# Effect of increasing levels of undegradable intake protein on metabolic and endocrine factors in estrous cycling beef heifers[1,2]

**K. K. Kane*, D. E. Hawkins*[3], G. D. Pulsipher*[4], D. J. Denniston*[5], C. R. Krehbiel*[6], M. G. Thomas*, M. K. Petersen*, D. M. Hallford*, M. D. Remmenga†, A. J. Roberts‡, and D. H. Keisler§**

*Department of Animal and Range Sciences and †University Statistics Center, New Mexico State University, Las Cruces 88003; ‡USDA-ARS, Fort Keogh LARRL, Miles City, MT 59301; and §Department of Animal Sciences, University of Missouri, Columbia 65211

**ABSTRACT:** To determine the influence of three levels of undegradable intake protein (UIP) supplementation on metabolic and endocrine factors that influence reproduction, 23 yearling crossbred heifers (body condition score = 4.5 ± 0.5; initial BW = 362 ± 12 kg) were stratified by BW and assigned randomly to one of three supplements: 1) low UIP (1,135 $g \cdot heifer^{-1} \cdot d^{-1}$; 30% CP, 115 g UIP, n = 7); 2) mid UIP (1,135 $g \cdot heifer^{-1} \cdot d^{-1}$; 38% CP, 216 g UIP, n = 8); or 3) high UIP (1,135 $g \cdot heifer^{-1} \cdot d^{-1}$; 46% CP, 321 g UIP, n = 8). Heifers were estrually synchronized before initiation of supplementation. Supplement was individually fed daily for 30 to 32 d, at which time heifers were slaughtered (d 12 to 14 of the estrous cycle) and tissues collected. Heifers were fed a basal diet of sudan grass hay (6.0% CP) ad libitum. On d 28 of supplementation (d 10 of the estrous cycle), no differences were observed ($P > 0.10$) in serum insulin or IGF-I among treatments. At slaughter (d 10 to 12 of the estrous cycle), treatments did not influence corpus luteum weight, cerebral spinal fluid leptin, or IGFBP; serum estradiol-17$\beta$, progesterone, leptin, IGF-I, and IGFBP; or anterior pituitary content of IGFBP ($P > 0.10$). Follicular fluid IGFBP-2 and IGFBP-4 were greater in high-UIP heifers than low- or mid-UIP heifers on d 12 to 14 of the estrous cycle ($P < 0.05$). Basal serum LH concentrations and LH area under the curve (every 15 min for 240 min) did not differ ($P > 0.10$) following 28 d of supplementation (d 10 of the estrous cycle); however, basal serum FSH concentrations were greater ($P = 0.06$) in low- and mid- vs. high-UIP heifers (5.2 and 5.2 vs. 4.6 ng/mL, respectively), and FSH area under the curve was greater ($P = 0.03$) in low- vs. high-UIP heifers. At slaughter (d 12 to 14 of the estrous cycle), anterior pituitary LH and FSH content and steady-state mRNA encoding $\alpha$, LH$\beta$, and GnRH receptor did not differ ($P > 0.10$) among treatments. However, FSH$\beta$ mRNA was increased approximately twofold ($P = 0.03$) in mid vs. low UIP. In summary, low and mid levels of UIP supplements fed to estrous cycling beef heifers seemed to enhance pituitary expression and/or secretion of FSH relative to high levels of UIP. Moreover, high-UIP supplementation was associated with increased low-molecular-weight IGFBP compared with supplementation of low and mid levels of UIP. These data suggest that differing levels of UIP supplementation may alter pituitary and ovarian function, thereby influencing reproductive performance in beef heifers.

Key Words: Beef Heifers, Follicle-Stimulating Hormone, Insulin-Like Growth Factor-I, Insulin, Luteinizing Hormone, Undegradable Intake Protein

*©2004 American Society of Animal Science. All rights reserved.* J. Anim. Sci. 2004. 82:283–291

## Introduction

Protein has been targeted for use in supplementation programs, as it is the first-limiting nutrient in dormant-season forage diets consumed by beef cattle (Wallace, 1987). Protein supplementation in beef cattle can in-

[1]Research supported by the New Mexico Agric. Exp. Stn., Las Cruces. This research is a contribution of the Western Regional Project W112.

[2]The authors gratefully acknowledge the USDA Animal Hormone Program, National Hormone and Pituitary Program of NIDDK, and A. F. Parlow for LH and FSH assay materials.

[3]Correspondence: Box 30003, Dept. 3-I (phone: 505-646-4135; fax: 505-646-5441; e-mail: dhawkins@nmsu.edu).

[4]Present address: Eastern Ag. Res. Center, Oregon State University, P.O. Box E, Union 97883.

[5]Present address: Dept. of Animal Science, Colorado State University, Fort Collins 80523.

[6]Present address: Dept. of Animal Science, 208 Anim. Sci. Bldg., Oklahoma State Univ., Stillwater 74078.

Received April 21, 2003.

Accepted September 24, 2003.

**Table 1.** Ingredient composition of low-, mid-, and high-undegradable intake protein (UIP) supplements fed to cycling beef heifers[a]

| Ingredient, % as fed | Low UIP | Mid UIP | High UIP |
|---|---|---|---|
| Cottonseed meal | 53.41 | 34.51 | — |
| Wheat middlings | 32.10 | 25.80 | 37.65 |
| Feather meal | — | 20.30 | 41.91 |
| Fish meal, 60% | — | 3.50 | 5.30 |
| Dry molasses | 7.50 | 7.50 | 7.50 |
| Molasses | 5.80 | 5.60 | 5.50 |
| Dicalcium phosphate | — | 1.60 | 0.40 |
| Urea, 288% CP | 0.70 | 0.70 | 0.70 |
| Potassium chloride | 0.35 | 0.35 | 0.90 |
| Trace mineral | 0.10 | 0.10 | 0.10 |
| Vitamin A | 0.04 | 0.04 | 0.04 |

[a]Supplements were manufactured by a commercial feed mill (Hi Pro, Friona, TX).

**Table 2.** Daily nutrients delivered for low-, mid-, and high-undegradable intake protein (UIP) supplements[a]

| Nutrient | Low UIP | Mid UIP | High UIP |
|---|---|---|---|
| Supplement, $g \cdot heifer^{-1} \cdot d^{-1}$ | 1,135 | 1,135 | 1,135 |
| CP, % | 30 | 38 | 46 |
| CP, $g \cdot heifer^{-1} \cdot d^{-1}$ | 341 | 431 | 522 |
| DIP, $g \cdot heifer^{-1} \cdot d^{-1}$ | 226 | 215 | 201 |
| UIP, $g \cdot heifer^{-1} \cdot d^{-1}$ | 115 | 216 | 321 |
| TDN, $g \cdot heifer^{-1} \cdot d^{-1}$ | 777 | 719 | 692 |

[a]Nutrient composition data were provided by HiPro, Friona, TX.

crease forage intake and digestibility, improve lactation, and enhance reproduction (Caton et al., 1988; Short et al., 1990; Wheeler et al., 2002). After meeting requirements for degradable intake protein (**DIP**), additional protein supplied as undegradable intake protein (**UIP**) may decrease the duration of postpartum anestrus and BW loss (Wiley et al., 1991; Appeddu et al., 1996; Anderson et al., 2001) and improve conception rate (Triplett et al., 1995; McCormick et al., 1999). However, UIP may be detrimental to fertility and/or establishment of pregnancy (Elrod et al., 1993; Appeddu et al., 1997).

