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PERFILES METABÓLICOS, ENDÓCRINOS Y DE EXPRESIÓN GÉNICA HEPÁTICA ASOCIADOS A CAMBIOS EN EL BALANCE ENERGÉTICO DE VACAS DE CARNE PRIMÍPARAS EN CONDICIONES DE PASTOREO

por

Ana Laura ASTESSIANO DICKSON

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Tesis aprobada por:

Alejandro Rielling

Elsa Garófalo

Carolina Viñoles

Laura Astigarraga

Fecha: 21/12/2010

Autor: **----------**

ANA LAURA ASTESSIANO

Tutor: **----------**

RAQUEL PÉREZ-CLARIGET

Co-tutor: **----------**

MARIANA CARRIQUIRY

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RESUMEN

Este trabajo evaluó en vacas de carne primíparas en campo nativo la asociación entre la evolución de la reservas corporales, medidas a través de la condición corporal (CC escala 1-8) al parto CCB≤3,5 vs. CCM≥3,75 baja vs. moderada durante la gestación invernal (Trabajo 1; n=20) y/o el incremento en el plano nutricional (campo nativo, NP vs. campo nativo mejorado con *Lotus subbiflorous* cv.Rincón, LR) previo al entore (Trabajo 2; n=64), y los cambios endocrinos/metabólicos y de expresión génica hepática, asociados a las respuestas productivas y reproductivas. Los resultados del Trabajo 1 muestran que los cambios en las reservas corporales (2-2,5 unidades de pérdida de CC) y en el perfil metabólico/endocrino (elevados ácidos grasos no-esterificados y urea) reflejan que el balance energético negativo estuvo más asociado con la disminución en la cantidad y calidad de forraje ofrecido por las pasturas nativas en invierno, que con el inicio de la lactación. El perfil metabólico/endocrino y de expresión hepática de genes del eje somatotrófico se asociaron a la evolución de la CC durante el preparto. Las reservas corporales, reflejadas en una pequeña diferencia de CC (0,5 unidades), señalizaron una partición de nutrientes diferencial hacia la glándula mamaria (menor niveles de insulina, y de ARNm del receptor de hormona de crecimiento 1A y de factor de crecimiento similar a la insulina-I (IGF), al día 52: 1,16 vs. 2,46±0,6 uU/mL, 0,44 vs. 1,10±0,1 y 0,08 vs. 0,2±0,03 en CCB vs. CCM), incrementando la producción de leche y el peso del ternero (4,4 vs. 5,9 L/d y 55 vs. 64±3,2 kg en CCB vs. CCM). Los resultados del Trabajo 2 demostraron que el incremento en el plano nutricional previo al entore no mejoró la respuesta reproductiva pero modificó el perfil metabólico/endocrino (mayores niveles de glucosa y menores de urea al día 12 de tratamiento y mayores de insulina a partir del día 12 para NP que LR: 81 vs. 72±0,03 mg/dL, 3,7 vs. 5,4±0,3 mmol/L y 2,2 vs. 1,2±0,3 uU/mL) y de expresión génica hepática (mayor ARNm de receptor insulina y de proteína de unión de IGF-3: 9,4 vs. 5,5±1 y 6,9 vs. 4,7±0,7) en acuerdo con una mayor partición de nutrientes hacia la glándula mamaria, reflejado en una mayor performance de los terneros a los 103 días (138 vs. 148±3 kg en CCB vs. CCM).
Palabras clave: reservas corporales, nutrición posparto, ARNm

SUMMARY

Metabolic and endocrine profiles, and hepatic gene expression associated with changes in energy balance of primiparous beef cows in grazing conditions

This work evaluated in primiparous beef cows in grazing conditions the association between the evolution of body reserves, measured by body condition score (BCS at calving, Low ≤ 3.5 vs. Moderate ≥ 3.75), during the gestation period of winter (Study 1 n; = 20) and/or the increase in the nutritional plane (native pastures, NP vs. native pastures improved with Lotus subbiflorous cv Rincón, IP) before the mating period (Study 2; n = 64), and changes in endocrine/metabolic profiles and hepatic gene expression, associated with the productive and reproductive responses. The results obtained in Study 1 showed that changes in body reserves (2 a 2.5 units of BCS losses) and metabolite/endocrine profiles (greater concentration of no-esterified fatty acid and urea) that reflected negative energy balance were more associated with the decrease in forage quantity and quality of native pastures during winter than to onset of lactation. The metabolic/endocrine profiles as well as liver expression of the somatotrophic axis genes were associated with evolution of body reserves during the prepartum. Body reserves, reflected in a rather small difference in BCS (0.5 units), signal a differential nutrient partitioning towards mammary gland (reduced insulin levels and mRNA expression of growth hormone receptor 1A and insulin-like growth factor I IGF at 52 days: (1.16 vs. 2.46±0.6 uU/mL, 0.44 vs. 1.10±0.1, 0.08 vs. 0.2±0.03 in L vs. M cows), increasing milk production (4.4 vs. 5.9 l in BCSL vs. BCSM and calf growth (55 vs. 64±3.2 kg in L vs. M cows). The results obtained in Study 2 showed that the increase in nutritional plane before the mating period failed to improve reproductive performance, but changed the metabolic/endocrine profile (greater glucose and reduce urea concentration at 12 days of treatment and greater insulin levels from 12 days in NP than IP: 81 vs. 72±0.03 mg/dL, 3.7 vs. 5.4±0.3 mmol/L, 2.2 vs. 1.2±0.3 uU/mL) and hepatic mRNA expression (greater mRNA of insulin receptor and binding proteins IGF-3, 9.4 vs. 5.5±1 y 6.9 vs. 4.7±0.7) in accordance with a further partition of nutrients to the mammary gland, reflected in increased performance of calf at 103 days (138 vs. 148±3 kg in L vs. M cows).

Key words: body reserves, pospartum nutrition, RNAm

1. INTRODUCCIÓN

En Uruguay la cría de bovinos de carne involucra 6,7 millones de cabezas y 8,3 millones de hectáreas, que significan el 48% de los 14,3 millones de hectáreas de pastoreo con bovinos y ovinos de carne; el 52% de la superficie agropecuaria nacional y 20.000 productores con un fuerte predominio de producción familiar. Datos nacionales de la Dirección de Estadísticas Agropecuarias (DIEA) en el 2010, muestran que el valor de su principal producto (terneros) fue de más de 750 millones de dólares. El contexto actual está cambiando, tanto a escala global como nacional, con la presencia de rubros altamente competitivos por la tierra (i.e agricultura, forestación), desplazando a la ganaderia vacuna. Por lo que los sistemas criadores de producción deben evolucionar hacia modelos más productivos y sustentables y así, responder a los desafíos actuales.

La cría vacuna es un proceso de largo período de maduración e ineficiente en el uso de la energía, ya que destina el 70% de la energía consumida al mantenimiento de sus funciones vitales (Dickerson, 1978). Dado que la cría se lleva a cabo principalmente sobre campo nativo, la producción estacional, las variaciones interanuales y las diferencias en la calidad y cantidad de la pastura ofrecida (Berretta *et al.*, 2000), determinan que el aporte de nutrientes a la vaca resulte la principal limitante del proceso. En particular, la baja producción invernal de forraje, coincide con el momento en el que las vacas se encuentran en gestación avanzada o inicio de lactancia y determina un período de balance energético negativo. Esto se ve reflejado en un pobre estado nutricional de las vacas al parto e inicio del entore, determinando un largo período de anestro posparto (de 92 días en promedio en vacas adultas y mayores a 120 días en vacas primíparas; Quintans *et al.* 2004, Quintans y Vázquez, 2002) y una baja probabilidad de preñez (Orcasberro 1991). Es así que, durante las últimas tres décadas, la tasa de destete (terneros destetados/vaca entorada) del rodeo nacional se ha mantenido en 64% (DIEA, 2010)

con una gran variación entre años en la cantidad de terneros que destetan con relación a las vacas entoradas.

El estado metabólico puede definirse como la cantidad de nutrientes y energía que están disponibles para el animal en un determinado momento, y depende de la cantidad y calidad de alimento consumido, de la cantidad de reservas corporales y del ritmo de utilización de la energía (Blache *et al.,* 2006). La condición corporal (CC) es una herramienta de fácil aplicación que mediante apreciación visual se estiman las reservas corporales y permite conocer el estado nutricional y energético del rodeo de cría (Vizcarra *et al.,* 1986). La investigación nacional ha enfatizado a través de la "Propuesta de Manejo del Rodeo de Cría", para lograr una CC al parto e inicio del entore de 4 en vacas adultas y 4,5 en vacas de primera cría (escala de 1-8) y así obtener una probabilidad de preñez entre 75 y 90% en el siguiente entore (Soca y Orcasberro, 1992). La asignación de forraje durante el otoño-invierno es clave en la evolución de la CC de las vacas de cría durante la gestación avanzada (Orcasberro *et al.,* 1992, Trujillo *et al.,* 1996) e impacta sobre la CC al parto.

En gestación avanzada, las demandas de energía y proteína aumentan del 30 a 50% con respecto a los requerimientos de mantenimiento, lo que se cumple en parte por una mayor ingesta voluntaria y en parte por una serie de adaptaciones metabólicas maternas que incluyen no sólo cambios en el metabolismo de carbohidratos y proteínas, sino también en el metabolismo lipídico (Bell, 1995). Estos cambios se caracterizan por aumento de la gluconeogénesis hepática, disminución de la utilización de glucosa en los tejidos periféricos, y una moderada movilización de ácidos grasos no esterificados (AGNE) del tejido adiposo, asociada a un aumento en la utilización periférica de los mismos y de su metabolito hepático, Beta-Hidroxibutirato (Bell, 1995). Los cambios específicos en el metabolismo de los aminoácidos no se han caracterizado, pero pueden incluir aumento de la síntesis de proteínas y reducción del catabolismo de aminoácidos en el hígado junto a una mayor predisposición a la

proteólisis muscular. Todas estas adaptaciones metabólicas resultan consistentes con la promoción de la disponibilidad de glucosa y aminoácidos para el metabolismo del feto, creciente dependencia de los tejidos maternos en AGNE y cuerpos cetónicos para el metabolismo oxidativo (Bell, 1995).

El aumento de los niveles circulantes de AGNE sobre el final de gestación se hace más evidente y exagerada si la ingesta de energía es restringida voluntaria o involuntariamente (Reid y Hinks 1962, Radloff *et al.,* 1966, Petterson *et al.,* 1994). Resultados nacionales de investigación en vacas de cría primíparas (Gestido *et al.* 2008) y multíparas (Quintans *et al.*, 2010) en condiciones de pastoreo de campo nativo, mostraron mayores descensos de la CC y aumentos de los niveles de AGNE durante el pre (últimos 30 a 60 días de gestación) que el posparto. Asimismo, estos resultados indicaron una asociación entre la CC al parto y evolución en los niveles de AGNE durante el pre y posparto. Las vacas de baja CC al parto (<4 unidades) presentaron mayores concentraciones de AGNE en gestación avanzada, evidenciando la mayor movilización de reservas para suplir las demandas de energía de gestación; mientras que las vacas con CC al parto moderada (>4 unidades) presentaron mayores concentraciones de AGNE en el posparto temprano, lo cual podría evidenciar una mayor producción de leche en estos animales. Mayores concentraciones de AGNE, asociadas a mayores producciones de leche, se han observado en vacas de carne con mejor alimentación posparto (Vizcarra *et al.,* 1998) o en vacas de leche a pastoreo con mejor CC al parto en condiciones de pastoreo (Meikle *et al.,* 2004, Chagas *et al.*, 2006).

En vacas lecheras, la transición desde el estado de preñez avanzada a no preñada-lactante y el inicio de lactación, están caracterizados por un período de balance energético negativo debido a que aumento de los requerimientos nutricionales para la producción de leche, no está acompañado de un aumento inmediato o suficiente en el consumo de alimentos (Bauman, 2000). Este período implica importantes cambios internos para la vaca, y se

caracteriza por una utilización prioritaria de la glucosa en la glándula mamaria, de manera que el déficit energético en el resto de los tejidos, provoca la movilización de los depósitos de grasa. Por otro lado, existe proteólisis en las primeras semanas posparto, con el fin de movilizar aminoácidos que contribuyan a la gluconeogenesis hepática y a la síntesis de proteína láctea en la glándula mamaria (Bauman 2000, Lucy 2008). Estos cambios se reflejan en alteraciones a nivel de los metabolitos (disminución de glucosa, aumento de AGNE) y hormonas (aumento de hormona de crecimiento (GH) y disminución de insulina, factor de crecimiento similar a la insulina tipo-I (IGF-I) y leptina) en sangre (Meikle *et al.,* 2004, Lucy 2008).

La capacidad del animal para adaptarse y sobrellevar el balance energético negativo (de gestación avanzada e inicio de lactación) depende de la capacidad de los mecanismos endócrinos y metabólicos de mantener la homeostasis (equilibrio de las condiciones internas; Chilliard *et al.,* 1998) y no sólo afecta la capacidad productiva de la vaca (kilogramos de terneros destetados) sino también compromete la respuesta reproductiva en el siguiente ciclo productivo ya que puede incrementar la duración del anestro postparto (Meikle *et al.,* 2004, Chagas *et al.,* 2006, Lucy 2008), y por lo tanto afectar la eficiencia de uso del forraje y rentabilidad del sistema de producción criador.

La hormona de crecimiento (GH) tiene un rol importante en la partición de nutrientes (homorhesis: flujo constante; Bauman and Currie 1980). Las alteraciones en esta hormona conjuntamente con los cambios en la síntesis hepática de glucosa, y en las concentraciones de insulina e IGF-I en la sangre, son indicativos de la disponibilidad de energía y del estatus metabólico de los animales.

