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Research Article

Effect of Protein Restriction During Mid-to Late Gestation of Beef Cows on Female Offspring Fertility, Lactation Performance and Calves Development

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Abstract

Cow calf operation in Argentina are managed under extensive grazing condition and the quality of forages is often poor during second half of gestation. Protein restriction in bovine gestation affects production traits in progeny. The present work investigated the effects of two levels of crude protein (CP) provided to mature dams during late gestation on subsequent heifer's growth postweaning, reproductive performance, milk production and grand offspring evolution. At 121 d prepartum, 68 multiparous Angus cows were randomly assigned to low protein (LP, 6% CP) or high protein (HP, 12% CP) at 12 pens per treatment group. Following calving, all cow/calf pairs were housed together until weaning, then female progeny were removed and maintained on pasture as a single group. At puberty (P = 0.01) and pregnancy determination (P = 0.05) the HP heifers were heavier than LP heifers. The LM area was greater at 20 mo of age in HP compared to LP heifers (P = 0.01) and the HP heifers had greater 12th rib fat thickness at 30 and 35 mo of age (P = 0.02). Serum IGF-1 concentration was greater in HP heifers compared to LP heifers (P = 0.05). No dam nutrition effects were found on offspring age at puberty (P = 0.98), final pregnancy rate (P = 0.28) or milk yield (P = 0.76) but heifers born to LP dams had greater milk protein percentage (P = 0.04) and tended to produce increased milk fat percentage (P = 0.08) compared with HP heifers. The LP grand offspring grew faster from birth until weaning compared with HP grand offspring (P < 0.01) with reduced insulin concentrations (P = 0.03) and tended to have increased glucose concentrations than HP calves (P = 0.09). Protein supplementation during late gestation does not affect reproductive performance of the offspring heifers but did impact their BW evolution, milk quality and grand offspring performance. Implications: The use of diet with low amount of protein which the female fetus is exposed in utero can affect her subsequent development and ability to nourish subsequent generations.

Keywords: *Fetal Programming; Offspring Puberty; Milk Yield; Progeny Growth*

Introduction

Cow-calf operations in Argentina are managed under extensive grazing conditions. In winter, the quality of forages is low and therefore cows experience periods of undernutrition that coincides with the second half of gestation. Nutritional restriction during late gestation

could affect postnatal growth and physiology [1]. In beef cattle, fetal nutrition has also been observed to influence muscle fiber size and growth, adipogenesis [2,3] and meat quality [4,5]. Little is known about environmental impacts on the reproductive performance of female offspring or how endocrine function could be affected. Previous studies indicate that protein supplementation during the last third of gestation increased heifer offspring BW and fertility. Funston., *et al*. [6] supplemented heifers with protein during late gestation, concluded that prenatal nutrition tended to affect age at puberty. Previous research [7] indicates that protein supplementation during the last third of gestation resulted in increased BW at prebreeding and pregnancy diagnosis, as well as greater pregnancy rate of daughters from supplemented dams compared with daughter of non -supplemented dams. However, it remains unclear if the responses observed in the previous experiments were due specifically to supplemented protein or an overall increase in total energy intake associated with increased rumen available protein supplementation. Conversely, Shoup., *et al*. [8] concluded that supplementing dams during late gestation with dried distiller's grains and soybean hull did not affect growth and reproductive performance of subsequent female progeny. In sheep, Jenkinson [9] observed that ewe nutrition during gestation can impact fetal mammary gland development and, therefore, future milk production and these factors could impact the BW of their grand-offspring [10].

Objective of the Study

The objective of the current study was to determine the effects of mid to late gestation protein supplementation on BW gain, reproduction and milk production and composition in female offspring, serum concentration of insulin, IGF-1, and whole blood glucose in female offspring and grand offspring.

Materials and Methods

Animals and experimental design

All animal's procedures were conducted in accordance with procedures approved by CIUCAE INTA-CERBAS nº87 (Institutional committee for Care and Use of Experimental Animals of South Buenos Aires region) Buenos Aires, Argentina.