Nutritionally influenced mediators of energy balance and reproductive function, such as insulin, the IGF system, and leptin (Gutierrez et al., 1997; Snyder et al., 1999; Sansinanea et al., 2001) may be modified by protein supplementation (Hunter and Magner, 1988; Wiley et al., 1991; Noguchi, 2000). The hypothalamo-hypophyseal-ovarian axis appears to play a critical role in the integration of nutritional status and reproduction (Schillo, 1992; Keisler and Lucy, 1996; Wiltbank et al., 2002). Decreased secretion of LH and FSH is associated with undernutrition in beef cows (Nolan et al., 1988; Richards et al., 1989). Supplementation with 335 g UIP $\cdot cow^{-1} \cdot d^{-1}$ increased GnRH-induced LH secretion in postpartum beef cows (Kane et al., 2002). Thus, reproductive events may be altered by UIP supplementation via endocrine and metabolic factors. However, the level of UIP supplementation at which changes in reproductive function occur has not been clearly defined.

The objective of this study was to determine whether UIP supplementation at three levels alters metabolic and endocrine factors associated with reproductive function in nulliparous estrous cycling beef heifers.

## Materials and Methods

### *Animals and Treatments*

Twenty-three estrous cycling Angus × Hereford yearling heifers (14 to 16 mo; initial body condition score = 4.5 ± 0.5; initial BW = 362 ± 12 kg) were stratified by BW and randomly assigned to one of three treatments. Treatments consisted of three levels of UIP supplement (Tables 1 and 2) as follows: 1) low UIP (1,135 $g \cdot heifer^{-1} \cdot d^{-1}$; 30% CP, 115 g UIP; n = 7); 2) mid UIP (1,135 $g \cdot heifer^{-1} \cdot d^{-1}$; 38% CP, 216 g UIP; n = 8); and 3) high UIP (1,135 $g \cdot heifer^{-1} \cdot d^{-1}$; 46% CP, 321 g UIP; n = 8). These supplements were designed to provide ruminally degradable protein in excess of NRC requirements for beef heifers and to differ in quantity of protein fed as UIP. Supplements were fortified with vitamins and macro- and microminerals to meet or exceed NRC requirements. The supplements were manufactured by a commercial feed mill (Hi Pro, Friona, TX). Nutrient composition data for the supplements were provided by the commercial feed mill (Tables 1 and 2). Two heifers in each treatment were randomly assigned to one of four collection periods. A collection period consisted of 1 wk of acclimation to supplementation followed by 30 to 32 d of supplementation and culminated in slaughter and the collection of tissues. Supplements were individually fed daily as loose meal at 0530 for 30 to 32 d. One week before the supplementation period, 550 g of an equal mix of all supplements was offered for 4 d, followed by 750 g of the assigned supplement for 3 d to acclimate heifers to supplements and feeding procedures. Heifers received a basal diet of chopped (5 cm) sudan grass hay (6.0% CP) ad libitum to simulate late-winter to early-spring dormant forage conditions. Sudan grass hay was fed for 10 d before and throughout the supplementation period. Hay intake was determined by weighing the hay fed each day and weighing orts before the next day's feeding. No differences were observed among treatments in hay intake ($P = 0.57$; 1.84, 1.86, and 1.77 ± 0.04% BW for low, mid, and high UIP, respectively). A commercial salt block (United Salt Corp., Houston, TX) and water were available free choice.

Heifers had at least two normal estrous cycles immediately before initiation of the study as confirmed by the presence of elevated (>1 ng/mL) concentrations of progesterone in at least three consecutive serum samples collected twice weekly. Estrus was synchronized using two injections of $PGF_{2\alpha}$ (25 mg, i.m., Lutalyse, Pharmacia & Upjohn, Kalamazoo, MI) administered 11 d apart before collection periods. Supplementation was

initiated on d 4 (estrus = d 0) of the first estrous cycle following synchronization. Heifers were slaughtered on d 12 to 14 of the second estrous cycle (corresponding with d 30 to 32 of supplementation) by captive bolt stunning and exsanguination. The study was conducted at the New Mexico State University Livestock Research Center, Las Cruces, NM, and all animal procedures were approved by the NMSU Institutional Animal Care and Use Committee and conformed with accepted guidelines (FASS, 1999).

*Sample Collection*

To evaluate basal serum LH and FSH concentrations, and serum insulin and IGF-I, an intensive blood collection was conducted on d 28 of supplementation, corresponding to d 10 of the estrous cycle. At 0500 on d 28, in-dwelling jugular cannulas were inserted and blood samples for serum LH and FSH were collected at time of supplementation (time 0) and then every 15 min for 240 min. Blood samples for insulin and IGF-I were collected at time 0 and every 6 h thereafter for 24 h. Following collection, blood samples were placed in 10-mL glass tubes, and blood separation crystals (Spin-Quik; Oti Specialties, Santa Monica, CA) were added to separate serum. Samples were allowed to coagulate for 1 h at ambient temperature and were centrifuged at 1,300 × *g* (25 min, 4°C) and serum stored at −20°C.

Immediately following slaughter on d 30 to 32 of the trial (d 12 to 14 of the second estrous cycle), blood samples, cerebral spinal fluid (**CSF**), anterior pituitary glands, follicular fluids, and corpora lutea (**CL**) were collected. Cerebrospinal and follicular fluid samples were obtained by needle and syringe aspiration from the occipital condyle area of the spinal column and follicles ≥7 mm, respectively, and placed on ice. Follicular fluid was aspirated and pooled by heifer from follicles for determination of IGFBP content. In addition, CSF samples were collected for leptin concentrations and relative amounts of IGFBP. Blood samples were collected for determination of serum progesterone, leptin, estradiol-17$\beta$, and IGFBP. Serum, CSF, and follicular fluid samples were stored at −20°C. Anterior pituitary glands were obtained to determine the content of LH, FSH, and IGFBP, as well as mRNA encoding GnRH receptor (**GnRH-R**), LH$\beta$, FSH$\beta$, and $\alpha$-subunits. Immediately following removal, the anterior pituitary gland was decapsulated, snap-frozen in liquid $N_2$, and stored at −80°C. Corpora lutea were obtained and immediately weighed.

*Measurements*

Radioimmunoassays. Serum insulin concentrations were measured using a commercial RIA kit purchased from Diagnostic Products Corp. (**DPC**; Los Angeles, CA; Reimers et al., 1982), with an intraassay CV of 6%. Serum concentrations of IGF-I were determined by RIA (Berrie et al., 1995), with an intraassay CV of 7%. Serum and CSF samples were analyzed for leptin using RIA (Delavaud et al., 2000), with an intraassay CV of 7%. Serum progesterone concentrations were quantified with a DPC progesterone RIA kit with modifications described by Schneider and Hallford (1996; intraassay CV = 5%).

Anterior pituitary gland LH and FSH were extracted for analysis by homogenizing 100 mg of anterior pituitary tissue midsagittal slices in 1 mL of 0.01 *M* phosphate-buffered saline (PBS; pH 7.4). Homogenates were centrifuged at 29,500 × *g* (30 min, 4°C) and supernatant decanted and stored at −20°C. Serum and pituitary homogenate samples were analyzed for LH content by RIA using procedures described by Hoefler and Hallford (1987; intraassay CV = 16%).

