregnancy decreased. There was a high correlation between PUN and MUN concentrations (r^2= 0.97, P≤0.001). The results of this study indicate that an increase in PUN or MUN can exert direct or indirect effects in reproduction, impairing the conception of grazing dairy cows.

KEY WORDS dairy cattle, milk urea nitrogen, plasma urea nitrogen, pregnancy rate.

INTRODUCTION

Over the last decades, declining fertility among dairy cows has been associated with nutritional strategies to increase milk production. Many studies (Blanchard *et al.* 1990; Canfield *et al.* 1990; Elrod and Butler, 1993; Ferguson *et al.* 1993; Butler *et al.* 1996), but not all (Howard *et al.* 1987; Carrol *et al.* 1988; Barton *et al.* 1996) have concluded that feeding dairy cows with large amounts of degradable protein, excess of requirements, increases blood urea which can reduce pregnancy rates per insemination. Besides the mechanism by which feeding large amounts of protein affects fertility is not completely known, De Wit *et al.* (2001) speculated that high concentrations of urea associated with excess feeding of crude protein can disrupt oocyte growth or maturation, fertilization or further development. Feeding large amounts of protein can also alter the uterine environment by reducing concentrations of magnesium, potassium and phosphorus in uterine secretions (Jordan *et al.* 1983) and by reducing uterine pH (Elrod and Butler, 1993).

A review published by Ferguson and Chalupa (1989) on protein intake and reproduction in dairy cows has been useful in identifying several mechanisms that underlie the discrepancies in effects on reproductive performance observed among various studies such dietary protein fractions (rumen-degradable protein, RDP and rumen undegradable protein, RUP). Excess RDP may exacerbate negative energy balance during early lactation and, thereby, reducing fertility levels. In relating dietary protein degradability to fertility, Ferguson *et al.* (1988) and Ferguson *et al.* (1993) reported that blood urea nitrogen (BUN) concentrations

exceeding 20 mg/dL were associated with reduced conception rates in lactating cows. Overall, Ocon and Hansen (2003) showed that the proportion of oocytes that developed to blastocysts was reduced by the addition of 21 mg/dl of urea to maturation conditions.

Studies previously developed by Borba (1992) showed that in some periods of the year the amount of nitrogen used to fertilize grass in the region is too high which in some situations can even be responsible for cattle mortality. Therefore, the aim of the present study was to evaluate the correlation between plasma urea nitrogen (PUN) and milk urea nitrogen (MUN) concentrations immediately after artificial insemination (AI) and the pregnancy rates at first breeding in a commercial dairy herd of Holstein-Friesian cows in the Azores, fed mainly with fresh grass. Plasma progesterone was measured on the AI's day to determine if the cow was inseminated on the right moment.

MATERIALS AND METHODS

A total of 177 lactating Holstein-Friesian dairy cows were used in the present experiment. On the day of AI, blood samples were taken from the coccygeal vein into heparinised and evacuated tubes for latter analysis of urea and progesterone, also milk samples were taken in sterility cups at the same time. Blood samples for progesterone analysis were centrifuged at 1,200 x g for 15 min. and plasma aliquots were frozen at -20 ºC until analysis. Milk samples were centrifuged at 1,120 x g for 20 min. The fat layer was aspirated and aliquots of supernatant were frozen at -20 ºC until the time of analysis.

Plasma and milk concentration of urea were determined using an autoanalizer (Cobas Integra; Roche Diagnostics, Rotkreuz, Switzerland) as previously described by Kenny et al. (2002). The method was based on two reactions: first, the hydrolysis of urea with urease and second, the reaction of the resulting ammonia with 2-oxoglutarate in the presence of glutamate dehydrogenase and NADH to form L-glutamayte and NAD^+. The amount of NADH oxidized was considered directly proportional to the amount of urea present.

Progesterone levels on the day of AI was determined using an automated quantitative test (VIDAS instruments, BioMérieux, France), by means of the ELFA technique (Enzyme Linked Fluorescent Assay), as previously described by Sauer et al. (1986). Cows were considered pregnant if six weeks after AI, did not return to estrus.

To relate pregnancy result to PUN and MUN, chi-square analysis (SPSS 14.0 Chicago, Illinois, USA) was used to analyse pregnancy rates for dairy cows with PUN and MUN concentrations greater than and less than the mean of the sample population. To discriminate the relationship between PUN/MUN and fertility rate of a cow data were categorized into incremental ranges (3 mg/dL) for calculation of pregnancy rate likelihood ratios (Ferguson et al. 1993). The likelihood ratio is expressed as the probability of finding in a PUN/MUN group a cow which is pregnant divided by the probability of finding in the same PUN/MUN group a cow which is not pregnant. Mean concentrations of MUN/PUN at AI for pregnant and non pregnant dairy cows were compared with Student's t-test. Cows which progesterone levels were higher than 0.5 ng/mL were not considered in the present study.

