Relationships of Dietary Protein and Fertility

W. Ronald Butler

Department of Animal Science, Cornell University, 203 Morrison Hall, Ithaca, NY 14853
Email: wrb2@cornell.edu

Take Home Message

- Feeding high dietary protein to lactating cows has been associated with decreased fertility.
- Feeding high protein diets increases urea and ammonia levels in ovarian follicles and the uterus. High urea concentrations exert direct effects on the uterine luminal environment.
- The combined effect of elevated urea and negative energy balance impairs the viability of embryos in lactating cows and contributes to lower fertility.

Introduction

Dietary strategies for meeting the nutritional requirements of high producing dairy cows have been adjusted in response to genetic gains in milk yield. Diets high in crude protein (CP; 17 to 19%) are typically fed during early lactation to both stimulate and support high milk production. Although high dietary protein stimulates milk production, high protein has often been associated with decreased reproductive performance (Butler, 1998; Westwood et al., 1998; Laven and Drew, 1999). Previous reviews on protein intake and reproduction have identified several mechanisms that underlie the discrepancies in effects on reproductive performance observed among various studies:

- Dietary protein fractions of rumen degradable protein (RDP) and rumen undegradable protein (RUP) fed at optimum ratios relative to requirements must be considered rather than simply using crude protein %.
- The effects of negative energy balance and excess protein interact during early lactation.

Conception and establishment of pregnancy are an ordered progression of interrelated events and processes within the reproductive tract: follicular development resulting in ovulation, fertilization of the oocyte, embryo transport and development, maternal recognition, and implantation. Hypothetically, ammonia, urea, or some other toxic product of protein metabolism may intercede at one or more of these steps to impair reproductive efficiency. This paper focuses on the detrimental effects of high dietary protein that may be exerted in the ovary and uterus.

Monitoring Protein Metabolism

The biological value of dietary protein for the lactating dairy cow is directly related to the energy status of the cow and the balance of absorbed amino acids relative to their requirements. In lactating cows, dietary CP comprises rumen RDP and RUP fractions. Through normal ruminal fermentation, RDP provides a source of ammonia for microbial protein synthesis. Some of the ammonia escapes incorporation by the microorganisms, diffuses out of the rumen into portal blood, and is detoxified in the liver by conversion to urea. The quantity of rumen ammonia produced and the amount that escapes for conversion to urea directly reflects both dietary RDP and the availability of fermentable carbohydrates in the rumen to support microbial growth and protein synthesis.

A second source of urea produced by the liver is from deamination and metabolism of amino acids. Circulating amino acids originate from RUP, microbial protein, and body protein stores. Amino acids not taken up for utilization in milk protein synthesis are deaminated by the liver to yield energy substrates and urea. Although the production of ammonia and urea can be minimized by balancing RDP and RUP, high dietary intake to support milk production and variation in rumen microbial protein yield make accurate prediction of the availability of amino acids very difficult. Consequently, most high producing cows consume protein in excess of requirements, and blood urea concentrations are increased.

Urea circulating in the bloodstream is measured as urea nitrogen in either plasma (PUN) or serum fractions and is often referred to generically as blood urea nitrogen (BUN). Typically, BUN peaks about 4 to 6 h after meals because of RDP catabolism, while metabolism of RUP contributes to BUN continuously throughout the day. Urea passes easily from blood into the milk within the mammary gland. Milk urea nitrogen (MUN) provides a rapid, noninvasive, and less expensive means of assessing BUN and of monitoring overall protein metabolism in lactating cows.

Measurements of PUN or MUN have provided a useful index for studying the association between metabolism of dietary protein and reproductive efficiency. Across many studies increased PUN or MUN concentrations were correlated with decreased fertility in dairy cows both in confinement and grazing herds (Butler, 1998; Westwood et al., 1998; Laven and Drew, 1999; Wittwer et al., 1999). Pregnancy rates were decreased by about 20% when PUN or MUN was >19 mg/dl (Butler et al., 1996). Most recently a large field study suggested that pregnancy rate is reduced at even lower MUN levels (>15.4 mg/dl; Rajala-Schultz et al., 2001).

