SYMPOSIUM: OPTIMIZING PROTEIN NUTRITION FOR REPRODUCTION AND LACTATION

Review: Effect of Protein Nutrition on Ovarian and Uterine Physiology in Dairy Cattle

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ABSTRACT

Milk production and dry matter intake of dairy cows are stimulated in response to increased intake of dietary protein, but, unfortunately, decreased fertility is often associated with this nutritional strategy. Ruminally degradable protein or ruminally undegradable protein in excess of requirement can contribute to reduced fertility in lactating cows. Dietary protein nutrition or utilization and the associated effects on ovarian or uterine physiology have been monitored with urea nitrogen in plasma or milk; concentrations above 19 mg/dl have been associated with altered uterine pH and reduced fertility in dairy cows. The uterine pH changed dynamically and inversely with plasma urea nitrogen, signaling possible changes in the uterine milieu. Mechanisms for reduced fertility include exacerbation of negative energy balance and reduced plasma progesterone concentrations when cows were fed rations that were high in ruminally degradable intake protein. Alternatively, changes in uterine secretions that are associated with high protein intake and elevated plasma urea nitrogen might be detrimental to embryos. Bovine endometrial cells in culture respond directly to increasing urea concentrations with alteration in pH gradient but respond most notably with increased secretion of prostaglandin F$_{2\alpha}$ (PGF$_{2\alpha}$). Increased uterine luminal PGF$_{2\alpha}$ interferes with embryo development and survival in cows, thus providing a plausible link between elevated plasma urea nitrogen concentrations and decreased fertility. Poor fertility in high producing dairy cows reflects the combined effects of a uterine environment that is dependent on progesterone and rendered suboptimum by the antecedent effects of negative energy balance or postpartum health problems and that is further compromised by the effects of urea resulting from intake of high dietary protein.

(Key words: protein, uterus, urea, progesterone)

Abbreviation key: BUN = blood urea nitrogen, MUN = milk urea nitrogen, PUN = plasma urea nitrogen, SUN = serum urea nitrogen.

INTRODUCTION

Increased genetic potential for milk production has been associated with a decline in fertility of lactating dairy cows [(6); Figure 1]. Strategies for meeting the nutritional requirements of high producing cows has necessarily changed in conjunction with genetic gains. Diets that are high in protein (17 to 19% CP) both support and stimulate high milk production in early lactation(20, 26, 32). Currently, the NRC (30) recommends that diets contain 18 to 19% CP for high producing cows. Although high dietary protein stimulates milk production, increased protein has been found to be detrimental to reproductive performance in most, but not all, studies (15, 38). Concerns about high dietary protein and impaired fertility are not new (23, 25, 35) but have become investigated more intensively in the last decade.

Previous reviews (15, 38) on protein intake and reproduction in dairy cows have been useful in identifying several mechanisms that underlie the discrepancies in effects on reproductive performance observed among various studies:

1. Dietary protein fractions (RDP and RUP) fed at optimum ratios relative to requirements must be considered rather than simply using CP percentage.
2. Any detrimental effects of high dietary protein may be confounded with or exacerbate reproductive health problems (1, 10).
3. Excess RDP may exacerbate negative energy balance during early lactation and thereby reduce fertility.
Conception and the establishment of pregnancy are an ordered progression of interrelated events involving all of the various tissues of 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 review focuses on the detrimental effects of high dietary protein that may be exerted on the physiological mechanisms of the ovary and uterus.

MONITORING PROTEIN METABOLISM

The biological value of protein substrates 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 protein comprises RDP and RUP fractions. Through normal ruminal fermentation, RDP provides a source of ammonia for microbial protein synthesis. Some of the ammonia that is produced escapes incorporation by the microorganisms, diffuses out of the rumen into the portal blood, and is detoxified in the liver by conversion to urea. The quantity of ammonia that is produced and the amount that escapes for conversion to urea directly reflects both dietary RDP and the availability of fermentable carbohydrates to support microbial growth and protein synthesis.

A second source of urea produced by the liver is from the deamination and metabolism of amino acids. Circulating amino acids originate from RUP, microbial protein, and body stores. Amino acids such as glutamine and arginine are important interorgan transporters of ammonia nitrogen in a nontoxic form. These and other available amino acids that are not taken up for utilization in milk protein synthesis or deposited elsewhere are deaminated by the liver to yield energy substrates and urea. As the urea that is produced by the liver circulates through the blood, it equilibrates into all tissues, is recycled through the rumen, and is excreted in urine. 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 the requirements, and urea concentrations are increased in accordance with intake (16). Urea is the metabolic end product of protein catabolism in the body and is easily measured by the nitrogen content (i.e., urea nitrogen concentration).

