Reduction of Fertility and Alteration of Uterine pH in Heifers Fed Excess Ruminally Degradable Protein

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ABSTRACT: The objective of these studies was to elucidate the causes underlying the reduction of fertility associated with feeding of excess ruminally degradable protein to cattle. Holstein heifers were fed total mixed rations that either met (Normal) or exceeded (High) ruminally degradable protein requirements. In Exp. 1 heifers (n = 80) were inseminated at estrus after being fed the experimental diets for 4 wk. First-service conception rates were 82 and 61% for the Normal and High groups, respectively (P < .05). Plasma urea nitrogen (PUN) levels were elevated (10.2 ± 17 vs 14.8 ± .19 mg/dL) in the High group throughout the experiment (P < .05). In Exp. 2, heifers (n = 32) receiving the same diets as in Exp. 1 were fitted with catheters in a jugular vein, in the bladder, and in the uterus on either the day before expected estrus or d 6 after estrus. On estrus or d 7, plasma and urinary NH4 and urea nitrogen and uterine pH were determined at 4-h intervals for 24 h beginning just before feeding. Plasma NH4 was variable and did not differ between treatments or days of the estrous cycle. During the 24-h period, urinary urea nitrogen and PUN were elevated (P < .05) in the High groups and did not differ between days of the cycle. Uterine NH4 excretion was elevated (P < .05) during the 24-h period only in the High group on d 7. Uterine pH did not change over time after feeding. On d 7 uterine pH was lower in the High group (P < .05) and a similar trend between groups approached significance (P < .1) at estrus. We conclude that excess degradable protein acts through some undefined mechanism to decrease uterine pH during the luteal phase (Exp. 2), which may play a role in the observed reduction of fertility.

Key Words: Cattle, Protein Excess, Fertility, Uterine pH


Introduction

In an effort to sustain milk production during early lactation, dairy producers often feed protein in excess of requirements and recommendations. Unfortunately, overfeeding protein may be associated with a decline in fertility, as documented by Jordan and Swanson (1979a), Kaim et al. (1983), and Canfield et al. (1990). The detrimental effect of excess protein has been linked to the ruminally degradable fraction (degradable intake protein, DIP) of dietary protein (Folman et al., 1981; Ferguson et al., 1988; Canfield et al., 1990). Jordan and Swanson (1979a) reported that excess dietary protein decreased fertility and significantly altered the ionic composition of uterine fluid during the luteal phase, but not the follicular phase, in lactating dairy cows (Jordan et al., 1983). Carroll et al. (1988) suggested that reproductive management of postpartum cows (i.e., treatment of uterine disorders and estrus detection programs), rather than dietary protein, could account for variations in fertility reported in various studies (Jordan and Swanson, 1979a; Howard et al., 1987; Carroll et al., 1988; Canfield et al., 1990). To avoid these confounding effects, virgin heifers were chosen for the present study, and we conducted pilot studies to determine that restriction of DMI and ME to 70% of NRC requirements was necessary to achieve the peak (6 to 8 h after feeding) plasma urea nitrogen (PUN) values in the range of 20 to 30 mg/dL that were observed in studies with lactating cows (Howard et al., 1987; Carroll et al., 1988; Canfield et al., 1990). To avoid these confounding effects, virgin heifers were chosen for the present study, and we conducted pilot studies to determine that restriction of DMI and ME to 70% of NRC requirements was necessary to achieve the peak (6 to 8 h after feeding) plasma urea nitrogen (PUN) values in the range of 20 to 30 mg/dL that were observed in studies with lactating cows (Howard et al., 1987; Carroll et al., 1988; Canfield et al., 1990). Thus, the current studies had two objectives: 1) to test...
Table 1. Composition and analysis of diets

