UNIVERSIDAD DE LA REPÚBLICA
FACULTAD DE AGRONOMÍA

NUTRICIÓN DURANTE LA ETAPA FETAL Y PRE-DESTETE DEL TERNERO EN SISTEMAS PASTORILES: PERFILES METABÓLICO-ENDÓCRINOS, COMPOSICIÓN CORPORAL Y CARACTERÍSTICAS DEL MÚSCULO *SEMITENDINOSO* DURANTE LA FASE DE CRECIMIENTO

por

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TESIS presentada como uno de los requisitos para obtener el título de Magíster en Ciencias Agrarias opción Ciencias Animales

MONTEVIDEO
URUGUAY
DICIEMBRE 2013

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AGRADECIMIENTOS

Quiero agradecer a la Ing. Agr. PhD Mariana Carriquiry y la Ing. Agr. PhD Ana Carolina Espasandin por su constante asesoramiento y apoyo incondicional durante estos 3 años de trabajo.

Quiero agradecer a la DCV. Paula Machado, al Ing. Agr. Federico Pereyra, al Br. Fernando Bideau DCV. Alberto Casal DMV. Carlos López-Mazz Ing. Agr. Ana Laura Astessiano Lic. Jimena Laporta por su colaboración y apoyo en todas las instancias de toma de muestras en el campo y en el procesamiento de las mismas en el laboratorio. En especial quiero agradecer al Sr. Óscar Cáceres y a la Téc. Agr. Victoria Cal por su colaboración en el cuidado, monitoreo y manipulación de los animales en el campo experimental.

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RESUMEN

Se estudió el efecto de la nutrición materna durante los períodos fetal y de lactancia (2 ofertas de forraje: alta AOF y baja BOF) y el genotipo materno sobre el crecimiento potencial y desarrollo muscular de terneros cruza (PUF1 y CRRC), y su relación con los cambios en el perfil metabólico y la composición corporal durante el primer año de vida. Durante este período, se registró mensualmente el peso vivo (PV) y se colectaron muestras de sangre para medir la concentración de glucosa, proteína total, albúmina, insulina e IGF-I. Al nacimiento, destete (142±15 días) y al año de vida, se tomaron muestras del músculo *Semitendinoso* para estudiar diámetro y densidad de fibras y la expresión génica de *GHR, IGF1R, IGFBP3, IGFBP5, IGF1, PAX3, PAX7, PPARG* y *SREBF1*. Al destete y al año de vida, se estudió la composición corporal estimando los porcentajes de grasa, proteína y agua en la carcasa y corporal. La información fue analizada utilizando los días postparto como efecto repetido, y a la raza de la madre, oferta de forraje, raza y sexo del ternero como efectos fijos. El PV fue menor para los terneros *BA-PUF1* al destete y al año de vida, asociado a menores concentraciones plasmáticas de IGF-I, glucosa e insulina durante la lactancia. Los terneros *BA-CRBC* presentaron PV similares a los animales en *AOF*, sin embargo al nacer se observó menor tasa de deposición de tejido magro, mayor diámetro de fibra y mayor expresión muscular de *IGFBP5*, pero menor expresión de *PPARG* y de *SREBF1*. Al año de vida, la Insulina plasmática fue mayor en terneros en *AL-PUF1* que en el resto de los grupos, asimismo se observó mayor expresión de *IGF1R* e *IGFBP5* en el tejido muscular comparado con los terneros *AL-CRBC*. La oferta de forraje durante la etapa fetal y de lactancia interactuó con el genotipo materno afectando a corto, mediano y largo plazo afectando el PV, la composición corporal, los perfiles endócrinos, el diámetro de fibra muscular y la expresión de genes relacionados al sistema IGF-I y de adipogénesis.

Palabras clave: ganado bovino, pastura, desarrollo del ternero, histología muscular, ARNm del sistema de IGF.

Calf fetal and pre-weaning nutrition in grazing systems: metabolic-endocrine profiles, body composition and *Semitendinosus* muscle characteristics during growing phase

SUMMARY

In this research it was evaluated the effect of maternal nutrition during fetal and lactation periods (2 Forage allowances: High HiFA and Low LoFA) and maternal genotype, on growth and muscle potential development in crossbred calf (PUF1 and CRBC), and their relationship with changes in the metabolic profile and body composition during the first year of life. During this period, monthly body weight (BW) were recorded and blood samples were collected to measure the concentration of glucose, total protein, albumin, insulin and IGF -I. At birth, weaning (142 ± 15 days) and a year of life (380 ± 15 days, *Semitendinosus* muscle samples were taken to study fiber diameter and density, and gene expression of GHR, IGF1R, IGFBP3, IGFBP5, IGF1, PAX3, PAX7, PPARG and SREBF1. At weaning and at 380d, body composition was studied by estimating body and carcass fat, protein and water percentage. Data were analyzed using days postpartum as repeated measures, and dam breed, forage allowance, calf genotype and sex as fixed effects. BW was lower in Lo-PUF1 calves at 142d and at 380d associated with lower plasma concentrations of IGF -I, glucose and insulin during lactation. Lo-CRBC calves had similar BW compared to HiFA calves, however at birth lean tissue deposition rate and expression of *PPARG* and *SREBF1* was lower while muscular fiber diameter and expression of *IGFBP5* was greater. At 380d plasma insulin was higher in Hi-PUF1 calves than in the other groups and showed higher expression of *IGF1R* and *IGFBP5* in muscle tissue compared to Hi-CRBC calves. Forage allowance during fetal and lactation periods interacted with maternal genotype affecting the short, medium and long term, affecting BW, body composition , endocrine profiles , muscle fiber diameter and the expression of genes related to IGF-system and adipogenesis.

Keywords: cattle, pasture development calf muscle histology, mRNA IGF syste

1.INTRODUCCIÓN

1.1.PLANTEO DEL PROBLEMA

El Uruguay es un país productor de carne bovina, utilizando para su explotación un 61,8% de la superficie total del país (11.925 millones de ha), con un stock bovino de 11,4 millones de cabezas y una producción anual de 997 mil toneladas de carne vacuna, que representan 1.600 millones de dólares anuales para el país según la Dirección de Estadísticas Agropecuarias del Ministerio de Ganadería, Agricultura y Pesca (DIEA, 2012). Las exportaciones de carne bovina, aproximadamente 250 mil ton., ascienden a 924 millones de dólares anuales. Las divisas generadas por los países que conforman Unión Europea (24,6%), Nafta (16,3%) y Mercosur (15,0%), junto con República Popular China (18,6%) e Israel (10,7%) representan el 85% del total exportado en dólares de acuerdo al Instituto Nacional de Carnes (INAC, 2012).

La cría bovina es el inicio de la producción de carne, y en Uruguay representa el 49% de la superficie total utilizada en los sistemas de producción ganadera e involucra el 53% de los establecimientos ganaderos (INAC, 2012), siendo la mayoría (65,2%) de ellos establecimientos familiares de los cuales 63% son criadores basados en la utilización del campo natural como recurso forrajero A su vez, nuestros sistemas de cría están basados principalmente en el uso de ganado Hereford y Angus de acuerdo al Instituto Nacional de Investigación Agropecuaria (INIA, 2011). La investigación nacional (Espasandin *et al.*, 2006; Gimeno *et al.*, 2002; Franco *et al.*, 2002) e internacional (Cundiff *et al.*, 1992; Long and Gregory, 1974) ha demostrado que el uso de las cruzas recíprocas entre estas razas (Angus y Hereford) incrementó, entre otros, la producción de carne, al mejorar la performance reproductiva, el peso al destete, y rendimientos de carcasa.

En los sistemas de cría en condiciones pastoriles, como los de Uruguay, las variaciones climáticas anuales e interanuales, particularmente precipitaciones y temperatura, y las diferencias en el crecimiento estacional, y en la cantidad y calidad de la pastura ofrecida (Berretta *et al*., 2000) determinan períodos de balance

energético negativo en los animales debido a que el consumo de energía no es suficiente para satisfacer los requerimientos en épocas críticas de las vacas y terneros (Laporta *et al.*, 2011; Astessiano *et al.*, 2008; Soca *et al*., 2007). En particular, los momentos de mayor déficit coinciden con el último tercio de la gestación, y/o las primeras semanas de lactancia (Laporta *et al.*, 2011; Astessiano *et al.*, 2008; Soca *et al*., 2007). Durante la gestación y/o lactación temprana, la nutrición materna puede alterar el crecimiento y desarrollo fetal debido al disminuido aporte de sustratos a través de la placenta (Funston *et al*., 2010), pudiendo repercutir o no en el peso vivo (PV) al nacimiento (Greenwood y Cafe, 2007; Holland y Odde, 1992), y/o destete y "programar el desarrollo" del ternero (Funston *et al.,* 2010).

El concepto de programación del desarrollo refiere a la programación en el largo plazo de varios sistemas del organismo y procesos por stress materno durante la preñez o período postnatal temprano que determina pobre crecimiento, alteración en la composición corporal, disfunciones metabólicas y pobre productividad de la cría durante toda su vida e incluso a través de generaciones (Reynolds y Canton, 2012; Funston *et al.,* 2010). Si bien está menos documentado, la programación del desarrollo afecta al ganado de carne, especialmente en sistemas extensivos de producción donde las vacas de cría son sometidas a ambientes nutricionales pobres (Reynolds, 2012).

Es así que este trabajo busca generar conocimiento que nos permita conocer el impacto (o no) que el manejo de la nutrición durante la gestación y lactancia temprana de las vacas de cría puras (Angus y Hereford) y su cruzas recíprocas tiene sobre el crecimiento y desarrollo de los terneros desde etapas tempranas, estudiando algunos de los mecanismos posiblemente involucrados (ie. metabólicos-endócrinos y de expresión génica). Este conocimiento permitirá planificar intervenciones (ie. suplementaciones estratégicas) durante la etapa fetal o manejos diferenciales de la progenie comprometida, que permitan corregir durante la etapa fetal o postnatal temprana los posibles efectos de la programación del desarrollo, de manera de incrementar el potencial de cantidad y calidad de carne producida. Más aún, el contexto actual ha cambiado y se debe evolucionar hacia modelos más productivos

para responder a las demandas más globales de los productos cárnicos, justificado por el valores actuales de la carne, el abastecimiento global de los mismos y por las buenas condiciones ambientales, institucionales y sanitarias del país (Secco, 2008). En esta situación, se requieren carcasas de mayor tamaño y de composiciones tales que aseguran altas calidades en la carne obtenida, en especial en parámetros como marmoreo y terneza por su alta influencia en las cualidades organolépticas percibidas por el consumidor (sabor, terneza, jugosidad; Millar *et al*., 1994).

1.2. CRECIMIENTO Y DESARROLLO MUSCULAR EN BOVINOS

La producción de carne está basada en el proceso de crecimiento y desarrollo del músculo esquelético de los animales (Lefaucheur *et al.,* 1998). El crecimiento es el producto de la regulación e integración del metabolismo a distintos niveles, teniendo como resultado final la retención de energía en forma de tejido proteico y graso, determinando la composición corporal del animal. Dado que en los mamíferos, el 55% del PV es debido al tejido muscular esquelético (Bonnet *et al.,* 2010), el crecimiento y desarrollo del animal va a estar altamente asociado al crecimiento y desarrollo del tejido muscular. Esta relación entre tejidos es el resultado de un balance dinámico entre el número y tamaño de fibras musculares y de las células adiposas (Bonnet *et al.,* 2010; Rehfeldt *et al.,* 1999).

La estructura básica del músculo esquelético se compone de tres tipos celulares, miocitos o fibras musculares, adipocitos y fibroblastos, que derivan del mismo pool de células mesenquimatosas progenitoras originadas en el músculo durante la etapa embrionaria. El desarrollo muscular o miogénesis se divide en tres etapas: miogénesis primaria momento en el que se forman las miofibras primarias que en el bovino ocurre durante los primeros 2 meses de gestación, una miogénesis secundaria que ocurre en el bovino entre los 2 hasta los 7-8 meses de gestación, y finalmente una fase de hipertrofia muscular que ocurre a partir de los 6-7 meses de gestación y continúa durante el crecimiento postnatal. Asimismo, durante la segunda miogénesis

también se superpone la adipogénesis intramuscular y algunas células progenitoras se van a diferenciar en adipocitos en lugar de miotúbulos (**Figura 1**; Du *et al.*, 2010).

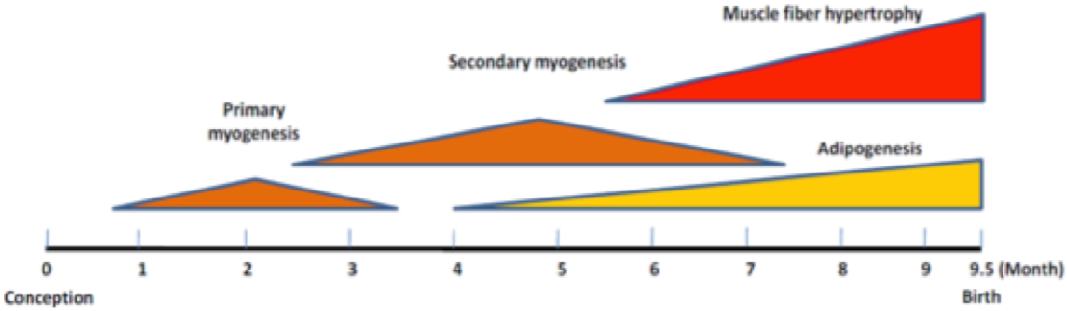

Figura 1. Desarrollo del tejido muscular en terneros durante la etapa fetal (en meses). Adaptado de Du *et al.* 2010.

Las células progenitoras del tejido muscular provienen de la diferenciación de las células paraxiales mesodérmicas durante las etapas tempranas de embriogénesis (Bonnet *et al.*, 2010). En la primer miogénesis (**Figura 2**; Bonnet *et al.*, 2010), se genera el tejido muscular temprano mediante la diferenciación de los primeros mioblastos mononucleares. Durante la segunda miogénesis nuevamente ocurre una diferenciación de las células progenitoras para dar lugar a nuevos mioblastos mononucleares, siendo esta etapa la más crítica en el desarrollo muscular ya que se producen la mayor cantidad de fibras (Russell y Oteruelo, 1981). Durante la etapa fetal, los mioblastos formados previamente van a proliferar y transformarse en células pos-mitóticas mediante la elongación y fusión de las mismas, con el objetivo de formar los miotúbulos multinucleados (fibras musculares). Durante este período, previo al nacimiento, gracias a las ondas miogénicas se determina la cantidad total de fibras que va a tener el tejido, haciendo a la etapa fetal crucial en el desarrollo del músculo esquelético, ya que va a determinar la capacidad de crecimiento del músculo en el período posnatal (Du *et al*., 2010).

Durante el crecimiento postnatal, las fibras musculares formadas previamente durante la gestación adquieren su capacidad de contracción, por lo que ya no pueden dividirse y únicamente podrán aumentar de diámetro (hipertrofiarse). Para poder

lograr esta hipertrofia, las fibras necesitan un incremento en el número de núcleos (Campion, 1984) que son aportados por las células miogénicas mononucleares llamadas células satélite ubicadas en la periferia de las fibras. Estas células satélite se fusionan con la fibra muscular previamente formada, permitiendo el incremento de tamaño del conjunto (Campion, 1984).

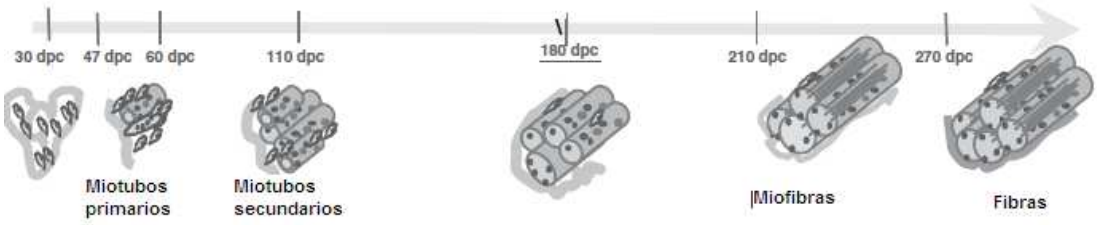

Figura 2. Desarrollo de las fibras musculares (Adaptado de Bonnet *et al.,* 2010)

Por otra parte, la formación de células adiposas intramusculares a partir de tejido mesenquimatoso comienza desde la mitad de la gestación (Figura 1; Du *et al.,* 2010). Las células satélite comienzan a incrementar su potencial adipogénico con la edad (Taylor-Jones *et al.,* 2002) por lo cual un pequeño porcentaje de estas células se diferencian en adipocitos o fibroblastos en vez de células miogénicas (Aguiari *et al.,* 2008). Los adipocitos tienen la capacidad de seguir incrementando su número en el período posnatal (Du *et al.,* 2010). Sin embargo, al igual que las fibras musculares, la etapa fetal es crucial en la determinación de la cantidad de adipocitos intramusculares ocurriendo en el período posnatal la acumulación de triacilglicéridos dentro de los adipocitos formados con el consecuente incremento de tamaño (Tong *et al.,* 2009). El número y tamaño de los adipocitos intramusculares tienen un importante rol en las características del marmoreo (Yang *et al.,* 2006).

