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RESTRICCIÓN PROTEICA DURANTE EL ÚLTIMO TERCIO DE GESTACIÓN EN BOVINOS: EFECTOS SOBRE CRECIMIENTO Y CALIDAD DE CARNE DE LA DESCENDENCIA

por

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RESUMEN

El objetivo del estudio fue determinar si la cantidad de proteína dietaria suministrada a vacas durante el último tercio de gestación puede afectar el crecimiento postnatal generando cambios en la regulación de la glucemia, características de carcasa y calidad de carne de la descendencia. A los 121 días preparto, 68 vacas multíparas Angus fueron asignadas aleatoriamente a dos tratamientos: bajo nivel de proteína (BP, 6% PC) o alto nivel de proteina (AP, 12% PC) y fueron alojadas en 12 corrales por tratamiento. Posparto, las vacas se manejaron juntas en pasturas hasta el destete. Se tomaron muestras de sangre al nacimiento y cada 30 días hasta el destete. Después del destete, los terneros machos fueron mantenidos como un grupo sobre pastizal natural hasta los 23 meses de edad y luego fueron alimentados con una dieta de terminación durante 84 días hasta la faena. El peso vivo tendió a ser mayor (P = 0.06) y el índice de masa corporal fue mayor (P = 0,04) en los novillos AP que en BP. El nivel de proteína no tuvo influencia en el peso vivo y tampoco en la ganancia de peso durante la lactación, recría y terminación (P > 0,10). El área de ojo de bife fue mayor en AP que en BP al inicio (P = 0,01) y al final (P = 0,04) de la etapa de terminación. La concentración de glucosa fue mayor (P < 0,001) en terneros BP que en AP desde el nacimiento hasta el destete. Durante el test de tolerancia a glucosa, la concentración de glucosa descendió más rápidamente (P = 0,002) en BP que en AP. El peso de la carcasa caliente fue similar entre tratamientos (P = 0,69), sin embargo, el rendimiento de la res fue mayor (P = 0,01) en novillos AP que en BP. La terneza del musculo *Longissimus* fue mayor (P < 0,001) en novillos AP comparado con BP. El diámetro de las fibras musculares fue similar en novillos BP y AP (P = 0,20), sugiriendo que el incremento en el área de ojo de bife en novillos AP pudo ser debido a hiperplasia muscular. Estos resultados demuestran que el bajo nivel de proteína dietaria durante la gestación tardía en vacas de cría puede afectar el crecimiento fetal, comprometiendo la regulación de la glucemia en la vida temprana. Los resultados también permiten concluir que el nivel de proteína durante la gestación tardía no afecta el crecimiento posnatal de la descendencia, pero impacta sobre la composición de la carcasa y calidad de carne.
Palabras clave: subnutrición proteica, crecimiento posnatal, glucosa, calidad de carne.

PROTEIN RESTRICTION DURING LATE GESTATION IN BEEF COW: EFFECTS ON GROWTH AND MEAT QUALITY OF PROGENY

SUMMARY

At 121 d prepartum, 68 multiparous Angus cows were randomly assigned to low protein level (LP, 6% CP) or high protein level (HP, 12% CP) and were allotted in 12 pens per treatment. After calving, cows were managed together on improved pastures until weaning. Calves were weighed and body measurements were recorded at birth. Blood samples were taken at birth and each 30 d until weaning at 180 d of age. Male calves were maintained as a group after weaning on native range until 23 month of age when individual steers were fed a finishing diet during 84 d before slaughter. Body weight at birth tended to be great (P = 0.06) and body mass index was greater (P = 0.04) on HP than LP progeny. Maternal dietary protein level had no influence on offspring body weight and growth rate during lactation, rearing or finishing phase (P > 0.10). *Longissimus muscle* area was greater in HP steers than LP steers at entrance into the feedlot (P = 0.01) and end of finishing phase (P = 0.04). Glucose concentrations was greater (P < 0.001) on LP calves than HP calves from birth to weaning without any change in insulin concentrations (P > 0.10). Insulin-like growth factor concentrations was lower on LP calves at birth (P < 0.05). During the glucose tolerance test at 24 month of age, glucose concentration decreased faster (P = 0.002) in LP compared to HP steers. Hot carcass weight was similar between treatments (P = 0.69), however dressing percentage was increased in HP relative to LP steers (P = 0.01). Tenderness of *Longissimus* muscle was increased in HP compared to LP steers (P < 0.001). Muscle fiber diameter was similar in LP and HP steers (P = 0.20), suggesting that increase of LM area in HP steers could be due to muscle hyperplasia. These data demonstrate that low protein during late gestation in bovine dams may affect fetal growth, compromising glucose regulation in early life. The results indicated that level of protein during mid to late gestation does not affect offspring growth but has impacts on carcass composition and meat quality of steer progeny.

Keywords: protein sub-nutrition, postnatal growth, glucose, meat quality.

1. INTRODUCCIÓN

Una de las principales limitantes de la ganadería para aumentar la producción de carne a nivel nacional es la baja eficiencia reproductiva de los rodeos de cría. Dentro de los índices reproductivos, se conoce que el porcentaje de preñez es uno de los más bajos y variables en función del clima. Un estudio realizado en la Cuenca del Salado, Buenos Aires, Argentina, durante 5 años en 83 establecimientos reveló que el 53% de las vacas llegan al parto flacas, con estado corporal inferior a 3 en la escala de 1 a 5 (Maresca et al. 2015).

Los rodeos de cría en la Argentina, tienen predominantemente servicios estacionados en los meses de octubre, noviembre y diciembre con pariciones concentradas en julio, agosto y septiembre. La mayoría de los rodeos se encuentran en situaciones de producción extensiva sobre pastizales naturales que tienen muy bajo crecimiento durante el invierno y principios de primavera por lo que la restricción nutricional de los vientres se da principalmente durante el último tercio de gestación. Durante esta época el forraje además de ser escaso es de baja calidad en cuanto a contenido de materia seca digestible y proteína bruta, por lo tanto, existen ambos tipos de restricción energética y proteica.

La restricción nutricional durante la gestación genera un bajo estado corporal al parto alargando el intervalo parto primer celo y disminuyendo las posibilidades de lograr buenos índices de preñez en el próximo servicio (Wiltbank et al., 1962). Numerosos estudios se han focalizado sobre los aspectos nutricionales que afectan el desempeño reproductivo de las vacas, sin evaluar posibles efectos negativos sobre el desempeño productivo del ternero. Recientes estudios han demostrado que la subnutrición durante la gestación genera un retardo del crecimiento y desarrollo fetal (Wu et al., 2006). Esto tendría efectos negativos a largo plazo en la descendencia afectando el desarrollo del aparato gastrointestinal, la eficiencia de utilización del forraje (Fowden et al., 2005) y la calidad de la carne (Greenwood et al., 2009).

La disponibilidad de nutrientes durante el desarrollo fetal puede alterar el metabolismo de la glucosa y la secreción de insulina durante la vida posnatal. Ford et al. (2007) y Long et al. (2010) observaron una alteración en la regulación de la

glucemia después de un test de tolerancia a glucosa (TTG) en desencintes de ovejas y vacas que fueron sub-nutridas durante primera y segunda mitad de gestación. La restricción de nutrientes durante el último tercio de gestación ovejas generó como resultado corderos con una alta concentración de glucosa e insulina después de un TTG (Gardner et al., 2005), sin embargo, las consecuencias que podría generar la subnutrición en el último tercio de gestación no ha n sido estudiadas. Hay evidencias que indican que el crecimiento y desarrollo del páncreas es crítico durante el final de la gestación y los primeros meses de vida en ovinos (Fowden, 1980) y equinos (Fowden et al., 2005), y puede ser sensible a bajos niveles de proteína dietaria (Petrik et al., 1999).

La nutrición fetal también es crucial para el desarrollo muscular porque el número de fibras musculares no se incrementa después del nacimiento (Zhu et al., 2004). La restricción nutricional durante la gestación puede resultar en un reducido número de fibras musculares y reducida masa muscular impactando en la performance animal. Durante los 2 a 8 meses de gestación se produce la miogénesis secundaria donde se forman la mayoría de las fibras musculares. Una reducción del número de fibras musculares durante este periodo por causa de una subnutrición maternal podría traer una larga y perdurable consecuencia irreversible en la descendencia (Du et al., 2010).

Los adipocitos intramusculares, los cuales determinan el marmóreo, también pueden ser afectados durante el desarrollo fetal (Du et al., 2010). La grasa intramuscular es crucial para la palatabilidad de la carne porque el marmoreo determina el sabor y la jugosidad. La cantidad de grasa intramuscular está determinada por el número y tamaño de los adipocitos intramusculares.

Estudios realizados en otras especies (humanos, ovejas y roedores) permiten hipotetizar que la nutrición durante la gestación puede afectar el desarrollo del tejido muscular y adiposo. Sin embargo, existe escasa evidencia en bovinos que demuestre las consecuencias directas de la subnutrición durante el último tercio de gestación sobre las características de la carcasa y calidad de carne. Tampoco es conocido el efecto de la deficiencia de componentes específicos de la dieta, como lo son las proteínas, sobra la productividad de la descendencia.

El objetivo de este trabajo es evaluar si la mejora del estatus nutricional de la vaca mediante la suplementación proteica de forrajes de baja calidad durante el último tercio de gestación afectará el desarrollo y las características de la carcasa en la descendencia.

1.1 HIPÓTESIS DE TRABAJO

La restricción proteica durante el último tercio de gestación afecta el desarrollo y crecimiento del feto con efectos negativos a largo plazo.

- Reduce el peso al nacimiento y afecta el crecimiento posnatal.
- Afecta el metabolismo de la glucosa y secreción de insulina.
- Afecta el desarrollo de tejido muscular y graso.
- Afecta las características de carcasa y calidad de carne.

1.2 OBJETIVO GENERAL

Estudiar el efecto de la restricción proteica durante la gestación sobre el desarrollo, crecimiento y el desempeño productivo de la progenie.

1.3 OBJETIVOS ESPECÍFICOS

Estudiar el efecto de la restricción proteica durante el último tercio de la gestación sobre:

- Comportamiento productivo de los vientres.
- Desarrollo y crecimiento de la progenie durante la lactancia y recría.
- Metabolismo de la glucosa.
- Performance productiva en terminación y calidad de carcasa de la progenie.

2. EFECTO DE LA RESTRICCIÓN PROTEICA DE VACAS DURANTE LA GESTACIÓN TARDÍA SOBRE EL CRECIMIENTO POSNATAL, EL METABOLISMO DE GLUCOSA - INSULINA Y LA CONCENTRACIÓN DE IGF-1 DE LA DESCENDENCIA

2.1. RESUMEN

El objetivo de este estudio fue determinar si la cantidad de proteína proporcionada a las vacas durante la gestación tardía afectaría el crecimiento postnatal y provocaría cambios en las concentraciones de glucosa, insulina e IGF-1. A los 121 días previos al parto, 68 vacas Angus multíparas fueron asignadas aleatoriamente a un nivel bajo de proteína (BP, 6% CP) o alto nivel de proteína (AP, 12% CP). Al parto, las vacas fueron manejadas juntas en pasturas mejoradas hasta el destete. Se pesaron los terneros y se registraron las medidas corporales al nacer. Se tomaron muestras de sangre al nacer y cada 30 días hasta el destete a los 180 días de edad. El peso corporal al nacer en la progenie AP tendió a ser mayor que la progenie BP ($P = 0.06$). La relación circunferencia de la cabeza / peso al nacer ($P = 0.04$), la relación circunferencia del corazón / peso al nacer ($P = 0.01$), la relación longitud del cuerpo / peso al nacer ($P = 0.05$) y la relación de altura / peso al nacer ($P = 0.01$) fueron mayores en terneros BP. El índice de masa corporal fue mayor en terneros AP ($P = 0.04$). No se encontraron diferencias en PV de terneros al destete, PV ajustado a 205 días y GDPV durante la lactancia ($P> 0,10$). Las concentraciones de glucosa fueron mayores en terneros BP que en terneros AP desde el nacimiento hasta el destete ($P <0.001$) sin ningún cambio en las concentraciones de insulina durante el crecimiento previo al destete ($P> 0,10$). Las concentraciones de IGF-I fueron menores en terneros BP al nacer ($P <0.05$) y similares a los terneros AP durante el crecimiento postnatal ($P> 0.10$). Estos datos demuestran que las deficiencias proteicas durante la gestación tardía en las madres bovinas pueden afectar el crecimiento fetal con un aumento proporcional de la circunferencia de la cabeza y un bajo índice de masa corporal. El nivel de proteína comprometió la regulación de la glucosa en la vida temprana y afectó la concentración de IGF-I de los terneros recién nacidos.

Palabras Clave: gestación, subnutrición, crecimiento posnatal, glucosa, IGF-I

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Effect of protein restriction of bovine dams during late gestation on offspring postnatal growth, glucose-insulin metabolism and IGF-1 concentration

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ABSTRACT

The objective of this study was determine whether the amount of protein provided to cows during late gestation would affect postnatal growth and lead to changes on glucose, insulin and insulin-like growth factor concentrations. At 134 d prepartum, 68 multiparous Angus cows were blocked by BW and expected calving date and randomly assigned to low protein level (LP, 6% CP) or high protein level (HP, 12% CP) and were allotted in 12 pens per treatment. At calving, cows were managed together on improved pastures until weaning. Calves were weighed and body measurements were recorded at birth. Blood samples were taken at birth and each 30 d until weaning at 180 d of age. Body weight at birth on HP progeny tended to be great than LP progeny ($P = 0.06$). At birth, calves from LP dams had greater head circumference ($P < 0.01$), heart girth ($P = 0.01$) and cannon bone circumference ($P = 0.02$). However, head circumference /birth BW ratio ($P = 0.04$), heart girth /birth BW ratio ($P = 0.01$), body length /birth BW ratio ($P = 0.05$) and height /birth BW ratio ($P = 0.01$) was greater on LP calves. Body mass index was greater in HP calves ($P = 0.04$). No differences were found on BW of calves at weaning, adjusted 205d BW and ADG during lactation ($P > 0.10$). Glucose concentrations were greater on LP calves than HP calves from birth to weaning (81.0 ± 1.5 vs. 76.4 ± 1.2 mg/dl; $P < 0.001$) without any change in insulin concentrations during preweaning growth (LP = 1.61 ± 0.04 ng/ml; HP = 1.61 ± 0.04 ng/ml; $P > 0.10$). Insulin-like growth factor concentrations was less on LP calves at birth ($P < 0.05$) and similar to HP calves during postnatal growth ($P > 0.10$). These data demonstrate that low protein during late gestation in bovine dams may affect fetal growth with proportional increasing of head circumference and low body mass index. Level of protein compromised glucose regulation in early life and affected the IGF-I concentration of newborn calves.