<table>
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<tr>
<th>Ingredient</th>
<th>Normal protein</th>
<th>High protein</th>
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<tbody>
<tr>
<td>Corn silage</td>
<td>66.0</td>
<td>64.2</td>
</tr>
<tr>
<td>Legume/grass hay</td>
<td>26.4</td>
<td>25.7</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>6.6</td>
<td>6.4</td>
</tr>
<tr>
<td>Urea</td>
<td>.5</td>
<td>.2</td>
</tr>
<tr>
<td>Limestone</td>
<td>.07</td>
<td>.06</td>
</tr>
<tr>
<td>Trace mineral salt</td>
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</tbody>
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Analysis

- CP
- Degradable protein
- TDN, %

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<thead>
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<th>Normal</th>
<th>High</th>
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</thead>
<tbody>
<tr>
<td>CP</td>
<td>15.45</td>
<td>21.8</td>
</tr>
<tr>
<td>Degradable protein</td>
<td>73.0</td>
<td>82.5</td>
</tr>
<tr>
<td>TDN, %</td>
<td>72</td>
<td>71</td>
</tr>
</tbody>
</table>

*Percentage of ration DM.

*Percentage of CP.

The effects of excess ruminally degradable protein on fertility of virgin heifers and 2) to determine effects of excess ruminally degradable protein on intrauterine pH at two stages of the estrous cycle.

Materials and Methods

Experiment 1

Eighty Holstein heifers > 14 mo old and weighing 373 ± 13.6 kg were randomly assigned to receive diets that were formulated to meet 70% of ME requirements (based on .8 kg/d gain), met ruminally undegradable protein requirements, and either met (Normal) or exceeded by 50% (High) ruminally degradable protein requirements (Table 1). Heifers, penned 10 per group, were fed a total mixed ration once daily (1000). To restrict energy intake adequately, DMI was limited to 70 kg/pen·d⁻¹. The experiment was conducted in four replicates during a 1-yr period with approximately 10 heifers·treatment·replicate⁻¹ (range of 9 to 11). Four animals were removed from the study before breeding because of injuries or disposition.

 Estrous cycles of heifers were synchronized by giving two injections of prostaglandin F₂α (30 mg, Lutalyse, Upjohn, Kalamazoo, MI) administered 11 d apart. The first injection was given 4 d before the 1st d of feeding the experimental diets. Heifers were monitored for estrous activity four times per day for 30 min, and by farm personnel as they worked in the barn, beginning approximately 19 d after the second PGF₂α injection (i.e., approximately d 16 of the synchronized estrous cycle). Approximately 12 h after observed spontaneous standing estrus (i.e., 20 to 28 d after the second injection of PGF₂α) each heifer was inseminated by one technician using semen from a single ejaculate of an active AI sire. Occurrence of pregnancy to that one insemination was determined by transrectal palpation of the uterus approximately 45 d after breeding.

Before feeding, blood was sampled from a coccygeal vessel twice weekly from the initiation of the experiment until approximately 5 wk after insemination. Plasma was stored frozen (−20°C) until it was assayed for urea nitrogen by the diacetylmonoxamine method (Technicon Industrial Method 339-01, Technicon Industries, Tarrytown, NY) and progesterone by RIA (Staigmiller et al., 1979; Nara and First, 1981). Intra- and interassay CV for PUN were 1.9 and .9%, and for progesterone were 9.6 and 12.2%, respectively. During the week in which the animals were inseminated, additional blood samples were taken at 4-h intervals from prefeeding until 24 h after feeding on one day to characterize the PUN profile during a 24-h period.

Experiment 2

Concurrent with the third replicate of Exp. 1, Holstein heifers (363 ± 11 kg and > 14 mo of age) were assigned randomly in a 2 x 2 factorial arrangement to dietary treatments (same diets as in Exp. 1), and to either estrus (d 0) or d-7 measurements (n = 32; eight diet⁻¹·d⁻¹). The animals were started on the experimental diets (group-fed) and their estrous cycles were synchronized as in Exp. 1. All animals were maintained on the experimental diets for a minimum of 2 wk before measurements were taken to allow them to adapt to their diets (Fenderson and Bergen, 1976; Jordan et al., 1983). Either on the day before the expected synchronized estrus or on d 6 after estrus, animals were fitted with jugular vein catheters (Abbocath-T, 16 gauge x 13.3 cm, Abbott Hospitals, North Chicago, IL) and bladder catheters (Foley, 18 gauge, 75-mL balloon, C. R. Bard, Covington, GA). On the day of estrus or d 7, a modified Foley catheter (18-gauge, 30-mL balloon; see Figure 1) was inserted into
the uterus and the balloon was inflated with sterile saline to facilitate passage of the uterine pH probe.