Analysis of pituitary homogenate samples for FSH concentration was performed by RIA (L'Hermite et al., 1972) with an intraassay CV of 10%. Concentration of FSH in serum was quantified by double-antibody RIA. Rabbit anti-oFSH (NIDDK-anti-oFSH-1) was diluted to 1:10,000. Radioiodination of purified bFSH (USDA-bFSH-I-2) was performed by the chloramine T method described for oLH by Niswender et al. (1969), except that 125I was used. Standards were prepared with appropriate dilutions of bFSH (USDA-bFSH-I-2) in 0.01 *M* PBS containing 1% bovine serum albumin (PBS + 1% BSA, pH 7.0). The second antibody (anti-rabbit gamma globulin) was produced in sheep using the method described by Swanson et al. (1972) and suspended at a working dilution of 1:6 in 0.01 *M* PBS containing 0.05 *M* EDTA (pH 7.0). The assay was performed by pipetting standard or serum (0.2 mL) into 12- × 75-mm disposable plastic culture tubes, and then the volume in each tube was normalized to 0.5 mL with PBS + 1% BSA. Rabbit anti-oFSH (0.2 mL in PBS-EDTA containing 1:400 normal rabbit serum) was added to each tube, followed immediately by addition of $^{125}$I-bFSH (0.1 mL containing approximately 24,000 cpm). Tubes were vortexed and incubated overnight at 4°C. Second antibody (0.2 mL) was added followed by a second incubation overnight at 4°C. On the third day, tubes were centrifuged at 3,100 × *g* for 15 min at 4°C. Supernatant was decanted and the radioactivity of the bound fraction determined with a Packard Cobra II gamma counter (Packard Bioscience Co., Downers Grove, IL). The addition of 1.0 ng of oFSH to the assay resulted in an approximately 27% reduction in the amount of $^{125}$I-bFSH bound to the antibody. When 12.5 ng of oFSH were added to wether serum, 93% was recovered. All samples were quantified in a single assay with an intraassay CV of 13%.

Serum estradiol-17$\beta$ was quantified by solid-phase RIA using components of a kit supplied by DPC. The assay utilized polypropylene tubes coated with an antibody against estradiol-17$\beta$ and $^{125}$I- estradiol-17$\beta$ as the tracer. The stated sensitivity of the assay for quantifying estradiol-17$\beta$ directly in serum was 7 pg/mL; a preliminary extraction step was therefore required before the RIA could be used for cattle/sheep serum. Du-

plicate 0.5-mL serum samples were extracted by vigorous vortexing with 4 mL of ethyl acetate:hexanes (2:1, vol:vol) in 13- × 100-mm borosilicate glass culture tubes. After freezing the serum, the solvent was transferred to 12- × 75-mm borosilicate glass tubes and evaporated to dryness at 80°C under a stream of nitrogen gas. Using this procedure, an extraction efficiency greater than 90% was routinely obtained. Tubes to contain estradiol-17$\beta$ standards received extract from charcoal-stripped steer or wether serum. Glass tubes containing the dried serum extract then received 0.8 mL of phosphate-buffered saline + 0.1% gelatin (**PBS + gel**), and the tubes were vortexed. The PBS + gel was subsequently transferred to the DPC antibody-coated tubes. A standard solution was prepared by suspending estradiol-17$\beta$ (Sigma) at 100 pg/mL in PBS + gel. This stock standard solution was pipetted into the antibody-coated tubes in amounts to provide a standard curve of 0, 2.5, 5, 10, 20, 40, and 80 pg of estradiol-17$\beta$ per tube. All tubes were then normalized to 1.3 mL using PBS + gel. Each tube then received 1 mL of DPC tracer, after which tubes were vortexed and incubated at room temperature for 4 to 5 h. Tubes were subsequently decanted and counted for 1 min in a Packard Cobra II gamma counter. The cross-reactivity at 50% displacement of the anti-estradiol-17$\beta$ was less than 0.005% with estradiol-17$\alpha$, androstenedione, testosterone, progesterone, cortisol, and zeranol. Cross-reactivities with estriol, estrone, trenbolone acetate, trenbolone-17$\alpha$, and trenbolone-17$\beta$ were 0.4, 2.8, 0.01, 0.04, and 0.04%, respectively. Detection limit (5% displacement) of the assay was 1 pg/mL, and limit of quantitation (close interval spike) was 2 pg/mL. When 4, 8, or 12 pg of estradiol-17$\beta$ was added to serum, between 100 and 110% was recovered. Within- and between-assay coefficients of variation were 10% and 12%, respectively.

*Western Ligand Blot Procedure.* Relative amounts of IGFBP in serum, CSF, anterior pituitary homogenate, and follicular fluid were characterized by one-dimensional SDS-PAGE and Western ligand blot (Roberts et al., 2001). Samples were loaded on a volume basis using 1.0 μL of serum and follicular fluid, 1.5 μL of CSF, and 5 μL of anterior pituitary homogenate. Identity of specific IGFBP in the different samples was based on migration patterns and apparent mass of individual bands obtained in samples subjected to immunoprecipitation with antibodies specific for each binding protein before ligand blotting (Funston et al., 1996; Roberts et al., 2001).

*Slot-Blot Analyses.* Total RNA was extracted from anterior pituitary glands for analysis of steady-state levels of mRNA encoding $\alpha$-, LH$\beta$-, and FSH$\beta$-subunits, GnRH-R, and tubulin using Tri Reagent RNA/DNA/protein isolation reagent (Molecular Research Center, Inc., Cincinnati, OH). Concentration and purity of RNA samples were determined by spectrophotometric analysis of absorbance at 260 and 280 nm. To determine steady-state levels of mRNA encoding $\alpha$-, LH$\beta$-, and FSH$\beta$-subunits (Nett et al., 1990); GnRH-R (Turzillo et al., 1994); and tubulin (Hawkins et al., 1993), slot blot analysis was performed with $^{32}$P-labeled cDNA probes (Maniatis et al., 1989). The cDNA for $\alpha$-, LH$\beta$-, and FSH$\beta$-subunits were provided by Dr. Richard Maurer (University of Iowa, Iowa City); GnRH-R by Dr. Colin Clay (Colorado State University, Ft. Collins); and tubulin by Dr. Terry Nett (Colorado State University, Ft. Collins). For $\alpha$-, LH$\beta$-, FSH$\beta$-subunits and GnRH-R cDNA probes, 10 μg of total RNA was applied in duplicate to nylon filters (Gene Screen, New England Nuclear, Boston, MA) and prehybridized at 55°C for 2 h in 12.5 mL of hybridization buffer (5× Denhardts, 0.1% SDS, 5× SSPE, and 50% formamide). Hybridization was carried out at 55°C for 18 h in 12.5 mL of fresh hybridization buffer with 2 mg of denatured, fragmented salmon sperm DNA and $1.5 \times 10^7$ cpm of cDNA probe. To verify equal loading of sample for each of the $\alpha$-, LH$\beta$-, FSH$\beta$-subunits and GnRH-R cDNA probes, a $^{32}$P-labeled cDNA probe for tubulin was hybridized to duplicate samples on an additional nylon filter prepared as described above. Filters were washed by gentle agitation in 250 mL of wash buffer (0.1% SDS, 2× SSC) for 15 min, three times at room temperature and a final wash at 55°C. Washed filters were air-dried, enclosed in plastic wrap and placed on a phosphorimager screen (Molecular Dynamics, Sunnyvale, CA) for 21 h at room temperature. Binding of $^{32}$P-labeled cDNA probe to mRNA on the filter was visualized with phosphorimagery (Storm 860, Version 4.00, Molecular Dynamics) and quantified by ImageQuant (Version 1.0 for Macintosh, Molecular Dynamics).