El crecimiento y desarrollo del tejido muscularse encuentra regulado endocrinamente (**Figura 3**; Bass et al. 2000). Se han propuesto a la hormona de crecimiento (GH), a los factores de crecimiento similares a la insulina tipos I y II plasmáticos (IGF-I e IGF-II) y a la insulina como unos de los principales mediadores en la regulación la miogénesis (Hyatt *et al.,* 2007). La GH, secretada por la adenohipófisis, promueve la ganancia de PV por medio del incremento de la masa muscular al estimular la

síntesis de proteína y ácido nucleicos por medio de la estimulación del transporte de aminoácidos hacia adentro del músculo esquelético (Lawrence y Fowler, 1998). Se ha sugerido que estos efectos de la GH sobre el músculo están mediados por el IGF-I (Nkrumah *et al.*, 2007), mientras que tiene efectos directos sobre el tejido adiposo.

El IGF-I es considerado como uno de los factores más importantes promotores del crecimiento muscular, la IGF-I circulantes producido principalmente en el hígado por el estímulo de la GH pero se expresa y produce también en forma localizada en el tejido muscular, sugiriendo un rol tanto endócrino como parácrino y/o autócrino de este factor de crecimiento en el desarrollo muscular (Le Roith *et al.,* 2001; Nkrumah *et al.*, 2007). Este factor de crecimiento, en conjunto con el IGF-II, estimula la proliferación y diferenciación de las células satélites, además de estimular la síntesis e inhibir la degradación proteica (Sjorgen *et al.,* 1999). Nkrumah et al. (2007) demostraron que la concentración de IGF-I en sangre está asociada con características productivas como crecimiento, características y calidad de carcasa pudiendo ser explicada por su influencia sobre el marmoreo de la carne (Gardan *et al.,* 2006).

Diversos estudios demuestran que IGF-I en sangre puede cumplir las funciones específicas del sistema endócrino, ya que la aplicación exógena de IGF-I estimuló el crecimiento de ratas jóvenes y la síntesis de proteínas en el músculo esquelético en cerdos recién nacidos (Davis *et al.,* 2002). Asimismo, la subnutrición induce un retraso en el crecimiento debido a un enlentecimiento de la estimulación de la vía hepática del eje GH-IGF (Hornick *et al*., 2000). Louveau y Dividich (2002) encontraron menores ganancias diarias en animales lactantes subnutridos y ésto fue asociado a una disminución en la concentración sanguínea de IGF-I. En contraste, durante un período de crecimiento compensatorio la concentración de IGF-I en sangre está incrementada (Ellenberger *et al.,* 1989). Sin embargo, ha sido reportado (Clemmons, 2009; Philippou *et al.,* 2007) que el IGF-I producida localmente en el músculo tiene un efecto mayor que el IGF-I circulante sobre el crecimiento del músculo en ratones. Chen *et al.* (2011) informaron que la expresión de ARNm de *IGF1* era menor en músculo *Longissimus* de lechones en crecimiento limitado, en

comparación con animales de crecimiento normal y Keady *et al.* (2011) reportaron que era menor en el músculo de toros con menor potencial de crecimiento y desarrollo muscular.

Las funciones endocrinas, autócrinas y parácrinas del IGF-I están mediadas a través de la unión con su receptor tipo 1 (IGF1R) que activa procesos intracelulares que afectan la proliferación y diferenciación celular (Duan *et al.,* 2010; Philippou *et al.,* 2007), estando la acción de esta hormona así como la de GH relacionada directamente a la cantidad y afinidad de sus receptores (Lawrence y Fowler, 1998). Se ha observado que el incremento del Ácido Ribonucleico mensajero (ARNm) de *GHR* o *IGF1R* a nivel del tejido muscular esquelético tiene efectos beneficiosos en el crecimiento y desarrollo del tejido muscular. Por ejemplo, a mayor abundancia de ARNm de *GHR* e *IGF1*en el tejido muscular, aumenta el número de las células precursoras de las fibras musculares y de los adipocitos (células satélite) (Lewis *et al.,* 2002). Asimismo, Coleman *et al.* (1995) observaron hipertrofia e incremento en la regeneración muscular con un correspondiente incremento en la fuerza muscular debido a una sobreexpresión de IGF-I en ratones transgénicos con una acción directa sobre el tejido muscular por medio de su interacción con el IGF1R.

Adicionalmente, seis proteínas de unión o transportadora de los IGF (IGFBP tipos 1 a 6) se han identificado, y a nivel tisular estas IGFBP pueden inhibir o potenciar la acción de los IGF prohibiendo su unión con el IGF1R o liberando los IGF para que puedan unirse al IGFR1 (Duan *et al.,* 2010). Se ha reportado que IGFBP3 e IGFBP5 están altamente expresadas en músculo y afectan su crecimiento a través de modular la acción de la IGF-I o exhibiendo acciones independientes a este ligando (Duan *et al.,* 2010). Se ha asociado mayor expresión de ARNm de *IGFBP3* con menor crecimiento muscular en fetos porcinos (Tilley *et al.,* 2007) y toros bovinos (Keady *et al.,* 2011). En contraste, Sadkowski *et al.* (2009) reportó mayor expresión de ARNm de *IGFBP3* en el músculo de toros con mayor potencial genético de crecimiento y desarrollo muscular. La IGFBP5 es la principal IGFBP secretada por el músculo esquelético y sus efectos sobre este tejido no son consistentes. A pesar de que se ha sugerido que esta IGFBP tiene efectos inhibitorios cuando es usada en

exceso en cultivos *in vitro* de mioblastos o en estudios de sobre-expresión en animales de laboratorio, investigaciones más recientes han demostrado un rol crítico de esta IGFBP en la diferenciación de los mioblastos (Duan *et al.,* 2010). Investigaciones comparando toros bovinos de diferente potencial genético, han mostrado que el ARNm de *IGFBP5* fue menor (Sadkowski *et al.,* 2009) o no cambió (Keady *et al*., 2011) en animales con mayor potencial para crecimiento y desarrollo muscular. Se ha indicado que los resultados contradictorios sobre el efecto de las IGFBP sobre el crecimiento muscular responden al patrón de expresión de las IGFBP durante el desarrollo muscular así como a la compensación funcional que puede ocurrir entre ellas (Keady *et al.,* 2011; Lehnert *et al.,* 2006)

Como se mencionó anteriormente, las células satélite cumplen un rol fundamental en el crecimiento de las fibras musculares mediante el aporte de Ácido Desoxirribonucleico (ADN) en el núcleo. Bajo condiciones normales, éstas células están quiescentes pudiendo ser reconocida por la expresión de factores de transcripción específicos de la familia paired-box: Pax3 y Pax7 (Zammit y Relaix, 2006). Asimismo estos factores se expresan en las células fetales progenitoras de la línea miogénica (Zammit y Relaix, 2006). Durante el período fetal, el Pax3 es un regulador de la cascada miogénica requerida para la miogénesis primaria por medio de la activación de otro factor de transcripción (Myf5) (Bonnet *et al.,* 2010). Está comprobado que la ausencia de estas proteínas, Pax3 y Pax7, generan que las células progenitoras no inicien el programa de miogénesis durante el período de embriogénesis (Relaix *et al.,* 2005). Durante el crecimiento postnatal, Pax3 y Pax7 regulan el inicio de la miogénesis a partir de las células satélite, activando otro factor de transcripción (MyoD) (Bonnet *et al.,* 2010). En particular, Pax7 es imprescindible para la supervivencia de estas células satélite (Kassar-Duchossoy *et al.,* 2010).

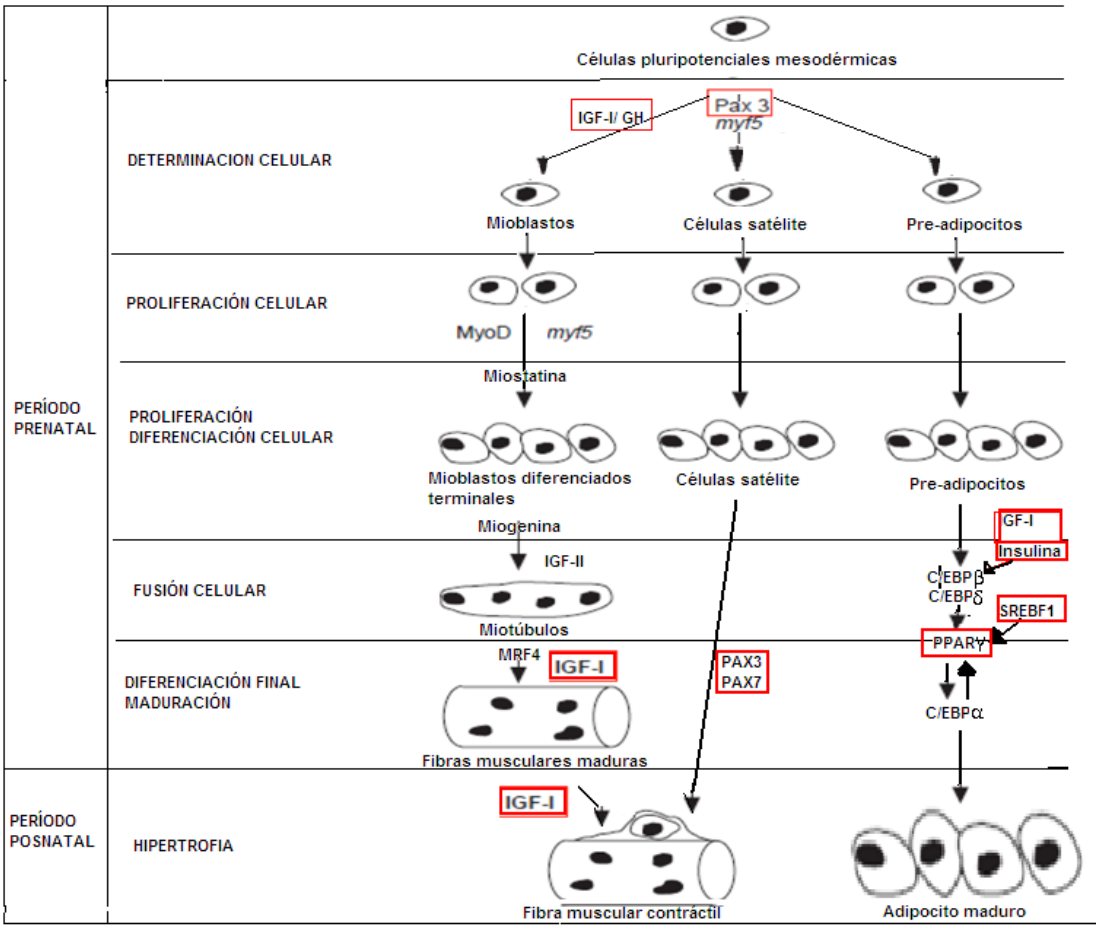

Figura 3. Determinación, proliferación, fusión y diferenciación de las células miogénicas, células satélite y células adiposas, y factores de control. Adaptado de Bass *et al.* 2000.

Por otra parte, durante el proceso de adipogénesis, una serie de factores de transcripción se activan e inducen de manera organizada para promover la síntesis de proteínas que confieren al adipocito las capacidades metabólicas que le son propias, principalmente lipogénesis, lipólisis regulada, producción y sensibilidad a hormonas (Wu *et al.*, 1999; Clarke *et al.*, 1997). Se ha determinado que la diferenciación temprana de los adipocitos está determinada por la expresión de las proteínas específicas (*Lipoproteína lipasa*) de los adipocitos y es regulada por dos factores de transcripción: proteínas de unión al enhancer/CAAT (C/EBP-α) y el receptor de la activación de la proliferación de peroxisomas - γ (PPARG) (Azain, 2004). Durante la

primera fase de la adipogénesis, C/EBPα es inducida y se une directamente al promotor de *PPARG* e induce su expresión (Wu *et al.,* 1999; Clarke *et al.,* 1997, Figura 3). La expresión de PPARG promueve aún más la expresión de C/EBPα, generando ciclos de feed-back positivos. La diferenciación terminal de un adipocito requiere la acción conjunta de PPARG y C/EBP (Castillo *et al.,* 1999; Hwang *et al.,* 1997). La activación de PPARG promueve la diferenciación terminal a través de la inducción de una serie de genes importantes para la absorción y almacenamiento de triglicéridos, tales como proteínas de unión a ácidos grasas, acil-CoA sintetasa, proteínas transportadoras de ácidos grasos, la lipoproteína y otros (Rosen y MacDougald, 2006). Existe evidencia de correlaciones positivas entre la expresión de ARNm de estos genes/factores de transcripción (*PPARG* y *C/EBP*) y el contenido de grasa intramuscular (Wang *et al.,* 2009). Se han identificado también a las Proteínas de Unión al Elemento Regulador de Esteroles (SREBP/*SREBF1*) como factores de transcripción que controlan el metabolismo lipídico regulando genes implicados en las rutas de síntesis y captación de colesterol, síntesis de ácidos grasos, triglicéridos y fosfolípidos, y genes que controlan el metabolismo glucídico (Bobard *et al.,* 2005). Se ha sugerido que este factor de transcripción también podría regular la diferenciación de los adipocitos (Yu *et al.,* 2006). En este sentido, se ha reportado que SREBP, pueden inducir la transcripción *PPARG* a través de la unión a regiones promotoras (Fajas *et al.,* 1999). La manipulación en estos factores de transcripción podría potencialmente ser usada para incrementar la deposición de grasa intramuscular (Wang *et al.,* 2009). Finalmente, tanto la insulina como IGF-I actúan sobre la diferenciación de adipocitos (Smith *et al.,* 1988), estimulando la acumulación de lípidos.

1.3. NUTRICIÓN EN ETAPA FETAL O POSTNATAL TEMPRANA Y CRECIMIENTO Y DESARROLLO MUSCULAR

Durante la gestación, el crecimiento de un animal no ocurre de igual manera en todos los tejidos del animal. Por ejemplo, durante el desarrollo fetal el tejido muscular tiene una prioridad baja en la partición de nutrientes en comparación con otros órganos

como el cerebro, corazón e hígado. Como resultado, el desarrollo del músculo esquelético es particularmente vulnerable a la disponibilidad de alimentos (Zhu *et al.*, 2006). Por lo tanto, una subnutrición materna durante este período puede alterar el crecimiento y desarrollo de este tejido, por un menor traspaso de alimentos a través de la placenta, con efectos a largo corto y plazo en estos animales (Reynolds y Canton, 2012; Funston *et al.*, 2010; Greenwood y Cafe, 2007). Confirmando el efecto a corto plazo de la nutrición durante el período fetal, se reportaron disminuciones en el PV al nacer, la tasa de crecimiento y la relación músculo/grasa (Jurie *et al.*, 1995), debido a un aumento en la adipogénesis provocando así la modificación en la composición corporal de los terneros (Bispham *et al.*, 2005). Contrariamente, en terneros nacidos de vacas suplementadas con proteína durante el último trimestre presentaron mayores tasas de crecimiento durante el primer año de vida (Ciminski, 2002). Asimismo, la restricción nutricional afecta las características del músculo esquelético. En ovejas gestadas, la subnutrición resulta en una reducción en el número de fibras musculares y aumento en el diámetro de las mismas en corderos de 8 meses de edad (Zhu et al., 2006). En bovinos se observó que la restricción nutricional durante el primer trimestre de la gestación alteró el tejido muscular de su descendencia, aumentando el área de corte de las fibras musculares y disminuyendo la concentración de proteínas (Long et al., 2010; Phillipou et al., 2007).