1. Introduction

Cow calf operations in Argentina are managed under extensive conditions on grazing systems. The quality of forages and roughages is often poor, particularly in the winter seasons leading to many spring calving cows having periods of undernutrition at the time that often corresponds to the second half of gestation. Nutrient restriction during late gestation can cause intrauterine growth retardation (IUGR) in bovine fetuses with long-term effect in offspring growth, reproductive development (Funston et al., 2010; Lemaster et al., 2016) and carcass quality (Underwood et al., 2010). Nutrition during late gestation may affect condition score at calving with consequences on both milk production and quality and calf growth during lactation (Lemaster et al., 2016). Protein supplementation during late gestation has been associated with increased weaning weight of offspring when forages base diet contain between 5% and 8% of CP (Martin et al., 2007; Stalker et al., 2006; Funston et al., 2010). However, milk yield and quality has been not evaluated and long-term consequences of fetal undernutrition could be confounded with maternal milk production and composition during postnatal growth up to weaning.

Nutrient availability during fetal development may alter glucose metabolism and insulin secretion during postnatal growth. Ford et al. (2007) and Long et al. (2010) report altered glucose clearance after glucose tolerance test (GTT) in offspring of ewes and cows that were undernourished during early to mid-gestation. Nutrient restriction during late gestation in ewes resulted in lambs with increased concentration of glucose and insulin after GTT (Gardner et al., 2005), but consequences in bovine progeny have been scarcely studied. There is evidence that pancreas growth and development is critical during late gestation and first months of life in fetal sheep (Fowden, 1980) and fetal

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horses (Fowden et al., 2005) and may be sensible to low protein levels (Petrik et al., 1999).

Insulin–like growth factor I (IGF-I) concentrations are critical for the development of the fetus and birth weight (Brameld et al., 2000). Pancreas growth, maturation and function are modulated by IGFs in rats (Hogg et al., 1994). Bovine maternal IGF-I is particularly sensitive at dietary protein levels during the second trimester of gestation (Perry et al., 2002).

The objective of this study was to understand the impact of maternal protein nutrition level during late gestation on offspring growth associated to changes in glucose, insulin and IGF-I concentrations.

2. Materials and methods

The institutional Animal Care and Use Committee of INTA-CERBAS approved all animal procedures used in this study (Approval No. 87).

2.1. Cow and calf management

A detailed description of management practices is available (Lopez Valiente et al., 2018), and only a brief description will be presented here. Sixty-eight Angus multiparous cows were synchronized using a controlled internal drug-realizing device (Cronipres®, Biogénesis-Bago, Argentina) for 7 d, and upon removal of the device, 500 μg of cloprostenol (Ciclase DL®, Syntex, Argentina) and 1 mg of estradiol benzoate (Benzoato de Estradiol Syntex®, Argentina) were administrated intramuscularly and AI 48 h after, using semen from a single Angus sire. Fifteen d after AI, a single Angus bull were used for a15 d natural breeding period. At 30 d after the end of the natural mating period, pregnancy to AI and natural service were identified by transrectal ultrasonography. Cows were managed on fescue pastures during early to mid-gestation. When cows were 134 ± 14 d of gestation, they were stratified by BW into 24 pens and pen was allotted to either a low protein (LP) or high protein (HP) diet to prove 6% and 12% CP, DM basis. Cows were feed to meet 100% of NEm requirements in both treatments and provide 64% and 121% of CP requirements for LP and HP treatments respectively (NRC, 2000). 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 pelleted sunflower meal, 1% of urea and 1.5% of mineral premix. 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 of calves at 180 days of age. Body weights and BCS of cows were recorded at calving (less than 12 h after calving) and at weaning. Bull calves were castrated at 5 month of age. Calf BW was recorded at birth and every 30 d until weaning. Adjusted 205-day weaning weight was computed on the basis of daily gain from birth to weaning. Milk production was assessed with a portable milking machine equipped with a milk meter in line (TrueTest, Auckland, New Zealand) at d 20, 34, 47, 75, 103, 135, 165 and 221 of lactation. At ~12:00 p.m. cows were separated from calves and each cow was injected intramuscularly with 10 IU of oxytocin (Over®, Argentina) to facilitate milk letdown. Cows were milked 5 min after injection and calves were fitted with strong nose plates and remained with their dams in the same paddock. The following day, at ~06:00 a.m., cows were milked again with the same protocol (Quintans et al., 2010). Homogenized milk samples were collected from each cow at each milking to determine milk 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.

2.2. Newborn body measurement

Less than 12 h postpartum, calf sex, birth weight, and the following measurements on the calf were recorded: head circumference (measurement collected around parietal bone and mandible just posterior to eye orbits), heart girth (posterior to foreleg), cannon bone circumference (narrowest point of metacarpus), body length (linear distance along the vertebral column from the occipital bone to the first coccygeal vertebra) and height (linear distance from the trochanter major of the femur to the floor). All body measurements were taken when the animal was standing naturally with head raised and weight on all four feet. Head measurements were taken while the animal was restrained. The ratio of newborn measurement and body weight at birth was calculated to determine if fetal growth is affected asymmetrically. The ratio of birth weight to head circumference is used in human epidemiological studies (Martyn et al., 1996) to determine effects on the growth of the brain which affects the measurements of the head and can serves as a marker for "brain sparing" (during intrauterine growth retardation the growth of the fetal brain is privileged over others organs) and to find out the pattern of fetal growth. The body mass index of newborn calves was calculated dividing the birth weight of each calf by the square root of body length.

2.3. Blood collection and assays

Glucose, insulin and IGF1 concentrations were determinate in calves at birth and monthly until 180 d of age. Calves were separated of theirs mothers for 16 h without feed and water before blood samples (6 ml) were obtained from the jugular vein, placed in ice for < 3 h, centrifuged at 2500 x g for 15 min and serum was removed and stored at −20 °C until further analysis. Glucose concentration was determinate in whole blood with a hand-held electronic glucometer (Abbott©, UK) immediately after blood sample was taken (Wittrock et al., 2013).

Serum Insulin concentration was measured by RIA 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. Intra- and inter-assay coefficients of variation were lower than 8% and 11%, respectively. Serum IGF-1 was quantified in one assay via RIA performed after acid ethanol extraction as described in Lacau Mengido et al. (2000). The IGF-1 antibody (UB2-495) of the NIDDK was used. Intra assay coefficient of variation was 8% and minimum detectable concentration was 2.5 ng/ml.

2.4. Statistical analyses

Cows were blocked according to BW and expected calving date and pens were considered the experimental unit. Offspring data, including BW and body measurements, were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The model included the fixed effects of maternal dietary treatment, blocks and sex and all possible interactions. The random effect was pen nested into the block per treatment interaction. Whole blood concentration of glucose, serum insulin and serum IGF-1 were analyzed as repeated measures using MIXED procedure of SAS with treatments, age and their interaction in the model. In all cases, pens were considered as experimental unit and data are presented as least squares means and polled MSE, and differences considered significant at $P \leq 0.05$, with a tendency at $P \leq 0.10$.

3. Results

The data regarding cow performance in response to different levels of protein during late gestation has been reported previously (Lopez Valiente et al., 2018), and only a brief description will be provided here. Cow BW and BCS was similar for LP and HP dams at the initiation of treatment (407.5 ± 54.1 kg BW; $P = 0.11$ and 4.32 ± 0.23 BCS $P = 0.79$). During treatment, HP dams gained 18.9 ± 18.4 kg whereas LP dams loss 3.2 ± 11.8 kg ($P < 0.01$). Cow BCS change during late gestation tended to be different between treatment ($P = 0.06$), HP dams gained 1.04 ± 0.15 and LP dams gained 0.60 ± 0.16 BCS. Cow BW and BCS at weaning were not

Table 1
Effects of low or high protein nutrition level of dams during late gestation on postnatal BW and ADG of offspring[a].

Item	Treatment					*P*-value		
	LP		HP					
	Steers	Heifers	Steers	Heifers	MSE[c]	Trt[b]	Sex	Trt × sex
BW, kg								
Birth	27.8	25.4	29.3	27.9	1.1	0.06	0.08	0.64
Adj. 45 d	76.5	65.8	76.3	73.3	2.4	0.13	<0.01	0.12
Weaning	227	212	223	220	8	0.79	0.17	0.36
Adj. 205 d	219	202	218	213	8	0.45	0.11	0.39
ADG, kg/d								
Birth to 45 d	1.08	0.90	1.00	1.03	0.04	0.55	0.07	0.02
45 d to weaning	0.89	0.86	0.87	0.88	0.03	0.98	0.74	0.39
Birth to weaning	0.93	0.86	0.92	0.90	0.03	0.66	0.14	0.42

[a] Dams were fed low protein (LP = 6% CP, DM basis) or high protein (HP = 12% CP DM, basis).
[b] Trt = Treatment.
[c] Pooled SEM presented.

different between treatments (426.8 ± 28.6 kg BW; $P = 0.47$ and 4.88 ± 0.19 BCS $P = 0.55$). Change of BW and BCS during lactation were not different between treatments (10.3 ± 27.8 kg; $P = 0.15$ and −0.21 ± 0.16 BCS $P = 0.17$).

Milk production was not affected by level of protein during late gestation ($P = 0.30$). Average daily milk yield was 5.7 ± 0.3 kg for LP dams and 5.3 ± 0.3 for HP dams. None of the components of milk measured were affected by nutritional treatment during late gestation. Average fat was 2.7 ± 0.2% ($P = 0.31$), protein 3.4 ± 0.1% ($P = 0.12$), urea 11.1 ± 0.2% ($P = 0.33$), lactose 4.9 ± 0.1% ($P = 0.92$) and total solids 12.0 ± 0.2% ($P = 0.16$).

There was a tendency to increase BW at birth for HP compared with LP calves ($P = 0.06$; Table 1). Late gestation nutrition treatment did not influence calf weaning BW ($P = 0.79$) and adjusted 205 d BW ($P = 0.45$). Male calves 45d BW was greater ($P < 0.01$), however, weaning BW ($P = 0.17$) and adjusted 205 d BW ($P = 0.11$) were similar than female calves. Calf ADG from birth to weaning was not influenced by treatment ($P = 0.66$), sex ($P = 0.14$) or treatment × sex ($P = 0.42$). There was a treatment × sex effect ($P = 0.02$) on ADG from birth to 45 d.

Calves born of HP cows had increased head circumference ($P < 0.01$; Table 2), heart girth ($P = 0.01$) and cannon circumference ($P = 0.02$) compared to calves born of LP cows. Body length ($P = 0.49$) and height ($P = 0.74$) were not different between treatments. Male calves had increased head girth ($P = 0.02$), cannon bone circumference ($P = 0.01$), and a tendency to increased body length ($P = 0.07$) than female calves. Head circumference ($P = 0.20$) and height ($P = 0.28$) were not different between sex. There was not treatment × sex interaction for any variables.

Protein nutrition level of dams affected fetal growth asymmetrically. Low protein newborn calves had increased the head circumference / birth BW ratio ($P = 0.04$; Table 3), heart girth / birth BW ratio ($P = 0.01$), height / birth BW ratio ($P = 0.01$) and body length / birth BW ratio ($P = 0.05$). Cannon bone circumference / birth BW ratio ($P = 0.28$) were not different between treatments. There were no sex effect on any of these variables ($P > 0.10$). The body mass index was reduced in LP calves ($P = 0.04$) and female calves ($P = 0.01$). There was no treatment × sex interaction for any of these variables ($P > 0.10$).

Whole blood glucose concentrations from birth to weaning were

Table 2
Effects of low or high protein nutrition level of dams during late gestation on body measurements of newborn calves[a].

Item	Treatment					*P*-value		
	LP		HP					
	Steers	Heifers	Steers	Heifers	MSE[c]	Trt[b]	Sex	Trt x sex
Head circ[d]., cm	45.6	44.2	46.6	46.5	0.6	<0.01	0.20	0.28
Heart girth, cm	72.5	68.6	73.7	72.6	1.0	0.01	0.02	0.17
Cannon circ[d]., cm	10.9	10.6	11.5	10.8	0.2	0.02	0.01	0.24
Body length, cm	75.9	71.7	75.1	74.3	1.4	0.49	0.07	0.21
Height, cm	61.0	58.9	60.3	60.3	0.9	0.74	0.29	0.30

[a] Dams were fed low protein (LP = 6% CP, DM basis) or high protein (HP = 12% CP DM, basis).
[b] Trt = Treatment.
[c] Pooled SEM presented.
[d] Circ. = circumference.

greater for LP calves compared with HP calves (81.0 ± 1.5 vs. 76.4 ± 1.2 mg/dl; Fig. 1). Calves born from LP dams had increased glucose concentration during the first 60 d of life but the concentration returned to similar levels from HP calves from 60 days until weaning. There was a significant effect of age on glucose concentrations ($P < 0.001$). Serum insulin concentrations from birth to weaning were not affected ($P = 0.96$) by nutritional treatment (LP = 1.61 ± 0.04 ng/ml; HP = 1.61 ± 0.04 ng/ml; Fig. 2).