### *Statistical Analyses*

All data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) for a randomized block design (Littell et al., 1996) with collection period as the random blocking factor. Hormone data from intensive blood sampling were analyzed as repeated measures. Fixed effects included treatment, time, and treatment × time interaction with the subject of the repeated measures specified as the animal within each treatment. The following covariance structures for the repeated measurements were compared: compound symmetry, autoregressive order one, and heterogeneous autoregressive order one. The appropriate covariance structure was determined utilizing Akaike's information criterion. All other response variables were analyzed with supplement as the only fixed effect. When significant differences were detected in mRNA encoding tubulin, mRNA encoding $\alpha$-, LH$\beta$-, FSH$\beta$-subunits and GnRH-R were adjusted for tubulin by determining the sample mRNA:tubulin ratios and the ratios were analyzed as a one-way fixed effects model. When differences were observed among treatments ($P \le 0.10$), pairwise comparisons of least squares means were performed with PDIFF. In addition, serum LH and FSH concentrations were analyzed by computing area under the curve (**AUC**) with SAS using a trapezoidal summation

**Table 3.** Serum, cerebrospinal fluid (CSF), anterior pituitary, and follicular fluid IGFBP in beef heifers fed three levels of undegradable intake protein (UIP)[a,b]

| IGFBP[c] | Low UIP | Mid UIP | High UIP | SE[d] |
|---|---|---|---|---|
| Serum | | | | |
| IGFBP-2 | 48.7 | 48.5 | 45.1 | 10.2 |
| IGFBP-3[a] | 50.8 | 43.2 | 43.0 | 7.9 |
| IGFBP-3[b] | 18.6 | 18.4 | 18.2 | 3.0 |
| IGFBP-4[a] | 9.6 | 9.2 | 9.3 | 1.9 |
| IGFBP-4[b] | 14.6 | 14.0 | 14.1 | 2.5 |
| CSF | | | | |
| IGFBP-2 | 20.4 | 13.4 | 20.2 | 5.2 |
| IGFBP-3[a] | 19.1 | 7.5 | 9.4 | 6.2 |
| IGFBP-3[b] | 9.0 | 6.5 | 6.5 | 2.8 |
| IGFBP-4[a] | 8.1 | 6.7 | 7.7 | 2.5 |
| IGFBP-4[b] | 13.0 | 7.5 | 9.4 | 3.5 |
| Follicular fluid | | | | |
| IGFBP-2 | 42.3[e] | 45.4[e] | 65.6[f] | 7.5 |
| IGFBP-3[a] | 33.9 | 30.1 | 35.4 | 5.6 |
| IGFBP-3[b] | 20.4 | 19.0 | 22.4 | 2.9 |
| IGFBP-4[a] | 16.9[e] | 14.1[e] | 32.8[f] | 5.6 |
| IGFBP-4[b] | 12.1[e] | 9.8[e] | 20.8[f] | 3.6 |
| Anterior pituitary | | | | |
| IGFBP-2 | 25.5 | 24.8 | 19.3 | 3.3 |
| IGFBP-3[a] | 13.0 | 12.8 | 9.4 | 1.9 |
| IGFBP-3[b] | 16.1 | 14.8 | 10.4 | 2.3 |
| IGFBP-5 | 52.1 | 55.5 | 39.9 | 8.7 |

[a]Treatments were low-UIP (1,135 $g \cdot heifer^{-1} \cdot d^{-1}$; 30% CP, 113 g UIP), mid-UIP (1,135 $g \cdot heifer^{-1} \cdot d^{-1}$; 38% CP, 216 g UIP), or high-UIP (1,135 $g \cdot heifer^{-1} \cdot d^{-1}$; 46% CP, 321 g UIP) supplements fed daily.
[b]Samples were collected at slaughter on d 12 to 14 of the estrous cycle, corresponding to d 30 to 32 of supplementation.
[c]IGFBP: relative amounts expressed in arbitrary units. The two forms of IGFBP-3 and IGFBP-4 are labeled a and b, for larger and smaller forms, respectively.
[d]Most conservative standard error; low UIP: n = 7; mid UIP: n = 8; high UIP: n = 6.
[e,f]Within a row, means without a common superscript differ ($P < 0.05$).

**Table 4.** Anterior pituitary gland LH and FSH content, serum LH and FSH concentrations, and area under the curve (AUC) in beef heifers supplemented with three levels of undegradable intake protein (UIP)[a,b]

| Gonadotropin[c] | Low UIP | Mid UIP | High UIP | SE[d] |
|---|---|---|---|---|
| LH | | | | |
| Anterior pituitary content | 71.1 | 73.8 | 53.5 | 12.7 |
| Serum concentration | 3.2 | 3.6 | 3.1 | 0.3 |
| AUC | 749 | 869 | 756 | 74 |
| FSH | | | | |
| Anterior pituitary content | 174.2 | 195.5 | 138.1 | 26.5 |
| Serum concentration | 5.2[e] | 5.2[e] | 4.6[f] | 0.2 |
| AUC | 1,278[g] | 1,207[gh] | 1,090[h] | 56 |

[a]Treatments were low-UIP (1,135 $g \cdot heifer^{-1} \cdot d^{-1}$; 30% CP, 113 g UIP), mid-UIP (1,135 $g \cdot heifer^{-1} \cdot d^{-1}$; 38% CP, 216 g UIP), or high-UIP (1,135 $g \cdot heifer^{-1} \cdot d^{-1}$; 46% CP, 321 g UIP) supplements fed daily.
[b]Tissue samples were collected at slaughter on d 12 to 14 of the estrous cycle, corresponding to d 30 to 32 of supplementation. Serum samples were collected on d 10 of the estrous cycle, corresponding to d 28 of supplementation.
[c]Anterior pituitary content, ng/100 mg tissue; serum concentration, ng/mL; AUC, units.
[d]Most conservative standard error; low UIP: n = 7; mid UIP: n = 8; high UIP: n = 6.
[e,f]Within a row, means without a common superscript differ ($P = 0.06$).
[g,h]Within a row, means without a common superscript differ ($P = 0.03$).

method from time 0 (time of supplementation) to time 240 min after supplementation and analyzing AUC with a one-way fixed-effects model. Two heifers in the high-UIP treatment group became anestrous during the study and were excluded from analysis of reproductive data yet were included in the analyses for serum insulin and IGF-I, as well as serum, CSF, and anterior pituitary IGFBP concentrations.