El hígado puede considerarse como el principal regulador e integrador del estatus metabólico de los animales y es el sitio primario de síntesis de IGF-I, en respuesta a la unión

de la GH, con su receptor (GHR). En contraste con la típica asociación directa y positiva entre GH e IGF-I, mientras GH en sangre comienza a aumentar previo al parto, IGF-I disminuye, disociación que coincide con el establecimiento del balance energético negativo de inicio de lactación (Rhoads *et al.*, 2004). Este desacople de eje GH-IGF ha sido atribuido a un estado de resistencia a la GH en el hígado y a una reducción de la expresión hepática de ARNm de GHR (Lucy 2008). Particularmente, en vacas lecheras al inicio de la lactación, la disminución en las concentraciones de IGF-I en sangre se ha asociado con una menor expresión de los transcriptos de la isoforma 1A del GHR (GHR1A) y de la IGF-I (Kobayashi *et al.* 1999). Trabajos posteriores (Rhoads *et al.*, 2004, Loor *et al.*, 2005, Carriquiry *et al.*, 2009) sugieren que la disminución de las concentraciones de insulina posparto (Rhoads *et al.* 2004) y de la expresión hepática de ARNm de la proteína de unión de IGF-3 (IGFBP-3; Loor *et al.*, 2005; Carriquiry *et al.*, 2009) serían clave en este mecanismo.

La mayoría de la información científica relacionada con el mecanismo molecular hepático responsable del desacople del eje GH-IGF ha sido generada durante el periparto en vacas lecheras alimentadas *ad-libitum* y en condiciones de estabulación. Sin embargo, investigaciones recientes (Jiang *et al.*, 2005, Rhoads *et al.*, 2007, Lucy *et al.*, 2009), demostrarían que los mecanismos que explican los cambios en las concentraciones de GH e IGF-I, el desacople de este eje y la movilización de reservas durante el periparto aún no están del todo claros y parecen estar más relacionados con la selección por producción de leche que con el balance energético negativo. La expresión de ARNm de GHR1A y/o IGF-I no se modificó en vacas lecheras en lactación tardía sometidas a una restricción alimenticia (Rhoads *et al.*, 2007), durante el periparto de vacas de carne alimentadas *ad libitum* (Jiang *et al.*, 2005), o en vacas de leche de baja producción (Lucy *et al.*, 2009). En contraste, Wang *et al.* (2003) reportaron en novillos con alimentación restringida, los niveles de IGF-I en sangre y la expresión hepática de ARNm de IGF-I se asociaron con menores niveles de ARNm de GHR (isoformas 1A y 1C).

El correcto funcionamiento del eje hipotálamo-hipofisiario-gonadal, eje con rol dominante en la regulación de la reproducción, requiere la integración de las señales periféricas (metabolitos: glucosa, AGNE y hormonas: GH, insulina, IGFI, sus 6 proteínas de unión -IGFBP1 a IGFBP6- y leptina) que indican el estatus fisiológico y nutricional de la vaca e identifica a la misma como pronta para concebir y llevar adelante una gestación. Gestido *et al.* (2008) en vacas de cría en pastoreo de campo nativo, encontró asociación entre los niveles de NEFA entre el día -30 y 15 posparto con la probabilidad de preñez temprana en el primer tercio del entore. De manera similar, el largo de anestro ha sido asociado con menores concentraciones de IGF-I y mayores concentraciones de IGFBP-2 en sangre tanto en vacas de leche (Meikle *et al.*, 2004) como de carne (Roberts *et al.*, 1997) y Sinclair (2008) reportaron que los niveles circulantes de insulina eran responsables de una mayor proporción de la variación entre el intervalo parto-primera ovulación que la CC al parto o el consumo de energía posparto.

La investigación nacional ha concentrado esfuerzos en mejorar el porcentaje de preñez en base a cambios en la alimentación y control de amamantamiento (Rovira y Frachia, 2005). Las propuestas tecnológicas han permitido incrementar el porcentaje de destete e ingreso neto de la cría vacuna con bajos costos, en base al manejo de la alimentación de campo natural de manera de controlar la CC de vacas primípras y multíparas (Soca *et al.*, 2007) lo cual interactúa con diversas opciones de control de amantamiento (Soca *et al.*, 2007, Quintans 2008). Adicionalmente, en la última década, se han estudiado cambios en el aporte energético durante 20-28 días, antes o durante el entore, en combinación con manejo del amamantamiento (destete temporario con o sin separación del ternero) y se ha demostrado que el mismo incrementa el porcentaje de preñez de vacas primíparas con CC sub-óptima durante la primera mitad del entore (Pérez-Clariget *et al.*, 2007, Soca *et al.*, 2007). Sin embargo, los cambios en el metabolismo asociado con esta respuesta no están claros en vacas de cría. Mas

aún, a nivel internacional, la información sobre el impacto de la suplementación en el metabolismo hepático y sus impactos en la respuesta productiva-reproductiva en vacas de carne es escasa (Cooke *et al.*, 2008).

Si bien los mecanismos fisiológicos y moleculares responsables de ligar el estatus metabólico (reservas energéticas y consumo de energía) con la respuesta productiva y reproductiva deberían ser similares en los rumiantes, el amamantamiento y la presencia de la cría, las demandas energéticas debido al pastoreo, los drásticos cambios en las reservas energéticas corporales que ocurren a lo largo del año, son alguno de los factores que podrían modificar los mecanismos básicos. Es así que, este trabajo de tesis tuvo como hipótesis que las variaciones del peso vivo y condición corporal, así como en los cambios temporales en los niveles de hormonas y metabolitos en sangre y en la expresión hepática de genes del eje GH-IGF-I, reflejan la relación entre el balance energético (medido a través de la evolución de las condición corporal preparto o debido a un incremento del plano nutricional previo al entore) y la fisiología productiva y reproductiva de la vaca de primera cría en pastoreo durante el pre y posparto. El objetivo general fue generar información que incremente el conocimiento de los mecanismos fisiológicos y moleculares responsables de ligar el estatus metabólico (condición corporal e incremento del plano nutricional) con la respuesta productiva y/o reproductiva en vacas de carne primíparas en condiciones de pastoreo de campo nativo.

La estructura central de la tesis consiste en dos artículos científicos, el primer artículo, titulado "***Metabolic and endocrine profiles and hepatic gene expression in periparturient, grazing primiparous beef cows with different body reserves"*** constituye el segundo capítulo de esta tesis. En este artículo, escrito para enviar a Domestic Animal Endocrinology, se evaluaron los cambios en los perfiles metabólicos, endocrinos y de expresión génica hepática durante el periodo de periparto en vacas de carne de primera cría con diferente balance energético, medido a través de la evolución de la condición corporal, y se relacionaron estos cambios con

la respuesta en producción de leche y crecimiento del ternero. El segundo artículo se titula ***"Effects of a short-term increase in the nutritional plane before the mating period on metabolic and endocrine parameters, hepatic gene expression, and reproduction in primiparous beef cows on grazing conditions"*** y constituye el tercer capítulo. En este artículo, aceptado para publicar en Journal of Animal Physiology and Animal Nutrition, se evaluaron los cambios en los perfiles metabólicos, endocrinos y de expresión hepática de genes relacionados con el eje somatotrófico en respuesta a un incremento en el plano nutricional de corto plazo mediante el pastoreo de campo nativo mejorado con Lotus subbiflorous cv. Rincón en el posparto de vacas de carne de primera cría con diferente balance energético y crecimiento del ternero. En el cuarto capítulo de esta tesis se presenta una discusión general y conclusiones globales del problema aquí estudiado.

2. PERIPARTURIENT PERIOD IN PRIMIPAROUS BEEF COWS WITH DIFFERENT BODY RESERVES

Metabolic and endocrine profiles and hepatic gene expression in periparturient, grazing primiparous beef cows with different body reserves

Astessiano, A. L.[A], R. Pérez-Clariget[A], G. Quintans[B,] P. Soca[A], A. Meikle[C], B.A. Crooker[D], M. Carriquiry[A]

[A] Facultad de Agronomía, Universidad de la República, Montevideo, Av. E. Garzón 780, Uruguay.

[B] Instituto Nacional de Investigación Agropecuaria Treinta y Tres, Ruta Nº 8 km 281, Treinta y Tres, Uruguay.

[C] Facultad deVeterinaria, Universidad de la República, Montevideo, Lasplaces 1550

[D] Department of Animal Sciences, University of Minnesota, 1364 Eckles Ave, St. Paul 55108-6118

Corresponding author: Ana Laura Astessiano. Departamento de Producción Animal y Pasturas, Facultad de Agronomía, Av. Garzón 780, Montevideo, Uruguay.

Tel: (+598)23559636; Fax: (+5982)3543460. E-mail: lauaste@gmail.com

2.1 SUMMARY

The aim of this study was to determine effects of prepartum body condition on hepatic gene expression of GH-IGF axis and associations among gene expression, milk production, and metabolite and hormone concentrations in grazing suckled-primiparous beef cows from -49 to 49 days postpartum (DPP). Twenty crossbred cows Aberdeen Angus x Hereford of BCS of 6 (scale 1 to 8) at -112 DPP (end of fall) were managed as a contemporary group and grazed together on a native pasture paddock. Cows were classified at -35 DPP into thin (BCS < 4.5) or moderate (BCS ≥ 4.5) BCS groups and blocked by calving date. Milk yield was determined at 14 and 35 DPP, blood samples were collected weekly for metabolite and hormone analyses and liver biopsies were collected for gene expression analysis at −11, 7, 31, and 52 DPP. During the prepartum period all cows mobilized body reserves as reflected in losses of BCS and elevated concentrations of non-esterified fatty acids (NEFA) and urea in blood. Cow BW and BCS were greater in moderate than thin BCS cows throughout the peripartum and early lactation period. Calf BW and ADG were greater in moderate than thin BCS cows and were associated with greater milk production by the moderate BCS cows at 35 DPP (6.0 vs. 5.0 ± 0.65 kg/d). Serum insulin concentrations were low and relatively stable throughout the peripartum period but increased by 21 DPP in the thin BCS group (1.0 vs 2.6 ± 0.6 uUI/mL). Total growth hormone receptor (GHR) mRNA expression was 2-fold greater at -11 DPP while GHR1A and IGF-I mRNA were 2.5-fold less at 52 DPP in moderate than thin BCS cows. Expression of IGFBP-2 mRNA decreased in moderate but increased in thin BCS cows from -11 to 52 DPP. Metabolic/endocrine profiles and liver expression of somatotropic axis genes were associated with changes of body reserves during the prepartum period and may indicate that prepartum differences in BCS lost can affect nutrient partitioning towards the mammary gland, and subsequent milk production and calf weight.

Key words: cattle, pastures, mRNA, metabolic profile.

2.2. INTRODUCTION

The adaptability of ruminants to periods of nutritional restriction depends on the capacity of their endocrine and metabolic mechanisms to maintain homeostasis [1]. In spring-calving cows in rangeland conditions, moderate to severe nutritional restrictions occur during the last months of gestation and calving in winter or early spring when quantity and quality of pastures limit energy intake and increased the energy costs of grazing [2]. This initiates a prepartum onset of negative energy balance and loss of BCS [3] in order to meet the greater energy demands of the developing fetus and mammary gland [4]. Wiltbank *et al.* [5] suggested that low-energy diets during the last trimester could adversely affect calf survivability and performance. Moreover, greater pre and postpartum body reserves (BCS) increased milk yield and calf performance in grazing conditions [3] and increased an overall profits from the cow-calf operations.

Adaptive mechanisms during periods of dietary restriction and/or negative energy balance include increased blood concentrations of GH, which in turn stimulates a state of insulin resistance in the peripheral tissues and redirects nutrients – especially glucose - to the fetus and/or milk production [4, 6, 7]. This increased GH is accompanied with increased fat mobilization (increased NEFA) and reduced insulin concentrations which is believed to be part of the consequence of uncoupling the GH-IGF axis [6, 8]. Reduced IGF-I concentrations in dairy cows during early lactation are associated with reduced expression of the liver-specific GH receptor-1A (GHR1A) and IGF-I mRNA [9]. These changes have been associated with negative energy balance, decreased concentrations of insulin [10] and reduced hepatic expression of IGFBP-3 mRNA [11, 12]. However, hepatic expression of GHR1A and IGF-I mRNA did not change after calving in *ad libitum*-fed beef cows [13] or in a strain of low-producing dairy cattle [14], indicating that this mechanism is related to genetic potential for milk production.

Effects of nutritional manipulation on the GH-IGF axis in ruminants have been contradictory. Lake *et al.* [15] reported elevated GH and reduced IGF-I concentrations in serum from postpartum beef cows managed nutritionally during gestation to achieve a low rather than a high BCS (4 vs. 6 on a 1 to 9 unit scale) at calving. Reduced plasma IGF-I concentration and hepatic mRNA expression of IGF-I in underfed ewes were not associated with changes in hepatic GHR or GHR1A mRNA [16]. Depressed GHR abundance in the liver did not involve reduced GHR1A mRNA in feed- restricted dairy cows in late lactation [17]. However, decreased serum IGF-I and hepatic IGF-I mRNA were linked to decreased expression of GHR1A and 1C mRNA in the liver of feed-restricted steers [18].

Beef cows with different body reserves (BCS) have distinctive productive and reproductive performances associated with differential metabolic/endocrine profiles (REFs), but only a few studies have been performed to exam the hepatic mechanisms related to uncoupling the GH-IGF axis during the peripartum period. An improved understanding of the mechanisms that regulate nutrient partitioning could help improve nutritional management of the peripartum cow. Thus, the objective of this study was to evaluate hepatic expression of somatotropic axis genes and their associations with changes in BCS, milk production, and metabolite and hormone concentrations in grazing, suckled, primiparous beef cows during the peripartum period.