▪ Dietary Protein and Associated Changes in Ovarian Follicular Fluid

In a recent study, ovarian follicular fluid was sampled at estrus and day 7 of the estrous cycle in early lactation cows (Hammon et al., 2004). The cows were divided into two groups based on PUN concentrations (≥ 20 *vs.* < 20 mg/dl). Both urea and ammonia were elevated in follicular fluid from the high PUN cows. These results confirm a previous study in heifers that were fed diets to generate high rumen ammonia and resulted in elevated ammonia levels in follicular fluid (Sinclair et al., 2000). Oocytes collected from heifers fed high ammonia generating diets had lower cleavage rates compared to heifers fed diets generating low ammonia.

▪ Linkages Between Protein Metabolism and the Uterine Environment

Successful development of the embryo during early pregnancy depends upon the uterine environment. The uterine luminal environment is dynamic and exhibits marked secretory differences across the estrous cycle due to ovarian steroid regulation. During early pregnancy the local signaling from the blastocyst further modifies the milieu and induces secretion of specific proteins by the uterine epithelium.

The effects of dietary CP on constituents of uterine secretions have been examined in high producing dairy cows at various stages of the estrous cycle (Jordan et al., 1983). BUN and urea in uterine fluid were higher for cows fed 23% CP than in cows fed 12% CP. High protein intake altered the concentrations of minerals in uterine secretions, but only during the luteal phase and not at estrus. Differential effects of high dietary protein on uterine luminal pH were also observed across the estrous cycle (Elrod and Butler, 1993; Elrod et al., 1993). Uterine pH normally increases from about 6.8 at estrus up to 7.1 on d 7 of the estrous cycle (luteal phase), but this increase failed to occur in both heifers and lactating cows fed excess RDP or RUP (high PUN).

In a recent study, uterine pH and PUN concentrations were inversely related across animals during 36 hours of observation (Figure 1). The sequential measurement of PUN and uterine pH demonstrated that uterine pH is dynamic and changes in response to PUN, given a time lag of several hours (Figure 2). Continuous intravenous infusion of urea to lactating cows significantly reduced uterine pH after 12 h (Rhoads et al., 2004). These results strongly suggest that PUN concentrations throughout the range of 12 to 24 mg/dl can exert direct effects on uterine secretion.

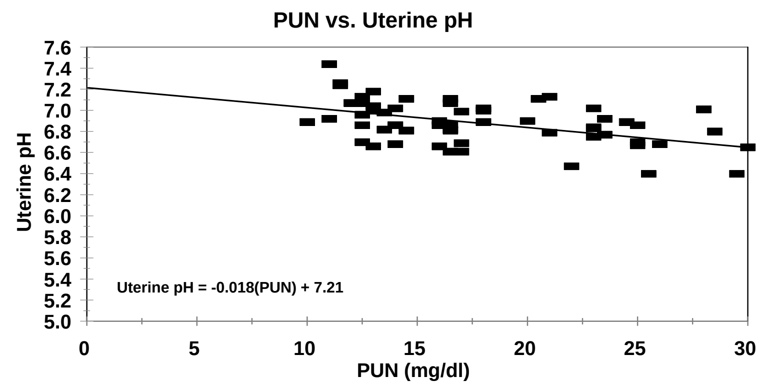

Figure 1. Uterine pH varied inversely with PUN in lactating dairy cows (n=8) over 36 hours of sequential measurements at 6-hour intervals.

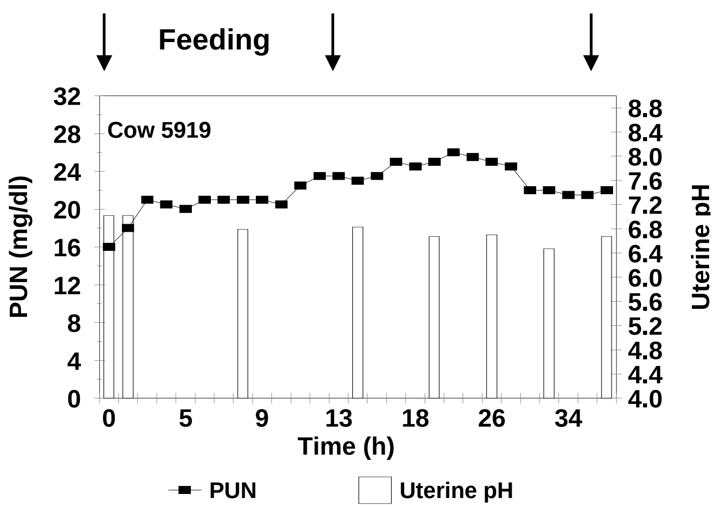

Figure 2. Uterine pH and PUN measured sequentially in a lactating cow over 36 h.