A detailed description of management practices is available in López Valiente., *et al.* [11]. Briefly, multiparous Angus cows of similar genetic background were inseminated using semen from a single sire via a timed artificial insemination protocol, then cows were exposed to a bull for 15d. Pregnant cows were enrolled in a randomized complete block design. Treatment was initiated at 121 ± 14d of gestation, when cows were stratified by BW and expected calving date and were allocated in 24 pens to either a low protein (LP; 6% CP) or high protein (HP; 12% CP) diet at 12 pens per treatment. Cows were fed to meet 100% of NEm requirements in both treatments and provide 64% and 121% of CP requirements for LP and HP respectively. The LP diet consisted of 98.5% corn silage and 1.5% of mineral premix and HP diet consisted of 87.5% of corn silage, 10% of sunflower pellet, 1% of urea and 1.5% of mineral premixed. All cows were allowed to calve naturally, and after calving, calves and their dams were managed as one group on oat grass and mixed grass pasture until weaning. After weaning, heifers were separated from males progeny and were managed as a group to commercial farming practices of National Institute of Agricultural Technology (INTA).

Heifers and calf management, BW and ultrasound measurement recording

Treatments were limited to the dam from mid to late gestation CP supplementation diet; no further treatments were applied to calves following birth. After weaning, heifers (n = 28; offspring form the original dams at LP n = 15 heifers and HP n = 13 heifers) were managed as one group until the end of trial. Heifers grazed a native and winter pasture throughout the study.

All heifers were weighed every 3.2 mo (± 0.8) until 20th mo of age (corresponding with pregnancy diagnosis, Period 1). At 16.5 mo of age, heifers were exposed to one bull for 60 d and pregnancy diagnosis was performed via transrectal ultrasonography 45 d after completion of the breeding period. All pregnant heifers were weighed every 1.9 ± 0.7 mo until weaning (Period 2). Fourteen calves comprised the grand offspring from the original cows (LP n = 9; HP n = 5) were tagged, identified to their mother, and weighed within 24h after birth, 3.8,

5.7 and 7.6 (± 0.3) mo of age. Ultrasound measurements of 12th rib fat thickness, and longissimus muscle (LM) area were taken between the 12th and 13th rib from the right side of the heifers, every 5 mo from 10 mo of age until weaning. Ultrasound measurements of grand offspring were collected at 3.8, 5.7 and 7.6 (± 0.3) mo of age using an Aquila pro, Esaote Europe B.V. Maastricht, NL; 3.5-MHz probe.

Blood collection and assays

Blood samples were collected via jugular venipuncture in heifers and calves at the same time BW were registered to determine blood glucose, serum insulin and IGF-1 concentrations. Blood samples were cooled immediately on ice and within 1 h, serum was collected via centrifugation at 3,000 x g for 12 minutes. Aliquots of serum were stored at -20°C until assayed for insulin and IGF-1 concentrations. Glucose concentrations were measured using a hand-held glucometer (Abbott ©, UK) as described previously [12]. Serum IGF-1 concentrations were determined via a radioimmunoassay (RIA) performed after acid ethanol extraction [13]. Briefly, the IGF-1 antibody (UB2-495) of the NIDDK was used. Assay sensitivity was 2.5 ng/mL with an intra and inter-assay CV of less than 8% and 12% respectively. Serum insulin concentration were quantified via RIA [13] with use of antibovine insulin antibody (Sigma, St. Louis, Missouri, USA) and standard human insulin provided by Laboratorios Beta (Buenos Aires, Argentina). The minimum detectable concentration was 0.05 ng/mL with an intra and inter-assay CV of lower than 8% and 11%, respectively.

Age at puberty and progesterone curve

To determine time of puberty onset in offspring, coccygeal venipuncture was used to collect blood samples weekly from 13.2 until 16.5 mo of age in order to determine serum progesterone concentrations. Serum from blood samples were obtained as previously described. Onset of ovarian luteal activity was considered to have occurred at the first of two successive weekly bleeding dates with a concentration of progesterone ≥ 1 ng/mL. To determine progesterone concentrations during an estrus cycle, once estrus was observed, 6 LP and 6 HP randomly selected heifers were synchronized for estrus using a controlled internal drug-releasing device (Cronipres®, Biogenesis-Bago, Argentina) for 7 days, and upon removal of the device, 500 µg of cloporostenol (Ciclase DL®, Syntex, Argentina) and 1 mg of oestradiol benzoate (Benzoate de oestradiol Syntex®, Argentina) were administrated intramuscularly. Blood samples were collected via jugular venipuncture on day 0 and 7 (day of placement and removal of the device) and every two days until day 35 to determine the progesterone concentrations curve over an estrus cycle. Serum progesterone was determined by chemiluminescent enzyme immunoassay (IMMULITE@ 2000) with an intra and inter-assay CV of less than 7% and 9.5%, respectively.

