Supplementation based on protein or energy ingredients to beef cattle consuming low-quality cool-season forages: II. Performance, reproductive, and metabolic responses of replacement heifers[1]

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ABSTRACT: This experiment evaluated the influence of supplement composition on performance, reproductive, and metabolic responses of Angus × Hereford heifers consuming a low-quality cool-season forage (8.7% CP and 57% TDN). Sixty heifers (initial age = 226 ± 3 d) were allocated into 15 drylot pens (4 heifers/pen and 5 pens/treatment) and assigned to 1) supplementation with soybean meal (PROT), 2) supplementation with a mixture of cracked corn, soybean meal, and urea (68:22:10 ratio, DM basis; ENER), or 3) no supplementation (CON). Heifers were offered meadow foxtail (*Alopecurus pratensis* L.) hay for ad libitum consumption during the experiment (d –10 to 160). Beginning on d 0, PROT and ENER were provided daily at a rate of 1.30 and 1.40 kg of DM/heifer to ensure that PROT and ENER intakes were isocaloric and isonitrogenous. Hay and total DMI were recorded for 5 consecutive days during each month of the experiment. Blood was collected every 10 d for analysis of plasma progesterone to evaluate puberty attainment. Blood samples collected on d –10, 60, 120, and 150 were also analyzed for plasma concentrations of plasma urea N (PUN), glucose, insulin, IGF-I, NEFA, and leptin. Liver samples were collected on d 100 from 2 heifers/pen and analyzed for mRNA expression of genes associated with nutritional metabolism. No treatment effect was detected ($P = 0.33$) on forage DMI. Total DMI, ADG, and mean concentrations of glucose, insulin, and IGF-I as well as hepatic mRNA expression of IGF-I and IGFBP-3 were greater ($P \leq 0.02$) for PROT and ENER compared with CON and similar between PROT and ENER ($P \geq 0.13$). Mean PUN concentrations were also greater ($P < 0.01$) for PROT and ENER compared with CON, whereas PROT heifers had greater ($P < 0.01$) PUN compared with ENER. Plasma leptin concentrations were similar between ENER and PROT ($P \geq 0.19$) and greater ($P \leq 0.03$) for ENER and PROT compared with CON on d 120 and 150 (treatment × day interaction, $P = 0.03$). Hepatic mRNA expression of mitochondrial phosphoenolpyruvate carboxykinase was greater ($P = 0.05$) in PROT compared with CON and ENER and similar between CON and ENER ($P = 0.98$). The proportion of heifers pubertal on d 160 was greater ($P < 0.01$) in ENER compared with PROT and CON and similar between PROT and CON ($P = 0.38$). In conclusion, beef heifers consuming a low-quality cool-season forage had a similar increase in DMI, growth, and overall metabolic status if offered supplements based on soybean meal or corn at 0.5% of BW.

Key words: beef heifers, gene expression, low-quality cool-season forage, metabolism, performance, supplementation

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INTRODUCTION

Supplementation is often required in heifer development programs based on low-quality forages (Schillo et al., 1992). Although forages typically represent the main energy source for forage-fed cattle and energy is the primary dietary consideration for heifer development (Mass, 1987), protein is traditionally considered the limiting nutrient in western U.S. cow–calf operations

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(DelCurto et al., 2000). Indeed, protein supplementation generally improves digestibility and DMI of low-quality warm-season forages, resulting in increased energy utilization from the forage and cattle BW gain (DelCurto et al., 1990; Lintzenich et al., 1995). However, Bohnert et al. (2011a) reported that protein supplementation did not increase digestibility and DMI of low-quality cool-season forages. Hence, inclusion of energy ingredients into supplements may be beneficial for growth and reproductive development of heifers consuming such forages.

Beef heifers, particularly *Bos taurus*, should attain puberty by 12 mo of age to maximize lifetime productivity (Lesmeister et al., 1973). Energy intake influences puberty attainment in heifers by other mechanisms besides BW gain, including modulation of hormones known to mediate the puberty process such as insulin and IGF-I (Schillo et al., 1992). Accordingly, Ciccioli et al. (2005) reported that feeding starch-based supplements hastened puberty attainment in beef heifers independently of BW gain. Hence, inclusion of energy ingredients, such as starch, into supplements may further benefit reproductive development of heifers consuming low-quality cool-season forages by favoring circulating concentrations of nutritional mediators of puberty. To test this hypothesis, this experiment compared the effects of supplements based on protein or energy ingredients on performance, plasma metabolites and hormones, expression of hepatic genes associated with nutritional metabolism, and puberty attainment of beef heifers consuming a low-quality cool-season forage.

MATERIALS AND METHODS

This experiment was conducted at the Oregon State University – Eastern Oregon Agricultural Research Center (Burns; 43°29′31″ N, 119°42′40″ W, and 1,425 m elevation) from November 2012 to April 2013 (d –10 to 160). All heifers used were cared for in accordance with acceptable practices and experimental protocols reviewed and approved by the Oregon State University Institutional Animal Care and Use Committee.

Hay (meadow foxtail [*Alopecurus pratensis* L.]) and supplement ingredients used in this experiment originated from the same field and batch, respectively, as the dietary ingredients used in the companion manuscript (Cappellozza et al., 2013). A sample of hay (according to Bohnert et al., 2011b) and each supplement ingredient was collected prior to the beginning of the experiment reported herein and those described in Cappellozza et al. (2013) and analyzed by nutrient content by a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY) also as described by Cappellozza et al. (2013). Hay nutritive value was (DM basis) 57% TDN, 58% NDF, 37% ADF, 1.12 Mcal/kg of NEm, 0.57 Mcal/kg of NEg, 8.7% CP, 6.0% RDP, and 2.1% ether extract.

Heifers and Diets

Sixty Angus × Hereford weaned heifers (initial age 226 ± 3 d; initial BW 200 ± 2 kg) were used in this experiment. On d –10 of the study, heifers were ranked by initial BW and age and allocated to 15 drylot pens (7 by 15 m; 5 pens/treatment and 4 heifers/pen), in a manner in which all pens had equivalent initial average BW and age. Pens were randomly assigned to receive 1 of 3 treatments: 1) supplementation with soybean [*Glycine max* (L.) Merr.] meal (**PROT**), 2) supplementation with a mixture of cracked corn (*Zea mays* L.), soybean meal, and urea (68:22:10 ratio, DM basis; **ENER**), or 3) no supplementation (**CON**). Heifers were offered meadow foxtail hay for ad libitum consumption during the entire experiment (d –10 to 160). Beginning on d 0, PROT and ENER treatments were fed once daily (0800 h) at a rate of 1.30 and 1.40 kg of DM/heifer, respectively, to ensure that PROT and ENER intakes were isocaloric and isonitrogenous (Table 1). Urea was included into ENER to result in isocaloric and isonitrogenous intakes of PROT and ENER. Furthermore, treatment intakes were formulated at 0.50 and 0.54% of the expected average heifer shrunk BW during the experiment for PROT and ENER, respectively, to achieve the same treatment intake as percent of BW used by Cappellozza et al. (2013). Average heifer shrunk BW during the experiment was estimated based on initial shrunk BW (d –9) and expected final shrunk BW (d 161). Expected final shrunk BW was projected based on previous research from our group (Cooke et al., 2012, 2013), which was conducted at the same research station and using the same cowherd as the experiment described herein.