Durante la etapa posnatal, el crecimiento del animal, medido como la ganancia de peso, también depende directamente de la nutrición, y va a ser el principal factor en determinar la proporción de proteína y grasa corporal formada (Di Marco, 2000). En particular, durante los primeros meses de vida de un ternero, la leche materna es su principal fuente de alimentación, estando por tanto determinando su crecimiento por la producción y composición de la leche materna (Jenkins y Ferrel, 1992). La leche materna no solamente determinaría la relación entre los tejidos magro y graso, sino también las características de la carne y la composición y distribución de la grasa (Di Marco, 2000). Sin embargo, la producción de leche varía con el grupo genético, la alimentación y edad de la vaca (Blanc et al., 2000). Ante una mejor cantidad/calidad

de alimentación mayor será la producción de leche materna (Jenkins y Ferrel, 1992), lo que a su vez incrementa la ganancia de peso al destete, la ganancia de peso promedio en el feedlot, el peso y el marmoreo logrado al mercado (Tong *et al*., 2009; Fiss y Wilton, 1993).

Los cambios en el PV están asociados a cambios en las características del tejido muscular. Un aumento en el PV en los terneros se ha asociado a incrementos en el diámetro de las fibras musculares (Cornforth *et al.,* 1980) ya que a mayor cantidades y diámetros de estas fibras, mayor es la acumulación de ADN, ARN y proteínas en el tejido muscular (Di Marco, 2000). La nutrición afecta la expresión de diversos genes a nivel del tejido muscular (Lindsey et al., 2007). Lehnert et al. (2006) hallaron menor abundancia en el tejido muscular de genes involucrados en el desarrollo de este tejido (ej. MyoD) en animales alimentados en base a pasturas de mala calidad comparado con animales bien alimentados. Contrariamente, una restricción proteica reduce la expresión de ARNm de *IGF1* en el tejido muscular en ovinos (Hua et al., 1993), e incrementa la expresión de ARNm de *IGF1R* (Tilley et al., 2007; Tomita et al. 2001) y de *SREBF1* (Nadeau et al., 2006). En efecto, Micke et al. (2011) reportó que el incremento en la expresión muscular de ARNm de *IGF1R* en los terneros con menor PV al nacer actuaba como un efecto compensatorio para promover el desarrollo muscular. Asimismo, Bayol et al. (2004) reportó que existen incrementos en la expresión de las *IGFBP* en ratones sometidos a una subnutrición moderada durante el período fetal como compensación para incrementar la acción local de la IGF-I plasmática. Estos resultados sugieren que frente a una subnutrición existen mecanismos de respuesta que incrementan la sensibilidad del tejido muscular a la IGF-I (Tomita et al., 2001) de forma de promover la hipertrofia muscular mediante el incremento de las células satélite, el incremento de la síntesis proteica y disminuyendo la degradación (Oksbjerg et al., 2004).

Por otra parte, la expresión de ARNm de *PPARG* se incrementa en el tejido muscular fetal y por ende la adipogénesis intramuscular frente a sobre-nutriciones (150% de los requerimientos; Tong et al., 2009). No hay estudios que demuestren un efecto sobre PPARG y SREBP1 en subnutrición.

En el caso particular de la expresión de ARNm de *PAX3* y *PAX7*, no hay estudios sobre el efecto de la nutrición, no teniendo información sobre qué ocurre en el tejido muscular en los animales sometidos a subnutrición durante el crecimiento y desarrollo del tejido muscular.

1.4.GENOTIPO MATERNO Y CRECIMIENTO DEL TERNERO

Experimentos nacionales que han evaluado las razas A. Angus, Hereford y sus cruzas recíprocas (F1), han demostrado que los terneros hijos de vacas cruzas presentan mayores PV al destete, siendo explicado esto por la heterosis materna y la heterosis individual (Espasandin *et al*., 2006). El ternero cruza posee por causa de su heterosis, mayores habilidades para crecer y para aprovechar la leche producida por su madre a través de su diferente comportamiento de amamantamiento (Casal *et al.,* 2009). Asimismo, resultados nacionales muestran un efecto a largo plazo debido a una mejor habilidad materna de las vacas cruza, observándose mayores PV al destete en terneros criados por vacas cruzas en comparación a puras (Espasandin *et al*., 2006; Gimeno *et al*., 2002), posiblemente asociado a las diferencias en producción de leche y ganancia de los terneros (Casal *et al*., 2009). A su vez estos resultados se han asociado con mayores rendimientos al momento de la faena, con mayores PV de carcasa y con mayor área de ojo de bife (Espasandin *et al*., 2006; Franco *et al.,* 2002).

Resultados similares han sido obtenidos en experimentos de cruzamiento a nivel internacional (Cundiff *et al.,* 1992; Long *et al.,* 1974). A su vez, recientemente, Oxford *et al.* (2009) indicaron que los terneros cruza (F1) presentan una ventaja en relación a los terneros puros (Angus and Hereford) contemporáneos en términos de PV al nacer, ganancia diaria y PV al destete en ambientes desfavorables., dado por la heterosis y por los efectos del genotipo materno y paterno.

1.5.HIPÓTESIS Y OBJETIVOS DEL TRABAJO

1.5.1.Hipótesis

El control de la intensidad de pastoreo del campo natural mediante el cambio (alta vs. baja) de la oferta de forraje impacta sobre la nutrición de la vaca de cría durante la gestación y lactancia y altera el crecimiento y desarrollo del ternero en el largo plazo (programando su desarrollo), lo cual podría afectar el potencial de producción de carne (en términos de cantidad y calidad). El impacto de la nutrición sobre el crecimiento y desarrollo del ternero podría depender o interaccionar con la heterosis materna.

1.5.2.Objetivo general

Estudiar efecto de la oferta de forraje del campo natural desde la concepción al destete sobre con el crecimiento y desarrollo durante el primer año de vida de terneros hijos de vacas puras (A. Angus y Hereford) y de la cruzas recíprocas (F1), contribuyendo al conocimiento de los mecanismos involucrados en la respuesta.

1.5.3. Objetivos específicos

1. Evaluar el efecto de la oferta de forraje de campo natural (alta vs. baja) desde la concepción al destete en el crecimiento del ternero, perfiles metabólicos y endócrinos, y composición corporal durante el primer año de vida del terneros hijos de vacas puras (A. Angus y Hereford) y cruzas recíprocas (F1).
2. Evaluar el efecto de la oferta de forraje de campo natural (alta vs. baja) desde la concepción al destete sobre la densidad y diámetro de las fibras musculares y expresión génica (sistema IGF y adipogénesis) en m. *Semitendinoso* durante el primer año de edad de terneros hijos de vacas puras (A. Angus y Hereford) y cruzas recíprocas (F1).

1.6. ESTRUCTURA GENERAL DE LA TESIS

Para la realización de esta tesis se utilizó el formato de "Tesis con dos artículos científicos como cuerpo central (opción B).

El primero titulado "**Calf fetal and early life nutrition on grazing conditions: metabolic and endocrine profiles and body composition during the growing phase"** fue publicado en la revista *Journal of Animal Physiology and Animal Nutrition* (J. Anim. Physiol. Anim. Nutr., 2013 Aug; 97(4):720-31) y se presentan los resultados de PV y composición corporal así como el perfil de metabólico-endócrino a lo largo del primer año de vida de terneros cruza hijos de madres puras (A. Angus y Hereford) o cruzas recíprocas (PUF1 vs. CRRC) pastoreando alta o baja oferta de forraje de campo natural (AOF vs. BOF) durante la gestación y lactancia.

El segundo artículo, titulado "**Calf fetal and early life nutrition: muscle fiber characteristics and gene expression during the growing phase"**, *fue enviado* a la revista *Livestock Science* y actualmente se encuentra en revisión. En el mismo se presentan los resultados de número y tamaño de las fibras y expresión génica (genes candidatos para crecimiento/diferenciación de la fibra muscular, adipogénesis y de las células satélite) del músculo *Semitendinoso* al nacimiento, destete y al año de vida de terneros cruza hijos de madres puras (A. Angus y Hereford) o cruzas recíprocas (PUF1 vs. CRRC) pastoreando alta o baja oferta de forraje de campo natural (AOF vs. BOF) durante la gestación y lactancia.

2. CALF FETAL AND EARLY LIFE NUTRITION ON GRAZING CONDITIONS: METABOLIC AND ENDOCRINE PROFILES AND BODY COMPOSITION DURING THE GROWING PHASE

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Summary

The aim of this study was to determine the effect of nutrition during fetal and lactation periods on calf growth and body composition, and their association with changes in metabolic and endocrine profiles during the calf first year of life on purebred (Hereford and Angus) and crossbred (F1) dam offspring. Forty crossbred calves and their dams (purebred-PU: Hereford and Angus, and crossbred-CR: F1) were used in a randomized block design with a factorial arrangement of herbage allowance of native pastures (High: Hi-HA and Low; Lo-HA, 4 vs. 2.5 kg dry matter/kg body weight (BW) and dam genotype (PU vs. CR) Calf BW and blood samples were collected monthly from birth to 380 ± 15 days of age and body composition was estimated by the urea dilution technique at weaning (142 ± 15 days) and 380 days. Calf birth weight did not differ among groups but from birth to 380 days and BW was reduced (P= 0.046) in Lo-PU offspring. Although Lo-CR calves achieved similar BW than Hi-PU and Hi-CR offspring, they showed an increased fat in detriment of lean tissue deposition. At birth, plasma total protein was less (P=0.04) while plasma glucose, insulin or IGF1 tended or were greater (P<0.072) in Hi-HA than Lo-HA calves. Greater (P<0.03) plasma total protein

and/or glucose concentrations during the first months of lactation were observed in CR offspring associated to the greater dam milk production. Although glucose concentrations did not differ among calf groups after weaning, plasma insulin was greater (P=0.004) in Hi-PU than other groups at 380 days. Consistent with the reduced BW, Lo-PU offspring presented the lowest (P=0.026) plasma IGF1 from birth to 380 days. Herbage allowance of native grasslands during calf fetal and lactation periods interacted with maternal heterosis to affect, in the short and/or long-term, calf BW or body composition, and metabolic and endocrine profiles.
Keywords: cattle, pasture, calf development

Introduction

Nutrition at critical developmental windows, both during the pre- and early post-natal periods, may alter growth, body composition, and metabolic function of the livestock offspring (Wu et al., 2006; Funston et al., 2010; Bach, 2011). Maternal undernutrition during gestation may alter fetal growth and development by moderating fetal substrate delivery through the placenta (Funston et al., 2010), which may or may not affect calf birth weight (Holland and Odde, 1992; Greenwood and Cafe, 2007). In addition, independent of changes in body weight (BW) at birth, maternal undernutrition could have long-term metabolic consequences, as altered glucose-insulin-insulin-like growth factor-I (IGF1) metabolism (Ford et al., 2007), that would impact on the offspring performance (i.e. increased adiposity, reduced muscle growth, reduced meat quality and reduced reproductive performance; Wu et al., 2006; Ford et al., 2007; Martin et al., 2007). In addition, dam genotype and/or the interaction between maternal and fetal genotype, can affect placental growth, vascularity, and function (Ferrell, 1991; Funston et al., 2010), impacting on the offspring growth (Ferrell, 1991; Greenwood and Cafe, 2007).

During the first months of age, milk is the main source of calf nutrition, which determines that quantity and quality of the milk produced by the dams would have important influence on calf growth (Jenkins and Ferrel, 1992). Moreover, greater dam milk production and milk feeding levels increased calf average daily gain

(ADG) and BW at weaning and slaughter, and may alter nutrient partitioning, modifying calf body composition (increased lean to fat tissue ratio) and organ size (Fiss and Wilton, 1993; Kamiya et al., 2009). Milk production is modified, among other factors, by nutrition during pregnancy and lactation (Jenkins and Ferrel, 1992; Quintans et al., 2010). Undernourished ewes during pregnancy showed a reduction in mammary gland weight and therefore, in milk production during the lactation period (Funston et al., 2010). Both, increased prepartum (Quintans et al., 2010; Martin et al., 2007), and postpartum (Astessiano et al., 2011) nutrition have been reported to increase milk production, milk energy output or calf ADG in beef cows in rangelands conditions. Dam genotype also affected milk yield and composition (Notter et al., 1978; Jenkins and Ferrel, 1992; Freetly and Cundiff, 1998). Notter et al. (1978) reported that crossbred F1 Angus x Hereford dams produced more milk than Angus or Hereford dams.

In the last years there has been an increased interest in the effects of nutrition during pre- and early post-natal life on producing animals (Funston et al., 2010; Bach, 2011). Particularly, nutrient and energy intake of beef cows in rangeland conditions depends on quantity and quality of herbage produced by native pastures, which are subjected to large intra and inter-annual climate variations, determining that cows experience poor nutritional environments during variable time periods during pregnancy and lactation. Our hypothesis was that an increased herbage allowance of native pastures, and therefore herbage mass and height, during the gestation and lactation periods, would increase calf BW and ADG, and lean to fat tissue ratio. In addition, this effect of nutrition could interact with maternal heterosis. Therefore, the objective of this study was to evaluate the effects of herbage allowance of native pastures from conception to weaning on calf growth, metabolite and endocrine profiles, and body composition during the calf first year on purebred (Hereford and Angus) and crossbred (F1) dam offspring.

Materials and methods

Location, animals and experimental design

The experiment was conducted on 90 ha of native grasslands (Campos biome) located at the Prof. Bernardo Rosengurtt Experimental Station (School of Agronomy, Universidad de la República, Uruguay; 32ºS, 54ºW) from December 2008 to November 2010. Native pastures were dominated by summer-growing C4 grasses, with few C3 grasses associated with the winter cycle, being the main species of genus *Paspalum, Coelorachis, Piptochetium, Andropogon, Cynodon* and *Bothriochloa*. Animal procedures were approved by the Animal Experimentation Committee of Universidad de la República (CHEA, Uruguay).

Forty calves and their dams were used in a randomized block design with two replications (block 1: sandy loam soil, 60 ha and block 2: clay loam soil, 35 ha) and a factorial arrangement of herbage allowance and dam genotype. Herbage allowance (HA) treatments were estimated according to Sollenberger et al. (2005) and represented 4 and 2.5 kg dry matter (DM)/ kg BW of annual mean (Hi-HA: high herbage allowance and Lo-HA: Low herbage allowance, respectively) that varied among seasons (Table 1). Herbage allowance was adjusted monthly after measuring the herbage mass available in each plot (Haydock and Shaw, 1975) by the put-and-take method (Mott, 1960). Experimental cows (calf dams) were maintained in the plot throughout the experiment, and "put and take" cows of similar genotype and physiological status than experimental ones, were added or removed based on herbage mass available. Herbage allowance treatments determined changes of herbage mass and height (Table 1). Chemical composition throughout the year averaged 100 ± 16 and 746 ± 37 g/kg of crude protein (CP) and neutral detergent fiber (NDF) for Hi-HA and 112 ± 22 and 722 ± 30 g/kg of CP and NDF for Lo-HA. Herbage allowance treatments represented 90, 100 and 110%, and 80, 90 and 90% of estimated NRC daily requirements (NRC, 2000) for second and third trimester of gestation and lactation, for Hi-HA and Lo-HA, respectively.

Experimental dams were purebred (Hereford, n=13 and Angus, n=10; n=5 to 7 per plot; PU) or crossbred (F1-HxA, n=9 and F1-AxH, n=10; n=4 to 5 per plot; CR)

multiparous cows (5 to 6 year-old) that belonged to a group of experimental animals generated as part of a diallel crossbreeding experiment between Angus and Hereford breeds conducted for 10 years at the Experimental Station, which design was based in the optimized crossbreeding scheme proposed by Sölkner (1993). These dam biological types were characterized (averages of 10-year evaluation) by differences in heifer BW at first service (267b, 276ab, 285a, 287a ± 9.8 kg for HH, AA, AxH, HxA, respectively) without differences in cow BCS at calving (3.6 ± 0.01 units) and calf birth weight (32.1 ± 3.6 kg) but greater BW at weaning in F1 than HH and AA offspring (132c, 156b, 167a, 169a ± 9.8 kg for HH, AA, AxH, HxA, respectively). Calf sires were Hereford or Angus, determining that calves from PU dams were crossbred (F1: HxA and AxH) while calves from CR dams were backcross (BC: H-HxA, H-AxH, A-HxA, and A-AxH) progeny (Table 2). Dams were maintained in the same plot (same herbage allowance treatment) since May 2007 and gestated and lactated one calf every year from 2007 to 2009. The present study included calves that were born during the spring calving season of 2009 (October to November). Thus, calves were subjected to the effect of herbage allowance treatments from conception (breeding season 2009-2010) to weaning (April 2010; 142 ± 15 days) and no further treatments were applied. Ten calves (n = 5 for males and females) were evaluated per treatment (Hi-PUF1, Lo-PUF1, Hi-CRBC, and Lo-CRBC).