Urea nitrogen concentrations circulating in the bloodstream are measured in either plasma or serum fractions (PUN or SUN, respectively) and are often referred to generically as blood urea nitrogen (BUN). Typically, BUN peaks about 4 to 6 h after meals because of RDP catabolism, and the metabolism of RUP contributes to BUN continuously throughout the day (13). The fluctuations in BUN during the day are usually smaller (2 to 3 mg/100 dl) in cows fed a TMR than in cows fed concentrates and forages separately (11, 13, 21). Urea is a small, water-soluble molecule that permeates all cells and tissues in the body and passes easily between the blood and milk within the mammary gland (21). With a lag time of less than 1 h for equilibration with blood concentrations, milk urea nitrogen (MUN) provides a rapid, noninvasive, and inexpensive means of assessing the dynamics of BUN (21, 31) and of monitoring overall protein metabolism in lactating cows (33). Also, MUN provides an integrated measure of metabolism and utilization of RDP and RUP, reflecting both intake and energy availability (8, 9, 31, 33).

Measurements of BUN or MUN have provided a useful index for studying the association between metabolism of dietary protein and reproductive efficiency. Conception rate decreased when SUN concentrations exceeded 20 mg/dl on the day of insemination, suggesting that degradation of excessive amounts of dietary protein in the rumen contributed to infertility (14). A more detailed field trial (16) with nine commercial dairy herds confirmed the inverse relationship of high SUN on conception rate and emphasized the detrimental effects of fertility when SUN exceeded 20 mg/dl. More recently, both PUN

Figure 1. The inverse relationship between conception rate (CR) and annual milk production of Holstein dairy cows in New York.
and MUN concentrations were used to monitor pregnancy rate, and the range of urea nitrogen concentrations in high producing cows and the inverse relationship between PUN (>19 mg/dl) and fertility were both confirmed (5). Most reports (38) have found that elevated BUN concentrations are associated with some deficiency in the reproductive performance of cows.

**OVARIAN CYCLES AND CIRCULATING PROGESTERONE CONCENTRATIONS**

The intake of high CP diets by lactating cows following parturition has had inconsistent effects on the reinitiation of ovarian activity (Table 1). Diets containing 20% CP prolonged the interval of days to first ovulation when RDP was particularly in excess [72.5% of CP; (38)] or when reproductive health status was included in the statistical assessment (1). Others (7, 10, 23, 25), however, have reported that diets with 19 to 21% CP did not delay the postpartum interval to first ovulation or estrus. Follicular development in nonlactating cows was not perturbed by high CP diets (18), but, thus far, no effects during the development of ovarian follicles in postpartum lactating cows has been reported. Overall, high CP in the diet does not appear to have a strong impact on the reinitiation of ovarian activity in the postpartum period.

Jordan and Swanson (24) were the first to report that cows fed low CP (12.7%) during the breeding period had higher serum progesterone concentrations than did cows fed 16.3 or 19.3% CP. In other studies, progesterone concentrations in circulation were lower (36, 38) or were not affected (1, 18) in dairy cows fed high protein diets (Table 2). One explanation for these conflicting responses is the difference in the lactational status of the cows in the various studies.

**TABLE 1. High dietary CP and postpartum ovarian activity in dairy cows.**

<table>
<thead>
<tr>
<th>CP in diet (%)</th>
<th>Effect on days to first estrus or ovulation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>Delay of 13 d</td>
<td>(38)</td>
</tr>
<tr>
<td>20</td>
<td>Delay of 4 d</td>
<td>(1)</td>
</tr>
<tr>
<td>19</td>
<td>NS</td>
<td>(7)</td>
</tr>
<tr>
<td>19.3</td>
<td>Reduced 9 d</td>
<td>(23)</td>
</tr>
<tr>
<td>19.4</td>
<td>NS</td>
<td>(25)</td>
</tr>
<tr>
<td>20</td>
<td>NS</td>
<td>(10)</td>
</tr>
</tbody>
</table>

1Excessive RDP (72.5% of CP).
2Effect of reproductive health status included.