Serum IGF-1 concentration at birth was greater in HP compared to LP calves ($P < 0.05$) but there was not effect ($P > 0.10$) of maternal diets after birth and until weaning (LP = 361 ± 12 ng/ml; HP = 358 ± 13 ng/ml; Fig. 3). Serum IGF-1 concentration changed with the age of the calves. Serum IGF-1 concentrations were lower at birth and increasing until day 90 in both groups ($P < 0.01$), then there was a decline in concentrations up to d 180 of age.

4. Discussion

Restriction of energy intake during late gestation has been shown to decrease calf birth weight for cows (Wiltbank et al., 1962; Corah et al., 1975; Houghton et al., 1990; Freetly et al., 2000) and heifers (Corah et al., 1975; Bellows and Short 1978; Warrington et al., 1988; Spritzer et al., 1995). In contrast, when dams were fed a protein deficient diet with adequate energy intake during the second half of gestation, calf birth weight was not influenced. These results are consistent for cows (Stalker et al., 2006; Larson et al., 2009) and heifers (Cartens et al., 1987; Martin et al., 2007). In agreement with those studies, we observed only a tendency to decrease birth weight on LP calves. Nutritional restriction appears to be more severe when energy intake was limited (Lemaster et al., 2016). Cows fed with low protein forage during late gestation did not have a drastic decrease of BW, and BCS has been near to five or higher (Cartens et al., 1987; Larson et al., 2009). Protein supplementation appears no effect fetal growth but rumen available protein source could affect the DM digestibility leading to increased AA available for fetal development (Bohnert et al., 2013; Lemaster et al., 2016).

Lactational performance of dams may be affected by both, nutrition during late gestation and BCS at calving, and can affect preweaning calf growth. Thus, long-term effects of fetal programming could be confounded with altered nutrition of calves during lactation. The association between body condition score at calving and milk production and quality has been extensively studied in dairy cows. Several studies had

Table 3
Effects of low or high protein nutrition level of dams during late gestation on body measurements/ birth weight ratio[a].

Item	Treatment					*P*-value		
	LP		HP					
	Steers	Heifers	Steers	Heifers	MSE[c]	Trt[b]	Sex	Trt x sex
Head circ.[d] / birth BW, cm/kg	1.67	1.78	1.59	1.63	0.06	0.04	0.17	0.57
Heart girth / birth BW, cm/kg	2.65	2.68	2.51	2.38	0.08	0.01	0.52	0.34
Cannon circ.[d]/ birth BW, cm/kg	0.41	0.41	0.40	0.39	0.01	0.28	0.95	0.68
Body length / birth BW, cm/kg	2.80	2.77	2.53	2.70	0.11	0.05	0.52	0.37
Height / birth BW, cm/kg	2.24	2.31	2.09	2.14	0.06	0.01	0.35	0.85
Body mass index, kg/m^2	3.19	2.93	3.45	3.14	0.1	0.04	0.01	0.77

[a] Dams were fed low protein (LP = 6% CP, DM basis) or high protein (HP = 12% CP DM, basis).
[b] Trt = Treatment.
[c] Pooled SEM presented.
[d] Circ. = circumference.

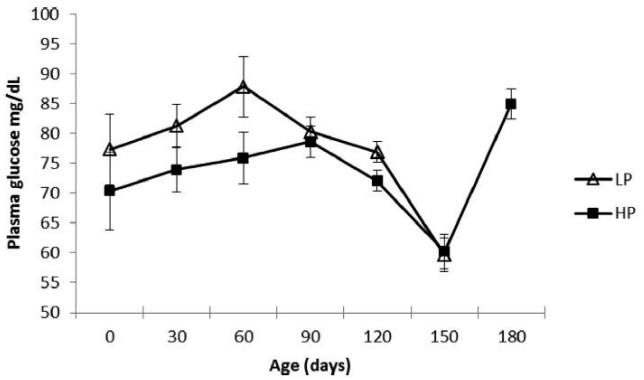

Fig. 1. Effect of low or high protein level during late gestation on progeny glucose concentration. *Indicates treatment differences (P < 0.05) Values are means ± SEM. (treatment, $P = 0.009$; age, $P < 0.001$; treatment x age, $P = 0.90$).

demonstrated that this association is nonlinear, rising from thin to moderate BCS and decreasing in over conditioned dairy cows (Roche et al., 2009). However, response does not appear be similar in beef cows. In this study, the different BCS generated with different level of protein nutrition during late gestation did not affect milk production or quality. The concept that beef cows are different than dairy cows is supported by Lake et al. (2005), who observed similar milk production on cows that calving had a BCS between 4 and 6. This is further

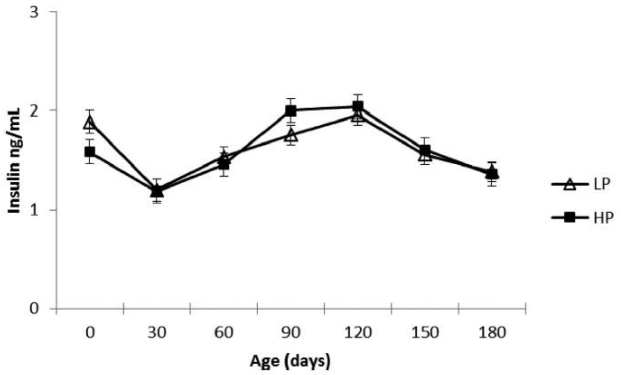

Fig. 2. Effect of low or high protein level during late gestation on progeny serum insulin concentration. Values are means ± SEM. (treatment, $P = 0.96$; time, $P = < 0.001$; treatment x time, $P = 0.24$).

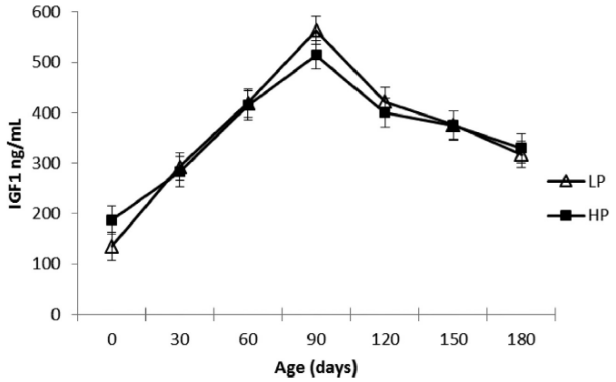

Fig. 3. Effect of low or high protein level during late gestation on serum IGF-1 concentration. *Indicates treatment differences ($P < 0.05$) Values are means ± SEM (treatment, $P = 0.36$; time, $P = < 0.001$; treatment x time, $P = 0.02$).

supported by Larson et al. (2009), who didn't find differences on milk production when protein supplement was offered at beef cows during last third month of gestation. Additionally, Van Emon et al. (2014) observed no effects of metabolizable protein supplementation during late gestation on milk production in ewes.

Overall milk yield has been correlated with daily gain of calves and weaning weight in beef cows (Gleddie and Berg, 1968; Arthur et al., 1997). However, only a few studies evaluated the postnatal growth after nutritional treatments of beef cows during second half of gestation and no consistent effect on weaning weight has been observed. Milk production has not been correlated with ADG of calves during lactation and weaning weight (Corah et al., 1975; Larson et al., 2009).

Intrauterine growth retardation can result in onset of various morphometric signs (Barker, 1998) and might have long-term effects without any change in birth weight (Harding and Johnston, 1995). Thoracic girth was greater in fetuses from nutrient restricted ewes on day 45 of gestation (Osgerby et al., 2002). In this case only a tendency for greater body weight at birth in HP calves was observed, however, HP calves had significant increases in head circumference, heart girth and a tendency for increased cannon bone circumference. Thus, on a body dimension basis, calves born of LP dam were smaller than calves born to HP dams. This data confirms previous research (Long et al., 2009) that fetal weight can be unreliable as an indicator of IUGR even if it occurs during late gestation.

Disproportionate organ growth is indicative of intrauterine growth retardation (McMillen et al., 2001; Platz and Newman, 2008). Nutritional restriction during early gestation followed by realimentation of cows generated smaller fetal exhibited increased brain and heart weight as a percentage of fetal weight, as well as a decreased abdominal circumference (Long et al., 2009). Our study demonstrates that fetal growth can be affected disproportionately by level of protein nutrition of dams in late gestation. Under conditions of intrauterine growth retardation, a phenomenon known as brain sparing occurs when the development of the fetal brain is prioritized over other organs such as skeletal muscle, liver, kidney or viscera (Barker, 1998). A greater head circumference-birth weight ratio in LP newborn calves compared with HP calves suggest that brain sparing has occurred. Similar effect has been shown previously in newborn lambs that developed in a restricted uterine environment (Sharma et al., 2012). The reduced body mass index observed in LP calves also supports the notion that they have experienced growth retardation in utero.

Prenatal nutrient availability may influence the ability of calves to regulate glucose blood concentration during postnatal growing. Gardner et al. (2005) reported that nutrient restriction ewes during late gestation, resulted in lambs with increased concentration of glucose and insulin in plasma after glucose tolerance test and no major change in glucose-insulin homeostasis was found in the offspring of undernourished ewes during early gestation. However, Ford et al. (2007) and Long et al. (2010) report altered glucose clearance after IVGTT in offspring of ewes and cows undernourished during early to mid-gestation. The likely mechanism of this altered Glucose and insulin dynamics during a challenge model is due to both altered insulin stimulated glucose uptake into tissue as observed by Gardner et al. (2005) along with pancreatic insufficiency discussed below.

Immediately after birth, endocrine pancreas function must control the glycaemia of a neonate transition from parenteral to enteral nutrition. Therefore, fetal and neonatal pancreas development is critical to control glucose homeostasis during the first months of life. Studies in fetal sheep (Fowden, 1980) and fetal horses (Fowden et al., 2005), demonstrated that sensibility of pancreatic β cell to glucose and arginine exogenous was increased during late gestation. Recent works have shown that the fetal bovine pancreas is sensitive to maternal nutrient restriction either during the first or second half of gestation (Washburn et al., 2016; Keomanivong et al., 2017). In our work, calves born from LP dams were hyperglycemic during the first 60 days of life but glucose concentrations returned to similar levels from HP calves from 60 days until weaning. The low glucose concentration on LP could be related to insulin resistance and not insulin deficiency as indicated by similar levels of insulin between diet treatments. Experimental induced IUGR in rat resulted in alterations of the endocrine pancreas, reduced pancreatic weight and β cell mass at birth, and lower insulin secretion in adult life. Studies showed that offspring of rat feed with a low protein (8% CP) isocaloric diet induced not only growth retardation, but disturbed the balance between β cell proliferation and β cell death. Low protein diet generated significantly increased rates of islet cell apoptosis during later gestation and the first 21 days of life (Petrik et al., 1999). Similar reduction in fetal pancreatic β cell mass and increased in β cell apoptosis has been observed in bovine fetuses that have been exposed to maternal nutrient restriction (Washburn et al., 2016). The observed hyperglycemia in the first 60 days of LP calves could reflect retarded pancreas development caused by maternal undernutrition during late gestation. However, similar glucose concentration between treatments after 60 d could be indicating that the pancreas may have been subjected to compensatory development. Keomanivong et al. (2017) observed that realimentation of caws during gestation was able to reverse the impact of restriction increasing the concentration of fetal pancreatic

protein and enzyme activity.

The effects of nutritional restriction on fetal development may be mediated through alteration in the IGF axis (Bauer et al., 1995). The IGF-I and IGF-II are mitogenic peptides that have a fundamental role in regulating fetal growth due to their ability to stimulate proliferation and differentiation of multiple cell types (Brameld et al., 1998). Reduction of energy intake to 25% of requirements to pregnant ewes between 110d and 124d of gestation increased plasma GH concentration and decreased plasma IGF-I concentration in both dams and her fetus (Bauer et al., 1995). Studies have shown that fetal serum IGF-I concentration is positively correlated to fetal weight, growth rate, crown-rump length and hip height in cattle (Holland et al., 1997), and birth weight in sheep (Owens et al., 1994). During late gestation and postnatal growth, IGF-I positively modulates protein synthesis rate and inhibits protein degradation rates contributing to myofiber hypertrophy (Oksbjerg et al., 2004). In our experiment, increased IGF-1 concentration on HP calves may be associated with increased body mass index at birth.

There are evidence in rats that IGFs contribute to pancreas growth, maturation and β cell growth (Hogg et al., 1993). Offspring of rats given low protein diet during gestation had a reduced pancreatic β cell mass and pancreatic insulin content associated to reduced pancreatic expression of IGF-II compared with control fed animals (Petrik et al., 1999). Therefore, is possible that in this study alteration of prenatal nutrition during late gestation of gestation has affected β cell ontogeny and compromised pancreas functionality and contributing to glucose dysregulation during early life.

In conclusion, our experiment demonstrates that low level of protein during late gestation in bovine dams may affect fetal growth. Body weight at birth was slightly affected whereas several changes in body measurement at birth on LP calves suggest that body weight may not be the best measure to detect if fetal growth is affected in bovine dams due to late gestation nutrient restriction. The relation between body measurements and weight at birth are able of detect disproportionate fetal growth. Proportional increase of head circumference and low body mass index in LP calves demonstrate that protein restriction of dams generate IUGR. Level of protein intake by the dam affected the IGF-I concentration of newborn calves. It is therefore possible that bovine prenatal undernutrition will lead to inappropriate pancreas development associated with a decreased concentration of IGF-I and result on a compromised glucose regulation in early life. No differences on milk production and quality of dam allow to conclude that effects observed on glucose regulation during early lactation could be attributed to fetal programming.