Heifer´s milk production and composition

Milk production of female offspring was recorded on days 20, 55, 90, 125, 160, 195 and 230 (± 10) of lactation. At 0:200 p.m., female offspring were separated from calves and each female offspring was injected intramuscularly with 10 IU of oxytocin (Over®, Argentina) to facilitate milk letdown. Female offspring were milked using a portable milking machine 5 min after oxytocin injection. Calves were fitted with strong nasal plates and remained with their dams in the same paddock. The following day, at ~06:00 a.m., female offspring were milked again using the described protocol by Quintans., *et al* [14]. Milk yield was measured throughout lactation using in line milk meters (TrueTest, Auckland, New Zealand). Nose plates were removed from calves after milking and cow-calf pairs returned to the paddock. Milk samples were collected in order to measure protein, fat, lactose, total solid (IDF 141C:2000 Bentley Instruments, Chaska, MN, USA) and urea (Chemspec 150, Bentley Instruments, Chaska, MN, USA) in Dairy Laboratory LABVIMA, Buenos Aires, Argentina. The equation used to estimate the milk yield in 24h was proposed by Restle., *et al* [15].

Statistical analyses

The experimental design was a randomized complete block design, where the originals cows (heifer's mothers) were blocked according to BW and expected calving date. For all data, pen was considered the experimental unit. All data was analyzed using the mixed linear procedures of SAS (SAS Institute, Cary, NC, USA) where treatment and block were the fixed effect while pen nested with in block was the random effect. Growth performance, concentrations of hormones, glucose and protein, milk fat, fat 4% corrected milk, urea, total solids

and lactose content in milk were analyzed as a repeated measures analysis using the MIXED procedure of SAS with treatment, day and their interaction in the model. The least squared means method was used for post hoc mean separation following a significant ($P < 0.05$) preliminary F test. The final pregnancy rate was analyzed by Fisher Test. The Wood function [16] was used to describe lactation curves and was analyzed by NLMIXED procedure of SAS. In all cases, least square means and polled MSE are reported, and differences considered significant at $P \leq 0.05$, with a tendency at $P \leq 0.10$.

Results

Cows and calves performance up to weaning in response to different protein amounts during mid to late gestation has been previously reported [11,17]. During treatment, HP dams gained 22.1 ± 15.1 kg ($P < 0.01$) more than LP dams and BCS changes tended to be greater for HP compared to LP dams (0.6 vs 0.1; respectively $P = 0.06$). BW and BCS during lactation were not different between treatments ($P \leq 0.15$). Milk production and quality were not affected by level of protein during late gestation ($P > 0.12$). Offspring born from HP cows had a tendency to be heavier at birth than LP ($P = 0.06$), but no differences were found on BW of offspring at weaning ($P = 0.75$).

Heifers BW and ultrasound measurement

The evolution of BW of heifers is presented in figure 1. Dam prepartum protein level did not affect BW during period 1, defined as the period prior to the diagnosis of pregnancy ($P = 0.16$) but heifers from HP dams had greater ADG than LP heifers (0.45 ± 0.02 vs. 0.35 ± 0.02 kg/d; $P = 0.03$). During period 2 from pregnancy determination to weaning of the grand offspring, HP heifers were heavier than LP heifers ($P = 0.05$; Figure 1). The LM area at 20 mo of age was greater in HP heifers compared to LP heifers (treatment x period $P = 0.01$, Figure 2) and was affected by maternal nutritional treatment during period 2 (LP 50.7 ± 2.8 cm^2; HP = 54.0 ± 3.3 cm^2 $P = 0.02$, Figure 2). Level of protein fed during gestation did not effect 12th rib fat thickness during period 1($P = 0.75$; Figure 3) however, during period 2, the HP heifers had greater 12th rib fat thickness at 30 and 35 mo of age (treatment x period $P = 0.02$; Figure 3).