High dietary CP reduced plasma progesterone concentrations in lactating cows in three of four studies (4, 24, 36, 38), but did not reduce concentrations in nonlactating cows (4, 18) or heifers (12). Plasma progesterone concentrations progressively increase over the first three ovarian cycles during early lactation, and the rate of increase is reduced or moderated by the effects of negative energy balance (37, 39). When cows are fed excess RDP during early lactation, the negative energy balance is exacerbated because of the energy cost of detoxifying ammonia escaping from the rumen (38), but changes in metabolic clearance rate of progesterone should also be considered. Therefore, the deleterious effects of a negative energy balance that is more extreme might explain the lower plasma progesterone concentrations that were observed in studies with postpartum cows fed high CP (24, 36, 38). Reduced concentrations of plasma progesterone during the early breeding period appears to be a likely component of the reduction in fertility associated with feeding high dietary protein.

**EMBRYO DEVELOPMENT**

The effects of high protein intake on early embryo development have been studied in dairy cows. Early degeneration and poor development of embryos occurred in lactating cows that were fed excess RDP (3), but a similar, well-controlled study of nonlactating cows found that excess intake of CP failed to affect the health and number of embryos (18). Because the energy status of the cows preceding each of these studies was markedly different, depending on whether or not they were lactating, those researchers (18) suggested that the consequence of increasing

**TABLE 2. High dietary CP and plasma progesterone concentrations (PPC) during the estrous cycle of lactating and nonlactating dairy cows.**

<table>
<thead>
<tr>
<th>CP in diet (%)</th>
<th>Effects on PPC</th>
<th>Lactating</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>19.3</td>
<td>Reduced 25%1</td>
<td>Yes</td>
<td>(24)</td>
</tr>
<tr>
<td>20</td>
<td>Reduced 30%1</td>
<td>Yes</td>
<td>(36)</td>
</tr>
<tr>
<td>20</td>
<td>Reduced 50%1</td>
<td>Yes</td>
<td>(38)</td>
</tr>
<tr>
<td>20</td>
<td>NS2</td>
<td>Yes</td>
<td>(1)</td>
</tr>
<tr>
<td>27.4</td>
<td>NS</td>
<td>No</td>
<td>(18)</td>
</tr>
<tr>
<td>25</td>
<td>NS</td>
<td>No</td>
<td>(4)</td>
</tr>
<tr>
<td>21.84</td>
<td>NS</td>
<td>No</td>
<td>(12)</td>
</tr>
</tbody>
</table>

1During more than one estrous cycle.
2Excessive RDP (72.5% of CP).
3Nonsignificant (P > 0.05).
4Heifers >14 mo of age fed diet containing 70% ME required.
dietary CP content or degradability in lactating cows might be to exacerbate one or more factors affecting nutrition, metabolism, or energy balance that would result in lower reproductive performance (i.e., impaired embryo development). Indeed, increased embryo losses were reported for heifers that were fed an energy-restricted diet containing high amounts of degradable protein (12). Furthermore, negative energy balance during the early postpartum period might exert residual effects during the 40 to 60 d required for follicular development that might impair the health of preovulatory follicles later during the breeding period (27). Therefore, the combined effects of excess RDP and energy status might explain why the embryo quality of lactating cows was compromised (3).

A recent study with sheep supports the detrimental effects of high RDP on embryo development. Bishonga et al. (2) suggested that elevated circulating concentrations of urea and ammonia resulting from feeding high RDP exerted an adverse effect on early embryo development (d 4 to 11). However, because their study of dietary treatments followed by insemination were conducted within 30 d after termination of lactation, the effects attributed to high RDP might be confounded with the possible residual effects of antecedent energy status of the ewes during lactation (e.g., negative energy balance).

Given the results of studies of dairy cows involving embryo collection and evaluation, no evidence exists that high dietary protein has an impact on ovarian follicular development, ovulation, or fertilization of oocytes (3, 18). However, there is an interaction between the effects of CP and energy status during lactation that may impair embryo development. The relative significance of the separate effects and their interactions are not yet fully resolved.

