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Effect of protein restriction of Angus cows during late gestation: Subsequent reproductive performance and milk yield

S. López Valiente,*[1] S. Maresca,* A. M. Rodríguez,* R. A. Palladino,†‡ I. M. Lacau-Mengido,§ N. M. Long,# and G. Quintans‖

*National Institute of Agricultural Technology (INTA), Experimental Station of Cuenca del Salado, Rauch, 7203, Buenos Aires, Argentina; †Facultad de Agronomía, Universidad de Buenos Aires, 1417, Buenos Aires, Argentina; ‡Facultad de Ciencias Agrarias, Universidad Nacional de Lomas de Zamora, 1836, Buenos Aires, Argentina; §Laboratorio de Regulación Hipofisaria, Instituto de Biología y Medicina Experimental (CONICET), 1428, Buenos Aires, Argentina; #Department of Animal and Veterinary Sciences, Clemson University, Clemson, SC 29634; and ‖National Institute for Agricultural Research, 33000, Treinta y tres, Uruguay

ABSTRACT

The effect of level of CP fed during late gestation on reproductive performance and milk production was studied in multiparous cows. Sixty-eight pregnant Angus cows were used. At 121 d prepartum, cows were blocked by BW (409 ± 57 kg) and expected calving date, randomly assigned to a low-protein (LP = 6% CP) or high-protein diet (HP = 12% CP), and allocated to 12 pens per treatment. After parturition, all cows were managed in a single group until weaning. Body weight and BCS were determined at the start of the experiment, at calving, and at weaning. Nonesterified fatty acids, insulin, IGF-1, and glucose were determined every 24 d prepartum and nonesterified fatty acids and glucose every 38 d postpartum. Progesterone was quantified weekly to indicate luteal activity and estimate interval to first estrus. Milk production was measured until weaning. The HP cows had greater BW gain during the prepartum period ($P < 0.01$) and tended to gain more BCS ($P = 0.06$) than LP cows. The prepartum diet did not affect gestation length ($P = 0.44$) or interval from calving to the onset of luteal activity ($P = 0.35$). Pregnancy rates, milk quality, and production were not influenced by dietary treatments. Cows in the HP treatment had greater prepartum serum urea concentrations than LP treatment ($P < 0.05$). In conclusion, protein level prepartum in multiparous beef cows affected the BW change at calving, without consequences on reproductive performance and milk quality and yield.

Key words: multiparous cow, protein restriction, late gestation, milk yield, postpartum reproductive performance

INTRODUCTION

Cow-calf operations in Argentina are managed under extensive conditions on grazing systems. The quality of forages and roughages is often poor (Sala et al., 1981), particularly in winter, leading to many spring-calving cows having periods of undernutrition during the second half of gestation. Protein supplementation during late gestation has been shown to lead to positive BW and BCS changes in cows and heifers (Stalker et al., 2006; Wilson et al., 2015a,b). Nutrition during the prepartum period is one of the most important factors affecting postpartum anestrous length and subsequent pregnancy rates in beef cows (Wettemann et al., 2003). For instance, diets low in CP from 150 d prepartum to 40 d postpartum negatively affected reproductive performance in heifers (Sasser et al., 1988).

Previous studies in dairy cattle have shown that BCS at calving and during early lactation are associated with milk quality (Roche et al., 2007), but this response does not appear to be similar in beef cows, although there is little research in beef cattle (Corah et al., 1975). Lake et al. (2005) supported the concept that the milk response in beef cows is different from that in dairy cows. The prepartum nutrition also affects some metabolites such as urea and nonesterified fatty acid (**NEFA**) concentrations (Konigsson et al., 2008). There are few studies on the effect of prepartum nutrition on milk quality and production in beef cattle and on its relationship with metabolites and hormonal concentrations, particularly when the level of dietary protein has been altered. The objective of this experiment was to determine the effect of protein nutrition level during the last 120 d before calving on BW and BCS, milk production and composition, reproductive performance, and blood metabolites and hormonal concentrations in mature Angus cows during the last 4 mo of gestation until 6 mo after calving. The hypothesis of this experiment was that a reduction of 35% of the CP require-

The authors declare no conflict of interest.

[1] Corresponding author: lopez.valiente@inta.gob.ar

ment in the prepartum diet of cows will decrease maternal BW and BCS and lead to alterations in milk production and postnatal reproduction compared with nonrestricted cows.

