



Removal of ruminal contents followed by restricted feeding does not affect the frequency of luteinizing-hormone pulses in steers¹

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ABSTRACT

Our objective was to determine the effects of acute nutrient restriction on secretion of luteinizing hormone (LH) in serum of male cattle. Angus × Hereford steers (402 ± 17 kg) with rumen cannulas were randomly assigned to treatment. Control (n = 4) steers had ruminal contents completely removed and immediately replaced, whereas restricted (n = 4) steers had ruminal contents completely removed and only 15% (5 L) of rumen contents replaced. Beginning at 1600 h

on d 0, control steers were fed 9 kg of prairie hay and 1 kg of a grain supplement each day, whereas restricted steers were fed 1.8 kg of prairie hay daily. Blood samples were collected twice daily for determination of plasma concentrations of glucose, nonesterified fatty acids, and urea nitrogen. Serial blood samples were collected at 10-min intervals for 8 h on d -1, 0, 1, and 3 to determine serum concentrations of LH. Concentrations of LH (P < 0.01) were greater on d -1 compared with after removal and replacement of ruminal contents on d 0 and 1. Amplitude of LH pulses was greater on d -1 (P < 0.02) compared with d 1 and 3. Concentrations of glucose in plasma were not influenced by treatment or day, but concentrations of nonesterified fatty acids and urea nitrogen in plasma after d 0 were greater (P < 0.01) in restricted than control steers. It is concluded that secretion of LH in steers is resistant to rapid changes in nutrient availability.

INTRODUCTION

During beef production, cattle can experience periods of acute nutrient deprivation such as during snow storms, transportation, or flooding. Inadequate nutrition can have negative consequences for reproduction in cattle by suppressing secretion of luteinizing hormone (LH; Imakawa et al., 1986; Bossis et al., 1999). The secretory pattern of LH from the anterior pituitary gland stimulates secretion of testosterone from the testes (Desjardins, 1981). Adequate nutrition is important for maintenance of normal reproductive function in bulls (Brown, 1994; Barth et al., 2008). Prolonged but moderate nutritional restriction, as might occur during extended drought, was found to suppress LH secretion in cows (Richards et al., 1989) and bulls (Gauthier and Berbigier, 1982). Inadequate LH in bulls leads to reduced serum concentrations of testosterone, reduced testicular weight, and decreased sperm production (Schanbacher, 1984a,b,

¹Approved for publication by the director, Oklahoma Agricultural Experiment Station. This research was supported under project H 2331.

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Key words: cattle, luteinizing hormone, nutrient restriction, physiology, steer

1985). It is unknown how acute nutritional restriction affects these mechanisms in male cattle. This is important because impairments in sperm morphology or production resulting from nutritional-induced reductions in LH cannot be resolved by simply refeeding bulls (Brown, 1994), because it takes approximately 61 d for the affected sperm to be ejaculated (Amann and Schanbacher, 1983).

Pulsatile secretion of LH in heifers was suppressed with a 2- to 3-d fast (Amstalden et al., 2000; Maciel et al., 2004), whereas others report that fasting heifers for 3 d had no effect on pulsatile LH secretion (Kadokawa and Yamada, 1999), or that LH secretion of heifers was affected only after chronic (7-d) fasting (McCann and Hansel, 1986). There are no data, which we know of, about how acute nutritional restriction affects LH in male cattle. The objective of this study was to determine the effects of removal of ruminal contents and restricted feeding on concentrations of LH in serum of steers. Steers were used because gonadectomy removes the inhibitory feedback of testosterone and results in increased LH pulse frequency, allowing the potential suppressive effects of nutritional restriction on LH secretion to be clearly measured. The hypothesis was that removing rumen contents and limiting nutrient intake would suppress mean concentrations of LH by reducing LH pulse frequency.

