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COMPOSICIÓN CORPORAL Y MASA, COMPOSICIÓN Y ACTIVIDAD METABÓLICA DE LAS VÍSCERAS EN VACAS DE CRÍA EN PASTOREO

por

Alberto CASAL SPERA

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Tesis aprobada por: el Ing. Agr. (MSc) Pablo Soca, la DMTV. (PhD) Cecilia Cajerville y la DMTV. (PhD) Carolina Viñoles.

Autor: DCV. Alberto Casal Spera

Tutora: Ing. Agr. (PhD) Mariana Carriquiry

Co-tutora: Ing. Agr. (MSc) Ana Ines Trujillo

Co-tutora: Ing. Agr. (DSc) Ana Carolina Espasandin

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RESUMEN

El objetivo de este trabajo fue identificar los efectos del control de la intensidad de pastoreo del campo natural, a través del manejo de la oferta de forraje, sobre características asociadas al requerimiento de mantenimiento [composición corporal y de masa, composición, índices de celularidad y expresión de genes que codifican proteínas mitocondriales en las vísceras del tracto gastrointestinal (TGI) e hígado] de vacas de cría puras (Angus/Hereford; PU) y sus cruzas reciprocas (F1; CR). Se utilizaron 32 vacas adultas en un diseño de bloques completos al azar con un arreglo factorial de oferta de forraje (OF; promedio 2.5 vs. 4 kg MS/d; BOF vs. AOF) y grupo genético (GG; PU vs. CR). El experimento comenzó en junio 2007 y durante el último año (2009), a los 150, 210 y 240 ± 10 días de gestación invernal y 190 ± 10 días posparto (otoño; mayo 2010) se estimó la composición corporal mediante la técnica de dilución de urea. A su vez, al final del tercer año (192 ± 10 días posparto) las vacas fueron sacrificadas y los órganos, la sangre y la carcasa fueron pesados (kg; peso absoluto) y se colectaron muestras para el análisis de su composición química (MS, N, lípidos y cenizas) y ácidos nucléicos (ADN, ARN). Las vacas pastoreando AOF presentaron mayor condición corporal (CC) y reservas corporales en términos de masa lipídica (pero no proteica) tanto durante la gestación invernal como en el otoño siguiente (al sacrificio) que las vacas en BOF. Sin embargo, presentaron menor deposición de grasa visceral (mas grasa mesentérica/omasal o mayor concentración de lípidos en órganos del TGI en CR-BOF o PU-BOF, respectivamente). A su vez, el peso de las vísceras del TGI e hígado expresado como proporción del peso corporal vacío (g/kg peso cuerpo vacio; peso relativo) no difirió entre vacas en AOF y BOF. Las vacas CR presentaron mayores CC y reservas corporales, asociadas a una mayor masa lipídica y proteica absoluta y una mayor masa proteica relativa de proteínas. A su vez, la movilización de reservas corporales durante el invierno (balance energético negativo) difirió entre CR y PU ya que las primeras movilizaron más masa proteica que las PU. Además, presentaron un mayor peso relativo del hígado explicado por mayor hiperplasia e hipertrofia en vacas AOF-CR pero solo por hipertrofia en vacas BOF-CR. Las expresión de genes que codifican para proteínas mitocondriales del complejo I y/o III fue mayor en AOF y en vacas CR en el intestino delgado y en BOF-CR en hígado. Estos resultados sugerirían que el mejor estado nutricional de las vacas en AOF que en BOF no se acompañaría de mayores requerimientos de energía para el metabolismo basal y que las vacas CR tendrían una mayor plasticidad de adaptación que las vacas PU a ambientes y/o momentos restrictivos.

Palabras clave: ganado de carne, pastoreo, composición corporal, vísceras.

SUMMARY

The aim of this study was to identify the effects of controlling the intensity of grazing natural pastures, through forage allowance on characteristics associated with maintenance energy requirements [body composition and mass, composition, cellularity indexes and expression of genes encoding mitochondrial protein on viscera of the gastrointestinal tract (GIT) and liver] in purebred (Angus/Hereford; PU) and reciprocal crosses (F1; CR). Adult cows n=32 were used in a design of randomized complete block with a factorial arrangement of herbage allowance (HA; mean 2.5 vs. 4 kg DM/ d; LO vs. HI) and cow genotype (CG; PU vs. CR). The experiment began in June 2007 and during the last year (2009), at 150, 210 and 240 ± 10 days of gestation in winter and 190 ± 10 days postpartum (fall; May 2010) body composition was estimated by the urea dilution technique. In addition, at the end of the third year (192 ± 10 days postpartum) cows were sacrificed and individual organs, blood and carcass were weighed (kg; absolute weight) and samples for chemical (DM, N, lipids and ash) and nucleic acids (DNA, RNA) composition analyses were collected. Cows grazing HI-HA had greater body condition score (BCS) and body reserves in terms of fat mass (but not protein mass) during pregnancy in winter and in the following fall (sacrifice) than LO-HA cows. However, they had less deposition of visceral fat (more mesenteric/omasal mass or greater fat concentrations in GIT organs in LO-CR or LO-PU cows, respectively). In turn, the weight of the GIT viscera and liver weights as a proportion of empty body weight (g/kg empty body weight; relative weight) did not differ between HI and LO cows. The CR cows had greater BCS and body reserves, associated with increased total lipid and protein mass and greater relative protein mass. Mobilization of body reserves during winter (negative energy balance) differed between CR and PU cows as CR cows mobilized more protein mass than PU cows. They also had a greater relative liver weight explained by increased hyperplasia and hypertrophy in HI-CR cows but only by hypertrophy in LO-CR cows. The expression of genes encoding for mitochondrial proteins of I and/or III complexes was greater in HI cows and CR cows in the small intestine and in LO-CR cows in the liver. These results suggest that the better nutritional status of HI than LO cows was not accompany by greater energy requirements for basal metabolism and that CR cows would have greater adaptive plasticity to restrictive environments and/or peridos than PU cows.

Keywords: beef cattle, body composition, grazing, viscera.

1. INTRODUCCIÓN

1.1. PLANTEO DEL PROBLEMA

La producción de carne basada en ecosistemas pastoriles (campo nattivo), resulta en importantes aportes a la economía nacional (segundo rubro en importancia en las exportaciones, totalizando (1626 millones de dólares en venta de carne y animales en pie ejercicio 2012-2013; DIEA, 2013). La cría de bovinos de carne (primer eslabón de la cadena cárnica) en Uruguay involucra 7,1 millones de cabezas y 7,6 millones de hectáreas, que significan el 51% de las hectáreas de pastoreo con bovinos de carne y ovinos. Sin embargo, el promedio nacional del porcentaje de destete en los últimos años ha sido de 64% (DIEA, 2013), y se ha mantenido por debajo del 65% durante las 3 últimas décadas. A su vez, el sector criador presenta una baja productividad (96 kg de carne/ha; IPA, 2013). Estos resultados reproductivos y productivos obtenidos por la cría vacuna en el país constituyen una de las principales limitantes para su desarrollo y expansión, al reducir el potencial de ingreso económico de los productores. Por otro lado, si bien la coyuntura de precios actual es favorable para la producción de carne, la expansión de la forestación y de la agricultura ha provocado aumentos en la competencia entre rubros y en el precio de la tierra (DIEA, 2010; 2013). En este contexto, ha ido incrementando la vulnerabilidad de los sistemas, especialmente en lo referido a la respuesta productiva frente a los cambios climáticos (Prins y Van Langevelde, 2008). Es así, que incrementos en la producción por unidad de superficie acompañados de reducciones o mantenimiento de los costos unitarios de producción, constituyen estrategias centrales para continuar con la competitividad del sistema criador.

En nuestros sistemas de producción la cría vacuna se lleva a cabo principalmente en pastoreo sobre campo nativo, un tipo de cobertura vegetal formada por una gran diversidad de gramíneas y plantas herbáceas que comprende más del 70% del territorio nacional (Berretta y Do Nascimento, 1991; bioma Campos). La diversidad florística que caracteriza al campo nativo en interacción con la variabilidad en los suelos, la topografía y el clima, determinan un ambiente pastoril con un alto de grado de heterogeneidad en la distribución de los recursos forrajeros

(Rosengurtt et al., 1939; Chapman et al., 2007). Así mismo, la producción estacional, las variaciones climáticas intra e inter anuales, y las diferencias en la calidad y cantidad de la pastura (Berretta et al., 2000), determinan que el aporte de nutrientes ofrecidos a la vaca resulte la principal limitante del proceso. Además, las necesidades de nutrientes de ganado de carne también fluctúan a lo largo del año debido a la presión de la reproducción y la lactancia. En este contexto, las vacas de carne pastoreando campo nativo transitan por cambios en el peso vivo (PV) y condición corporal (CC) a través del ciclo de producción anual (Quintans et al., 2010; Soca et al., 2013a; Laporta et al., 2014) que impactan negativamente sobre el comportamiento reproductivo de los rodeos de cría (Soca el al. 2013a).

Freetly et al. (2008) sugirieron que la capacidad de la vaca para adaptar su metabolismo a períodos de restricción alimenticia y realimentación ("weight-cycling") permitiría el desarrollo de estrategias de manejo de los recursos alimenticios (qué, cuánto y cuándo) con el fin de reducir los costos de alimentación. Sin embargo, el momento y la severedad de la restricción durante el ciclo anual de producción de ganado de carne podrían afectar el comportamiento productivo y reproductivo de la vaca y su ternero (Freetly et al., 2005; Houghton et al., 1990a; 1990b; Funston et al., 2010).

Por lo tanto, en sistemas criadores bajo pastoreo de campo nativo, el manejo de la intensidad de pastoreo mediante el control de la oferta de forraje estacional permitiría controlar la disponibilidad de forraje por vaca, con el fin de beneficiarse de la capacidad de "weight-cycling" de la vaca, geneando efectos mínimos sobre su comportamiento productivo y reproductivo. Se ha reportado que la oferta de forraje modificó la producción de forraje (Soares et al., 2003), el consumo (Chapman et al., 2007) y los requerimientos de energía para mantenimiento de los animales (ej. costos energéticos pastoreo en costos de energía; Brosh et al., 2007). En sistemas de cría, el aumento de la oferta de forraje se ha asociado con un mayor PV y CC de la vaca, así como con aumentos en la producción de leche de la madre y PV del ternero (Nicol, 1979; Baker et al., 1981).