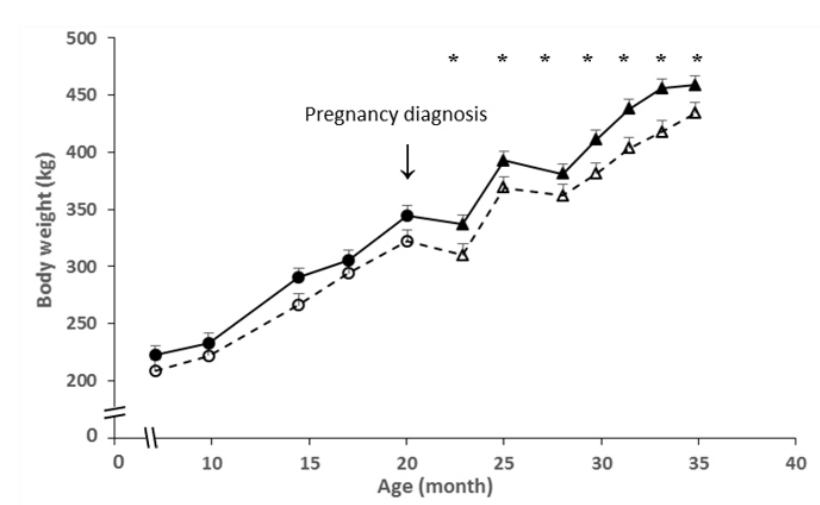

Figure 1: *Effect of low (6% CP, open symbols) or high (12% CP, solid symbols) protein level during late gestation on BW evolution during period 1 previous pregnancy diagnosis (circles) or period 2 post pregnancy diagnosis (triangles). Only pregnant animals are represented in period 2. *Indicates treatment differences ($P \leq 0.05$). Values are means ± SEM. (period 1: treatment, $P = 0.16$; time, $P < 0.001$; treatment x time, $P = 0.44$; period 2: treatment, $P = 0.05$; time, $P < 0.001$; treatment x time, $P = 0.65$).*

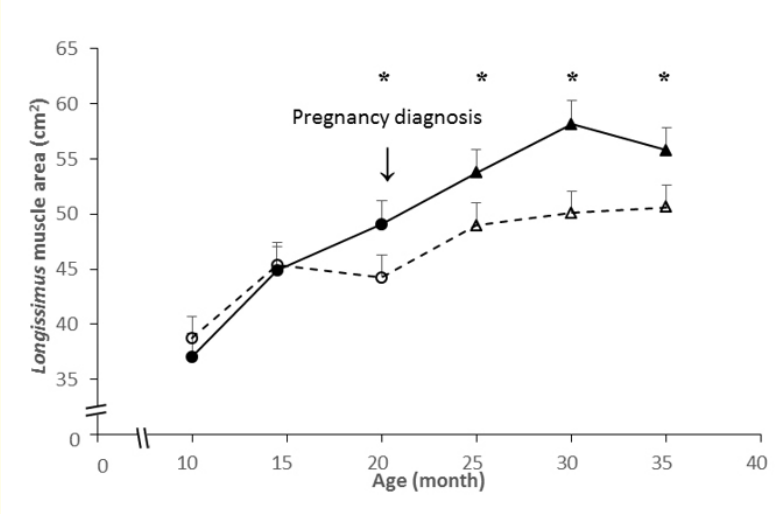

Figure 2: *Effect of low (6% CP, open symbols) or high (12% CP, solid symbols) protein level during late gestation on longissimus muscle area during period 1 (circles) previous pregnancy diagnosis or period 2 post pregnancy diagnosis (triangles). * Indicates treatment differences (P ≤ 0.05). Values are means ± SEM (period 1: treatment, P = 0.38; time, P < 0.001; treatment x time, P = 0.01; period 2: treatment, P = 0.02; time, P = 0.51; treatment x time, P = 0.60).*