RESULTS AND DISCUSSION

The average PUN concentrations in lactating cows (n=177) was of 15.7 ± 0.35 mg/dL on the day of AI. The pregnancy rate of cows with PUN concentrations greater than the average was less than that for cows with PUN less than the mean (13% different, P<0.05, Figure 1). The relationship between PUN and pregnancy rate was discriminated further with likelihood ratio test based on increments of 3 mg/dl of PUN (Table 1).
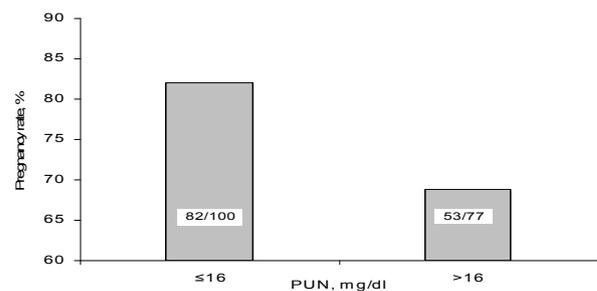

Figure 1 The relationship of plasma urea nitrogen (PUN) to pregnancy rate at artificial insemination (AI) in lactating cows (n=177). Pregnancy rate was significantly reduced (P<0.05) in cows with PUN≥16 mg/dl. The number of pregnant cows is indicated within each category

Table 1 Pregnancy rate (PR) likelihood ratios for dairy cows (n=177) categorized by plasma urea nitrogen (PUN) concentration on the day of AI (n=177)

PUN (mg/dL)	Cows, (n)	PR (%)	Likelihood ratio[a]
< 16	100	82	1.08
16 – 18.9	34	75	0.92
19 – 21.9	26	65	0.45
22 – 24.9	12	58	0.33
≥ 25	5	24	0.16

[a] Percentage of cows pregnant divided by the percentage of cows not pregnant.

As PUN increased to greater than 16 mg/dl, the likelihood ratio for pregnancy decreased.

The average MUN concentrations in lactating cows (n=170) was of 15.8 ± 0.31 mg/dL (n=170) on the day of AI. For cows later determined to be pregnant (n=127) and

non pregnant (n=43), MUN concentrations were different (P<0.05, 15.4 ± 0.34 and 16.9 ± 0.76 mg/dL, respectively). The overall relationship between MUN and pregnancy rate was discriminated using likelihood ratio test based on increments of 3 mg/dL of MUN (Table 2).

Table 2 Pregnancy rate (PR) likelihood ratios for dairy cows (n=170) categorized by milk urea nitrogen (MUN) concentration on the day of artificial insemination (AI).

MUN (mg/dL)	Cows n	PR (%)	Likelihood ratio [a]
< 16	94	81	1.02
16 – 18.9	40	72	0.64
19 – 21.9	24	67	0.48
22 – 24.9	8	50	0.24
≥ 25	4	50	0.24

[a] Percentage of cows pregnant divided by the percentage of cows not pregnant.

As MUN increased to greater than 16 mg/dL, pregnancy decreased clearly and likewise to the results for PUN. The pregnancy rate of cows with MUN concentrations higher than 16 mg/dL was lower (14% difference, P<0.05) than in cows with less MUN (Figure 2).
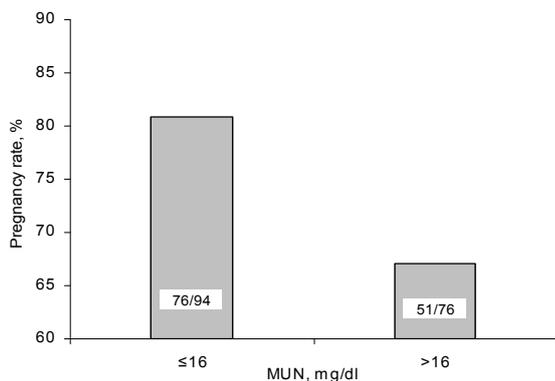

Figure 2 The relationship of milk urea nitrogen (MUN) to pregnancy rate at artificial insemination (AI) in lactating cows (n=170). Pregnancy rate was significantly reduced (P<0.05) in cows with MUN≥16 mg/dL. The number of pregnant cows is indicated within each category

High dietary protein intake resulting in high concentrations of urea nitrogen, in plasma and milk, have been associated with decreased fertility in dairy cattle (Jordan and Swanson, 1979; Kaim *et al.* 1983; Blanchard *et al.* 1990; Canfield *et al.* 1990; Ferguson *et al.* 1993; Butler *et al.* 1996).