Por otra parte, la productividad del sistema criador (peso al destete y eficiencia reproductiva) está influenciado por componentes genéticos. El genotipo de la vaca está representado por la o las razas que la compongan (efectos raciales), así como por la interacción que se genera entre ellas, vigor híbrido o heterosis y complementariedad (Koch et al., 1985). Se ha reportado que el uso de vacas cruza (Hereford/Angus) puede mejorar la producción física hasta 30% en su vida útil, sin incrementar los costos de producción (Morris et al., 1987). A su vez, la eficiencia global en el uso del alimento por vacas de cría, depende de la interacción entre el genotipo y el ambiente. Jenkins y Ferrell (1994) revelan diferencias a nivel de consumo, producción de leche, composición corporal y resultados productivos de diversos materiales genéticos frente a cambios en la cantidad de alimento ofrecido.

En este marco, desde el año 2007 en la Estación Experimental Bernando Rosengurtt [(EEBR), Facultad de Agronomía, Cerro Largo] se ha llevado adelante un experimento con un diseño de bloques al azar con repetición en el espacio y un arreglo factorial de oferta de forraje y grupo genético. Vacas adultas (multíparas, n=60) de dos grupos genéticos (puras: Aberdeen Angus y Hereford y cruzas: sus respectivas cruzas F1, PU vs. CR fueron asignadas a dos tratamientos de oferta de forraje (4 vs. 2.5 kgMS/kg PV en promedio, AOF vs. BOF; respectivamente). Resultados de este experimento (Carriquiry et al., 2012; Soca et al., 2013b) muestran que control de intensidad de pastoreo a través del cambio en la oferta de forraje permitió en AOF incrementar la producción de forraje sin modificaciones en la capacidad de carga del sistema. La AOF mejoró la respuesta productiva y reproductiva de las vacas de cría, incrementando la eficiencia global del sistema criador (ej. +19% en kg ternero destetado/vaca entorado/año). Las vacas CR (F1) presentaron una superioridad (heterosis) productiva y reproductiva (ej. +15% en kg ternero destetado/vaca entorado/año) frente a las vacas PU (Angus y Hereford). Esta mejor respuesta productiva y reproductiva de las vacas en AOF y vacas CR se asoció a un mejor balance energético, reflejado no solo en la CC sino también en el perfil de hormonas metabólicas o de expresión génica en hígado a lo largo del ciclo gestación-lactación (Laporta et al., 2014). Esta mejora en el balance de energía de las vacas en AOF y vacas CR podría ser el resultado no solo de un mayor consumo sino también

de una reducción en los costos energéticos de mantenimiento (metabolismo basal y actividad). Estas respuestas diferenciales probablemente estén asociadas a diferencias en los mecanismos de partición de la energía, priorizando diferentes funciones ante cambios ambientales.

Es así que, este trabajo busca investigar sobre los mecanismos biológicos que podrían explicar reducciones en el costo de mantenimiento (a nivel del metabolismo basal) modificando la partición de la energía y por lo tanto la productividad de vacas CR y PU pastoreando AOF y BOF de campo nativo. Este trabajo aportará información sobre características asociadas al costo energético de mantenimiento que contribuyan a incrementar la eficiencia biológica de producción. Una disminución de estas exigencias energéticas (menores gastos energéticos para el mantenimiento de los vientres) contribuirá a una mayor eficiencia de alimentación (ej. para vacas de cría, kg terneros destetados/kg alimento consumido), disminuyendo los costos de producción.

1.2. ENERGÍA DE MANTENIMIENTO EN VACA DE CRÍA

En la producción de carne vacuna, sólo el 5% del total de energía consumida durante el ciclo de vida es utilizado para la deposición de proteínas, mientras que la producción de carne de cerdo y aves de corral son más eficientes (14 y 22% respectivamente del total de energía consumida; Ritchie, 2000). Las principales razones de la ineficiencia de la producción de carne vacuna, puede deberse a la relativamente baja y lenta tasa de reproducción y el alto costo energético de su mantenimiento.

Es asi que, la cría vacuna se caracteriza por ser un proceso largo e ineficiente en el uso de la energía, en donde el costo de mantenimiento significa entre el 40 a 50% del total de energía ingerida por el animal (desde la concepción a la faena; Montaño-Bermudez et al., 1990). En el ciclo de cría vacuna, más del 70% de los costos energéticos son debidos al mantenimiento de los vientres (Ferrell y Jenkins, 1985). El mantenimiento es el estado fisiológico en cual no hay cambio neto en la energía corporal o alternativamente cuando el balance de energía es cero (Baldwin, 1995), o sea la energía de mantenimiento de un animal es la fracción de la energía

neta consumida necesaria para mantener el equilibrio energético del animal (NRC, 2000). Ésta comprende a la energía destinada a mantener constantes los tejidos corporales y actividades vitales básicas. Según NRC (2000), la estimación de la energía requerida para el mantenimiento incluye: la energía requerida para el metabolismo basal (**MB**), la termorregulación y la actividad voluntaria del animal, en confinamiento y en un ambiente no estresante. Sin embargo, para animales en pastoreo se le debe sumar la energía necesaria para el pastoreo, la rumia y la búsqueda del alimento.

Dentro del MB están incluídas las funciones de servicio y el mantenimiento celular (Baldwin, 1995). Las funciones de servicio están relacionadas a la propia existencia del individuo, e incluyen todas aquellas vinculadas al funcionamiento de los sistemas respiratorio y circulatorio, actividades de excreción y la transmisión nerviosa, fundamentalmente. Éstas representan del 40 al 50% de la energía utilizada para el MB y están muy poco influenciadas por factores externos al animal (Smith y Baldwin, 1974; Baldwin et al., 1980). La energía destinada al mantenimiento celular incluye el transporte de iones y la renovación de proteínas y lípidos que representan del 50 al 60% de la energía utilizada para el MB y son dependientes de factores propios y externos al animal (Smith y Baldwin, 1974; Baldwin et al., 1980).

La transferencia de iones través de la membrana es fundamental para el mantenimiento del gradiente de concentración extra e intracelular (ej. movimiento de entrada y salida de Na^{+} de las células nerviosas durante el impulso nervioso, reabsorción de Na^{+} filtrado por los riñones, y movimiento hacia dentro y fuera del Ca^{++} del retículo sarcoplasmico durante la actividad muscular). Existe una íntima relación entre el movimiento de iones y la transducción energética. El resultado es un circuito contínuo de transporte de iones que sirve como el enlace entre la utilización de la energía (ATP), con la conservación de la energía en una forma útil desde el punto de vista metabolico (Milligan y Mc Bride 1985, Gill et al., 1989). Estudios realizados por Wang et al. (2009) demostraron que el nivel de alimentación modificó la expresión de la $Na^{+}K^{+}$-ATPasa en el páncreas, pero no en otros órganos del TGI. Milligan y Mc Bride (1985) estimaron que por ejemplo, la actividad de la $Na^{+}K^{+}$-ATPasa representa del 20 al 70% del consumo de energía *in vitro* de varios tejidos, y

está muy ligada al crecimiento y proliferación celular. Estos autores en ensayos de proliferación celular en presencia de mitógenos, demostráron que la activación de $Na^{+}K^{+}$-ATPasa es esencial, tanto para el crecimiento hiperplásico como hipertrófico de las células.

Por otra parte, la energía necesaria para el mantenimiento está correlacionada con la cantidad de proteínas y grasa presentes en el animal. La distribución, ubicación y cantidad de proteína y grasa depositadas afecta los requerimientos de mantenimiento. Se ha propuesto que el costo asociado con el mantenimiento de los tejidos resulta de un proceso continuo de síntesis, degradación y remplazo de parte del tejido corporal que se renueva, existiendo una considerable diferencia en la energía retenida por unidad de peso (eficiencia de síntesis) en la deposición de proteína y grasa (10 al 40% vs. 60 al 80%, respectivamente; Ferrell y Jenkins, 1985), así como en la tasa de renovación de la proteína y grasa corporal. Determinaciones de la síntesis proteica *in vitro* en homogenizado de distintos tejidos demuestran que el músculo, el TGI, el hígado y la piel contribuyen 12 a 16%, 38 a 46%, 7 a 8% y del 14 a 20% al total de síntesis proteica corporal, respectivamente. Lobley et al. (1980) estudiando vacas Frisian y Hereford x Frisian que consumían una dieta basada en concentrados, estimaron que el recambio total de las proteínas del TGI se lleva a cabo aproximadamente cada 3 días. Es así que, si bien en los distintos sistemas de alimentación de rumiantes (NRC, CSIRO, AFRC), el costo energético de mantenimiento se estima como constante en relación al PV adulto ajustado por diferencias en tamaño corporal (peso metabólico; $PV^{0.75}$) existe cada vez mas evidencia en ratas, cerdos, ovinos y vacas lecheras que la tasa metabólica depende más de la masa proteica corporal que del PV total de los animales (Agnew y Yan, 2000).

Existen otros factores que modifican los requerimientos de mantenimento como lo son: el consumo de materia seca (MS) (Burrin et al., 1989; Reynolds et al., 1992; Freetly and Ferrell, 1995), actividad y conducta de pastoreo (Brosh et al., 2006), la masa y composición corporal (Kock y Preston, 1979; Ferrell y Jenkins, 1985; Goodrich et al., 1985; Agnew et al., 2005), el tamaño de vísceras del tracto gastrointestinal (TGI) y otros órganos (McLeod, y Baldwin, 2000; Baldwin et al.,

2004) y la actividad metabólica de los mismos (Milligan y Mc Bride, 1985; Herd y Arthur, 2009; Wang et al., 2009).