At 4-h intervals from prefeeding until 24 h after feeding, uterine pH was determined by inserting a flexible pH electrode (1.6-mm diameter, 3 m in length, MI-506, Microelectrodes, Londonderry, NH) through the uterine catheter to extend approximately 5 cm into the uterine lumen. A Ag-AgCl skin patch electrode applied to a shaved area of the animal’s flank was used as the reference electrode. The pH electrode was left in place until a stable pH reading was obtained. After removal from the uterus, the pH electrode was rinsed extensively in distilled, deionized water, calibrated with buffers of pH 4 and 7, disinfected in .013% benzalkonium chloride, and rinsed in sterile, distilled, deionized water. Between the 4-h measurements, the electrode was cleaned with Terg-a-zyme (Alconox, New York, NY) to remove any protein and disinfected again. Concurrent with pH measurements, jugular blood was sampled to determine PUN and plasma NH₄.

Total urine collection was performed for the 24-h period starting just before feeding. Urine was collected into polyallomer bags containing 20 mL of 6 N HCl, which served as a preservative. At 4-h intervals, the volume of urine was recorded and two 5-mL aliquots were frozen for later determinations of urea nitrogen and NH₄, and a new bag was then attached to the catheter.

Blood for NH₄ determinations was prepared following the procedures of McCullough (1967). A 10-mL sample was drawn from the jugular catheter by syringe and placed into a glass tube containing 150 IU of sodium heparin. After gentle mixing, a 2-mL aliquot was added to a tube containing 1 mL of 10% (wt/vol) sodium tungstate and mixed. To this was added 1 mL of 1 N sulfuric acid followed by thorough mixing. The sample was then placed on ice until it was centrifuged (15 min at 4°C) and the deproteinized plasma was separated and frozen within 30 min of collection.

Plasma and urinary NH₄ determinations were made within 36 h of collection using a modification of the methods of Chaney and Marbach (1962). To the frozen 1-mL aliquots was added .5 mL of phenol reagent (.53 M phenol and .001 M sodium nitroferricyanide). After the sample thawed, the mixture was mixed, followed by the addition of .5 mL of hypochlorite reagent (.625 M sodium hydroxide and .03 M sodium hypochlorite). The solution was mixed and incubated at 39°C for 15 min. After dilution with 5 mL of distilled, deionized water, the absorbance was measured on a spectrophotometer at 630 nm. The NH₄ values were extrapolated from the standard curve and adjusted for dilution. Urinary NH₄ and urea nitrogen (assayed as in Exp. 1) concentrations were multiplied by the urine volume to calculate NH₄ and urea nitrogen excretion during a 4-h period.

Statistical Analysis

Experiment 1. Conception rate data were analyzed by chi-square test of association. Prefeeding PUN concentrations for each heifer were analyzed for effects of days on the experimental diets using regression analysis. There was no significant effect of time (days) on PUN concentrations; therefore, the mean PUN level for each animal was used in ANOVA with treatment and replicate (season) in the model (Minitab, 1988). The prebreeding estrous cycle length was determined as the number of days between the synchronized estrus and the return to estrus at which insemination occurred and was analyzed by ANOVA. The area under the progesterone profiles in prebreeding estrous cycles was calculated and analyzed by ANOVA.

Experiment 2. Uterine pH measurements for each animal were analyzed for effects of time after feeding using regression analysis. Because there was no effect of time, a mean uterine pH was calculated for each animal, which was then analyzed by ANOVA. Profiles for PUN, plasma NH₄, urinary urea nitrogen, and urinary NH₄ were analyzed by split-plot ANOVA.

