Influence of nitrogen and sulfur intake on bovine uterine pH throughout the luteal phase

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ABSTRACT: Previous research has reported that diets high in protein and sulfur decreased uterine pH in cattle. The objective of this study was to determine the effect of high N and high S intake on uterine pH. Holstein (n = 15) and Angus-cross (n = 5) heifers (337.5 ± 8.4 kg of BW) were randomly assigned to 1 of 4 diets: control (CON; 13.4% CP and 0.17% S); high nitrogen (HN; CON plus urea supplement); high sulfur (HS; CON plus calcium sulfate); or both high nitrogen and sulfur (HNS). Diets were individually fed at 2.6% of BW on a DM basis using Calan gates and estrus was synchronized to occur on d 13 (d 0 = start of dietary treatment). Blood samples were collected on d –2 and daily (d 1 to 28) at 1400 h to determine concentrations of plasma urea nitrogen (PUN), sulfate (d 1, 4, 8, 12, 16, 20, 24, and 28), and progesterone. Uterine pH was measured on d 16, 20, 24, and 28 (d 3, 7, 11, and 15 of the estrous cycle). There was a treatment, time, and treatment by time interaction (P < 0.01) on concentrations of PUN. There was an effect of treatment (P < 0.01) on concentrations of sulfate, with concentrations being increased in HS compared with CON, HN, and HNS (P < 0.01), and HNS increased compared with CON (P < 0.01) and HN (P < 0.01). Uterine pH was increased in HN and HNS compared with CON (P < 0.02), whereas HS was not different from any treatment (P > 0.11). There was no effect of time (P = 0.26) or treatment by time interaction (P = 0.71) on uterine pH. In summary, uterine pH was increased in HN and HNS compared with CON, whereas HS was intermediate and was associated with increased concentrations of PUN.

Key words: nitrogen, sulfur, uterine pH

INTRODUCTION

In the dairy industry, increased milk production within the last 60 yr has been associated with decreased reproductive efficiency in lactating dairy cattle (Butler, 2000; Chagas et al., 2007). This decrease in reproductive efficiency is attributed to low fertilization rates of 76%, with <50% of embryos establishing pregnancy and fetal death rates as great as 60% (Santos et al., 2004). The primary cause of this phenomenon remains unknown; however, it may be due in part to the common practice of overfeeding dietary protein to high producing dairy cows to sustain milk production (Elrod and Butler, 1993).

Specifically, research has reported that high protein diets increased concentrations of blood urea nitrogen (BUN) and milk urea nitrogen (MUN), and decreased pregnancy rates, conception rates, and embryonic development (Ferguson et al., 1988, 1993; Blanchard et al., 1990; Butler et al., 1996; Rajala-Schultz et al., 2001). This decrease in fertility may be mediated through changes in the uterine environment. Research by Elrod et al. (1993) and Elrod and Butler (1993) found that excess protein increased concentrations of
plasma urea nitrogen (PUN) and decreased uterine pH on d 7 of the estrous cycle compared with those fed a balanced diet. Similar to high dietary protein intake, high dietary sulfur intake has been reported to decreased uterine pH during the luteal phase (Perry et al., 2009). In cattle, Hugentobler et al. (2007) reported a positive correlation between concentrations of sulfate anion in serum and uterine fluid. As serum sulfate concentrations increased, uterine sulfate concentrations also increased.

Changes in uterine pH during the midluteal phase are important due to its effect on embryo development and survival. Ocon and Hansen (2003) reported that in vitro culture of bovine embryos at a pH less than 7.0 resulted in decreased cleavage rates and development to blastocyst stage. It was our hypothesis that the high nitrogen and high sulfur in feedstuffs fed to breeding females both contribute to early embryonic loss, due to alterations in uterine pH. Therefore, the objective of this study was to determine if alterations in bovine uterine pH was due to the effect of increased dietary nitrogen and/or sulfur.

**MATERIALS AND METHODS**

All animal work was approved by the North Dakota State University (NDSU) Animal Care and Use Committee.