Particularmente, los costos de energía para el mantenimiento varían entre vacas de diferentes genotipos (Jenkins y Ferrell, 1994). Estos autores indican que existe un 16% de diferencia entre los costos de requerimientos energéticos de mantenimiento entre razas. Vacas con mayor potencial de producción de leche tienden a tener requerimientos de mantenimiento más altos, asociado a una mayor masa de los órganos del TGI e higado (Ferrell y Jenkins, 1984; Montaño-Bermudez et al., 1990; Nielsen, 1995).

A su vez, se ha reportado que la variación genética de las necesidades energéticas de mantenimiento del ganado es de moderada a alta, con heredabilidadades de 0,22 a 0,71, sugiriendo la posibilidad de obtener pregreso genético al seleccionar por animeles más eficientes (Carstens et al,. 1989; Bishop, 1992). El consumo de alimento neto (o residual; CRA) o la entrada neta de alimentos se ha utilizado como una característica de eficiencia alimenticia independiente de tamaño corporal (Koch et al., 1963). Se ha reportado que los mecanismos biológicos que explican el CRA están muy relacionados mecanismos que determinan las necesidades de energía de mantenimiento, fundamentalmente MB (Richardson y Herd, 2004; Herd et al., 2004).

1.2.1. Composición corporal

La composición corporal es la anatomía química del animal, y es la suma de los diversos componentes (agua, grasa, proteína, minerales) de los tejidos y sistemas que conforman el organismo (lo cual difiere de la anatomía morfológica). Varios experimentos en vacas de carne han demostrado que los requerimientos de mantenimiento por unidad de peso metabólico son menores en vacas con mayor proporción de grasa (Klosterman et al., 1968; Thompson et al., 1983; Goodrich et al., 1985; Houghton et al., 1990a; DiCostanzo et al., 1990;1991). Sin embargo, Ferrell et al. (1986) trabajando con corderos alimentados en distintos planos nutricionales, sugieren que las diferencias en los costos de mantenimiento no son el resultado de la composición corporal *per se,* sino del incremento en el gasto de energía

correlacionado, pero no directamente asociado, con la síntesis de proteína de corporal, indicando que la masa de los órganos viscerales [ej. del tracto gastrointestinal (TGI) e hígado] es determinante del gasto energético de mantenimiento. Es así que, la composición corporal y el tamaño de las vísceras contribuyen de forma importante a los costos energéticos de mantenimiento. Se ha estimado que diferencias en la composición corporal de los animales explican aproximadamente 5% de las variaciones en CRA entre animales, fundamentalmente relacionado a la acumulación de proteínas corporales (Richardson et al., 2001; Basarab et al., 2003; Richardson y Herd, 2004; Herd et al., 2004).

1.2.2. Masa y actividad de las vísceras del TGI e hígado

Existe considerable evidencia que indica que el peso de las vísceras difiere entre genotipos, entre estados fisiológicos y entre planos nutricionales (Smith y Baldwin, 1974; Baldwin, 1995; Jenkins et al., 1986; Baldwin et al., 2004) y que estas diferencias pueden traducirse en variaciones en las necesidades de energía de mantenimiento (Jenkins et al., 1986; Baldwin, 1995).

Si bien el músculo representa del 30 al 50% del cuerpo, las vísceras del TGI e hígado presentan una alta irrigación con un importante flujo de sangre y son responsables de una porción sustancial (40%) del total de consumo de oxígeno de los animales (Baldwin et al., 2004). Es así que, las vísceras del TGI (y el hígado) juegan un rol crítico en la determinación de los requerimientos energéticos de mantenimiento, ya que son tejidos que presentan una tasa muy alta de recambio celular, con una tasa metabólica muy alta (intercambio de iones y recambio proteico). Se ha estimado que de los costos energéticos del mantenimiento la mitad está representada por la actividad del hígado y de los intestinos, aunque sólo representan del 10 al 13% de la masa corporal (Seal y Reynolds, 1993). Ksiazek et al. (2004) reportaron en líneas de ratones con selección divergente por tasa metabólica (alto vs. bajo tasa de MB) que si bien los animales no diferían en PV, los de alto MB presentaban mayor consumo de alimento y mayor peso de los órganos (hígado, riñones, intestino delgado y corazón). En particular el hígado presentó una diferencia del 17% entre las líneas genéticas divergentes, siendo mayor en las de alto

MB. De manera similar, DiCostanzo et al. (1990) determinaron que la energía que se requiere para el mantenimiento de vacas Angus tiene una correlación positiva con el peso del hígado y el corazón y el hígado en relación al peso de cuerpo vacío (PCV).

Los gastos energéticos derivados de los procesos mecánicos y metabólicos asociados con la ingestión y digestión de los alimentos así como con la absorción de nutrientes, contribuyen a los gastos de mantenimiento y están fuertemente influenciados por la cantidad y tipo de alimento ingerido. El consumo de oxígeno *in vivo* de vísceras se eleva con el aumento de la ingesta de energía alimentaria (Burrin et al., 1989; Reynolds et al., 1992; Freetly y Ferrell, 1995). Es así que, el plano nutricional tiene un impacto directo sobre las actividades de digestión y de absorción de nutrientes afectando el requerimiento de energía de las vísceras. Por ejemplo, Kozloski et al. (2001) sugiere que un aumento en la ingestión de alimento, llevaría a un mayor gasto energético debido a una mayor actividad metabólica de las células epiteliales y a una mayor actividad contráctil de las células musculares en el tracto digestivo. Por otra parte, el tipo de alimento y la concentración energética del mismo influencian el tamaño de las vísceras del tracto digestivo, el desarrollo de la capa epitelial de todo el TGI, llevando a cambios en la masa de estos órganos (McLeod y Baldwin, 2000). Susenbeth et al. (1998) comprobaron que el requerimiento de energía para comer y rumiar es alto y varía según el tipo de alimento. Cuando se alimentan novillos con forrajes muy fibrosos (paja sin tratar), aproximadamente el 30% de la energía proporcionada por el alimento se utiliza para cubrir la demanda energética de las actividades de ingesta y rumia. A su vez, cuando se suministra un forraje de alta calidad, sólo el 10% de la energía que aporta el alimento es utilizado para estas funciones fisiológicas. A su vez, el consumo de forraje provoca el incremento del tamaño de las vísceras frente a dietas basadas en concentrados. Webster et al. (1975; 1980) determinaron que el consumo de forrajes provoca un mayor aumento del calor de fermentación y actividad de las vísceras en comparación con el consumo de granos. Efecto similar se observa frente al incremento en la cantidad de alimento ingerido. El costo energético de la ingestión de una misma cantidad de forraje por el animal es del doble si se compara el pastoreo directo con el suministro del forraje picado suministrado *in situ* (Holmes et al., 1978).

1.2.3. Energía de mantenimiento y metabolismo mitocondrial

En las mitocondrias se produce el 90% de la energía celular y están integradas en numerosas funciones de diferentes redes metabólicas y señalización con otros compartimentos celulares. Rolfe y Brand (1997) estimaron que aproximadamente el 90% del consumo de oxígeno en los mamíferos ocurre en la mitocondria, siendo este organelo un lugar apropiado para estudiar la variación de los requerimientos de energía de mantenimiento entre animales. Esta variación puede contribuir a explicar las diferencias fenotípicas observadas entre los animales (Herd y Arthur, 2009).

El ciclo del ácido cítrico (o del ácido tricarboxílico) es el eje central del metabolismo celular, representando la vía final común de oxidación de las moléculas energéticas. De hecho, todos los compuestos energéticos (carbohidratos, lípidos y proteínas) acaban siendo metabolizados hasta acetil-CoA o a algún componente del ciclo del ácido cítrico. Este ciclo también es fuente de precursores para la biosíntesis de otras moléculas, e incluye una serie de reacciones de óxido-reducción que conducen a la oxidación de un grupo acetilo hasta dos moléculas de dióxido de carbono. Esta oxidación genera electrones de alta energía que se utilizarán para la síntesis de ATP. La función específica del ciclo del acido cítrico es proporcionar electrones a partir de compuestos carbonados para la formación de compuestos transportadores de electrones (NADH y $FADH_2$) que luego son oxidados nuevamente mediante O_2 en la fosforilación oxidativa. Los electrones liberados del NADH y/o $FADH_2$ fluyen a través de una serie de proteínas de membrana denominada cadena de transporte de electrones o cadena respiratoria situada en la membrana interna de la mitocondria y que consiste en cinco complejos enzimáticos (multiproteicos): Complejo I (NADH: ubiquinona reductasa), Complejo II (succinato: ubiquinona reductasa), Complejo III (ubiquinol: citocromo C reductasa), Complejo IV, (ferrocitocromo c: oxígeno oxidoreductasa) y Complejo V (ATP sintasa). A través de los Complejos I al IV se da el transporte de electrones, que se acopla al bombeo de protones hacia el espacio intermembrana (a través de los complejos I, III y IV), generando un gradiente de protones a través de la membrana.

Los protones luego fluyen a través de la ATPsintasa (Complejo V) y la disipación del gradiente se acopla a la síntesis de ATP (Berg y Tymoczko, 2008).