## Results

Concentration of insulin and IGF-I in serum did not differ among treatments on d 28 of supplementation (insulin: 0.39, 0.34, and 0.46 ± 0.05 ng/mL, $P = 0.26$; IGF-I: 114.9, 119.3, and 119.3 ± 7.9 ng/mL, $P = 0.91$, for low, mid, and high UIP, respectively). In addition, serum and CSF leptin concentrations on d 30 to 32 of supplementation did not differ among treatments (serum: 1.7, 1.9, 2.1 ± 0.3 ng/mL, $P = 0.89$; CSF: 1.0, 1.2, 1.2 ± 0.2 ng/mL, $P = 0.90$, for low, mid, and high UIP, respectively).

Two heifers in the high-UIP treatment group that were estrous cycling before onset of supplementation, failed to ovulate and develop a CL during the supplementation period and were therefore excluded from analyses on reproductive data. On day of slaughter, follicular fluid IGFBP-2 and -4, but not IGFBP-3, were elevated in the high-UIP heifers compared with those in the low- and mid-UIP treatment groups ($P < 0.05$; Table 3). However, relative amounts of individual IGFBP in serum, CSF, or anterior pituitary gland content did not differ due to treatments ($P > 0.10$).

Following 30 to 32 d of supplementation, no differences were observed in serum estradiol-17$\beta$ among treatments (2.0, 1.6, 1.7 ± 0.4 pg/mL, for low, mid, and high UIP, respectively; $P = 0.86$). Moreover, serum progesterone and CL weight at slaughter were similar among treatments (progesterone: 5.0, 5.5, and 6.4 ± 0.9 ng/mL, $P = 0.52$; CL weight: 3.7, 3.8, and 4.0 ± 0.3 g; $P = 0.71$; for low, mid, and high UIP).

Basal LH secretion and AUC were similar among treatments during intensive sampling period on d 28 of UIP supplementation, corresponding to d 10 of the estrous cycle ($P \geq 0.28$; Table 4). Although there were no differences among treatments, anterior pituitary gland LH content on d 30 to 32 of supplementation (d 12 to 14 of the estrous cycle) was numerically lower ($P = 0.18$) in heifers consuming the highest level of UIP.

Serum FSH concentrations on d 10 of the estrous cycle were greater following 28 d of UIP supplementation, in the low- and mid-UIP heifers than in the high-UIP treatment group ($P = 0.08$; Table 4). Serum FSH AUC was greater in the low UIP than the high UIP ($P = 0.03$) and tended to be elevated in mid vs. high

**Table 5.** Steady state levels of anterior pituitary gland mRNA encoding $\alpha$, LH$\beta$, FSH$\beta$, and GnRH receptor (GnRH-R) in beef heifers fed three levels of undegradable intake protein (UIP)[a,b]

| mRNA[cde] | Low UIP | Mid UIP | High UIP | SE[f] |
|---|---|---|---|---|
| $\alpha$ | 29,743 | 32,019 | 30,794 | 6,556 |
| LH$\beta$ | 21,225 | 21,667 | 31,029 | 6,548 |
| FSH$\beta$ | 5,680[d] | 12,822[e] | 8,660[de] | 2,751 |
| GnRH-R:tubulin | 1.5 | 1.5 | 1.2 | 0.3 |

[a]Treatments were low-UIP (1,135 g·heifer$^{-1}$·d$^{-1}$; 30% CP, 113 g UIP), mid-UIP (1,135 g·heifer$^{-1}$·d$^{-1}$; 38% CP, 216 g UIP), or high-UIP (1,135 g·heifer$^{-1}$·d$^{-1}$; 46% CP, 321 g UIP) supplements fed daily.
[b]Anterior pituitaries were collected at slaughter on d 12 to 14 of the estrous cycle, corresponding to d 30 to 32 of supplementation.
[c]Arbitrary units for $\alpha$, LH$\beta$, and FSH$\beta$. Data for GnRH-R are reported as a ratio to tubulin due to unequal loading of samples.
[de]Within a row, means without a common superscript differ ($P$ = 0.03).
[f]Most conservative standard error; low: n = 7; mid: n = 8; high: n = 6.

UIP ($P$ = 0.14), yet was similar in the low- vs. mid-UIP-supplemented heifers. On d 30 to 32 of supplementation, anterior pituitary gland FSH content was similar among treatments ($P$ = 0.26), yet showed a comparable trend to that observed with LH content with mid-UIP heifers numerically highest in FSH content.

Steady-state levels of mRNA encoding $\alpha$- and LH$\beta$-subunits did not differ among treatments ($P \geq 0.49$; Table 5). Quantity of mRNA encoding FSH$\beta$ was greater in the mid-UIP treatment group compared with the low-UIP group ($P$ = 0.03). However, no differences were observed between mid- vs. high-UIP heifers or low- vs. high-UIP heifers ($P \geq 0.20$). Loading differences were detected in GnRH-R mRNA, and, therefore, data were analyzed as a ratio of GnRH-R mRNA to tubulin mRNA. There were no differences in GnRH-R mRNA among treatments ($P$ = 0.76).

## Discussion

Insulin has been associated with enhanced reproductive function throughout the hypophyseal-hypothalamic-ovarian axis (reviewed by Butler, 2000; Gong, 2002). However, no differences in serum insulin were observed among treatments in this study. Previous studies have shown that postpartum cows and prepubertal heifers supplemented with UIP have increased serum insulin, but not until 51 and 125 d of supplementation, respectively (Wiley et al., 1991; Lalman et al., 1993). When UIP supplementation was initiated at 7 mo of gestation and continued through the first 3 mo of lactation, serum insulin concentrations were immediately increased and maintained at increased concentrations throughout the supplementation period in beef cows fed 412 g UIP·cow$^{-1}$·d$^{-1}$; UIP compared with cows supplemented with 53 or 223 g UIP·cow$^{-1}$·d$^{-1}$ (Sletmoen-Olson et al., 2000). Collectively, present data and previous studies suggest that insulin response to UIP supplementation may differ depending on age, production state, and duration of supplementation.

Leptin, a hormone secreted by adipocytes, has been implicated as a signal of metabolic status to the reproductive axis (reviewed by Williams et al., 2002). Heifers in this study did not differ in CSF or serum leptin concentrations regardless of level of UIP supplementation. Previous data regarding the influence of protein supplementation in the ruminant on leptin concentrations are lacking. However, body condition score and BW were similar among heifers (body condition score = 4.5 ± 0.5; initial BW = 362 ± 12 kg); therefore, these data agree with Delavaud et al. (2000), who reported a positive correlation between body fatness and body condition score and plasma leptin concentrations in sheep. Vernon et al. (2001) suggested that leptin concentrations are primarily a signal of too little adipose tissue and are most dramatically influenced by fasting and undernutrition. Therefore, leptin concentrations in the serum and CSF may not be responsive to changes in level of UIP supplementation in beef heifers in a moderate-to-good plane of nutrition.

Insulin-like growth factor-I and its binding proteins have an integral function in energy metabolism and have been implicated as metabolic mediators in nutritional regulation at all levels of the reproductive axis in the bovine female (Zulu et al., 2002). In the present study, no differences were observed in serum IGF-I concentrations among heifers following 28 d of supplementation with low, mid, or high UIP. These data are similar to findings by Alderton et al. (2000), who observed no differences in serum IGF-I in postpartum beef cows fed supplements to meet DIP requirements. Moreover, although 30 d of prepartum UIP supplementation in first-calf heifers increased serum IGF-I at calving, this difference was not sustained throughout 90 d of postpartum UIP supplementation (Strauch et al., 2001), suggesting that physiological state and/or duration of supplementation may influence the serum IGF-I response to UIP supplementation.