MATERIALS AND METHODS

Animals

The experiment was conducted at Experimental Farm Cuenca del Salado INTA (Buenos Aires, Argentina: 37°05′S 57°52′W) during 2013 to 2014. All procedures were approved by CICUAE INTA- CERBAS nº 87 (Institutional Committee for Care and Use of Experimental Animals of South Buenos Aires region) Buenos Aires, Argentina.

Sixty-eight multiparous Angus cows (initial BW of 409 ± 57 kg) that had just calved in late winter or early spring were synchronized for estrus using a controlled internal drug-releasing device (Cronipres, Biogenesis-Bago, Buenos Aires, Argentina) for 7 d, and upon removal of the device, 500 μg of cloprostenol (Ciclase DL, Syntex, Buenos Aires, Argentina) and 1 mg of oestradiol benzoate (Benzoate de oestradiol Syntex) was administrated intramuscularly. Timed AI was conducted 48 h after oestradiol injection, using semen from a single Angus sire. Fifteen days after AI, a single Angus bull was used for a 15-d natural breeding period. Thirty days after the end of the natural breeding period, pregnancy and fetal age were determined by transrectal ultrasonography. Cows were managed on fescue pastures during early to mid-gestation (62.7% IVDMD, 14.1% CP). At 121 d prepartum, cows were blocked into 4 blocks by BW and expected calving date and randomly assigned to 1 of 2 treatments: a low-protein (6% CP on DM basis; **LP**) or high-protein diet (12% CP on DM basis; **HP**) (Table 1). Cows were allocated to 24 pens (12 pens per treatment) at a rate of 2 or 3 cows per pen. Cows were fed to meet 100% of their ME requirements (NRC, 2000). Rations were fed in plastic feeders as a TMR daily at 0900 h. After parturition all cows were managed in a single group and grazed oats grass (81.4% IVDMD, 16.3% CP) and mixed grass pasture (51.7% IVDMD, 10.3% CP) until weaning.

Ninety-two days after calving, 51 cycling cows were subjected to AI using the synchronization protocol as previously described. Fifteen days after the end of AI, all the cows were exposed to fertile bulls at a ratio of approximately 1 bull per 30 cows for 90 d. The pregnancy rate to timed AI and to natural service was determined by transrectal ultrasonography 28 d after the end of natural service.

BW, BCS, and Gestation Length

The BW and BCS (1 = emaciated to 9 = obese; Wagner et al., 1988) were recorded at the time of group assignment, at calving (less than 12 h after calving), and at weaning. The gestation length was determined only in AI pregnant cows because date of breeding was accurately recorded (LP: 18 cows, HP: 17 cows).

Milk Production and Composition

Milk production was recorded on the same cow per pen on d 20, 34, 47, 75, 103, 135, 165, and 221 (±10.9) postpartum. At 1200 h, cows were separated from calves and each cow was injected intramuscularly with 10 international units of oxytocin (Over, San Vicente, Santa Fe State, Argentina) to facilitate milk letdown. Cows were milked using a portable milking machine 5 min after injection. Calves were fitted with nose plates to prevent suckling (San Miguel, Bahia Blanca, Argentina) and remained with their dams in the same paddock. The following day, at approximately 0600 h, cows were milked again using the protocol described by Quintans et al. (2010). Milk yield was measured throughout lactation using an in-line milk meter (TrueTest, Auckland, New Zealand), and samples were collected to evaluate protein, fat, lactose, total solids (IDF 141C:2000 Bentley Instruments, Chaska, MN), and urea (Chemspec 150, Bentley Instruments). The equation used to estimate milk yield over a 24-h period was proposed by Restle et al. (2004):

$$MY = MMY \times 60/IM \times 24,$$

Table 1. Nutrient content of low-protein and high-protein dietary treatment rations fed to multiparous cows for 120 d before expected parturition

	Prepartum treatment[1]	
Item	**LP**	**HP**
Ingredient (% DM)		
Maize silage	98.5	87.5
Sunflower pellet	—	10.0
Urea	—	1.0
Mineral mix	1.5	1.5
DM (%)	22.1	29.6
Diet composition		
IVDMD (% of DM)	68.1	68.2
NDF (% of DM)	63.2	60.1
ADF (% of DM)	32.7	31.9
Ash (% of DM)	6.5	6.5
CP (% of DM)	6.2	11.7
CP[2] (% NRC)	64.0	121.0
RDP[3] (% CP)	69.0	74.5
RUP (% CP)	31.0	25.5
DMI (kg/d)	7.47	7.53
ME (Mcal/kg)	2.38	2.36

[1]LP = low protein (6% CP); HP = high protein (12% CP).