In addition to urea, ammonia has also been found to be elevated in the uterine fluid in dairy cows with high PUN (Hammon et al., 2004). The high levels of ammonia probably come from local metabolism and utilization of the amino acid, glutamine, for energy rather than diffusion from the blood stream. Ammonia inside the uterine lumen increases during the luteal phase and could further add to the negative effects of urea on pH and other secretions. Results from all these studies suggest that increased urea from feeding high dietary protein may reduce fertility by interfering with the normal inductive effects of progesterone on the uterine microenvironment, thereby providing suboptimal conditions for the support of embryo development (Butler, 2001).

▪ Embryo Development

The effects of high protein intake on early embryo development have been studied in dairy cows. Lactating cows (40-60 days postpartum) were fed diets resulting in either moderate or high PUN (19 mg/dl) and embryos were flushed and recovered 7 days after superovulation and artificial insemination (AI) (Bode et al., 2001). Embryos were transferred to recipient dairy heifers also fed diets for either low or high PUN, but positive energy status. Pregnancy rates were lower in recipients after transfer of embryos recovered from high PUN cows vs. moderate PUN cows (11 vs. 35 %; Figure 3). These results indicate that detrimental effects of high PUN occur before day 7 after AI and may be directed at oocyte development in ovarian follicles or aspects of embryo development and transport through the oviduct. Recent *in vitro* studies have demonstrated that urea acts during oocyte maturation and not after fertilization to impede embryo development (De Wit et al., 2001; Ocon and Hansen, 2003). Urea levels used were the same as those that reduce fertility in cows (Butler et al., 1996; Bode et al., 2001). Urea also advances blastocyst hatching (Roland and Butler, unpublished; Figure 4) that could result in asynchrony between the embryo and uterine environment. In addition elevated ammonia in the uterine lumen of cows fed high dietary protein may directly impair embryo development (Hammon et al., 2000; 2004).

Our embryo transfer results also showed that pregnancy rate was not affected by high PUN in recipients when good quality embryos were transferred to heifers in positive energy balance (*i.e.,* PUN effect in donors, but not recipients; Figure 3). Perhaps the uterine environment is made sensitive to the detrimental effects of urea described above by the predisposing effects of negative energy balance (NEBAL). This interpretation would explain why embryo transfer and superovulation experiments in beef heifers found no detrimental effect of high dietary CP and urea on fertilization rate, embryo quality, or embryo viability (Gath et al., 1999; Kenny et al., 2002). Furthermore, since the energy balance status of the embryo donor dairy and beef cattle were also different, the effects of high dietary protein in lactating dairy cows may be to exacerbate metabolic or hormonal mediated processes related to NEBAL that would result in impaired embryo development. For example, NEBAL during the early postpartum period may exert residual effects during the 40 to

60 d required for follicular development that may impair the health of oocytes later during the breeding period (Britt, 1991; Lucy et al., 1992; Sartori et al., 2002), in addition to which some aspect of protein metabolism would further compromise successful embryo development. Indeed, increased embryo losses were reported for dairy heifers that were fed an energy restricted diet containing high amounts of RDP (Elrod and Butler, 1993). Early degeneration and poor development of embryos also occurred in lactating dairy cows fed excess RDP (Blanchard et al., 1990), but a similar, well-controlled study in nonlactating cows found that excess intake of CP failed to affect the health and number of embryos (Garcia-Bojalil et al., 1994). Because the ongoing energy status of the dairy cows in each of these studies was markedly different depending on whether or not they were lactating, the combined effects of excess RDP and energy status may explain why embryo quality of lactating cows was compromised (Blanchard et al., 1990; Bode et al., 2001, Sartori et al., 2002). Additional research on the interactions of NEBAL and dietary protein metabolism that may impact embryo developmental processes should aid our understanding of poor fertility in high producing dairy cows. In regard to future research, it is interesting that higher plasma progesterone levels ameliorate some of the adverse effects of PUN on fertility after AI in dairy cows (Butler, 2001; Figure 5). This observation is in keeping with the idea that detrimental carry-over effects of NEBAL such as lower plasma progesterone may increase the sensitivity of the uterus to urea.