2.3 MATERIALS AND METHODS

The experiment was carried out at Palo a Pique Experimental Unit of the Instituto Nacional de Investigacion Agropecuaria (Treinta y Tres, Uruguay; 33º S, 56º W) from May to November 2007. Animal procedures were approved by the Animal Experimentation Committee of Universidad de la República (UdelaR, Montevideo, Uruguay).

Animals and Experimental Design

Twenty primiparous Aberdeen Angus x Hereford cows (471 ± 6.5 kg of BW) with a BCS of 6 (scale 1 to 8 units; [19]) at 16 weeks prepartum (May, 2007) were selected from a group (n = 60) according to calving date (all calved within a 28-days period) for the study. Cows were classified at -35 d postpartum (DPP) into thin (BCS < 4.5) or moderate (BCS ≥ 4.5) BCS groups and blocked by calving date. A BCS of 4.5 has been reported as the critical BCS at calving because subsequent reproductive performance of primiparous cows is reduced if their BCS at calving is < 4.5 [2]. Changes in BCS were not due to dietary treatments, because all 20 cows were managed together in the same pasture.

During the pre and postpartum periods, cows were managed as a contemporary group in a herd of 60 cows and grazed a native pasture paddock (60 ha) with good access to water. Available forage was determined by cutting squares (0.3 x 0.3 m, n=60; [20]) every 28 d from -49 to 49 DPP. Forage provided 453 ± 24 kg dry matter (DM)/ha with 132 ± 19 g of CP and 244 ± 6 g of ADF per kg DM during the prepartum (-49 to 0 DPP) and 552 ± 61 kg DM with 144 ± 7 g of CP and 251 ± 1g of ADF per kg DM during the postpartum (0 to 49 DPP) periods. From 17 to 28 ± 9 DPP, cows were offered an additional 4.7 kg DM/cow/day of pasture hay (137 g/kg of CP and 300 g/kg of ADF).

During the prepartum period (May to August, end of fall) monthly precipitation was 154 ± 65 mm, average daily temperature was 10 ± 1 ºC (5 and 15 ºC for minimum and maximum), and there was an average of 6 ± 1 d with frost per month. During the postpartum period (September to November, spring) monthly precipitation was 108 ± 25 mm, average daily temperature was 17 ºC (11 and 23 ºC for minimum and maximum), and there was an average of 1 ± 0.3 d with frost per month.

Data and Sample Collection

Body weight and BCS were measured every 14 day from -49 to 49 DPP. Body condition scores, at 0.25 unit intervals, from 1.0 (very thin) to 8.0 units (very obese), were assessed using a modified scale [19]. Blood samples were collected weekly from -49 to 49 DPP via jugular venipuncture using Vacutest® tubes (8 mL, Vacutest Kima, Arzergrande, Italy) that contained clot activator gel. Samples were centrifuged (2000 X g for 15 min at 4°C) within 2 h after collection and serum was stored at -20° C until assayed. Milk samples and liver biopsies were obtained from a subset of 12 cows (n = 6 per BCS group). Milk production was determined at 14 and 35 ± 4 DPP using a mechanical milking device [3]. Samples from these milkings were preserved with potassium dichromate upon collection and analyzed for fat, protein, and lactose by infrared analyses (Bently 2001; Bently, USA; Dairy Laboratory; INIA-La Estanzuela, Colonia, Uruguay). Calf weight was determined at birth and at 40 ± 9 days of age.

Liver biopsies (approximately 0.5 g of tissue) were obtained at -11 ± 4, and 7, 31, and 52 ± 2.5 DPP using a 14-gauge biopsy needle (Tru-Core®-II Automatic Biopsy Instrument; Angiotech, Lausanne, Suitzerland) as described by Carriquiry *et al.* [12]. Liver samples were placed in a screw-cap microcentrifuge tube, immediately frozen in liquid nitrogen and stored at −80°C until total RNA was extracted.

Serum Analyses

Metabolites and insulin concentrations were measured weekly in all cows of the study. Non-esterified fatty acids, cholesterol, total protein, albumin, and urea concentrations were determined spectrophotometrically using commercial kits (Wako NEFA-HR(2) from Wako Pure Chemical Industries, Ltd. Osaka, Japan, and Biuret, Bromocresol Green, and Ureasa/Salicilato from BioSystems S.A., Barcelona, Spain; respectively) with volume of samples and reagents adjusted to a 96-well microplate and read in a Multiskan EX (Thermo

Scientific, Waltham, MA, USA). All samples were determined in a single assay for each metabolite. The intra-assay CV values for low, medium and high controls were not greater than 7.6, 8.6, and 12.3 %; respectively.

Insulin concentrations were quantified by solid-phase RIA (Coat and Count, Diagnostic Products Co, Los Angeles, CA, USA). The sensitivity of the assay was 1.6 uUI/mL; intra and inter-assay CV for low (2.85 uUI/mL), medium (16 uUI/mL) and, high (35.5 uUI/mL) controls were not greater than 7, 6.3, and 10.7% respectively.

Isolation and Purification of RNA

Total RNA was isolated using TRIzol (Invitrogen, Life Technologies, Carlsbad, CA, USA), followed by precipitation with lithium chlorideand by DNase-treatment with a DNA-Free Kit (Applied Biosystems/Ambion, Austin,TX, USA). Concentration of RNA was determined by measuring absorbance at 260 nm (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 elecrophoresis in 1% agarose gel. Isolated RNA was stored at −80°C until analyzed by quantitative real time PCR.

Quantitative Real Time PCR

The SuperScript®III First-Strand Synthesis System kit (Invitrogen) using random hexamers and 1 μg of total RNA as a template was used to conduct the reverse transcription (in a single run). The cDNA was stored at -20 ºC until use in the quantitative reverse transcription PCR (RT-PCR). Primers (Table 1) were designed (Primer Express Software; Applied Biosystems, Foster City, CA, USA) specifically to amplify cDNA for GHR, GHR1A, IGF-I, IGFBP-2, and IGFBP-3 as target genes of interest and for hypoxanthine phosphoribosyltransferase (HPRT) and ß-actin as endogenous controls. Before use, primer

product sizes (1% agarose gel separation) and sequence (capillary electrophoretic DNA analyzer ABI3130xl; Applied Biosystems) were determined to ensure that the primers produced the desired amplification products. Hypoxanthine phosphoribosyltransferase and ß-actin have been used before as an endogenous control in tissues from ruminants [11, 12] and their expression was stable between BCS groups and across time points in the samples of this study.

Real time PCR reactions were performed using 10 µL SYBR®Green mastermix (Quantimix EASY SYG kit, Biotools B&M Labs, Madrid, Spain), 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 20 µL . Samples were analyzed in duplicate in a 72-disk Rotor-GeneTM 6000 (Corbett Life Sciences, Sydney, Australia). Standard amplification conditions were 10 min at 95 ºC and 40 cycles of 15 s at 95 ºC, 45 s at 60 ºC, and 20 s at 72 ºC. Dissociation curves were generated after the last cycle. Each disk included duplicate wells of water (no template) for each set of primers and cDNA standard curves for the genes being analyzed in the plate. Plasmids that encoded the GHR, GHR1A, IGF-I, IGFBP2, IGFBP3, HPRT or ß-actin genes were diluted in yeast cDNA to achieve a starting concentration of 10^6 gene copies/µL for subsequent serial dilutions (n = 6 dilutions from 10^6 to 10^1). Linear regression was used to estimate the number of copies of target and control gene message in the samples. Expression of each target gene was normalized with average expression of HPRT and ß-actin controls genes. Intra and inter-assay CV values were 1.9 and 4.2 %, respectively, and the efficiency of quantification for mRNA expression was 99, 118, 101, 124, 102, 135, and 109%, for GHR, GHR1A, IGF-I, IGFBP-2, IGFBP-3, HPRT, and ß-actin, respectively.

Table 1 Primers used for the quantification of target and endogenous control gene cDNA

Gene[1]	Accession no.[2]		Primer sequence	Lenght (bp)	Source
GHR	NM_176608	Sense	TCTGGGAATCCTAAATTCACCAA	91	[40]
		Antisense	CTGTAAACTGTGATTAGCCCCATCT		
GHR 1A	AY748827	Sense	AGCCTGGAGGAACCATACGA	94	[40]
		Antisense	GCTGCCAGAGATCCATTCTGTA		
IGF-I	XM_612412	Sense	CCAGACAGGAATCGTGGATG	89	[40]
		Antisense	ACTTGGCGGGCTTGAGAG		
IGFBP-2	NM_174555	Sense	ATGCGCCTTCCGGATGA	83	This paper
		Antisense	GTTGTACAGGCCATGCTTGTCA		
IGFBP-3	NM_174556	Sense	AGCACAGACACCCAGAACTTCT	86	[40]
		Antisense	TTCAGCGTGTCTTCCATTTCC		
ACTB	BT030480	Sense	CGTGGC TACAGCTTCA CC	53	This paper
		Antisense	GAA ATCGTCCGTGACATCAA		
HPRT	XM_580802	Sense	TGGAGAAGGTGTTTATTCCTCATG	105	[12]
		Antisense	CACAGAGGGCCACAATGTGA		

[1]GHR = growth hormone receptor, GHR1A= growth hormone receptor 1A, IGF-I = insulin-like growth factor-I, IGFBP-2 = IGF-binding protein-2; IGFBP-3 = IGF-binding protein-3, ACTB= ß-actin (endogenous control gene), HPRT = hypoxanthine phosphoribosyltransferase (endogenous control gene)

[2]GeneBank bovine sequences.

Energy Calculations

All estimates of energy requirements were calculated according to NRC [22]. Milk energy output was calculated as NEL = milk yield × [(0.0929 × fat %) + (0.0563 × true protein %) + (0.0395 × lactose %)], using composition data derived from analysis of the samples collected.

Statistical Analyses

Data were analyzed in a randomized block design (cows of different BCS were blocked by calving date) 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. Cow BW, BCS, milk production and composition, serum metabolite and hormone concentrations, and hepatic mRNA expression were analyzed as repeated measures using the MIXED procedure with DPP as the repeated effect and first-order autoregressive (for evenly spaced data) or spatial power law (for unevenly spaced data) as the covariance structure. The Kenward-Rogers procedure was used to adjust the denominator degrees of freedom. The model included BCS group, DPP, and their interaction as fixed effects, and cow and block as random effects. Tukey-Kramer tests were conducted to analyze differences between cow groups and DPP. In addition, BCS response was fitted to appropriate order-polynomial curves. Correlation analysis was used to describe relationships between variables using the CORR procedure (SAS Institute Inc). Results are expressed as lsmeans ± SE. For all results, means were considered to differ when $P \leq 0.05$, and trends were identified when $0.05 < P < 0.10$.

2.4. RESULTS

Cow and calf performances

Moderate cows had greater ($P < 0.001$) BW (413 vs. 394 ± 2.5 kg) and BCS (4 vs. 3.6 ± 0.03 units) than thin cows throughout the 98-days period and there was an effect of DPP ($P < 0.001$) on both variables (Figure 1A and B). Evolution of BCS fit a

quadratic model (P < 0.001) for both BCS groups (Figure 1B). Intercept values indicate BCS was 0.5 greater (P < 0.001) in moderate than thin cows (3.4 vs. 3.9 ± 0.05) at calving. Linear coefficients did not differ (-0.012 ± 0.001; P = 0.334) but the quadratic coefficient tended (0.00014 vs. 0.00021 ± 0.00002; P = 0.095) to be less for moderate than thin cows which is consistent with a similar rate of peripartum reduction in BCS but a slower postpartum recovery of BCS in the moderate BCS cows. Cow BCS nadir was greater (P < 0.001) in moderate than thin cows (3.6 vs. 3.3 ± 0.04) but the moment of nadir did not differ between groups (28 vs. 42 ± 5 DPP for moderate and thin cows, respectively). Calf BW at birth (34.2 vs. 29.0 ± 1.0 kg) and at 40 days of age (64.4 vs. 55.4 ± 3.2 kg), and ADG (0.81 vs. 0.65 ± 0.05 kg/day) from birth to 40 days of age were greater (P < 0.05) for calves from moderate than thin cows.

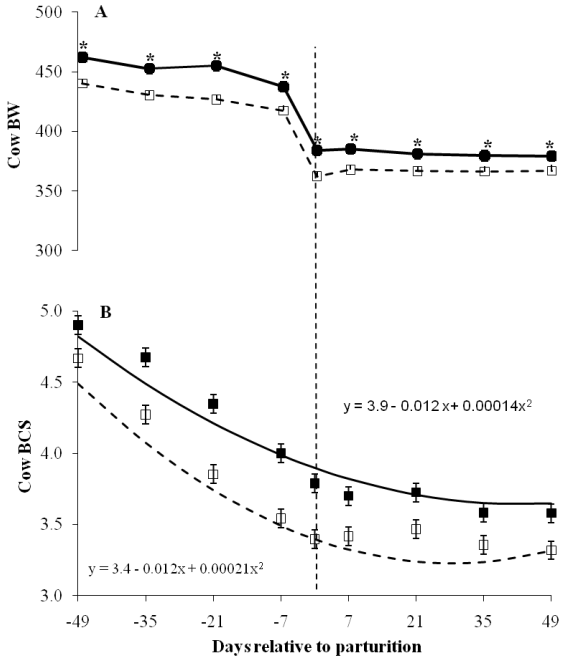

Figure 1 Evolution of BW (A) and BCS (B) during peripartum period (from -49 to 49 days relative to parturition) in primiparous beef cows classified according to BCS at -35 days prior to parturition (10 moderate ■ vs. 10 thin □). Day of calving is indicated by the dashed vertical line. Differences between cow groups are indicated with * when $P \leq 0.05$.