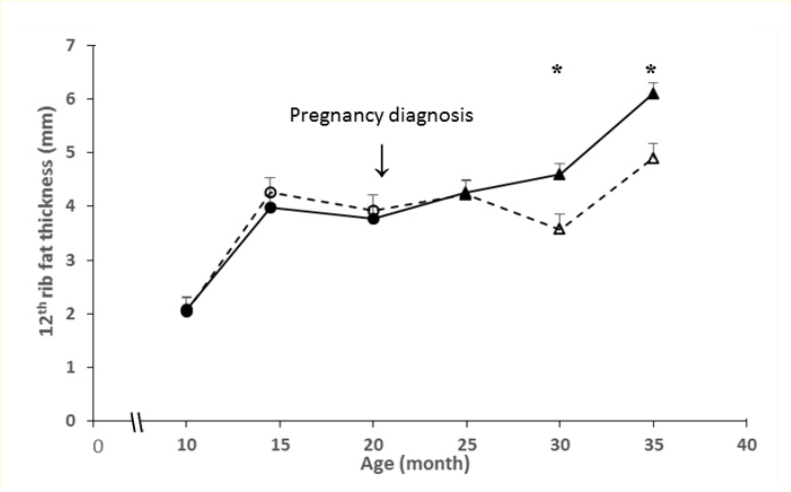

Figure 3: *Effect of low (6% CP, open symbols) or high (12% CP, solid symbols) protein level during late gestation on 12th rib fat thickness during period 1 previous pregnancy diagnosis (circles) or period 2 post pregnancy diagnosis (triangles). * Indicates treatment differences (P ≤ 0.05). Values are means ± SEM (Period 1: treatment, P = 0.75; time, P < 0.001; treatment x time, P = 0.44; Period 2: treatment, P = 0.05; time, P < 0.001; treatment x time, P = 0.02).*

Hormones and glucose

Serum IGF-1 concentrations during period 1 were greater in HP heifers compared to LP heifers (P = 0.05). Concentrations of serum insulin and blood glucose were not influenced by maternal protein level at any sampling time (P > 0.19). Similar to period 1, only serum IGF-1 concentration were affected in period 2, with HP heifers having increased serum IGF-1 concentrations compared to LP heifers (P = 0.01; Table 1).

	Treatments[1]		P-value		
Item[2]	**LP**	**HP**	**Treatment**	**Period**	**Treatment x period**
Period 1					
No.	15	13			
IGF-1 ((ng/mL)	193 ± 16	240 ± 18	0.05	0.01	0.46
Insulin (ng/mL)	1.01 ± 0.08	0.96 ± 0.09	0.68	0.02	0.51
Glucose (ng/dL)	71.5 ± 2.4	73.8 ± 2.7	0.51	0.22	0.13
Period 2					
No.	9	5			
IGF-1 (ng/mL)	135 ± 10	174 ± 12	0.01	0.87	0.28
Insulin (ng/mL)	0.64 ± 0.08	0.74 ± 0.09	0.42	<0.001	0.69
Glucose (ng/dL)	55.6 ± 1.7	58.7 ± 2.1	0.19	0.12	0.76
Calves					
No.	9	5			
IGF-I (ng/mL)	94 ± 26	159 ± 35	0.18	0.09	0.76
Insulin (ng/mL)	0.74 ± 0.10	1.06 ± 0.14	0.03	0.14	0.82
Glucose (ng/dL)	90.9 ± 4.2	74.9 ± 8.1	0.09	<0.001	0.66

Table 1: *Effect of protein consumption during late gestation on blood variables for cows daughters and their calves.*

[1]LP, Low Protein (6% CP) High Protein (12% CP).

[2]Period 1: period before pregnancy diagnosis, Period 2: period after pregnancy diagnosis.

Reproductive performance

The effect of dam nutritional treatment during mid to late gestation on heifers reproductive performance is presented in table 2. Pre-partum dam nutrition did not affect age at puberty or final pregnancy rate between treatments (P = 0.98 and P = 0.28 respectively), but LP heifers were 30.8 kg lighter (P = 0.01) than HP heifers at puberty. The level of dam nutrition had no effect (P > 0.11) on the progesterone curves collected over an estrus cycle in the 6 subsampled heifers per treatment.

