Supplementation based on protein or energy ingredients to beef cattle consuming low-quality cool-season forages: I. Forage disappearance parameters in rumen-fistulated steers and physiological responses in pregnant heifers[1]

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ABSTRACT: Two experiments evaluated the influence of supplement composition on ruminal forage disappearance, performance, and physiological responses of Angus × Hereford cattle consuming a low-quality cool-season forage (8.7% CP and 57% TDN). In Exp. 1, 6 rumen-fistulated steers housed in individual pens were assigned to an incomplete 3 × 2 Latin square design containing 2 periods of 11 d each and the following treatments: 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). Steers were offered meadow foxtail (*Alopecurus pratensis* L.) hay for ad libitum consumption. Treatments were provided daily at 0.50 and 0.54% of shrunk BW/steer for PROT and ENER, respectively, to ensure that PROT and ENER intakes were isocaloric and isonitrogenous. No treatment effects were detected on rumen disappearance parameters of forage DM ($P \geq 0.33$) and NDF ($P \geq 0.66$). In Exp. 2, 35 pregnant heifers were ranked by initial BW on d –7 of the study, allocated into 12 feedlot pens (4 pens/treatment), and assigned to the same treatments and forage intake regimen as in Exp. 1 for 19 d. Treatments were fed once daily at 1.77 and 1.92 kg of DM/heifer for PROT and ENER, respectively, to achieve the same treatment intake as percent of initial BW used in Exp. 1 (0.50 and 0.54% for PROT and ENER, respectively). No treatment effects ($P = 0.17$) were detected on forage DMI. Total DMI was greater ($P < 0.01$) for PROT and ENER compared with CON and similar between PROT and ENER ($P = 0.36$). Accordingly, ADG was greater ($P = 0.01$) for PROT compared with CON, tended to be greater for ENER compared with CON ($P = 0.08$), and was similar between ENER and PROT ($P = 0.28$). Heifers receiving PROT and ENER had greater mean concentrations of plasma glucose ($P = 0.03$), insulin ($P \leq 0.09$), IGF-I ($P \leq 0.04$), and progesterone ($P = 0.01$) compared to CON, whereas ENER and PROT had similar concentrations of these variables ($P \geq 0.15$). A treatment × hour interaction was detected ($P < 0.01$) for plasma urea N (PUN), given that PUN concentrations increased after supplementation for ENER and PROT (time effect, $P < 0.01$) but did not change for CON (time effect, $P = 0.62$). In conclusion, beef cattle consuming low-quality cool-season forages had similar ruminal forage disappearance and intake, performance, and physiological status if offered supplements based on soybean meal or corn at 0.5% of BW.

Key words: beef cattle, low-quality forage, performance, physiology, ruminal forage disappearance, supplementation

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INTRODUCTION

Supplementation is often required in heifer development programs based on low-quality forages (Schillo et al., 1992). Protein is traditionally considered the limiting nutrient in western U.S. cow–calf systems (DelCurto et al., 2000), although energy is the primary dietary consideration for female development (Mass, 1987) and forages typically represent the main energy source for forage-fed cattle. Indeed, supplemental

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protein has been shown to improve digestibility and DMI of low-quality warm-season forages, resulting in increased energy utilization from the forage and cattle performance (DelCurto et al., 1990; Lintzenich et al., 1995). However, supplemental protein did not increase forage digestibility and DMI of low-quality cool-season forages (Bohnert et al., 2011a). Hence, inclusion of energy ingredients into supplements may be beneficial for growth and reproduction of heifers consuming such forages.

After the first breeding season, pregnant heifers still need to grow while maintaining the pregnancy. Energy intake modulates BW gain and circulating concentration of progesterone ($\mathbf{P_4}$), a steroid required for pregnancy establishment and maintenance (Spencer and Bazer, 2002). The hormones associated with the metabolism of energy substrates, particularly starch, increase P_4 concentration by reducing hepatic P_4 catabolism (Cooke et al., 2012) and stimulating ovarian steroidogenesis (Spicer and Echternkamp, 1995). Hence, inclusion of energy ingredients into supplements may further benefit reproductive performance of pregnant heifers consuming low-quality cool-season forages by increasing circulating P_4 concentration. However, supplements based on energy ingredients often impair forage digestibility and DMI in cattle (DelCurto et al., 2000). Therefore, 2 experiments compared the effects of supplements based on protein or energy ingredients on ruminal forage disappearance in steers (Exp. 1) and performance and physiological parameters of pregnant beef heifers (Exp. 2).