Milk Production

Milk production did not differ between BCS groups, but was affected by DPP (P = 0.020) and an interaction (P = 0.037) of BCS group and DPP (Figure 2). While milk production was maintained in moderate BCS cows, it declined 1.9 kg in thin cows from 14 to 35 DPP. Milk composition did not differ between BCS groups and averaged 2.70 ± 0.30, 2.96 ± 0.11, and 4.95 ± 0.6% for fat, protein, and lactose, respectively. Milk protein and fat percentages tended to decreased (P < 0.080) while lactose percentage increased (P < 0.001) from 14 to 35 DPP in both groups (data not shown). Consistent with the impact on milk yield, there was a trend for an interaction (P < 0.080) of BCS group and DPP on protein and lactose yield and milk energy output as they decreased milk energy output decreased (P < 0.05) only in thin cows (0.17 vs. 0.13 ± 0.02 kg, 0.30 vs. 0.23 ±0.03 kg, 16.0 to 14.0 ± 1.3 MJ/d and 16.7 to 10.2 MJ/d from 14 to 35 DPP for protein and lactose yield and milk energy output in moderate and thin cows, respectively).

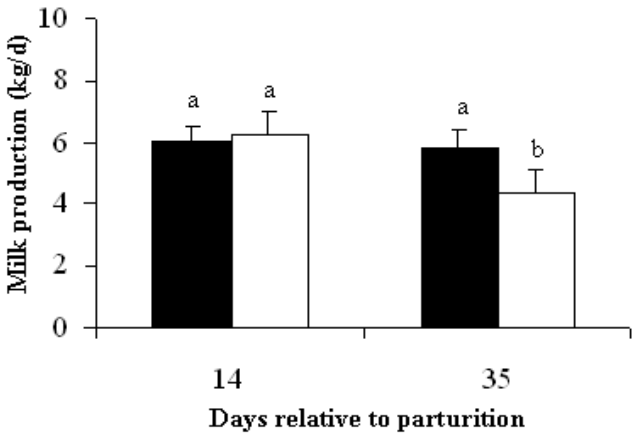

Figure 2 Milk production of primiparous beef cows classified according to BCS from -35 days to parturition (moderate ■ vs. thin □) at 14 and 35 days of lactation**.** [a, b]Letters above the columns denote least square means differences $P \leq 0.05$.

Metabolites and Hormones

Serum NEFA concentration (Figure 3A) did not differ between BCS groups but was affected by DPP ($P < 0.001$). Concentrations of NEFA were elevated during the prepartum, peaked at -7 DPP, decreased until 21 DPP, and were maintained thereafter. However, the peak in NEFA at -7 DPP was only evident in moderate BCS cows. Serum cholesterol (Figure 3B) did not differ between BCS groups, but was affected by DPP ($P < 0.001$) as concentrations remained low from -49 to 21 DPP and increased thereafter. Although the interaction between BCS group and DPP was not significant, serum cholesterol appeared to increase after 21 DPP in moderate BCS cows.

Serum urea concentration (Figure 3C) did not differ between BCS but was affected by DPP ($P < 0.001$) and by the interaction ($P = 0.033$) between BCS group and DPP. Concentrations of urea in moderate BCS cows were increased at -7 DPP and decreased at 7 DPP whereas serum urea remained constant in thin cows. Total serum protein concentration (Figure 3D) did not differ between BCS groups but was affected by DPP ($P < 0.001$) and by the interaction ($P = 0.018$) between BCS group and DPP. Total serum protein concentrations in moderate BCS cows decreased from -21 to 7 DPP, were reduced until 35 DPP, and increased at 49 DPP. In contrast, total serum protein in thin cows increased at -7 DPP and decreased thereafter until 49 DPP. Total protein concentrations were greater ($P < 0.05$) in moderate than thin BCS cows at -21 and 49 DPP (Figure 3D). Serum albumin concentration (Figure 3E) was greater ($P < 0.001$) for moderate than thin BCS cows (42.3 vs 32.0 ± 1.8 g/L) and there was an effect of DPP ($P = 0.033$). Albumin concentrations were greater from -49 to -7 DPP and decreased thereafter until 49 DPP, but this effect was only evident in moderate BCS cows as serum albumin in thin cows were stable throughout the period evaluated.

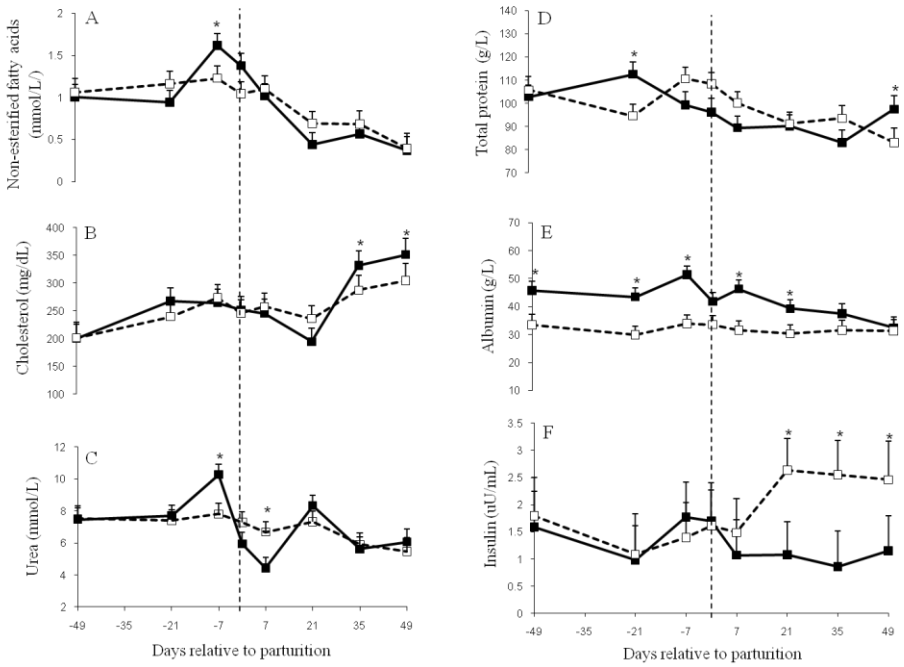

Figure 3 Concentrations of NEFA (A), cholesterol (B), urea (C), total protein (D), albumin (E), and insulin (F) during peripartum period (from -49 to 49 days relative to parturition) in primiparous beef cows classified according to BCS from -35 days to parturition (10 moderate ■ vs. 10 thin □). Calving is indicated with dashed lines. Differences between cow groups are indicated with * when $P \leq 0.05$.

Serum insulin concentrations (Figure 3F) were relatively stable in thin and moderate BCS cows during the prepartum period and remained stable in the moderate BCS cows postpartum. However, insulin concentrations increased in thin cows after 7 DPP and this resulted in an overall lower ($P = 0.024$) concentration in moderate than in thin cows (1.28 vs. 1.88 ± 0.38 uU/mL).

Hepatic mRNA expression

Expression of GHR mRNA tended to be greater (P = 0.075) for moderate than thin cows (1.4 vs. 1.1 ± 0.1) but was not affected by DPP or their interaction. Hepatic GHR mRNA was greater (P = 0.012) for moderate than thin cows only at -11 DPP (Figure 4A). Expression of GHR1A was not affected by BCS group or DPP but there was an interaction between BCS group and DPP (P = 0.050) as GHR1A mRNA increased (P < 0.050) at 52 DPP only in thin cows (Figure 4B). Hepatic IGF-I mRNA expression were reduced (P = 0.041) in moderate than thin cows, but was not affected by DPP or their interaction. Expression of IGF-I mRNA was less (P = 0.020) in moderate than thin cows only at 52 DPP (Figure 4C). Expression of IGFBP-2 mRNA was affected by the interaction of BCS group by DPP (P = 0.045) as abundance of this transcript decreased from -11 to 52 DPP in moderate BCS cows, while it tended to increase in thin cows during the same period (Figure 4D). Expression of IGFBP-3 mRNA was not affected by BCS group, DPP or their interaction (Figure 4E).

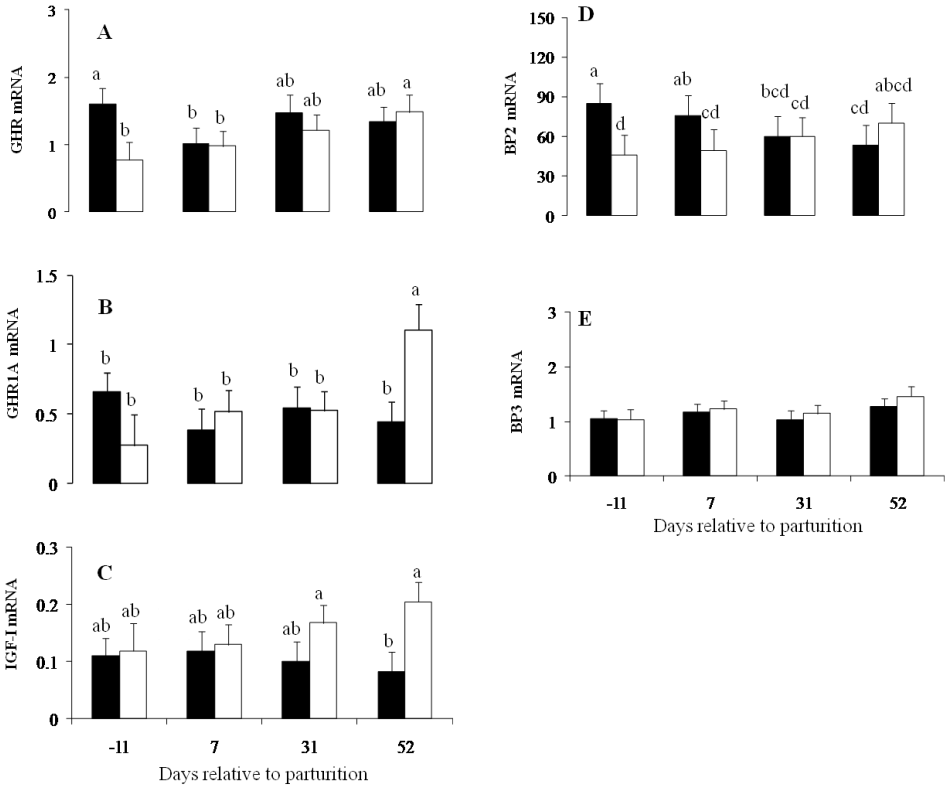

Figure 4 Hepatic expression of genes related to the GH-IGF-I axis during peripartum period (from -49 to 49 days relative to parturition) in primiparous beef cows classified according to BCS from -35 days to parturition (10 moderate ■ vs. 10 thin □). Amount of mRNA expressed relative to expression of endogenous control genes (ß-actin and hypoxanthine phosphoribosyltransferase). [a, b.] Letters above the columns denote least squares means differ $P \leq 0.05$.

Independent of BCS groups or DPP, expression of GHR and GHR1A mRNA were correlated (r = 0.47, P = 0.003). In addition, expression of GHR1A mRNA was correlated with the expression of IGF-I (r = 0.56, P <0.001) and IGFBP-3 (r = 0.43, P = 0.007) mRNA and hepatic IGF-I mRNA were correlated with IGFBP-3 mRNA (r = 0.52, P < 0.001). Expression of IGF-I mRNA was positively correlated (r = 0.45, P = 0.010) while IGFBP2 mRNA was negatively correlated (r = -0.37, P = 0.036) with serum insulin concentrations.

2.5 DISCUSSION

Loss of body condition during the prepartum period can vary among cows and magnitude of this loss has affected subsequent cow performance [2, 23]. Our moderate and thin cows had the same BCS (6.0 units) at -112 DPP (end of fall) and were managed together under the same grazing conditions. The moderate cows lost less body condition (1.3 vs. 1.8 ± 0.07 units) from -112 to -35 DPP and both groups loss an additional 0.8 ± 0.09 units during the last month of gestation. Actual reasons for the differential loss in BCS between moderate and thin cows prior to -35 DPP are unknown but it could be due to a combination of individual animal variation in intake (i.e. better grazing ability, greater social hierarchy) or efficiency (reduced demand for energy maintenance) [24] when quantity and quality of forage in native pasture is limiting (winter). The moderate BCS cows calved with 0.5 units more BCS (3.9 vs. 3.4 units) and produced more milk at 35 DPP (5.9 vs. 4.2 kg/d) than the thin cows. The moderate BCS cows also produced heavier calves that grew more rapidly than the calves from the thin cows. These differences in milk yield and calf performance were associated with an increase in insulin concentrations and hepatic mRNA expression of GHR1A and IGF-I mRNA during the second month of lactation in the thin BCS cows.

Cows in both groups lost more BCS during the pre than the postpartum interval (2.1 vs. 0.3 units and 2.6 vs. 0.1 units for pre and postpartum intervals in moderate and thin cows, respectively). The larger prepartum losses reflected the reduced forage availability in native pastures during winter which prevented cows from consuming enough feed to meet the energy demands of the last trimester of gestation. Cows that lost less condition during the prepartum interval were classified as having a moderate BCS at calving and these cows also maintained a greater postpartum BCS than cows that lost more condition during the prepartum period. Similarly, Ciccioli *et al.* [25] and Lake *et al.* [26] determined in cows fed during gestation to achieve different BCS at calving and differences in BCS between groups were greater than in the present study, that cows that calved with greater BCS maintained greater BCS during the postpartum.