**Experimental Design**

Fifteen pubertal, nulliparous Holstein heifers and 5 Angus-cross beef heifers (337.5 ± 8.4 kg BW), housed at the NDSU Animal Nutrition and Physiology Center, were equally allotted to 1 of 4 dietary treatments: control (CON; 13.4% CP and 0.17% S; n = 4 Holstein and 1 Angus); high nitrogen [HN; CON plus urea (1%) and a coated, slow-releasing urea (Optigen, Alltech Inc., Nicholasville, KY; 1%); n = 4 Holstein and 1 Angus]; high sulfur [HS; CON plus calcium sulfate (1.5%); n = 3 Holstein and 2 Angus]; or both high nitrogen and sulfur (HNS; n = 4 Holstein and 1 Angus). At 0700 h, diets were individually fed at 2.6% of BW on a DM basis, using Calan gate individual feeding system (Northwood, NH) and estrus was synchronized to occur on d 13 of the experimental period. Heifers received PGF2α [Lutalyse, 25 mg intramuscularly (i.m.); Pfizer Animal Health, Madison, NJ] on d 1, GnRH (100 μg as 2 mL of Cysto-relin i.m.; Merial, Athens, GA) and insertion of a controlled internal drug releasing device (CIDR; Pfizer Animal Health) on d 4, PGF2α and CIDR removal on d 10, and GnRH on d 13. At 1400 h, BW and blood samples were collected on d –2 before the experimental period and daily beginning on d 1 (d –12 of the estrous cycle) for determination of concentrations of PUN, sulfate, and progesterone. Uterine pH was measured on d 16, 20, 24, and 28 (d 3, 7, 11, and 15 of the estrous cycle).

**Diet Composition, Acclimation, and Feeding**

Ingredient composition and analyzed nutrient composition are presented in Tables 1 and 2 for the CON, HN, HS, and HNS dietary treatments. Heifers were acclimated to CON for 7 d before the start of the experimental period. Heifers were subsequently acclimated to their allotted dietary treatment from d 1 to 13 of the experimental period (d –12 to 0 of the estrous cycle). Diets consisted of hay and a supplement.

**Table 2. Nutrient composition of control, high nitrogen, high sulfur, and high nitrogen and sulfur dietary treatments**

<table>
<thead>
<tr>
<th>Item1</th>
<th>Control2</th>
<th>High nitrogen2</th>
<th>High sulfur2</th>
<th>High nitrogen and sulfur2</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP, %</td>
<td>10.8</td>
<td>15.9</td>
<td>11.0</td>
<td>16.3</td>
</tr>
<tr>
<td>S, %</td>
<td>0.14</td>
<td>0.15</td>
<td>0.45</td>
<td>0.40</td>
</tr>
<tr>
<td>NDF, %</td>
<td>55.4</td>
<td>54.7</td>
<td>56.6</td>
<td>55.4</td>
</tr>
<tr>
<td>Fat, %</td>
<td>2.36</td>
<td>2.27</td>
<td>2.71</td>
<td>2.44</td>
</tr>
<tr>
<td>TDN, %</td>
<td>55.21</td>
<td>54.99</td>
<td>56.02</td>
<td>55.62</td>
</tr>
<tr>
<td>Calcium, %</td>
<td>1.10</td>
<td>1.13</td>
<td>0.80</td>
<td>0.76</td>
</tr>
<tr>
<td>Phosphorus, %</td>
<td>0.22</td>
<td>0.22</td>
<td>0.22</td>
<td>0.22</td>
</tr>
<tr>
<td>Magnesium, %</td>
<td>0.18</td>
<td>0.20</td>
<td>0.17</td>
<td>0.18</td>
</tr>
<tr>
<td>Potassium, %</td>
<td>1.46</td>
<td>1.46</td>
<td>1.50</td>
<td>1.50</td>
</tr>
<tr>
<td>Iron, mg/kg</td>
<td>299</td>
<td>303</td>
<td>274</td>
<td>255</td>
</tr>
<tr>
<td>Sodium, %</td>
<td>0.13</td>
<td>0.12</td>
<td>0.12</td>
<td>0.11</td>
</tr>
<tr>
<td>Chloride, %</td>
<td>0.58</td>
<td>0.45</td>
<td>0.55</td>
<td>0.44</td>
</tr>
<tr>
<td>Manganese, mg/kg</td>
<td>46.3</td>
<td>46.7</td>
<td>34.0</td>
<td>38.7</td>
</tr>
<tr>
<td>Zinc, mg/kg</td>
<td>43.0</td>
<td>40.3</td>
<td>41.0</td>
<td>40.1</td>
</tr>
<tr>
<td>Copper, mg/kg</td>
<td>8.49</td>
<td>7.55</td>
<td>8.49</td>
<td>7.15</td>
</tr>
<tr>
<td>Molybdenum, mg/kg</td>
<td>1.29</td>
<td>1.55</td>
<td>1.32</td>
<td>1.59</td>
</tr>
<tr>
<td>DCAD, mEq/100 g of DM</td>
<td>18.0</td>
<td>20.6</td>
<td>0.1</td>
<td>5.8</td>
</tr>
</tbody>
</table>