MATERIALS AND METHODS

Both experiments were conducted at the Oregon State University – Eastern Oregon Agricultural Research Center (Burns) from August to September 2012 (43°31′06″ N, 119°01′21″ W, and 1,370 m elevation). All cattle 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.

Supplement ingredients provided during Exp. 1 and 2 were from the same batch, whereas the hay (meadow foxtail [*Alopecurus pratensis* L.]) provided during both experiments was harvested from the same field in June 2012. A sample of hay (according to Bohnert et al., 2011b) and each supplement ingredient was collected before the beginning of both experiments and analyzed for nutrient content by a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY). Samples were analyzed in triplicates by wet chemistry procedures for concentrations of CP (method 984.13; AOAC, 2006), RDP (Roe et al., 1990, for supplement ingredients and Coblentz et al., 1999, for hay), ADF (method 973.18 modified for use in an Ankom 200 fiber analyzer; Ankom Technology Corp., Fairport, NY; AOAC, 2006), and NDF (Van Soest et al., 1991; modified for use in an Ankom 200 fiber analyzer; Ankom Technology Corp.). Calculations for TDN used the equations proposed by Weiss et al. (1992), whereas NEm and NEg were calculated with the equations proposed by the NRC (1996). 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.

Experiment 1

Steers and Diets. Six Angus × Hereford steers (initial shrunk BW 494 ± 11 kg), housed in individual pens (8 by 20 m) and fitted with a ruminal cannula, were assigned to an incomplete 3 × 2 Latin square design containing 2 periods of 11 d each (2 steers/treatment in each period) and the following 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**). Steers were offered meadow foxtail hay for ad libitum consumption during the entire experiment. The PROT and ENER treatments were provided daily (0800 h) at 0.50 and 0.54% of steer shrunk BW recorded at the beginning of each period, 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. Treatment intake during the experiment averaged at 2.20 and 2.37 kg of DM/steer for PROT and ENER, respectively. Treatments were inserted directly into the ruminal cannula of each steer to ensure readily supplement consumption. All steers had ad libitum access to water and a mineral and vitamin mix (Cattleman's Choice; Performix Nutrition Systems, Nampa, ID) containing 14% Ca, 10% P, 16% NaCl, 1.5% Mg, 3,200 mg/kg of Cu, 65 mg/kg of I, 900 mg/kg of Mn, 140 mg/kg of Se, 6,000 mg/kg of Zn, 136,000 IU/kg of vitamin A, 13,000 IU/kg of vitamin D3, and 50 IU/kg of vitamin E throughout the experimental period.

Sampling. Within each period (d 0 to 11), steer shrunk BW was recorded on d 0 after 16 h of feed and water restriction to determine steer initial BW. From d 1 to 7 of each period, voluntary forage DMI was recorded daily by collecting and weighing refusals. Samples of the offered and nonconsumed forage were collected daily from each pen and dried for 96 h at 50°C in forced-air ovens for DM calculation. From d 8 to 11 of each period, steers were offered 90% of their voluntary forage DMI determined from d 1 to 7. Immediately before treatments were provided on d 8, Dacron bags (50 ± 10 μm pore size and 10 by 20 cm bag size; Ankom Technology

Corp.) containing 4 g (DM basis) of ground dietary hay (2-mm screen; Wiley Mill, Model 4; Arthur H. Thomas, Philadelphia, PA) were suspended into the ruminal ventral sac of each steer and incubated in triplicates for 0, 1, 3, 5, 8, 12, 24, 36, 48, 72, and 96 h. Before ruminal incubation, all bags were soaked in warm water (39°C) for 15 min. After ruminal incubation, bags were washed repeatedly with running water until the rinse water was colorless and subsequently dried for 96 h at 50°C in a forced-air oven. The 0-h bags were not incubated in the rumen but were subjected to the same soaking, rinsing, and drying procedures applied to the ruminally incubated bags. Dried samples were weighed for residual DM determination, and triplicates were combined and analyzed for NDF (Robertson and Van Soest, 1981) using procedures modified for use in an Ankom 200 Fiber Analyzer (Ankom Technology Corp.).