MATERIALS AND METHODS

All animal procedures were approved by the Institutional Animal Care and Use Committee of Oklahoma State University. Angus \times Hereford steers in the current study were castrated by banding at birth. At initiation of the experiment, steers weighed 402 ± 17 kg and had moderate BCS (4.6 ± 0.2 ; 1 = emaciated, 9 = obese; Wagner et al., 1988). Animals had rumen cannulas and were confined in stalls in a barn at $21 \pm 4^\circ\text{C}$ with 14 h of light. Steers were blocked by BW and randomly assigned to treatment ($n = 4/\text{treat-}$

ment). On d 0, between 0900 and 1100 h, ruminal contents were completely removed. For control (**CON**) steers, rumen contents were immediately placed back into the same steer. For restricted (**RST**) steers, only 15% (5 L) of rumen contents were replaced. Steers were not fed the morning rumen contents were removed (d 0), but all steers had ad libitum access to water throughout the experiment. Beginning at 1600 h on d 0, CON steers were fed 9 kg of prairie hay (CP = 5.8% DM basis) and 1 kg of a supplement (40% CP, 2 Mcal/kg NE_m, 1.36 Mcal/kg NE_g on a DM basis), whereas RST steers were only fed 1.8 kg/d of prairie hay. Restricted steers did not receive a supplement. Under these conditions, CON steers were fed 211% and RST steers were fed 32% of their daily maintenance energy requirements. Steers were fitted with a polyvinyl jugular catheter (i.d., 1.68 mm; o.d., 2.39 mm; BB 317 v11, BioLab, Lake Havasu City, AZ) 2 d before collection of blood samples (10 mL; d -3). Serial blood samples were collected at 10-min intervals for 8 h commencing at 1200 h on d -1, 0, 1, and 3. Samples were allowed to clot for 24 h at 4°C and centrifuged at $2,500 \times g$ for 20 min. Serum was decanted and stored at -20°C . Blood samples were collected twice daily (0800 and 1600 h) on d -1 to 3 into tubes containing EDTA (0.1 mL of a 15% solution), placed on ice, and centrifuged ($2,500 \times g$ for 15 min) to obtain plasma.

Concentrations of glucose in plasma were quantified by an enzymatic colorimetric procedure (No. 510, Sigma Chemical Co., St. Louis, MO). Intra- and interassay CV were 4 and 14%, respectively. Concentrations of non-esterified fatty acids (**NEFA**) in plasma were determined by an enzymatic colorimetric procedure (Wako-NEFA C, Wako Chemicals Inc., Dallas, TX) with modifications (McCutcheon and Bauman, 1986) and expressed as microequivalents of palmitate per liter. Intra- and interassay CV were 6 and 11%, respectively. Concentrations of urea nitrogen in plasma were quantified with an enzymatic proce-

dures (640-B, Sigma Chemical Co.). Concentrations of LH in serum were quantified by RIA (Bishop and Wettemann, 1993) using NIH LH-B9 as the standard. Intra- and interassay CV were 8 and 19%, respectively.

Frequency and amplitude of LH pulses were determined using the PC pulsar program (Merriam and Wachter, 1982). A mixed model ANOVA (PROC MIXED, SAS Institute Inc., Cary, NC) for repeated measures was used to determine effects of treatment on concentrations of glucose, NEFA, plasma urea nitrogen (**PUN**), and LH, and on frequency and amplitude of LH pulses. The model included the fixed effect of treatment, with day and sample as the repeated units. The within-animal covariance structure for the repeated measure was modeled by a first-order autoregressive function with lag equal to one. Fisher's least significant difference was used to compare means when *F*-tests for day effects were significant.

RESULTS AND DISCUSSION

Increasing world population and limited natural resources drive an ever greater need to increase the efficiency of animal protein production. Beef production will need to adapt to increased volatility in weather patterns driven by climate change. This has sparked renewed interest in increasing the understanding of how nutrition effects reproductive efficiency of bulls (Martin et al., 2010). Most research has focused on how minimal but long-term nutritional restriction, such as might occur under prolonged drought, affects the hypothalamic-pituitary-gonadal axis of the bull (Gauthier and Berbigier, 1982). During beef production, cattle can experience periods of acute nutrient deprivation such as during snow storms, transportation, or flooding. Acute nutritional restriction can affect LH secretion and reproduction in female cattle (Amstalden et al., 2000; Maciel et al., 2004; Lents et al., 2013), but there is no information on the effects of acute nutritional restriction on the reproductive hypothalamic-

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