Conflict of interest

The authors whose names were listed previously certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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3. LA RESTRICCIÓN PROTEICA DURANTE EL FINAL DE LA GESTACIÓN IMPACTA EN EL CRECIMIENTO DEL MUSCULO Y LA REGULACIÓN DE LA GLUCOSA DE LOS NOVILLOS

3.1 RESUMEN

El objetivo de este estudio fue determinar si la cantidad de proteína proporcionada a las vacas durante la gestación tardía afectaría el crecimiento postnatal y provocaría cambios en las concentraciones de glucosa e insulina. A los 129 días de gestación, 10 vacas Angus multíparas se asignaron a bajo nivel de proteína (BP, 6% de proteína cruda (CP)) o alto nivel de proteína (AP, 12% CP). Después del parto, las vacas fueron manejadas juntas en pasturas mejoradas hasta el destete. Los terneros machos se mantuvieron en grupo después del destete en campo natural hasta los 23 meses de edad, cuando los novillos fueron colocados en corrales individuales y alimentados con una dieta de terminación durante 84 días. Veinte días antes de finalizar la fase de terminación, los novillos fueron sometidos a una prueba de tolerancia a la glucosa intravenosa. Los novillos fueron faenados y las características de la carcasa recolectadas. Las vacas AP ganaron 21 kg, mientras que las vacas BP perdieron 7 kg ($P = 0.04$). La nutrición proteica no influyó en el peso al nacer, el peso al destete y la ganancia diaria durante la lactancia ($P > 0,10$). El área de ojo de bife fue mayor ($P = 0.02$) en novillos AP al comienzo y al final de la fase de finalización. El espesor de grasa dorsal no fue diferente ($P > 0,10$) entre los tratamientos. La concentración de glucosa después de la administración intravenosa disminuyó ($P = 0.002$) en BP en comparación con los novillos AP. El pico de concentración de insulina en suero fue mayor ($P = 0.04$) y la concentración de insulina en suero tendió a disminuir ($P = 0.08$) más rápidamente en BP en comparación con los novillos AP después de la infusión de glucosa. En la faena, el peso de la canal caliente fue similar entre los tratamientos, pero el rendimiento fue mayor ($P = 0.05$) en AP en comparación con los novillos BP. Estos datos demuestran que un bajo nivel de nutrición proteica de las vacas durante la gestación tardía afecta las características de la canal y altera la regulación de la glucosa, incrementando la secreción de insulina en la progenie.

Palabras clave: subnutrición, crecimiento posnatal, glucosa, insulina.

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Late-gestation protein restriction negatively impacts muscle growth and glucose regulation in steer progeny

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ABSTRACT

The objective of this study was to determine whether the amount of protein provided to cows during late gestation would affect postnatal growth and lead to changes in glucose and insulin concentrations. At 129 d of gestation, 10 mature multiparous Angus cows were stratified by body weight (BW) and body condition score (BCS) and allotted to either low protein level (LP, 6% crude protein [CP]) or high protein level (HP, 12% CP) groups. After calving, cows were managed together on improved pastures, which provided forage in excess of requirements until weaning. Male calves were maintained as a group after weaning on native range until 23 mo of age when individual steers were placed in single pens and fed a finishing diet for 84 d. The 12th rib fat thickness and *longissimus* muscle area were measured during finishing phase by ultrasound. Twenty days before the end of the finishing phase, steers were subjected to an intravenous glucose tolerance test. Steers were harvested and carcass characteristics collected. Cows' BW and BCS were similar at the initiation of treatment. During treatment HP dams gained 21 kg, whereas LP dams lost 7 kg ($P = 0.04$). Protein nutrition during late gestation did not influence calf birth weight, BW at weaning, adjusted 205 d BW, or average daily gain during lactation ($P > 0.10$). *Longissimus* muscle area measure by ultrasound was greater ($P = 0.02$) in HP steers at the beginning and end of finishing phase. Fat thickness of the 12th rib was not different ($P > 0.10$) between treatments. Glucose concentration after intravenous administration decreased ($P = 0.002$) in LP compared with HP steers. Peak of serum insulin concentration was greater ($P = 0.04$) and serum insulin concentration tended to decrease ($P = 0.08$) more rapidly in LP compared with HP steers after glucose infusion. At harvest, hot carcass weight was similar between treatments, but dressing percentage was increased ($P = 0.05$) in HP compared with LP steers. These data demonstrate that a lower protein nutrition level of dams during late gestation affect carcass characteristics and alter glucose regulation enhancing insulin secretion in steer progeny.

1. Introduction

The quality of forages and roughages is commonly poor in Argentina's cow calf operation, particularly in the winter seasons, leading many spring calving cows to periods of undernutrition during the second half of gestation. Nutrient restriction during late gestation can cause

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intrauterine growth retardation (IUGR) in bovine fetuses with long-term effects in offspring growth and carcass quality. However, protein supplementation during late gestation has been associated with increased weaning weight of offspring [1–3]. In addition, cows on improved pasture during mid-gestation to late gestation produced steers with increased weaning weight, hot carcass weight (HCW), and 12th rib fat thickness than steer offspring of cows feed on native range pasture [4]. These management practices may be used to alleviate the consequences of IUGR in offspring.

During the 1990s, retrospective human studies found a correlation between low birth weight and a risk of developing type 2 diabetes later in life [5,6]. Since then, several rodent studies have confirmed that IUGR increases the risk of metabolic disorders during postnatal life [7,8]. Studies in sheep have revealed that prenatal growth restriction has long-term impact on postnatal growth and glucose-insulin regulation. Nutrient restriction during late gestation in ewes resulted in lambs with increased concentration of glucose and insulin after intravenous glucose tolerance testing (IVGTT) [9]. Similarly, a 50% nutrient restriction of ewes during early to mid-gestation, resulted in subsequent offspring with increased growth rate, decreased skeletal muscle mass, and greater area under curve for glucose after an IVGTT [10].

In contrast with the studies in sheep, Long et al [11] reported that concentration of glucose decreased more rapidly after glucose infusion in offspring of cows that were undernourished during early to mid-gestation. The effects of nutritional restriction during late gestation on offspring glucose metabolism have been scarcely studied and are relatively unknown. We hypothesized that prenatal protein restriction of dams alters regulation of glucose and secretion of insulin in postnatal offspring. The objective of this study was to determine the effect of protein restriction during late gestation in bovine on offspring growth associated with changes in glucose and insulin secretion.

2. Materials and methods

2.1. Animal care and treatments

The Institutional Animal Care and Use Committee of INTA-CERBAS approved all animal procedures used in this study. Angus multiparous cows were synchronized using a controlled internal drug-releasing device (Cronipres; Biogénesis-Bago, Argentina) for 7 d. On removal of the device, 500 μg of cloprostenol (CICLASE DL; Syntex, Argentina) and 2 mg of estradiol benzoate (Benzoato de Estradiol Syntex, Argentina) were administrated intramuscularly and bred via artificial insemination (AI) 48 h later, using semen from a single Angus sire. At 30 d after AI, 68 pregnant cows were identified by transrectal ultrasonography and used in an alternative study. Pregnant dams were managed on improvement pastures during early to mid-gestation. At 129 d of gestation, dams were stratified by body weight (BW) and BCS and placed in 10 pens (10 × 20 m). Cows that subsequently produced the steers utilized for postnatal investigations were fed to meet 100% of NEm requirements [12] and allotted to either a low-protein (LP, n = 5) or high-protein (HP, n = 5) diet to provide 6% and 12% crude protein (CP) and DM basis, 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 premix. Cows' BCS and BW were determined after 12 h feed and water withdrawal.

All cows were allowed to calve naturally, and after calving, dams and calf pairs were managed as one group on improved pastures until weaning when calves reached 220 ± 12 d of age. Body weight of calves was recorded at birth (within 24 h after calving). After weaning, LP and HP steers (n = 5 per treatment) were maintained as a group on native range until 688 ± 12 d of age, when individual steers were placed in single pens (10 × 20 m) and fed an *ad libitum* finishing diet (75% whole corn grain, 15% corn silage, and 10% CP supplement [88.4% DM, 75.9% DM digestibility, 13.1% CP]). After a feeding period of 84 d, steers were transported to a commercial abattoir (95 km) 24 h before harvest and allowed free access to water and feed withdrawal. Body weight was recorded during growing and finishing phase with 12 h of feed and water withdrawal. Ultrasound measurement (Aquila pro; Esaote Europe B.V., Maastricht, NL; 3.5-MHz probe) of 12th rib subcutaneous fat thickness and *longissimus* muscle (LM) area was conducted at the beginning and end of finishing phase. Dry matter intake was recorded 3 times a week as the difference between offer and refusal during the finishing phase.

After 64 d of finishing diet, steers (478 ± 32 kg of BW) were catheterized via the jugular vein without anesthesia using aseptic procedures. The steers were allowed approximately 30 min from the catheterization until initiation of the intravenous glucose tolerance test. To establish the baseline values of glucose and insulin, blood samples were collected at −15 and 0 min before steers were given a bolus intravenous infusion of a sterile 50% aqueous glucose solution at 0.3 g of glucose/kg of BW [13].

2.2. Hormone and metabolite analysis

Blood samples were obtained from the jugular vein for 120 min every 15 min after infusion. Glucose concentration was determined in whole blood immediately after a sample was taken with a hand-held electronic glucometer (Abbott, UK). Samples for insulin determination were placed on ice for < 3h, centrifuged at 2,500 × *g* for 15 min, and serum was removed and stored at −20°C. Serum insulin concentration was measured by RIA [14] using an antibovine insulin antibody (Sigma-Aldrich, St. Louis, MO) and standard human insulin provided by Laboratorios Beta (Buenos Aires, Argentina); the minimum detectable concentration was 0.05 ng/mL. Intra- and interassay coefficients of variation were <8% and 11%, respectively.

2.3. Statistical analyses

Maternal BW and BCS changes, steers BW, growth rates, feedlot performance, and carcass characteristic were analyzed as an ANOVA analysis using the PROC GLM of SAS with treatment in the model. Concentrations of glucose in whole blood and insulin in serum from −15 to 120 min

relative to infusion for the IVGTT were analyzed as a repeated measures analysis using the MIXED procedure of SAS. The statistical model included prenatal nutritional treatment, time, and their interaction as fixed effects, with insulin laboratory assay block as a random variable. Concentrations of insulin and glucose were plotted, and area under the curve (AUC) was determined using the trapezoidal rule with SigmaPlot software (SPSS Inc, Chicago, IL) and analyzed using MIXED Proc of SAS. Samples collected at −15 and 0 min were averaged and used as a basal concentration for calculating AUC. For IVGTT, samples collected between 0 and 90 min, relative to infusion of glucose, were used to calculate AUC for glucose, and samples collected between 0 and 60 min were used for insulin. Glucose and insulin concentration were analyzed from 15 to 120 min after IVGTT. Regression coefficients for each animal were determined and analyzed using GLM (SAS) with treatment in the model. Data are presented as least squares means and root-mean-square error, and differences considered significant at $P \leq 0.05$, with a tendency at $0.051 < P \leq 0.10$.

3. Results

Cows' BW and BCS at the initiation of treatments were similar for LP and HP dams ($P = 0.59$ and $P = 0.27$, respectively; Table 1). Dams' BW and BCS at calving were similar for LP and HP dams ($P = 0.83$ and $P = 0.27$, respectively). However, BW change during late gestation differed with LP dams losing 7 kg and HP dams gaining 21 kg ($P < 0.05$). Body condition score change during late gestation was affected by protein level with LP dams losing 0.5 and HP dams gaining 0.5 BCS ($P < 0.05$). No effect of late gestation treatment on dam BW and BCS at weaning ($P = 0.66$ and $P = 0.66$ respectively) were observed.

Maternal prenatal nutrition treatment did not influence calf BW at birth ($P = 0.38$; Table 2), weaning weight ($P = 0.73$), average daily gain (ADG) during lactation ($P = 0.46$), or ADG during rearing phase ($P = 0.77$). During finishing phase, there was no effect of treatment on initial BW ($P = 0.98$), final BW ($P = 0.42$), or ADG ($P = 0.78$). *Longissimus* muscle area was greater in HP steers than LP steers during initial ($P < 0.05$) and final ($P < 0.05$) phase of finishing. No difference in initial ($P = 0.44$) or final ($P = 0.73$) 12th rib fat thickness was observed. Maternal prenatal protein level did not affect DM intake ($P = 0.58$) or feed:gain ratio ($P = 0.50$) during finishing phase. At harvest, HCW was similar ($P = 0.60$) in LP and HP steers; however, dressing percentage was greater in HP steers than LP steers ($P = 0.05$).

Table 1
Effects of low- or high-protein nutrition level of dams during late gestation on body weight and body condition score.

	LP[a]	HP[a]	RMSE	*P*-value
Body weight, kg				
At start of treatments	431	412	51	0.59
At calving	424	433	61	0.83
Change during treatment	−7	21	17	0.04
At weaning	441	449	25	0.66
Body condition score[b]				
At start of treatments	5.1	4.5	0.8	0.27
Al calving	4.6	5.0	0.5	0.27
Change during treatment	−0.5	0.5	0.6	0.03
At weaning	5.5	5.4	0.6	0.66

Abbreviation: RMSE, root-mean-square error.
[a] Dams were fed low protein (LP = 6% crude protein DM basis) or high protein (HP = 12% crude protein DM basis); n = 5 by treatment.
[b] Scale 1 to 9; 1 = emaciated and 9 = obese [15].

Table 2
Effects of low- or high-protein nutrition level of dams during late gestation on postnatal growth and carcass characteristics of steers progeny.

	LP[a]	HP[a]	RMSE	*P*-value
Body weight at birth, kg	28.3	30.6	3.9	0.38
Body weight at weaning, kg	222	226	18	0.73
Body weight Adj. 205 d, kg	205	215	15	0.35
ADG during lactation, kg/d	0.865	0.899	0.069	0.46
ADG during rearing, kg/d	0.359	0.348	0.064	0.77
Finishing phase				
Body weight initial, kg	386	385	21	0.98
Body weight final, kg	492	510	34	0.42
ADG, kg/d	1.462	1.366	0.494	0.78
Initial LM area, cm^2	50.23	58.26	4.21	0.02
Final LM area, cm^2	68.32	77.86	4.27	0.02
Initial 12th rib fat thickness, cm	3.30	3.68	0.75	0.44
Final 12th rib fat thickness, cm	7.76	8.18	1.74	0.73
Dry matter intake, kg/d	11.91	11.30	1.56	0.58
Feed:Gain, kg/kg	7.71	8.56	1.83	0.50
After slaughter				
Hot carcass weight, kg	288	297	25	0.60
Dressing, %	55.9	59.9	2.5	0.05

Abbreviations: ADG, average daily gain; LM, *longissimus* muscle; RMSE, root-mean-square error.
[a] Dams were fed low protein (LP = 6% crude protein DM basis) or high protein (HP = 12% crude protein DM basis); n = 5 by tratment.