The ENER and PROT treatments were not mixed with hay and were readily consumed by heifers. All heifers had ad libitum access to water and the same mineral and vitamin mix described by Cappellozza et al. (2013) throughout the experimental period.

Sampling

Heifers were weighed on 2 consecutive days to determine both full and shrunk (after 16 h of feed and water restriction) BW at the beginning (d –10 and d –9) and end of the experiment (d 160 and 161). Shrunk BW was used to determine heifer ADG during the study. Blood samples were collected at 10-d intervals throughout the entire experiment (d –10 to 160), starting 4 h after the ENER and PROT treatments were offered, to determine onset of puberty according to plasma progesterone concentration. Heifers were considered pubertal when plasma progesterone concentration was equal or greater than 1.0 ng/mL for 2 consecutive samplings (Perry et al., 1991), and puberty attainment was declared at the second sampling of elevated progesterone. In addition, blood samples collected on d –10, 60, 120, and 150 were

also analyzed for plasma urea N (**PUN**), glucose, insulin, NEFA, IGF-I, and leptin concentrations.

Hay and total DMI were evaluated from each pen by collecting and weighing refusals from d 12 to 16, d 53 to 57, d 71 to 75, d 93 to 97, d 112 to 116, and d 143 to 147 of the experiment, which were classified as periods (periods 1 to 6, respectively). Samples of the offered and nonconsumed hay were collected daily from each pen and dried for 96 h at 50°C in forced-air ovens for DM calculation. Hay, concentrate, and total daily DMI of each pen were divided by the number of heifers within each pen and expressed as kilograms per heifer per day. In addition, daily intake/heifer of NEm, NEg, CP, RDP, and starch were estimated based on DMI of each pen and nutritive value of hay and treatments (Table 1).

On d 100 of the experiment, 2 heifers/pen were randomly assigned for liver sample collection via needle biopsy (Arthington and Corah, 1995), which began 4 h after supplements were offered. Immediately after collection, liver samples (average 100 mg of tissue, wet weight) were placed in 1 mL of RNA stabilization solution (RNAlater; Ambion Inc., Austin, TX), maintained at 4°C for 24 h, and stored at –80°C. Samples were analyzed via real-time quantitative reverse transcription (**RT-**) PCR for IGF-I, IGFBP-3, pyruvate carboxylase (**PC**), cytosolic phosphoenolpyruvate carboxykinase (**PEPCK-C**), mitochondrial phosphoenolpyruvate carboxykinase (**PEPCK-M**), and cyclophilin mRNA expression.

Table 1. Ingredient composition and nutrient profile of treatments offered during the experiment

	Treatment[1]	
Item	PROT	ENER
Ingredients, % DM		
Cracked corn	–	68
Soybean meal	100	22
Urea	–	10
Nutrient profile, DM basis		
TDN,[2] %	85.4	77.0
NEm,[3] Mcal/kg	2.02	1.91
NEg,[3] Mcal/kg	1.37	1.31
CP, %	50.1	45.0
RDP, %	28.3	36.0
NFC,[4] %	33.5	59.0
NDF, %	8.6	9.0
Starch, %	5.4	48.4
Ether extract, %	1.5	2.9
Daily intake[4]		
DM, kg	1.30	1.40
TDN,[2] kg	1.11	1.08
NEm,[3] Mcal	2.63	2.67
NEg,[3] Mcal	1.78	1.83
CP, kg	0.65	0.63
RDP, kg	0.37	0.50
Non-fiber carbohydrates, kg	0.44	0.83
NDF, kg	0.11	0.13
Starch, kg	0.07	0.68
Ether extract, kg	0.02	0.04

[1]PROT = supplementation with soybean meal; ENER = supplementation with a mixture of cracked corn, soybean meal, and urea (68:22:10 ratio, DM basis). Values obtained from a commercial laboratory wet chemistry analysis (Dairy One Forage Laboratory, Ithaca, NY).

[2]Calculated according to the equations described by Weiss et al. (1992).

[3]Calculated with the following equations (NRC, 1996): NEm = 1.37 ME – 0.138 ME^2 + 0.0105 ME^3 – 1.12; NEg = 1.42 ME – 0.174 ME^2 + 0.0122 ME^3 – 0.165, given that ME = DE × 0.82 and 1 kg of TDN = 4.4 Mcal of DE.

[4]Estimated from the concentrate consumption of individual experimental unit.

Laboratory Analysis

Blood Samples. Blood samples were collected via jugular venipuncture into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ) containing 158 United States Pharmacopeia units of freeze-dried sodium heparin for plasma collection. All blood samples were placed immediately on ice, subsequently centrifuged (2,500 × *g* for 30 min at 4°C) for plasma harvest, and stored at –80°C on the same day of collection. Plasma concentrations of glucose, PUN, insulin, progesterone, and IGF-I were determined as described by Cappellozza et al. (2013). Plasma concentration of NEFA was determined using a colorimetric commercial kit (HR Series NEFA-2; Wako Pure Chemical Industries Ltd. USA, Richmond, VA) with the modifications described by Pescara et al. (2010). Plasma concentration of leptin was determined according to procedures described by Delavaud et al. (2000). The intra- and interassay CV were, respectively, 4.82 and 3.53% for NEFA, 0.93 and 5.69% for glucose, 10.31 and 6.54% for PUN, 6.17 and 3.37% for IGF-I, 7.92 and 4.27% for insulin, and 5.01 and 4.97% for progesterone. All samples were analyzed for leptin concentration within a single assay, and the intra-assay CV was 4.40%. The minimum detectable concentrations were 0.02 μIU/mL for insulin and 0.056, 0.10, and 0.10 ng/mL for IGF-I, leptin, and progesterone, respectively.

Tissue Samples. Total RNA was extracted from tissue samples using TRIzol Plus RNA Purification Kit (Invitrogen, Carlsbad, CA). Quantity and quality of isolated RNA were assessed via UV absorbance (NanoDrop 2000; Thermo Fisher Scientific, Minneapolis, MN) at 260 nm and 260:280 nm ratio, respectively (Fleige and Pfaffl, 2006). Extracted RNA was stored at –80°C until further processing.