Calves were weaned at 142 ± 15 days by definitive separation of their dams. All calves (males and females) were put together in a native pasture paddock and supplemented with 1% of BW (approximately 1.2 kgDM) of a commercial concentrate (150 g/kgDM of CP, 11.7 MJ/kgDM of metabolizable energy, 20 g/kgDM of ether extract) until they reached 150 ± 15 kg BW (approximately 210 days) and no further supplement was used. Male calves were castrated one week after weaning (149 ± 15 days). After weaning calves were managed as a contemporary group grazing on a native pasture (102 ha, 1596 ± 185, 1072 ± 114, and 1606 ± 74 kgDM/ha of estimated herbage mass for fall, winter and spring, respectively) with good access to water.

Table 1. Herbage allowances, and forage mass and height, and cow physiological status through the year.

		Fall	Winter	Spring	Summer
Herbage allowance (kg DM/kgBW)					
	Hi-HA[1]	5	3	4	4
	Lo-HA	3	3	2	2
Herbage mass (kg DM/ha)[2]					
	Hi-HA	1592 ± 189 bc	821 ± 189 de	1695 ± 189 bc	3547 ± 189 a
	Lo-HA	928 ± 189 cde	476 ± 189 e	1098 ± 189 cde	2514 ± 189 b
Height (cm)[2]					
	Hi-HA	2.9 ± 0.4 cd	2.4 ± 0.4 de	3.4 ± 0.4 c	11.0 ± 0.39 a
	Lo-HA	1.5 ± 0.4 e	1.6 ± 0.4 e	2.7 ± 0.4 de	8.0 ± 0.39 b
Days of gestation		60 to 150	150 to 240	240 to 282	
Days of lactation				0 to 50	50 to 142

[1]Hi-HA = High herbage allowance treatment; Lo-HA= Low herbage allowance treatment. [2]lsmeans ± s.e.
[a,b] Means with different literals differed with $p<0.05$

Table 2. Schematic representation of calf genotypes included in the study

Genotypes[1]		
Dams	Sires	Calves
Purebred		
HH	AA	AxH
AA	HH	HxA
Crossbred, F1		
HxA	AA	A-HxA
HxA	HxA	H-HxA
AxH	AA	A-AxH
AxH	HxA	H-HxA

[1]H= Hereford, A=Angus

Data and Sample Collection

Dam milk yield and milk composition were individually measured at 15 days postpartum and from 30 days to weaning (142 ± 15 days) in 30 d-intervals by machine-milking according to Quintans et al. (2010). Milk samples were collected for protein, fat and lactose determinations.

Calves were weighted and blood samples were collected in 30-d intervals from birth (6 to 12 h after birth, after first colostrum intake) to 380 ± 15 days. Blood samples were obtained by jugular venipuncture using tubes with sodium fluoride and potassium oxalate (Vacutest®; Arzergrande, Italy) to extract plasma by centrifugation (2,000 Xg, 15 min). Plasma was stored at -20ºC for metabolite and hormone analyses. Subcutaneous fat thickness (SFT) was measured every 30 days from weaning (142 ± 15 days) to 380 ± 15 days by ultrasound (Ambivision Digital AV-3018V Notebook, AMBISEA Technology Corp., Ltd., China; bimodal probe of 5.0 and 7.5 MHf) according to Schröder and Staufenbiel (2006).

Body composition at weaning (142 ± 15 days) and 380 ± 15 days was estimated by the urea dilution technique (Wells and Preston 1998). Briefly, calves were infused with 0.75 mL/kg BW of a urea solution (20% urea in 0.9% saline solution, wt/vol.) by jugular venipuncture and blood was collected in tubes with heparin (Vacutest®) before and 12 min after the mean infusion time. Samples were centrifuged (2,000 Xg, 15 min), and plasma was harvested. The plasma was stored at -20 C in order to determine the difference in plasma urea-nitrogen between blood samples (ΔU) and to calculate urea space (US %). Percentages of empty body water (EBW %) and fat (EBF %), and carcass water (CW %), fat (CF %) and protein (CP %) were estimated by the following multiple regressions described by Rule et al. (1986) using US% and BW as predictors:

Urea space (US%) = (urea volume x urea concentration)/ΔU x BW x 10

% Empty Body Water (EBW%) = 59.1 + 0.22 x US% - 0.04 x BW

% Empty Body Fat (EBF%) = 19.5 – 0.31 x US% + 0.05 x BW

% Carcass Water (CW%) = 59 + 0.18 x US% - 0.04 x BW

% Carcass fat (CF%) = 21- 0.32 x US% + 0.05 x BW

$$\% \text{ Carcass Protein (CP\%)} = 16.7 + 0.07 \times \text{US\%} - 0.01 \times \text{BW}$$

where urea volume is in mL units of urea injected per animal, urea concentration in mg/mL, and ΔU is the difference between urea in blood post and pre urea-infusion in mg/100mL.

Metabolite and hormone analyses

Metabolite concentrations were determined by spectrophotometry with commercial kits (glucose: Oxidase/Peroxidase, total protein: Biuret, albumin: Bromocresol green, UREA/BUN-Color: Urease/Salicilate; BioSystems S.A., Barcelona, Spain, respectively) according to Astessiano et al. (2011). All samples were determined in a duplicate assay for each metabolite. The intra-assay and inter-assay CV for low and high controls were less than 9 and 17%, respectively. Concentrations of insulin were measured using a bovine immunoradiometric assay (IRMA; INS-IRMA; DIA Source ImmunoAssays S.A., Belgium) and concentrations of IGF1 were determined with an IRMA (IGF1-RIACT Cis Bio International, GIF-SUR-YVETTE CEDEX, France) previously used in ruminants (Adrien et al., 2012). All samples were determined in a single assay for each hormone. For insulin, the assay detection limit was 2.2 µIU/mL, and intra-assay CV for control 1 (22.3 µIU/mL) and 2 (55.5 µIU/mL) were 7.4 and 9.7%, respectively. For IGF1, the assay detection limit was 0.9 ng/mL, and intra-assay CV for control 1 (41.1 ng/mL) and control 2 (521.5 ng/mL) were 12.9 and 11.3%, respectively.

Statistical analyses

Data were analyzed using the SAS Systems programs (SAS 9.0V; SAS Institute Inc., Cary, NC, USA). Univariate analyses were performed on all variables to identify outliers and inconsistencies and to verify normality of residuals. Milk yield and composition, calf BW and body composition, and metabolite and hormone concentrations were analyzed with a mixed model with repeated measures using the MIXED procedure. The model included herbage allowance treatment, dam genotype, calf age (repeated measure), their interactions, and calf sex as fixed

effects, block, sire genotype, and calf as random effects, and birth date as a covariable. The original model also included the interaction between herbage allowance treatment and block but as it was not significant ($P > 0.20$) it was removed, and therefore, calf was used as the experimental unit. The appropriate covariance structure (unstructured (UN), compound symmetry (CS), or autoregressive of first order (AR (1)) and the Kenward-Rogers procedure to adjust the degrees of freedom of denominator were specified. Mean separation was performed using the Tukey test, and differences were considered significant at $P \leq 0.05$ and trend when $0.05 < P \leq 0.10$. Pearson correlation coefficients to describe relationships between variables were estimated using the CORR procedure. Results were presented as least square means ± pooled standard error.

Results

Cow BW, BCS, milk yield and composition

Cow BW during the period evaluated (-165 to +142 days postpartum) was greater ($P \leq 0.043$) for Hi-HA than Lo-HA (449 vs. 408 ± 11 kg) and for CR than PU (442 vs. 415 ± 11 kg) dams. Cow BCS during gestation (4.4 vs. 3.8 ± 0.1 units) and lactation (4.1 vs. 3.6 ± 0.1 units) was greater in Hi-HA than Lo-HA cows but did not differ between dam genotypes. Cow BCS decreased ($P< 0.001$) 0.5 units during gestation, reached nadir at calving, and remained low stable thereafter until 142 days.

Milk yield decreased ($P < 0.001$) from 7.2 to 4.5 ± 0.3 L/day from 15 to 90 days of lactation and remained stable thereafter until weaning (142 days). Milk yield during lactation was greater ($P \leq 0.028$) for Hi-HA than Lo-HA and for CR compared to PU dams), being milk yield lower ($P < 0.05$) for Lo-PU dams than other dam groups (Table 3). Milk composition did not differ due to herbage allowance treatments or

Table3. Effect of herbage allowance (HA) and dam genotype (DG) on milk yield and composition

	Cows					p-value[1]		
	Hi-PU[2]	Lo-PU	Hi-CR	Lo-CR	SE	HA	DG	HAxDG
Milk yield (kg/d)[2]	5.7ab	4.3b	6.3a	6.0a	0.4	0.028	0.003	0.096
Fat (%)	3.03	2.82	3.16	2.9	0.17	0.176	0.534	0.89
Protein (%)	2.97	2.88	2.87	2.93	0.06	0.816	0.678	0.179
Lactose (%)	4.99	4.94	4.93	4.95	0.05	0.771	0.562	0.404
Fat (kg/d)	0.17	0.13	0.21	0.16	0.02	0.029	0.11	0.946
Protein (kg/d)	0.16	0.13	0.19	0.16	0.01	0.043	0.037	0.95
Lactose (kg/d)	0.27	0.22	0.32	0.27	0.02	0.039	0.049	0.887
Milk energy output (MJ/d)	11.2	9.1	13.5	11.1	1	0.039	0.046	0.941

[1] For all variables, day effect $p < 0.001$, HA: high and low: 4 and 2.5 kg DM/kg BW in average, respectively), DG: purebred: Hereford and Angus vs. F1-crossbred; PU vs. CR.

[a,b] Means with different literals differed with $p<0.05$

dam genotypes, however milk fat, protein and lactose yields were greater ($P \leq 0.043$) for Hi-HA than Lo-HA dams (Table 3) and protein and lactose yields were greater ($P \leq 0.049$) observed for CR than PU dams. Milk energy output decreased ($P < 0.001$) from 15 days to weaning (from 14.6 to 8.6 ± 0.89 MJ/d), was greater ($P \leq 0.046$) for Hi-HA than Lo-HA, and for CR than PU dams (Table 3).

Calf growth

Calf birth weight was not affected by herbage allowance treatment, dam genotype, or their interaction but males were heavier ($P = 0.001$) than females (42.3 vs. 37.2 ± 2.6 kg) calves. Body weight showed a linear increase ($P = 0.001$) from birth to weaning (142 days), did not change ($P > 0.13$) from weaning to 300 days (end of winter to beginning of spring), and increased ($P = 0.001$) thereafter until the end of the experiment (380 days, end of spring) (Figure 1). At weaning, calf BW tended ($P \leq 0.061$) to be greater for Hi-HA than Lo-HA and was greater ($P \leq 0.030$) for CR than PU offspring and for males than females (143.5 *vs.* 127.3 ± 16.3 kg). These differences of BW at weaning were associated with greater ($P \leq 0.028$) ADG from birth to weaning for Hi-HA than Lo-HA (0.795 *vs.* 0.720 ± 0.024 kg/d), for CRBC than PUF1 (0.793 *vs.* 0.723 ± 0.023 kg/d), and for males than females offspring (0.802 *vs.* 0.713 ± 0.024 kg/d). Calf BW from weaning to 380 days tended ($P \leq 0.095$) to be greater for Hi-HA than Lo-HA and for CRBC than PUF1 offspring. However, there was an interaction ($P = 0.046$) between herbage allowance treatment and dam genotype on calf BW from birth to 380 days as it was less for Lo-PUF1 than Hi-PUF1, Hi-CRBC and Lo-CRBC calves (Figure 1).

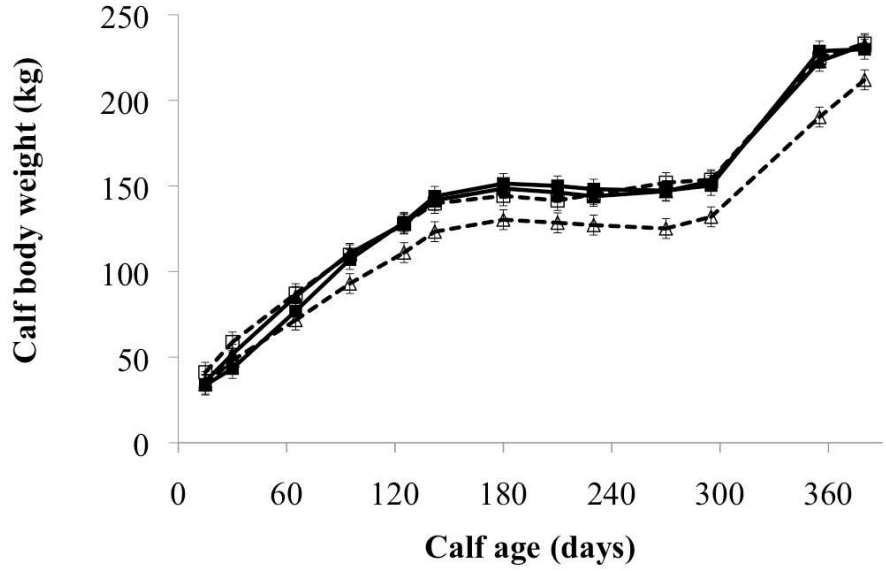

Figure 1. Body weight (BW) during the first year of age of calves from purebred (PUF1 Hereford and Angus; triangles) and crossbred (CREC; squares) dams grazing high (solid symbols and lines) and low (open symbols and dashed lines) herbage allowances (4 and 2.5 kg dry matter (DM)/kg BW of annual mean, respectively) during gestation and lactation (n = 10 calves per group). Data are least squares means and SEM.

Calf ADG was correlated with milk yield during the first three months of lactation (r = 0.44 to 0.66 for 1st and 3rd month; $P \leq 0.05$), with fat yield during the first four months of lactation (r = 0.50 to 0.70 for 1st and 4th month; $P < 0.001$), and with protein and lactose yields during the first two months of lactation (r = 0.68 to 0.71; $P < 0.001$).

Metabolites and hormones

Plasma total protein concentrations from birth to 380 days were not affected by herbage allowance treatment, dam genotype, or their interaction (Figure 2A). However, plasma total protein was affected ($P \leq 0.005$) by calf age and by the interaction between herbage allowance and calf age, and tended ($P = 0.09$) to be affected by the interaction between dam genotype and calf age. Concentrations of total protein decreased from 30 to 142 days, remained low until 270 days, and increased thereafter until 380 days. Plasma total protein was greater ($P = 0.04$) at birth for Lo-HA than Hi-HA calves but did not differ between herbage allowance

treatments thereafter. Concentrations of total protein were greater ($P = 0.03$) during the first 60 days for CRBC than PUF1 offspring.

Concentrations of albumin from birth to 380 days were not affected by herbage allowance treatment, dam genotype, or their interaction but varied ($P = 0.001$) with calf age. Plasma albumin increased from birth to 30 days, remained high until 142 days, decreased from 142 to 180 days, to remain low thereafter until 380 days (data not shown).

Plasma glucose concentrations from birth to 380 days were not affected by herbage allowance treatment, dam genotype, or their interaction. However, plasma glucose was affected ($P = 0.001$) by calf age and tended ($P = 0.055$) to be affected by the interaction between dam genotype and calf age (Figure 2B). Concentrations of glucose were elevated during the first 120 days, decreased from 120 to 180 days, remained low until 270 days, and increased thereafter until 380 days. Plasma glucose tended to be greater ($P \leq 0.072$) for Hi-HA than Lo-HA offspring at birth. But, during the first three months of lactation glucose concentrations tended to be greater or were greater for CRBC than PUF1 offspring due to less ($P \leq 0.019$) plasma glucose in Lo-PUF1 compared to other calf groups.

Concentrations of plasma insulin from birth to 380 days were not affected by herbage allowance treatment, dam genotype, or their interaction. However, there was an effect ($P < 0.022$) of calf age and of the interactions ($P \leq 0.055$) between herbage allowance and calf age, dam genotype and calf age, and among herbage allowance, dam genotype and calf age on plasma insulin. Plasma insulin decreased from birth to 60 days, remained stable from 60 to 240 days, and increased at 380 days (Figure 2C). Concentrations of insulin were only greater for Hi-HA than Lo-HA at 380 days, and tended ($P = 0.057$) to be less at birth and were less ($P = 0.059$) at 380 days for CRBC than PUF1 offspring, without other differences during the period evaluated. These differences between calf groups at birth and 380 days were associated to reduced ($P = 0.016$) plasma insulin at birth for Hi-CRBC than other groups and increased ($P = 0.004$) plasma insulin at 380 days for Hi-PUF1 than other groups.