Blood glucose concentrations during IVGTT were lower ($P < 0.001$; Fig. 1A) in LP steers (109.7 ± 2.7 mg/dL) than HP steers (127.1 ± 3.1 mg/dL). A main effect of time ($P < 0.001$) was observed for glucose concentrations. Peak of blood glucose concentration 15 min after glucose bolus administration was similar ($P = 0.75$; Table 3) for LP and HP steers (197.6 vs 193.4 ± 20.0 mg/dL). The AUC (0 to 90 min after infusion) for blood glucose concentration tended to be lower ($P = 0.07$; Fig. 1B) in LP (11,874 ± 673) than HP steers (14,076 ± 2,167). Blood glucose concentration decreased from infusion to 15 min after infusion more rapidly ($P = 0.002$) in LP (−0.84 ± 0.12) than HP steers (−0.58 ± 0.12).

Serum insulin concentrations during IVGTT was similar ($P = 0.28$; Fig. 2A) for LP steers (6.30 ± 0.42 ng/mL) and HP steers (6.97 ± 0.46 ng/mL). A main effect of time ($P < 0.001$) was observed for serum insulin concentrations. Peak of serum insulin concentration 15 min after glucose bolus administration was greater ($P = 0.04$; Table 3) for LP than HP steers (19.2 vs 13.6 ± 2.3 ng/mL). The AUC (0 to 60 min after infusion) for serum insulin concentration was not different ($P = 0.31$; Fig. 2B) for LP (607 ± 118) and HP steers (497 ± 248) after glucose infusion. The decrease of serum insulin concentration from infusion to 15 min after infusion tended to be greater ($P = 0.08$) in LP (−0.075 ± 0.12) than HP steers (−0.022 ± 0.12).

4. Discussion

Dams fed a protein-deficient diet during late gestation resulted in a 2% decrease in BW, whereas BW increased 6%

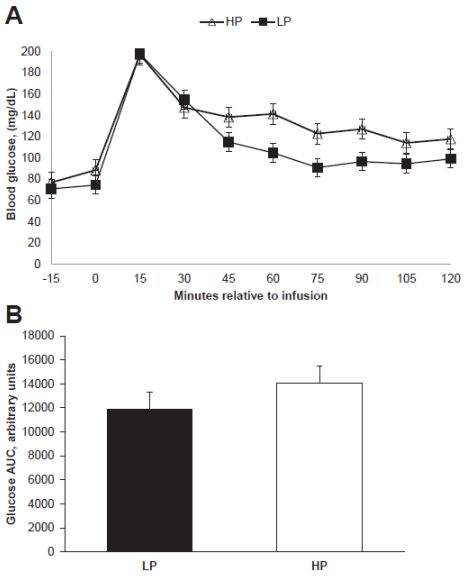

Fig. 1. Effects of low-protein (LP; n = 5) or high-protein (HP, n = 5) nutrition level of dams during late gestation on blood glucose concentration during intravenous glucose tolerance test of steers progeny at 24 mo of age (A). Blood samples were collected at −15, 0, 15, 30, 45, 60, 75, 90, 105, and 120 min relative to administration of 50% glucose solution (0.3 g of glucose/kg of BW) at 0 min. Data are presented as least squares mean ± standard error of the mean. Blood glucose concentrations was lower ($P < 0.001$) in LP steers (109.7 ± 2.7 mg/dL) than HP steers (127.1 ± 3.1 mg/dL). The area under curve (AUC) for glucose concentration was calculated between 0 and 90 min relative to infusion of glucose (B). The AUC tended to be lower ($P = 0.07$) in LP than HP steers.

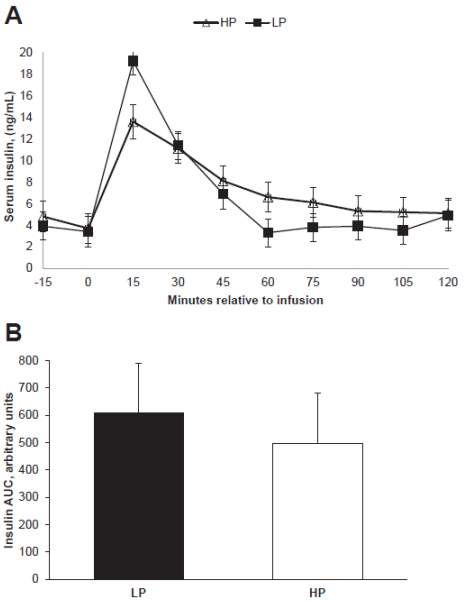

Fig. 2. Effects of low-protein (LP; n = 5) or high-protein (HP, n = 5) nutrition level of dams during late gestation on serum insulin concentration during intravenous glucose tolerance test of steers progeny at 24 mo of age (A). Blood samples were collected at −15, 0, 15, 30, 45, 60, 75, 90, 105, and 120 min relative to administration of 50% glucose solution (0.3 g of glucose/kg of BW) at 0 min. Data are presented as least squares mean ± standard error of the mean. Serum insulin concentrations was similar ($P = 0.28$) for LP steers (6.30 ± 0.42 ng/mL) and HP steers (6.97 ± 0.46 ng/mL). The area under curve (AUC) for serum insulin concentration was calculated between 0 and 60 min relative to infusion of glucose (B). The AUC was not different ($P = 0.31$) for LP and HP steers.

in HP dams. However, calf birth weight was not influenced by treatments. These results are consistent with other studies by Stalker et al [1] and Larson et al [16], who reported that cows grazing winter rangeland and receiving CP supplementation during late gestation had no differences in calf birth weight. Similar results were reported when heifers were fed a protein-deficient diet for the last 149 d of gestation [17]. The negative nutritional impact on fetal growth appears to be more evident when caloric intake is restricted, as seen when cows were fed with low-protein forage during late gestation and did not experience a drastic decrease of BW and BCS [1,16,17]. Restriction of dietary energy during late gestation in beef cows severely decreased BCS, and birth weight of calves was lower than nonrestricted cows [3,17]. Protein supplementation appears to have no effect on fetal growth, while BW at birth may not be the best method to detect if fetal growth is affected in bovine dams because of late-gestation nutrient restriction. Calf BW at birth was slightly affected, whereas several changes in calf body morphology were detected in calves from protein-restricted cows during late gestation [18].

Postnatal growth and final BW of steers were not influenced by protein restriction of prenatal nutrition in the present study. This is consistent with previous findings of maternal nutrition during mid-gestation to late gestation of both protein [1,16] and energy restriction [19].

Table 3
Effects of low- or high-protein nutrition level of dams during late gestation on glucose and insulin kinetics after an intravenous glucose tolerance test in beef steers progeny.

	LP[a]	HP[a]	RMSE	*P*-value
Glucose				
Baseline, mg/dL	72.1	82.6	8.6	0.13
Peak at 15 min, mg/dL	197.6	193.4	20.0	0.75
AUC (0–90 min)	11,874	14,076	1,420	0.07
Regression (15–20 min)	−0.84	−0.58	0.12	0.002
Insulin				
Baseline, ng/mL	4.8	3.7	2.6	0.58
Peak at 15 min, ng/mL	19.2	13.6	2.3	0.04
AUC (0–60 min)	607	497	183	0.31
Regression (15–20 min)	−0.075	−0.022	0.12	0.08

Abbreviations: AUC, area under curve; RMSE, root-mean-square error.
[a] Dams were fed low protein (LP = 6% crude protein DM basis) or high protein (HP = 12% crude protein DM basis); n = 5 by tratment.

Subcutaneous fat thickness at 12th rib was similar during finishing period for both treatments. High-protein steers entered and finished the feedlot period with increased LM area; however, protein level had no effect on HCW at slaughter. Previous studies investigating nutrient-restricted beef cows during mid-gestation to late gestation have not had consistent results regarding offspring carcass composition. Offspring of dams grazing low-quality forage (native range) had lower HCW and 12th rib fat thickness than offspring of dams grazing improved pasture [4]. Cows supplemented 3 times a week with crude protein during late gestation had no differences in carcass weight or fat cover [1,16]. Although steers from both treatments exhibited similar HCW, dressing percentage was decreased for LP steers. The body composition after harvest was not determined in this study, however, we believe that lower dressing percentage could be attributed to high abdominal fat deposition in LP steers. Lambs from nutrition-restricted ewes had increased adiposity, mainly kidney and mesenteric fat depots [10,19], but this effect remained unclear in bovine species.

Prenatal nutrient restriction may affect the ability of calves to regulate blood glucose concentration during postnatal life by altering pancreatic function or composition. Studies in fetal sheep and fetal horses demonstrated that sensitivity of pancreatic β cell to glucose and exogenous arginine was increased during late gestation [20,21]. Recent work has shown that the fetal bovine pancreas is sensitive to maternal nutrient restriction during either the first or second trimester of gestation [22]. Hyperglycemia during the first 60 d of life was observed in calves from dams fed a low-protein diet, reflecting altered pancreas functionality caused by maternal undernutrition during late gestation [18]. Epidemiological studies in many human populations worldwide have confirmed that intrauterine growth retardation is strongly linked with impaired glucose tolerance and eventual development of type II diabetes, although the molecular mechanisms involved are not fully understood [23]. Animal studies have replicated these hypotheses and demonstrated that undernutrition may have an impact on glucose–insulin metabolism. Gardner et al [9] reported that nutrient-restricted ewes during late gestation produced lambs with increased concentrations of plasma glucose and insulin after IVGTT, but no major alterations in glucose-insulin homeostasis were observed in the offspring of ewes undernourished during early gestation. Ford et al [10] reported that ewes exposed to 50% of nutritional requirements during early to mid-gestation resulted in offspring that had greater concentrations of plasma glucose in response to IVGTT at 63 and 250 d of age. We observed that the rate of glucose clearance from the blood of steers at 24 mo of age was correlated with protein availability of dams during late gestation. However, in contrast with Gardner et al [9] and Ford et al [10], we observed that glucose in whole blood decreased more rapidly in LP steers after glucose infusion compared with HP steers. Similar results were reported by Long et al [11] in steers and heifers at 15 mo of age when dams were restricted at 55% of requirement during early gestation. The differences observed in the ability to remove blood glucose concentration after glucose infusion in the cited studies [9–11] may be related to the age of the offspring at glucose tolerance testing. Insulin action and secretion continue to develop and mature with age [23–25]. A study of infants small for gestational age showed increased insulin sensitivity at birth compared with average-sized for gestational age infants; however, by 3 yr of age, small infants were more insulin-resistant than average-sized infants [26]. Studies in rats showed that offspring whose dams were fed a low-protein diet had enhanced glucose tolerance and increased insulin sensitivity during early life [7,8]. Rats born to a mother given a restricted protein diet during pregnancy had a better glucose tolerance than controls when reaching adulthood at 2.5 mo of age, but poorer tolerance in old age at 15 mo of age [25]. Body insulin sensitivity was improved during early life in low-protein offspring [27,28]. However, by 15 mo of age, they have impaired glucose tolerance, and at 17 mo of age, the offspring have frank diabetes [29]. We observed that peak serum insulin concentration was greater in LP than HP steers 15 min after glucose infusion. Similar to the results of the present study, Camacho et al [30] observed that glucose-stimulated insulin secretion and insulin sensitivity for glucose disposal are enhanced in IUGR lambs during their first 2 wk after parturition, predisposing IUGR lambs to excessive glucose utilization and hypoglycemia.

5. Conclusion

Carcass characteristics could be affected without evident changes of growth rate and final BW of offspring born from dams that were protein-restricted during late gestation. Although the association between maternal nutrient restriction and metabolic alteration in adulthood of progeny is well known in humans, scarce information is available on the metabolic consequences of IUGR in domestic animals. There is evidence in humans and rats that glucose tolerance may be age specific in prenatal restricted offspring. Hyperinsulinemia of human infants usually continues into childhood and is followed by a progressive decrease in insulin secretion and glucose intolerance in later life. Studies in farm animals have been performed at an early age, and long-term dynamics of insulin action and secretion in IUGR progeny remains unclear. Differences observed between ovine and bovine species could be attributed to different physiological maturity at the time of study. An analysis of a possible biphasic insulin secretion response should be elucidated in bovine models, especially in females which tend to live to an older age than steers in most production settings.

CRediT authorship contribution statement

S. Maresca: Conceptualization, Methodology, Validation, Investigation, Resources, Writing - original draft, Visualization, Project administration, Funding acquisition. **S.L. Valiente:** Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing - review and editing, Visualization. **A.M. Rodriguez:** Validation, Investigation, Resources, Writing - review and editing, Visualization. **E. Pavan:** Validation, Writing - review and editing. **G. Quintans:** Validation, Writing - review and

editing. **N.M. Long:** Conceptualization, Methodology, Software, Validation, Writing - review and editing, Supervision, Project administration.