**TABLE 3. Changes in plasma urea nitrogen (PUN) and ammonia concentrations following feeding in dairy cows.**

<table>
<thead>
<tr>
<th>Cow</th>
<th>Ammonia (mg/dl)</th>
<th>PUN (mg/dl)</th>
<th>Ammonia (mg/dl)</th>
<th>PUN (mg/dl)</th>
<th>Ammonia (mg/dl)</th>
<th>PUN (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4783</td>
<td>42</td>
<td>24</td>
<td>33</td>
<td>25</td>
<td>12</td>
<td>25</td>
</tr>
<tr>
<td>4788</td>
<td>76</td>
<td>20.5</td>
<td>82</td>
<td>22</td>
<td>24</td>
<td>21</td>
</tr>
<tr>
<td>4890</td>
<td>76</td>
<td>20</td>
<td>115</td>
<td>22.5</td>
<td>39</td>
<td>23</td>
</tr>
<tr>
<td>5322</td>
<td>21</td>
<td>24</td>
<td>21</td>
<td>26</td>
<td>1</td>
<td>26</td>
</tr>
<tr>
<td>5311</td>
<td>15</td>
<td>28</td>
<td>9</td>
<td>28.5</td>
<td>1</td>
<td>28</td>
</tr>
<tr>
<td>5331</td>
<td>36</td>
<td>23</td>
<td>24</td>
<td>25.5</td>
<td>1</td>
<td>22</td>
</tr>
<tr>
<td>5339</td>
<td>145</td>
<td>23</td>
<td>27</td>
<td>25.5</td>
<td>1</td>
<td>23</td>
</tr>
<tr>
<td>5345</td>
<td>12</td>
<td>21.5</td>
<td>1</td>
<td>21.5</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>5359</td>
<td>70</td>
<td>26</td>
<td>49</td>
<td>28</td>
<td>3</td>
<td>27</td>
</tr>
<tr>
<td>X</td>
<td>55.0</td>
<td>23.3</td>
<td>40.0</td>
<td>24.9</td>
<td>9.2</td>
<td>23.9</td>
</tr>
</tbody>
</table>

**UTERINE PHYSIOLOGY**

Successful development of the embryo during early pregnancy depends upon the nature of the uterine luminal environment (29). The luminal environment is dynamic and exhibits marked differences between the stages of the estrous cycle, and further evolution occurs during early pregnancy in support of the developing embryo. The cyclic nature of the microenvironment of the lumen is a consequence of ovarian steroidal regulation of endometrial secretion (17, 29). The local signaling effects of secretions from the blastocyst further modify the milieu and induce the secretion of specific proteins by the uterine epithelium (29). Early embryonic mortality (by d 7 of pregnancy) in lactating dairy cows has been found (40) to be associated with concentrations of ions and protein in the uterine environment that were significantly \( P < 0.05 \) different from those in cows with normal embryos.

Jordan et al. (22) examined the effects of dietary CP on the constituents of uterine secretions at various stages of the estrous cycle in high producing dairy cows. Blood ammonia and urea concentrations and urea in uterine secretion were all higher for cows fed 23% CP than for cows fed 12% CP. Intake by cows of 23% CP altered the concentrations of magnesium, potassium, and phosphorus in uterine secretions, but only during the luteal phase and not at estrus. Differential effects during the estrous cycle of high CP on the uterine environment were also observed for uterine luminal pH (12, 13). Uterine pH normally increases from about 6.8 at estrus to 7.1 on d 7 of the estrous cycle, but this increase failed to occur in both heifers and lactating cows fed excess RDP or RUP (12, 13). Uterine pH was inversely related to PUN, regardless of diet (13). The combined results from those studies suggest that high dietary CP may reduce fertility by interfering with the normal induc-
Figure 2. The concurrent measurements and correlation of plasma urea nitrogen (PUN) and uterine luminal pH in lactating dairy cows (n = 8) sampled during 36 h. For the regression equation, $R^2 = 0.22$.

Figure 3. The time course of inverse changes in plasma urea nitrogen (PUN) and uterine luminal pH in a lactating cow (cow 5919). Feeding occurred at the times indicated by arrows during the 38-h period of study.

IRREVERSIBLE EFFECTS OF PROGESTERONE ON THE MICROENVIRONMENT OF THE UTERUS, THEREBY PROVIDING SUBOPTIMAL CONDITIONS FOR THE SUPPORT OF EMBRYO DEVELOPMENT.