[2]Ration as a percentage of NRC recommended nutrient requirements of beef cattle (NRC, 2000).

[3]Ration as a percentage of RDP of total CP.

where MY = estimated 24-h milk yield in kilograms per day, MMY = observed milk extracted by milking machine in kilograms, and IM = time interval in minutes between the 2 milkings. Individual lactation curves for each cow were obtained by quadratic equation the week of peak yield and lactation persistence (g/d), which was defined as the linear average daily change in milk production (g/d) between peak lactation and weaning (Jenkins et al., 2000).

Blood Sampling, Metabolites, and Hormone Determinations

Blood samples were collected via jugular venipuncture every 24 d until calving and every 38 d until weaning. Blood was collected into 10-mL vacutainer tubes (Becton, Dickinson and Co., Franklin Lakes, NJ) and centrifuged at 3,000 × *g* for 12 min at 4°C, within 1 h of collection. Aliquots of serum were stored at −20°C until they were assayed for insulin, IGF-1, NEFA, and urea. Coccygeal venipuncture was used to collect blood samples weekly from calving until 130 d postpartum to determine progesterone concentrations. Onset of ovarian luteal activity was considered to have occurred at the first of 2 successive weekly bleeding dates when concentration of progesterone was ≥1 ng/mL. Progesterone was determined by chemiluminescent enzyme immunoassay (IMMULITE 2000, Siemens Healthcare GmbH, Erlangen, Germany), and the intra- and interassay CV were less than 7 and 9.5%, respectively. Concentrations of NEFA were determined by enzymatic method (kit NEFA, Randox Laboratories Ltd., Crumlin, Antrim, UK), and urea was determined by colorimetric method (kit Uremia, Wiener lab S.A.I.C., Rosario, Argentina). The absorbance of both metabolites was assessed in the UV-visible spectrophotometer F3560 (Hitachi, Tokyo, Japan). The intra- and interassay CV were less than 5 and 10%, respectively, for both metabolites. Glucose concentrations were measured using a hand-held glucometer (Abbott, Maidenhead, UK) as described previously by Wittrock et al. (2013). The IGF-1 concentrations in samples were determined with a RIA performed after acid ethanol extraction as described by Lacau-Mengido et al. (2000). Briefly, the IGF-1 antibody (UB2–495) of the NIDDK was used. Assay sensitivity was 2.5 ng/mL, and intra- and interassay CV were less than 8 and 12%, respectively. The insulin concentration was measured via RIA as previously described (Lacau-Mengido et al., 2000) with use of antibovine insulin antibody (Sigma, St. Louis, MO) and standard human insulin provided by Laboratorios Beta (Buenos Aires, Argentina); the minimum detectable concentration was 0.05 ng/mL. The intra- and interassay CV were lower than 8 and 11%, respectively.

Statistical Analyses

The experimental arrangement was a randomized complete block design, where cows were blocked according to BW and expected calving date. For all data, pen was considered the experimental unit. All data analysis was performed using the mixed linear procedures of SAS software (SAS Institute Inc., Cary, NC), where treatment and block were the fixed effect and pen nested into block was the random effect. Concentrations of hormones and metabolites and content of protein, fat, urea, total solids, and lactose in milk were analyzed by ANOVA for repeated measures using the MIXED procedure of SAS. The insulin *P*-value corresponds to the logarithmic transformation. The models included the effects of treatment, pregnancy date (AI or bulls), and time of measurement, and interactions. The AI pregnancy and final pregnancy were analyzed by Fisher test. The significance was declared at $P \leq 0.05$, with a tendency at $P \leq 0.10$.