Extracted hepatic RNA (2.5 μg) was incubated at 37°C for 30 min in the presence of RNase free DNase (New England Biolabs Inc., Ipswich, MA) to remove contaminant genomic DNA. After inactivating the DNase (75°C for 15 min), samples were reverse transcribed using the High Capacity cDNA Reverse Transcription Kit with

Table 2. Primer sequences and accession number for all gene transcripts analyzed by quantitative real-time reverse transcription PCR

Target gene	Primer sequence[1]	Accession no.
IGF-I		
Forward	CTC CTC GCA TCT CTT CTA TCT	NM_001077828
Reverse	ACT CAT CCA CGA TTC CTG TCT	
IGFBP-3		
Forward	AAT GGC AGT GAG TCG GAA GA	NM_174556.1
Reverse	AAG TTC TGG GTG TCT GTG CT	
Pyruvate carboxylase		
Forward	CCA ACG GGT TTC AGA GAC AT	NM_177946.3
Reverse	TGA AGC TGT GGG CAA CAT AG	
Cytosolic phosphoenolpyruvate carboxykinase		
Forward	CAA CTA CTC AGC CAA AAT CG	NM_174737.2
Reverse	ATC GCA GAT GTG GAC TTG	
Mitochondrial phosphoenolpyruvate carboxykinase		
Forward	GCT ACA ACT TTG GGC GCT AC	XM_583200
Reverse	GTC GGC AGA TCC AGT CTA GC	
Cyclophilin		
Forward	GGT ACT GGT GGC AAG TCC AT	NM_178320.2
Reverse	GCC ATC CAA CCA CTC AGT CT	

[1]All primer sequences were obtained from Cooke et al. (2008).

Table 3. Performance and puberty parameters of replacement beef heifers consuming a low-quality cool-season forage (meadow foxtail [*Alopecurus pratensis* L.]) and receiving no supplementation (CON; $n = 5$) or supplementation with soybean meal (PROT; $n = 5$) or supplementation with a mixture of cracked corn, soybean meal, and urea (68:22:10 ratio, DM basis; ENER; $n = 5$)[1]

	Treatment				
Item	CON	PROT	ENER	SEM	*P*-value
ADG,[2] kg/d	0.36[a]	0.76[b]	0.72[b]	0.04	<0.01
DMI,[3] kg/d					
Hay	5.94	5.79	5.51	0.20	0.33
Total	5.92[a]	7.10[b]	6.91[b]	0.19	<0.01
Daily nutrient intake[4]					
NEm, Mcal	6.54[a]	9.00[b]	8.74[b]	0.22	<0.01
NEg, Mcal/d	3.27[a]	4.97[b]	4.87[b]	0.11	<0.01
CP, kg	0.51[a]	1.15[b]	1.11[b]	0.02	<0.01
RDP, kg	0.35[a]	0.71[b]	0.83[c]	0.01	<0.01
Starch, kg	0.10[a]	0.17[b]	0.77[c]	0.003	<0.01
Pubertal heifers on d 160,[5] %	10 (2/20)[a]	5 (1/20)[a]	25 (5/20)[b]	4	<0.01

[1]All heifers were offered meadow foxtail hay for ad libitum consumption. Treatments were offered and consumed daily (d 0 to 160) at 1.30 and 1.40 kg of DM for PROT and ENER, respectively, to ensure that PROT and ENER intakes were isocaloric and isonitrogenous. Within rows, values with different superscript differ ($P \leq 0.05$).

[2]Calculated using initial and final shrunk BW (after 16 h of feed and water restriction) obtained on d –9 and 161 of the experiment.

[3]Recorded monthly from each pen during 5 consecutive days but divided by the number of heifers within each pen and expressed as kilograms per heifer per day.

[4]Estimated based on total DMI of each pen and nutritive value of hay and treatments.

[5]Estimated based from blood samples collected every 10 d during the experimental period (d –10 to 160). Heifers were considered pubertal once plasma progesterone concentration was equal or greater than 1.0 ng/mL for 2 consecutive wk (Perry et al., 1991), and puberty attainment was declared at the second week of elevated progesterone. Values within parenthesis represent pubertal heifers/total heifers.

random hexamers (Applied Biosystems, Foster City, CA). Quantity and quality of cDNA were again assessed via UV absorbance at 260 nm and 260:280 nm ratio, respectively (NanoDrop 2000; Thermo Fisher Scientific). Real-time RT-PCR was completed using the Rotor-Gene SYBR Green PCR Kit (Qiagen Inc., Valencia, CA) and specific primer sets (25 ng/mL; Table 2), with a Rotor-Gene Q real-time PCR cycler (Qiagen Inc.) according to procedures described by Yoganathan et al. (2012). At the end of each RT-PCR, amplified products were subjected to a dissociation gradient (95°C for 15 s, 60°C for 30 s, and 95°C for 15 s) to verify the amplification of a single product by denaturation at the anticipated temperature. Responses were quantified based on the threshold cycle ($\mathbf{C_T}$), the number of PCR cycles required for target amplification to reach a predetermined threshold. All C_T responses from genes of interest were normalized to cyclophilin C_T examined in the same sample and assessed at the same time as the targets. Results are expressed as relative fold change ($2^{-\Delta\Delta CT}$), as described by Ocón-Grove et al. (2008).

Statistical Analysis. All data were analyzed using pen as experimental unit and Satterthwaite approximation to determine the denominator df for the tests of fixed effects. Performance, plasma variables, and gene expression data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The model statement used for BW, ADG, and gene expression contained only the effects of treatment. Data were analyzed using heifer(pen) and pen(treatment) as the random variables. The model statement used for plasma variables contained the effects of treatment, day, the treatment × day interaction, and values obtained on d –10 as covariate. Data were analyzed using heifer(pen) and pen(treatment) as random variables, with day as the specified term for the repeated statement and heifer(pen) as subject. The model statement used for feed and nutrient intake contained the effects of treatment, day, period, and all the resultant interactions. Data were analyzed using pen(treatment) as the random variable, given that DMI was recorded daily from each pen as well as day(period) as the specified term for the repeated statement and pen(treatment) as subject. For both intake and plasma variables, the covariance structure used was first-order autoregressive, which provided the smallest Akaike information criterion and hence the best fit for all variables analyzed. Puberty data were analyzed using the GLIMMIX procedure of SAS (SAS Inst. Inc.). The model statement used contained the effects of treatment, day, and the resul-

tant interaction. Data were analyzed using heifer(pen) and pen(treatment) as the random variables. Results are reported as least square means or covariately adjusted means for plasma variables and separated using PDIFF. Significance was set at $P \leq 0.05$ and tendencies were denoted if $P > 0.05$ and $P \leq 0.10$. Results are reported according to main effects if no interactions were significant or according to highest-order interaction detected.

RESULTS AND DISCUSSION

As previously stated, inclusion of energy ingredients into supplements may benefit growth and reproductive performance of replacement heifers consuming low-quality cool-season forages (Schillo et al., 1992; Ciccioli et al., 2005; Bohnert et al., 2011a). To test this theory, a series of experiments evaluated productive and biological responses in beef cattle consuming a low-quality cool-season forage and receiving CON, PROT, or ENER. The experiments reported in the companion manuscript (Cappellozza et al., 2013) evaluated forage disappearance parameters in rumen-fistulated steers as well as performance and physiological responses in pregnant heifers provided PROT and ENER at 0.50 and 0.54% of shrunk BW, respectively. The experiment reported herein compared growth, puberty attainment, and metabolic responses of beef heifers assigned to CON or to PROT and ENER after weaning. It is important to note that average shrunk BW during the present experiment was 227, 257, and 264 kg for CON, ENER, and PROT, respectively (SEM = 3.3), which resulted in an average treatment intake of 0.54 and 0.49% of shrunk BW for ENER and PROT, respectively. Hence, average intake of ENER and PROT during the present experiment as percent of shrunk BW was similar to that of Cappellozza et al. (2013). These supplementation rates were adopted to yield adequate ADG of beef heifers, either nonpregnant or pregnant, consuming low-quality cool-season forages (NRC, 1996).