Nutrient intake of cows during gestation has [28, 29] and has not [25, 26] altered calf BW at birth. Although both the thin and moderate cows had less than the critical 4.5 BCS at calving [2], the small difference in BCS at calving (0.5 units) and/or other differences in metabolism between the groups appears to have been sufficient to elicit differences in calf birth weight. Presumably this difference in calf birth weight was due to a greater supply of nutrients to the growing calf [4]. Calves from the moderate BCS cows grew more rapidly during the first 40 days of age which is consistent with previous reports [30, 31] of a positive correlation between milk yield, and calf ADG and BW in beef cattle.

Milk production and composition from our thin and moderate BCS cows did not differ at 14 DPP. This is consistent with reports that milk yield in early lactation was not influenced by BCS at calving [26] or energy intake during the prepartum period [32]. However, moderate BCS cows had greater milk yield and energy output, associated with greater milk protein and lactose yields at 35 DPP. Lake *et al.* [26] reported greater milk protein percentages but not fat percentages in cows with greater BCS at calving, and suggested that this increase was probably associated with increased availability of tissues used for mobilization in cows that maintained greater body energy reserves.

The delayed replenishment of body reserves (reflected in a greater quadratic coefficient of the BCS regression) in moderate cows is consistent with greater partitioning of dietary nutrients and energy toward milk production. Alternatively, greater milk production at 35 DPP in moderate BCS cows could be associated with greater feed intake [33]. However, thin BCS cows had a faster postpartum recovery of BCS when forage availability and quality increased as temperature rise in spring [35] associated with an increase in serum insulin after 21 DPP, which would suggest an increase feed intake in this group of cows. In contrast, greater cholesterol and total protein concentrations at 35 and/or 49 DPP associated to a better nutritional status [34] were detected in moderate BCS.

Concentrations of NEFA were elevated during the prepartum period in both cows groups reflecting mobilization of lipid stores during the negative energy balance period [36]. A peak in NEFA and urea concentrations were observed only in moderate BCS cows at -7 DPP and could reflect greater mobilization reserves from adipose tissue [37] and muscle [38]. This greater mobilization could probably be caused by greater energy requirements for conceptus growth [4] and maintenance of maternal non-uterine tissues [28] as estimated energy requirements (maintenance and gestation; estimated by NRC [22]) were 11% greater in moderate than thin cows during this period. With the milk production observed in this study, estimated energy requirements did not appear to increase dramatically from late gestation to early lactation (in average 46.4 vs. 51.0 MJ/d NEm for both groups; estimated by NRC [22]) which could explain the postpartum decrease in serum NEFA.

The onset of lactation did not modified insulin concentration profiles as insulin did not decrease from pre- to postpartum as typically observed in dairy cows [37]. Low insulin concentrations favor gluconeogenesis and lipolysis [7]. However, while serum insulin in moderate BCS cows remained low during the entire period evaluated, insulin concentrations increased in thin cows by 21 DPP and remained elevated through 49 DPP. The insulin increase in thin cows during the pospartum period is an interesting finding without an obvious explanation. Adiponectin and other compounds from adipose depots are involved with regulation of energy homeostasis (39, 40).Adiponectin has been correlated negatively with adiposity and increases after weight reduction, and promotes insulin secretion and sensibility, increases food intake and reduces energy expenditure [39, 40]. This increase in insulin concentrations were associated with a faster recovery of BCS (reflected in a greater quadratic coefficient of the BCS regression) and could explain the reduction in milk yield and energy output at 35 DPP in thin cows as a negative association between insulin and milk yield have been reported previously for beef cows [3, 41].

In agreement with the insulin profile around parturition, and in contrast with pattern of expression of GH-IGF axis gene in dairy cows [8], in this study there were no changes in hepatic GHR1A and IGF-I mRNA expression from pre to postpartum (-11 vs. 7 DPP). Similarly, Jiang *et al.* observed no differences in hepatic expression of these transcripts around parturition in beef cows fed *ad libitum* (-21 vs. 21 DPP) [13] or during lactation (0 to 40 DPP) in beef cow grazing native pastures [42]. However, we found a differential expression of GHR1A and IGF-I at 52 DPP associated with BCS at calving, as expression of these transcripts were less in moderate than thin cows. The increase in GHR1A and IGF-I mRNA in thin cows at 52 DPP was associated with the increase in serum insulin concentrations and reduction in milk production observed in this group after 21 DPP. Schneider *et al.* [42] and Spicer *et al.* [43] determined greater plasma IGF-I in crossbred cows (*Bos indicus* x *Bos Taurus*) compared with Angus cows and the difference in plasma IGF-I become more evident as lactation progressed, suggesting that this differences would be associated to lactational demand since *Bos indicus* produce less milk. In addition, the positive correlation between IGF-I mRNA and insulin concentrations in our study was in agreement with the key role of insulin in regulating hepatic GHR1A and IGF-I expression [10]. Cows that maintained greater body energy reserves (moderate BCS cows) during the pre- and postpartum were able to maintain milk yield and energy outputs for a longer interval and did not increase GHR1A and IGF-I mRNA abundance at 52 DPP. Reduced expression of this transcripts has been associated with a state of GH resistance in the liver as circulating GH concentration increase [8], has been linked with an antagonism between GH and insulin actions, characteristic of partitioning of nutrients towards the mammary gland [7].

Expression of IGF-I and GHR1A mRNA were correlated, whereas no relationship with total GHR mRNA was found. This is consistent with previous report in dairy cows that total GHR mRNA has proven not to be correlated with GHR activity [17]. Hepatic expression of GHR mRNA was greater at -11 DPP in moderate than thin cows probably associated with their better metabolic status. The greater total GHR

mRNA expression in moderate BCS cows at -11 DPP was not only due to a greater expression of GHR1A mRNA, as ratio of GHR to GHR1A mRNA was similar between BCS groups, which would suggest that other transcripts of the GHR gene were involved [18]. On the contrary, the increase in total GHR mRNA in thin cows at 52 DPP was associated with the greater expression of GHR1A and IGF-I transcripts.

In contrast with the postpartum decrease in hepatic IGFBP-2 mRNA expression in dairy cows [11, 12], no changes were observed in the expression of this transcript in the present study. The decrease IGFBP-2 mRNA in moderate BCS cows towards 52 DPP would agree with their greater milk production and cholesterol concentrations during the postpartum, and indicate a better nutritional status [44] as reflected in the negative correlation between circulating insulin concentration and IGFBP2 mRNA determined in this study. In addition, the trend to an increase IGFBP-2 mRNA during the postpartum in thin cows could be associated with the reduced reproductive performance reported previously [3] in thin beef cows as delayed return to ovarian cyclicity ocurred in cows with greater blood concentrations of IGFBP-2 [45].

In summary, in spring-calving primiparous beef cows changes in body reserves and metabolite/endocrine profiles that reflected negative energy balance were more associated with the decrease in forage quantity and quality of native pastures during winter than to onset of lactation. Results indicate that the molecular mechanism of uncoupling of GH-IGF axis in the liver around parturition described in dairy cows did not occur in beef cows in rangeland conditions. However, metabolic/endocrine profiles as well as liver expression of the somatotropic axis genes were associated with evolution of body reserves during the prepartum and indicated that body energy reserves, reflected in a rather small difference in BCS (0.5 units), signal a differential nutrient partitioning towards mammary gland, increasing milk production and calf growth.

2.6 ACKNOWLEDGMENTS

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3. INCREASED NUTRITIONAL PLANE BEFORE MATING PERIOD IN BEEF COWS

Effects of a short-term increase in the nutritional plane before the mating period on metabolic and endocrine parameters, hepatic gene expression, and reproduction in primiparous beef cows on grazing conditions

Astessiano, A. L.[A], R. Pérez-Clariget[A], G. Quintans[B,] P. Soca[A], M. Carriquiry[A]

[A] Facultad de Agronomía, Universidad de la República, Montevideo, Uruguay
[B] Instituto Nacional de Investigación Agropecuaria Treinta y Tres, Treinta y Tres, Uruguay.

Corresponding author: Ana Laura Astessiano. E-mail: lauaste@gmail.com

Increased nutritional plane before mating period in beef cows

3.1 SUMMARY

Sixty-four spring-calved primiparous crossbred cows paired by calving date, and body condition score (BCS) at calving were used to study the effect of a short-term increase in the nutritional plane before the mating period on cow and calf performance, changes in metabolic and endocrine parameters, and hepatic gene expression. At 48 ± 10 days postpartum (onset of nutritional treatment = day 0), cows were assigned to two treatments during 23 days: control (grazing of native pastures; NP; n= 31) and increased nutritional plane (native pastures improved with *Lotus subbiflorous* cv Rincón; IP; n=33). Cow body weight (BW), BCS and total protein and albumin concentrations increased while urea and NEFA levels decreased from the beginning of the nutritional treatment in both groups, indicating the animal positive energy balance as forage growth and availability of pastures increased during spring. In addition cow BW and BCS, as well as calf average daily gain and BW were greater in IP than NP cows groups. Insulin concentrations were less in IP than NP (1.37 vs. 2.25 ± 0.26 uU/mL) because insulin increased due to nutritional treatment only in NP cows. Hepatic insulin receptor mRNA at day 23 tended to be 1.5- fold greater while insulin growth factor binding protein-3 mRNA expression was 1.7-fold greater in NP than IP cows. Reproductive responses were not affected by nutritional treatment but days to initiation of ovarian ciclicity (108 ± 10 days) were positively correlated with insulin concentrations. Grazing of improved native pastures for 23 days before the mating period did not improve cow reproductive performance but modified metabolic, endocrine, and gene expression parameters, in agreement with greater nutrient and energy partitioning towards milk production, reflected in better calf performance.

Key words: cattle, pastures, metabolites, hormones, mRNA.

3.2 INTRODUCTION

In extensive rangeland cow-calf systems, variability in quantity and quality of pastures limits the energy intake, increases the grazing costs, and impacts negatively on reproductive performance of beef cows. Prepartum nutrition, reflected in body condition score (BCS) at calving, is the most important factor determining the duration of the postpartum anestrus period in primiparous beef cows (Perry *et al.* 1991; Lalman *et al.* 1997). However, postpartum nutrition may overcome partially the negative effects of a restriction in prepartum nutrition in thin to moderate BCS first-calf cows (Perry *et al.* 1991; Lalman *et al.* 1997). Increased energy intake for 20 to 25 days, before or during the mating period, in combination with suckling management (temporary weaning, with or without separation of the calf) has shown to increase pregnancy rates during the first half of the mating period in primiparous cows with low to moderate BCS (Pérez-Clariget *et al.* 2007). However, changes in metabolism associated with this response are not clear.

Blood concentrations of glucose, insulin, and insulin-like growth factor -I (IGF-I) are indicative of the availability of energy, provide short- or long-term signals that mediate the effects of nutrition on reproduction (Bossis *et al.* 2000), and are increased with supplementation in beef females (Cooke *et al.* 2007; 2008). The IGF-I plays a pivotal role in cattle fertility, acting as a monitoring signal that allows reproductive events to occur when nutritional conditions for successful reproduction are reached (Velazquez *et al.* 2008). The hepatic secretion of IGF-I is stimulated by the union of growth hormone with its receptor (GHR) and its action is modulated by different binding proteins (IGFBP1-6) that can inhibit or potentiate IGF-I role. In beef cows, dietary restriction or periods of negative energy balance are associated with increased circulating IGFBP-2 and decreased IGFBP-3 (Rajaram *et al.* 1997) and it has been determined that IGFBP-3 was suppressed and IGFBP-2 was elevated in cows that failed to resume ovarian cyclicity early in the postpartum (Roberts *et al.* 1997).

Nutritional status is reflected in changes in body weight (BW) and BCS as well as in levels of metabolites and hormones in blood, and these changes can be used to discuss the relationship between energy balance and postpartum reproductive physiology. The objective of this study was to evaluate the effect of a short-term increase in the nutritional plane, by grazing of a native pasture improved with *Lotus subbiflorous* cv Rincón, before the mating period on production and reproduction responses, and changes in metabolic, endocrine, and hepatic gene expression parameters of primiparous beef cows.

3.3 MATERIALS AND METHODS

The experiment was carried out at Palo a Pique Experimental Unit of the Instituto Nacional de Investigaciones Agropecuarias Treinta y Tres, Uruguay (34ºS, 54ºW) from August 2007 to January 2008. Animal procedures were approved by the Animal Experimentation Committee of the Universidad de la República.

Animals and Experimental Design

Sixty-four spring-calved primiparous crossbred beef cows (Aberdeen Angus x Hereford) with BCS of 3.6 ± 0.04 units and BW of 392 ± 5 kg at calving, and in anestrus, were used. Cows were paired by calving date and BCS at calving, and at 48 ± 10 days postpartum (onset of nutritional treatment = day 0) were assigned to two treatments during 23 days before the mating period: control (NP; grazing of native pastures; 60 ha, 1301 ± 362 kg dry matter (DM)/ha, 6.4 ± 1.5 cm of sward height; n=31, Table 1), and increased nutritional plane (IP; native pastures improved with *Lotus subbiflorous* cv Rincón; 50 ha, 2846 ± 733 kg DM/ha, 16% Lotus Rincón, 14.9 ± 3.2 cm of sward height; n=33, Table 1). Before and after the nutritional treatments, cows grazed together on a native pasture paddock (60 ha), with an average forage mass available of 453 ± 24 kg DM/ha, and 1306 ± 189 kg DM/ha, respectively (Table 1). Cows were naturally mated at 71 ± 10 days postpartum (end of the nutritional treatment = day 23) with two bulls with previous andrological evaluation (McGowan *et al.* 1995) and the mating period lasted 80 days.