LINKAGES BETWEEN PROTEIN METABOLISM AND THE UTERINE ENVIRONMENT

The intake of high dietary CP results in elevated blood concentrations of ammonia and urea. Conceivably, one or both of these metabolites is responsible for altering the luminal microenvironment of the uterus. When diets with 22 to 27% CP were fed, the ammonia in blood was increased by 25 to 50% (18, 22) or not at all (12). In the latter study, blood ammonia was low even though the animals were fed an energy-restricted diet. From recent measurements in cows fed a 19% CP TMR, plasma ammonia concentrations were low (Table 3) and unrelated to elevated PUN concentrations, which peaked 4 to 8 h after feeding (11, 13). Because accurate measurements of blood ammonia require special procedures and rapid analysis (12), differences in methodology may account for the discrepancies in ammonia values among the studies. It remains uncertain whether blood ammonia concentrations in lactating cows are sufficient to affect fertility. Furthermore, ammonia would be directly involved only when RDP, not RUP, was in excess.

Across the literature, an excess amount of either RDP or RUP results in lower fertility; consideration of protein fractions, rather than CP, explained much of the variation in conception rate that was observed among the studies (15). Because RDP and RUP are metabolized and utilized separately and by different organs in the lactating cow, the common element of their metabolism when in excess of requirement is the formation of urea. Excess of either RDP or RUP increased PUN and altered uterine pH to a similar degree (13). Those observations further support the potential for urea as the common mediator of uterine effects of excess RDP or RUP. In addition, PUN varies inversely with uterine pH (13) and, thereby, is a possible mediator of decreased fertility associated with elevated PUN or MUN (5).

The active involvement of urea in uterine physiology is being investigated in further studies (W. R. Butler and R. O. Gilbert, unpublished). Lactating cows (n = 8) were fitted with intrauterine Foley catheters (12) to allow the frequent monitoring of uterine pH in association with PUN concentrations. Cows were fed a TMR containing 18% CP with RDP and RUP balanced for requirements (30). Uterine pH and PUN concentrations across cows during 36 h of observation were correlated (Figure 2). The sequential measurement of PUN and uterine pH demonstrated that uterine pH is quite dynamically attuned to changes in PUN, given a time lag of several hours (Figures 3 and 4). These results strongly suggest that PUN concentrations throughout the range of 12 to 24 mg/dl can exert direct effects on uterine function.

A system of endometrial cell culture has been developed to study the effects of urea on secretory function (19). In this three-dimensional system, the endometrial cells become polarized and establish a pH
The gradient between apical and basal compartments as soon as the cells reach confluence. The pH gradient is sensitive to both estradiol and progesterone. The presence of urea significantly ($P < 0.05$) diminished the effects of progesterone in maintaining a pH differential between apical and basal compartments. Large amounts of PGF$_{2\alpha}$ and PGE$_2$ are secreted by the endometrial cultures. Treatment of the cultures with both estradiol and progesterone suppressed PGF production, but the presence of urea significantly ($P < 0.01$) increased the secretion of PGF$_{2\alpha}$ and PGE$_2$. The importance of further studies on the effects of urea on the production of endometrial prostaglandin stems from the evidence that PGF$_{2\alpha}$ interferes with embryo development (28) and viability (34).

**CONCLUSIONS**

To support and stimulate high milk production, dairy cows are usually fed diets that are high in protein (>17% CP), which appears to result in decreased reproductive performance. Investigations of this inverse relationship have indicated that excesses of either RDP or RUP relative to requirements result in lower fertility and that high amounts of dietary protein exacerbate the effects of negative energy balance and reproductive health problems to impair reproduction further. Because the metabolism of excess RDP or RUP results in high urea production, monitoring of BUN or MUN concentrations has proved to be useful in associating decreased conception rate with BUN or MUN concentrations above 19 to 20 mg/dl. As part of the progression of events involved in establishing pregnancy, high dietary RDP seems not to impact follicle development or ovulation, but results in reduced concentrations of plasma progesterone in lactating cows, which appears to be linked to the effects of exacerbated negative energy balance. The effects of RDP on energy status may also impair embryo development. Embryo survival and growth depend upon the quality of the uterine luminal environment, and the intake of high dietary protein alters uterine secretions. Blood urea concentrations, rather than ammonia, seems to be related to the detrimental effects on fertility as shown by the dynamic changes in uterine pH with PUN and in vitro evidence that urea alters pH and prostaglandin production in endometrial cell cultures. In conclusion, the poor fertility of high producing dairy cows reflects the combined effects of a uterine environment that is dependent on progesterone, but has been rendered suboptimal by antecedent effects of negative energy balance or postpartum health problems and has been further compromised by the effects of urea resulting from intake of high dietary protein.

**REFERENCES**