No treatment effects were detected ($P = 0.33$) on forage DMI (Table 3). Accordingly, rumen-fistulated steers receiving CON, ENER, or PROT had similar ruminal disappearance and estimated degradability of the same forage used herein (Cappellozza et al., 2013), whereas ruminal forage digestibility is positively associated with intake (Allen, 1996). In addition, Cappellozza et al. (2013) also reported similar hay intake among pregnant replacement heifers receiving CON, ENER, and PROT. These results support that protein supplementation does not impact DMI of a low-quality cool-season forage (Bohnert et al., 2011a) and that supplements based on energy ingredients can be fed at approximately 0.5% of BW without impacting forage intake (Bowman and Sanson, 1996). Total daily DMI and estimated daily intake of NEm and NEg were greater ($P < 0.01$) for PROT and ENER compared with CON heifers and similar ($P \geq 0.41$) between PROT and ENER heifers (Table 3). Estimated daily intake of CP, RDP, and starch were greater ($P < 0.01$) for PROT and ENER compared with CON heifers, whereas ENER had greater ($P < 0.01$) RDP and starch intake and tended ($P = 0.09$) to have less CP intake compared to PROT heifers (Table 3). Hence, PROT and ENER had greater overall nutrient intake compared with CON heifers, although starch was the main energy source provided by ENER. The greater RDP intake of ENER compared with PROT heifers can be attributed to the inclusion of urea into the ENER treatment (Horn and McCollum, 1987) and consequent RDP content of treatments (Table 1). In addition, the slightly greater CP intake of PROT compared with ENER heifers, despite similar CP content of treatments (Table 1), can be attributed to the numerical difference in hay intake between PROT and ENER heifers. However, CP and RDP intakes were not limited in ENER or PROT heifers, based on supplement formulation and intake rate (NRC, 1996).

A treatment effect ($P < 0.01$) was detected for ADG (Table 3), which was greater ($P < 0.01$) for PROT and ENER compared with CON heifers and similar between ENER and PROT ($P = 0.52$). Cappellozza et al. (2013) also reported that pregnant heifers receiving ENER and PROT had similar ADG, which were greater compared with CON cohorts. Collectively, these results provide evidence that beef heifers consuming low-quality cool-season forages can equally utilize nutrients provided by supplements based on protein or energy ingredients to support BW gain. These results also indicate that differences in CP and RDP intakes between ENER and PROT in the present experiment were minimal and not sufficient to impact heifer ADG. Supporting this rationale, equivalent treatment effects were detected ($P \leq 0.05$) for the plasma variables associated with dietary energy and protein metabolism evaluated herein (Table 4; Fig. 1; Hammond, 1997; Huntington, 1997; Hess et al., 2005).

A treatment effect was detected ($P < 0.01$) for plasma NEFA (Table 4). Values obtained on d –10 were significant covariates ($P < 0.01$) but did not differ ($P = 0.93$) among treatments (0.121, 0.124, and 0.119 μEq/L for CON, PROT, and ENER, respectively; SEM = 0.01). During the experiment, mean NEFA concentration was greater ($P < 0.01$) for CON compared with PROT and ENER heifers and similar ($P = 0.13$) between PROT and ENER heifers (Table 4). Accordingly, circulating NEFA concentration in cattle was negatively associated with nutrient intake and ADG, whereas elevated NEFA is often associated with negative energy balance (Lucy et al., 1991; Peters, 1986). Nevertheless, it is important to note that heifers from all treatments were in a positive nutritional status based on their ADG (Table 3). Hence, the elevated NEFA concentration in CON heifers were

Table 4. Plasma concentrations of plasma urea N (PUN), glucose, insulin, IGF-I, leptin, and NEFA of replacement beef heifers consuming a low-quality cool-season forage (meadow foxtail [*Alopecurus pratensis* L.]) and receiving no supplementation (CON; $n = 5$) or supplementation with soybean meal (PROT; $n = 5$) or supplementation with a mixture of cracked corn, soybean meal, and urea (68:22:10 ratio, DM basis; ENER; $n = 5$)[1,2]

	Treatment				
Item[3]	CON	PROT	ENER	SEM	*P*-value
NEFA, μEq/L	0.412[a]	0.194[b]	0.241[b]	0.022	<0.01
PUN, mg/dL	3.57[a]	20.07[b]	17.87[c]	0.54	<0.01
Glucose, mg/dL	59.3[a]	65.1[b]	65.0[b]	1.1	<0.01
Insulin, μIU/mL	5.20[a]	6.72[b]	6.69[b]	0.35	0.02
IGF-I, ng/mL	79.5[a]	159.4[b]	149.5[b]	5.5	<0.01

[1]All heifers were offered meadow foxtail hay for ad libitum consumption. Treatments were offered and consumed daily (d 0 to 160) at 1.30 and 1.40 kg of DM for PROT and ENER, respectively, to ensure that PROT and ENER intakes were isocaloric and isonitrogenous. Within rows, values with different superscript differ ($P \leq 0.05$).

[2]Blood samples were on d –10, 60, 120, and 150, starting at 4 h after supplements were offered.

[3]Results covariately adjusted to samples collected on d –11 of the study.

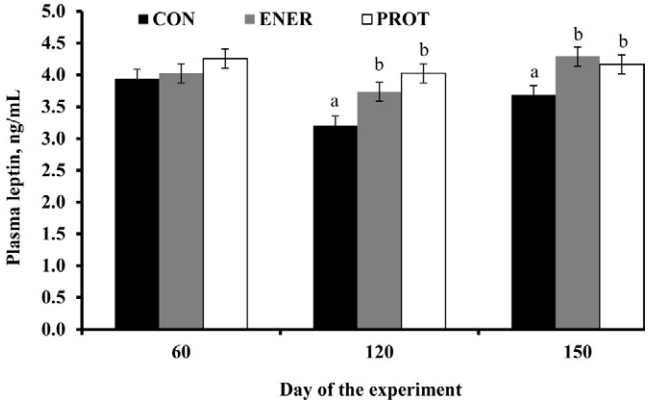

Figure 1. Plasma concentration of leptin in replacement beef heifers consuming a low-quality cool-season forage (meadow foxtail [*Alopecurus pratensis* L.]) and receiving no supplementation (CON; $n = 5$) or supplementation with soybean meal (PROT; $n = 5$; 100% soybean meal on DM basis) or supplementation with a mixture of cracked corn, soybean meal, and urea (68:22:10 ratio, DM basis; ENER; $n = 5$). Treatments were fed and consumed daily (d 0 to 160) at 1.30 and 1.40 kg of DM for PROT and ENER, respectively, to ensure that PROT and ENER intakes were isocaloric and isonitrogenous. Blood samples were collected on d –10, 60, 120, and 150, starting at 4 h after supplements were offered. Results are covariately adjusted to values obtained on d –10. A treatment × hour interaction was detected ($P < 0.01$). Within day, letters indicate differences between treatments ($P \leq 0.03$).

somewhat unexpected, given that Bossis et al. (2000) and Ellenberger et al. (1989) reported similar NEFA concentrations in beef cattle being managed to achieve different but positive rates of ADG.