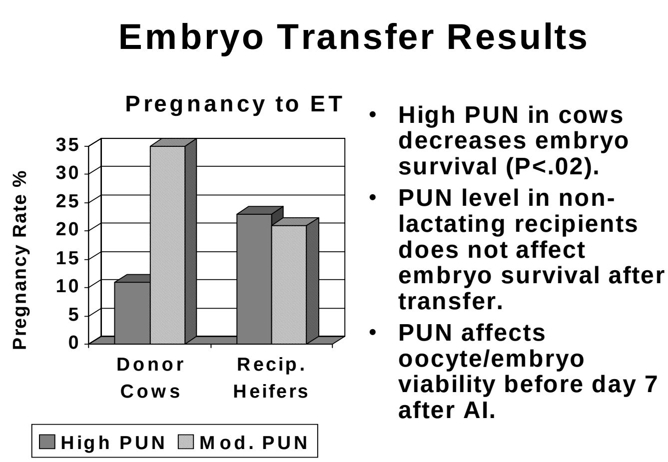

Figure 3. The effect of PUN levels in donor lactating cows or recipient heifers on pregnancy rate after embryo transfer.

Effects of urea on bovine embryos

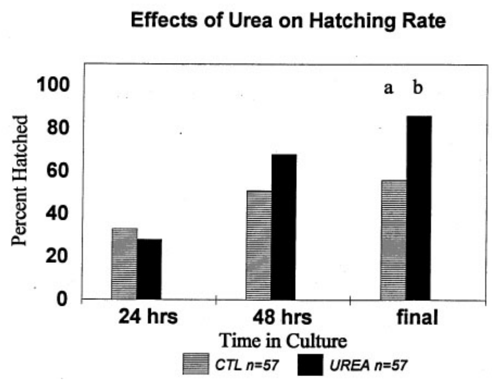

Figure 4. The effect of urea on hatching rate of bovine blastocysts in culture.

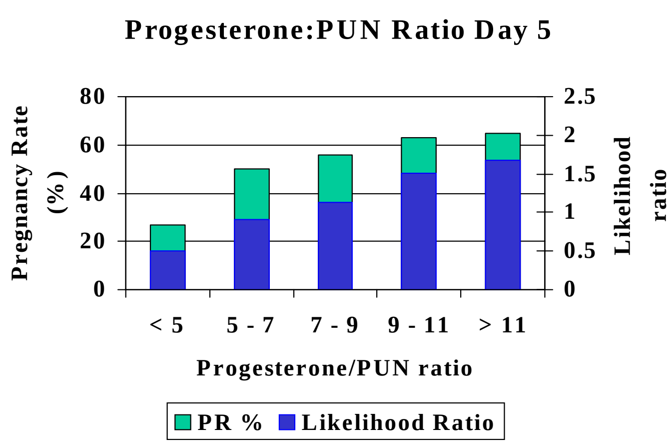

Figure 5. The positive relationship between the plasma Progesterone:PUN ratio at day 5 post-AI on pregnancy rate and likelihood ratio in lactating dairy cows.

▪ Feeding Strategies Related to Dietary Protein and Fertility

When prepartum diets contain higher levels of protein than that recommended by NRC, there are usually improvements in metabolic status of the cows. This type of response should be beneficial to early postpartum ovarian activity and subsequent fertility. Feeding diets containing 14-16% crude protein seems most effective in maintaining or increasing prepartum dry matter intake (DMI) (VandeHaar et al., 1999; Phillips et al., 2003). Considering carry-over effects on postpartum DMI and reduced accumulation of triglycerides by the liver, feeding prepartum diets with higher energy density (1.6-1.65 Mcal NE_L/kg) seems more important than high % crude protein (VandeHaar et al., 1999; Doepel et al., 2002). This may be the case because our knowledge of amino acid requirements, especially for branched chains, under a range of nutrient intakes is still inadequate.

Feeding high producing cows to support peak lactation requires high protein diets. Since it remains difficult to predict specific amino acid balances based on diet composition, diets will necessarily be formulated with somewhat of an excess of total protein. However, carefully formulating diets according to NRC guidelines for RDP and RUP as well as energy density, should minimize urea and ammonia concentrations in the ovary and uterus to reduce the negative effects on fertility.

▪ Summary

Overall, the interactions of nutrition on reproductive performance in dairy cattle involve the most important dietary components, energy and protein, and their adequacy relative to requirements for high milk yield. The observed decline in fertility may be attributed to the combined effects of a uterine environment that is dependent on progesterone, but has been rendered suboptimal for embryo development by antecedent effects of negative energy balance and can be further compromised by the effects of urea or ammonia resulting from intake of high dietary protein.

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