Los complejos mitocondriales no son entidades separadas, sino que están formados por subunidades multiprotéicas, que tienen una gran dependencia estructural y funcional de las subunidades individuales, donde la alteración de uno de ellos puede causar perturbación parcial o global de toda la funcionalidad de uno, varios o todos los complejos. La cadena de transporte de electrones es también un sitio reconocido de la producción de especies reactivas de oxígeno (ROS), principalmente complejos I y III (Bottje y Carstens, 2009). Una elevada producción de ROS implica una alta susceptibilidad de los diversos componentes celulares al daño oxidativo. Este daño puede determinar un aumento en el recambio de componentes corporales, fundamentalmente proteínas, que al estar alteradas por el daño oxidativo no cumplen su función y es necesario renovarlas con el eventual gasto de energía que conlleva. Se ha demostrado que la actividad de los complejos respiratorios mitocondriales y la expresión de varias proteínas de estos complejos (Sandelin et al., 2005), así como la expresión de varios genes codificadores de proteínas de estos complejos (Connor et al., 2009), es mayor en novillos de alta vs. baja eficiencia alimenticia. Recientes hallazgos sugieren que la actividad de la cadena respiratoria puede tener impactos críticos sobre el estrés oxidativo, lo que resulta en cambios en el equilibrio metabólico contribuyendo a las diferencias fenotípicas en la eficiencia alimenticia en bovinos de carne (Kolath et al., 2006). Varios estudios han identificado posibles regiones en el genoma relacionadas con las diferencias entre animales en eficiencia de alimentación (Moore et al., 2009). Parte de las diferencias en CRA se ha asociado a cambios en el equilibrio metabólico, fundamentalmente a la actividad de los complejos respiratorios mitocondriales y la expresión de varias proteínas de estos complejos y al daño oxidativo (Kolath et al., 2006; Bottje y Carstens, 2009). Barendse et al. (2007) determinaron que el mayor número de polimorfismos de una sola base (SNPs) asociados con eficiencia alimenticia se encontraban en regiones no-codificantes del genoma bovino, muy frecuentemente en elementos promotores y motivos de microARN. Estos resultados junto con los anteriores sugieren que una proporción sustancial de la variación entre

animales en eficiencia alimenticia, es debido a diferencias en la regulación de la expresión génica, en particular de los complejos respiratorios mitocondriales. Sin embargo, a pesar de su importancia potencial, la información disponible en esta área en bovinos es escasa y la existente se ha generado en novillos en crecimiento, mientras que no se dispone de información en vacas de carne bajo condiciones pastoriles.

En resumen, el costo de mantenimiento tiene un impacto significativo entre la partición de la energía metabolizable entre pérdidas de calor y energía neta de producción y por lo tanto puede afectar la eficiencia biológica y económica de la producción de carne (Montaño-Bermudez et al., 1990; Johnson et al., 2003). Es así que, una mejor comprensión de los factores asociados al costo de mantenimiento y balance energético de la vaca de cría pastoreando campo nativo, como son la nutrición, la genética y la etapa fisiológica en interacción con el momento del año, permitirá generar manejos/tecnologías que aumenten la eficiencia global del sistema criador.

1.3. HIPÓTESIS Y OBJETIVOS

1.3.1. Hipotesis

Este trabajo tiene como hipótesis que el control de la intensidad de pastoreo de campo nativo, a través del manejo de la oferta de forraje, afecta la composición corporal y la masa, celularidad y actividad metabólica de los órganos y vísceras. Estos efectos, podrían diferir en vacas de cría puras (Hereford y Angus) y cruzas (F1) y podrían contribuir a explicar diferencias en el comportamiento productivo-reproductivo entre vacas de carne PU y CR pastoreando AOF y BOF de campo nativo (Carriquiry et al., 2012; Soca et al., 2013b; Gutiérrez et al., 2013; Laporta et al., 2014).

1.3.2. Objetivo general

Conocer los efectos del control de la intensidad de pastoreo del campo nativo, a través del manejo de la oferta de forraje, sobre características asociadas al requerimiento de mantenimiento (composición corporal y de la masa, celularidad, composición y expresión de genes que codifican proteínas mitocondriales en las vísceras del TGI e hígado) de vacas de cría puras (Herford y Angus) y sus cruzas recíprocas (F1), contribuyendo a la comprensión de mecanismos biológicos que explican diferencias en la eficiencia biológica de producción del sistema criador.

1.3.3. Objetivos específicos:

1- Evaluar el efecto de dos ofertas de forrajes de campo nativo sobre sobre la composición corporal durante el ciclo anual de produccion en vacas de cría puras (Hereford y Angus) y cruzas (F1) (Capitulo 2; *Articulo 1*).

2- Evaluar el efecto de dos ofertas de forrajes de campo nativo sobre la masa, composición, índices de celularidad y expresión de genes que codifican proteínas de los complejos respiratorios mitocondriales de las vísceras del TGI e hígado en vacas de cría puras (Hereford y Angus) y cruzas (F1) (Capitulo 3; *Artículo 2*).

1.4. ESTRUCTURA GENERAL DE LA TESIS

Consiste en dos artículos científicos, el primer artículo, titulado **"Body composition of mature beef cows grazing different herbage allowances of native grasslands"** constituye el segundo capítulo de esta tesis, en el mismo se presenta la evaluación del efecto de la oferta de forraje del campo nativo sobre la composición corporal (agua, proteína, lípidos y energía bruta) predicha a partir la técnica de dilución de la urea en vacas de cría puras (Hereford y Angus) y sus cruzas reciprocas (F1) durante el período de gestación invernal (Objetivo específico 1). Este artículo será enviado a la revista *Animal Production Science*, y

El segundo artículo, titulado **"Visceral organ mass, cellularity and expression of genes encoding for mitochondrial respiratory chain proteins in pure and crossbred mature beef cows grazing different forage allowances of native**

pastures”, constituye el capítulo 3 de esta tesis y se presenta la evaluación del efecto de la oferta de forraje del campo nativo sobre la masa, celularidad, composición y expresión de genes que codifican proteínas mitocondriales de las vísceras del TGI e hígado en vacas de cría puras (Hereford y Angus) y sus cruzas reciprocas (F1) (Objetivo específico 2). Este artículo, fue enviado a la revista *Livestock Science*, y está en proceso de revisión.

En el cuarto capítulo de esta tesis se presenta una discusión general y conclusiones globales de los dos artículos.

2. CHANGES IN BODY COMPOSITION DURING THE WINTER GESTATION PERIOD IN MATURE BEEF COWS GRAZING DIFFERENT HERBAGE ALLOWANCES OF NATIVE GRASSLANDS

A. Casal*[1], A.L. Astessiano*, A.C. Espasandin†, A.I. Trujillo*, P. Soca†, M. Carriquiry*

*Departamento de Producción Animal y Pasturas, Facultad de Agronomía, Universidad de la República (UdelaR). Av. E. Garzón 780, 12900, Montevideo, Uruguay.

†Departamento de Producción Animal y Pasturas, EEMAC Facultad de Agronomía, Universidad de la República (UdelaR). Ruta 3 km 363, 60000, Paysandú, Uruguay.

[1]Corresponding author: A. Casal; alcas@adinet.com.uy

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2.2. **ABSTRACT**

The aim of this study was to evaluate the effect of controlling the grazing intensity of native pastures, through the herbage allowances (HA) on body composition (water, protein and fat) of beef cows of different cow genotype (CG; purebred: Angus and Hereford; PU, and crossbred: reciprocal F1; CR). Mature beef cows (n = 32) were used in a complete randomized block design with a factorial arrangement of HA (2.5 vs. 4 kg DM/d; LO vs. HI) and CG (PU vs. CR). The experiment was conducted during three years and at the end of the third year at 150, 210 and 240 ± 10 days of gestation and 190 ± 10 days pospartum body composition was estimated using the urea dilution technique. At 192 ± 10 days postpartum cows were slaughtered. Samples including all tissue were collected for chemical composition analyses. At slaughter cow live weight (LW) and empty body weight (EBW) tended to be greater ($P = 0.07$) in HI than LO cows and in CR than PU cows. Body water mass did not differ between HA, but tended ($P = 0.10$) to be greater in CR than PU cows. Body fat mass was not affected by HA, CG or their interaction, but body protein mass tended ($P = 0.06$) to be greater in HI than LO cows and was greater ($P = 0.03$) in CR. During the winter gestation period, maternal LW tended ($P = 0.09$) to be greater in HI than LO cows and was greater ($P = 0.02$) in CR than PU cows. Body water mass was greater ($P = 0.05$) in CR than PU cows and decreased ($P < 0.01$) from 150 to 210 days to gestation, remaining stable thereafter at 240 days to gestation. Relative body water (g/kg of EBW) was greater ($P \leq 0.02$) in HI than LO cows and CR than PU cows while relative body protein did not differ between HI and LO but tended ($P = 0.10$) to be greater in CR than PU. Relative body water increased ($P < 0.01$) while relative body fat decreased ($P < 0.01$) from 150 to 210 days of gestation, remaining constant at 240 days. Body fat mass tended ($P = 0.10$) to be greater HI than LO cows and was greater ($P = 0.03$) in CR than PU cows. Body fat per unit of EBW tended ($P = 0.10$) to be greater in HI than LO cows, while it did not differ between CR and PU cows. Body protein mass was greater ($P = 0.02$) in CR than PU cows and tended ($P = 0.06$) to decrease from 150 to 210 days to gestation, remaining stable thereafter at 240 days to gestation. Relative body fat and protein, as the body fat decrease and the body protein increase from 150 to 210 days of gestation were more sharply for PU

than CR cows. Body fat mass was not affected by HA, CG or their interaction, but body protein mass tended (P = 0.06) to be greater in HI than LO cows and was greater (P = 0.03) in CR than PU cows. However, these differences disappeared when body composition was expressed in relative terms. These results suggest that HI maintained greater body fat mass and retained GE increased when compared with LO cows, and CG affected not only body composition (greater body fat and protein in CR than PU cows) but also composition of mobilized/retained weight during the winter gestation period with a greater protein tissue mobilization in CR than PU cows.

Key words: beef cattle, body composition, rangelands, urea space.

2.3. INTRODUCTION

Beef cow-calf systems transform less than 30% of the annual metabolizable energy (**ME**) intake into calf product as the greater proportion of the total energy consumed is used for maintenance of the cow herd (Ferrell and Jenkins, 1985). Total energy expenditure has been related to animal metabolic weight (live weight (**LW**) adjusted by body size; $LW^{0.75}$; NRC, 1996). However, evidence in different species (rats, pigs, sheep, and dairy cows) indicates maintenance metabolic rate depends more on body protein mass rather than total animal LW (Agnew and Yan, 2000). Thus, body composition could affect maintenance energy requirement (Thompson et al., 1983). In the mature beef cow, body composition is not static but may vary in response to genotype (Laurenz et al., 1992; Jenkins and Ferrell, 1994) and nutrition (Ball et al., 1997).