A treatment effect was detected ($P < 0.01$) for PUN (Table 4). Values obtained on d –10 were not significant covariates ($P = 0.40$) and did not differ ($P = 0.22$) among treatments (22.74, 20.26, and 22.28 mg/dL for CON, PROT, and ENER, respectively; SEM = 1.06). During the experiment, mean PUN concentrations were greater ($P < 0.01$) for PROT and ENER compared with CON, whereas PROT also had greater ($P < 0.01$) PUN concentration compared with ENER heifers (Table 4). Concentration of PUN is positively associated with intake of CP, RDP, and ruminal ammonia concentration (Broderick and Clayton, 1997). In addition, optimal PUN concentrations in growing beef heifers range from 15 to 19 mg/dL (Hammond, 1997). Hence, the greater PUN concentrations of PROT and ENER compared with CON heifers can be directly attributed to their greater CP and RDP intake and suggest that CON heifers required supplemental CP and RDP. Differences in PUN concentrations between ENER and PROT heifers can also be attributed to the slightly greater CP intake of PROT heifers as well as improved N utilization by ruminal microbes in ENER heifers (Hall and Huntington, 2008). Although RDP intake was greater in ENER compared with PROT heifers, the ENER treatment also contained a greater proportion of starch and non-fiber carbohydrates, which are known to optimize the synchrony in energy and protein utilization by rumen microbes and reduce the amount of ammonia and subsequent PUN in the circulation (Hammond, 1997; Hall and Huntington, 2008). Moreover, PUN concentrations in ENER and PROT heifers further corroborates that CP and RDP intakes were not limiting in ENER or PROT heifers (Hammond, 1997). Hence, differences between ENER and PROT heifers on the parameters evaluated herein, besides PUN concentrations, should not be associated with CP and RDP intake.

A treatment effect was detected ($P < 0.01$) for plasma glucose (Table 4). Values obtained on d –10 tended to be significant covariates ($P = 0.08$) but did not differ ($P = 0.52$) among treatments (58.3, 61.4, and 58.1 mg/dL for CON, PROT, and ENER, respectively; SEM = 2.2). During the experiment, mean glucose concentrations were greater ($P < 0.01$) for PROT and ENER compared with CON heifers and similar ($P = 0.91$) between PROT and ENER heifers (Table 4). A similar treatment effect was also detected in plasma glucose concentrations of pregnant heifers, as described by Cappellozza et al. (2013). Supporting these results, glucose concentration was positively associated with feed intake and rates of BW gain (Vizcarra et al., 1998; Hersom et al., 2004), as observed herein based on the greater nutrient intake and ADG of PROT and ENER compared with CON heifers (Table 3). However, starch is the major dietary precursor for glucose in ruminants (Huntington, 1997); hence, it would be expected that ENER heifers had greater plasma glucose concentration compared to PROT. Nevertheless, blood glucose concentrations in cattle are fairly stable due to the role of insulin, which may have prevented proper assessment of treatment effects on glucose flux herein (Marston

Table 5. Expression of hepatic genes associated with nutritional metabolism and growth of replacement beef heifers consuming a low-quality cool-season forage (meadow foxtail [*Alopecurus pratensis* L.]) and receiving no supplementation (CON; $n = 5$) or supplementation with soybean meal (PROT; $n = 5$) or supplementation with a mixture of cracked corn, soybean meal, and urea (68:22:10 ratio, DM basis; ENER; $n = 5$)[1,2]

	Treatment				
Item[3]	CON	PROT	ENER	SEM	*P*-value
PC	3.64	2.77	2.66	0.45	0.28
PEPCK-C	5.00	4.68	3.92	0.68	0.52
PEPCK-M	2.92[a]	4.19[b]	2.90[a]	0.42	0.08
IGF-I	3.71[a]	8.31[b]	6.75[b]	1.00	0.02
IGFBP-3	1.62[a]	2.46[b]	2.38[b]	0.21	0.03

[1]All heifers were offered meadow foxtail hay for ad libitum consumption. Treatments were offered and consumed daily (d 0 to 160) at 1.30 and 1.40 kg of DM for PROT and ENER, respectively, to ensure that PROT and ENER intakes were isocaloric and isonitrogenous. Within rows, values with different superscript differ ($P \leq 0.10$).

[2]Liver samples collected on d 100 of the experiment from 2 heifers per pen. Values are expressed as relative fold change (Ocón-Grove et al., 2008; Cooke et al., 2008).

[3]PC = pyruvate carboxylase; PEPCK-C = cytosolic phosphoenolpyruvate carboxykinase; PEPCK-M = mitochondrial phosphoenolpyruvate carboxykinase.

et al., 1995). In addition, Huntington (1997) reported that growing cattle are highly capable of synthesizing glucose from amino acids, such as those provided in the PROT treatment or produced by rumen microbes.

Supporting this latter rationale, PROT heifers had greater ($P = 0.05$) mRNA expression of liver PEPCK-M compared with ENER and CON, which was similar ($P = 0.98$) between ENER and CON (treatment effect, $P = 0.08$; Table 5). Although liver PEPCK-M is considered constitutive and not highly responsive to hormones and nutritional state (Agca et al., 2002), it may account for up to 61% of glucose synthesis in ruminant hepatocytes (Aiello and Armentano, 1987). Moreover, Cooke et al. (2008) also reported that PEPCK-M mRNA expression was influenced by supplementation and reflective of overall nutritional status of beef heifers. No treatment effects were detected ($P \geq 0.28$; Table 5) for mRNA expression of PC and PEPCK-C, although mRNA expression of these enzymes are modulated by nutrient intake (Cooke et al., 2008) and are positively associated with glucose synthesis in cattle (Greenfield et al., 2000; Bradford and Allen, 2005). Nevertheless, circulating NEFA are known to stimulate mRNA expression of hepatic PC and PEPCK-C, but not PEPCK-M, to preserve gluconeogenesis in cattle with insufficient nutrient intake (Agca et al., 2002; White et al., 2011). Hence, the greater NEFA concentration in CON heifers may have maintained mRNA expression of hepatic PC and PEPCK-C similar to that of ENER and PROT heifers. In addition, it may be speculated that a greater gluconeogenesis through hepatic PEPCK-M in PROT heifers contributed to their greater glucose concentration compared with CON and to the similar glucose concentration compared with ENER heifers despite treatment differences in starch intake.