1Values listed on a DM basis.
2Calculated values based on laboratory analysis of diet composite.
3DCAD = dietary cation-anion difference, calculated as (Na + K) – (Cl + S).
for each dietary treatment. The supplement was fed before the hay and hay was not fed until ~90% or more of the supplement was consumed. Subsamples of each dietary treatment were collected daily and composited for nutrient analysis. Orts were collected daily before feeding, which occurred daily at 0700 h, and weighed. Feed and orts were frozen at –20°C until further nutrient analyses. Orts were dried in a forced air oven at 60°C for 48 h to determine DM (AOAC, 1990) and ground using a Wiley Mill (Author H. Thomas, Philadelphia, PA) through a 1-mm-mesh screen. Orts were combined by week to determine weekly average intake of N and S. Composite samples of the dietary treatments and weekly orts subsamples were analyzed by Dairyland Laboratories, Inc. (Arcadia, WI).

**Blood Collection, Concentrations of Plasma Urea Nitrogen, Sulfate, and Progesterone, and Uterine pH**

Blood samples were collected on d –2 before the experimental period and daily at 1400 h starting on d 1 (d –12 to 15 of the estrous cycle) to determine concentrations of PUN, sulfate anion, and progesterone. Samples were collected through venipuncture of the jugular vein into 10-mL Vacutainer tubes with EDTA (Fisher Scientific, Pittsburg, PA) and were immediately placed on ice. Samples were centrifuged at 1,200 × g for 30 min at 4°C and plasma was collected and stored at –20°C until blood analyses were performed.

For determination of concentrations of PUN, all plasma samples were deproteinized using 1:1 dilution of plasma to 6% trichloroacetic acid solution. The sample solution was vortexed and centrifuged at 4°C for 10 min at 5,000 × g and supernatant collected for analysis. Supernatant collected was frozen overnight at –20°C, thawed, centrifuged for 5 min at 5,000 × g at 4°C, and diluted using a 1:3 dilution of supernatant to double-distilled H₂O. Plasma urea nitrogen was quantified in duplicate using the methods of Rahmatullah and Boyde (1980), and using a spectrophotometer (UVmini-1240; Shimadzu, Columbia, MD). Intra-assay and interassay CV for the PUN assays were 1.7% and 8.4%, respectively. Assay sensitivity was 1.4 mg/dL.

Plasma samples collected on d 1, 4, 8, 12, 16, 20, 24, and 28 (d –12, –9, –5, –1, 3, 7, 11, and 15 of the estrous cycle) were analyzed for concentrations of sulfate anion by ion chromatography performed by The Oscar E. Olson Biochemistry Laboratory (Brookings, SD).

All plasma samples collected during the experimental period were analyzed for circulating concentrations of progesterone using methodology described by Engel et al. (2008). Intra-assay and interassay CV for the progesterone assays were 3.3% and 13.8%, respectively. Assay sensitivity was 0.4 ng/mL.

Uterine pH was measured on d 16, 20, 24, and 28 (d 3, 7, 11, and 15 of the estrous cycle). Using the method similar to that described by Perry and Perry (2008), a sterile, plastic sheath was placed on an AI gun and was inserted through the cervix and into the uterus. Once placed in the uterine body, a flexible pH electrode (1.4 mm diam.; Microelectrodes, Bedford, NH) was passed through the inside of the AI gun and into the body of the uterus. A reference electrode was inserted into the vagina of the animal and both probes were left in place until a stable pH reading was obtained.