In contrast to Alderton et al. (2000), concentrations of IGFBP in serum, CSF, or anterior pituitary glands on the day of slaughter were not influenced by level of UIP supplementation. However, IGFBP-2 and -4 were elevated in the follicular fluid of heifers supplemented with high UIP with no differences observed in IGFBP-3 among treatments. Follicular growth and development are associated with decreases in IGFBP of <40 kDa (IGFBP-2, 4, and 5), whereas follicular atresia is characterized by increases in the relative abundance of these proteins (Monget et al., 1996). Moreover, two heifers in the high-UIP treatment group became anestrous during the supplementation period. Although serum estradiol-17$\beta$ concentrations did not differ among treatments, these data suggest that supplementation with high levels of UIP in the beef heifer may alter follicular dynamics.

Supplementation of UIP at any level in the present study did not affect serum progesterone concentrations

or luteal weight on d 12 to 14 of the estrous cycle (day of slaughter following 30 to 32 d of supplementation). In addition, postpartum beef cows supplemented with UIP ranging from 293 to 573 *g* UIP·cow$^{-1}$·d$^{-1}$ also had similar serum progesterone concentrations on d 6, 8, 10, and 12 of the estrous cycle (Triplett et al., 1995). These data suggest that, although follicular functions may be altered by high-UIP supplementation, once ovulation has occurred UIP supplementation may not affect luteal growth and function in the beef heifer.

Basal LH secretion and anterior pituitary gland LH content on d 10 of the estrous cycle, as well as mRNA for $\alpha$- and LH$\beta$-subunits on d 12 to 14 of the estrous cycle, were similar among all heifers. Previous data are lacking on the effect of UIP supplementation on basal LH secretion and anterior pituitary gland content of $\alpha$ and LH$\beta$ mRNA in cycling heifers. However, postpartum cows supplemented with 182 g UIP·cow$^{-1}$·d$^{-1}$ did not differ from control-supplemented cows in GnRH-induced LH secretion on d 20 and 38 postpartum (Albertini et al., 1996). In contrast, heifers receiving high UIP in the present study tended to have decreased anterior pituitary gland LH content compared with low- and mid-UIP-supplemented heifers. These findings are similar to data by Rusche et al. (1993), who reported a tendency toward decreased mean basal LH concentrations on d 35 postpartum in high-UIP (600 g UIP·cow$^{-1}$·d$^{-1}$) cows. These findings suggest that basal LH serum concentrations and anterior pituitary gland LH content may be decreased with higher levels of UIP supplementation. These observations combined with the two heifers in the high-UIP treatment group that became anestrous during the supplementation period suggest a change in function of the hypothalamo-hypophyseal-ovarian axis at high levels of UIP supplementation. Similar responses may play a role in delayed puberty and impaired fertility observed in heifers and postpartum cows supplemented with high UIP (Elrod et al., 1993; Lalman et al., 1993; Appeddu et al., 1996, 1997). The observation that LH$\beta$ mRNA tended to be greater in high-UIP-supplemented heifers compared with low and mid UIP is opposite from what was expected based on trends observed for concentrations of LH in serum and anterior pituitary, suggesting that high-UIP supplementation may alter posttranscriptional processing of LH.

Basal serum concentration of FSH on d 28 of supplementation was greater in heifers supplemented with low and mid UIP compared with high UIP. Moreover, low- and mid-UIP heifers had numerically greater anterior pituitary gland content of FSH than the high-UIP treatment group. In addition, FSH$\beta$ mRNA on d 12 to 14 of the estrous cycle was greater in mid-UIP heifers compared with those receiving the low-UIP supplement, suggesting that supplementation at a rate of 216 g UIP·heifer$^{-1}$·d$^{-1}$ may enhance FSH synthesis, storage, and secretion. Nutritional regulation of FSH in estrous cycling beef cattle, particularly in cycling heifers, has not been well characterized. Pituitary FSH content and serum FSH concentrations were decreased in postpartum beef cows fed CP-restricted diets (Nolan et al., 1988). Moreover, suckled postpartum beef cows fed ad libitum or restricted amounts of low-quality roughage had prolonged (>70 d) suppression of plasma FSH and LH concentrations and LH pulse frequency (Jolly et al., 1991). Bays et al. (1994) reported enhanced basal FSH secretion in postpartum 3-yr-old cows fed 300 g UIP·heifer$^{-1}$·d$^{-1}$ compared with urea-supplemented cows. These authors attributed the response to the UIP supplementation as forage consumption was adjusted to be isoenergetic. Elevated FSH synthesis, storage, and secretion associated with moderate levels of UIP supplementation may play a role in improved reproductive performance in beef heifers and postpartum cows (Wiley et al., 1991; Dhuyvetter et al., 1993; Appeddu et al., 1996, 1997).

Heifers receiving UIP supplementation did not differ in steady-state levels of mRNA encoding GnRH-R on d 12 to 14 of the estrous cycle, indicating that alterations in gonadotropins discussed above are not mediated by alterations in steady-state levels of GnRH-R mRNA. However, synergistic interaction between GnRH and low levels of estradiol-17$\beta$ is required to increase GnRH-R mRNA and GnRH-R numbers (Kirkpatrick et al., 1998; Turzillo et al., 1998), and up-regulation of GnRH-R mRNA does not occur until circulating progesterone concentrations decrease during luteolysis (Turzillo et al., 1995). Possible alterations in GnRH-R mRNA associated with increasing levels of UIP supplementation may not be detectable at d 12 to 14 of the estrous cycle.

Collectively, these data suggest that supplementation with high levels of UIP (321 g·cow$^{-1}$·d$^{-1}$) in estrous cycling beef heifers may decrease mRNA encoding FSH$\beta$ subunit, and may tend to decrease anterior pituitary content of LH and FSH and serum FSH concentrations. These alterations in the synthesis, storage, and secretion of the gonadotropins may impair follicular growth and development (Mihm et al., 2002), as indicated by an increase in the low-molecular-weight IGFBP concentrations in the follicular fluid of heifers supplemented with high UIP, as well as the observed cessation of estrous cycles in two high-UIP-supplemented heifers. Further research is needed to determine effects of increasing UIP supplementation on the hypothalamo-hypophyseal-ovarian axis in estrous cycling beef heifers at various times in the estrous cycle.

## Implications

Undegradable intake protein supplementation has been reported to enhance reproductive performance in beef females by minimizing body weight changes, hastening the onset of puberty, decreasing postpartum anestrus, and increasing pregnancy rates in cattle consuming dormant forage. However, high levels of undegradable intake protein have also been associated with impaired fertility in both beef and dairy cattle. The regulatory role that undegradable protein supplemen-

tation has in reproductive function in beef cattle remains unclear, and further work is needed to establish the mechanisms involved in the nutritional modulation of reproduction. This study suggests that alterations in reproductive performance with undegradable intake protein supplementation in beef cattle may be associated with changes in anterior pituitary gland synthesis, storage, and secretion of gonadotropins and/or ovarian follicular dynamics.