Treatment effects were detected ($P \leq 0.05$) for plasma insulin and IGF-I (Table 4) as well as mRNA expression of liver IGF-I and IGFBP-3 (Table 5). Values obtained on d –10 were significant covariates for plasma insulin and IGF-I analyses ($P < 0.01$) but did not differ ($P \geq 0.66$) among treatments (5.77, 5.52, and 5.68 μIU/mL of insulin, SEM = 0.57, and 92.3, 85.8, and 85.8 ng/mL of IGF-I, SEM = 7.6, for CON, PROT, and ENER, respectively). During the experiment, mean insulin and IGF-I concentrations were greater ($P < 0.01$) for PROT and ENER compared with CON heifers and similar ($P \geq 0.21$) between PROT and ENER heifers (Table 4). In Cappellozza et al. (2013), ENER and PROT also increased plasma concentrations of insulin and IGF-I compared to CON in pregnant beef heifers. Expression of liver IGF-I and IGFBP-3 mRNA were also greater ($P \leq 0.05$) in PROT and ENER compared with CON and similar ($P \geq 0.29$) between PROT and ENER (Table 5). Collectively, these results corroborate with treatment effects detected for DMI, nutrient intake, and plasma glucose, given that circulating concentration of insulin is positively regulated by nutrient intake and blood glucose (Vizcarra et al., 1998; Nussey and Whitehead, 2001). In turn, availability of energy substrates and circulating insulin positively modulate the expression of liver IGF-I and IGFBP-3 mRNA and consequent hepatic synthesis of these proteins (McGuire et al., 1992; Thissen et al., 1994; Cooke et al., 2008). For these reasons, plasma insulin and IGF-I have been recognized as indicators of nutritional status of cattle (Yelich et al., 1995; Wettemann and Bossis, 2000; Hess et al., 2005), suggesting that ENER and PROT heifers in the present experiment had equivalent intake, utilization, and metabolism of dietary substrates despite differences in ingredients between treatments.

A treatment × day interaction was detected ($P = 0.03$) for plasma leptin (Fig. 1). Values obtained on d –10 were significant covariates ($P = 0.03$) but did not differ ($P = 0.19$) among treatments (4.34, 4.87, and 4.49 ng/mL for CON, PROT, and ENER, respectively; SEM = 0.20). Plasma leptin concentrations were similar between ENER and PROT throughout the experiment ($P \geq 0.19$) and greater for ENER and PROT compared with CON on d 120 ($P \leq 0.01$) and 150 ($P \leq 0.03$; Fig. 1). Circulating leptin concentration is regulated by body fat content, nutrient intake, and circulating insulin (Houseknecht et al., 1998). Hence, the similar plasma leptin concentrations between PROT and ENER corroborate with the similar nutrient intake, growth rates, and plasma insulin concentrations between treatments (Tables 3 and 4). Nevertheless,

the greater ADG, nutrient intake, and plasma insulin concentrations of PROT and ENER heifers compared with CON only resulted in a similar effect on plasma leptin beginning on d 120 of the experiment. The reason for this delay is unknown and deserves further investigation but may be associated with heifer age and rate of body fat accretion (Houseknecht et al., 1998).

No overall treatment effects were detected ($P = 0.25$) on puberty attainment (data not shown). However, a greater ($P < 0.01$) proportion of ENER heifers were pubertal at the end of the experiment (d 160) compared with CON and PROT cohorts, whereas no differences were detected ($P = 0.38$) between CON and PROT heifers (Table 3). The main hypothesis of the experiment was that replacement beef heifers consuming a low-quality cool-season forage and receiving a supplement based on an energy ingredient would have enhanced ADG and hastened puberty attainment compared with heifers receiving no supplementation or supplemented with a protein ingredient. This hypothesis was developed based on the premise that energy ingredients such as corn favor circulating concentrations of insulin, IGF-I, and leptin (Huntington, 1990; Molento et al., 2002; Lents et al., 2005), and these hormones are known to impact the puberty process by mediating synthesis and activity of GnRH and gonadotropin (Butler and Smith, 1989; Schillo et al., 1992; Maciel et al., 2004). Indeed, a greater proportion of ENER heifers were pubertal at the end of the experiment compared with PROT and CON, but this outcome disagrees with the similar ADG and metabolic status between PROT and ENER heifers. Supporting our findings, Ciccioli et al. (2005) also reported that heifers receiving a high-starch supplement had hastened puberty attainment but similar ADG compared with cohorts receiving an isocaloric and isonitrogenous low-starch supplement.

Nevertheless, puberty results reported herein should be adopted with caution, given that overall puberty attainment was lower than expected according to previous work from our research group (Cooke et al., 2012, 2013). Based on the mature BW of the cowherd used herein (535 kg; Bohnert et al. 2013), mean full BW and percent of mature BW at the end of the experiment (d 160) were greater ($P < 0.01$) for ENER and PROT compared to CON and similar ($P = 0.13$) between ENER and PROT (271, 335, and 348 kg of BW, SEM = 7, and 50.7, 62.6, and 65.1% of mature BW, SEM = 1.2, for CON, ENER, and PROT, respectively). Heifer age at the end of the experiment was also similar among treatments ($P = 0.97$) and averaged 396 ± 6 d. Hence, ENER and PROT heifers achieved the BW recommended for puberty achievement at 13 mo of age (Patterson et al., 2000). It is important to note that heifers used herein were reared in a 7 by 15 m drylot pens, whereas heifers used by Cooke et al. (2012) and Cooke et al. (2013) were reared on 6-ha pastures. Exercise may be required for adequate reproductive function in cattle (Lamb et al., 1979, 1981; Cooke et al., 2012) via endogenous opioids known to modulate gonadotropin secretion and consequent onset of puberty, cyclicity, and fertility (Harber and Sutton, 1984; Mahmoud et al., 1989). Accordingly, Mulliniks et al. (2013) reported that heifers reared in drylots had greater ADG but reduced pregnancy rates compared with cohorts reared on range pastures. Therefore, it may be speculated that the lack of exercise haltered puberty attainment in the present experiment, despite adequate growth rates and final BW of ENER and PROT heifers.

In summary, replacement beef heifers offered PROT and ENER had a similar increase in nutrient intake, ADG, and overall metabolic status compared with CON heifers, despite differences in ingredients between supplement treatments. Puberty attainment was enhanced in ENER heifers only, although this outcome should be interpreted with caution due to the reduced number of pubertal heifers across all treatments. Hence, replacement beef heifers consuming a low-quality cool-season forage equally use and benefit, in terms of growth and metabolic parameters, from supplements based on protein or energy ingredients provided as 0.5% of heifer BW/d at isocaloric and isonitrogenous rates.

LITERATURE CITED

Agca, C., R. B. Greenfield, J. R. Hatwell, and S. S. Donkin. 2002. Cloning and characterization of bovine cytosolic and mitochondrial PEPCK during transition to lactation. Physiol. Genomics 11:53–63.