RESULTS AND DISCUSSION

BW and BCS

The effect of CP supplementation during late gestation on cow performance is presented in Table 2. Different patterns in BW and BCS were observed between cows from both treatments during the prepartum period; HP cows presented greater daily BW gain during the treatment period ($P < 0.01$) and tended to gain more BCS ($P = 0.06$) than LP cows. The amount of protein during the prepartum period did not affect BW and BCS change during lactation ($P = 0.15$ and 0.17, respectively). Cows fed the higher protein level gained almost 19 kg from the start of the treatments to calving, whereas cows fed the lower protein level diet lost 3 kg during the same period. Supply of protein to the rumen bacteria enhances energy yield from fiber sources low in protein as maize silage and, additionally, improves the body nitrogen accretion through a greater microbial protein synthesis and subsequent increase in duodenal protein flow. Our results are similar to those reported by Stalker et al. (2006) and Larson et al. (2009), who reported that cows grazing winter rangeland and receiving 0.45 kg/d of a 42% CP supplement were heavier than cows fed a lower amount of proteins. In these studies, the supplement protein effect can be confounded with increased forage intake. In our case, the improved BW gain and BCS in HP cows may be entirely attributed to the greater level of protein consumed (62 vs. 117 g/kg), given that DMI was controlled during the experimental period (7.5 kg/d).

Gestation and Reproductive Performance

When dams are supplemented with CP during late gestation, gestation length may be decreased. Funston et al. (2010) and Stalker et al. (2006) noted that CP supplemented cows had calves that were born 3 or 4 d earlier than their unsupplemented counterparts. This could be attributed to an increase in fetal development during the supplementation period. This is contrary to the result observed in this experiment in which the level of CP in the diet during late gestation did not affect ($P = 0.44$) the gestation length of the cows on different nutritional treat-

Table 2. Body weight and BCS pre- and postpartum of multiparous cows fed diets with high or low protein content for 120 d before the expected calving date

	Prepartum treatment[1]			*P*-value
Item	LP (mean)	HP (mean)	MS error	Treatment
BW (kg)				
Initial	415	402	58.1	0.11
Parturition	411	423	55.2	0.23
Change during treatment	−3	19	14.3	<0.01
Weaning	430	423	46.9	0.47
Change during lactation	20	0	27.9	0.15
BCS				
Initial	4.3	4.3	0.5	0.79
Parturition	4.9	5.3	0.6	0.70
Change during treatment	0.6	1.0	0.3	0.06
Weaning	4.9	4.7	0.5	0.55
Change during lactation	0.0	−0.6	0.5	0.17

[1]LP = low protein (6% CP); HP = high protein (12% CP).

ments. Similar to our result, Amanlou et al. (2011) and Van Emon et al. (2014) reported that gestation length was not altered by maternal CP supplementation in ewes during late gestation. Means of reproductive parameters are presented in Table 3. Prepartum CP nutrition did not influence ($P = 0.35$) days from parturition to ovarian luteal activity. Also, the amount of prepartum protein did not affect AI pregnancy rate (48.9%, $P = 0.41$) nor final breeding season pregnancy rate (94.7%, $P = 0.48$). It has been shown that BCS at calving affects subsequent pregnancy rates and the interval from parturition to resumption of luteal activity (Wettemann et al., 2003). Richards et al. (1986), using 1 to 9 BCS scale, found that cows with a BCS of 5 or greater at calving become pregnant sooner than cows calving with a BCS of 4. In our experiment, BCS tended to change due to treatment, but we found no differences in pregnancy rates nor interval to first estrus when cows were fed with different protein levels during late gestation. This is probably because the cows on both treatments calved with BCS near to or greater than 5 and were mature multiparous cows. These results are in agreement with other authors who reported no effect of manipulated prepartum diets on reproductive performance, with cows calving with a BCS of 5 or greater (Stalker et al., 2006; Larson et al., 2009; Radunz et al., 2010; Wilson et al., 2015a,b).