In the present work, PUN concentrations greater than 16 mg/dl were associated with a decrease of pregnancy rate. The PUN and MUN concentrations measured in the same cows (n=164) were not different and were significantly (P<0.001; r^2=0.97) correlated (16.1±0.3 and 15.8±0.3

mg/dL, respectively). The equation describing the relationship between MUN (y) and PUN (x) was: y= 0.76 (x) + 6.3; r^2=0.98, showing that milk is a well-liked target for urea analysis. Our results are in agreement with those previously published by Oltner and Wiktorsson (1983) in which using only 8 cows and sampling blood 3 hours after feeding reported a correlation of 0.98. Nevertheless, our results are substantially higher than those obtained by Roseler *et al.* (1993) when they characterized the relationship of MUN to PUN using 15 cows sampled for PUN 4 hours after feeding obtaining a r^2=0.79 for PUN and MUN. Using data from 35 trials involving 482 cows fed 106 diets over a 15-year period, Broderick and Clayton (1997) reported a relationship between PUN and MUN with r^2=0.84. Differences between r^2 can be explained by the time which milk and blood was sampled after feeding. As known, significant feed intakes influence urea patterns in body fluids (Gustafsson and Palmquist, 1993; Geerts *et al.* 2004; Duinkerken *et al.* 2005)

The observed decrease in fertility may be a consequence of toxic effects of urea on the oocyte or the early embryo (Ferguson and Chalupa, 1989). The precise mechanisms are however unknown. However, it is known that stress due to exposure of preimplantation embryos to excess ammonium may also occur *in vivo*, particularly in domestic ruminants. This occurs when animals consume excess rapidly degradable nitrogen in the absence of readily fermentable carbohydrates (e.g. as found in spring pasture) or when feed is supplemented with excess urea (McEvoy *et al.* 1997). Urea is hydrolysed by the urease activity of rumen microorganisms, resulting in the production of ammonium (McDonald *et al.* 1995). In this case, the rumen microflora cannot maximise microbial protein synthesis from dietary nitrogen, urea or ammonium.

Furthermore, dietary protein may also be deaminated and used as a microbial energy source, thereby, releasing even larger amounts of ammonium into the circulation and increasing the risk of toxicity before it is converted to urea and removed by the kidneys (Papadopoulos *et al.* 2001). Effects on fertility are particularly evident when such dietary changes are implemented around the time of mating or insemination (Dawuda *et al.* 2002). Despite having no effect on ovulation rate (Fahey *et al.* 2001), elevated systemic urea adversely affects oocytes and/or the follicular environment, and leads to reduced embryo development and quality in terms of disrupted blastocyst metabolism, possibly through alterations in reproductive tract pH (McEvoy *et al.* 1997; Hammon *et al.* 2000; Papadopoulos *et al.* 2001). This may affect embryos in the long-term through reprogramming during the earliest stages of embryo development (McEvoy *et al.* 1997; Kwong *et al.* 2000). The mechanism by which early embryos dispose off ammonium has been

little investigated. Partridge and Leese (1996) and Donnay et al. (1999) suggested that pyruvate, after transamination to alanine, may potentially be used as an ammonium sink, thereby preventing the build-up of ammonium ions in the culture medium.

Some authors (Jordan *et al.* 1983; Elrod and Butler, 1993; Rhoads *et al.* 2004; Rhoads *et al.* 2006) proposed that greatest changes in the uterine environment occur during the mid-luteal phase, a critical period for early embryo development that ultimately determines long-term embryo survival.

In addition, PUN levels appear to influence ovarian follicular development and ovulation, oocyte quality and fertilization, embryo transport and development; maternal recognition of pregnancy (Blanchard *et al.* 1990).

Moreover, some toxic effect related to protein metabolism may interfere at one or more steps to impair fertility. An imbalance in the protein/energy relationship can also lead to a negative energy balance reducing the fertility by this way (Elrod and Butler, 1993; Butler *et al.* 1996).

The results of the present study demonstrated that urea nitrogen concentrations greater than 16 mg/dL in plasma or milk are associated with decreased pregnancy rate in grazing dairy cattle.

Therefore, it may be advantageous for milk producers to monitor urea concentrations in their herds in the effort to maintain or improve reproductive efficiency.

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