Plasma IGF1 concentrations from birth to 380 days were greater (P = 0.019) in Hi-HA than Lo-HA calves (120.8 *vs.* 97.0± 6.8 ng/ml) but were affected by the interaction between herbage allowance treatment and dam genotype as plasma IGF1 was less (P = 0.026) for Lo-PUF1 than the other groups. Plasma IGF1 was affected (P = 0.005) by calf age as plasma IGF1 decreased from birth to 60 days, remained low from 60 to 240 days, and increased at 380 days (Figure 2D). However, concentrations of IGF1 tended (P = 0.06) to be affected by the interaction between herbage allowance treatment and calf age as they were greater for Hi-HA than Lo-HA except at 240 days. Plasma insulin and IGF1 concentrations were correlated (r = 0.40, P < 0.001) and both were correlated with glucose (r > 0.26, P < 0.001).

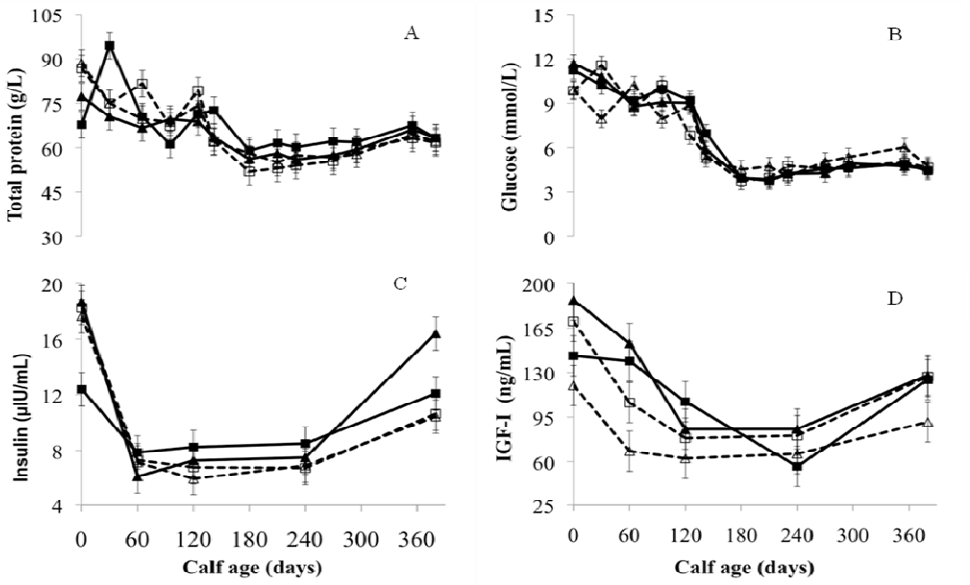

Figure 2. Plasma total protein (A), glucose (B), insulin (C), and insulin-like growth factor-I (IGF1, D) during the first year of age of calves from purebred (PUF1: Hereford and Aberdeen Angus; triangles) and crossbred (CRBC; squares) dams grazing high (solid symbols and lines) and low (open symbols and dashed lines) herbage allowances (4 and 2.5 kg dry matter (DM)/kg BW of annual mean, respectively) during gestation and lactation (n = 10 calves per group). Data are least squares means and SEM.

Subcutaneous fat thickness and estimated body composition

Calf SFT tended (P = 0.084) to be greater for Hi-HA than Lo-HA (14.9 *vs*. 14.2 ± 0.3 mm) and for CRBC than PUF1 (14.9 *vs*. 14.2 ± 0.3 mm) offspring. However, there was an interaction between herbage allowance treatment and dam genotype on calf SFT as it was less for Lo-PUF1 than the other groups (15.1, 14.7, 13.3, and 15.1

± 0.3 mm for Hi-PUF1, Hi-CRBC, Lo-PUF1, and Lo-CRBC, respectively). In addition, calf SFT was less (P = 0.017) in males than females (14.0 *vs*. 15.0 ± 0.3 mm). Calf SFT was affected (P < 0.001) by age as did not change from weaning to 300 days (on average 13.4 ± 0.3 mm) and increased thereafter until 380 days (up to 16.5 ± 0.4 mm).

Estimated EBW%, CW%, and CP% decreased (P < 0.001) from weaning (142 days) to 380 days (from 64.5 to 58.8 ± 0.7%, from 66.9 to 60.8 ± 0.8%, and from 19.6 to 17.9 ± 0.3%, respectively). In contrast, estimated EBF% and CF% increased (P = 0.001) during the same period of time (from 7.6 to 15.7 ± 1.1% and from 8.5 to 16.5 ± 1.2%, respectively). Estimated EBW% and CP% tended (P ≤ 0.097) to be greater for Hi-HA than Lo-HA offspring and this was more marked in CRBC than PUF1 offspring (Table 4). Estimated EBF% and CF% tended (P ≤ 0.089) to be less in Hi-HA than Lo-HA calves and this was more evident for CRBC than PUF1 offspring (Table 4).

Table 4. Effects of herbage allowance during the calf fetal and lactation periods and dam genotype on estimated body composition

	Calves					p-value[1]			
	Hi-PUF1[2]	Lo-PUF1	Hi-CRBC	Lo-CRBC	SE	HA	DG	HA×DG	Calfage
EBW%2	64.1 ab	63.8ab	65.8a	61.9b	1.7	0.097	0.369	0.14	<0.001
EBF%	11.4 ab	11.9ab	8.9b	14.4a	1.2	0.089	0.38	0.146	<0.001
CP%	18.8 ab	18.6ab	19.3a	18.1b	0.4	0.083	0.39	0.151	<0.001
CW%	61.8 ab	61.7ab	63.1a	60.0b	1	0.113	0.35	0.131	<0.001
CF%	11.9 ab	12.9ab	9.6b	15.5a	1.7	0.06	0.285	0.167	<0.001

[1]HA: Herbage allowance treatments (high and low: 4 and 2.5 kg DM/kg BW in average, respectively; Hi-HA vs. Lo-HA). DG: dam genotype (purebred: Hereford and Angus vs. F1-crossbred; PU vs. CR). 2Hi-PUF1 and Lo-PUF1 = F1 calves from PU dams grazing Hi-HA and Lo-HA, respectively; Hi-CRBC and Lo-CRBC = backcross calves from CR dams grazing Hi-HA and Lo-HA, respectively.

[2]Estimated body composition (expressed as percentage body weight) by urea dilution technique (Wells and Preston, 1998); EBW%: empty body water. EBF%: empty body fat. CP%: carcass protein. CW%: carcass water. CF%: carcass fat

a,b Means with different literals differed with p<0.05.

Discussion

Herbage allowance of native grasslands during calf fetal and lactation periods interacted with maternal heterosis to affect calf BW or body composition, and metabolic and endocrine profiles.

Neonatal period

Maternal nutrition during gestation has been associated or not to changes in calf birth weight (Holland and Odde, 1992; Greenwood and Cafe, 2007). In our study, calf birth weight was not affected by the degree of maternal undernutrition during gestation in agreement with Rasby et al. (1990) that suggested that major reductions in dam nutrient intake are needed to affect calf birth weight. In the present study, cows were in the same nutritional treatment for two years previous to the study; therefore, they could have adapted their metabolism to the nutritional input in order to supply enough nutrients for the developing fetus. Similar to previous reports (Freetly and Cundiff, 1998), calf birth weight was not affected by dam genotype when purebred (Angus and Hereford) and the reciprocal F1 dams were compared.

Although there were not differences in calf birth weight between treatments, dam nutrition during gestation and/or dam genotype affected plasma total protein, glucose, insulin, and IGF1 at birth. Plasma total protein concentrations at birth were greater in Lo-HA than Hi-HA offspring. Hammer et al. (2007) showed that undernutrition during gestation lead to an increase in calf serum total protein and immunoglobulin (IgG) concentrations during the first 24 h, as offspring of undernourished dams presented an increased intestine IgG transference during this period of time. This would suggest that their fetal gastrointestinal system may be programmed to be more efficient in extracting nutrients, specifically large molecules, in postnatal period (Funston et al., 2010).

In contrast, Hi-HA calves had greater glucose, insulin, and IGF1 concentrations at birth than Lo-HA calves. Bell (1995) reported that energy-deprived ewes are susceptible to hypoglycemia during pregnancy which leads to reductions in uterine and fetal uptake of glucose. Moreover, fetal hypoglycemia develops to help to sustain

the maternal-fetal gradient in glucose concentration by restricting the reverse transfer of glucose to the placenta, and reducing placental glucose consumption (Hay, 1995). In agreement with this, plasma insulin concentrations were greater in Hi-HA than Lo-HA dams during gestation (Laporta, J., unpublished data), which were in turn associated to the greater plasma glucose at birth in Hi-HA than Lo-HA offspring reported here. Plasma insulin was also greater in Hi-HA than Lo-HA offspring, and this increased insulin in calves better nourished is consistent with central role of this hormone in glucose homeostasis. However, Hi-CRBC offspring showed reduced plasma insulin concentrations at birth when compared with the other calf groups, indicating that maternal nutrition during gestation could interact with maternal heterosis on fetal glucose-insulin metabolism.

In addition, the maternal IGF system can modulate the delivery of substrates to the fetus, particularly influencing glucose and amino acid transference across the placenta to the fetus (Kniss et al., 1994). Serum IGF1 concentrations were greater in Hi-HA than Lo-HA dams (Laporta, J., unpublished data), which is consistent with the greater plasma glucose at birth in Hi-HA offspring reported here. It has been demonstrated in sheep that glucose influence fetal IGF1 concentrations through insulin-mediate effects (Oliver et al., 1993; 1996). In agreement with these results, in the present study, the greater plasma glucose and insulin in Hi-HA calves were associated with greater IGF1 concentrations at birth. In contrast, Rehfeldt et al. (2004) showed that although maternal over-nutrition caused increased maternal IGF1 concentrations, it did not affect fetal IGF1 concentrations in pigs.

Post-natal pre and post-weaning periods

In agreement with previous studies (Jenkins and Ferrel, 1992; Funston et al., 2010; Quintans et al., 2010), Hi-HA dams showed greater milk and solid yields than Lo-HA dams. This greater milk production could be explained by greater mammary gland development during gestation (Funston et al., 2010), body reserves at calving (Quintans et al., 2010) and nutrient and energy intake during the lactation period (Jenkins and Ferrel, 1992). In addition, milk production is influenced by dam genotype (Jenkins and Ferrel 1992) and similar to previous reports (Notter et al.,

1978) milk yield was greater in CR than PU dams in both herbage allowances.

Milk and solid yields were associated with calf ADG during the first months of lactation consistent with the positive correlation between milk yield, and calf ADG reported for beef cattle (Totusek et al., 1973; Beal et al., 1990). Alencar (1989) reported when calves are more than 60-day old, forage intake starts to be significant in terms of energy. Thus, differences in milk and solid yields as well as herbage mass and height during lactation were reflected in differences in calf BW at weaning which was less in Lo-PUF1 calves than other groups.

After weaning, calf ADG decreased for all groups which could be associated to a short-period of stress provoked by calf-cow separation (Weary and Jasper, 2008), a period of calf adaptation to a diet composed only by forage and to the reduced herbage mass and height of native pastures as temperature decreased in winter which affects DM intake (Chapman et al., 2007). After the end of winter, at the beginning of spring (240 to 270 days), calf BW started to increase again with the increased herbage mass and height of native pastures. However, calf BW remained less for Lo-PUF1 calves than other groups, indicating a long-term effect of calf nutrition during gestation and/or lactation (Stalker, 2006; Caton and Hess, 2010).

Probably, the greater milk and solid yields of CR compared to PU dams, allowed, a period of compensatory growth (Sainz et al., 1995; Abdelsamei et al., 2005) in Lo-CRBC calves, after a moderate restriction (80% of NRC requirements in this study) during the fetal period. Compensatory growth may provoke changes in body composition. Sainz et al. (1995) reported that fat distribution is modified in compensating animal (less subcutaneous and more internal fat), and Hornick et al. (1998) demonstrated that they had greater adipose tissue contents but less meat fat. Similarly, Wu et al. (2006) indicated that fat deposition started earlier or was increased in restricted animals. These results are in agreement with our study in which Lo-CRBC calves reached similar BW at weaning and 380 days but presented more estimated EBF% and CF% and less SFT than Hi-CRBC calves. In addition, Bartlett et al. (2006) showed that pre-ruminant calves fed with increasing amounts of dietary protein had decreased fat percentage while increased protein and water

percentages. Thus, greater milk protein yields for Hi-CR dams could explain the greater estimated EBW% and CP%, which would indicate a greater lean tissue deposition of Hi-CRBC calves. However, EBW% and CP% for Hi-CR calves were neither different than for Hi-PUF1 nor Lo-PUF1 offspring which could be associated to the intermediate milk protein yields for Hi-PU dams that allowed an intermediate lean tissue deposition in Hi-PUF1 calves but probably to a delay in time to reach physiological maturity in Lo-PUF1 offspring as these calves did not showed compensatory growth.

Metabolic and endocrine profiles during the postnatal pre and post-weaning periods were associated to changes in calf BW and ADG. Thus, plasma total protein, albumin, and glucose were elevated and highly variable during the first months of lactation associated to the high efficiency of use of milk nutrients for body tissue deposition, reflected in high calf ADG, as milk nutrients are processed directly by abomasum and small intestine where they are absorbed. Greater plasma total protein and/or glucose concentrations during the first months of lactation in Hi-HA and CRBC offspring reflected the better nutritional status (Ndlovu et al., 2007) due to greater milk and solid yields of their dams and greater herbage mass and height, and were associated to the greater calf ADG and BW at weaning. Similarly, Bartlett et al. (2006) reported that calf plasma glucose concentrations and calf BW were higher as milk-feeding level increased. During winter, after weaning, when calf ADG decreased to a minimum (100 g/d or less), metabolite concentrations remained low and stable, reflecting a period of reduced nutrient intake as well as the continuous release of nutrients from ruminal microbial fermentation (Hugi and Blum, 1997). Finally, with the beginning of spring and the increase in herbage mass and height, plasma total protein, albumin, and glucose increased when calf BW started to increase again.

It has been proposed that insulin and IGF1 are two of the main endocrine factors associated to calf growth and development, and circulating concentrations of these hormones are directly related to nutritional/metabolic status (Hugi and Blum, 1997; Hyatt et al., 2007). Insulin and IGF1 profiles were highly associated with each other.

In agreement with metabolic profiles reported here, both plasma insulin and IGF1 were elevated early in lactation, decreased around weaning and during the post-weaning period in winter, and increased again with the beginning of the spring, reflecting probably changes in daily energy and protein intake as reported previously (Kamiya et al., 2009; Bartlett et al., 2006; Abdelsamei et al., 2005).

Although glucose concentrations did not differ among calf groups after weaning, plasma insulin were greater in Hi-PUF1 than other groups at 380 days which could suggest some degree of insulin resistance as plasma insulin is elevated in order to compensate the lack of tissue response to its action to increase the use of glucose by tissues (Ozzane et al., 2003). Plasma IGF1 concentrations were greater in Hi-HA calves, not only during lactation, when probably nutrient intake was greater in these calves associated with greater dam milk and solid production, but also at 380 days when all calves were in the same nutritional plane. Differences in calf individual feed intake (not measured in the study) when calves were in the same nutritional plane could have contributed to changes in endocrine profiles also after weaning. Muscle and protein deposition is stimulated by IGF1 (Nkrumah et al., 2007), which is consistent with the greater estimated CP% in Hi-HA calves. In agreement with the lowest calf BW, and consistent with the role of IGF1 in growth and development, Lo-PUF1 offspring presented the lowest plasma IGF1 from birth to 380 days. These effects on insulin and IGF1 profiles, that remained after weaning when all calves were in the same nutritional plane, would indicate a mid or long-term effect of calf nutrition during fetal and lactation periods on glucose-insulin-IGF metabolism and/or feed intake.

In conclusion, although calf birth weight did not differ, postnatal calf BW was reduced in Lo-PUF1 offspring while Lo-CRBC calves increased fat in detriment of lean tissue deposition, and these changes were associated to altered glucose-insulin-IGF profiles.

Acknowledgments

The authors thank Bernardo Rosengurtt Experimental Unit staff (O. Cáceres, M. Cáceres, J. Cáceres, and V. Cal), for animal care and assistance throughout the field study and Lic. (MSc) J. Laporta and Bach. P. Machado, F. Pereyra and F. Bideau, for their help during the experiment. This study was supported by research project INIA-FPTA/2007 #262 awarded to M. Carriquiry and CSIC-Iniciación/2009 awarded to V. Gutiérrez.