Statistical Analysis. All data were analyzed using steer as the experimental unit and Satterthwaite approximation to determine the denominator df for the tests of fixed effects. Kinetic parameters of forage DM and NDF disappearance were estimated using nonlinear regression procedures of SAS (SAS Inst. Inc., Cary, NC), as described by Vendramini et al. (2008). Effective degradability of forage DM and NDF were calculated by fixing ruminal passage rate at 0.046/h (Poore et al., 1990) and using the model proposed by Ørskov and McDonald (1979), whereas treatment effects on these parameters were analyzed using the MIXED procedure of SAS (SAS Inst. Inc.). The model statement contained the effects of treatment and period as independent variables. Data were analyzed using steer(treatment × period) as the random variable. Results are reported as least square means and separated using PDIFF. Significance was set at $P \leq 0.05$ and tendencies were denoted if $P > 0.05$ and $P \leq 0.10$.

Table 1. Ingredient composition and nutrient profile of treatments offered during Exp. 1 and Exp. 2.

	Exp. 1[1]		Exp. 2	
Item	PROT	ENER	PROT	ENER
Ingredient, % DM				
Cracked corn	–	68	–	68
Soybean meal	100	22	100	22
Urea	–	10	–	10
Nutrient profile, DM basis				
TDN,[2] %	85.4	77.0	85.4	77.0
NEm,[3] Mcal/kg	2.02	1.91	2.02	1.91
NEg,[3] Mcal/kg	1.37	1.31	1.37	1.31
CP, %	50.1	45.0	50.1	45.0
RDP, %	28.3	36.0	28.3	36.0
NFC,[4] %	33.5	59.0	33.5	59.0
NDF, %	8.6	9.0	8.6	9.0
Starch, %	5.4	48.4	5.4	48.4
Ether extract, %	1.5	2.9	1.5	2.9
Daily intake[5]				
DM, kg	2.20	2.37	1.77	1.92
TDN,[2] kg	1.88	1.82	1.51	1.48
NEm,[3] Mcal	4.44	4.53	3.58	3.67
NEg,[3] Mcal	3.01	3.10	2.42	2.52
CP, kg	1.10	1.08	0.89	0.86
RDP, kg	0.62	0.85	0.50	0.69
NFC, kg	0.74	1.40	0.59	1.13
NDF, kg	0.19	0.21	0.15	0.17
Starch, kg	0.12	1.15	0.10	0.93
Ether extract, kg	0.03	0.07	0.03	0.06

[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]NFC = non-fiber carbohydrates.

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

Experiment 2

Heifers and Diets. Thirty-five pregnant Angus × Hereford heifers (initial shrunk BW 354 ± 4 kg and initial age = 508 ± 4 d) were used in the study. Heifers were concurrently exposed and became pregnant to a fixed-time AI protocol (CO-Synch + controlled internal progesterone-release device; Larson et al., 2006) 90 d before the beginning of the experiment. Pregnancy status to AI was verified by detecting a fetus via transrectal ultrasonography (5.0-MHz transducer, 500 V; Aloka, Wallingford, CT) 80 d after AI (d –10 of the experiment). On d –7, all heifers were ranked by initial shrunk (after 16 h of feed and water restriction) BW and allocated to 12 feedlot pens (4 pens/treatment: 11 pens with 3 heifers and 1 pen with 2 heifers; 8 by 20 m) in a manner in which all pens had equivalent initial average shrunk BW. Pens were randomly assigned to receive the same treatments described in Exp. 1. Heifers were offered meadow foxtail hay for ad libitum consumption during the entire experiment (d –7 to 19). Beginning on d 1, PROT and ENER treatments were fed once daily (0700 h) at a rate of 1.77 and 1.92 kg of DM/heifer, respectively, to achieve the same treatment intake as percent of initial shrunk BW used in Exp. 1 (0.50 and 0.54% of initial BW for PROT and ENER, respectively) and to ensure isocaloric and isonitrogenous intakes (Table 1). The ENER and PROT treatments were not mixed with hay and were readily consumed by heifers. Water availability and mineral and vitamin mix supplementation were the same as in Exp. 1.