## Literature Cited


Albertini, H. L., D. E. Hawkins, M. K. Petersen, D. M. Hallford, T. T. Ross, R. A. Renner, K. K. Kane, and L. A. Appeddu. 1996. Undegradable intake protein (UIP) and fat on reproductive and endocrine function of young beef cows. Pages 197–199 in Proc. West. Sec. Am. Soc. Anim. Sci., Rapid City, SD.

Alderton, B. W., D. L. Hixon, B. W. Hess, L. F. Woodard, D. M. Hallford, and G. E. Moss. 2000. Effects of supplemental protein type on productivity of primiparous cows. J. Anim. Sci. 78:3027–3035.

Anderson, L. P., J. A. Paterson, R. P. Ansotegui, M. Cecava, and W. Schmutz. 2001. The effects of degradable and undegradable intake protein on the performance of lactating first-calf heifers. J. Anim. Sci. 79:2224–2232.

Appeddu, L. A., M. K. Petersen, J. S. Serrato-Corona, L. F. Gulino, I. Tovar-Luna, G. B. Donart, D. E. Hawkins, J. R. Strickland, E. E. Parker, and S. Cox. 1996. Postpartum reproductive responses of two year old range cows supplemented with protein, fat, or energy. Pages 2–6 in Proc. West. Sec. Am. Soc. Anim. Sci., Rapid City, SD.

Appeddu, L. A., J. E. Sawyer, J. S. Serrato-Corona, L. Knox, H. Albertini, E. E. Parker, D. E. Hawkins, G. B. Donart, D. M. Hallford, and M. K. Petersen. 1997. Different uses of protein supplements by two-year old range cows during a drought. Pages 45–48 in Proc. West. Sec. Am. Soc. Anim. Sci., San Luis Obispo, CA.

Bays, T. R., D. E. Hawkins, M. K. Petersen, I. Tovar, and D. M. Hallford. 1994. Protein and exogenous insulin in postpartum beef cows: 1. Effects on metabolic hormones and pituitary stores of LH and FSH. Pages 263–265 in Proc. West. Sec. Am. Soc. Anim. Sci., Las Cruces, NM.

Berrie, R. A., D. M. Hallford, and M. L. Galyean. 1995. Effects of zinc source and level on performance, carcass characteristics, and metabolic hormone concentrations of growing and finishing lambs. Prof. Anim. Sci. 11:149–156.

Butler, W. R. 2000. Nutritional interactions with reproductive performance in dairy cattle. Anim. Reprod. Sci. 60–61:449–457.

Caton, J. S., A. S. Freeman, and M. L. Galyean. 1988. Influence of protein supplementation on forage intake, in situ forage disappearance, ruminal fermentation and digesta passage rates in steers grazing blue grama rangeland. J. Anim. Sci. 66:2262–2271.

Delavaud, C., F. Bocquier, Y. Chilliard, D. H. Keisler, A. Gertler, and G. Kann. 2000. Plasma leptin determination in ruminants: Effect of nutritional status and body fatness on plasma leptin concentration assessed by a specific RIA in sheep. J. Endocrinol. 165:519–26.

Dhuyvetter, D. V., M. K. Petersen, R. P. Ansotegui, R. A. Bellows, B. Nisley, R. Brownson, and M. W. Tess. 1993. Reproductive efficiency of range beef cows fed different quantities of ruminally undegradable protein before breeding. J. Anim. Sci. 71:2586–2593.

Elrod, C. C., M. Van Amburgh, and W. R. Butler. 1993. Alterations of pH in response to increased dietary protein in cattle are unique to the uterus. J. Anim. Sci. 71:702–706.

FASS. 1999. Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching. Fed. Anim. Sci. Soc., Savoy, IL.

Funston, R. N., G. E. Seidel, Jr., J. Klindt, and A. J. Roberts. 1996. Insulin-like growth factor I and insulin-like growth factor-binding proteins in bovine serum and follicular fluid before and after the preovulatory surge of luteinizing hormone. Biol. Reprod. 55:1390–1396.

Gong, J. G. 2002. Influence of metabolic hormones and nutrition on ovarian follicle development in cattle: Practical implications. Domest. Anim. Endocrinol. 23:229–241.

Gutierrez, C. G., J. Oldham, T. A. Bramley, J. G. Gong, B. K. Campbell, and R. Webb. 1997. The recruitment of ovarian follicles is enhanced by increased dietary intake in heifers. J. Anim. Sci. 75:1876–1884.

Hawkins, D. E., C. J. Belfiore, J. P. Kile, and G. D. Niswender. 1993. Regulation of messenger ribonucleic acid encoding $3\beta$-hydroxysteroid dehydrogenase/$\Delta^5$-$\Delta^4$ isomerase in the ovine corpus luteum. Biol. Reprod. 48:1185–1190.

Hoefler, W. C., and D. M. Hallford. 1987. Influence of suckling status and type of birth on serum hormone profiles and return to estrus in early-postpartum spring-lambing ewes. Theriogenology 27:887–895.

Hunter, R. A., and T. Magner. 1988. The effect of supplements of formaldehyde-treated casein on the partitioning of nutrients between cow and calf in lactating *Bos indicus* × *Bos taurus* heifers fed a roughage diet. Aust. J. Agric. Res. 39:1151–1162.

Jolly, P. D., Q. S. McSweeney, M. J. D'Occhio, and K. W. Entwistle. 1991. Nutritional, suckling and ovarian feedback effects on plasma LH and FSH during post-partum anoestrus in Brahman-cross cattle. Proc. Aust. Soc. Reprod. Biol. 23:152.

Kane, K. K., K. W. Creighton, M. K. Petersen, D. M. Hallford, M. D. Remmenga, and D. E. Hawkins. 2002. Effects of varying levels of undegradable intake protein on endocrine and metabolic function of young post-partum beef cows. Theriogenology 57:2179–2191.

Keisler, D. H., and M. C. Lucy. 1996. Perception and interpretation of the effects of undernutrition on reproduction. J. Anim. Sci. 74(Suppl. 3):1–17.

Kirkpatrick, B. L., E. Esquivel, G. E. Moss, D. L. Hamernik, and M. E. Wise. 1998. Estradiol and gonadotropin-releasing hormone (GnRH) interact to increase GnRH receptor expression in ovariectomized ewes after hypothalamic-pituitary disconnection. Endocrine 8:225–229.

Lalman, D. L., M. K. Petersen, R. P. Ansotegui, M. W. Tess, C. K. Clark, and J. S. Wiley. 1993. The effects of ruminally undegradable protein, propionic acid and monensin on puberty and pregnancy in beef heifers. J. Anim. Sci. 71:2843–2852.

L'Hermite, M., G. D. Niswender, L. E. Reichert, Jr., and A. R. Midgley, Jr. 1972. Serum follicle-stimulating hormone in sheep as measured by radioimmunoassay. Biol. Reprod. 6:325–332.

Littell, R. C., G. A. Milliken, W. W. Stroup, and R. D. Wolfinger. 1996. SAS® System for Mixed Models. SAS Institute, Inc., Cary, NC.

Maniatis, T., E. Frisch, and J. Sambrook. 1989. Molecular Cloning: A Laboratory Manual. 2nd ed. C. Nolan, N. Ford, M. Ferguson, and M. Ockler, ed. Cold Spring Harbor Press, Cold Spring Harbor, NY.