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4. INFLUENCIA DE LA RESTRICCIÓN PROTEICA EN EL ÚLTIMO TERCIO DE GESTACIÓN SOBRE EL CRECIMIENTO, CARACTERÍSTICAS DE CARCASA Y CALIDAD DE CARNE DE LA DESCENDENCIA

4.1 RESUMEN

El objetivo de este estudio fue determinar si la ingesta de proteína durante los últimos tres meses de gestación afecta el crecimiento, las características de la canal y la calidad de la carne de la progenie. A 134 ± 14 días de gestación, 68 vacas Angus multíparas fueron bloqueadas por PV y fecha de parto esperada y asignadas aleatoriamente a dietas que contenían alta (AP) o baja (BP) concentración de proteína cruda en la dieta. Después del parto, las vacas fueron manejadas juntas en pasturas mejoradas durante la lactancia. Después del destete a los 219 ± 13 días de edad, los terneros se recriaron en pastos naturales hasta 687 ± 13 días de edad y luego se colocaron en un corral de engorde durante 83 días antes del sacrificio. La concentración de proteína en la dieta materna no tuvo influencia en el peso vivo y la tasa de crecimiento de las crías durante las fases de recría o terminación (P> 0,10). El espeso de grasa dorsal (GD) de los novillos no se vio afectado (P = 0.38) por los tratamientos de nutrición materna, sin embargo, el área de ojo de bife (AOB) fue mayor en novillos AP que novillos BP al inicio (P = 0.01) y al final de la fase de terminación a corral (P = 0.04). El peso de la canal caliente fue similar entre los tratamientos (P = 0,69), sin embargo, el rendimiento aumentó en AP en relación con los novillos BP (P = 0,01). La terneza del músculo *Longissimus* aumentó en AP en comparación con novillos BP después de 3 y 14 días (P <0.001) de maduración. No se observaron diferencias en la degradación de troponina-t (P = 0,77) y el contenido de colágeno (P = 0,58). El diámetro de las fibras musculares fue similar en novillos BP y AP (P = 0.20), lo que sugiere que el aumento del AOB en novillos AP podría deberse a hiperplasia muscular. Estos datos indicaron que el nivel de proteína durante la gestación media a tardía no afecta el crecimiento de la descendencia, pero tiene un impacto en la composición de la canal y la calidad de la carne de los novillos.

Palabras clave: proteína, crecimiento, calidad de carne, musculo.

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The influence of protein restriction during mid- to late gestation on beef offspring growth, carcass characteristic and meat quality

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ABSTRACT

The objective of this study was to determine whether crude protein intake during the last three months of gestation affects growth performance, carcass characteristics and meat quality of steer progeny. At 134 ± 14 d of gestation, 68 multiparous Angus cows were blocked by BW and expected calving date and randomly assigned to diets that contained either low or high dietary crude protein concentrations and were allotted in 12 pens per treatment. After calving, cows were managed together on improved pastures during lactation. After weaning at 219 ± 13 d of age, steers calves were stockered on natural pastures until 687 ± 13 d of age then placed into a feedlot for 83d before slaughter. Maternal dietary protein concentration had no influence on offspring body weight and growth rate during rearing or finishing phases ($P > .10$). Rib fat thickness of steers was not affected ($P = .38$) by maternal nutrition treatments, however, LM area was greater in HP steers than LP steers at entrance into the feedlot ($P = .01$) and end of finishing phase ($P = .04$). Hot carcass weight was similar between treatments ($P = .69$), however dressing percentage was increased in HP relative to LP steers ($P = .01$). Tenderness of *Longissimus* muscle was increased in HP compared to LP steers after 3 and 14d ($P < .001$) of aging. No treatment differences in troponin-t degradation ($P = .77$) and collagen content ($P = .58$) were observed. Muscle fiber diameter was similar in LP and HP steers ($P = .20$), suggesting that increase of LM area in HP steers could be due to muscle hyperplasia. These data indicated that level of protein during mid to late gestation does not affect offspring growth but has impacts on carcass composition and meat quality of steer progeny.

1. Introduction

Cow calf operations in Argentina are managed under extensive grazing conditions. The quality of forages and roughages is often poor, particularly in the winter seasons, leading many spring calving cows to undergo periods of undernutrition during the second half of gestation. Mid- to late gestation is a critical period for muscle, adipose and connective tissue development in the fetuses; therefore, nutrient restriction during this period can alter carcass characteristics and meat quality of offspring (Du et al., 2010). Previous studies about nutritionally restricted beef cows during mid- to late gestation have reported inconsistent results on offspring carcass composition. Offspring of dams grazing low quality forage (Native range) had reduced hot carcass weight (HCW) and 12 rib fat thickness than offspring of dams grazing improved pasture (Underwood et al., 2010). When cows were supplemented three times weekly with crude protein during late gestation, no differences were found in offspring's HCW and fat cover (Larson, Martin, Adams, & Funston, 2009; Stalker, Adams, Klopfenstein, Feuz, & Funston, 2006). The above-mentioned experiments did not control a specific component of the diet nor total dry matter intake, therefor no clear conclusion about the effect of dietary protein level during late gestation on the progeny performance and carcass or meat quality could be reached. Considering the importance of mid- to late gestation maternal nutrition on muscle, fat and connective tissue development, the purpose of this study was to determine the influence of dietary protein concentration during mid- to late gestation of beef cows on offspring carcass characteristics and meat quality.

2. Materials and methods

All procedures used in this study were approved by the Institutional Animal Care and Use Committee of the National Institute of Agricultural Technology (CICUAE INTA – CERBAS; Approval No. 87).

2.1. Animals

Cow management has been previously published (Lopez Valiente et al., 2018), and only a brief description is presented here. One

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hundred and twenty suckled Angus multiparous cows were synchronized with a drug-releasing device contained progestogen (Cronipres®, Biogénesis-Bago, Argentina) for 7 d, and upon removal of the device, 500 μg of cloprostenol (Ciclase DL®, Syntex, Argentina) and 2 mg of estradiol benzoate were administrated intramuscularly (Benzoato de Estradiol Syntex®, Argentina). Timed AI was conducted 48 h after estradiol injection, using semen from a single Angus sire. Fifteen days after AI, a single Angus bull was used during a 15 d natural breeding period. Sixty-eight cows pregnancy to AI and natural mating were identified by transrectal ultrasound at 30 d after the end of the natural breeding period. During early to mid-gestation, pregnant cows were managed on improved pastures. At 134 ± 14 d of gestation, cows were stratified by BW and expected calving date into 24 pens. The pens were allotted to either a low protein (LP) or high protein (HP) diet to provide 6% or 12% dietary CP on a DM basis. The LP diet was composed of 89% corn silage and 2% mineral premix to provide an estimated 100% requirements for NE_m and 64% for CP (NRC, 2000). The HP diet consisted of 70% of corn silage, 10% sunflower pellet, 1% urea and 2% of mineral premix to provide an estimated 100% requirements for NE_m and 121% for CP (NRC, 2000). All cows were allowed to calve naturally and birth date of male calves ranged from July 22 to September 5 (mean date of birth = Aug 6). A total of 28 male calves (LP = 15 calves; HP = 13 calves) were born from pregnant cows and all pens had at least one male calf. Calf body weight was recorded within 24 h post-calving. After calving, dams and their calves (male and female) were managed as one group on fescue pastures until weaning when male calves were 219 ± 13 d of age.

At weaning, 28 male calves were separated of female calves. During the rearing period, LP and HP steers were maintained as a group on native range until 687 ± 13 d of age, when steers were placed in individual pens (10 × 20 m) and fed an *ad-libitum* finishing diet (Table 1) for 84 d. Body weight was recorded at the beginning of the rearing phase and at the initial and end of finishing phase, after a 12 h withdrawal from feed and water. Ultrasound measurements (Aquila pro, Esaote Europe B.V. Maastricht, NL; 3.5-MHz probe) were recorded to determine 12th rib subcutaneous fat thickness and LM area at the beginning and end of finishing phase. The slaughter time was determined when the steers reached at less an average of 0.7 cm 12th rib fat thickness. Dry matter intake was determined 3 times per week by calculating the difference between feed offered and orts during the finishing phase.

2.2. Carcass characteristics and sample collection

After 24 h without feed, steers were weighed and transported 95 km to a commercial slaughterhouse, where they were allowed free access to fresh water with a 24 h feed withdrawal before slaughter. All steers were slaughtered as a single skill group. Twenty minutes after slaughter, HCW was recorded and a sample of subcutaneous adipose tissue (1.5 cm × 1.5 cm) and *Longissimus* muscle tissue (1 cm × 1 cm) were collected from the left side of the carcass at 10 cm from the midline at the 9th rib. Tissue samples were processed and fixed in 4% paraformaldehyde for histological analysis. At 3 and 24 h after slaughter, pH and carcass temperature were recorded. A rib section was removed from the left carcass side by cutting between the 9th and 13th rib. The rib sections were transported to the Meat Quality Laboratory at Instituto Nacional de Tecnología Agropecuaria, Balcarce (BA, Argentina), and stored at 4 °C until fabrication a day later. The whole rib block was separated into subcutaneous fat, *Longissimus* muscle, intramuscular fat and bone, and each section was weighed.

Table 1
Nutrient composition of diets in feedlot period.

Item	%
Ingredient, % of DM	
Whole Corn	75
Corn silage	10
Pellet supplement[a]	15
Diet composition[b]	
DM, %	88.4
CP, %	13.1
TDN, %	75.9
NDF, %	15.5
ADF, %	6.7
Ether extract, %	4.4

[a] Pellet sourced by Nutra Alimentos Balanceados (BA, Argentina). Composition: 50.9% CP, 1.2% Ca, 0.7% P, 5.6% ether extract.
[b] All values are from laboratory analyses and are presented on a 100% DM basis (except DM).

2.3. Warner-Bratzler shear force

Two 2.5 cm-thick *Longissimus* muscle steaks were removed from the 12-13th rib section of each carcass and randomly assigned to one of two aging periods (3 or 14 d) for the analysis of Warner–Bratzler shear force (WBSF). The analysis of WBSF was conducted according to AMSA (1995) guidelines. Steaks were thawed overnight at 4 °C, and cooked on an electric clam shell grill (Farberware, Bronx, NY, USA) to an internal temperature of 71 °C. Peak internal temperature was recorded for each steak using a multiscan digital thermometer (Scanning Thermometer, Digi-Sense, Cole Parmer, Vernon Hills, IL, USA). Cooked steaks were cooled to room temperature and ten cores were removed in parallel to the muscle fiber using a sharp 1.27 cm diameter coring device. Peak shear force was measured with a Warner-Bratzler machine (G-R Manufacturing CO., Manthattan, KS, USA) equipped with a digital force gauge (BFG500N, Quantrol™, Dillon/Quality Plus, Inc., Kansas City, MO, USA), using a crosshead speed of 200 mm/min.

2.4. Histology

Longissimus muscle and subcutaneous fat tissue samples were sectioned (10 um thick) using Leica RM2125RT microtome (Leica Microsystems Inc., Bannockburn, IL, USA). Sections were stained with Hematoxylin/eosin. Digital images were taken with a digital camera (PowerShot 450, Canon, Tokyo, Japan) mounted on an optical microscope (40× magnification level; Olympus CX31, Tokyo, Japan). Muscle fiber and adipocyte diameter were determined from 5 fields per animal using Image J software (National Institutes of Health, Bethesda, MD, USA). Fields were chosen so that the field was composed mostly of regularly shaped muscle fibers or adipocytes. A solution of latex beads 40 μm (Beckman Coulter, Miami, FL, USA) and graded slice were used as a diameter standard. Adipocyte diameter was measured by averaging the widest diameter and the narrowest diameter for at least 200 cells per animal. Muscle fiber diameter was measured in the shortest section of the fibers for at least 200 fibers per animal. Muscle fiber area was calculated from fiber diameter and total fiber number of *Longissimus* muscle was estimated as: LM area/fiber area. Whereas, the fat thickness to adipocyte diameter ratio was used as an indicator of absolute number of adipocytes.

2.5. Sarcomere length

The sarcomere length was determined on *Longissimus* muscle samples according to the procedure described by Cross, West, and Dutson (1981) using a helium-neon laser diffraction method. Twenty myofibril fragments were measured from each sample to determine the sarcomere average length.

2.6. Total and soluble collagen content

Total collagen, soluble and insoluble fraction was evaluated on

Longissimus muscle samples. Ten grams of muscle were used for thermal treatment according to the procedure described by Latorre, Lifschitz, and Purslow (2016). Solid residue and supernatant fluids were separated by centrifugation (2324 x g 10 min at 25 °C) and were dried at 60 °C. The fractions were hydrolyzed in 5 ml HCl (6 N) at 110 °C for 16-20 h. The fractions were neutralized and hydroxyproline concentrations was determined by spectrophotometric determination according to the procedure described by Bergman and Loxley (1963) procedure. The % of soluble collagen was calculated by dividing the hydroxyproline content of the soluble phase by the total hydroxyproline in both the soluble phase and the solid residue and multiplying by 100.

2.7. Troponin -T (tn-T)

Longissimus muscle samples that were previously aged for 3 days were used for Tn-T degradation analysis. Intact Tn-T was determined according to the procedure described by Huff-Lonergan, Mitsuhashi, Parrish, and Robson (1996) using western blotting procedures. Briefly, 1 g of frozen muscle samples were homogenized in 10 ml of deionized water and protein content was determined using Microplate Spectrophotometer equipped with reader type Epoch (No.257878; Biotek Instruments Inc., Winooski, Vermont, USA). Samples were adjusted to a constant protein concentration of 3 mg/ml using distilled deionized water. Protein from the whole muscle preparation (33 µg) were loaded into 12% poly acrylamide slab separating gels with a 5% polyacrylamide stacking gel (Mini Protean TGX, Bio-Rad, California, USA). Gels were run at a constant voltage of 200 and 60 mA per gel for 45 min at room temperature (20 °C). Gels were transferred to Immuno-Blot PVDF Transfer membranes (No.55518; Thermo Scientific) for 1 h at 100 V and 340 mA using a Mini Trans-Blot Cell (Bio-Rad). Complete transfer of proteins (30 kDa molecular weight range) was verified using a Kaleidoscope prestained molecular weight marker (Bio-Rad) and subsequent staining of membranes after transfer. Western blotting procedure used as the primary antibody anti-troponin-TJLT-12 (Sigma, St. Louis, MO, USA; 1:7000) and as the secondary antibody anti-mouse IgG labeled with peroxidase (Sigma; 1:7000), and a chemiluminescent detection system (Pierce Super Signal Substrate; Pierce, Rockford, IL, USA). A common sample was used as an internal standard for reference in each blot. Protein bands were quantified using the ImageQuant 400 digital analysis system (GE Healthcare Bio-Science, UK). Bands of 41.7 and 39.9 kDa molecular weights were considered to be intact Tn-T and the sum of their intensities was quantified according to the procedure described by Weaver, Bowker, and Gerrard (2008). Content of intact Tn-T was estimated by expressing the density of the intact Tn-T band from each sample in a given blot relative to the density of the intact Tn-T band of internal reference standard in the same blot.