Beef production is often conducted in extensive grazed forage-based production systems (ie. Campos region) where nutrient availabilty flucutates throughout the year, as forage quantity and quality vary according with seasonal changes in rainfall and temperature (Berretta et al., 2000). Beef cow nutrient requirements also fluctuate throughout the year due to the pressure of reproduction and lactation. Thus, rangeland beef cows undergo changes in LW, body condition score (**BCS**) and body composition (Laurenz et al., 1992) througout the annual production cycle (Quintans et al., 2010; Soca et al., 2013). Freetly et al. (2008) suggested cow ability to adapt its energy metabolism during periods of feed

restriction and re-feeding (weight cycling) would allow the development of management strategies of feed resources in order to reduce feed costs. However, timing and severity of restriction during the beef cow annual production cycle could affect cow and calf productive and reproductive performance (Freetly et al., 2005; Houghton et al., 1990a; 1990b; Funston et al., 2010). Therefore, in beef cow-calf management of grazing intensity by controlling seasonal herbage allowance (**HA**) would allow to control feed availability per cow, in order to benefit on weight cycling without or with minimal effects on beef cow (and calf) productive and reproductive performance.

Our hypothesis was that HA of native grasslands would affect body composition and the priorities for body protein and fat mobilization or recovery through the annual production cycle in spring-calved beef cows. In addition, the impact of HA on body composition could differ according with beef cow genotype (**CG**). The objective of this research was to evaluate the effect of two HA of native grasslands (Campos region) on body composition during the winter gestation period and after weaning in fall of rangeland purebred (**A**: Angus and **H**; Hereford; **PU**) and the reciprocal F1 crossbred (AxH and HxA; **CR**) mature beef cows.

2.4. MATERIALS AND METHODS

Location, animals and experimental design

Animal procedures were approved by the Animal Experimentation Committee of Universidad de la República.

The experiment was carried out at the Experimental Station Bernardo Rosengurtt (School Agronomy, Universidad de la República, Uruguay, 32°S 54°W) from June 2007 to May 2010. Thirty-two multiparous cows (4 to 5 year-old), were used in a randomized block design with two replications (block 1: sandy loam soil, 60 ha and block 2: clay loam soil, 35 ha) and four plots in each block to which a 2 x 2 factorial arrangement of HA and CG (PU vs. CR) was allocated. Native pastures were dominated by summer-growing C4 grasses (*Poaceae*), with few C3 grasses associated with the winter cycle. The main forb families included *Asteraceae*, *Fabaceae*, *Rubiaceae* and *Umbelliferae*.

Herbage allowance, the ratio between forage mass and stocking rate (kg of dry matter (**DM**) per kg of LW; Sollenberger et al., 2005), represented 4 and 2.5 kg DM/kg LW of annual mean (**HI** and **LO**, respectively) and varied with season of the year (Table 1). Herbage allowance was adjusted monthly after measuring the herbage mass available in each plot (Haydock and Shaw, 1975) by the "put-and-take" method (Mott, 1960). Experimental cows were maintained in the plot throughout the experiment, and 'put-and-take' cows of similar genotype and physiological status to the experimental ones were added or removed based on the herbage mass available. Herbage allowance treatments differed in forage mass and height throughout the year but did not differ in chemical composition (Table 1).

Table 1. Herbage allowances (HA), forage mass, height and chemical composition, by season, during the three experimental years.

	Season[1]				
	Fall	**Winter**	**Spring**	**Summer**	**se**
HA (kg DM/kgBW)[2]					
HI	5	3	4	4	
LO	3	3	2	2	
Forage mass (kg DM/ha)					
HI	2556	1459	1892	2382	160
LO	1422	971	1587	1371	160
Height (cm)					
HI	5.4	4.1	5.0	6.2	0.3
LO	3.4	2.9	3.7	3.6	0.3
Chemical composition					
DM, %					
HI	53.1	55.0	41.7	53.9	2.5
LO	53.6	52.5	38.9	46.6	2.5
CP, % of DM					
HI	7.5	8.3	10.4	8.4	0.5
LO	7.0	9.0	10.6	8.7	0.5
ADF, % of DM					
HI	42.6	39.9	35.5	41.2	1.6
LO	42.7	43.0	36.5	39.2	1.6
ME, MJ/kg DM[3]					
HI	8.4	8.7	9.3	8.6	0.2
LO	8.4	8.4	9.1	8.7	0.2

[1]Fall (April, May, and June), Winter (July, August, and September), Spring (October, November, and December), Summer (January, February and March).[2]HA:Herbage allowance treatments. Mean annual allowance represented 4 and 2.5 kg DM/kg BW (HI and LO, respectively) and changed seasonally. [3]Estimated according to Rohweder, (1984).

Experimental cows belonged to a group of experimental animals generated as part of a diallel crossbreeding experiment carried out for 10 years at the Experimental Station (Espasandin et al., 2010). Eight cows were evaluated per treatment (**HI-CR**, **HI-PU**, **LO-CR**, and **LO-PU**; n = 4 for each individual genotype: H and A for PU or HxA and AxH for CR). Cows grazed on the same HA (HI or LO) since June 2007, and gestated and lactated one calf every year from 2007 to 2010. At the beginning of the experiment (June 2007) cow LW and BCS (scale 1 to 8; Vizcarra et al., 1986) did not differ among groups and average 447 ± 58 kg and 4.1 ± 0.5 units, respectively.

Data and sample collection

During the pregnancy-lactation cycle 2009-2010 at 150, 210 and 240 ± 10 days to gestation (middle and end of winter and beginning of spring, respectively) and 190 ± 10 days from calving (late fall; 45 days after calf weaning), LW (after a 16 h-fasting) and BCS were determined and body composition was estimated using the urea dilution technique (Kock and Preston, 1979). Briefly, cows were infused with 0.65 ml/kg LW of a urea solution (20% urea in 0.9% saline solution, wt/vol) by jugular venipuncture, and blood was collected in tubes with heparin (BD Vacutainer tubes; Becton Dickinson, NJ, USA) before and 12 min after the mean infusion time. Samples were centrifuged (2000 x *g*, 15 min), and plasma was harvested. The plasma was stored at -20º C to determine the difference in plasma urea–nitrogen (**PUN**) between blood samples (before and after infusion) and to calculate urea space (USV or US). Urea space volume (**USV**, kg) was calculated by dividing the amount of urea (mmol of urea) infused by the difference in PUN between samples (mmol of urea-N/L) while urea space as a proportion of LW (% LW; **US**) was calculated by dividing the USV by LW.

All cows were slaughtered at 192 ± 10 days postpartum (48 h after the last day of measurements) in a commercial abattoir (PUL S.A; Cerro Largo, Uruguay; 10 km from the Experimental Station). Prior to transport, cow BW and BCS were recorded. Cows were stunned with a captive bolt gun and exsanguinated. Blood volume was estimated according to Hansard et al. (1953) and blood samples were

collected for chemical composition analysis. Exsanguinated cow bodies were divided into hide, feet, head, gastrointestinal tract (**GIT**) (including omental/mesenteric fat), pluck (trachea, lungs, heart, diaphragm, liver, kidneys, and tail), and carcass, and individual organs of the GIT and pluck were dissected. The weight of each component or individual organ was recorded and representative samples (100 to 200 g) samples were collected and stored at -20°C for chemical composition analyses. Carcass samples were collected for the right half carcass at the 9^{th} to 11^{th} rib section. This section was dissected into soft tissues and bone, which were individually weighed and frozen at -20°C for chemical composition.

Chemical analyses

Samples of all organs/tissues were ground in liquid nitrogen and analyzed for DM, nitrogen, total lipid, and ash according to AOAC (2000). Concentrations of PUN were determined by a colorimetric assay using a commercial kit (Urea UV cinética AA líquida, Wiener Laboratorios, Rosario, Argentina) on Vitalab Selectra 2 autoanalyzer (Vital Scientific, Dieren, The Netherlands). The intra-assay coefficient of variation did not exceed 11%.

Calculations and statistical analyses

During the winter gestation period, maternal LW was calculated as the difference between cow LW and estimated weight of the gravid uterus adjusted for the actual calf birth weight (Ferrell et al., 1976). Cow empty body weight (**EBW**) was estimated as carcass weight plus total offal weight and maternal LW x 0.891 (NRC, 2000). Body composition (water, protein, and lipids) was estimated from the weighted average of the each individual organ/tissue weight and its composition. Gross energy (**GE**) was calculated using retained combustion values of 23.85 MJ/kg protein and 39.75 MJ/kg fat (Brouwer, 1965).

Data were analyzed using the SAS Systems program (SAS 9.0V; SAS Inst., Cary, NC, USA). Prediction equations for body composition, and GE content were developed using either USV or US as a single predictor in the linear regression (equation [i]) or using USV or US together with other animal factors (e.g., maternal

LW, BCS, and milk yield and calf weights obtained by Gutiérrez et al., 2013) as predictors in multiple regression (equation [ii]). Single and multiple regressions were adjusted using the REG procedure. Model in multiple regressions was selected using the maximum adjusted r^2 (ADJRSQ) method.

$y = a + bx$ [i]

$y = a + b1\ x1 + b2\ x2 + ... + bn\ xn$ [ii]

Body composition during gestation was analyzed using a mixed-model repeated measures analysis using the MIXED procedure. The model included the HA, CG, days of gestation (repeated measure) and their interactions as fixed effects, block as a random effect, and the spatial power law (SP(POW)) as the covariance structure. The paddock was used as the experimental unit to evaluate HA effect and cow as the experimental unit to evaluate CG and the HA by CG interaction. The interaction between HA and block was included in the model as a random effect and when covariance parameter estimates were zero or close to zero it was removed from the model. Body composition at slaughter was analyzed with the same model without the effect of days. Mean separation was performed using the Tukey test ($\alpha = 0.05$), Changes in LW, BCS and body composition between 240 days gestation and 192 days postpartum (at slaughter) were assessed from a comparison of confidence intervals ($\alpha = 0.05$) within each group. Differences were considered significant when $P \leq 0.05$ and a trend when $0.05 < P \leq 0.10$. Results were presented as least square means ± pooled standard error.