Aiello, R. J., and L. E. Armentano. 1987. Gluconeogenesis in goat hepatocytes s affected by calcium, ammonia and other key metabolites but not primarily through cytosolic redox state. Comp. Biochem. Physiol. 88B:193–201.

Allen, M. S. 1996. Physical constraints on voluntary intake of forages by ruminants. J. Anim. Sci. 74:3063–3075.

Arthington, J. D., and L. R. Corah. 1995. Liver biopsy procedures for determining the trace mineral status in beef cows. Part II. (Video, AI 9134). Extension TV. Dep. Commun. Coop. Ext. Serv., Kansas State Univ., Manhattan, KS.

Bohnert, D. W., R. F. Cooke, B. I. Cappellozza, C. Trevisanuto, and V. D. Tabacow. 2011a. A procedure to reduce collected sample size for nutrient analysis of hay cores. J. Anim. Sci. 89(E-Suppl. 2):192.

Bohnert, D. W., T. DelCurto, A. A. Clark, M. L. Merrill, S. J. Falck, and D. Harmon. 2011b. Protein supplementation of ruminants consuming low-quality cool- or warm-season forage: Differences in intake and digestibility. J. Anim. Sci. 89:3707–3717.

Bohnert, D. W., L. A. Stalker, R. R. Mills, A. Nyman, S. J. Falck, and R. F. Cooke. 2013. Late gestation supplementation of beef cows differing in body condition score: Effects on cow and calf performance. J. Anim. Sci. 91:5485–5491.

Bossis, I., R. P. Wettemann, S. D. Welty, J. Vizcarra, and L. J. Spicer. 2000. Nutritionally induced anovulation in beef heifers: Ovarian and endocrine function during realimentation and resumption of ovulation. Biol. Reprod. 62:1436–1444.

Bowman, J. G. P., and D. W. Sanson. 1996. Starch- or fiber-based energy supplements for grazing ruminants. In. Proc. 3rd Grazing Livestock Nutrition Conf. M. B. Judkins and F. T. McCollum III, ed. Proc. West. Sect. Am. Soc. Anim. Sci. (Suppl. 1):118-135.

Bradford, B. J., and M. S. Allen. 2005. Phlorizin administration increases hepatic gluconeogenic enzyme mRNA abundance but not feed intake in late-lactating dairy cows. J. Nutr. 135:2206–2211.

Broderick, G. A., and M. K. Clayton. 1997. A statistical evaluation of animal and nutritional factors influencing concentrations of milk urea nitrogen. J. Dairy Sci. 80:2964–2971.

Butler, W. R., and R. D. Smith. 1989. Interrelationships between energy balance and postpartum reproductive function in dairy cattle. J. Dairy Sci. 72:767–783.

Cappellozza, B. I., R. F. Cooke, T. A. Guarnieri Filho, and D. W. Bohnert. 2014. Supplementation based on protein or energy ingredients to beef cattle consuming low-quality cool-season forages: I. Forage degradability parameters in rumen-fistulated steers and physiological responses in pregnant heifers. J. Anim. Sci. 92:2716–2724.doi:10.2527/jas.2013-7441.

Ciccioli, N. H., S. L. Charles-Edwards, C. Floyd, R. P. Wettemann, H. T. Purvis, K. S. Lusby, G. W. Horn, and D. L. Lalman. 2005. Incidence of puberty in beef heifers fed high- or low-starch diets for different periods before breeding. J. Anim. Sci. 83:2653–2662.

Cooke, R. F., J. D. Arthington, D. B. Araujo, G. C. Lamb, and A. D. Ealy. 2008. Effects of supplementation frequency on performance, reproductive, and metabolic responses of Brahman-crossbred females. J. Anim. Sci. 86:2296–2309.

Cooke, R. F., D. W. Bohnert, B. I. Cappellozza, C. J. Mueller, and T. DelCurto. 2012. Effects of temperament and acclimation to handling on reproductive performance of *Bos taurus* females. J. Anim. Sci. 90:3547–3555.

Cooke, R. F., D. W. Bohnert, C. L. Francisco, R. S. Marques, C. J. Mueller, and D. H. Keisler. 2013. Effects of bovine somatotropin administration on growth, physiological, and reproductive responses of replacement beef heifers. J. Anim. Sci. 91:2894–2901.

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. 165:519–526.

DelCurto, T., R. C. Cochran, D. L. Harmon, A. A. Beharka, K. A. Jacques, G. Towne, and E. S. Vanzant. 1990. Supplementation of dormant tallgrass-prairie forage: I. Influence of varying supplemental protein and(or) energy levels on forage utilization characteristics of beef steers in confinement. J. Anim. Sci. 68:515–531.

DelCurto, T., B. W. Hess, J. E. Huston, and K. C. Olson. 2000. Optimum supplementation strategies for beef cattle consuming low-quality roughages in the western United States. J. Anim. Sci. 77:1–16.

Ellenberger, M. A., D. E. Johnson, G. E. Carstens, K. L. Hossner, M. D. Holland, T. M. Nett, and C. F. Nockels. 1989. Endocrine and metabolic changes during altered growth rates in beef cattle. J. Anim. Sci. 67:1446–1454.

Fleige, S., and M. W. Pfaffl. 2006. RNA integrity and the effect on the real-time qRT-PCR performance. Mol. Aspects Med. 27:126–139.

Greenfield, R. B., M. J. Cecava, and S. S. Donkin. 2000. Changes in mRNA expression for gluconeogenic enzymes in liver of dairy cattle during the transition to lactation. J. Dairy Sci. 83:1228–1236.

Hall, M. B., and G. B. Huntington. 2008. Nutrient synchrony: Sound in theory, elusive in practice. J. Anim. Sci. 86:E287–E292.

Hammond, A. C. 1997. Update on BUN and MUN as a guide for protein supplementation in cattle. In: Proc. Florida Ruminant Nutr. Symp., Univ. of Florida, Gainesville, FL. p. 43-52.

Harber, V. J., and J. R. Sutton. 1984. Endorphins and exercise. Sports Med. 1:154–171.

Hersom, M. J., R. P. Wettemann, C. R. Krehbiel, G. W. Horn, and D. H. Keisler. 2004. Effect of live weight gain of steers during winter grazing: III. Blood metabolites and hormones during feedlot finishing. J. Anim. Sci. 82:2059–2068.

Hess, B. W., S. L. Lake, E. J. Scholljegerdes, T. R. Weston, V. Nayigihugu, J. D. C. Molle, and G. E. Moss. 2005. Nutritional controls of beef cow reproduction. J. Anim. Sci. 83:E90–E106.

Horn, G. K., and F. T. McCollum. 1987. Energy supplementation of grazing ruminants. In: Proc. Grazing Livest. Nutr. Conf., Univ. of Wyoming, Jackson, WY. p. 125-136.

Houseknecht, K. L., C. A. Baile, R. L. Matteri, and M. E. Spurlock. 1998. The biology of leptin: A review. J. Anim. Sci. 76:1405–1420.