Table 1 Pasture chemical composition before, during, and after nutritional treatments

Chemical Composition	Native pasture			Improved native pasture[1]
	Before[2]	During	After	
Dry matter (%)	28.8 ± 4.8	26.3 ± 2.2	48.6 ± 11.1	19.5 ± 2.2
Crude protein (%, DM basis)	13.2 ± 1.9	12.8 ± 1.4	8.0 ± 0.9	13.2 ± 1.5
Neutral detergent fiber (%, DM basis)	51.1 ± 4.8	55.9 ± 2.8	63.9 ± 4.8	52.9 ± 2.7
Acid detergent fiber (%, DM basis)	24.4 ± 0.6	29.3 ± 4.3	34.1 ± 1.3	29.9 ± 1.4
Metabolizable energy (MJ/kg DM)	6.2 ± 0.1	7.3 ± 0.1	6.0 ± 0.1	8.3 ± 0.1

[1]Native pastures improved with *Lotus subbiflorous* cv Rincón

[2] Before (from calving to day 0), during (days 0 to 23), and after (days 24 to 103)

Data and Sample Collection

Cow BW and BCS (scale 1–8; Vizcarra *et al.* 1986), and calf BW were measured at 14 day-intervals from days -9 to 103. Blood samples were collected at the same hour in the morning at days -2, 12 and 26 by jugular venipuncture using Vacutest® tubes (Vacutest Kima, Arzergrande, Italy) that contained clot activator gel. Samples were centrifuged (2000 x g for 15 min at 4 °C) within 2 h after collection and serum was stored at -20 °C until assayed.

Liver biopsies obtained from a subset of 12 cows (6 complete pairs) at the end of the nutritional treatment (day 23) were collected using a 14-gauge biopsy needle (Tru-Core®-II Automatic Biopsy Instrument; Angiotech, Lausanne, Suitzerland). After local intramuscular administration of 3 mL of 2% lidocaine HCl, a sample was collected with the biopsy instrument through a stab incision made through the skin at the intersection of a line running from the tuber coxae to the shoulder joint with the 9th or 10th intercostal space. Liver samples were immediately frozen in liquid nitrogen and stored at −80 °C until total RNA was isolated.

Transrectal ultrasonography (Aloka SSD 500 Echo Camera, Overseas Monitor Corp. Ltd., Richmond, BC with a 5 MHz linear-array transrectal transducer) was used at days -12 and -2 to examine ovaries and determine presence of corpus luteum (CL) to confirm anestrus. In addition, ovarian structures were scanned at day 23 to determine presence of CL and at days 64, 94 and, 140 to determined presence of CL and/or pregnancy. At 140 days (final pregnancy diagnosis) if cows were pregnant, embryo age was determined (DesCôteaux *et al.* 2010), and days to pregnancy was estimated subtracting embryo age to 140.

Serum Analyses

Metabolite and insulin concentrations were measured in samples from days -2, 12, and 26 in a subset of 28 cows (14 complete pairs). Non-esterified fatty acids

(NEFA), glucose, total protein, albumin, and urea concentrations were determined spectrophotometrically using commercial kits (Wako NEFA-HR(2), Wako Pure Chemical Industries Ltd., Osaka, Japan; Oxidase/Peroxidase, Biuret, Bromocresol Green, Ureasa/Salicilato, BioSystems S.A., Barcelona, Spain, respectively) with volume of samples and reagents adjusted to a 96-well microplate and read in a Multiskan EX (Thermo Scientific, Waltham, MA, USA). All samples were determined in the same metabolite assay; intra-assay coefficients of variation (%CV) were not greater than 12.3%.

Insulin concentrations were quantified by solid-phase radioimmunoassay (RIA) (Coat and Count, Diagnostic Products Co. Los Angeles, CA, USA). All samples were determined in the same assay; the sensitivity was 1.6 uUI/mL, and the intra-assay %CV were not greater than 10.7%.

Concentrations of progesterone (P4) were measured in all samples by solid-phase RIA (Coat and Count, Diagnostic Products Co. Los Angeles, CA, USA). All samples were analyzed in a single assay; the sensitivity was 0.01 ng/mL, the intra-assay %CV were not greater than 10.6%. Days to first ovulation were considered when plasma P4 increased above the threshold value of 1 ng/mL.

Isolation and purification of RNA

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 260 nm (NanoDrop ND-1000 Spectrophotometer; Nanodrop Technnologies Inc., Wilmington, DE, USA), and purity and integrity of RNA isolates were assessed from 260/280 and 260/230 absorbance ratios and by elecrophoresis in 1% agarose gel. Isolated RNA was stored at −80 °C until analyzed by quantitative real-time PCR.

Real time PCR

The SuperScript®III First-Strand Synthesis System kit (Invitrogen) was used to conduct the reverse transcription using random hexamers and 1 μg of total RNA as a template. The cDNA was stored at -20 ºC until its use in the real time PCR. Primers (Table 2) designed specifically to amplify cDNA of GHR, IGF-I, IGFBP-2, IGFBP-3, and insulin receptor (INSR) as target genes of interest and for hypoxanthine phosphoribosyltransferase (HPRT) as an endogenous control were used.

Hypoxanthine phosphoribosyltransferase has been used before as an endogenous control in tissues from ruminants (Carriquiry *et al.* 2009) and its expression was determined in the samples of this study and it proved a good housekeeping gene since there was no variation between treatments. Before use, primer product sizes (1% agarose gel separation) and sequence (capillary electrophoretic DNA analyzer ABI3130xl; Applied Biosystems) were determined to ensure that the primers produced the desired amplification products.

Real time PCR reactions were performed using 10 μL SYBR®Green mastermix (Quantimix EASY SYG kit, Biotools B&M Labs, Madrid, Spain), 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 20 μL . Samples were analyzed in duplicate in a 72-disk Rotor-GeneTM 6000 (Corbett Life Sciences, Sydney, Australia). Standard amplification conditions were 10 min at 95 ºC and 40 cycles of 15 s at 95 ºC, 45 s at 60 ºC, and 20 s at 72 ºC. Dissociation curves were generated after the last cycle. Each disk included duplicate wells of water (no template) for each set of primers and cDNA standard curves for the genes being analyzed in the disk. Plasmids that encoded the GHR, IGF-I, IGFBP-2, IGFBP-3, INSR, and HPRT genes were diluted in yeast cDNA to achieve a starting concentration of 10^6 gene copies/μL for subsequent serial dilutions (n = 6 dilutions from 10^6 to 10^1). Linear

regressions were used to estimate the number of copies of target and control gene mRNA in the samples.

Expression of each target gene was normalized to expression of the HPRT control gene. Intra and inter-assay %CV were 1.16 and 3.59%, respectively, and the efficiency of quantification for mRNA expression was 109, 99, 94, 85, and 97%, for GHR, IGF-I, IGFBP-2, IGFBP-3, and INSR, respectively.

Table 2 Primers used for the quantification of target and endogenous control gene cDNA

Gene[1]	Accession no.[2]		Primer sequence	Length (bp)	Source
GHR	NM_17768	Sense	TCTGGGAATCCTAAATTCACCAA	91	Carriquiry *et al.* 2009
		Antisense	CTGTAAACTGTGATTAGCCCCATCT		
IGF-I	XM_61412	Sense	CCAGACAGGAATCGTGGATG	89	Carriquiry *et al.* 2009
		Antisense	ACTTGGCGGGCTTGAGAG		
IGFBP-2	NM_17455.1	Sense	ATGCGCCTTCCGGATGA	83	This paper
		Antisense	GTTGTACAGGCCATGCTTGTCA		
IGFBP-3	NM_174556	Sense	AGCACAGACACCCAGAACTTCT	86	Carriquiry *et al.* 2009
		Antisense	TTCAGCGTGTCTTCCATTTCC		
INSR	XM_590552.4	Sense	CTGAAGCCAAGGCAGATGATATT	77	This paper
		Antisense	GCCACATCAAGTGAACAACGTT		
HPRT	XM_580802	Sense	TGGAGAAGGTGTTTATTCCTCATG	105	Carriquiry *et al.* 2009
		Antisense	CACAGAGGGCCACAATGTGA		

[1]GHR = growth hormone receptor, IGF-I = insulin-like growth factor-I, IGFBP-2 = IGF-binding protein-2; IGFBP-3 = IGF-binding protein-3, INSR = insulin receptor, HPRT = hypoxanthine phosphoribosyltransferase (endogenous control gene)

[2]GeneBank sequences.

Calculations and Statistical Analyses

Individual energy intake was estimated using energy requirements (NRC 2000) of the cow-calf pair (Smit *et al.* 2005)

Data were analyzed in a randomized block design using the SAS Systems program (SAS Institute Inc., Cary, NC, USA). Data of BW, BCS, serum metabolite, and hormone concentrations were analyzed by repeated measures using the MIXED procedure with day of the experiment as the repeated effect, and the first-order autoregressive as a covariance structure. The Kenward-Rogers procedure was used to adjust the denominator degree of freedom. The model included treatment, days of experimental period, and their interaction as fixed effects and block as random effect. Hepatic mRNA expressions were analyzed without the repeated effect of day. Pretreatment values for BW, BCS, metabolite, and hormone concentrations were used as covariates in their respective data analyses. Impact of pre-treatment alterations were assessed from a comparison of confidence intervals ($\alpha = 0.05$) between covariate values and post-treatment data. Tukey–Kramer tests were conducted to analyze differences between nutritional treatments and days. Data for reproductive variables were analyzed using generalized model (GENMOD procedure, SAS Institute) specifying the binomial distribution and logit transformation of data (anestrus and pregnancy) or the Poisson distribution (days to initiation of ovarian activity) and log transformation of data, with a model that included treatment as fixed effect. Correlation coefficients used to describe relationships between variables evaluated were estimated using the CORR procedure (SAS Institute). Data are presented as least square means ± pooled standard errors. For all results, means were considered to differ when $P \leq 0.05$, and trends were identified when $0.05 < P \leq 0.10$.

3.4 RESULTS

Cow and Calf Performances

Estimated energy intake during the nutritional treatment was greater ($P < 0.001$) in IP than NP cows (15.1 vs. 13.7 ± 0.2 Mcal/d).

On average, BW and BCS were greater ($P < 0.03$) for IP than NP cows (421 vs. 415 ± 2 kg and 3.9 vs. 3.8 ± 0.03 units, respectively), however, there was an effect of days ($P < 0.01$) and an interaction between treatment and days for both variables (Fig. 1A, B). Cow BW increased from days -9 to 19 in NP but to day 33 in IP cows, and tended ($P = 0.10$) to be greater at day 19 and was greater ($P < 0.01$) from days 33 to 75 in IP than NP cows. Cow BCS increased from days -9 to 5 in NP but to day 19 in IP and was greater ($P < 0.01$) in IP than NP cows from days 19 to 61 and in both groups, BCS decreased from 61 to 74 days, and then remained constant until the end of the experiment. In average, during the nutritional treatment period (from -9 to 33 days), IP gained 0.25 units of BCS more than NP cows.

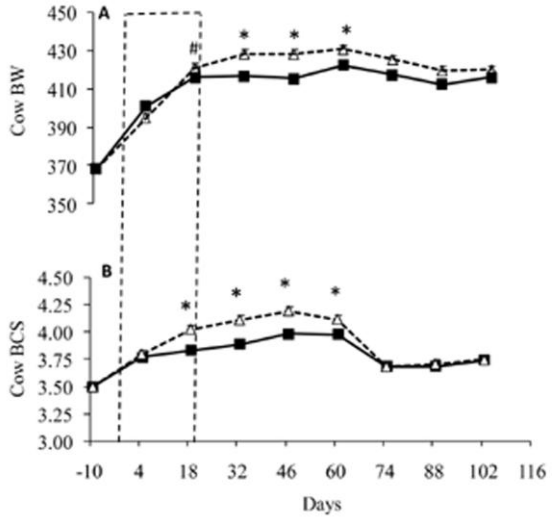

Figure 1. Cow BW and BCS during the experimental period (days -9 to 103). Cows grazed native pastures (NP, ■; n=31) or native pastures improved with *Lotus subbiflorous* cv Rincón (IP, Δ; n=33) during 23 days before the mating period. Period of nutritional treatment is indicated with dashed lines. Differences between treatments are indicated with * when $P \leq 0.05$ or # when $0.05 < P \leq 0.10$.

Calf BW was greater ($P < 0.01$) in the IP than NP group from days 19 to 103 (87.6 vs. 95.4 ± 2.6 kg and 138.6 vs. 148.2 ± 3.0 kg for NP and IP at 33 and 103 days, respectively). This was associated with greater ($P < 0.01$) average daily gain from days

- 9 to 33 for the IP than NP group (0.75 vs. 0.88 ± 0.02 kg/d).

Metabolites and Hormones

Total protein and albumin concentrations increased ($P < 0.05$) from days -2 to 26 and did not differ between treatments (Fig. 2A and B). In average, concentrations of urea did not differ between treatments, but there was an interaction ($P < 0.01$) between treatment and days (Fig. 2C). Serum urea concentration decreased ($P < 0.05$) from days -2 to 26

for both treatments but at day 12, concentrations were greater ($P < 0.01$) in IP than NP cows.

Non-esterified fatty acid concentrations decreased ($P < 0.05$) from days -2 to 12 and increased slightly at day 26. Concentration of NEFA tended ($P = 0.06$) to be greater for IP than NP due to increased ($P < 0.05$) NEFA at day 26 in IP cows (Fig. 2D). Glucose concentrations did not differ between treatments, however, tended ($P = 0.06$) to be less in IP than NP cows at day 12 (Fig. 2E).