Results

Experiment 1

Feeding high levels of ruminally degradable protein to nonlactating Holstein heifers receiving 70% of recommended ME daily reduced (χ² = 4.334, P < .05) first-service pregnancy rate from 82% in the Normal group to 61% in the High group (Table 2). Average daily gain was 623 ± 120 g and did not differ between treatments. Neither preinsemination nor postinsemination (for nonpregnant animals) estrous cycle lengths differed (Table 2). Of the 16 animals in the

![Figure 2. Plasma progesterone, before insemination and during early pregnancy, in heifers fed Normal (n = 32) or High (n = 25) levels of dietary protein. Each point represents the mean ± SEM.](image-url)
High group that were not pregnant at palpation, nine exhibited normal estrous cycle lengths (18 to 22 d), as did all seven heifers in the Normal group that did not conceive. However, seven heifers of the High group exhibited extended luteal phases, which resulted in lengthened estrous cycles ranging from 26 to 36 d. As shown in Figure 2, progesterone profiles for pregnant heifers did not differ between treatments in the prebreeding estrous cycle and early pregnancy. Plasma progesterone profiles after insemination in heifers that were not pregnant at palpation and with differing estrous cycle lengths are compared in Figure 3.

Concentrations of PUN (Table 2) peaked at 8 to 12 h after feeding (Table 2) and were elevated \((P < .05)\) at all times during the 24-h sampling period in the High protein group (Figure 4). Mean prefeeding PUN levels were elevated \((P < .05)\) in the High group compared with the Normal group (Table 2).

Categorizing PUN data with breakpoints one SD above and below the overall mean of 12.9 mg/dL yields categories of < 9.9, 9.9 to 16, and > 16 mg/dL. The conception rates within these categories were 87.5, 72.5, and 42.8%, respectively \((\chi^2 = 7.45, P < .05)\), with the lowest conception rate associated with the highest PUN values. Similarly, mean prefeeding PUN in pregnant animals was lower (12.35 ± 0.39 mg/dL) than in heifers that were not pregnant (14.32 ± 0.64 mg/dL, \(P < .05\)).

**Experiment 2**

As in Exp. 1, PUN levels were elevated by the High protein diet and peaked at 8 h after feeding, regardless of the day of the estrous cycle (Figure 5a, b). Plasma NH₄, however, was variable and did not differ with either treatment or day of the cycle (Figure 5c, d). At estrus, urinary urea nitrogen excretion in the High group peaked at 12 h after feeding and was

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Table 2. First-service conception rate, estrous cycle lengths, and plasma urea nitrogen levels for heifers fed either normal or high levels of protein

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal protein</th>
<th>High protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>First-service conception rate % (no. preg/no. inseminated)</td>
<td>82% (32/39)ᵃᵇ</td>
<td>61% (25/41)ᵇ</td>
</tr>
<tr>
<td>Preinsemination estrous cycle length, d</td>
<td>20.1 ± .28ᶜᵉ</td>
<td>20.4 ± .33</td>
</tr>
<tr>
<td>Postinsemination estrous cycle length, d</td>
<td>21.9 ± .52</td>
<td>24.4 ± 1.2</td>
</tr>
<tr>
<td>Plasma urea nitrogen, mg/dL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prefeeding</td>
<td>10.2ᵃ ± .17</td>
<td>14.8ᵇ ± .19</td>
</tr>
<tr>
<td>Peak</td>
<td>17.5ᵃ ± .22</td>
<td>26.6ᵇ ± .23</td>
</tr>
</tbody>
</table>

ᵃᵇConception rate data within the same row with differing superscripts differ \((P < .05)\) by \(χ^2\) test of association.
ᶜMean ± SE.
ᵈMeans within the same row with differing superscripts differ \((P < .05)\) by one-way ANOVA.
elevated \((P < .05)\) at all times except at 24 h (Figure 6a). On d 7, urinary urea nitrogen excretion was elevated \((P < .05)\) at all times throughout the 24-h sampling period (Figure 6b). Urinary NH$_4$ excretion did not differ between treatments at estrus (Figure 6c). On d 7, urinary NH$_4$ excretion was elevated \((P < .05)\) at all times in the High group compared with the Normal group (Figure 6d).