Sampling. Heifer shrunk BW was collected before the beginning (d –7) and at the end of the study (d 20 and also after 16 h of feed and water restriction) for ADG

calculation. Hay DMI was evaluated daily from each pen from d 1 to 19 by collecting and weighing refusals daily. Samples of the offered and nonconsumed feed 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 total DMI of each pen and nutritive value of hay and treatments (Table 1).

Blood samples were collected immediately before and 2, 4, 6, and 8 h after treatment feeding (h 0) on d 13, 15, 17, and 19 of the experiment and analyzed for plasma concentrations of glucose, plasma urea N (**PUN**), insulin, IGF-I, and P_4. Blood samples were also collected on d 0 of the experiment, immediately before and 4 and 8 h after hay feeding (h 0), to determine if ENER, PROT, and CON heifers had similar P_4 concentrations before the beginning of treatment administration (d 1 to 19). All 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. After collection, 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 P_4 and insulin were determined using Coat-A-Count solid phase ^{125}I RIA kits (Siemens Healthcare Diagnostics, Los Angeles, CA) previously used for bovine samples (Moriel et al., 2008). Plasma glucose and PUN concentrations were determined using quantitative colorimetric kits (number G7521 and B7551, respectively; Pointe Scientific, Inc., Canton, MI). Concentration of IGF-I was determined in samples collected at 0 and 4 h after feeding, using a human-specific commercial ELISA kit (SG100; R&D Systems, Inc., Minneapolis, MN) with 100% cross-reactivity with bovine IGF-I and previously validated for bovine samples (Cooke et al., 2012). The intra- and interassay CV were, respectively, 1.94 and 3.30% for glucose, 8.55 and 8.64% for PUN, 2.34 and 4.74% for IGF-I, 2.98 and 3.29% for insulin, and 6.87 and 7.19% for P_4. The minimum detectable concentrations were 0.02 μIU/mL for insulin and 0.056 and 0.07 ng/mL for IGF-I and P_4, respectively.

Statistical Analysis. All data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc.), using pen as experimental unit and Satterthwaite approximation to determine the denominator df for the tests of fixed effects. The model statement used for ADG contained only the effect of treatment. Data were analyzed using pen(treatment) and heifer(pen) as random variables. The model statement used for feed and nutrient intake contained the effects of treatment, day, and the treatment × day interaction. Data were analyzed using pen(treatment) as the random variable, given that DMI was recorded from each pen. The specified term for the repeated statement was day and subject was pen(treatment). The model statement used for plasma variables contained the effects of treatment, hour, day, and all the resultant interactions. The model statement for P_4 also contained the average P_4 concentration on d 0 as covariate. Data were analyzed using pen(treatment) and heifer(pen) as random variables. The specified term for the repeated statement was hour(day), whereas heifer(treatment × day) was the 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. Results are reported as least square means or as covariately adjusted means for plasma P_4 concentration 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 beef heifers consuming low-quality cool-season forages (Bohnert et al., 2011a; Cooke et al., 2012), although supplemental energy ingredients may impair forage digestibility and intake (DelCurto et al., 2000). To address these theories, the experiments reported herein evaluated performance and physiological responses in pregnant heifers provided PROT and ENER at 0.50 and 0.54% of BW as well as in situ forage disappearance in rumen-fistulated steers to estimate supplementation effects on ruminal forage degradability parameters. Similar treatments were applied to replacement heifers following weaning, and these results are being reported in a companion manuscript (Cappellozza et al., 2014). These supplementation rates were adopted to yield adequate ADG of beef heifers, either nonpregnant or pregnant, consuming low-quality cool-season forages (NRC, 1996).