2.8. Statistical analysis

Cows were blocked according to BW (four blocks) and expected calving date (two blocks). The averages of the BW blocks were 317 ± 22 kg, 389 ± 17 kg, 440 ± 10 kg and 480 ± 32 kg and the averages of the expected calving date blocks were July 30 ± 2 d for AI cows and August 21 ± 3 d for natural breeding cows. This resulted in 4, 4, 5, and 5, calves per block 1–4 that calved to AI and 2, 3, 2, and 3, calves per block 1–4 that calved the natural breeding. Dam's pens were considered the experimental unit and each treatment were allotted to 2 pens for each BW*AI blocks and 1 pen for each BW*natural breeding block. All offspring data, including, rearing and finishing growth, carcass measurement, meat quality, muscle fiber, and adipocyte diameter were analyzed using the MIXED procedure of SAS (Version 9.4, SAS Inst., Inc., Cary, NC, USA). Treatment and block were the fixed effect and pen into block was the random effect. Data are presented as least squares means and MSE, and differences considered significant at $P \leq .05$, with a tendency at $0.05 < P \leq .10$.

Table 2
Effect of maternal dietary protein concentration during mid- to late gestation on rearing and feedlot performance of steer progeny.

Item	Treatments[a]		
	LP[b]	HP[b]	*P*-value
Rearing period			
BW initial, kg	259.7 ± 6.9	265.4 ± 8.3	0.61
BW final, kg	371.0 ± 7.4	371.8 ± 8.8	0.94
ADG kg/d	0.355 ± 0.02	0.347 ± 0.02	0.75
Initial 12th rib fat thickness[c], cm	0.20 ± 0.01	0.20 ± 0.01	0.96
Final 12th rib fat thickness[c], cm	0.33 ± 0.02	0.36 ± 0.03	0.38
Initial LM area[c], cm^2	39.3 ± 1.2	39.4 ± 1.5	0.95
Final LM area[c], cm^2	48.55 ± 1.29	53.56 ± 1.47	0.01
Finishing period			
BW Final, kg	493.6 ± 12.5	480.5 ± 16.0	0.52
Final 12th rib fat thickness[c], cm	0.75 ± 0.05	0.75 ± 0.06	0.97
Final LM area[c], cm^2	63.74 ± 1.65	69.39 ± 2.22	0.04
ADG kg/d	1.45 ± 0.10	1.29 ± 0.23	0.31
DMI kg/d	11.56 ± 0.66	10.45 ± 0.68	0.27
G:F kg/kg	7.52 ± 0.58	7.98 ± 0.60	0.59

[a] LP, Low Protein (6% CP) High Protein (12% CP). Treatments were applied from 134 d of gestation until partum.
[b] $n = 12$.
[c] The measurements of 12th rib fat thickness and LM area were taken *via* ultrasound.

3. Results and discussion

Cow and calf performance have been reported previously (Lopez Valiente et al., 2018; Maresca et al., 2018). At the beginning of rearing period, body weight, 12th rib fat thickness and LM area were similar between treatments ($P > .10$; Table 2), showing that maternal dietary protein concentration during mid- to late gestation had no effect on animal growth during early life. At the end of the rearing period, body weight, ADG and 12th rib fat thickness were not different between treatments ($P > .10$). However, HP steers had a greater LM area than steers born from LP cows ($P = .01$). Growth performance and carcass composition during rearing have been scarcely described on beef cattle fetal programming studies and only few studies have recorded daily gain. Long, Prado-Cooper, Krehbiel, Desilva, and Wettemann (2010) did not find differences in ADG of steers whose dams were exposed to 55% of energy restriction during early gestation compared to steers whose dams were fed to meet requirements. Ewes exposed to 50% of restriction energy requirements from d110 of gestation to term had lambs with similar growth rate at 1 year of age (Gardner et al., 2005).

Steers from both treatments were finished with a similar body weight and 12th rib fat thickness ($P > .10$; Table 2). This is consistent with previous studies of Larson et al. (2009) and Stalker et al. (2006) who evaluated steer progeny performance of protein supplemented dams during mid- to late gestation compared to steer progeny from unsupplemented dams. In contrast, Greenwood and Cafe (2007) showed that cattle with low birth weights due to severe undernutrition during gestation had reduced daily gain and body weight at slaughter. In addition, Underwood et al. (2010) observed that steers born to cows grazing native grass had reduced daily growth and body weight than steers from cows grazing improvement pastures during late gestation. The conflicting results in the literature may be attributed to differences across studies in the degree of nutrient restriction or specific nutrient manipulated. Most protein supplementation studies are confounded by increased dry matter intake, and thus a resulting increase in energy intake due to increased dietary protein, which was not the case in our study as energy content of the ration was the same between our treatments.

The HCW, 12 th rib fat thickness and marbling score showed no differences between treatments ($P > .10$; Table 3). Previous studies involving nutritionally restricted beef cows during mid- to late

Table 3
Effect of maternal dietary protein concentration during mid- to late gestation on carcass characteristics of steer progeny.

Item	Treatments[a]		
	LP[b]	HP[b]	*P*-value
HCW, kg	284.3 ± 7.7	289.5 ± 9.9	0.69
Dressing, %	57.6 ± 0.6	60.2 ± 0.8	0.01
12th-rib fat, cm	0.67 ± 0.07	0.57 ± 0.08	0.38
Marbling score[c]	455 ± 15	435 ± 20	0.44
Rib Block[d]			
LM, %	33.6 ± 0.7	34.1 ± 0.9	0.29
Subcutaneous fat, %	8.0 ± 0.5	7.2 ± 0.7	0.55
Bone, %	24.2 ± 0.9	25.2 ± 1.2	0.75
Subcut. fat/LM ratio	25.6 ± 1.5	21.1 ± 1.8	0.08
Shear force			
3d, N	46.09 ± 0.88	42.07 ± 1.07	< 0.001
14d, N	29.91 ± 0.49	27.55 ± 0.58	< 0.001

[a] LP, Low Protein (6% CP) High Protein (12% CP). Treatments were applied from 134 d of gestation until partum.
[b] $n = 12$.
[c] 500 = Modest[00], 400 = Small[00], 300 = Slight[00], 200 = Traces[00].
[d] Rib section removed between 9th and 13th rib.

gestation have been inconsistent in offspring carcass composition. Offspring of dams grazing low quality forage (native range) had decreased HCW and 12th rib fat thickness compared to offspring of dams grazing improved pasture (Underwood et al., 2010). When cows were supplemented three times weekly with crude protein during late gestation, no differences were found in offspring HCW and 12th rib fat thickness (Larson et al., 2009; Stalker et al., 2006). Although steers in our current study from both treatments show similar BW and HCW at slaughter, dressing percentage was lower ($P = .01$) for LP steers compared to HP steers. Although a complete analysis of body composition was not conducted in this experiment, we hypothesize that the lower dressing percentage could be attributed to increased abdominal fat deposition in LP steers. This is due to the practice of removing abdominal fat as part of the evisceration step in Slaughter facilities in Argentina, thus removing the weight of the abdominal fat before HCW was recorded. Research in sheep show that lambs from nutritionally restricted ewes have increased adiposity, primarily in the kidney and mesenteric fat depots, however this effect remain unclear in bovine specie (Ford et al., 2007; Zhu et al., 2006).

To determine if carcass composition was affected by nutritional treatment, proportional weight of LM, subcutaneous fat and bone were calculated from the whole rib block that was collected from each carcass. Pre-partum protein concentration did not affect the percentage of LM ($P = .29$; Table 3), subcutaneous fat ($P = .55$) or bone ($P = .75$); however the subcutaneous fat to LM ratio tended ($P = .08$) to be greater in LP steers compared to HP steers in the current experiment.

The pH of LP and HP steers carcasses were similar at 3 h postmortem (6.27 ± 0.06 v. 6.18 ± 0.08, respectively; $P = .39$) and 24 h postmortem (5.71 ± 0.02 v. 5.70 ± 0.02, respectively; $P = .74$). The Temperature of LP and HP steers carcasses were similar at 3 h postmortem (26.2 ± 0.6 v. 25.9 ± 0.7, respectively; $P = .79$) and 24 h postmortem (8.0 ± 0.01 v. 7.9 ± 0.01, respectively; $P = .18$).

Dietary protein intake during mid- to late gestation did not affect the marbling score of steer progeny ($P = .44$; Table 3). In addition, ether extract and moisture of the LM at the 12th rib was similar between LP and HP steers ($P > .10$; Table 4). These results support previous studies on cows that were restricted during late gestation (Mulliniks et al., 2016; Shoup et al., 2015; Stalker et al., 2006 and Underwood et al., 2010), mid-gestation (Mohrhauser et al., 2015) or early to mid-gestation (Long et al., 2010). It has been hypothesized that the fetal stage is the most efficient period of development to increase marbling in beef offspring (Du et al., 2010). However, there are evidence that adipocyte hyperplasia not only occurs during fetal life, it is

Table 4
Effect of maternal dietary protein concentration during mid- to late gestation on proximate analysis of the *Longissimus* muscle of steer progeny.

Item	Treatments[a]		
	LP[b]	HP[b]	*P*-value
Moisture, %	72.4 ± 0.3	72.8 ± 0.3	0.32
Protein, %	21.6 ± 0.8	21.1 ± 0.7	0.41
Ether extract, %	4.6 ± 0.4	4.0 ± 0.5	0.44

[a] LP, Low Protein (6% CP) High Protein (12% CP). Treatments were applied from 134 d of gestation until partum.
[b] $n = 12$.

also important during postnatal life and can arise during adult life in cattle. In cattle fetus, the chronology appearance of adipocyte tissue depends of anatomical localization. Perirenal adipocyte appears at 80 days of gestation, subcutaneous and intermuscular adipocyte tissue appear from 180 days of gestation, while intramuscular fat only appears after birth (Vernon, 1980). Post-natal growth through adipocyte hyperplasia depends of anatomical location being higher in intramuscular than perirenal, omental or subcutaneous areas. Therefore, early postnatal life nutrition probably could have more effect than fetal programming on intramuscular adipogenesis because the critical intramuscular adipogenesis window is greater after calving (Bonnet, Cassar-Malek, Chilliard, & Picard, 2010; Du et al., 2013; Mangrum et al., 2016).

To determine if observed differences in LM area during the growing and finishing phases were due to muscle hyperplasia or hypertrophy, the diameter of muscle fibers were measured. Diameters of fibers in *Longissimus* muscle were similar ($P = .19$; Table 5) between treatments, indicating that increased LM area in HP steers may be due to greater muscle fiber number. Estimated total fiber number in LM was greater ($P = .03$) for HP steers when compared to LP steers. It has been postulated that the number of muscle fibers is fixed from the end of the second third of gestation (180d), suggesting that the third generation of fibers which appears later is not quantitatively important in this species (Picard, Lefaucheur, Berri, & Duclos, 2002). However, the influence of fetal nutrition on musculogenesis has been scarcely documented on bovine specie. Only the study of Long et al. (2010) reported greater muscle fiber area in steers from dams exposed to a globally restricted nutrient intake during early gestation without a difference in carcass composition, which could be interpreted as decreased fiber number since muscle weight was similar between treatments.

To determine if protein level during mid- to late gestation affected adipogenesis, we investigated whether subcutaneous adipocyte size was altered. Subcutaneous adipocyte diameter was similar ($P = .66$; Table 5) for LP and HP steers. As the subcutaneous fat thickness and subcutaneous adipocytes diameter were similar between treatments, we hypothesize that number of adipocyte was also not affected by treatments (fat thickness/adipocyte diameter ratio, $P = .44$). Adipogenesis of subcutaneous fat occurs between late gestation and early post-natal life in ruminant animals (Bonnet et al., 2010), thus manipulation of nutrition in this stage could affect the total number of adipocyte and final subcutaneous fat cover. Underwood et al. (2010) suggested that an increase of 12th fat thickness in offspring of cows placed on an improved plane of nutrition during late gestation may be attributed to increased adipocyte number. The lack of effect on subcutaneous fat cover in our study could be attributed to only CP being restricted not energy as all cows were fed to meet energy requirements.

Steaks from high protein steers had lower shear force values than LP steers after 3 d or 14 d ($P < .001$) of postmortem aging period. Few studies had evaluated the effect of bovine maternal nutrition on offspring meat shear force or tenderness and results are not consistent (Alvarenga et al., 2016; Mohrhauser et al., 2015; Underwood et al., 2010). Sarcomere length tended to be greater ($P = .07$; Table 6) for HP

Table 5
Effect of maternal dietary protein concentration during mid- to late gestation on *Longissimus* muscle fiber and subcutaneous fat adipocyte diameter of steer progeny.

Item	Treatments[a]		
	LP[b]	HP[b]	*P*-value
Adipocyte diameter (μm)	90.5 ± 3.1	86.5 ± 4.0	0.66
Fat thickness/adipocyte diameter ratio	0.083 ± 0.005	0.090 ± 0.007	0.44
Muscle fiber diameter (μm)	43.1 ± 2.1	38.9 ± 2.3	0.19
Total fiber number[c]	$4{,}77 \times 10^6 \pm 0.38 \times 10^6$	$6{,}22 \times 10^6 \pm 0.49 \times 10^6$	0.03

[a] LP, Low Protein (6% CP) High Protein (12% CP). Treatments were applied from 134 d of gestation until partum.
[b] $n = 12$.
[c] Estimated as: LM area/fiber area.

Table 6
Effect of maternal dietary protein concentration during mid- to late gestation on longissimus muscle sarcomere length, content of intact troponin-T (Tn-T) and collagen content of steer progeny.