**Statistical Analysis**

Differences in ADG, DMI, N intake, and S intake were determined by ANOVA using PROC GLM (SAS Inst. Inc., Cary, NC). When a significant \( P \leq 0.05 \) effect of treatment was detected, least squares means were separated by the PDIFF option of SAS. Concentrations of PUN, sulfate anion, progesterone, and uterine pH were analyzed by repeated measures using the MIXED procedure of SAS as described by Littell et al. (1998). All covariance structures were modeled in the initial analysis. The indicated best fit covariance structure, heterogeneous compound symmetry, was used for the final analysis. The model was setup as a 2 × 2 factorial arrangement of treatments (with and without added S; and with and without added N), repeated over time. When the effect of time was not significant \( P > 0.05 \), data are reported by the main effect of treatment. When the effect of time was significant \( P < 0.05 \), data are reported as the treatment by time interaction. All data are reported as means ± SEM.

**RESULTS**

Throughout the experimental period, ADG did not differ \( P = 0.27 \) among the CON, HN, HS, and HNS dietary treatments \( (0.71 \pm 0.14, 0.62 \pm 0.08, 0.30 \pm 0.24, \text{and} \ 0.48 \pm 0.09 \text{kg/d, respectively} \). High nitrogen, HS, and HNS dietary treatments did not inhibit \( P > 0.20 \) heifer ADG over the 28-d experimental period compared with the CON diet. Heifers in the high N treatments (HN and HNS) consumed more \( P < 0.01 \) CP each day compared with heifers in the low N treatments (HS and CON), and heifers in the high S treatments (HS and HNS) consumed more \( P < 0.01 \) S each day compared with heifers in the low sulfur treatments (HN and CON; Table 3).

There was an effect of N \( P < 0.01 \) and time \( P < 0.01 \), but no effect of S \( P = 0.93 \) on concentrations of PUN. Furthermore, there was a N by time interaction \( P < 0.01 \) but no S by time interaction \( P = 0.53 \) on concentrations of PUN (Fig. 1). Concentrations of PUN were similar \( P > 0.10 \) before treatment (Fig. 1);
however, concentrations of PUN were greater ($P < 0.01$) in high N heifers 7 h after feeding on d 1 compared with low N heifers and remained increased for the entire study (Fig. 1).

There was an effect of S ($P < 0.01$) and tended to be an effect of time ($P = 0.06$), but no effect of N ($P = 0.13$) on plasma concentrations of sulfate. Furthermore, there was no N by time ($P = 0.89$) or S by time ($P = 0.41$) interaction on concentrations of sulfate, but there was a N by S interaction ($P = 0.03$; Fig. 2). Across d 1, 4, 8, 12, 16, 20, 24, and 28, concentrations of sulfate were greater in HS (209.45 ± 10.29 mg/kg) compared with CON (108.0 ± 10.3 mg/kg; $P < 0.01$), HN (113.4 ± 10.3 mg/kg; $P < 0.01$), and HNS (155.8 ± 10.3 mg/kg; $P < 0.01$; Fig. 2).

Additionally, concentrations of sulfate were increased in HNS compared with CON ($P < 0.01$, respectively) and HN ($P = 0.01$, respectively). Concentrations of sulfate in CON and HN were not different ($P = 0.71$).

There was no effect of N ($P = 0.81$) or S ($P = 0.53$) and no N by time ($P = 0.13$) or S by time ($P = 0.85$) interaction on plasma concentrations of progesterone. There was an effect of time ($P < 0.01$), with increasing concentrations after estrus consistent with normal corpus luteum formation (Fig. 3).

There was an effect of N ($P = 0.02$; Fig. 4) on uterine pH. Luteal phase uterine pH was increased ($P = 0.02$) in high N heifers (7.24 ± 0.03) compared with low N heifers (7.12 ± 0.03). However, there was no effect of S ($P = 0.23$), time ($P = 0.25$), and no N by time ($P = 0.33$) or S by time ($P = 0.57$) interaction on uterine pH.

**DISCUSSION**

Regulation of intracellular pH is important for proper embryonic growth and development (see review by Bavister, 2000). Research has reported that alteration of intracellular pH greater than (Zhao et al., 1995) or less than (Leclerc et al., 1996; Edwards et al., 1998; Lane et al., 1998) normal physiological intracellular pH can result in loss of embryonic developmental competence. This is because most cellular processes are pH sensitive and deregulation of intracellular pH can result in loss of normal cell function (FitzHarris and Baltz, 2009), impaired cell growth and proliferation (Grinstein et al., 1989; Kapus et al., 1994), and decreased cell survival (Pouysségur et al., 1984). In preimplantation hamster embryos, mitochondrial distribution is highly correlated with developmental competence (see review by Bavister, 2000). Changes in intracellular pH using weak acids or bases disrupted normal mitochondrial distribution and decreased embryo development (Lane et al., 1998). Furthermore, changes in the postinsemination culture environment are critical for the development and survival of the embryo (see review by Lonergan et al., 2006).