On d 7, uterine pH was lower \((P < .05)\) in the High protein group than in the Normal group, whereas at estrus uterine pH did not differ between treatments (Table 3) but approached significance \((P < .1)\). Uterine pH was unaffected by time after feeding (Figure 7).

**Discussion**

Low fertility is a major constraint to overall efficiency in high-producing dairy herds. High levels of milk production are often maintained by feeding excess protein to ensure an adequate flow of amino acids to the small intestine. Although still somewhat controversial, there is increasing evidence that the negative effects of excess protein on fertility may be attributable to the DIP fraction of the crude protein (Folman et al., 1981; Ferguson et al., 1988; Canfield et al., 1990); however, Carroll et al. (1988) suggested that reproductive management rather than protein quantity or degradability could account for differences in reproductive variables reported in various studies. In the present study, we have demonstrated the detrimental effects of excess degradable protein on fertility under conditions in which reproductive management was optimized.

Urea is one metabolite of dietary protein that is formed from detoxification of NH$_4$ by the liver. The level of urea in the plasma or serum (PUN or SUN) is reflective of the quantity and degradability of the protein consumed, of the severity of negative energy balance in fasted animals, or of the combination of protein feeding and negative energy balance. Plasma urea nitrogen or SUN concentrations have often been used as a correlate between dietary protein level and fertility. Ferguson et al. (1991) suggested that when SUN concentrations are \(> 20\) mg/dL, fertility will be impaired. Kaim et al. (1983) reported that in cows fed a 20% CP diet with PUN of 16.8 mg/dL, pregnancy rate was lower than in cows fed a 15% CP diet with PUN of 9.0 mg/dL. Likewise, Canfield et al. (1990) reported that PUN was lower \((15.7 \text{ vs } 18.6 \text{ mg/dL})\) in cows that conceived than in cows that did not. In the

![Figure 5](image_url)

**Figure 5.** Plasma urea nitrogen (a and b) and NH$_4$ (c and d) at estrus and d 7 from before feeding to 24 h after feeding in heifers fed Normal (○) or High (■) levels of dietary protein. Each point represents the mean (+ SEM) of eight determinations. *Values for a given time differ \((P < .05)\).
current study, heifers with prefeeding PUN concentrations ≥ 16 mg/dL had conception rates that were at least 30% lower than those of heifers with PUN levels < 16 mg/dL (42% vs 72.5 and 87.5%, for > 16, 9.9 to 16, and < 9.9 mg/dL, respectively). Nevertheless, Carroll et al. (1988) and Howard et al. (1987) reported elevated PUN (> 24 mg/dL) in cows fed high-protein diets but in which a concomitant decline in fertility did not occur. These discrepancies, which Carroll et al. (1988) attributed to reproductive management rather than to level of dietary protein, cannot be explained in light of the current experiment in which reproductive variables were tightly controlled.

Another protein metabolite, NH₄, which escapes detoxification by the hepatic urea cycle system, is usually prevented from entering the general circulation by a very high affinity perivenous glutamine synthetase system (Haussinger, 1990). Glutamine, which then acts as the nitrogen carrier in the blood, is

<table>
<thead>
<tr>
<th>Day of estrus cycle</th>
<th>Normal protein</th>
<th>High protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrus</td>
<td>pH 6.87 ± .02ᵃ</td>
<td>pH 6.75 ± .03</td>
</tr>
<tr>
<td>Day 7</td>
<td>pH 7.09ᵇ ± .06</td>
<td>pH 6.79ᶜ ± .01</td>
</tr>
</tbody>
</table>

ᵃMean ± SE, n = 8.
ᵇ,cMeans within the same row with differing superscripts differ (P < .05) by one-way ANOVA.