Experiment 1

No treatment effects were detected for ruminal disappearance rate or effective ruminal degradability of hay DM ($P \geq 0.33$) and NDF ($P \geq 0.66$; Table 2), indicating that PROT and ENER did not impact rumen in situ disappearance parameters of a low-quality cool-season forage. Supporting these results, Caton and Dhuyvetter (1997) suggested that ruminal disappearance rate of

Table 2. Ruminal in situ disappearance parameters of meadow foxtail (*Alopecurus pratensis* L.) hay incubated in forage-fed steers receiving no supplementation (CON; $n = 4$) or supplementation with soybean meal (PROT; $n = 4$) or supplementation with a mixture of cracked corn, soybean meal, and urea (68:22:10 ratio, DM basis; ENER; $n = 4$)[1]

	Treatment				
Item	CON	PROT	ENER	SEM	*P*-value
Ruminal disappearance rate, %/h					
DM	2.88	3.36	3.67	0.35	0.33
NDF	3.64	4.24	4.06	0.51	0.71
Effective degradability,[2] %					
DM	60.7	60.8	60.3	1.1	0.95
NDF	55.4	55.5	53.7	1.5	0.66

[1]All steers were offered meadow foxtail hay for ad libitum consumption. Treatments were provided daily at 0.50 and 0.54% of BW/steer for PROT and ENER, respectively, to ensure that PROT and ENER intakes were isocaloric and isonitrogenous.

[2]Calculated by fixing ruminal passage rate at 0.046/h (Poore et al., 1990) and using the model proposed by Ørskov and McDonald (1979).

Table 3. Performance parameters of pregnant beef heifers consuming a low-quality cool-season forage (meadow foxtail [*Alopecurus pratensis* L.]) and receiving no supplementation (CON; $n = 4$) or supplementation with soybean meal (PROT; $n = 4$) or supplementation with a mixture of cracked corn, soybean meal, and urea (68:22:10 ratio, DM basis; ENER; $n = 4$)[1]

	Treatment				
Item	CON	PROT	ENER	SEM	*P*-value
ADG,[2] kg/d	0.49[a]	0.89[b]	0.75[b]	0.09	0.03
DMI,[3] kg/d					
Hay	8.60	8.42	8.84	0.14	0.17
Total	8.60[a]	10.19[b]	10.50[b]	0.22	<0.01
Daily nutrient intake[4]					
NEm, Mcal	9.46[a]	12.84[b]	12.89[b]	0.35	<0.01
NEg, Mcal/d	4.73[a]	7.06[b]	7.03[b]	0.22	<0.01
CP, kg	0.74[a]	1.62[b]	1.51[b]	0.07	<0.01
RDP, kg	0.51[a]	1.00[b]	1.12[b]	0.06	<0.01
Starch, kg	0.146[a]	0.239[a]	0.950[b]	0.075	<0.01

[1]All heifers were offered meadow foxtail hay for ad libitum consumption. Treatments were offered and consumed (d 1 to 19) daily at 1.77 and 1.92 kg of DM for PROT and ENER, respectively, to ensure that PROT and ENER intakes were isocaloric and isonitrogenous. Within rows, values with different superscripts differ ($P \leq 0.05$).

[2]Calculated using initial and final shrunk BW (after 16 h of feed and water restriction) obtained on d −7 and 20 of the experiment.

[3]Recorded from each pen from d 1 to 19 of the experiment, 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.

low-quality forages is not impacted by energy or protein-based supplementation. Nevertheless, supplements based on protein and energy ingredients are often associated, respectively, with improved and decreased ruminal forage digestibility in beef cattle (DelCurto et al., 2000). However, protein supplementation is generally beneficial to forage digestibility when the CP content of the basal forage is less than 8% (DelCurto et al., 2000), whereas the forage used herein had 8.7% CP (DM basis). Supplements based on energy ingredients can be provided to forage-fed cattle at 0.5% of BW without major impacts on forage digestibility and intake (Bowman and Sanson, 1996), whereas the ENER treatment was provided at 0.54% of steer BW.