RESULTS

Cow BCS, LW and chemical body composition at slaughter

Cow LW and EBW at slaughter tended to be greater (P = 0.07) in HI than LO cows and in CR than PU cows (Table 2). Cow BCS at slaughter was not affected by HA, CG or their interaction (Table 2). Body water mass (kg) did not differ between HA, but tended (P = 0.10) to be greater in CR than PU cows (Table 2). Body fat mass was not affected by HA, CG or their interaction, but body protein mass tended (P = 0.06) to be greater in HI than LO cows and was greater (P = 0.03) in CR than PU cows. The retained GE tended (P = 0.08) to be greater in HI than LO and CR than PU cows. However, when body composition was expressed in relative terms (g/kg EBW), these differences disappear except for relative protein mass that tended (P = 0.06) to be greater in CR than PU cows (Table 2).

Tabla 2. General data and chemical body composition (absolute and relative) at slaughter.

	Main effects[1]						**P-value**	
	HA			**CG**				
	HI	**LO**	**se**	**CR**	**PU**	**se**	**HA**	**CG**
General data								
LW, kg[2]	433	406	15.5	436	403	15.5	n.s.	0.02
EBW, kg[3]	356	334	10.8	356	334	10.8	0.07	0.07
BCS[4]	3.9	3.8	0.1	3.9	3.8	0.1	n.s.	n.s.
Milk production at 120 days, kg/day	5.7	4.6	0.6	5.9	4.4	0.6	0.01	<0.01
Calf weight at birth, kg	35	36	1.2	34	34	1.3	n.s.	n.s.
Calf weight at weaning, kg	115	107	4.4	118	104	4.5	n.s.	0.03
-Body composition-								
Absolute composition								
Water, kg	248	236	7.9	250	233	7.9	n.s.	0.10
Lipids, kg	37	34	2.5	36	34	2.5	n.s.	n.s.
Protein, kg	75	68	3.2	75	68	3.2	0.06	0.03
Gross energy, MJ	3241	2963	171.4	3237	2966	171.4	0.08	0.08
Relative composition								
Water, g/kg EBW	693	699	7.4	701	691	7.4	n.s.	n.s.
Lipids, g/kg EBW	103	102	4.7	102	102	4.7	n.s.	n.s.
Protein, g/kg EBW	208	203	3.6	210	201	3.6	n.s.	0.06
Gross energy, MJ/kg EBW	9.1	8.9	0.3	9.1	8.9	0.3	n.s.	n.s.

[1]HA: Herbage allowance (High and Low: 4 and 2.5 kg DM / kg BW on average; HI vs. LO). CG: Cow genotype (Pure Breed: Hereford and Angus vs. Crosses F1, PU vs. CR). [2]Live weight. [3]EBW: Empty body weight estimated as carcass weight plus total offal weight. [4]BCS: Body condition score by visual assessment (scale of 1 = very thin to 8 = very fat, Vizcarra et al., 1986).

Prediction equations of body composition

At slaughter, the correlations between the actual chemical composition (water, fat, protein, GE) in absolute (kg) or relative (g/kg EBW) terms and USV or US, respectively, were significant ($P \leq 0.03$ data not shown). Prediction equations for body water, fat, protein and retained GE using USV or US as the sole predictors were all significant ($P \leq 0.03$; Table 3). Multiple regressions (Table 4) were all significant ($P < 0.01$) and each predictor had an effect ($P < 0.15$) on the relationship. Using USV or US with other predictors (ie. LW, calf weight, etc.) for the prediction of body components improved the relationships, which showed greater r^2.

Table 3. Linear relationships between urea space volume (USV) or absolute chemical composition (kg or MJ) and urea space (US) or relative chemical composition (g or MJ/kg EBW) (values in parentheses are SE).

	Equations[1,2]	σ	$\mathbf{r^2}$
Absolute composition			
Water, kg	$= 0.775_{(0.08)}$ (USV) + $79.83_{(18.01)}$	13.6	0.79
Lipids, kg	$= 0.152_{(0.03)}$ (USV) + $3.11_{(6.61)}$	10.7	0.48
Protein, kg	$= 0.255_{(0.04)}$ (USV) + $16.36_{(8.16)}$	7.2	0.63
Gross energy, MJ	$= 10.086_{(1.52)}$ (USV) + $864.591_{(330.8)}$	0.3	0.64
Relative composition			
Water, g/kg EBW	$= 1.5_{(0.66)}$ (US) + $575.97_{(32.5)}$	13.3	0.16
Lipids, g/kg EBW	$= -1.1_{(0.40)}$ (US) + $148.87_{(20.2)}$	5.0	0.24
Protein, g/kg EBW	$= 0.773_{(0.25)}$ (US) + $153.95_{(12.4)}$	6.1	0.25
Gross energy, MJ/kg EBW	$= -0.033_{(0.01)}$ (US) + $10.103_{(0.67)}$	259.4	0.20

[1]USV: Urea space volume (kg). [2]US: Urea space as percent body weight.

Tabla 4. Regression equations for absolute chemical composition using urea space and live weight with other variables (values in parentheses are SE)

		US (%)[1]	USV (kg)[2]	Maternal LW (kg)[3]	BCS (units)[4]	Milk yield (kg)[5]	Calf weight(kg)			Constant	σ	r^2
							birth	weaning	Gain[6]			
Absolute composition												
Water, kg	=	-	$0.1_{(0.06)}$	$0.4_{(0.04)}$	-	-	$0.3_{(0.2)}$	-	-	$22.4_{(8.8)}$	10.2	0.97
Lipids, kg	=	-	$0.05_{(0.05)}$	$0.06_{(0.03)}$	-	-	-	-	$0.1_{(0.06)}$	$-7.7_{(7.5)}$	9.7	0.65
Protein, kg	=	-	$0.07_{(0.03)}$	$0.14_{(0.02)}$	-	-	-	-	-	$-3.6_{(5.6)}$	6.9	0.88
Gross energy, MJ	=	-	$2.2_{(1.5)}$	$6.5_{(0.9)}$	-	-	-	-	-	$-128.3_{(245.6)}$	0.3	0.89
Relative composition												
Water, g/kg EBW	=	$2.5_{(0.6)}$	-	$-0.06_{(0.05)}$	$-26.1_{(11.1)}$	$-3.2_{(2.1)}$	$1.44_{(0.5)}$	$-0.35_{(0.2)}$	-	$661.2_{(43.5)}$	4.8	0.65
Lipids, g/kg EBW	=	$-1.4_{(0.4)}$	-	$0.08_{(0.04)}$	-	$-1.9_{(1.4)}$	-	-	$0.3_{(0.1)}$	$115.9_{(23.2)}$	4.6	0.47
Protein, g/kg EBW	=	$0.9_{(0.03)}$	-	-	-	$1.1_{(1.1)}$	-	-	$-0.14_{(0.09)}$	$154.4_{(12.5)}$	3.7	0.40
Gross energy, MJ/kg EBW	=	$-0.05_{(0.01)}$	-	$0.002_{(0.001)}$	-	-	-	-	$0.006_{(0.005)}$	$9.5_{(0.8)}$	153.1	0.41

[1]US: Urea space as percent body weight. [2]USV: Urea space volume. [3]Body weight: cow live weight minus gravid uterine weight estimated by Ferrell et al. (1976). [4]BCS: Body condition score by visual assessment (scale of 1 = very thin to 8 = very fat, Vizcarra et al., 1986). [5] Milk production at 120 days, kg/day. [6]Gain pre weaning.

Maternal LW, BCS and body composition during the winter gestation period

During the winter gestation period (150 to 240 days of gestation), maternal LW tended (P = 0.09) to be greater in HI than LO cows and was greater (P = 0.02) in CR than PU cows (Table 5). Maternal LW decreased (P < 0.01) 18 ± 5 kg from 150 to 210 days remaining stable at 240 days (Figure 1A, 1B). Cow BCS was greater (P ≤ 0.02) for HI than LO cows and CR than PU cows (Table 5) and decreased (P = 0.01) 0.5 ± 0.1 units during the winter gestation period (Figure 1C, 1D).

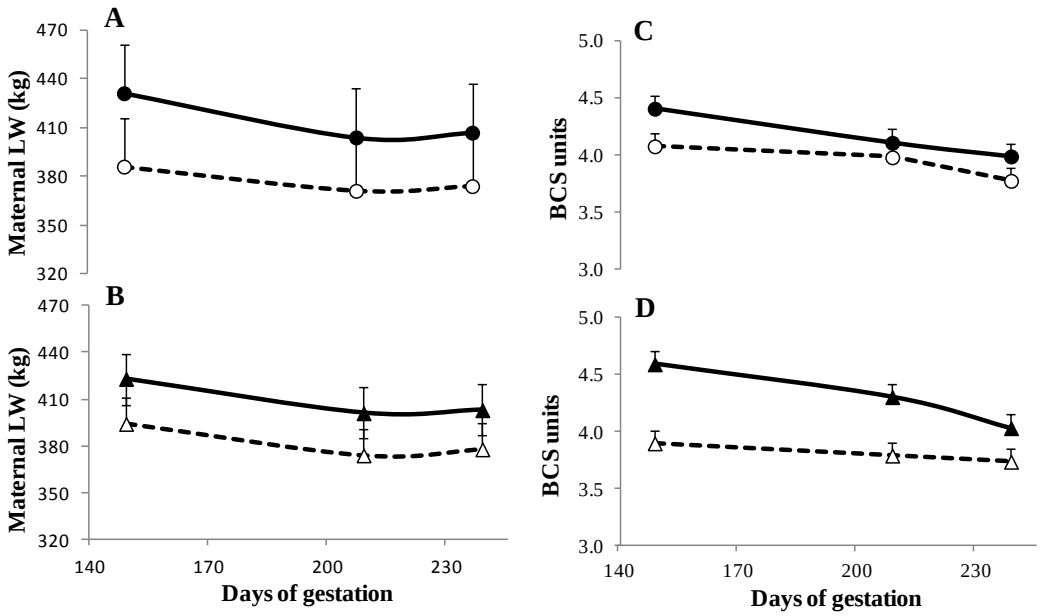

Figure 1: Maternal LW (A) (B) and BCS (C) (D) throughout the gestation (150 to 240 days of gestation) in purebred and crossbred beef cows (n = 32) and herbage allowances (HA) treatments grazing high and low (4 and 2.5 kg DM/kg LW in average, respectively herbage allowances of native pastures). Purebred cows (Hereford and Angus; dash lines and open circles), Crossbred cows (reciprocal cross F1; solid lines and circles), High HA cows (solid lines and triangles), and Low HA cows (dash lines and open triangles).