Milk Production and Quality

Milk production and quality results are presented in Table 4. Dams that received the HP diet during the last 4 mo of gestation had similar daily milk production and total adjusted 210-d milk yield to LP cows ($P = 0.30$ and $P = 0.77$). Peak yield and week of peak yield were similar for cows in both treatment ($P = 0.38$ and $P = 0.92$). The lactation persistence was similar for LP and HP cows ($P = 0.93$). Indeed, the milk curve was similar for cows in both treatments ($P > 0.65$). Milk composition of fat, protein,

Table 3. Postpartum reproductive performance of multiparous cows fed low-protein and high-protein dietary treatments for 120 d before expected parturition

	Prepartum treatment[1]			*P*-value
Item	LP (mean)	HP (mean)	MS error	Treatment
Gestation length (d)	276.3	274.9	3.56	0.44
Interval to first estrus (d)	64.3	59.8	10.22	0.35
AI pregnancy rate (%)	40.5	56.0		0.41
Final pregnancy rate (%)	99.3	90.1		0.48

[1]LP = low protein (6% CP); HP = high protein (12% CP).

Table 4. Milk production and quality in multiparous cows fed low-protein and high-protein dietary treatments for 120 d before expected parturition

	Prepartum treatment[1]			*P*-value		
Item	LP (mean)	HP (mean)	MS error	Treatment	Period	Treatment × period
Milk yield (kg/d)	5.7	5.3	1.33	0.30	—	—
TY210d[2] (kg)	1,162	1,130	1.2	0.77	—	—
Peak yield (kg)	6.5	6.1	0.87	0.38	—	—
Week of peak yield	14.5	14.2	1.14	0.92	—	—
Lactation persistence (g/d)	−22.2	−22.6	1.26	0.93	—	—
Fat (%)	2.8	2.6	0.61	0.31	<0.001	0.15
Protein (%)	3.4	3.3	0.11	0.12	<0.001	0.82
Urea (%)	11.2	11.0	1.43	0.33	<0.001	0.91
Lactose (%)	4.9	4.9	0.16	0.92	<0.001	0.33
Total solids (%)	12.0	11.8	1.12	0.16	<0.01	0.15

[1]LP = low protein (6% CP); HP = high protein (12% CP).

[2]TY210d = total adjusted 210-d milk yield.

urea, lactose, and total solids was unaffected by dietary treatment during the trial ($P \geq 0.12$).

Multiple studies have evaluated the effect of nutrition during gestation on reproductive performance of beef cows. However, few have investigated the effects of nutrition during gestation on milk production and quality, and even less have worked with protein nutrition. Milk production of dams may be affected by nutrition during late gestation, BCS at calving, and postpartum nutrition. An increase of prepartum protein intake has been associated with a differential response in terms of milk production and quality in dairy cattle (Chew et al., 1984; Bell et al., 2000; Park et al., 2002; Kokkonen, 2014). However, a similar response does not appear to happen in beef cows as was observed in our experiment. This is in agreement with Larson et al. (2009), who found no differences in milk production when beef cows were supplemented with 0.45 kg/d of a 42% CP range cube during late gestation. Body condition score achieved at calving on this experiment with beef cows could explain the differences found with dairy cow studies. The association between BCS at calving and milk production and quality has been extensively studied in dairy cows. Several studies have demonstrated that this association is nonlinear, rising from thin to moderate BCS and decreasing in overconditioned dairy cows (Roche et al., 2007). On the other hand, Lake et al. (2005) and Radunz et al. (2010) observed similar milk production in beef cows with thin to moderate BCS at calving. However, Quintans et al. (2010) found that cows with moderate BCS (4.8) produced more milk than cows with low BCS (3.9), possibly due to the fact that the beef cows were handled separately to maintain the differences in BCS until weaning. The lack of consistent milk results in beef cattle could be due to the lack of genetic selection to mobilize reserves for milk production that are observed in modern dairy cattle. It has been previously shown in dairy cows that differences in prepartum protein levels lower than our experiment have resulted in differences in milk production (Chew et al., 1984).

Postpartum protein level can negate the effects of prepartum protein restriction. Bell et al. (2000) concluded that relatively high protein diets during lactation may mask the effect of insufficient prepartum protein diets. High levels of CP in the forage grazed during early lactation in this experiment (16.3% CP) could have mitigated the effects of prepartum nutritional level.

Blood Metabolites

Whole blood glucose concentrations from cows on both treatments reached the greatest concentration at 20 d before calving (86.5 ± 3.6 ng/dL), and no differences were found between treatments ($P = 0.62$; Figure 1A). Serum urea concentration during the prepartum period was greater in HP compared with LP cows ($P < 0.01$, Figure 1B), but values were similar between treatments after calving. Serum NEFA concentration during the prepartum period was lower ($P < 0.01$) compared with the lactation period (Figure 1C). There was a significant treatment × time interaction ($P = 0.05$) resulting from NEFA concentrations being increased in LP cows compared with HP cows only at 20 d before parturition.

