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**Uterine environment and pregnancy rate of heifers
with high blood urea concentrations¹**

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SUMMARY

Reports demonstrate that excess dietary protein significantly alters the ionic composition of uterine fluid during the luteal phase ultimately decreasing fertility. Since the early bovine embryo cannot adapt to changes in the uterine environment, changes in the concentrations of ions (pH) in the uterus can be unfavorable to embryo development and survival therefore having negative effects on fertility. In this study, heifers fed a high protein diet had elevated systemic concentrations of plasma urea nitrogen (PUN) compared to heifers fed a control diet. However, there was no deleterious effect on uterine pH or reproductive success. In summary, excess protein in a diet did increase PUNs to a concentration that has previously been reported to be detrimental to pregnancy success; however, there was no negative effect on uterine pH or pregnancy success.

INTRODUCTION

For both beef and dairy production, one of the most important indicators of success is reproductive efficiency. Multiple studies have reported that high protein intakes at the time of breeding have negative effects on fertility (Dyck, 1991; Cassar et al., 1994; Wallace et al., 1994; Gath et al., 2012). Dietary protein is comprised of rumen degradable protein (RDP) and rumen undegradable protein (RUP). Rumen degradable protein is either used for microbial protein synthesis or nitrogen released from the protein is absorbed through the rumen and converted to urea in the liver. Rumen undegradable protein may be absorbed by the small intestine, and excessive amino acids are catabolized at the liver producing urea. Elrod et al. (1993) determined that protein fed in excess, regardless of source or its degradability impacted uterine pH. Historically, uterine pH on d 7 has been reported to be lower in heifers fed high protein diets (Elrod and Butler, 1993; Elrod et al., 1993), whereas at time of estrus uterine pH does not differ between treatments and is usually decreased (Perry and Perry, 2008a; 2008b). However, Grant et al. (2013) reported an increase in uterine pH on d 7 of the estrous cycle when beef and dairy heifers were fed urea to increase PUN concentrations. We hypothesized that feeding a high protein diet would increase urea concentrations within the uterus causing an increase in uterine pH as well as decrease pregnancy success. Therefore, the objective of this experiment was to determine the effects of feeding a high protein diet on uterine environment and pregnancy success in beef heifers.

MATERIALS AND METHODS

All experiments were approved by the USDA Meat Animal Research Center Institutional Animal Care and Use Committee. Yearling heifers (n = 150) were utilized for this study. Heifers were blocked based on

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breed type, age, and body weight. Within blocks, 3 contemporary groups were established ($n = 50$ heifers/replicate) and heifers were then randomly assigned to 1 of 2 dietary treatments ($n = 25$ heifers/diet): Control (30% ground alfalfa hay, 64.8% corn silage, 0.2% salt, and 5% corn; 10% CP) or High Protein (29.79% ground alfalfa hay, 64.35% corn silage, 0.2% salt, 4.97% soybean meal, and 0.07% urea; 14% CP). Replicates were staggered by 1 week. Heifers were maintained on their treatment diets from 60 d prior to uterine pH determination through the entire breeding season. Heifers were injected with PGF_{2 α} (25 mg as 5 mL of Lutalyse i.m.; Zoetis Animal Health; Florham Park, NY) and HeatWatchTM patches were applied 53 d after initiation of treatment. The HeatWatchTM data were used to determine day of the estrous cycle when uterine pH was measured. Uterine pH was determined on d 7 of the estrous cycle.

Following uterine pH determination on d 60, the heifers were injected with PGF_{2 α} and housed with four 2- or 3-year-old bulls for a 21-d natural service-breeding season (2 bulls/treatment group). The same bulls were utilized in replicate 1 and 3; 4 different bulls were used in replicate 2. Prior to mating, bulls were adapted to the experimental diets. Thirty days after the breeding season pregnancy status was determined by transrectal ultrasonography. Plasma urea nitrogen, and uterine pH, percent observed in estrus, interval from PGF_{2 α} to estrus, duration of estrus, and number of mounts recorded were analyzed using the MIXED procedure of SAS (9.2 SAS Inst. Inc., Cary, NC) with diet (Control or High Protein), replicate (1,2, or 3), and the interaction as the fixed effects.

RESULTS AND DISCUSSION

During estrus, uterine pH has been observed to decrease from 7.0 to 6.7, and following estrus uterine pH returns to ~ 7.0 prior to ovulation (Perry and Perry, 2008a; Perry and Perry, 2008b). Concentrations of urea nitrogen in plasma and/or milk have been monitored and associated with effects on ovarian or uterine physiology. It has been reported that concentrations above 19 mg/dL PUN decreased uterine pH and consequently decreased fertility in dairy cows (Butler, 1998, 2000; Ferguson and Chalupa, 1989). Observations from the present study indicate that high protein diets generated higher PUN concentrations compared to Control heifers (23.48 ± 0.36 mg/dL vs 13.33 ± 0.36 mg/dL; $P < 0.001$; Table 1) but had no effect on uterine pH on d 7 of the estrous cycle ($P = 0.59$; Table 1). Similarly, Elrod et al. (1993) and Elrod and Butler (1993) found that excess dietary protein intake in cows and nulliparous heifers had increased PUN concentrations (> 20 mg/dL, respectively), but, in contrast to this study uterine pH was decreased on d 7 compared with animals fed a standard diet.

There was no effect of estrus status ($P = 0.40$), or interval to estrus ($P = 0.77$; Table 2) on uterine pH, and there was no effect of diet ($P = 0.90$; Table 2), replicate ($P = 0.51$), or the diet by replicate interaction ($P = 0.40$) on pregnancy success during the 21 d breeding season. Some research has reported no decline in fertility among cows fed diets with PUN concentrations > 24 mg/dL (Carroll et al., 1988; Howard et al., 1987). Nonetheless, studies that have tried to account for reproductive management (Elrod and Butler, 1993) have reported that heifers with prebreeding PUN concentrations of ≥ 16 mg/dL had conception rates approximately 30% lower than heifers with PUN concentrations of < 16 mg/dL. In the present study, there was no negative effect on conception rates even when heifers were fed a diet high in protein and PUN concentration were > 23 mg/dL. This evidence would suggest that something in a high-protein diet may contribute to decreased conception rates, but it is likely not urea. Further research is necessary to determine the mechanism through which excess protein in a diet can impair reproductive function and decreased uterine pH.

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Table 1. Effect of dietary protein concentration on plasma concentrations of urea and uterine pH.

	Diet		P-value
	Control	High protein	
Heifers, n=	60	60	
Urea, mg/dL	13.33 ± 0.36	23.48 ± 0.36	0.001
Uterine pH	6.85 ± 0.03	6.87 ± 0.03	0.68

Table 2. Effect of dietary protein concentration on estrus activity and pregnancy rate.

	Diet		P-value
	Control	High protein	
Heifers, n=	60	60	
Interval from PG to estrus, d	2.36 ± 0.17	2.43 ± 0.17	0.77
Pregnancy rate, %	43 ± 6	44 ± 6	0.90