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3. CALF FETAL AND EARLY LIFE NUTRITION: MUSCLE FIBER CHARACTERISTICS AND GENE EXPRESSION DURING THE GROWING PHASE

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Abstract

The aim of this study was to evaluate the effect of herbage allowance treatment of native pastures from calf conception to weaning on muscle fiber density and diameter and gene expression (IGF system and adipogenesis) during the first year of age of purebred (PU) (Hereford and Angus) and crossbred (F1) dams offspring. Forty crossbred calves offspring of purebred (PUF1) or crossbred (CRBC) dams were used in a randomized block design with a factorial arrangement of herbage allowance of native pastures (High: Hi-HA and Low; Lo-HA, 4 vs. 2.5 kg dry matter/kg body weight (BW)) and dam genotype (PU and F1) resulting in 4 calf groups (Hi-PUF1, Hi-CRBC, Lo-PUF1 and Lo-CRBC). Calf body weight (BW) were registered while blood and *Semitenidinosus* muscle samples were collected at birth, weaning and at 380 days old to measure plasma IGF concentrations and muscle expression of genes related with the insulin-growth factor (IGF) system and adipogenesis by quantitative realtime PCR. Calf BW at birth did not differ between calf groups but Lo-PUF1 calves were lighter ($P<0.05$) than the other three calf groups during the postnatal period. Lean to fat tissue ratio tended to be greater (P=0.08) in HiHA than in Lo-HA calves. Muscle fiber density did not differ among calf groups, but fiber diameter was greater in Lo-HA than Hi-HA

and in CRBC than PUF1 offspring. Plasma IGF-I concentration was lower (P<0.05) in Lo-PUF1 than in the other three calf groups. The *IGFBP5* mRNA expression was greater in Lo-CRBC as compared to the other three groups and *PPARG* mRNA expression was greater in Hi-HA than in Lo-HA and in PUF1 than in CRBC offspring at birth whereas *SREBF1* mRNA expression was greater in Hi-CRBC as compared to the other three groups at birth and at weaning. At 380 days, after winter restriction, *IGF receptor type 1 (IGF1R*) and *IGFBP5* mRNA expression were greater in Hi-PUF1 than in Hi-CRBC calves.

The environment provided by the dams during gestation and lactation is probably influenced by the nutritional plane and genotype of the cow. As a consequence, changes appeared between calf groups in calf BW, body composition, Semitendinosus muscle fiber diameter and expression of genes related with the IGF-I system and adipogenesis. The effects of nutrition during the fetal and pre-weaning periods varied according to dam genotype as well as calf age.

Keyword: muscle histology, IGF-system mRNA, cattle, grazing.

1. Introduction

Beef cows in rangeland extensive conditions experience poor nutritional environments for variable periods of time during pregnancy and lactation, as their intake depends on quantity and quality of herbage produced by native pastures, which is subjected to large intra and inter-annual climate variations. These periods of nutrient restriction (during gestation and/or lactation) can affect calf muscle growth and development as well as intramuscular fat (marbling) and compromise beef meat production (Du et al., 2010).

Muscle growth and its intrinsic properties determine, at least in part, the quantity and quality of the meat produced. Muscle mass is mainly determined by fiber number and diameter (Rehfeldt et al., 1999). In livestock, all muscle fibers are formed during the prenatal stage (early and mid-gestation), and muscular hyperplasia is completed at birth (Du et al., 2010). The postnatal skeletal muscle

development is mainly due to the increase in muscle fiber size (hypertrophy) with nuclei contribution of satellite cells (Bayol et al., 2004). In ruminants, undernutrition during early and mid fetal periods reduced fiber number whereas undernutrition during the end of fetal and during postnatal periods reduced muscle fiber diameter and may reduce calf body weight at birth (BW; Zhu et al., 2006; Greenwood et al., 1998). Similar to myogenesis, adipogenesis can be divided into preadipocyte hyperplasia and adipocyte hypertrophy which occurs by accumulation of triacylglyceride (Du et al., 2010). In ruminants adipogenesis is initiated around mid-gestation (Feve, 2005) and although adipocyte hyperplasia may occur during postnatal growth, the fetal period is a major stage for generation of intramuscular adipocytes and thereby for intramuscular fat accumulation potential later on life (Tong et al., 2008).

The somatotropic axis (ST-axis; growth hormone-insulin-like growth factor; GH-IGF) is critical in regulating growth, development and differentiation of skeletal muscle, via both mitogenic and myogenic processes and metabolic and anabolic actions (Clemmons, 1998; (Philippou et al., 2007; Duan et al., 2010). ST axis involves peptide hormones (GH, IGF-I and IGF-II), their receptors (GHR and IGF1R) and IGF binding proteins (IGFBP1 to 6), which can either potentiate or inhibit IGF action by modulation of their bioavailability to receptors (Clemmons, 1998). Recent studies have shown that many genes of the somatotropic axis were differentially expressed in animals selected for greater muscle growth and differentiation potential (Keady et al., 2011). In addition, muscle expression of several components of ST axisis modulated by pre- and postnatal nutrition in mice (Bayol et al., 2004) and cattle (Oksbjerg et al., 2004). On the other hand, adipocyte differentiation and lipid metabolism are controlled, among various factors, by the transcription factors *peroxisome proliferator activated-receptor-γ* (*PPARG*) and *sterol regulatory element-binding transcription factor 1* (*SREBF1*) through the induction of genes which are important for triglyceride uptake, synthesis and storage (Du et al., 2010; Yu et al., 2006). Nutrition in early life affected muscle expression of *PPARG* and *SREBF1* mRNA in cattle (Du et al.,

2010; Graugnard et al., 2009), which suggested different potential for intramuscular adipogenesis.

Our hypothesis was that control of grazing intensity of native pastures through changes in herbage allowance would impact on dam nutrition during gestation and lactation, thus, altering calf muscle fiber characteristics and gene expression during the growing period (pre and post-weaning). Therefore, our objective was to evaluate the effects of herbage allowance treatment of native pastures from calf conception to weaning on muscle fiber density and diameter and gene expression (IGF system and adipogenesis) during the first year of age of purebred (Hereford and Angus) and crossbred (F1 Hereford and Angus) dam offspring.

2. Materials and methods

2.1. Location, animals and experimental design

The experiment was conducted on 90 ha of native grasslands (Campos biome) located at the Prof. Bernardo Rosengurtt Experimental Station (School of Agronomy, Universidad de la República, Uruguay; 32ºS, 54ºW) from December 2008 to November 2010. Animal procedures were approved by the Animal Experimentation Committee of Universidad de la República (CHEA, Uruguay). Details of the experimental design have been reported previously (Gutiérrez et al., 2013). We determined that maternal nutrient intake (due to control of grazing intensity of native pastures through changes in herbage allowance) during gestation and lactation affected not only calf BW and body composition (Gutierrez et al., 2013) but also calf *Semitendinous* muscle fiber diameter and expression of genes related with the IGF-I system and adipogenesis. The effects of nutrition during the fetal and pre-weaning periods varied according to dam genotype as well as calf age.

Forty calves and their dams were used in a randomized block design and a factorial arrangement of herbage allowance and dam genotype. Herbage allowance treatments were estimated according to Sollenberger et al. (2005) and

represented 4 and 2.5 kg dry matter (DM)/ kg BW (Hi-HA and Lo-HA, respectively) on an annual mean basis that varied among seasons (5, 3, 4, and 4 kg DM/kg BW and 3, 3, 2, and 2 kg DM/kg BW for Hi-HA and Lo-HA in fall, winter, spring, and summer, respectively). Herbage allowance treatments determined changes of herbage mass and height (annual average 1914 kg DM/ha and 4.93 cm vs. 1254 kg DM/ha and 3.45 cm, for Hi-HA and Lo-HA, respectively). Herbage chemical composition throughout the year averaged 100 ± 16 and 746 ± 37 g/kg of crude protein (CP) and neutral detergent fiber (NDF) for Hi-HA and 112 ± 22 and 722 ± 30 g/kg of CP and NDF for Lo-HA. Herbage allowance treatments represented 90, 100 and 110% and 80, 90 and 90% of estimated NRC daily requirements (NRC, 2000) for second and third trimester of gestation and lactation, for Hi-HA and Lo-HA, respectively. Experimental dams were purebred (Hereford, n=13 and Angus, n=10; PU) or crossbred (F1-HxA, n=9 and F1-AxH, n=10; CR) multiparous cows (5 to 6 year-old) that belonged to a group of experimental animals generated as part of a diallel crossbreeding experiment between Angus and Hereford breeds conducted for 10 years at the Experimental Station. Calf sires were Hereford or Angus, determining that calves from PU dams were crossbred (PUF1: HxA and AxH offspring) while calves from CR dams were backcross (CRBC: H-HxA, H-AxH, A-HxA and A-AH) progeny. Dams were maintained in the same plot (same herbage allowance treatment) since May 2007 and gestated and lactated one calf every year from 2007 to 2009. The present study included calves that were born during the 2009 spring calving season (October to November). Thus, calves were subjected to the effect of herbage allowance treatments from conception (breeding season 2009-2010) to weaning (April 2010; 142 ± 15 days) and no further treatments were applied. Ten calves (n = 5 for males and females) were evaluated per treatment (Hi-PUF1, Lo-PUF1, Hi-CRBC, and Lo-CRBC). Calves were weaned at 142 ± 15 days of age by definitive separation from their dams. All calves (males and females) were put together in a native pasture paddock and supplemented with 1% of BW (approximately 1.2 kg DM) of a commercial concentrate (150 g/kg DM of CP,

11.7 MJ/kg DM of metabolizable energy, 20 g/kg DM of ether extract) until they reached 150 ± 15 kg BW (approximately 210 days) and no further supplement was used. Male calves were castrated one week after weaning (149 ± 15 days). After weaning, calves were managed as a contemporary group grazing on a native pasture (102 ha, 1596 ± 185, 1072 ± 114, and 1606 ± 74 kg DM/ha of estimated herbage mass for fall, winter and spring, respectively) with good access to water.

At birth (first 72 h), weaning and 380 ± 15 days of age, calf BW was recorded and blood and muscle samples were collected. Blood samples were obtained by jugular venipuncture using tubes containing sodium fluoride and potassium oxalate (Vacutest®; Arzergrande, Italy) and plasma was extracted by centrifugation (2,000 Xg, 15 min). Plasma was stored at -20ºC for IGF1 analysis according to Gutierrez et al. (2013). Muscle samples were obtained from the center of the left *Semitendinosus* muscle through a 3-cm skin incision after local anesthesia (5 mL, 2% Lidocaine). Muscle samples (1 to 2 g, 2 cm^3) were removed using a scalpel and divided in two to be conserved in 10 mL of 4 % paraformaldehide in 0.1 M phosphate buffer for histological analyses or immediately snap-frozen using liquid nitrogen to be stored at −80°C until RNA extraction. At weaning and 380 days of age, lean to fat tissue ratio was estimated using the urea dilution technique (Wells and Preston, 1998) as described by Gutiérrez et al. (2013).

2.2.Histological analyses

Histological analyses were performed in muscle samples at birth and weaning (142 days). Muscle samples from the mid-belly muscular region were paraffin-embedded, sectioned (10 μm thick; model 2030 Reichert-Jung, Germany) and stained with hematoxylin-eosin to evaluate muscle morphology (Bayol et al., 2004). Muscle images (16 images/sample; 4 areas in 4 sections) were captured using an optic microscope (Olympus BX50, Olympus, Tokyo, Japan) equipped with an INFINITY1-3c camera (3.1 Megapixel Color CMOS Camera, y LuSDK:

Software Developer's Kit, Lumenera Corporation, Otawa, Canada) and analyzed with the INFINITY Camera Software v5.0.3 (Lumenera Corporation). Fiber density was calculated by examining a minimum of 450 muscle fibers per animal and fiber diameters were determined by averaging the measure of both the smallest and longest apparent diameters of a minimum of 300 muscle fibers per animal. This was done to minimize errors associated with any fibers that may not have been cut exactly perpendicular to fiber direction. Muscle growth rate was calculated (µm) as the increment in diameter per fiber per day from birth to weaning.

2.3.Quantitative real time PCR

Total RNA was isolated using TRIzol (Invitrogen, Life Technologies, Carlsbad, CA, USA), followed by precipitation with lithium chloride and by DNase-treatment with a DNA-Free Kit (Applied Biosystems/Ambion, Austin,TX, USA). Concentration of RNA was determined by measuring absorbance at 260nm (NanoDrop ND-1000 Spectrophotometer; Nanodrop Technnologies Inc., Wilmington, DE, USA), and purity and integrity of all RNA isolates were assessed from 260/280 and 260/230 absorbance ratios and by electrophoresis in 1% agarose gel. Isolated RNA was stored at −80°C until analyzed by quantitative real time PCR. Reverse Transcription was conducted with SuperScript®III Transcriptase (Invitrogen) using random hexamers and 1 µg of total RNA as a template. The cDNA was stored at -20ºC until use in the quantitative real time PCR.

Primers (**Supplementary table S1**) specifically designed to amplify cDNA for the target genes of interest: *GHR*, *IGF1*, *IGF1R*, *IGFBP3*, *IGFBP5*, *PPARG*, *SREBF1*, and *paired box -3 and -7* (*PAX3* and *PAX7*), and endogenous control genes: *ß-actin* (*ACTB*) *hypoxanthine phosphoribosyltransferase* (*HPRT*) were used for real time RT-PCR. Before use, primer product size (1% agarose gel separation) and sequence (Macrogen Inc., Seoul, Korea) were determined to

ensure that the primers produced the desired amplification products (data not shown). Real time PCR reactions were performed using 7.5 µL KAPA SYBR® FAST Universal 2X qPCR Master Mix (Kapa Biosystems, inc. Woburn, MA, USA), equal amounts (200 nM) of forward and reverse primers (Operon Biotechnologies GmbH; Cologne, Germany), and 3 µL diluted cDNA (1:7.5 in RNase/DNase free water) in a final volume of 15 µL. Samples were analyzed in duplicate in a 72-disk Rotor-GeneTM 6000 (Corbett Life Sciences, Sydney, Australia). Standard amplification conditions were 5 min at 95°C and 40 cycles of 10 s at 95°C, 45 s at 60°C, and 20 s at 72°C. Dissociation curves were run on all samples to detect primer dimers, contamination, or presence of other amplicons. Each disk included a pool of total RNA from muscle samples analyzed in triplicate to be used as the basis for the comparative expression results (exogenous control) and duplicate tubes of water (non-template control).

Gene expression was measured by relative quantification (Pfaffl, 2009) to the exogenous control and normalized to the mean expression of the endogenous control genes (*ACTB* and *HPRT*) corrected by amplification efficiency. Both *ACTB* and *HPRT* have been used before as an endogenous control in skeletal muscle (Feng et al., 2010) and did not vary among samples in this study. Amplification efficiency for target and endogenous control genes was estimated by linear regression of a dilution cDNA curve (n = 5 dilutions, from 100 to 6.25 ng/tube; Table 1). The intra and inter-assays CV were less than 7.6 and 12.3%, respectively.

2.4.Statistical analyses

Data were analyzed using the SAS Systems programs (SAS 9.0V; SAS Institute Inc., Cary, NC, USA, 2009??). Univariate PROC were performed on all variables to identify outliers and inconsistencies and to verify normality of residuals. Calf BW, lean to fat tissue ratio, plasma IGF-I, muscle fiber diameter and density and gene expression data were analyzed with a mixed model with repeated measures using the MIXED procedure. The model included herbage allowance, dam

genotype, calf age (repeated measure), their interactions, calf sex and birth date (covariate) as a fixed effects, and block, sire genotype, and calf ID as a random effects. The appropriate covariance structure (unstructured (UN), compound symmetry (CS), or autoregressive of first order (AR (1), according to Bayesian Information Criteria (BIC) and the Kenward-Rogers procedure to adjust the degrees of freedom of denominator were specified. Mean separation was performed using the adjusted Tukey test. Pearson correlation coefficients to describe relationships between variables were estimated using the CORR procedure. Results are presented as least square means ± pooled standard error, and are considered as significantly different if $P<0.05$ and a tendency if $0.05<P<0.10$.

3.Results

3.1.Calf BW, lean to fat tissue ratio, and muscle characteristics

Calf BW at birth did not differ among calf groups but was less ($P<0.05$) in Lo-PUF1 than other three calf groups during the postnatal period (Table 1). Lean to fat tissue ratio decreased ($P<0.01$) from 142 to 380 days and tended to be greater ($P=0.08$) in Hi-HA than Lo-HA calves (Table 1).