The negative effects of high protein intake on fertility have been reported in gilts (Dyck, 1991; Cassar et al., 1994), ewes (Wallace et al., 1994), and cattle (Jordan and Swanson, 1979; Canfield et al., 1990; Butler et al., 1996). Specifically in lactating dairy cows, research by

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**Table 3.** Daily DMI, CP, and sulfur by the control, high nitrogen, high sulfur, and high nitrogen and sulfur dietary treatments

<table>
<thead>
<tr>
<th>Item</th>
<th>Control2</th>
<th>High nitrogen2</th>
<th>High sulfur2</th>
<th>High nitrogen and sulfur2</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMI, kg/d</td>
<td>8.66</td>
<td>8.66</td>
<td>8.66</td>
<td>8.66</td>
<td>0.001</td>
</tr>
<tr>
<td>CP, g/d</td>
<td>1,063.09a</td>
<td>1,522.89b</td>
<td>1,071.60a</td>
<td>1,666.33b</td>
<td>73.18</td>
</tr>
<tr>
<td>S, g/d</td>
<td>13.63a</td>
<td>14.31a</td>
<td>43.79b</td>
<td>41.16b</td>
<td>1.61</td>
</tr>
</tbody>
</table>

1Values listed on a DM basis.
2Calculated values based on laboratory analysis of diet composite for the entire experimental period and orts composited weekly.

abValues within a row having different superscripts are different ($P < 0.001$).
Jordan and Swanson (1979) reported that dairy cows fed a 19.3% CP diet exhibited an increased number of days open, services per conception, and, subsequently, calving interval compared with dairy cows fed a 12.7% CP diet. However, work by Howard et al. (1987) compared lactating dairy cows fed either a 14.9% or 19.4% CP diet, even though concentrations of PUN were greater in cows fed the 19.4% CP diet (>24 mg/dL) compared with the 14.9% CP diet (~16 mg/dL). Interval to first estrus, days open, services per conception, and percentage of pregnant cows did not differ between treatments.

Research has also reported that high protein diets increased concentrations of BUN and MUN. Additionally, when BUN or MUN were >19 mg/dL, pregnancy rates, conception rates, and embryonic development in cattle were decreased (Ferguson et al., 1988, 1993; Blanchard et al., 1990; Butler et al., 1996; Rajala-Schultz et al., 2001). In addition, BUN >19 mg/dL decreased preimplantation embryo development and survival in sheep (Butler et al., 1996; Meza-Herrera et al., 2006), compared with concentrations <10 mg/dL (Rajala-Schultz et al., 2001). Increased concentrations of BUN and MUN may impair fertility through alteration of the uterine environment. Research by Elrod et al. (1993) and Elrod and Butler (1993) found that excess protein intake in dairy heifers and cows increased concentrations of PUN (~20.75 and ~23.6 mg/dL, respectively), and decreased uterine pH (~6.9 and ~6.79, respectively) on d 7 compared with those fed a balanced diet (PUN: ~16.1 and ~10.2 mg/dL, respectively; uterine pH: ~7.13 and ~7.09, respectively). Additionally, lactating dairy cows fed a 23% CP diet had decreased uterine concentrations of Mg, K, and P on d 5 of the estrous cycle, compared with cows fed a 12% CP diet (Jordan et al., 1983). These changes in the ionic composition of the uterine fluid may negatively influence enzymes responsible for pH regulation to reduce uterine pH.