Glucose is the main energy source used by the neural system, and the neural-endocrine system is intimately involved in the control of reproduction and hormone secretion (Adams et al., 1987). The whole blood glucose concentrations of both treatments were not different during the trial, probably because similar energy concentrations

were supplied. This observation was confirmed by Park et al. (2002), who fed diets with different levels of CP (9.7 to 16.2%) but similar energy concentrations during the last 28 d before calving in dairy cows. Richards et al. (1989) found differences in plasma glucose concentration when energy supplied was strongly restricted. In contrast, Long et al. (2009) found no differences in blood glucose with contrasting energy restriction levels during early gestation in old cows; however, glucose concentration of old cows was less than young females, indicating that younger cows are more susceptible to nutrient restriction than older cows. Wallace et al. (2005) speculated that older animals have the ability to partition adequate nutrients to support fetal growth and the younger animal do not.

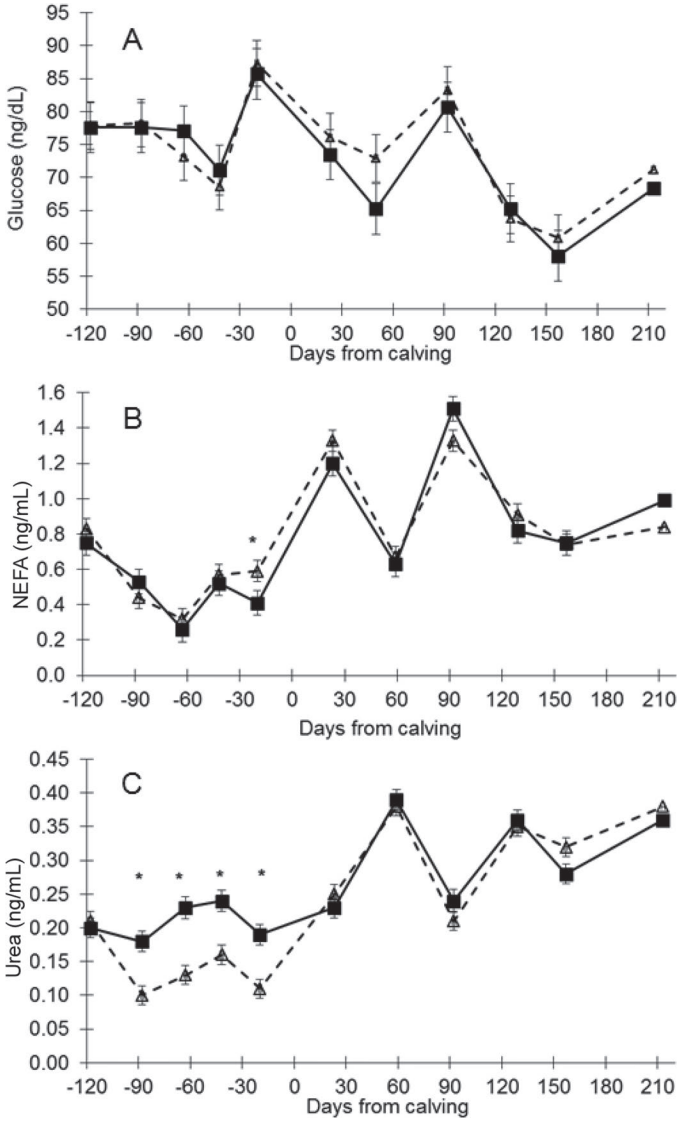

Figure 1. Whole blood glucose (A), serum nonesterified fatty acid (NEFA; B), and serum urea (C) concentrations of multiparous beef cows fed low-protein (6% CP, triangle symbols) and high-protein (12% CP, square symbols) dietary treatments for 120 d before expected parturition during treatment and lactation. Values are means ± SEM. *$P < 0.01$.

Increased concentrations of NEFA in plasma of cows are an indication of a negative energy balance (Richards et al., 1989). The concentrations of serum NEFA were similar in both treatments during all of the experiment except during the last blood collection. The concentrations of serum NEFA were greater ($P < 0.05$) during the last prepartum blood collection in the LP cows compared with the HP cows, suggesting a reserve mobilization in LP.