McCormick, M. E., D. D. French, T. F. Brown, G. J. Cuomo, A. M. Chapa, J. M. Fernandez, J. F. Beatty, and D. C. Blouin. 1999. Crude protein and rumen undegradable protein effects on reproduction and lactation performance of Holstein cows. J. Dairy Sci. 82:2697–2708.

Mihm, M., M. A. Crowe, P. G. Knight, and E. J. Austin. 2002. Follicle wave growth in cattle. Reprod. Domest. Anim. 37:191–200.

Monget, P., N. Besnard, C. Huet, C. Pisselet, and D. Monniaux. 1996. Insulin-like growth factor-binding proteins and ovarian folliculogenesis. Horm. Res. 45:211–217.

Nett, T. M., J. A. Flores, F. Carnevali, and J. P. Kile. 1990. Evidence for a direct negative effect of estradiol at the level of the pituitary gland in sheep. Biol. Reprod. 43:554–558.

Niswender, G. D., L. E. Reichert, Jr., A. R. Midgely, Jr., and A. V. Nalbandov. 1969. Radioimmunoassay for bovine and ovine luteinizing hormone. Endocrinology 84:1166–1173.

Noguchi, T. 2000. Protein nutrition and insulin-like growth factor system. Brit. J. Nutr. 84:S241–S244.

Nolan, C. J., R. C. Bull, R. G. Sasser, C. A. Ruder, P. M. Panlasigui, H. M. Schoenemann, and J. J. Reeves. 1988. Postpartum reproduction in protein restricted beef cows: Effect on the hypothalamic-pituitary-ovarian axis. J. Anim. Sci. 66:3208–3217.

Reimers, R. J., R. G. Cowan, J. P. McCann, and M. W. Ross. 1982. Validation of a rapid solid-phase radioimmunoassay for canine, bovine, and equine insulin. Am. J. Vet. Res. 43:1274–1278.

Richards, M. W., R. P. Wettemann, and H. M. Schoenemann. 1989. Nutritional anestrus in beef cows: Body weight change, body condition, luteinizing hormone in serum and ovarian activity. J. Anim. Sci. 67:1520–1526.

Roberts, A. J., R. N. Funston, and G. E. Moss. 2001. Insulin-like growth factor binding proteins in the bovine anterior pituitary. Endocrine 14:339–406.

Rusche, W. C., R. C. Cochran, L. R. Corah, J. S. Stevenson, D. L. Harmon, R. T. Brandt, Jr., and J. E. Minton. 1993. Influence of source and amount of dietary protein on performance, blood metabolites, and reproductive function of primiparous beef cows. J. Anim. Sci. 71:557–563.

Sansinanea, A. S., S. I. Cerone, I. Zonco, C. Garcia, and N. Auza. 2001. Serum leptin levels in cattle with different nutritional conditions. Nutr. Res. 21:1045–1052.

Schillo, K. K. 1992. Effects of dietary energy on control of luteinizing hormone secretion in cattle and sheep. J. Anim. Sci. 70:1271–1282.

Schneider, F. A., and D. M. Hallford. 1996. Use of a rapid progesterone radioimmunoassay to predict pregnancy and fetal numbers in ewe. Sheep Goat Res. J. 12:33–38.

Short, R. E., R. A. Bellows, R. B. Staigmiller, J. G. Berardinelli, and E. E. Custer. 1990. Physiological mechanisms controlling anestrus and infertility in postpartum beef cows. J. Anim. Sci. 68:799–816.

Sletmoen-Olson, K. E., J. S. Caton, K. C. Olson, D. A. Redmer, J. D. Kirsch, and L. P. Reynolds. 2000. Undegraded intake protein supplementation: II. Effects on plasma hormone and metabolite concentrations in periparturient beef cows fed low-quality hay during gestation and lactation. J. Anim. Sci. 78:456–463.

Snyder, J. L., J. A. Clapper, A. J. Roberts, D. W. Sanson, D. L Hamernik, and G. E. Moss. 1999. Insulin-like growth factor-I, insulin-like growth factor-binding proteins, and gonadotropins in the hypothalamic-pituitary axis and serum of nutrient-restricted ewes. Biol. Reprod. 61:219–224.

Strauch, T. A., E. J. Scholljegerdes, D. J. Patterson, M. F. Smith, M. C. Lucy, W. R. Lamberson, and J. E. Williams. 2001. Influence of undegraded intake protein on reproductive performance of primiparous beef heifers maintained on stockpiled fescue pasture. J. Anim. Sci. 79:574–581.

Swanson, L. V., H. D. Hafs, and D. A. Morrow. 1972. Ovarian characteristics and serum LH, prolactin, progesterone and glucocorticoids from first estrus to breeding size in Holstein heifers. J. Anim. Sci. 34:284–293.

Triplett, B. L., D. A. Neuendorff, and R. D. Randel. 1995. Influence of undegraded intake protein supplementation on milk production, weight gain, and reproductive performance in postpartum Brahman cows. J. Anim. Sci. 73:3223–3229.

Turzillo, A. M., C. E. Campion, C. M. Clay, and T. M. Nett. 1994. Regulation of gonadotropin-releasing hormone (GnRH) receptor messenger ribonucleic acid and GnRH receptors during the early preovulatory period in the ewe. Endocrinology 135:1353–1358.

Turzillo, A. M., J. L. Juengel, and T. M. Nett. 1995. Pulsatile gonadotropin-releasing hormone (GnRH) increases concentrations of GnRH receptor messenger ribonucleic acid and numbers of GnRH receptors during luteolysis in the ewe. Biol. Reprod. 53:418–423.

Turzillo, A. M., T. E. Nolan, and T. M. Nett. 1998. Regulation of gonadotropin-releasing hormone (GnRH) receptor gene expression in sheep: Interaction of GnRH and estradiol. Endocrinology 139:4890–4894.

Vernon, R. G., R. G. Denis, and A. Sorensen. 2001. Signals of adiposity. Domest. Anim. Endocrinol. 21:197–214.

Wallace, J. D. 1987. Supplemental feeding options to improve livestock efficiency on rangelands. Achieving Efficient Use of Rangeland Resources. R. S. White and R. E. Short, ed. Fort Keogh Research Symposium, Miles City, MT.

Wheeler, J. S., D. L. Lalman, G. W. Horn, L. A. Redmon, and C. A. Lents. 2002. Effects of supplementation on intake, digestion, and performance of beef cattle consuming fertilized, stockpiled bermudagrass forage. J. Anim. Sci. 80:780–789.

Wiley, J. S., M. K. Petersen, R. P. Ansotegui, and R. A. Bellows. 1991. Production from first-calf beef heifers fed a maintenance or low level of prepartum nutrition and ruminally undegradable or degradable protein postpartum. J. Anim. Sci. 69:4279–4293.

Williams, G. L., M Amstalden, M. R. Garcia, R. L. Stanko, S. E. Nizielski, C. D. Morrison, and D. H. Keisler. 2002. Leptin and its role in the central regulation of reproduction in cattle. Domest. Anim. Endocrinol. 23:339–349.

Wiltbank, M. C., A. Gumen, and R. Sartori. 2002. Physiological classification of anovulatory conditions in cattle. Theriogenology 57:21–52.

Zulu, V. C., T. Nakao, and Y. Sawamukai. 2002. Insulin-like growth factor-I as a possible hormonal mediator of nutritional regulation of reproduction in cattle. J. Vet. Med. Sci. 64:657–665.