Total body water, fat and protein mass (kg) were greater (P ≤ 0.05) in CR than PU cows (Table 5). Body fat and protein mass tended (P = 0.10) to be greater in HI than LO cows (Table 5). Total body water and protein mass decreased (P < 0.01) from 150 to 210 days of gestation, remaining stable thereafter at 240 days (Figure 2A, 2C). Body water was affected (P = 0.03) and body fat tended (P = 0.07) to be affected by the interaction between CG and days of gestation as they were greater (P < 0.05) in CR than PU cows only at 150 days of gestation (beginning of winter) and decreased from 150 to 210 days of gestation only in the former ones (Figure 2A, 2B).

The decrease of 0.5 units BCS between 150 and 240 days of gestation represented a decrease of 18 ± 5 kg of maternal LW (140 to 215g/kg EBW of protein and 95 to 200 g/kg EBW of fat. Maternal LW and BCS did not change between 240 days gestation and 192 days postpartum (at slaughter). However, relative body composition varied during this period; relative water and protein mass increased for HI and CR while only relative water mass increased in LO and PU cows.

Tabla 5. Effects of herbage allowance (HA) and cow genotype (CG) on LW, Maternal LW, EBW, BCS and chemical composition during the winter gestation period (150-240 days of winter beginning to spring beginning).

	Main effects[1]						**P-value**		
	HA			**CG**					
	HI	**LO**	**se**	**CR**	**PU**	**se**	**HA**	**CG**	**DG[2]**
LW, kg[3]	428	402	17.6	433	397	17.6	n.s.	0.04	<0.01
Maternal LW, kg[4]	409	382	17.2	414	377	17.2	0.09	0.02	<0.01
EBW, kg[5]	363	340	15.9	368	336	16.0	n.s.	0.04	<0.01
BCS[6]	4.3	3.8	0.1	4.2	3.9	0.1	<0.01	0.02	0.01
-Body Composition-									
Water									
kg	236	226	10.1	240	223	10.0	n.s.	0.05	<0.01
g/kg EBW	632	657	3.4	640	649	3.4	<0.01	0.02	<0.01
Lipids									
kg	35	33	1.5	35	32	1.4	0.10	0.03	n.s.
g/kg EBW	95	91	1.4	93	93	1.4	0.10	n.s.	0.01
Protein									
kg	68	64	3.1	69	63	3.1	n.s.	0.02	0.07
g/kg EBW	191	192	0.9	192	190	0.9	n.s.	0.10	n.s.
Gross energy									
MJ	2995	2797	135.5	3033	2759	135.5	n.s.	0.03	<0.01
MJ/kg EBW	8.7	8.6	0.04	8.6	8.6	0.04	<0.01	n.s.	0.05

[1]HA: Herbage allowance (High and Low: 4 and 2.5 kg DM / kg BW on average; HI vs. LO); CG: Cow genotype (Purebreed: Hereford and Angus vs. Crosses F1, PU vs. CR). [2]Days of gestation; Effect of CG * DG interaction was significant ($P \leq 0.03$) in Water (kg) and Gross energy (MJ) and tended ($P \leq 0.10$) in Lipid (kg) Protein (g / kg EBW) and Gross energy (MJ / kg EBW). [3]Live weight. [4]Cow live weigth minus gravid uterine weight estimated by Ferrell et al. (1976). [5]EBW: Empty body weight calculated according to NRC 2000. [6]BCS: Body condition score by visual assessment (scale of 1 = very thin to 8 = very fat, Vizcarra et al., 1986).

Relative body water (g/kg of EBW) was greater ($P \leq 0.02$) in HI than LO cows and CR than PU cows while relative body protein did not differ between HI and LO but tended ($P = 0.10$) to be greater in CR than PU cows (Table 5). In contrast body fat per unit of EBW tended ($P = 0.10$) to be greater in HI than LO cows, while it did not differ between CR and PU cows. Relative body water increased ($P < 0.01$) while relative body fat decreased ($P < 0.01$) from 150 to 210 days of gestation, remaining constant at 240 days (Table 5; Figure 2D, 2E). The interaction between CG and days of gestation tended ($P \leq 0.10$) to affect both, relative body fat and protein, as the body fat decrease and the body protein increase from 150 to 210 days of gestation were more sharply for PU than CR cows (Figure 2E, 2F).

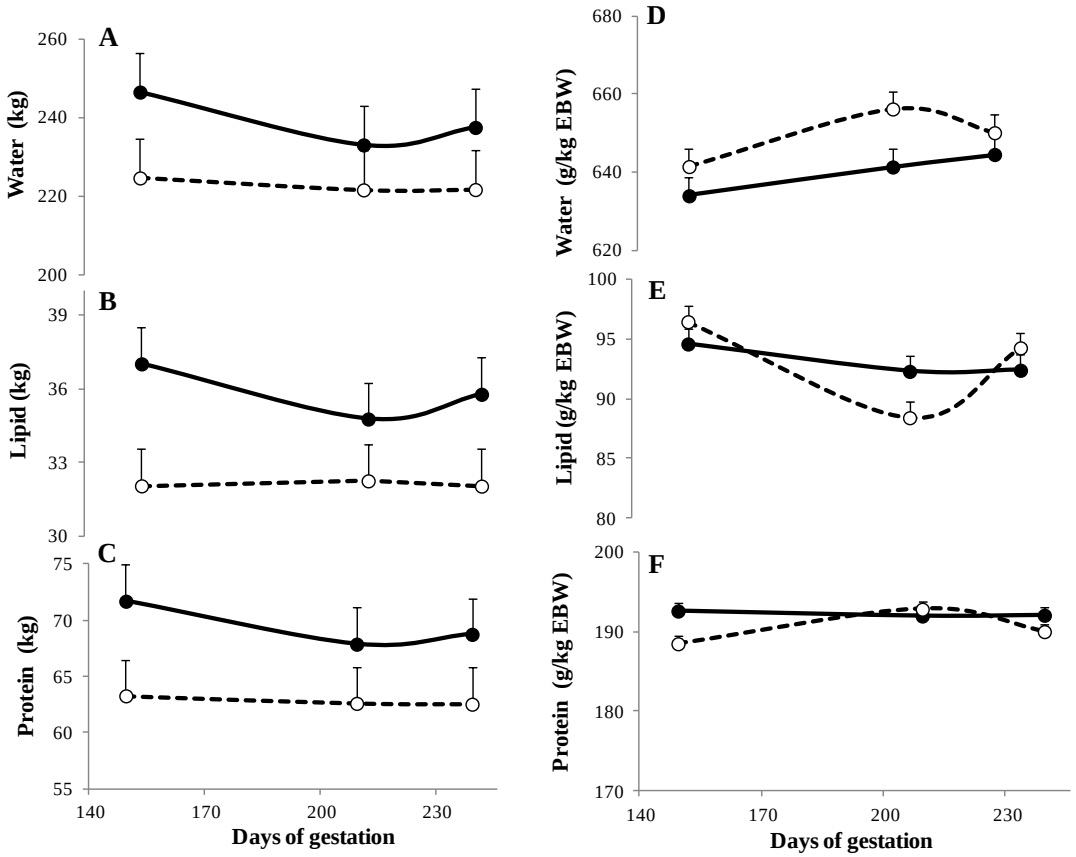

Figure 2: Evolution of body composition in absolute terms (kg) or relative [g/kg EBW] of water (A) [D], lipid (B) [E] and protein (C) [F]. In purebred (Hereford and Angus; dash lines and open circles) crossbred beef cows (reciprocal cross F1; solid line and circles) from 150 to 240 days of gestation.

Retained GE was greater (P = 0.03) in CR than PU cows (Table 5) and decreased (P < 0.01) from 150 to 210 days of gestation, remaining constant at 240 days of gestation. However, there was an interaction between CG and days of gestation (P = 0.03) on the retained GE as it was greater (P < 0.05) in CR than PU cows only at 150 days of gestation and decreased from 150 to 210 days of gestation only in the former ones (Figure 3A). Gross energy content per unit of EBW (MJ/kg EBW) was greater (P < 0.01) in HI than LO cows but did not differ between CR and PU cows (Table 5). Gross energy content per unit of EBW decreased (P = 0.05) from 150 to 210 days of gestation to be maintained at 240 days. However, the interaction between CG and gestation days tended (P = 0.08) to be significant as the content of GE per unit EBW decreased (P < 0.05) from 150 to 210 days of gestation and increased from 210 to 240 days of gestation only PU cows (Figure. 3B). Gross energy content was positive correlated with BCS (P = 0.01, r = 0.30, n = 84).

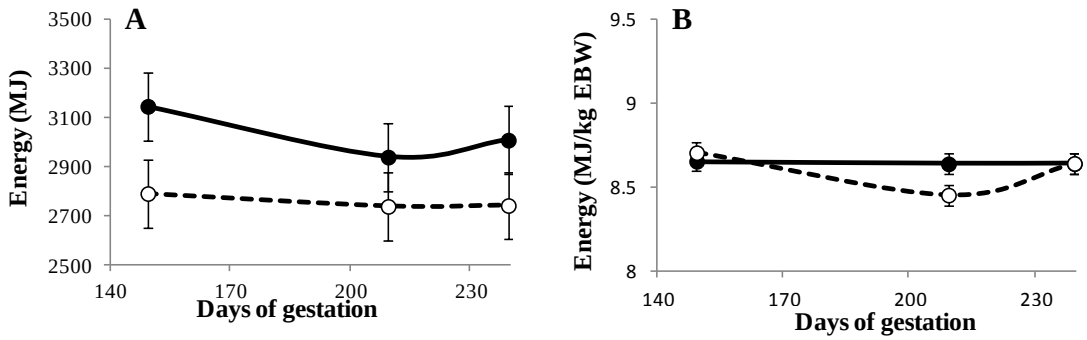

Figure 3: Evolution of gross energy in absolute terms MJ (A) or relative MJ/kg EBW [B]. In purebred (Hereford and Angus; dash lines and open circles) and crossbred beef cows (reciprocal cross F1; solid line and circles) from 150 to 240 days of gestation.

