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Uterine environment and pregnancy rate of heifers with elevated plasma urea nitrogen☆

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ABSTRACT

Diets high in protein are associated with lower reproductive performance and changes in the uterine environment. The objective of this study was to determine the effect of elevated systemic concentrations of urea nitrogen on the uterine environment and pregnancy success in beef heifers. Heifers (n = 150) were matched by breed, age, and body weight then randomly assigned to one of two dietary treatments: 1) Control (10% CP) or 2) High protein (14% CP) over three replicates (n = 40/replicate). Estrus was synchronized with an injection of $PGF_{2\alpha}$. Uterine pH, plasma urea nitrogen (PUN), ammonia, and glucose concentrations were determined on d 7 of the estrous cycle. Pregnancy status was determined by ultrasonography 30 d following the breeding season. In vitro fertilization was performed on heifers precluded from uterine analysis (n = 15/diet) to determine the effect of a High Protein diet on oocyte quality. Plasma urea concentrations were greater in the High Protein diet compared to Control ($P < 0.001$). There was no effect of diet on plasma ammonia ($P = 0.12$), plasma glucose ($P = 0.40$), uterine pH ($P = 0.67$), interval to estrus ($P = 0.54$), duration of estrus ($P = 0.38$), or pregnancy rate ($P = 0.83$). There was no effect of diet ($P > 0.40$) on the number of oocytes collected, number of oocytes cleaved, amount of blastocysts, percentage of oocytes cleaved and percentage of blastocysts present. In summary, high nitrogen diets increased PUN concentrations in heifers; however, there were no deleterious effects on reproduction.

1. Introduction

For both beef and dairy production, one of the most important indicators of success is reproductive efficiency. Increased milk production in the dairy industry has been dependent on feeding increased amounts of dietary protein in addition to energy (Butler, 2000). As milk production has increased, fertility has decreased, and diets with high protein levels have been associated with decreased pregnancies per AI in lactating cows (Lean et al., 2012). Elrod et al. (1993) proposed that this decrease in fertility was associated with a reduced uterine pOcon and Hansen (2003) found that decreasing pH in in vitro cul-

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Table 1
Nutrient composition of Control and High Protein dietary treatments.

Items[a]	Diets	
	Control	High Protein
Ground alfalfa hay%	30	29.79
Corn silage%	64.8	64.34
Salt%	0.2	0.2
Corn%	5	–
Soybean meal%	–	4.97
Urea%	–	0.7
CP%	10	14

[a] Value listed in DM basis.

tures decreased embryo development suggesting that the reduced fertility observed in cows fed high protein diets may be associated with a decrease in uterine pH. Elrod and Butler (1993) reported a decrease in uterine pH and fertility among heifers fed a high protein diet; however, Grant et al. (2013) reported an increase in uterine pH among heifers in a positive energy balance fed nitrogen supplements. However, all of these studies have been associated with an increase in circulating concentrations of urea. We hypothesized that high dietary protein would increase systemic urea and alter uterine pH as well as decrease pregnancy rates.

2. Materials and methods

2.1. Experimental design

All experiments were approved by the South Dakota State University (15-042A) and the USDA U.S. Meat Animal Research Center Institutional Animal Care and Use Committees. Yearling heifers (n = 150) born in the spring of 2011 were utilized for this study. Before allotment to diets, heifers received standard diets and management for 60 d. Following 60 d, heifers were blocked based on breed type, age, and BW. Within blocks, three contemporary groups were established (n = 40 heifers/replicate) and heifers were then randomly assigned to one of two dietary treatments (n = 20 heifers/diet): Control (10% CP; Table 1) or High Protein (14% CP; Table 1). Replicates were staggered by one week. Heifers were maintained on their respective diet for 60 d before uterine pH was determined and remained on their diet through the entire breeding season.

2.2. Blood collection, uterine pH, plasma urea concentrations

Heifers were injected with $PGF_{2\alpha}$ (5 mL of Lutalyse i.m.; Zoetis Animal Health; Florham Park, NY) and HeatWatch™ (CowChips; Manalapan, NJ) patches were applied 53 d after initiation of treatment. Estrus was defined as the period a female stood to be mounted for 3 s or longer. The HeatWatch™ data were used to determine day of the estrous cycle when uterine pH was measured. Ten days following the $PGF_{2\alpha}$ injection, uterine pH was determined (20 heifers/diet per replicate) as described by Perry and Perry (2008a). Briefly, a sterile, plastic sheath was placed over an AI gun then inserted through the cervix into the uterus. Once positioned in the uterine body, a flexible pH electrode (1.4 mm diam.; Microelectrodes, Bedford, NH) was passed through the inside of the sheath into the uterine body. A reference electrode was inserted into the vagina of the heifer and both probes remained in place until a stable pH reading was acquired. A 5-mL blood sample was collected by jugular venipuncture at the time that uterine pH was determined, in a heparinized tube, to measure circulating concentrations of plasma urea, ammonia, and progesterone. Blood samples were centrifuged for 25 min; plasma was removed and stored at −20 °C. Subsequently, plasma samples were analyzed for urea, ammonia, and glucose concentrations. All analyses were completed by automated procedures (Technicon Industrial Systems, Tarrytown, NY) as follows: ammonia-*N* by a hypochlorite method (Technicon Industrial Systems, 1974), urea-*N* by a diacetylmonoxime method (Technicon Industrial Systems, 1977), and glucose described by Huntington (1984).

Plasma samples were analyzed for progesterone concentrations by RIA with the use of the method described by Engel et al. (2008). Heifers with progesterone concentrations <1 ng/mL on d 7 were removed from analysis.

2.3. Pregnancy status

Following uterine pH determination, heifers were injected with $PGF_{2\alpha}$ (5 mL of Lutalyse i.m.; Zoetis Animal Health; Florham Park, NY) and housed with four 2- or 3-year-old bulls for a 21-d natural service-breeding season (2 bulls/diet). The same bulls were utilized in replicate 1 and 3, and four 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.

2.4. In vitro fertilization (IVF)

A subset of thirty heifers was identified (n = 15/diet) and not used for determination of uterine pH. Three weeks following the uterine pH experiment (Fig. 1), 15 of these heifers (Control = 8 and High Protein = 7) were given two injections of $PGF_{2\alpha}$ (5 mL, i.m. Zoetis Animal Health; Florham Park, NY) 11 d apart to synchronize estrous. At the second injection, heifers were fitted with heat detection patches (e.g., Estrotect™, Western Point, Inc, Apple Valley, MN) and detection of behavioral estrus was performed twice daily from 48- to 96-h post-injection. Four days following observed estrus, heifers were transported to a local abattoir where the reproductive tracts were harvested. The remainder of the heifers (Control = 7 and High Protein = 8) were handled in the same manner offset by one week. Heifers were handled in two groups for ease of management for the abattoir and setting up cultures.

At harvest, ovaries were collected, placed in a beaker containing physiological saline (0.9%) with antibiotics (100 I.U. penicillin and 100 μg streptomycin/mL) at 37 °C, and immediately transported to the laboratory where oocytes were collected to produce embryos by IVM-IVF as described by Hansen (2013). Briefly, follicles (1–8 mm in diameter) were aspirated and the contents