Corn intake above 0.25% of BW has been shown to impair forage use in cattle (Bowman and Sanson, 1996) by reducing ruminal pH, shifting rumen microbes from a cellulolytic population towards an amylolytic population, and decreasing ruminal NH_3 concentration (Chase and Hibberd, 1987; Sanson et al., 1990; Caton and Dhuyvetter, 1997). In the present experiment, ENER steers consumed corn at 0.37% of their BW. However, inclusion of a RDP source into corn-based supplements may offset the negative impacts of corn-based supplements on rumen function and digestibility (Olson et al., 1999). Hence, the inclusion of soybean meal and urea into the ENER treatment as well as the equivalent intake of CP and RDP by ENER and PROT steers may also have contributed to the similar ruminal forage digestibility among treatments.

In summary, results from this experiment suggest that ruminal in situ disappearance and estimated degradability parameters of a low-quality cool-season forage in beef steers is not impacted by supplements based on protein or energy ingredients provided as 0.5% of steer BW/d at isocaloric and isonitrogenous rates.

Experiment 2

No treatment effects ($P = 0.17$) were detected on forage DMI (Table 3). This outcome agrees with the lack of treatment effects on ruminal degradability parameters of the forage used herein reported in Exp. 1, given that ruminal forage digestibility is positively associated with intake (Allen, 1996). Bohnert et al. (2011a) also reported that protein supplementation did not impact DMI of a low-quality cool-season forage, whereas Bowman and Sanson (1996) suggested that supplements based on energy ingredients may be fed at 0.5% of BW without impacting forage intake. In Cappellozza et al. (2014), hay intake was also similar among growing replacement heifers receiving CON, ENER, and PROT. As expected due to the lack of treatment effects on forage intake as well as treatment design and intake rate, total daily DMI, NEm, NEg, CP, and RDP intake were greater ($P < 0.01$) for PROT and ENER compared with CON heifers and similar ($P \geq 0.18$) between PROT and ENER heifers (treatment effects, $P < 0.01$; Table 3). In addition,

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

	Treatment				
Item	CON	PROT	ENER	SEM	*P*-value
PUN, mg/dL	4.6[a]	16.3[b]	18.5[b]	1.9	<0.01
Glucose, mg/dL	62.2[a]	66.5[b]	66.6[b]	1.3	0.04
Insulin, μIU/mL	2.48[a]	3.65[b]	3.09[ab]	0.25	<0.01
IGF-I, ng/mL	112.9[a]	143.6[b]	137.3[b]	7.3	0.03
Progesterone,[3] ng/mL	6.38[a]	7.79[b]	7.75[b]	0.36	0.01

[1]All heifers were offered meadow foxtail hay for ad libitum consumption. Treatments were offered and consumed (d 1 to 19) daily at 1.77 and 1.92 kg of DM for PROT and ENER, respectively, to ensure that PROT and ENER intakes were isocaloric and isonitrogenous. Within rows, values with different superscripts differ ($P \leq 0.05$).

[2]Blood samples were collected on d 13, 15, 17, and 19 of the study immediately before and 2, 4, 6, and 8 h relative to supplement feeding (h 0).

[3]Covariately adjusted to samples collected on d 0, immediately before and 4 and 8 h relative to hay feeding (h 0).