In contrast, increased concentrations of PUN between HN and HNS in this study were associated with increased uterine pH compared with controls. The increased uterine pH reported in this study may have been due to increased concentrations of blood NH₃. The current study used both normal urea and a slow release urea in the diet. Digestion of this slow release urea may have resulted in a sustained release of NH₃ by the rumen and an increase in NH₃ could have resulted in the increase in uterine pH. This is supported by Jordan et al. (1983), who reported increased concentrations of blood NH₃ in lactating dairy cows fed excess protein. Furthermore, Hammon et al. (2005) reported increased concentrations of NH₃ in uterine secretions of lactating dairy cows fed excess protein. Increased concentrations of blood NH₃ may be produced during ruminal fermentation due to NH₃ escaping entrance into the urea cycle as a result of inadequate uptake by hepatic cells in the liver (Jordan et al., 1983) or by NH₃ diffusing across the peritoneal cavity and entering into peripheral circulation without passing through the liver (Visek, 1978). Additionally, excess protein intake can cause ruminal alkalosis and increased NH₃ production, which results in hepatic cell disorders and disruption of the urea cycle (Sommer, 1975).

In this study, intake of the high S diet resulted in increased concentrations of plasma sulfate. However, these increased concentrations of plasma sulfate were not associated with a decrease in uterine pH. This is different from previous research by Perry et al. (2009), who reported a negative correlation between concentrations of blood sulfate and uterine pH in heifers on d 7 and 11 of the estrous cycle. In this study, heifers were fed 18.1 or 31.3 g/d of dietary S had decreased uterine pH from...
d 7 to 11 compared with heifers fed 9.6 g/d of dietary S. Additionally, concentrations of blood sulfate increased from d 7 to 11 as uterine pH decreased. Mardon and others (2008) reported increased intake of sulfur AA by rats resulted in increased low grade metabolic acidosis due to sulfate. Calcium cations neutralize sulfate anions produced from catabolism of sulfur AA and rats fed the high protein diet had increased glomerular filtration rate and decreased tubular reabsorption of Ca cations (Alpern and Sakhaee, 1997). Goff et al. (2004) reported that CaSO4 could significantly reduce the pH of both blood and urine, but Ca had a small but significant alkalinizing effect when accompanying sulfate.

The effect of altering the dietary cation-anion difference (DCAD) on blood and urinary pH has been well established. Generally, as DCAD of the diet decreases, so does pH. In mature beef cattle, Hersom et al. (2010) reported a tendency for the pH of uterine flushes of cows consuming diets containing –0.9 mEq/100 g DM to be slightly lower than those of cows fed 25 mEq/100 g (6.13 and 6.39, respectively). This response would be consistent with research previously reported with high sulfur diets (Perry et al., 2009). Hersom et al. (2010) fed diets containing a broader range of DCAD values than those of the current experiment (–0.9 to 25 mEq/100 g vs. 0.1 to 20.6 mEq/100 g DM, respectively). However, the reason a decrease in uterine pH values in cows on low DCAD diets (HS and HNS) was not observed in the current experiment is unclear. As mentioned previously, it is possible that the additional dietary Ca from the CaSO4 used in the current experiment contributed additional buffering or alkalinizing capacity that mitigated the effect of decreased DCAD on uterine pH.

As discussed previously, high protein intake has been reported to impair embryo development and survival, and decrease pregnancy rates in cattle. Because increased concentrations of PUN and sulfate did not decrease uterine pH in this study, other mechanisms may be responsible for decreased fertility when cattle are fed excess N and S. A review by Promitzer and Krebs (2006) reported that intake of greater quantity of AA alters glucose metabolism through changes in the ratio of glucagon and insulin in the portal circulation. Glucose is the major energy substrate for preimplantation embryo growth and development, and disruption of glucose availability can result in increased embryo mortality (Brinster, 1965). Additionally, a review by Adams (1995) reported that legumes high in phytoestrogens, specifically isoflavones, have been reported to cause infertility in sheep (Bennetts et al., 1946). This is due to their similar chemical structure to estrogen that permits their binding to estradiol receptors in the endometrium to produce estrogenic activity (see review by Adams, 1995).

In summary, excess protein intake has been reported to decrease pregnancy rates due to increased concentrations of BUN and MUN that decrease uterine pH. Additionally, high S intake has been reported to decrease uterine pH. Results of this study found feeding excess N increased concentrations of PUN and feeding excess S increased plasma sulfate. However, neither excess N (increased PUN) nor S (increased plasma sulfate) decreased uterine pH. Therefore, further research is necessary to determine other mechanisms that may be decreasing uterine pH when high protein diets are fed and impairing fertility when protein and/or S are fed in excess.

**LITERATURE CITED**