Milking performance

Milk production and composition of heifer offspring are presented in table 3. Dam nutrition did not effect (P > 0.16) milk yield, milk yield corrected at 4%, fat, urea, lactose or total solids at any time point. However, LP heifers produced increased protein percentage (P = 0.04) compared to HP heifers.

Dam´s protein nutrition level during mid to late gestation did not affect heifers offspring estimated curve of milk production (LP yield model= 2.5891 * Day 0.2322 * EXP (0.00549 * Day; HP yield model= 2.4107 * Day0.2256 * EXP (-0.005342 * Day; P = 0.98).

Item	Treatments[1]		
	LP	HP	P-value
Age at puberty (d)	435 ± 10	435 ± 10	0.98
BW at puberty (kg)	278 ± 16	309 ± 16	0.01
Final pregnancy rate (%)	73.3 ± 11	53.8 ± 11	0.28
P4 Average (ng/mL)	4.41 ± 0.37	3.37 ± 0.37	0.23
Cycle time (d)	11.2 ± 0.5	10.6 ± 0.5	0.35
P4 Peak, (d)	7.30 ± 0.70	6.01 ± 0.69	0.11
P4 Max concentration (ng/mL)	8.00 ± 1.18	9.24 ± 1.16	0.48
P4 Area under the curve	52.3 ± 4.4	41.2 ± 4.2	0.21

Table 2: *Effect of protein nutrition level during late gestation on heifer reproductive performance.*
[1]LP, Low Protein (6% CP) High Protein (12% CP).

Item	Treatments[1]		P-value		
	LP	HP	Treatment	Period	Treatment x period
No.	9	5			
Milk yield (kg)	3.22 ± 0,25	3.32 ± 0,33	0.76	< 0.001	0.90
Milk 4 (%)	2.97 ± 0.33	3.11 ± 0.37	0.75	< 0.001	0.79
Fat (%)	3.00 ± 0.36	2.94 ± 0.37	0.08	< 0.001	0.91
Protein (%)	3.23 ± 0.06	3.09 ± 0.07	0.04	< 0.001	0.53
Urea (%)	10.3 ± 0.6	10.3 ± 0.6	0.98	0.39	0.92
Lactose (%)	4.64 ± 0.07	4.74 ± 0.08	0.16	0.007	0.65
Total solids (%)	11.7 ± 0.5	11.5 ± 0.5	0.77	0.006	0.36

Table 3: *Effect of protein nutrition level during late gestation on offspring milk yield and quality.*
[1]LP, Low Protein (6% CP) High Protein (12% CP).

Grand offspring evaluation

Grand offspring BW evolution are illustrated in figure 4. Grand dams protein nutrition showed a treatment by day interaction (P = 0.001) for grand offspring BW, where the LP grand calves were heavier than HP grand calves during lactation except at birth. However, no differences were observed in LM area (29.8 ± 1.8 cm^2; P = 0.25) between treatments. The IGF-1, insulin and glucose concentrations of grand offspring are presented in table 1. Prenatal protein treatment of grand dams did not influence IGF-1 concentration of grand calves (126.5 ± 30.5 ng/mL, P = 0.18), but insulin concentrations of HP calves were greater than those of LP calves before weaning (1.06 ± 0.14 vs 0.74 ± 0.10 ng/mL; P = 0.03). The LP grand calves tended to have greater whole blood glucose concentrations from birth to weaning compared with HP grand calves (P = 0.09).