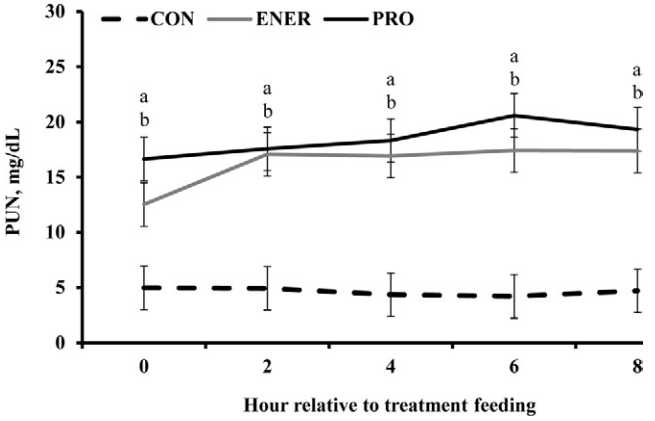

Figure 1. Plasma concentration of plasma urea N (PUN) in pregnant beef heifers consuming a low-quality cool-season forage (meadow foxtail [*Alopecurus pratensis* L.]) and receiving no supplementation (CON; $n = 4$) or supplementation with soybean meal (PROT; $n = 4$; 100% soybean meal on DM basis) or supplementation with a mixture of cracked corn, soybean meal, and urea (68:22:10 ratio, DM basis; ENER). Treatments were offered and consumed at 1.77 and 1.92 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 13, 15, 17, and 19 of the experiment immediately before and at 2, 4, 6, and 8 h relative to treatment feeding (h 0). A treatment × hour interaction was detected ($P < 0.01$) for PUN, given that PUN concentrations increased after supplementation for ENER and PROT heifers (time effect, $P < 0.01$) but did not change for CON (time effect, $P = 0.62$). Within hour, letters indicate the following treatment differences: [a]PROT vs. CON ($P < 0.01$) and [b]ENER vs. CON ($P < 0.02$).

estimated mean daily intake of starch was greater ($P < 0.01$) for ENER compared with PROT and CON and similar ($P = 0.40$) between PROT and CON (Table 3). Hence, PROT and ENER had a similar increase in energy and protein intake compared with CON heifers, although starch was the main energy source provided by ENER.

A treatment effect ($P = 0.03$) was detected for ADG (Table 3). In agreement with the treatment effects observed for DMI and nutrient intake, ADG was greater ($P = 0.01$) for PROT compared with CON, tended to be greater for ENER compared with CON ($P = 0.08$), and was similar between ENER and PROT ($P = 0.28$). Cappellozza et al. (2014) also reported that growing replacement heifers receiving ENER and PROT had similar ADG, which were greater compared with CON cohorts. 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. Supporting this rationale, similar treatment effects were detected for plasma concentrations of PUN ($P < 0.01$), glucose ($P = 0.04$), insulin ($P < 0.01$), and IGF-I ($P = 0.03$) in the present study (Table 4), which are hormones and metabolites associated with dietary protein and energy metabolism in cattle (Hammond, 1997; Huntington, 1997; Wettemann et al., 2003).

A treatment × hour interaction was detected ($P < 0.01$) for PUN (Fig. 1), given that PUN concentrations increased after supplementation for ENER and PROT heifers (time effect, $P < 0.01$) but did not change for CON (time effect, $P = 0.62$). In addition, mean PUN concentrations were greater ($P < 0.01$) for ENER and PROT heifers compared with CON and were similar ($P = 0.44$) between ENER and PROT heifers (Table 4). Concentration of PUN is positively associated with intake of CP, RDP, and concentration of ruminal ammonia (Broderick and Clayton, 1997). Therefore, treatment effects detected for PUN can be attributed to the equivalent treatment effects detected for CP and RDP intake (Table 3). In addition, PUN concentration has been shown to promptly increase after consumption of supplements containing RDP sources such as the ENER and PROT used herein (Cooke et al., 2007a,b), likely due to prompt degradation of soluble protein by rumen microbes and subsequent absorption of ammonia by ruminal tissues (Broderick and Clayton, 1997). Optimal PUN concentration in growing beef heifers range from 15 to 19 mg/dL (Hammond, 1997), which suggests that CON heifers in present study required supplemental CP and RDP. Conversely, PUN concentrations were similar between ENER and PROT heifers and within the optimal level proposed by Hammond (1997), suggesting that these heifers had adequate and equivalent protein intake, utilization, and metabolism despite differences in CP and RDP sources between treatments.