Huntington, G. B. 1990. Energy metabolism in the digestive tract and liver of cattle: Influence of physiological state and nutrition. Reprod. Nutr. Dev. 30:35–47.

Huntington, G. B. 1997. Starch utilization by ruminants: From basics to the bunk. J. Anim. Sci. 75:852–867.

Lamb, R. C., M. J. Anderson, and J. L. Walters. 1981. Forced walking prepartum for dairy cows of different ages. J. Dairy Sci. 64:2017–2024.

Lamb, R. C., B. O. Barker, M. J. Anderson, and J. L. Walters. 1979. Effects of forced exercise on two-year-old Holstein heifers. J. Dairy Sci. 62:1791–1797.

Lents, C. A., R. P. Wettemann, F. J. White, I. Rubio, N. H. Ciccioli, L. J. Spicer, D. H. Keisler, and M. E. Payton. 2005. Influence of nutrient intake and body fat on concentrations of insulin-like growth factor-I, insulin, thyroxine, and leptin in plasma of gestating beef cows. J. Anim. Sci. 83:586–596.

Lesmeister, J. L., P. J. Burfening, and R. L. Blackwell. 1973. Date of first calving in beef cows and subsequent calf production. J. Anim. Sci. 36:1–6.

Lintzenich, B. A., E. S. Vanzant, R. C. Cochran, J. L. Beaty, R. T. Brandt Jr., and G. St. Jean. 1995. Influence of processing supplemental alfalfa on intake and digestion of dormant bluestem-range forage by steers. J. Anim. Sci. 73:1187–1195.

Lucy, M. C., C. R. Staples, F. M. Michel, and W. W. Thatcher. 1991. Energy balance and size and number of ovarian follicles detected by ultrasonography in early postpartum dairy cows. J. Dairy Sci. 74:473–482.

Maciel, M. N., D. A. Zieba, M. Amstalden, D. H. Keisler, J. P. Neves, and G. L. Williams. 2004. Chronic administration of recombinant ovine leptin in growing beef heifers: Effects on secretion of LH, metabolic hormones, and timing of puberty. J. Anim. Sci. 82:2930–2936.

Mahmoud, A. I., F. N. Thompson, D. D. Peck, K. M. Mizinga, L. S. Leshin, L. A. Rund, J. A. Stuedemann, and T. E. Kiser. 1989. Difference in luteinizing hormone response to an opioid antagonist in beef heifers and cows. Biol. Reprod. 41:431–437.

Marston, T. T., K. S. Lusby, R. P. Wettemann, and H. T. Purvis. 1995. Effects of feeding energy or protein supplements before or after calving on performance of spring-calving cows grazing native range. J. Anim. Sci. 73:657–664.

Mass, J. 1987. Relationship between nutrition and reproduction in beef cattle. Vet. Clin. North Am. Food Anim. Pract. 3:633–646.

McGuire, M. A., J. L. Vicini, D. E. Bauman, and J. J. Veenhuizen. 1992. Insulin-like growth factors and binding proteins in ruminants and their nutritional regulation. J. Anim. Sci. 70:2901–2910.

Molento, C. F. M., E. Block, R. I. Cue, and D. Petilerc. 2002. Effects of insulin, recombinant bovine somatotropin, and their interaction on insulin-like growth factor I secretion and milk production in dairy cows. J. Dairy Sci. 85:738–747.

Mulliniks, J. T., D. E. Hawkins, K. K. Kane, S. H. Cox, L. A. Torrell, E. J. Scholljegerdes, and M. K. Petersen. 2013. Metabolizable protein supply while grazing dormant winter forage during heifer development alters pregnancy and subsequent in-herd retention rate. J. Anim. Sci. 91:1409–1416.

NRC. 1996. Nutrient requirements of beef cattle. 7th rev. ed. Natl. Acad. Press, Washington, DC.

Nussey, S. S., and S. A. Whitehead. 2001. Endocrinology: An integrated approach. BIOS Scientific, Oxford, UK.

Ocón-Grove, O. M., F. N. T. Cooke, I. M. Alvarez, S. E. Johnson, T. L. Ott, and A. D. Ealy. 2008. Ovine endometrial expression of fibroblast growth factor (FGF) 2 and conceptus expression of FGF receptors during early pregnancy. Domest. Anim. Endocrinol. 34:135–145.

Patterson, D. J., S. L. Wood, and R. F. Randle. 2000. Procedures that support reproductive management of replacement beef heifers. J. Anim. Sci. 77:1–15.

Perry, R. C., L. R. Corah, R. C. Cochran, J. R. Brethour, K. C. Olson, and J. J. Higgins. 1991. Effects of hay quality, breed and ovarian development on onset of puberty and reproductive performance of beef heifers. J. Prod. Agric. 4:13–18.

Pescara, J. B., J. A. A. Pires, and R. R. Grummer. 2010. Antilipolytic and lipolytic effects of administering free or ruminally protected nicotinic acid to feed-restricted Holstein cows. J. Dairy Sci. 93:5385–5396.

Peters, J. P. 1986. Consequences of accelerated gain and growth hormone administration for lipid metabolism in growing steers. J. Nutr. 116:2490–2503.

Schillo, K. K., J. B. Hall, and S. M. Hileman. 1992. Effects of nutrition and season on the onset of puberty in the beef heifer. J. Anim. Sci. 70:3994–4005.

Thissen, J. P., J. M. Ketelslegers, and L. E. Underwood. 1994. Nutritional regulation of the insulin-like growth factors. Endocr. Rev. 15:80–101.

Vizcarra, J. A., R. P. Wettemann, J. C. Spitzer, and D. G. Morrison. 1998. Body condition at parturition and postpartum weight gain influence luteal activity and concentrations of glucose, insulin, and nonesterified fatty acids in plasma of primiparous beef cows. J. Anim. Sci. 76:927–936.

Weiss, W. P., H. R. Conrad, and N. R. St. Pierre. 1992. A theoretically-based model for predicting total digestible nutrient values of forages and concentrates. Anim. Feed Sci. Technol. 39:95–110.

Wettemann, R. P., and I. Bossis. 2000. Energy intake regulates ovarian function in beef cattle. J. Anim. Sci. 77:1–10.

White, H. M., S. L. Koser, and S. S. Donkin. 2011. Characterization of bovine pyruvate carboxylase promoter 1 responsiveness to serum from control and feed-restricted cows. J. Anim. Sci. 89:1763–1768.

Yelich, J. V., R. P. Wettemann, H. G. Dolezal, K. S. Lusby, D. K. Bishop, and L. J. Spicer. 1995. Effects of growth rate on carcass composition and lipid partitioning at puberty and growth hormone, insulin-like growth factor I, insulin, and metabolites before puberty in beef heifers. J. Anim. Sci. 73:2390–2405.

Yoganathan, P., S. Karunakaran, M. M. Ho, and S. M. Clee. 2012. Nutritional regulation of genome-wide association obesity genes in a tissue-dependent manner. Nutr. Metab. (Lond.) 9:65.