Insulin concentrations were less ($P = 0.03$) in IP than NP cows (1.37 vs. 2.25 ± 0.26 uU/mL) because insulin increased ($P < 0.05$) from days -2 to 12 only in NP cows (Fig. 2F)

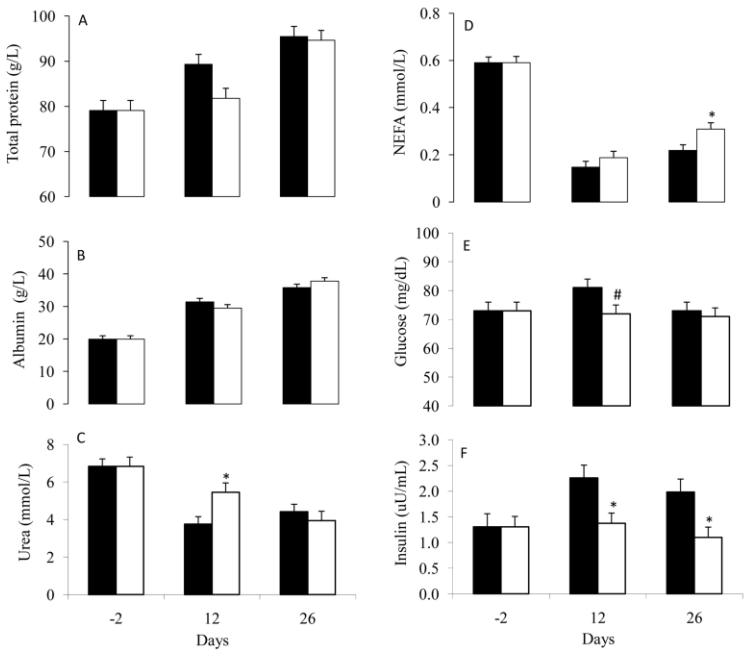

Figure 2. Metabolite and insulin concentrations during experimental period (from days -2 to 26). Cows were grazed native pastures (NP, ■; n=14) and native pastures improved with *Lotus subbiflorous* cv Rincón (IP, □; n=14) during 23 days before the mating

period. Initiation of nutritional treatment is indicated with dashed lines. Differences between treatments are indicated with * when $P \leq 0.05$ or # when $0.05 < P \leq 0.10$.

Hepatic mRNA expression

The hepatic expression of GHR, IGF-I, and IGFBP-2 mRNA were not affected by treatments (Table 3). Abundance of IGFBP-3 mRNA was less ($P = 0.04$) for IP than NP cows, with no effect of treatments on IGFBP-3/ IGFBP-2 mRNA ratio. Hepatic INSR mRNA tended ($P = 0.08$) to be reduced in IP than NP cows. Independent of nutritional treatment, expression of IGFBP-3 and INSR were positively correlated ($r = 0.74$, $P = 0.01$) and IGF-I expression tended to be positively correlated with serum insulin ($r = 0.67$, $P = 0.09$).

Table 3 Effect of a short-term increase in the nutritional plane[1] before the mating period on hepatic gene expression

Genes[1]	Nutritional treatments[2]			
	NP	IP	SE	P- value
GHR	9.23	12.23	2.21	0.282
IGF-I	0.26	0.23	0.04	0.592
INSR	6.90	4.67	0.77	0.077
IGFBP-2	51.84	40.74	4.41	0.114
IGFBP-3	9.40	5.60	1.02	0.040
IGFBP-3/IGFBP-2	0.18	0.14	0.02	0.175

[1]GHR = growth hormone receptor, GHR1A= growth hormone receptor 1A, IGF-I = insulin-like growth factor-I, IGFBP-2 = IGF-binding protein-2, IGFBP-3 = IGF-binding protein-3, INSR = insulin receptor.

[2] Cows grazed of native pastures (NP, n=6) or native pastures improved with *Lotus subbiflorous* cv Rincón (IP, n=6) during 23 day before the mating period.

Reproductive Performance

Days to initiation of ovarian activity and percentage of anestrous and pregnant cows did not differ between treatments (Table 4). Independent of nutritional treatment, days to initiation of ovarian activity and percentage of anestrous cows at the end of the first month of the mating period were negatively correlated with serum insulin at the end of the nutritional treatment period (r = -0.40, $P = 0.05$ and r = -0.45, $P < 0.03$, respectively) and with the increase in serum insulin levels during the nutritional treatment period (r = -0.62, $P < 0.01$ and r = -0.70, $P < 0.01$, respectively).

Table 4 Effect of a short-term increase in the nutritional plane[1] before the mating period on reproduction

	Nutritional treatmens[1]			
	IP	NP	SE	P-vaule
Days to first ovulation[2]	107	108	1.1	0.54
Anestrous cows at first month of mating season, % (no./no.)	54.5 (18/33)	61.3 (19/31)		0.54
Early pregnancy [3], % (no./no.)	36 (12/33)	23 (7/31)		0.25
Total pregnancy % (no./no.)	88 (29/33)	93 (29/31)		0.60
Interval between calvings (days)	422	418	7.5	0.41

[1] Cows grazed of native pastures improved with *Lotus subbiflorous* cv Rincón (IP, n=33) or native pastures (NP, n=31) during 23 day before the mating period.

[2] Days from calving.

[3] Pregnancy rate at the end of the first month of mating season.

3.5 DISCUSSION

In this study, although the increase in the nutritional plane before the mating period did not affect reproductive performance, grazing of improved native pastures resulted in increased calf ADG and BW and modified metabolic, endocrine, and gene expression parameters suggesting a greater nutrient and energy partitioning towards milk production.

The increase in BW and BCS in both treatments was associated with the increase in forage availability and quality from the pre-treatment (winter) to the nutritional treatment (spring) period (Berretta *et al.* 2000), indicating a positive energy balance in both groups. The greater BW and BCS gains of IP cows could be explained by increased intake of energy and/or nutrients, due to a greater nutritional plane, and/or selection of diet, as reported in previous research in primiparous suckled beef of low to moderate BCS (Ciccioli *et al.* 2003). In addition, increased forage availability and quality have been associated with reduced grazing energy cost by decreasing grazing time (Brosh *et al.* 2007).

The greater calf ADG and BW in IP, was probably associated with increased milk production (Totusek *et al.* 1973) due to increased forage offer as it has been previously reported that milk production and calf weights increased with energy intake (Perry *et al.* 1991; Ciccioli *et al.* 2003), and/or changes in diet quality (Waterman *et al.* 2006; 2007) (i.e. increasing rumen undegradable protein (RUP), 40% crude protein *Lotus subbiflorous* cv Rincón; Trujillo *et al.* 2009). Alternatively, in this study, IP calves may have consumed some forage (as forage availability and quality increased), which could have increased calf ADG and BW, independently of the greater cow milk production. However, it would be unlikely that calf forage intake would explain differences in calf ADG and BW between treatments since forage intake is minimal in terms of energy when calves are less than 60-day old (Alencar, 1989). In the present study, a short-term increase in nutritional plane before the mating period appeared to

increase cow body energy reserves as well as milk production as reported previously (Ciccioli *et al.* 2003).

In agreement with the BCS recovery from the beginning of the nutritional treatments in both groups, total protein and albumin concentrations increased and urea and NEFA decreased which would indicate a better nutritional status (Ndlovu *et al.* 2007) and decreased mobilization of reserves from muscle (Chimonyo *et al.* 2002) and adipose tissue (Meikle *et al.* 2004), respectively. Similarly, serum NEFA was reduced in beef cows as range forage quantity and quality improved (Waterman *et al.* 2007).

The greater urea levels observed in the IP group at day 12 may be associated with higher protein intake (Chimonyo *et al.* 2002) or with an asynchrony in ruminal release of availability of energy and nitrogen as suggested by degradation kinetics of DM and nitrogen of *Lotus subbliflorus* cv Rincón (Trujillo *et al.* 2009). Greater levels of NEFA at the end of the nutritional treatments were associated with heavier calves in IP cows. This is consistent with Vizcarra *et al.* (1998) and Quintans *et al.* (2010) that found greater NEFA levels, associated with greater milk production in cows in high compared with those in moderate postpartum nutrition.

Concentrations of glucose and insulin remained stable and unchanged in IP whereas in NP cows there was a transient increase in glucose at day 12 that was accompanied by a permanent increase in insulin levels. Glucose requirements of ruminants are largely met through hepatic gluconeogenesis which is a positive function of energy intake (Huntington *et al.* 2006). Therefore, it should not be expected that greater glucose levels at day 12 in NP cows were a result of greater glucose synthesis in the liver as estimated energy intake was less in these than in IP cows. Moreover, the greater blood insulin concentrations in NP cows would suggest reduced rates of gluconeogenesis as studies have shown that net uptake of glucose precursors as well as net hepatic glucose release were reduced by insulin (Huntington *et al.* 2006). The trend

for a greater hepatic abundance of INSR mRNA in NP cows would also suggest inhibition of gluconeogenesis as reported by Liu *et al.* (2010).

Glucose is the main precursor of lactose in cows and determines fluid milk produced (Vizcarra *et al.* 1998). Therefore, reduced glucose concentrations during the nutritional treatment period in IP cows were probably associated with the lactational stress as previously reported (Waterman *et al.* 2007). In addition, reduced circulating insulin levels in IP cows would also be in agreement with greater milk production (Quintans *et al.* 2010). Insulin does not appear to have a direct effect of the mammary gland but increases uptake of glucose for extra-mammary tissues (Chilliard, 1999) what would be in agreement with reduced abundance of INSR mRNA in the liver of IP cows. Therefore, in the present study, changes in circulating concentrations of insulin as well as in hepatic abundance of INSR mRNA would indicate greater partitioning of nutrients and energy towards milk production in IP than NP cows.

Similarly to our results, Cooke *et al.* (2008) did not find differences in hepatic IGF-I mRNA when supplementation frequency was increased in beef cows. Reduced abundance of hepatic IGFBP-3 mRNA have been reported (Carriquiry *et al.* 2009) in dairy cows as milk production increased in early lactation, and it has been suggested that insulin-induced alteration in IGFBP-3 or IGFBP-3/IGFBP-2 ratio could represent a mechanism by which insulin regulates circulating IGF-I concentrations (Carriquiry *et al.* 2009). In agreement with these results, in this study, reduced circulating insulin concentrations, and hepatic expression of INSR and IGFBP-3 mRNA, as well as greater calf ADG and BW were determined in IP cows. Also, there was a positive correlation between insulin and abundance of IGF-I mRNA consistent with Rhoads *et al.* (2004) who reported that insulin increased hepatic IGF-I synthesis in dairy cows in early and mid-lactation. Similarly, association between circulating insulin and IGF-I concentrations in beef cattle have been reported previously (Ciccioli *et al.* 2003). The hepatic expression of IGFBP-2 (although non-significant) and IGFBP3 mRNA were reduced in NP cows, which determined no changes in the ratio IGFBP-3/IGFBP-2

mRNA ratio. Increased circulating IGFBP-2 and decreased IGFBP-3 have been associated with dietary restriction (Rajaram *et al.* 1997), or delayed return to ovarian ciclicity in beef cows (Roberts *et al.* 1997).

The length of the interval from calving to first ovulation in this study (108 ± 10 days postpartum) is consistent with the reduced early pregnancy rates obtained, and was in agreement with Quintans and Vázquez (2002) for primiparous beef cows, and would indicate that the nutrient demands for maintenance, milk production and growth limits the proportion of primiparous cows cycling early in the mating period (Short *et al.* 1990). There was no effect of the short-term increase in the nutritional plane in reproduction responses in contrast with Pérez-Clariget *et al.* (2007) who reported short-term supplementation increased percentage of early pregnancy by 20% associated with shorter intervals between calving and first ovulation. Differences between those results and the present study could be due to the characteristics of the feed (concentrate vs. pastures), days of postpartum when treatments were applied (>60 vs. 48 days), temporary suckling management (with vs. without temporary weaning), and pretreatment nutritional management. In agreement with Sinclair (2008) who reported insulin concentrations accounted for a greater proportion of the variance associated with the interval form calving to first ovulation than either BCS at calving or postpartum energy intake in beef cows, serum insulin was negatively associated with days to first ovulation as well as percentage of anestrous cows at the end of the first month of the mating period.

In summary, differences in the metabolic signals or endocrine function due to short-term increase in nutritional treatment with improved native pastures before the mating period in primiparous suckled beef cows, probably influenced milk production, increasing calf ADG and BW, but failed to improve reproductive performance.

3.6 ACKNOWLEDGMENTS

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4. DISCUSIÓN Y CONCLUSIONES

4.1 DISCUSIÓN GENERAL

El estudio de las señales responsables de ligar el estatus metabólico (CC e incremento del plano nutricional) con los perfiles metabolicos/endocrinos y de expresión génica hepática en vacas de carne de primera cría en sistemas pastoriles (alimentación limitante), permitió comprender -en parte- la priorización de la partición de nutrientes hacia producción de leche y crecimiento del ternero en detrimento de la reproducción. Las vacas que perdieron menos CC durante la gestación y llegaron al parto con 0.5 unidades mas de CC (vacas en CC moderada), presentaron menores concentraciones de insulina y abundancia de ARNm de GHR1A e IGF-I durante el segundo mes de lactación, lo cual se asoció a una mayor producción de leche y crecimiento del ternero. El incremento en el plano nutricional previo al entore no afectó las respuestas reproductivas pero incrementó el PV y ganancia diaria del ternero y modificó los parámetros metabólicos, endócrinos y de expresión génica hepática sugiriendo una mayor partición de nutrientes y energía hacia la producción de leche.