Mean plasma glucose concentration was greater ($P = 0.03$) for ENER and PROT compared with CON heifers and were similar ($P = 0.96$) between ENER and PROT heifers (Table 4). A similar outcome was detected by Cappellozza et al. (2013), which was unexpected given

the difference in starch intake between ENER and PROT heifers (Table 3). Glucose concentration in beef cattle was positively associated with feed intake and rate 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, Huntington (1997) indicated 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. In addition, blood glucose concentration in cattle is fairly stable due to the role of insulin, which may have prevented proper assessment of treatment effects on glucose flux herein (Marston et al., 1995).

Mean plasma insulin concentration was greater ($P < 0.01$) for PROT compared with CON heifers, tended ($P = 0.08$) to be greater for ENER compared with CON heifers, and did not differ ($P = 0.15$) between PROT and ENER heifers (Table 4). Mean plasma IGF-I concentration was greater ($P \leq 0.04$) for PROT and ENER compared with CON heifers and did not differ ($P = 0.55$) between PROT and ENER heifers (Table 4). In cattle, circulating insulin is directly influenced by nutrient intake and blood glucose concentration (Vizcarra et al., 1998; Nussey and Whitehead, 2001) and is known to stimulate hepatic IGF-I synthesis (Molento et al., 2002). Hence, plasma insulin and IGF-I concentrations have been recognized as indicators of nutrient intake and nutritional status of cattle (Yelich et al., 1995; Wettemann and Bossis, 2000; Hess et al., 2005). Similar treatment effects were detected for plasma insulin and IGF-I in Cappellozza et al. (2014), which supports the results detected herein for plasma glucose concentration and suggests that ENER and PROT heifers had equivalent intake, utilization, and metabolism of dietary substrates despite differences in ingredients between treatments.

A treatment effect was also detected ($P = 0.01$) for plasma P_4 concentration. Progesterone concentrations on d 0 were significant covariates ($P < 0.01$) but did not differ ($P = 0.98$) among treatments (6.84, 6.84, and 6.99 ng/mL for CON, ENER, and PROT, respectively; SEM = 0.71), indicating that heifers from all treatment groups had similar plasma P_4 concentration before the beginning of treatment administration. Within samples collected on d 13, 15, 17, and 19, mean plasma P_4 concentrations were greater ($P \leq 0.01$) for PROT and ENER compared with CON heifers and did not differ ($P = 0.93$) between PROT and ENER heifers (Table 4). The main hypothesis of this experiment was that beef heifers consuming a low-quality cool-season forage and receiving a supplement containing an energy ingredient would have greater plasma P_4 compared with unsupplemented or cohorts receiving a supplement based on a protein ingredient. This hypothesis was developed based on the premise that energy ingredients such as corn favor circulating concentrations of glucose, insulin, and IGF-I (Huntington, 1990; Nussey and Whitehead, 2001; Molento et al., 2002), whereas insulin and IGF-I have been positively associated with circulating P_4 concentration. More specifically, IGF-I is known to stimulate luteal P_4 synthesis (Spicer and Echternkamp, 1995). Insulin also stimulates luteal P_4 synthesis (Spicer and Echternkamp, 1995) and alleviates hepatic P_4 catabolism by CYP2C and CYP3A enzymes (Murray, 1991; Cooke et al., 2012; Vieira et al., 2013). In the present experiment, the lack of differences in plasma P_4 concentrations between ENER and PROT heifers, which were greater compared with CON heifers, can be directly attributed to the equivalent treatment effects detected for insulin and IGF-I. Hence, the ENER and PROT treatments used herein equally increased plasma P_4 concentrations in pregnant beef heifers consuming a low-quality cool-season forage.

In summary, heifers offered PROT and ENER had a similar increase in nutrient intake, ADG, and plasma concentrations of hormones and metabolites associated with dietary protein and energy metabolism as well as plasma P_4 concentration compared with CON heifers, despite differences in ingredients between supplement treatments. Hence, pregnant beef heifers consuming a low-quality cool-season forage equally use and benefit, in terms of performance and physiological parameters, from supplements based on protein or energy ingredients provided as 0.5% of heifer BW/d at isocaloric and isonitrogenous rates.

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