Semitendinosus muscle fiber diameter was duplicated ($P<0.01$) from birth to 142 days whereas muscle fiber density decreased ($P<0.01$) to one third during the same period of time (Table 1). Muscle fiber diameter was less ($P=0.02$) in Hi-HA than in Lo-HA and greater ($P=0.04$) in CRBC than in PUF1 calves (Table 1). However, muscle fiber growth rate did not differ among calf groups (Table 1).

3.2.Plasma IGF-I and Semitendinosus muscle gene expression

Plasma IGF-I concentrations decreased ($P<0.01$) from birth to 142 days and increased ($P<0.01$) from 142 to 380 days, being less ($P<0.05$) in Lo-PUF1 than in the other three calf groups (Table 2).

Table 1. Effect of herbage allowance (HA) and dam genotype (G) on calf BW, lean to fat tissue ratio and *Semitendinous* muscle fiber characteristics

	Treatments[1]						P-value[2]			
	Hi-PUF1	Lo-PUF1	Hi-CRBC	Lo-CRBC	All[3]	s.e.	HA	G	D	HAxG
Calf performance										
Calf BW (kg)							0.09	0.05	<0.01	0.04
Birth	39.6^{a}	38.2^{a}	38.6^{a}	42.8^{a}	38.4^{a}	2.7				
142 days	138.1^{c}	122.0^{d}	142.6^{c}	139.0^{c}	139.3^{c}	16.7				
380 days	229.0^{a}	206.8^{b}	228.8^{a}	232.9^{a}	228.9^{a}	8.1				
Lean:fat tissue ratio							0.08	0.71	<0.01	0.97
142 days	3.9^{a}	2.7^{b}	4.1^{a}	2.9^{b}	3.4^{a}	0.7				
380 days	2.3^{b}	1.5^{c}	1.6^{bc}	0.9^{c}	1.6^{b}	0.6				
Muscle characteristics										
Fiber diameter (µm)							0.02	0.04	<0.01	0.19
Birth	42.2^{c}	44.4^{c}	40.9^{c}	52.1^{b}	44.9^{b}	2.7				
142 days	85.3^{a}	89.8^{a}	87.5^{a}	93.5^{a}	89.1^{a}	3.0				
Fiber density (cell/mm^{2})							0.21	0.83	<0.01	0.59
Birth	0.53	0.50	0.57	0.46	0.51^{a}	0.03				
142 days	0.16	0.14	0.14	0.15	0.15^{b}	0.01				
Fiber growth rate (µm/day)	0.32	0.31	0.35	0.31	0.32	0.02	0.45	0.58	-	0.60

[1]Herbage allowance treatments: high and low: 4 and 2.5 kg DM/kg BW in average, respectively; Hi vs. Lo. G: dam genotype (purebred: Hereford and Angus vs. F1-crossbred; PU vs. CR). D: days. [2]The interactions HAxD, GxD and HAxGxD were not significant (P>0.12) for all variables. [3]Lsmeans for day effect, including all treatments. [a,b] Denote lsmeans differences by Tukey analysis.

Muscle expression of *GHR* and *IGFBP5* mRNA increased (*P*<0.01) from birth to 142 days (weaning), remaining stable thereafter at 380 days while *IGF1* and *IGFBP3* mRNA did not change from birth to weaning and increased (*P*<0.01) from weaning to 380 days and *IGF1R* mRNA increased (*P*<0.01) from birth to 380 days (Table 2). Muscle expression of *GHR* and *IGFBP3* mRNA was not affected by herbage allowance treatment, dam genotype or their interaction (Table 2). Muscle *IGF1* mRNA was not affected by herbage allowance treatment, but was less (*P*=0.03) in CRBC than PUF1 calves (Table 2). Expression of *IGF1R* mRNA tended (*P*=0.09) to be affected and *IGFBP5* mRNA was affected (*P*=0.01) by the interaction between herbage allowance treatment, dam genotype and calf age as their expression was greater (*P*<0.05) in Lo-CRBC than Lo-PUF1 calves at birth and was less (*P*<0.05) in Hi-CRBC than Hi-PUF1 calves at 380 days.

Muscle expression of *PPARG* mRNA did not change from birth to weaning, but

increased (P<0.01) at 380 days. The expression of this transcript tended (P=0.07) to be less in CRBC than PUF1 offspring, due to its reduced (P<0.05) expression in Lo-CRBC than Lo-PUF1 calves. In addition, *PPARG* mRNA expression tended (P=0.09) to be affected by the interaction between herbage allowance and calf age as it was greater in Hi-HA than Lo-HA at birth (Table 2). Expression of *SREBF1* mRNA increased (P<0.01) from birth to 380 days, was greater (P=0.05) in CRBC than PUF1 offspring and tended (P=0.07) to be affected by the interaction between herbage allowance, dam genotype and calf age as its abundance was greater in Hi-CRBC offspring than other calf groups at birth and weaning (Table 2).

Muscle *PAX7* and *PAX3* mRNA did not change from birth to weaning but increased at 380 days (P=0.01) and were not affected by herbage allowance treatment, dam genotype, or their interaction (Table 2).

Independently of calf group and age, muscle *GHR* mRNA was positive correlated (P≤0.01) with *IGF1* (r=0.30), *IGF1R* (r=0.52), *IGFBP3* (r=0.59), and *IGFBP5* (r=0.56) mRNA. Expression of *IGF1R* and *IGFBP5* were also positively correlated (r=0.52; P<0.01). Muscle fiber density and diameter were negatively correlated

(P<0.01, r=-0.85). Muscle fiber density was negatively correlated while muscle fiber diameter was positively correlated with *IGF1* (P<0.01; r=-0.32 and +0.42, respectively), *IGF1R* (P<0.01; r=-0.32 and +0.38, respectively), and *IGFBP5* (P<0.01; r=-0.42 and +0.38, respectively), mRNA expression. In addition, muscle fiber diameter was positively correlated with *IGFBP3* to *IGBP5* mRNA ratio (P<0.01, r = +0.34).

Table 2. Effects of herbage allowance (HA) and cow genotype (G) on plasma IGF-I and *Semitendinous* gene expression at birth, weaning and 380 days of age of calves

		Treatments1						*P value*						
	Days	*Hi-PUFI*	*Lo-PUFI*	*Hi-CRBC*	*Lo-CRBC*	*All2*	*s.e*	*HA*	*G*	*D*	*HAxG*	*HAxD*	*GxD*	*HAxGxD*
Plasma IGF-I (ng/mL)								*0.02*	*0.38*	*<0.01*	*0.02*	*0.05*	*0.68*	*0.18*
	Birth	*186.9a*	*119.8bc*	*143.1ab*	*170.2a*	*155.0a*	*15.8*							
	142	*85.5d*	*62.5e*	*107.1cd*	*78.4de*	*83.4c*	*15.7*							
	380	*127.9bc*	*91.2d*	*124.3bc*	*126.7bc*	*117.5b*	*15.7*							
Muscle gene expression														
GHR3								*0.36*	*0.88*	*<0.01*	*0.62*	*0.68*	*0.25*	*0.5*
	Birth	*1.45*	*0.71*	*1.81*	*1.56*	*1.38b*	*0.25*							
	142	*2.52*	*2.46*	*2.88*	*2.25*	*2.53a*	*0.23*							
	380	*2.53*	*2.15*	*1.52*	*2.05*	*2.06a*	*0.23*							
IGF1								*0.19*	*0.03*	*<0.01*	*0.76*	*0.74*	*0.48*	*0.29*
	Birth	*1.18cd*	*0.85de*	*0.95cde*	*0.46e*	*0.86b*	*0.14*							
	142	*1.26bc*	*1.33bc*	*1.29 bc*	*0.74de*	*1.16b*	*0.13*							
	380	*2.23a*	*1.90ab*	*1.35bc*	*1.45bc*	*1.73a*	*0.13*							
IGF1R								*0.85*	*0.86*	*<0.01*	*0.75*	*0.86*	*0.07*	*0.09*
	Birth	*0.54d*	*0.74d*	*1.04cd*	*1.00cd*	*0.83c*	*0.12*							
	142	*1.30bc*	*1.59b*	*1.85ab*	*1.32bc*	*1.52b*	*0.13*							
	380	*2.34a*	*1.92ab*	*1.52b*	*1.86ab*	*1.91a*	*0.13*							
IGFBP3								*0.19*	*0.39*	*<0.01*	*0.34*	*0.87*	*0.18*	*0.67*
	Birth	*1.51*	*0.42*	*1.49*	*1.25*	*1.17b*	*0.35*							
	142	*2*	*1.1*	*1.03*	*1.33*	*1.36b*	*0.35*							
	380	*2.15*	*1.71*	*3.03*	*2.57*	*2.36a*	*0.35*							
IGFBP5								*0.94*	*0.94*	*<0.01*	*0.29*	*0.93*	*0.34*	*0.01*
	Birth	*0.93cd*	*0.53d*	*0.86cd*	*1.43bc*	*0.94b*	*0.21*							
	142	*1.98b*	*2.52ab*	*2.63ab*	*1.89b*	*2.25a*	*0.21*							
	380	*3.09a*	*2.18ab*	*1.62bc*	*2.69ab*	*2.39a*	*0.2*							
PPARG								*0.53*	*0.07*	*<0.01*	*0.12*	*0.09*	*0.48*	*0.19*
	Birth	*0.52bcd*	*0.40cd*	*0.61bc*	*0.26d*	*0.45b*	*0.12*							
	142	*0.62bc*	*0.72ab*	*0.56bcd*	*0.42cd*	*0.58b*	*0.12*							
	380	*0.80ab*	*1.02a*	*0.70ab*	*0.71ab*	*0.81a*	*0.12*							
SREBF1								*0.56*	*0.05*	*<0.01*	*0.36*	*0.4*	*0.39*	*0.06*
	Birth	*0.55e*	*0.68e*	*1.15bcd*	*0.86de*	*0.81b*	*0.11*							
	142	*0.90de*	*1.04cde*	*1.77a*	*1.03cde*	*1.19ab*	*0.11*							
	380	*1.48abc*	*1.35abc*	*1.31abc*	*1.67ab*	*1.45a*	*0.11*							
PAX3								*0.91*	*0.62*	*0.01*	*0.16*	*0.3*	*0.65*	*0.66*
	Birth	*1.72*	*0.52*	*1.5*	*1.52*	*1.32b*	*0.45*							
	142	*1.27*	*1.46*	*1.36*	*2.63*	*1.68b*	*0.44*							
	380	*3.5*	*2.25*	*2.01*	*3.39*	*2.79a*	*0.45*							
PAX7								*0.71*	*0.4*	*0.01*	*0.37*	*0.85*	*0.13*	*0.58*
	Birth	*1.6*	*0.78*	*1.22*	*1.75*	*1.33b*	*0.36*							
	142	*1.87*	*1.59*	*1.86*	*1.47*	*1.70b*	*0.35*							
	380	*3.37*	*2.95*	*1.62*	*2.19*	*2.53a*	*0.35*							

[1]Herbage allowance treatments: high and low: 4 and 2.5 kg DM/kg BW in average, respectively; Hi vs. Lo. G: dam genotype (purebred: Hereford and Angus vs. F1-crossbred; PU vs. CR). D: days. 2Lsmeans for day effect, including all treatments
[3]GHR=growth hormone receptor, IGF1=insulin-like growth factor 1, IGF1R=IGF receptor type 1, IGFBP3 and IGFBP5=IGF binding protein-3 and -5, PPARG= peroxisome proliferator activated-receptor-γ, SREBF1=sterol regulatory element-binding transcription factor 1, PAX3 and PAX7 = paired box -3 and -7, HPRT=hypoxanthine phosphoribosyl transferase, ACTB=ß-actin.
[a,b] Denote lsmeans differences by Tukey analysis.

4.Discussion

This study describes short and mid-term changes in calf skeletal muscle fiber characteristics and gene expression after exposure to different maternal nutrition plans during the fetal and pre-weaning periods. We determined that maternal

herbage allowance (due to control of grazing intensity of native pastures through changes in herbage allowance) during gestation and lactation affected not only calf BW and body composition (Gutierrez et al., 2013) but also calf *Semitendinosus* muscle fiber diameter and expression of genes related with the IGF-I system and adipogenesis. The effects of nutrition during the fetal and pre-weaning periods varied according to dam genotype as well as calf age.

4.1.Pre-weaning period

Growth in mammals is directly correlated to muscle mass, which is associated with fiber number and diameter, and both parameters can be modified by nutrition (Bayol et al., 2004; Du et al., 2010). In the present work, calf BW at weaning and thereafter, was smaller for Lo-PUF1, but not for Lo-CRBC, when compared with Hi-HA calves. However, in contrast to previous results (Wu et al., 2006), herbage allowance (i.e nutrition) did not affect *Semitendinosus* fiber density. Nevertheless, fiber diameter was greater in Lo-HA than Hi-HA calves during early growth period (fetal and lactation stages), being this difference more marked for Lo-CRBC than Lo-PUF1 calves. The finding of greater muscle fiber diameter has been reported previously in bovines and sheep when dam nutrient intake was restricted during gestation (Du et al., 2010). A greater skeletal muscle adipogenesis in Hi-HA calves could have provoked adipocytes to take up more space in muscle bundles of decreasing fiber diameter (Albrecht et al., 2006). Alternatively, nutrient restriction during miogénesis could have provoked lower fiber numbers, thus leading to compensatory growth in fiber diameter. Indeed, muscle *PPARG* mRNA at birth was greater in Hi-HA than Lo-HA calves and *SREBF1* mRNA at birth and weaning was greater in Hi-CRBC than the other three calf groups (Hi-PUF1, Lo-CRBC and Lo-PUF1). Previous reports (Tong et al., 2008) showed that overnutrition (150% requirements) enhanced muscle adipogenesis and *PPARG* mRNA expression in fetal muscle. Both, *PPARG* and *SREBF1* are key transcripts involved in adipogenesis, by stimulating adipocyte differentiation and promoting fatty acid, triglyceride and cholesterol uptake and

storage (Rosen et al., 2002; Horton et al., 2002).

Although, Lo-CRBC calves at weaning showed a similar BW and a greater muscle fiber diameter than both Hi-PUF1 and Hi-CRBC calves, the reduced estimated lean to fat ratio would indicate that muscle mass was decreased in these animals as well as in Lo-PUF1 calves. Indeed, prenatal nutritional restriction has been shown to increase subcutaneous, perirenal and omental adipose tissue deposition in steers and lambs (Long et al., 2010; Ford et al., 2007). In addition, the reduced lean to fat ratio at weaning together with the greater expression of transcripts involved in adipogenesis at birth or weaning in Hi-HA calves, particularly in Hi-CRBC calves, would show a potential to increase intramuscular fat, without an increase in other fat deposits in these animals.

The lower BW of Lo-PUF1 was associated to lower plasma IGF-I during pre-weaning period, probably as a result of their more restricted nutrition in terms of energy and protein (Breier et al., 1988) which agrees with the reduced milk production of their dams (Gutiérrez et al., 2013). Plasma IGF-I is one of the most important nutritional hormones involved in promoting muscle fiber hypertrophy (Bayol et al., 2004). However, it has been reported (Philippou et al., 2007; Clemmons, 2009) that muscle locally produced IGF-I have more effect than plasma IGF-I in promoting skeletal muscle growth actions in mice. However, our results showed differences in muscle diameter between Lo-CRBC and Hi-HA calves (both, PUF1 and CRBC) with no differences in plasma IGF-I and reduced local *IGF1* mRNA expression, indicating that other mechanisms would have been involved.

The endocrine, autocrine, and paracrine functions of IGF-I are mediated through binding to IGF1R that activate intracellular processes can affect cell proliferation and differentiation (Duan et al., 2010; Philippou et al., 2007). Greater abundance of *IGFR1* mRNA has been associated to nutrient restriction during fetal life in mice (Tomita et al., 2001), in pig (Tilley et al., 2007) and in bulls with reduced genetic potential to muscle growth and development (Keady et al., 2011). We did not detect differences in muscle *IGF1R* mRNA neither at birth nor at weaning.

These contrasting results could be explained as the result of the moderate-chronic nutrient restriction during calf fetal and pre-weaning stages in our study.