Item	Treatments[a]		
	LP[b]	HP[b]	*P*-value
Sarcomere length, μm	2.08 ± 0.01	2.10 ± 0.01	0.07
Intact Tn-T, %	2.49 ± 0.76	2.23 ± 0.54	0.77
Collagen, mg/g fresh tissue			
Total	2.49 ± 0.18	2.34 ± 0.18	0.58
Insoluble	1.96 ± 0.20	1.91 ± 0.20	0.87
Insoluble/Total ratio	0.78 ± 0.03	0.81 ± 0.03	0.52

[a] LP, Low Protein (6% CP) High Protein (12% CP). Treatments were applied from 134 d of gestation until partum.
[b] $n = 12$.

steers than LP steers. Amount of troponin-T was used to determine if the degree of tenderness during postmortem storage in HP steers was attributed to increased myofibrillar degradation. Inmunoblotting showed that the amount of troponin-T degradation at 2 days postmortem storage was not affected ($P = .77$) by nutritional treatment of dams (Table 6).

The generation of fibroblast and connective tissue in fetal skeletal muscle is highly active during late gestation (Du et al., 2010). Connective tissue is composed mainly of collagen, which is the responsible for the background toughness of meat, however, there is limited research to confirm that maternal undernutrition could affect fibrogenesis in skeletal muscle (Du et al., 2013). In the current study, the total collagen, insoluble collagen and insoluble/total collagen ratio was not influenced ($P > .10$; Table 6) by maternal dietary protein concentration during mid- to late gestation. Similar results were found by Underwood et al. (2010) in bovine offspring when diet of dams was improved during late gestation. On the other hand, Alvarenga et al. (2016) reported that low protein level during peri-conceptional and first trimester of gestation in cattle may increase the shear force and collagen content in *Semitendinosus* muscle, but not in *Longissimus* muscle.

4. Conclusions

The results of this study show that dietary concentration of protein during mid- to late gestation may affect the carcass composition of progeny without effects on growth during the rearing and finishing stages. Improved carcass quality of high protein steers was associated with higher dressing percentage and increased *Longissimus* muscle area. Consistently decreased shear force values were observed in offspring of dams fed an increased protein level. However, the underlying factors for these results have been not revealed in this study. More detailed investigations are needed to reveal how changes of specific components of maternal diet may affect bovine fetal development; specifically, myogenesis and fibrogenesis in skeletal muscle. Reduced maternal dietary protein status, similar to what could be encountered with extensive grazing cow/calf operations in Argentina, appears to have negative impact in carcass quality.

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5. DISCUSIÓN GENERAL Y CONCLUSIONES GLOBALES

Los resultados de este experimento han demostrado que un bajo nivel de proteína dietaria durante el final de la gestación en hembras bovinas puede afectar el crecimiento fetal, aunque no se observó un cambio marcado en el peso al nacer de los tereros. En la mayoría de los estudios donde se realizaron restricciones energéticas a vacas durante la segunda mitad de gestación, el peso de los terneros al nacer fue más bajo que en el grupo control sin restricción (Wiltbank et al., 1962; Corah et al., 1975; Houghton et al., 1990; Freetly et al., 2000). En contraste, cuando las madres gestantes son alimentadas con dietas deficientes en proteína, varios estudios coinciden en que el peso al nacer no es afectado (Stalker et al., 2006; Larson et al., 2009; Cartens et al., 1987). Según lo que muestra la bibliografía, las pérdidas de peso y condición corporal de las madres han sido mucho más severas en experimentos donde las restricciones son energéticas. Esto podría explicar las diferencias observadas en el crecimiento fetal entre los distintos tipos de restricción. La suplementación proteica parece no afectar el crecimiento fetal, sin embargo hay indicadores que confirman que la disponibilidad de aminoácidos para el feto pueden afectar el desarrollo y madurez de determinados órganos sin cambios aparentes en el peso vivo (Bohnert et al., 2013; Lemaster et al., 2016). En el presente estudio, se observaron varios cambios en la morfometría corporal que sugieren que el peso al nacer puede no ser el mejor indicador para detectar si el crecimiento fetal es afectado en bovinos debido a restricciones nutricionales durante la gestación. La relación entre medidas corporales y peso es capaz de detectar un crecimiento fetal desproporcionado. El incremento proporcional de la circunferencia cefálica y el bajo índice de masa corporal en los terneros hijos de vacas BP demuestra que la restricción proteica afecta el crecimiento intrauterino.

Los resultados de este estudio indican que la restricción proteica durante el último tercio de gestación afecta el metabolismo de la glucosa de la descendencia durante los primeros 60 días de vida. La falta de efecto de los tratamientos sobre la producción y calidad de leche materna permitieron concluir que las consecuencias observadas en la regulación de la glucemia de los terneros durante los primeros meses de vida se podrían atribuir específicamente a programación fetal y no a un cambio en

el consumo de nutrientes de los terneros. Durante los primeros dos meses de vida se observó una elevada concentración de glucosa sin modificaciones en la concentración de insulina, lo que indica que los terneros hijos de vacas restringidas en proteína podrían desarrollar resistencia a la insulina como consecuencia de un retardo en el desarrollo y maduración del páncreas. Inmediatamente después del parto, la función endocrina del páncreas debe controlar la glucemia del neonato que se encuentra en etapa de transición de una nutrición parenteral a una nutrición entérica. Por lo tanto, el desarrollo y maduración del páncreas durante la vida fetal y neonatal es crítica para el control de la homeostasis de la glucosa durante los primeros meses de lactancia. Estudios recientes en bovinos han demostrado que la subnutrición fetal durante la segunda mitad de gestación puede reducir la proliferación de la masa de células β e incrementar la apoptosis de los islotes de células pancreáticas (Washburn et al., 2016).

Contrariamente a lo observado por otros autores en estudios con la especie ovina, los novillos hijos de madres que sufrieron una restricción proteica durante el último tercio de gestación, tuvieron una baja concentración de glucosa en sangre y una alta concentración de insulina luego de una infusión de glucosa por via endovenosa a los 24 meses de edad. Aunque la asociación entre restricción maternal de nutrientes y alteraciones metabólicas es bien conocida en humanos, la información disponible sobre las consecuencias metabólicas del retraso en el crecimiento intrauterino en animales domésticos es escasa. Hay evidencias de estudios en humanos y roedores que indican que la tolerancia a la glucosa se puede manifestar de distintas maneras según la edad de los descendientes de madres restringidas nutricionalmente. Hay reportes de casos de hiperinsulenemia en infantes humanos que progresivamente se transforman en casos de reducida secreción de insulina e intolerancia a la glucosa en la vida adulta. Las diferencias observadas en la bibliografía respecto de la respuesta en bovinos y ovinos podrían ser atribuidas a diferencias en la madurez fisiológica en la que se realizan los experimentos. Se deberían profundizar estudios sobre una posible respuesta bifásica en la secreción de insulina en modelos bovinos, especialmente en hembras, las cuales perduran como madres hasta una edad mayor que los machos en la mayoría de los sistemas de producción. Los primeros estudios retrospectivos realizados en humanos, que dieron origen a la teoría de la programación fetal,

observaron una fuerte correlación entre bajo peso al nacimiento y desarrollo de enfermedades coronarias, obesidad y diabetes Tipo II en la edad adulta. Los estudios posteriores realizados con animales domésticos han sido de corta duración evaluando principalmente los efectos de la restricción gestacional en una temprana edad de la descendencia. Por lo tanto, el efecto a largo plazo en la dinámica de la secreción y acción de la insulina en la progenie de bovinos que sufrió retardo en el crecimiento intrauterino no se ha esclarecido. Existe un potencial campo de estudio mediante experimentos de largo plazo para evaluar posibles consecuencias de la restricción nutricional intrauterina en bovinos adultos.

Los resultados de este estudio también demostraron que la concentración de proteína dietaria durante el final de la gestación puede afectar la composición de la carcasa de la progenie sin efectos sobre el crecimiento durante la etapa de recría y terminación. Se han publicado estudios en ovinos (De Blasio at al., 2007) y más recientemente en bovinos (Tipton et al., 2018) que indican que una buena nutrición posnatal puede generar un crecimiento compensatorio en animales que han sido restringidos nutricionalmente durante la gestación, alcanzando pesos similares a la descendencia de madres bien nutridas. Aunque los mecanismos fisiológicos involucrados en esta respuesta no han sido del todo elucidados, en la progenie de ovejas sub-nutridas se han observado alteraciones del metabolismo de la glucosa y cambios en la composición de la canal, con aumentos de la deposición de tejido graso (Gardner et al., 2005; Ford et al., 2007). En este estudio, se observó una mejora en la calidad de carcasa en novillos hijos de vacas alimentadas con alto nivel de proteína asociada con un incremento en el rendimiento de la canal y una mayor área del músculo *Longissimus Dorsi*. Esto fue debido a un incremento en el número de fibras musculares estimadas en función del diámetro miofibrilar y el área de ojo de bife. Si bien, varios autores postulan que la mayor cantidad de fibras musculares se definen durante la segunda ola miogénica que ocurre principalmente durante el segundo trimestre de gestación, los resultados de este experimento indican que la hiperplasia muscular puede ser sensible a la nutrición materna durante el último tercio de gestación. Nosotros hipotetizamos que el total de fibras musculares puede ser afectado en el último tercio de gestación debido a un efecto positivo sobre la actividad de las células

miosatelites que se desarrollan en apoyo a la hipertrofia muscular luego de terminar la miogénesis secundaria (Greenwood et al; 1999).

También se observó en este estudio una mejora en la calidad de la carne asociada a una disminución en la fuerza de corte en novillos hijos de vacas AP. Sin embargo, los factores involucrados en esta respuesta no pudieron ser dilucidados, ya que no se observaron diferencias en la concentración de Troponina – T y composición del colágeno. Otro de los factores altamente relacionados con la calidad de carne que fue estudiado en este experimento es el contenido de grasa intramuscular, a través de la determinación del marbling score y la concentración de extracto etéreo en el músculo *Longuissimus Dorsi.* El nivel de proteína dietaria materna no afectó ninguno de estos factores asociados a deposición de grasa intramuscular, lo que permitió rechazar una de las hipótesis principales del trabajo. Esta hipótesis fue planteada en base a un postulado de Du et al. (2010), quienes mencionaron que en los bovinos, la adipogénesis comienza en el último tercio de gestación y es máxima al momento del parto. Sin embargo, estudios posteriores indicaron que el potencial de multiplicación de células grasa depende de su localización anatómica en el animal. Du et al. (2013) postula que la hiperplasia del tejido graso subcutáneo, perirenal y mesentérico es sensible durante el final de la gestación, sin embargo, la formación de tejido graso intramuscular, que es el que define el marmóreo, comienza después del parto y se extiende hasta los 250 días de vida del ternero. En situaciones reales de los sistemas de producción ganadera en Argentina, las restricciones nutricionales suelen exceder los tres meses. Los rodeos de vacas tienen pariciones concentradas en el mes de agosto y la restricción nutricional ocurre durante el último tercio de gestación y se extiende a los dos primeros meses de lactancia. Los recursos forrajeros predominantes en estos sistemas de cría son pastizales naturales que comienzan a aumentar sus tasas de crecimiento a fines del mes de octubre (Donzelli et al., 2014). La mayoría de los estudios sobre programación fetal han sido diseñados para identificar efectos de la subnutrición en trimestres específicos de la gestación. Como se mencionó anteriormente, los periodos restrictivos pueden exceder los estudiados, por lo tanto el diseño de estudios que abarquen los primeros meses de vida del ternero donde se completa el desarrollo del tejido graso intramuscular, podrían aportar conocimientos

sobre aspectos del desarrollo neonatal que influencian la calidad de carne. En este sentido, resultará necesario el diseño de nuevos experimentos para comprender como los cambios de componentes específicos de la dieta maternal pueden afectar el desarrollo fetal y neonatal con un concepto más amplio de periodo crítico para el desarrollo de la progenie.

Como conclusiones generales podemos decir que este estudio demostró que restricciones proteicas durante el último tercio de gestación, similares a las que pueden ocurrir en nuestros rodeos de cría en condiciones extensivas, pueden afectar el rendimiento y la calidad de carcasa de la progenie, lo cual podría tener un impacto negativo directo sobre el resultado económico de las empresas ganaderas. Es importante remarcar que se identifica un gran campo de trabajo en la investigación de distintas variables que deberían ser consideradas para futuros diseños de experimentos en programación fetal de bovinos. La condición corporal de las vacas previa al periodo restrictivo puede ser una fuente de variación importante de los resultados observados en la bibliografía que aún no ha sido estudiada. La capacidad de las vacas gestantes para movilizar reservas mediante la lipolisis o proteólisis durante un período de restricción nutricional podría amortiguar los efectos negativos de la falta de nutrientes a nivel placentario. Por lo tanto, vacas con diferente peso o condición corporal previa a al período de restricción podrían responder de distinta forma ante la carencia de distinto tipo de nutrientes. Otro aspecto que puede ser fuente de variación de los resultados en los experimentos de programación fetal es la edad materna. Este es un aspecto que no se ha estudiado lo suficiente en la especie bovinos, sin embargo, hay algunas evidencias que indican que las vacas durante su primera gestación pueden sufrir las restricciones nutricionales con mayor impacto sobre la progenie debido a que se trata de vientres que no han alcanzado su madurez fisiológica y tienen mayores requerimientos. Por último, cabe mencionar que los antecedentes históricos de los rodeos en distintos ambientes o bajo distintos manejos que generan restricciones nutricionales de distinta frecuencia o magnitud pueden tener incidencia en la respuesta de la progenie. Bajo las condiciones de manejo extensivo en Argentina, los rodeos de cría sufren restricciones severas cada año. De esta manera, los sistemas de cría funcionan bajo una alta presión de selección sobre madres que son capaces de persistir

bajo condiciones de restricción de nutrientes severas y permanentes pudiendo tener una respuesta diferencial con rodeos de vacas que sufren restricciones ocasionales. Entonces, el diseño y análisis de experimentos en programación fetal de bovinos debería contemplar posibles respuestas fisiológicas diferenciales frente a un periodo restrictivo en función de los distintos antecedentes históricos de manejo nutricional.

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