Hormones

No differences were found in serum insulin concentration between cows from LP and HP treatments ($P = 0.4$), and concentrations were greater at the start of treatments and declined as pregnancy progressed in both treatments ($P < 0.001$; Figure 1A). No differences were found between cows from LP and HP treatments for serum IGF-1 concentrations ($P = 0.9$). The highest concentrations were observed at 88 d before calving, and then they decreased from calving to weaning (Figure 2B, time effect $P < 0.01$).

From the data collected, it is not evident that protein restriction during the last 4 mo of gestation affected IGF-1 concentration. Several studies have reported that blood IGF-1 concentration is altered by nutritional status and in particular by protein supplementation in the first and sec-

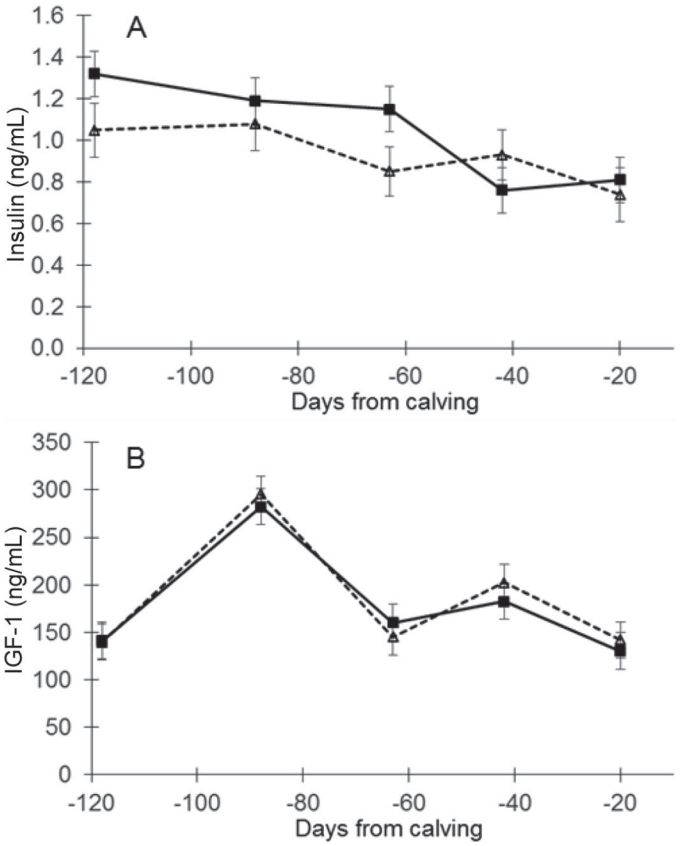

Figure 2. Serum insulin (A) and IGF-1 (B) concentrations of multiparous beef cows fed low-protein (6% CP, triangle symbols) and high-protein (12% CP, square symbols) dietary treatments for 120 d before expected parturition during treatment. Values are means ± SEM.

ond third of gestation, being lower at the end of pregnancy (Perry et al., 2002; Sullivan et al., 2009). However, the circulating IGF-1 concentration can vary with the stage of development. Protein restriction caused a decrease in serum IGF-1 in the younger animals, and this effect was progressively attenuated with increasing maternal age (Fliesen et al., 1989).

Insulin concentration decreased until calving in cows of both groups; this is probably a metabolic adaptation to cope with the energy demands of lactation (Taylor et al., 2003). No differences in serum insulin concentrations between treatments could be explained, because diets have no restrictive energy content (Rusche et al., 1993). Radunz et al. (2010) found that multiparous beef cows fed diets prepartum that were more energy dense had more blood insulin after feeding than cows fed less energy dense diets. A similar conclusion was postulated by Grimard et al. (1995) in postpartum beef cows.

IMPLICATIONS

In conclusion, protein restriction during late gestation did not affect subsequent reproductive performance and milk production and quality. Our results indicate that there is no decrease in production when cows are moderately (36%) protein restricted during the last gestation trimester provided energy in the diet is not limiting. If the cows had started in a lower BCS or had a greater milking ability, the results of this experiment may have been different. However, further research is necessary to better understand the effect of prepartum protein restriction in beef cows and its interaction with postpartum diet under grazing conditions.

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