Figure 7. Uterine pH from before feeding to 24 h after feeding at estrus and d 7 in heifers fed Normal or High levels of dietary protein. Each point represents the mean (± SEM) of eight determinations. *Values differ by treatment within day for a given time (P < .05).
recognized as a readily available source of NH₄⁺ (NRC, 1979). Normally, glutamine circulates in the blood and, upon presentation to the kidney, is deaminated via renal glutaminase and the resulting NH₄⁺ is excreted in the urine. Because glutamine is probably utilized for protein accretion under the anabolic influence of estriadiol (Wagner et al., 1988; Enright et al., 1990), the difference in urinary NH₄⁺ excretion between heifers in estrus and those at d 7 may be due to the anabolism of this active form of NH₄⁺ by the estrual heifer (compare Figures 6c and 6d). Because DMI was restricted and the daily ration was consumed within 4 h of feeding, the decreased NH₄⁺ excretion at estrus cannot be ascribed to decreased DMI in estrual animals. Because of the increased levels of urinary NH₄⁺ but not of urea, excreted on d 7, coincident with the aberrant uterine pH, it is proposed that the detrimental effects on fertility may be ascribed to excess NH₄⁺, possibly derived from glutamine.

Proper functioning of the corpus luteum is necessary as the source of progesterone for the maintenance of pregnancy. In heifers that conceived to the first service in Exp. 1, we found no differences between diets in plasma progesterone levels (area under progesterone profiles) either before or after breeding. This is consistent with the findings of Blauwiekel et al. (1986) but not with those of Jordan and Swanson (1979b).

By using the postbreeding progesterone profiles, we examined estrous cycle lengths among the nonpregnant heifers. Nine of the heifers fed high protein and all those fed normal levels of protein exhibited normal inter estrus intervals, whereas the other seven heifers fed high protein exhibited extended luteal phases and inter estrus intervals of 26 to 36 d. These prolonged luteal phases probably indicate that embryonic death occurred sometime after the critical period (d 15 to 16 postbreeding) for maternal recognition of pregnancy (Stewart, 1990). Therefore, the fertilization rate between the treatments was probably similar (32/39, Normal; 32/41, High) but the excess degradable protein resulted in death of embryos in seven of the High heifers sometime after d 20.

In Exp 2 we determined uterine pH at estrus and d 7 of the estrous cycle. Day 7 was selected for luteal phase intrauterine pH measurements because the corpus luteum is functional and the embryo has migrated into the uterus. At estrus, uterine pH was relatively low, 6.8, regardless of diet. The pH of ejaculated bull semen is also approximately 6.8 (Salisbury et al., 1978; Mann and Lutwak-Mann, 1981), and so the uterine pH observed at estrus should be compatible with sperm viability. In the Normal protein group, uterine pH was higher, 7.1, during the early luteal phase than at estrus. This increased uterine pH on d 7 parallels the luteal phase rise in uterine Mg, K, and P as reported for cows fed normal protein levels (Schultz et al., 1971; Jordan et al., 1983). In the High protein group, however, luteal phase uterine pH was significantly lower than in the Normal protein group and similar to that observed at estrus. Again, this is consistent with the decreased uterine Mg, K, and P observed during the luteal phase and at estrus in cows fed high-protein diets (Jordan et al., 1983).

Thus, it seems that intrauterine pH, as reported here, might reflect alterations in the uterine secretory activity. How these alterations might relate to tissue urea, glutamine, or NH₄⁺ and directly to embryo survival cannot be determined from the present studies. Although these studies were conducted with virgin heifers, not lactating cows, the data presented provide a basis for continuing experiments on these mechanisms in lactating cows.

Implications

Feeding excess ruminally degradable protein to dairy cattle can be deleterious to fertility, particularly if there is inadequate energy supplied to the rumen. This effect on fertility may be mediated through an alteration in the uterine environment in which the embryo must grow. Use of a relatively undegradable protein source may allow increased milk production as well as alleviation of the effects on fertility.

Literature Cited