Las vacas primíparas constituyen un desafío importante para el sistema de producción/investigación dado que aún no han completado el proceso de crecimiento, y por lo tanto son más sensibles a las restricciones alimenticias (Orcasberro *et al.*, 1992). El estres del parto y los efectos combinados de la primera lactancia y del crecimiento, imponen requerimientos nutricionales que en general no son satisfechos en pastoreo de campo nativo (Ciccioli *et al.*, 2003). Esto contribuye a explicar que el anestro postparto en esta categoría sea de mayor duración que en vacas adultas (Short *et al.*, 1990, Lalman *et al.*, 2000, Quintans y Vazquez, 2002).

La alimentación preparto, reflejada en la CC al parto, es el factor más importante que determina la duración del período de anestro posparto en vacas primíparas. Sin embargo, la

nutrición posparto puede atenuar en parte los efectos negativos de una restricción alimenticia preparto en las mismas (Short *et al.*, 1990; Hess *et al.*, 2005; Perry *et al.*, 1991, Lalman *et al.*, 1997). En este sentido la tesis incluyó dos fases experimentales complementarias, en las cuales se evaluó la asociación entre la evolución de la CC (periodo de gestación invernal; Trabajo 1) y/o el incremento en el plano nutricional previo al entore (Trabajo 2) y los cambios endocrinos/metabólicos y de expresión génica hepática en vacas de carne primíparas en condiciones de pastoreo, asociados a la respuesta en producción de leche, al crecimiento del ternero y a la reproducción.

El balance energético es de medición compleja a nivel de campo, debido a la dificultad de determinar el consumo de energía en animales en pastoreo, pero los cambios en CC (Vizcarra *et al.,* 1986) así como en los perfiles metabólicos y endócrinos (Ndlovu *et al.,* 2007) han sido utilizados como indicadores del estatus nutricional de los animales. En sistemas de producción basados en el pastoreo de campo nativo, la alta variabilidad climática entre años y estaciones y la intensidad de pastoreo que provoca cambios en la cantidad y/o calidad del forraje ofrecido, y determina que los animales pasen por períodos variables de consumo de energía que interaccionan con el estado fisiológico y el balance energético determinando fluctuaciones en la CC (Cassady *et al.*, 2009, Soca *et al.*, 2007).

En este sentido, en el presente estudio, a fines del otoño las vacas presentaron igual CC (6 unidades) perdiendo entre 2 y 2,5 unidades durante los últimos meses de gestación, indicando que las condiciones meteorológicas del invierno no permitieron una producción de forraje suficiente en cantidad y/o en calidad, para evitar esa caída de CC. Estos resultados coinciden con Trujillo *et al.* (1996) quienes reportaron que la CC al parto estuvo positivamente relacionada con la disponibilidad de forraje durante el otoño y la fase final de la gestación, momento en que se da el máximo crecimiento del feto (Bell, 1995). Sin embargo, a pesar de que las vacas de este estudio fueron manejadas todas juntas, existió una pérdida diferencial de CC, la cual podría explicarse por una mayor capacidad de consumo, por mejor capacidad de pastoreo o mayor jerarquía social, y/o reducción de las necesidades

de energía para mantenimiento en aquellos animales que llegaron al parto con mejor CC. Scarlato *et al* (sin publicar), reportan una relación negatva entre tiempo dedicado al pastoreo y la CC de vacas multíparas durante gestación tardía e inicio de lactacica lo cual se asociaría a mayores gastos por actividad de pastoreo y requerimientos de mantenimiento (Brosh *et al.*, 2007).

Independientemente de la CC al parto (Trabajo 1) y/o del tratamiento nutricional previo al entore (Trabajo 2), la evolución de la CC observada durante el posparto mostró claramente que a partir de los 45 a 50 días posparto (dpp) las vacas comienzan a recuperar CC y el PV. Esta recuperación posiblemente este dada a un mayor consumo de energía y/o de nutrientes, explicado por el incremento en la oferta y calidad de forraje asociado al comienzo de la (Berretta *et al*, 2000).

La evolución de la CC durante todo el período experimental (Trabajos 1 y 2), se asoció con cambios en las concentraciones de metabolitos en sangre. La pérdida de CC durante el preparto, coincide con las concentraciones elevadas de AGNE y urea indicando una mayor movilización de las reservas del tejido adiposo (Meikle *et al.*, 2004) y del músculo (Chimonyo *et al.*, 2002), respectivamente. Adicionalmente, la disminución en los niveles de urea en sangre registrada entre los 35 y 60 dpp podría estar asociada a un uso más eficiente de los compuestos nitrogenados a nivel del rumen debido a una mayor disponibilidad de energía en el forraje en primavera (Trujillo *et al.*, 2009).

Asimismo, las concentraciones de proteínas totales, albúmina y colesterol aumentaron a partir de los 35 dpp, lo cual reflejaría una recuperación del consumo (Cavestany *et al.*, 2005) y el mejor estatus nutricional de los animales (Agenas *et al.*, 2006, Ndlovu *et al.*, 2007). Ha sido reportado que, las concentraciones de proteína total y albúmina reflejan la disponibilidad de proteína y sus concentraciones están influenciadas por el estado nutricional, disminuyendo cuando existe déficit de proteína o desnutrición (Ndlovu *et al.*, 2007). Mas aún, las concentraciones de albúmina han asido sugeridas como un buen indicador nutricional

temprano del estatus proteico debido a su relativa vida media corta en plasma (16 días; Agenas *et al.*, 2006).

Estos cambios en la CC y los metabolitos en sangre durante el posparto, indican un balance energético positivo y la recuperación de las reservas corporales de las vacas previas al entore al comenzar la primavera.

La insulina juega un papel central en el control homeostático del metabolismo energético (Chilliard, 1999). Los niveles de insulina se mantuvieron estables y bajos durante el preparto y el inicio de la lactación no modificó los niveles de insulina como se observa en vacas de leche (Meikle *et al.*, 2004, Rhoads *et al.*, 2004). La recuperación del estatus nutricional durante el posparto, anteriormente mencionada, no se reflejó en cambios drásticos en los niveles de insulina. Sin embargo, las concentraciones de insulina fueron mayores a partir de los 21 dpp solamente en las vacas que llegaron al parto con menor CC (Trabajo 1) y a los 60 dpp en las vacas que pastorearon campo natural (Trabajo 2). La mayor concentración de insulina encontrada en estos grupos se asoció a una menor producción de leche y/o crecimiento del ternero (PV y ganancia diaria). La insulina no parecería tener un efecto directo sobre la glándula mamaria pero incrementa el uso de la glucosa por los tejidos extra-mamarios, disminuyendo la disponibilidad de la misma para la síntesis de lactosa, principal componente de la leche (Bauman, 2000). Varios autores han reportado una correlación positiva entre producción de leche, PV y ganancia diaria de los terneros en bovinos de carne (Totusek *et al.*, 1973) y han encontrado una asociación negativa entre la concentración de insulina, la producción de leche y el crecimiento del ternero en vacas de carne (Vizcarra *et al.*, 1998, Hammon *et al.*,. 2007, Quintans *et al.* 2010). Independientemente del plano nutricional, el PV de los terneros a partir de los 50 dpp (Trabajo 2) fue mayor para las vacas que parieron con mejor CC. De manera similar, estudios previos (Vizcarra *et al*. 1998, Quintans *et al.*, 2010) reportaron que vacas con CC al parto moderadas y/o altas, presentaron mayor PV y ganancia diaria del ternero, asociados a una mayor producción de leche.

De acuerdo con las concentraciones de insulina cercanas al parto, y en contraste con el desacople del eje GH-IGF en vacas lecheras durante el periparto (Lucy, 2008), en este estudio no hubo cambios en la expresión hepática de ARNm de GHR, GHR1A e IGF-I durante el pre y posparto inmediato (-11 vs 7 dpp). Estos resultados coinciden con Jiang *et al.* (2005) quienes no encontraron diferencias en la expresión hepática de estos transcriptos en el periodo de transición en vacas alimentadas *ad libitum* (-21 vs 21 dpp). Sin embargo, a los 52 dpp en las vacas con menores reservas corporales (Trabajo 1), encontramos una mayor expresión de estos transcriptos (GHR1A e IGF-I) asociada con el aumento en las cocentraciones de insulina y la reducción en la producción de leche. Estos resultados sugieren que el desacople del eje GH-IGF en vacas de carne ocurriría más tarde en la lactancia, coincidiendo con el pico máximo de producción de leche (alrededor del día 60 de lactancia; NRC 2000). Si bien la abundancia de estos transcriptos (GHR1A e IGF-I) no se modificó en respuesta al incremento en el plano nutricional (71 dpp; Trabajo 2), la expresión hepática de ARNm del receptor de insulina y de IGFBP-3 fue mayor en las vacas que pastoreaban campo nativo, lo cual podría asociarse a una menor partición de nutrientes hacia la glándula mamaria. Una reducción en la abundancia del transcripto de IGFBP-3 en hígado ha sido reportado en vacas lecheras con el aumento en la producción de leche en lactación temprana (Carriquiry *et al.*, 2009). En acuerdo con el rol clave que tiene la insulina en la regulación de la expresión hepática de ARNm de los genes del eje GH-IGF (Rhoads *et al.*, 2004), en ambos trabajos se observó una correlación alta y positiva entre las concentraciones de insulina en sangre y la abundancia de ARNm de IGF-I en hígado.

En rumiantes, restricciones nutricionales han sido asociadas con aumentos en los niveles circulantes de IGFBP-2 y disminuciones de IGFBP-3 (Rajaram *et al.*, 1997). Más aún en vacas de carne, menores concentraciones de IGFBP-2 y mayores de IGFBP-3 han sido asociadas con el reinicio de la ciclicidad ovárica (Robert *et al.*, 1997). De manera similar, en el presente trabajo la relación de ARNm de IGFBP-2/ IGFBP-3 disminuyó hacia los 52 dpp en las vacas con mayores reservas corporales (Trabajo 1), no modificándose en respuesta al incremento en el plano nutricional (Trabajo 2). En acuerdo con estos resultados y similar a lo

reportado por diversos autores (Vizcarra *et al.*, 1998, Ciccioli *et al.*, 2003, Quintans *et al.*, 2010), el porcentaje de vacas que estaban ciclando al final del primer mes de entore, independientemente del plano nutricional, fue mayor en los animales que presentaron una mejor CC al parto (68 vs. 51%, moderada vs. baja, respectivamente).

El retraso en el reinicio de la ciclicidad ovárica (108 dpp) y el alto porcentaje de vacas en anestro al final del primer mes de entore (58%) fue consistente con el bajo porcentaje de preñez temprana (30%) alcanzado en este estudio y con reportes previos (Quintans y Vázquez, 2002) que indican largos de anestro posparto mayores a 120 días en vacas primíparas. Independientemente del plano nutricional (Trabajo 2), la concentración de insulina se asoció negativamente con los días a primera ovulación, así como el porcentaje de vacas en anestro al final del primer mes de entore, consistente con el efecto directo de insulina reportado en el eje hipotálamo-hipófisis-ovario (Sinclair, 2008).

En contraposición con Pérez-Clariget *et al.* (2007) y Soca *et al.* (2007) que han demostrado incrementos en el porcentaje de preñez en vacas primíparas con CC sub-óptima durante la primera mitad del entore en respuesta al aporte energético durante 20-28 días, en el presente trabajo el incremento en el plano nutricional no mejoró la respuesta reproductiva. Las diferencias entre estos trabajos y el presente, podrían deberse al alimento utilizado (concentrado vs. pastura), momento en que el tratamiento fue aplicado (> 60 vs. 48 dpp) y/o manejo del amamantamiento (con vs. sin destete temporario). Asimismo, la ausencia de una mejor respuesta reproductiva al incremento del plano nutricional, podría deberse a que la disponibilidad de forraje, al aumentar la temperatura en primavera, se incrementó durante el período evaluado para ambos tratamientos (campo nativo y campo nativo mejorado con *Lotus subbiflorou*s cv. Rincón) implicando una mejora nutricional para todos los animales (Trabajo 2).

4.2 CONCLUSIONES GLOBALES

En sistemas de producción donde la cría se realiza a cielo abierto sobre campo nativo y los últimos meses de gestación e inicio de lactancia coinciden con el momento en que hay mayor déficit de forraje (invierno), los cambios en las reservas corporales y en los perfiles metabólicos/endocrinos y de expresión génica hepática reflejan que: a) el aumento en los requerimientos de gestación que ocurren cuando la cantidad y/o calidad del forraje es limitante durante el invierno tienen un mayor impacto sobre el balance energético que el inicio de la lactancia b) la evolución de la CC y de los perfiles metabólicos/endocrinos posparto indicaron una recuperación del estatus nutricional de los animales hacia la primavera previo al entore. c) las mayores reservas corporales y/o el incremento en el plano nutricional, permitieron una mayor producción de leche y/o crecimiento del ternero pero no modificó la respuesta reproductiva. d) el mecanismo molecular hépatico que explica el desacople del eje GH-IGF en las vacas lecheras durante el periparto no se evidencia en vacas de carne en pastoreo de campo nativo. Sin embargo, componentes de este mecanismo homeorhético se diferenciaron mas tarde en la lactancia, coincidiendo con el pico máximo de producción de leche, en vacas de carne con diferentes reservas corpororales.

Por lo tanto, el estudio de las señales responsables de ligar el estatus metabólico con los mecanismos fisiológicos y moleculares en vacas primíparas en sistemas pastoriles (alimentación limitante), permitió comprender -en parte- la priorización de la partición de nutrientes hacia producción de leche y crecimiento en detrimento de la reproducción.

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