In addition, six different IGFBP have been identified, and at a tissue level, IGFBP can both inhibit and potentiate IGF action by either prohibiting IGF from binding with IGF1R or by releasing IGF to bind IGF1R (Duan et al., 2010). In the present study, we measured muscle *IGFBP3* and *IGFBP5* mRNA expression as it has been reported that both are highly expressed in muscle and can affect its growth by modulating IGF-I action or by exhibiting independent-ligand actions (Duan et al., 2010). Greater *IGFBP3* mRNA expression was associated with less muscle growth in porcine fetus (Tilley et al., 2007) and bovine bulls (Keady et al., 2011). In contrast, Sadkowski et al. (2009) reported greater muscle expression *IGFBP3* mRNA in bulls with the greater genetic potential for muscle growth and development. The IGFBP5 is the main IGFBP secreted by skeletal muscle and its effects on muscle growth are not consistent. Research comparing bovine bulls of different genotypes has shown that *IGFBP5* mRNA expression was either down-regulated (Sadkowski et al., 2009) or unchanged (Keady et al., 2011) in bulls with the greater genetic potential for muscle growth and development. These contradictory results can be explained by the pattern of expression of IGFBP during muscle development (Lehnert et al., 2007) as well as the functional compensation that can occur between IGFBP (Keady et al., 2011).

In our study, *Semitendinosus IGFBP3* mRNA at birth or weaning did not differ among calf groups. However, *IGFBP5* mRNA expression was greater in Lo-CRBC calves at birth and it increased the most from birth to weaning in Lo-PUF1 calves. This greater *IGFBP5* mRNA expression in Lo-HA calves was associated with muscle fiber diameter. Similarly, Bayol et al. (2004) reported that *IGFBP5* mRNA expression increased in mice submitted to moderate undernutrition during fetal life as a compensation mechanism to increased local action of IGF-I. Moreover, we found a positive correlation between muscle fiber diameter and muscle expression of *IGFBP5* mRNA and *IGFBP3* to *IGFBP5* mRNA ratio expression. In agreement with our results, Sadkowski et al. (2009) suggested that

the greater muscle *IGFBP3* to *IGFBP5* mRNA ratio expression may facilitate muscle differentiation.

4.2. Post-weaning period

After weaning, calf ADG was reduced to near maintenance when herbage mass and height of native pastures decreased with low winter temperatures (142 to 240-270 days of age) to increase again with the beginning of spring (240-270 to 380 days of age; Gutierrez et al., 2013). Therefore, differences in calf BW lean to fat ratio, and *Semitendinosus* gene expression presented in this work at 380 days of age would be the results of early calf nutrition (fetal and lactation), dam genotype and compensatory growth (Gutierrez et al., 2013).

After winter restriction, in spite of being all calf groups under the same nutritional and management conditions, Lo-PUF1 calves were not able to compensate the difference in BW established at weaning between this and the other three calf groups. Moreover, lower BW could be associated to smaller muscle mass, reduced percentages of bone and greater percentages of fat in the whole body (Wu et al., 2006; Greenwood et al., 1998). Indeed, both Lo-PUF1 and Lo-CRBC maintained a smaller lean to fat ratio than Hi-HA calves at 380 days of age. Reduced BW and/or lean to fat ratio could have reduced maintenance requirements in Lo-HA calves (NRC, 2000), determining that the effects of post-weaning nutrient restriction would be more marked in Hi-HA calf groups which nutrition was better during fetal and pre-weaning stages.

Although not different in BW or lean to fat ratio, Hi-PUF1 and Hi-CRBC calves presented differences in skeletal muscle gene expression at 380 days of age (after winter restriction). Increased *IGF1R* and *IGFBP5* mRNA was determined in muscle of Hi-PUF1 when compared with Hi-CRBC calves. Neither plasma IGF-I nor muscle *IGF1* mRNA differ between these two calf groups, however, the greater local *IGF1R* mRNA expression in Hi-PUF1 calves, would suggest an increased muscle sensitivity to IGF-I in response to undernutrition (Tomita et al.,

2001) to promote muscle hypertrophy via satellite cell proliferation, increased protein synthesis and decrease protein degradation (Oksbjerg et al., 2004). Indeed, Micke et al. (2011) reported up-regulated muscle expression of *IGF1R* mRNA at slaughter in calves that were smaller at birth and suggested that this increase in gene expression acted as a compensatory effect to promote muscle growth. Similarly, increased muscle *IGFBP5* mRNA has been associated with increased IGF-stimulated myotube differentiation during regeneration (Clemmons, 2009; Ewton and Florin, 1995) that involves the activation of quiescent satellite cells, which participate in the reconstitution of damaged tissue that can occur after nutrient restriction such as in winter. Indeed, *PAX3* and *PAX7* mRNA expression, which are involved in activation of satellite cells (Bonnet et al., 2010), were increased at 380 days of age.

In conclusion, maternal nutrition through differences in herbage allowance during calf pre-weaning period (gestation and lactation) reduced BW in Lo-PUF1 and increased muscle fiber diameter but decreased estimated lean to fat tissue ratio in both Lo-PUF1 and Lo-CRBC calves. At birth and/or weaning, muscle expression of *PPARG* and *SREBF1* mRNA, was greater in Hi-HA calves, particularly Hi-CRBC, which could indicate a greater potential to increase intramuscular fat. Differences in *IGF1R* and *IGFBP5* mRNA were detected only between Hi-PUF1 and Hi-CRBC calves after post-weaning nutrient restriction in winter which could be related to a differential local response to IGF-I as a compensatory effect to promote muscle growth.

5. Acknowledgements

The authors thank Bernardo Rosengurtt Experimental Unit staff (O. Caceres, M. Caceres, J. Caceres, and V. Cal), for animal care and assistance throughout the field study, and Bach. Fernando Bideau and Ing. Agr. Federico Pereyra and for help in the fieldwork. This study was supported by research project INIA-FPTA/2007 #262 awarded to M. Carriquiry and CSIC-Iniciación/2009 awarded to V. Gutiérrez.

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4.DISCUSIÓN GENERAL Y CONCLUSIONES GENERALES

4.1.DISCUSIÓN GENERAL

La nutrición materna, debido al manejo de la oferta de forraje en campo natural durante la gestación y la lactancia (AOF. vs BOF), interactuó con la heterosis materna, afectando en el corto, mediano y largo plazo en el crecimiento y desarrollo de los terneros, con cambios en el PV, la composición corporal y el diámetro de las fibras en el músculo *Semitendinoso*, asociados a cambios en los perfiles endócrinos, metabólicos y de expresión muscular de genes relacionados con el sistema IGF y adipogénesis.

4.1.1.Período pre-natal

En nuestro trabajo, a pesar de no observar diferencias entre tratamientos en el PV al nacer de los terneros, el diámetro de las fibras del músculo, las concentraciones plasmáticas de proteína, glucosa, insulina e IGF-I y la expresión de ARNm de *IGF1*, *IGFBP5*, *PPARG* y *SREBF1* difirieron debido a la oferta de forraje y/o al genotipo materno.

Al nacer, los terneros en BOF presentaron mayor concentración plasmática de proteína total que los animales en AOF. Hammer *et al.* (2007) demostraron que la subnutrición durante la gestación puede llevar a incrementos en la proteína total e inmunoglobulinas (Ig) en suero de los terneros durante las primeras 24 h de nacidos, debido a una mayor transferencia intestinal de IgG. Estos resultados sugieren que el sistema gastrointestinal se programa para ser más eficiente en la extracción de nutrientes luego del nacimiento (Funston *et al*., 2010). Sin embargo, los terneros en AOF presentaron mayores concentraciones plasmáticas de glucosa, insulina e IGF-I que los terneros en BOF. De manera similar, Hay (1995) encontró que las ovejas sometidas a restricciones energéticas durante la gestación presentan hipoglicemia y una disminución de la transferencia de glucosa al útero y feto. En acuerdo con el mayor nivel plasmático de glucosa en AOF, también se observaron mayores

concentraciones de insulina, consistente con su rol como regulador de la homeostasis de la glucosa en mamíferos. Sin embargo, los terneros AOF-CRRC presentaron menores concentraciones de insulina al nacer que los otros tres grupos, lo que indicaría que la nutrición y la heterosis materna podrían interactuar sobre el metabolismo fetal de glucosa e insulina.

El sistema IGF maternal puede modular la partición de nutrientes hacia el feto, particularmente influencia la transferencia de glucosa y aminoácidos a través de la placenta hacia el feto (Kniss *et al.*, 1994). Consistentemente con las mayores concentraciones de glucosa reportadas en este trabajo en los terneros AOF al nacer, Laporta *et al.* (2011) reportó mayores concentraciones de IGF-I en las madres en AOF que BOF durante la gestación. En ovinos, se ha demostrado que la glucosa a su vez, tiene un efecto estimulante en la concentración de IGF-I plasmática a través de efectos mediados por la insulina (Oliver *et al.*, 1993; 1996). Es así que los mayores concentraciones de glucosa e insulina en terneros de AOF fueron asociados con mayores concentraciones de IGF-I.

Sin embargo, al nacer (y posteriormente) los terneros BOF-CRRC, presentaron concentraciones de IGF-I mayores a los terneros BOF-PUF1 y no diferentes de los terneros en AOF. Esta mayor concentración de IGF-I circulante de los terneros BOF-CRRC en comparación con BOF-PUF1 se asoció a una menor expresión local en el músculo *Semitendinoso* del ARNm de *IGF1* y mayor de ARNm de *IGFBP5* en los primeros. Bayol *et al.* (2004) encontraron un aumento de la expresión muscular de ARNm de *IGFBP5* en ratones sometidos a subnutrición durante el período fetal, y sugieren que sería un mecanismo de compensación para aumentar el efecto de la IGF-I sobre el tejido muscular. En efecto, en este trabajo hemos encontrado una correlación positiva entre la expresión de ARNm de *IGFBP5* y el diámetro de la fibra muscular. Existe evidencia en bovinos y ovino del incremento del diámetro de las fibras musculares de manera de compensar una disminución en el número de fibras en el tejido muscular debido a una restricción nutricional durante la gestación en el período de miogénesis (Du *et al.*, 2010). Asimismo, una mayor adipogenesis muscular en los terneros AOF podría haber incrementado el tamaño de los adipocitos

intermusculares e intramusculares disminuyendo el diámetro de las fibras musculares (Albrecht *et al*., 2006). Consistente con esto, la expresión de ARNm de *PPARG* y *SREBF1*, factores de transcripción claves en la diferenciación de adiposos y la acumulación de ácidos grasos y triglicéridos en los adipocitos (Rosen y MacDougald, 2006; Horton *et al.,* 2002), fue mayor en los terneros de AOF que BOF al nacer y en AOF-CRRC que en los otros tres grupos al nacer y destete, respectivamente.

4.2.Período post-natal

Durante la lactancia, la producción de leche, nutriente clave en el desarrollo del ternero durante sus primeros meses de vida, fue más abundante y de mejor calidad (mayor presencia de sólidos: proteína, lactosa y grasa) en las vacas en AOF y en vacas CR (Jenkins y Ferrel, 1992; Notter *et al.,* 1978). La menor producción de leche y sólidos de las vacas puras en BOF se asoció con menor PV del ternero al destete (Beal *et al.,* 1990; Totusek *et al*., 1973) y con menores concentraciones plasmáticas de glucosa, proteínas totales e IGF-I en los terneros BOF-PUF1 que en los otros tres grupos de terneros, indicando un peor estado nutricional en los mismos (Ndlovu *et al*., 2007).

El menor PV está asociado, por lo general, con una menor masa muscular, menor porcentaje de hueso y mayor porcentaje de grasa corporal (Wu *et al.,* 2006; Greenwood *et al*., 1998). Si bien al destete los terneros en BOF mantuvieron el mayor diámetro de la fibras musculares, los mismos presentaron una menor proporción de tejido magro:grasa y menor espesor de grasa subcutánea, y estas diferencias fueron más marcadas en los terneros BOF-CRRC en comparación a BOF-PUF1. Probablemente, la mejor alimentación de los terneros BOF-CRRC en comparación con BOF-PUF1 durante la lactancia, les permitió realizar un crecimiento compensatorio luego de la restricción moderada durante la etapa fetal (80% de los requerimientos de National Research Council: NRC, 2000), lo que llevó a los cambios en la composición corporal aumentado más rápidamente el contenido graso en detrimento del tejido magro (Wu *et al*., 2006; Abdelsamei *et al*., 2005;

Sainz *et al.,* 1995). Asimismo, la mayor proporción de tejido magro:grasa, el mayor espesor de grasa subcutánea y la mayor expresión muscular de ARNm de transcriptos involucrados en la adipogénesis (*PPARG* y *SREBF1*) en etapas tempranas (nacimiento y/o destete), indicarían que los terneros en AOF, particularmente AOF-CRRC, presentaron un mayor potencial de adipogénesis intramuscular y subcutánea sin incrementar otros depósitos grasos (grasa perirenal, omasal y mesentérica).

Luego del destete y hasta el primer año de vida, en comparación con los otros tres grupos, los terneros BOF-PUF1 mantuvieron un menor PV, asociado a una menor IGF-I plasmática al año de vida, hormona que estimula de deposición de músculo y proteína (Nkrumah *et al*., 2007). A su vez, al primer año, la proporción tejido magro:grasa se mantuvo menor en terneros BOF que AOF, especialmente en terneros BOF-CRRC que AOF-CRRC.

Una reducción en el PV o en la proporción de tejido magro:grasa puede reducir los requerimientos de mantenimiento en los terneros en BOF (NRC, 2000), determinando que los efectos de la restricción nutricional luego del destete (restricción invernal) pueden ser más marcados en los terneros en AOF ya que durante los períodos fetal y de lactancia tuvieron mejor alimentación. En efecto, luego de la restricción invernal, al primer año de vida, a pesar de no presentar diferencias en el PV ni en la composición corporal, la expresión de ARNm de *IGF1R* e *IGFBP5* fue mayor en el músculo *Semitendinoso* de AOF-PUF1 que AOF-CRRC. La mayor expresión local de estos dos transcriptos en los terneros AOF-PUF1 puede sugerir un incremento en la sensibilidad del músculo a la hormona IGF-I circulante en respuesta a la subnutrición como mecanismo de compensación (Micke *et al*., 2011; Tomita *et al*., 2001) de forma de promover la hipertrofia muscular por medio de la proliferación celular, incremento de la síntesis proteica y disminución de la degradación proteica (Oksbjerg *et al*., 2004). De manera similar, incrementos en el ARNm de *IGFBP5* ARNm han sido asociados con la estimulación de la IGF-I de la diferenciación de miotúbulos durante la regeneración muscular (Clemmons, 2009;

Ewton y Florini, 1995) que podría ocurrir para reconstituir el músculo dañado durante la restricción de nutrientes en invierno.

4.2. CONCLUSIONES GENERALES

La nutrición durante el período fetal y de lactancia tuvo impacto a mediano y largo plazo disminuyendo el PV en terneros BOF-PUF1 o incrementando la deposición de grasa frente al tejido magro en BOF-CRRC. Estos cambios en los terneros en BOF, tanto PUF1 como CRRC, se asociaron a una alteración en los perfiles de glucosa, insulina, e IGF-I y a un mayor diámetro de las fibras musculares pero a una menor expresión de los transcriptos *PPARG* y *SREBF1* en el m. *Semitendinoso.*

4.3.IMPLICANCIAS DE ESTE TRABAJO

Este trabajo fue el primero que se realizó en Uruguay evaluando el efecto de la nutrición en la etapa fetal y postnatal temprana del ternero sobre características que pueden influir sobre la cantidad y calidad de la producción de carne. Si bien existen trabajos internacionales que estudian el efecto de la subnutrición en etapas tempranas de la vida del ternero sobre la programación del desarrollo, este trabajo tiene la novedad de lograr distintos niveles nutricionales mediante el manejo del campo natural (que representa el 65% de la superficie dedicada a la cría bovina) y emplear las dos razas más utilizadas para la cría bovina en el país (A. Angus y Hereford, 90% del rodeo nacional) y sus cruzas recíprocas. A su vez, presenta una aproximación integrada al problema, evaluando conjuntamente cambios en el PV, composición corporal, perfiles hormonales y metabólicos y características histológicas y de expresión génica del tejido muscular. Pudimos verificar que la subnutrición durante la gestación y lactancia así como el genotipo materno tienen impacto sobre el crecimiento y desarrollo de los terneros en el corto y mediano-largo plazo, pudiendo afectar la cantidad y calidad de la carne producida. Estos estudios deberían continuarse a lo largo de la vida del animal hasta su faena, de manera de verificar si estos efectos persisten.

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6. ANEXO I: AUTHOR GUIDELINES OF JOURNAL OF ANIMAL PHYSIOLOGY AND ANIMAL NUTRITION y FOR LIVESTOCK SCIENCE

Ver archivos adjuntos