Discussion

We hypothesized that low protein nutrition of cows during mid to late gestation would affect circulating levels of glucose, insulin and IGF1 in subsequent offspring, impairing the development of muscles and fat, fetal ovaries, increasing the age of puberty and affecting

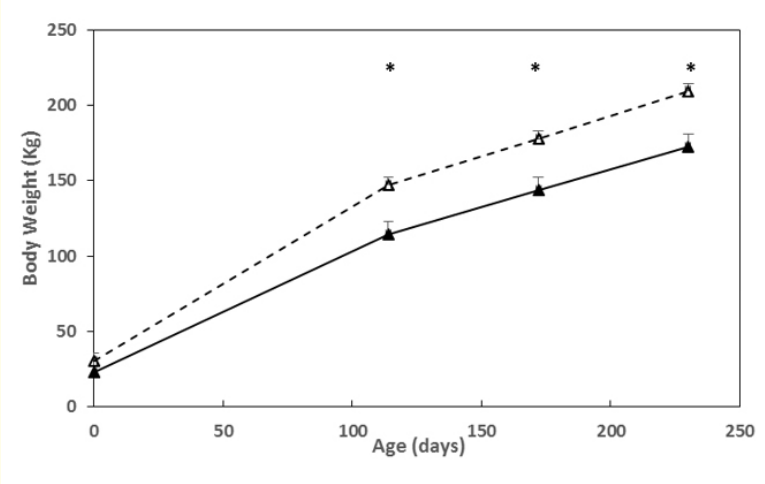

Figure 4: *Effect of low (6% CP, open symbols) or high (12% CP, solid symbols) protein level during late gestation on grand-offspring BW until weaning. *Indicates treatment differences (P ≤ 0.05). Values are means ± SEM. (treatment, P = 0.01; time, P < 0.001; treatment x time, P = 0.001).*

the development of the mammary gland. This would result in decreasing the milk production or milk component production and lighter offspring weight until weaning compared to offspring born to dams fed high protein during mid to late gestation. During period 1, BW, rump height (data not shown) and 12th rib fat thickness were similar between treatments (P > 0.10; Table 2). No differences in ADG were observed in heifer [6,7] or steer progeny [4,6,17] born to dams supplemented with protein during late gestation.

Other studies have also reported that feeding DDGS [8,18,19] or different energy diets [20] in late gestation and late gestation/early lactation did not alter ADG of heifer progeny. However, HP heifers achieved puberty at a heavier BW compared to LP heifers (P = 0.01). Then, at pregnancy diagnosis (period 2), HP heifers were heavier than LP heifers (P = 0.05). Similar results have been reported by different authors [6,7] who observed greater BW at pregnancy diagnosis in heifers from protein supplemented dams than heifers from un supplemented dams. This is in agreement with previous reports in lambs [21,22] and rat pups [23] born to dams on low planes of nutrition during gestation in which offspring were slower growing, had lower average daily gain, and lighter body weights than those from dams on greater planes of nutrition.

Fat thickness at the 12th rib was increased (P = 0.02) in HP heifers in period 2 when compared to LP heifers. These result are in agreement to previous studies in steers [24] where offspring of dams grazing low quality forage had reduced 12th rib fat thickness than offspring of dams grazing improved pasture. Underwood., *et al.* [24] indicated that increased fat thickness of steers from dams that grazed improved pastures compared to dams that grazed dormant native pastures may be due to increased number of adipocytes and possibly affected by gestational nutrition. As numbers of adipocytes was not measured in this current study, no conclusion can be made as to which factor or factors resulted in our observed differences in 12th rib fat thickness.

The IGF-1 is part of a hormonal system that is essential for regulation of animal growth and nutritional restriction on fetal development which may result in permanent alterations of the IGF axis [23,24]. Maresca., *et al.* [17] evaluated heifer and steer siblings and observed that serum IGF-1 concentrations at birth were greater in HP compared to LP calves (P < 0.05). Heifers from HP dams had greater IGF-1

concentrations than heifers born from LP dams (P = 0.05) during periods 1 and 2. Greater BW in HP heifers than LP heifers could be explained by a different post-natal endocrine control of growth, which was reflected in greater IGF-1 serum concentrations in HP heifers as compared to LP heifers during the study.

Protein nutrition of pregnant beef cows could also influence reproductive performance of heifer progeny. Martin., *et al*. [7] reported that the percentage of heifers that calved in the initial 21d of the calving season was greater for heifers from protein-supplemented dams compared to heifers from unsupplemented dams. A similar finding was reported by Cushman., *et al.* [20] when limiting nutrient availability during late gestation. Martin., *et al.* [7] reported overall pregnancy rate of 93 vs. 80% for heifers from protein-supplemented or unsupplemented dams, respectively. However, in this study the protein level did not affect final pregnancy rate. A similar result was reported by Warner., *et al.* [19] in which no differences were observed in pregnancy rates for heifers from dams grazing cornstalk residue and receiving protein supplement compared to dams grazing cornstalk residue and receiving no supplement during late gestation. These results agree with data presented by Funston., *et al.* [6] where pregnancy rates of heifers from protein supplemented dams were not affected by dam winter treatment. Cushman., *et al.* [20] did not find differences in pregnancy rate of daughters when their dams were assigned to either low (75% of maintenance), moderate (100% of maintenance) or high (125% of maintenance) nutrient intake during second or third trimester. Some authors found that under nutrition during gestation impacts age at puberty [6,26]. However, these results do not agree with Nepomuceno., *et al.* [27] and Martin., *et al.* [7]. In the current experiment, dam protein supplementation did not affect age at puberty or fertility of heifer offspring. One possible reason for the lack of differences is that mature cows were utilized and mature cows are less susceptible to nutrient restriction compared to younger cows [28]. Moreover, the small sample size and thus the reduced power together with the complex influences on reproduction in general likely limited detecting potential differences.

The factors, conditions and environmental exposure to one generation could affect the health, growth and development of the next generation [29]. Others authors have also observed no differences due to late gestation dam nutrition on heifer progeny´s first calf's BW at birth [6,8,19]. However, in the present study, grand offspring BW after birth until weaning were different. There has been speculation that maternal nutrition and other prenatal environmental factors may influence fetal mammary development and subsequent lactation performance [30]. Dam prepartum protein supplementation had no effect on the heifers milk yield. Similar results have been reported by Shoup., *et al.* [8], which is one of the few reports that evaluated the offspring milk production whose dams were nutritionally restricted during gestation. However in the current study, LP heifers had greater milk protein percentage and tended to had greater milk fat percentage than HP heifers. The greater milk protein concentration from LP heifers is consistent with van der Linden., *et al.* [31] who reported that ewes whose dams were fed at maintenance level during pregnancy produced greater protein yields than ewes dams were fed ad libitum during pregnancy. Dam nutrition may induce epigenetic alterations and may modify the development of the fetal mammary gland which in turn may affect milk composition in later life. The LP grand offspring calves had less serum insulin concentration and tended to have increased blood glucose concentration than HP grand offspring calves. It has been shown that prepartum nutrition in ewes [32] or cows [33] affects offspring glucose metabolism. Washburn., *et al.* [34] and Keomanivong., *et al.* [35] showed that the fetal pancreas is sensitive to maternal nutrient restriction during the first or second half of gestation. The lower BW during the pregnancy of LP heifers could have affected the development of the grand offspring pancreas and thus the production of insulin. This is in accordance with the Thrifty Phenotype Hypothesis [36], where came to the conclusion that events in fetal life play a major role in alterations of fetal organogenesis as a response to maternal and fetal nutrition. These adaptations may permanently alter the metabolism and one detrimental consequence would be reduced insulin secretion. Some studies have demonstrated other intergenerational effects on the endocrine pancreas following the use of a low protein, isocaloric diet [37]. Fetal undernutrition in rats can impact glucose homeostasis of the F2 generation [38].

Maternal nutrition restriction in mid to late gestation produce altered circulating levels of IGF 1 in female offspring including changes in muscle and adipose tissues development. The lack of response of reproductive characteristics could be due to the fact that these variables are commonly influenced by nutrition at other stages of pregnancy or by a combination of energy and protein restrictions.

Conclusion

In conclusion, the protein supplementation of cows during late gestation resulted in heifer progeny with increased ADG and BW, and modified serum IGF-1 concentrations. The HP heifers had altered growth and body composition, but age of puberty and reproductive performance during the first breeding season were similar. It is important to note that the low number of replicates has to be taken into account when conclusions are made. Our results indicate that dam nutrition may affect the development of heifer offspring and ability to adapt to be mothers of the next generation. Further studies are warranted to confirm our finding and also to explore the exact mechanism that a reduced concentration of maternal CP from mid to late gestation can influence the grand offspring.

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Conflicts of Interest

The authors declare no conflicts of interest.

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