2.5. DISCUSSION

Prediction equations of body composition using the urea dilution technique

The urea dilution technique is based on the principle that there is a relatively constant relationship between EBW and body components such as water, lipid, and protein (Reid et al., 1955). Previous research has reported significant relationships between USV and body components (kg of water, lipid and protein) in beef (Kock and Preston, 1979; Bennett et al., 1982; Bartle et al., 1987; Hammond et al., 1988) and dairy (Hammond et al., 1990; Agnew et al., 2005) cattle. In addition, validation

studies (Rule et al., 1986; Bartle et al., 1987; Wells and Preston, 1998; Agnew et al., 2005) have shown that the urea dilution technique can be used to predict body composition in terms of absolute (kg) or relative (g/kg) body composition in beef and dairy cows. In agreement with previous studies in beef and dairy cows (Bartle et al., 1987; Agnew et al., 2005), regression equations to estimate body total water, protein and fat mass as well as and GE retained were significant and had moderate to high r^2. Regressions to estimate relative body composition (g/kg EBW) were significant and had a moderate r^2 similar to those reported for beef cows (Bartle et al., 1987) but lower than those reported in dairy cows (Agnew et al., 2005).

Maternal LW, BCS and body composition during the winter gestation period

During the winter gestation period (150 to 240 days of gestation), the trend for greater maternal LW, associated with greater BCS in HI than LO cows, could be mainly explained by for greater body fat mass with no differences in body protein mass. This determined that HI cows presented less body water, and greater body fat and GE per unit of EBW than LO cows. Greater percentages of body fat in cows with greater BCS have been reported (NRC, 1996). An increased energy intake during the gestation and/or the annual production cycle (Laporta et al., 2014; Casal et al., unpublished), a decreased energy expenditure for grazing activity (Scarlato et al., 2011) or a combination of both factors, could explained the greater deposit of body fat and GE retained in HI than LO cows. Similar, beef cows fed a high-energy diet during the last 190 days of gestation had greater body fat and GE in comparison to those fed a low-energy diet (Thompson, et al., 1983; Houghton et al., 1990a, 1990b)

In addition, during this period maternal LW and BCS were also greater in CR than PU cows but these differences were accompanied by greater total body water, protein and fat mass. However, CR cows had greater relative body water and protein content but did not differ in body fat content when compared to PU cow. Gaines et al. (1967) evaluated the use of crossbreeding of British breeds reported heterosis values of 3 to 4% for carcass weight and *longissimus* muscle area adjusted for carcass weight but did not observed significant advantages in carcass fat content. In contrast others (Long and Gregory, 1975) reported significant heterosis not only in

longissimus muscle area but also in dressing percentage and fat thickness adjusted for carcass weight. Probably, sex, paternals breed and feeding regimes would explain differences in heterosis values reported (Long, 1980; Marshall, 1994).

Changes in maternal tissues and organs occurred during pregnancy, which are reflected an altered maternal body composition (ie. tissue hydration, fat deposition; Robinson, 1986). However, moderate sub-nutrition imposed by the environment, during pregnancy not only prevented the deposition of nutrients in the maternal body but actually induced substantial losses of fat and protein (Robinson, 1986). In this study, cows of all groups lost maternal LW and BCS during the winter gestation period. Nevertheless, this loss in maternal LW was accompanied by changes in body composition. Body water content (per unit weight) increased during this period indicating retention of water in the body and greater loss of energy per unit of weight mobilized. The increase in body water retention results from an increase in tissue hydration, and particularly in the amounts of extracellular fluid (i.e blood volume) that occurs during pregnancy in a number of species (sheep, Foot, 1969; cows, Degan and Young, 1980).

Changes in relative contents of body fat and protein during the winter gestation period depended on CG, which indicated that composition of mobilized weight during this period of negative energy balance was different between CR and PU cows. Purebred cows showed a preference towards fat mobilization while in CR cows both fat and protein were presented in mobilized weight in a similar proportion than the present in the body weight. Fat mobilization is expected in pregnant cows when feed/energy intake does not meet their requirements. Although, the fetus cannot take direct advantage of lipid substrates mobilized by its dam as a source of energy, they serve for decreasing maternal glucose utilization, and amino acids which are prioritized nutrients for fetal growth (Bell, 1995). In contrast, protein mobilization may imply a differential adaptation to negative energy balance in CR than PU cows.

In a dairy cows during the transition period, van der Drift et al. (2012) showed that protein tissue started before than fat mobilization (4 wk before calving vs. 1 wk after calving) and that the extent of protein tissue mobilization was

associated with muscle thickness. In agreement with these results, CR cows that mobilized more protein tissue presented greater body protein mass at 150 days of gestation (beginning of winter). Regulation mechanisms of protein tissue mobilization are poorly understood but it has been suggested that reduced concentrations of insulin as well as amino acid deficiency may favor it (Tesseraud et al., 2007). In agreement with this, CR cows had a greater fall in insulin concentrations from 150 to 240 days when compared to PU cows (Laporta et al., 2014), when forage CP content was low. Protein tissue mobilization may impact on animal health (i.e reduced ketogenesis as gluconeogenic precursors increase; van der Drift et al., 2012), as well as in the reduction of energy requirements for maintenance as energy cost for basal metabolism cost depends more on animal body protein mass than total LW or metabolic weight (Agnew and Yan, 2000). Changes in the composition of weight lost and retained can have a significant influence on the energy value of mobilized weight and in maintenance energy requirements.

In conclusion, HI-HA allowed beef cows to maintained greater body fat mass and retained GE increased when compared with LO-HA. However, CG affected not only body composition (greater body fat and protein in CR than PU cows) but also composition of mobilized/retained weight during the winter gestation period with a greater protein tissue mobilization in CR than PU cows. These differences could explain, at least partly, the greater milk yield and calf weight and shorter commencement of luteal activity in the postpartum period (Gutiérrez et al., 2013; Laporta el al., 2014) in HI and CR cows, improving efficiency of the cow-calf system.

2.6. REFERENCES

Agnew RE. and Yan T (2000) Impact of recent research on energy feeding systems for dairy cattle. *Livestock Production Science* **66**,197-215.

Agnew RE, Yan T, McCaughey WJ, McEvoy, JD, Patterson DC, Porter, MG, Steen, RW (2005) Relationships between urea dilution measurements and body weight and composition of lactating dairy cows. *Journal of Dairy Science* **88,** 2476-86.

AOAC (2000) Official methods of analysis. 17th edn. (Association of Official Analytical Chemists: Gaithersburg, MD)

Ball, AJ, Oddy, VH and Thompson JM (1997) Nutritional manipulation of body composition and efficiency in ruminants. In: Recent Advances in Animal Nutrition in Australia, Armidale, New South Wales, Australia. p 192-208.

Berretta E, Risso D, Montossi F, Pigurina G (2000) Campos in Uruguay. In Grassland ecophysiology and grazing ecology. (Eds G Lemaire, J Hogdson, A de Moraes, C Nabinger, PC Carvalho) pp. 377–394. (CAB International: Curitiba Parana, BR)

Bell, AW (1995) Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. *Journal of Animal Science* **73,** 2804-19.

Bartle SJ, Kock SW, Preston RL, Wheeler TL G, Davis, W (1987) Validation of urea dilution to estimate in vivo body composition in cattle. *Journal of Animal Science* **64**, 1024–30.

Bennett GL, Swiger LA, Preston RL, Cahill VR (1982) Evaluation of urea space and ultrasonic measurement as selection criteria for beef animal composition. *Journal of Animal Science* **54,** 553–58.

Brouwer E (1965) Report of sub-committee on constants and factors. pp 441–443 in Energy Metabolism: Proc. 3rd Symp. European Association of Animal Prodroduction. Publ. No. 11, K. L. Blaxter, ed. Academic Press, London, UK.

Degan AA, Young BA (1980). Live-weight, total body-water and maternal body-solid changes in pregnant and lactating beef cows. *The Journal of Agricultural Science*, **95**, 1-5.

Espasandin AC, Ciria M, Franco JB, Pereyra F, Gimeno D (2010) Heterosis and productive and reproductive performance in Angus, Hereford and F1 reciprocal crossed cows on grazing systems of Uruguay Internacional , XXVI World Buiatrics Congress 2010 , Santiago de Chile , 2010.

Ferrell CL, Garrett WN, Hinman, N (1976) Growth, development, and composition of the udder and gravid uterus of beef heifers during pregnancy. *Journal of Animal Science* **42,** 1477–89.

Ferrell CL, Jenkins TG (1985) Cow type and the nutritional environment: nutritional aspects. *Journal of Animal Science 61,* 725–41.

Foot JZ (1969) Body water in ovine pregnancy. *Journal of Reproduction and Fertility* **9,** Suppl. 9-16.

Freetly HC, Ferrell CL, Jenkins TG (2005) Nutritionally altering weight gain patterns of pregnant heifers and young cows changes the time that feed resources are offered without any differences in production. *Journal of Animal Science* **83,** 916-26.

Freetly HC, Nienaber JA, Brown-Brandl T (2008) Partitioning of energy in pregnant beef cows during nutritionally induced body weight fluctuation. *Journal of Animal Science* **8,**:370-77.

Funston RN, Martin JL, Adams DC, Larson DM (2010) Winter grazing system and supplementation of beef cows during late gestation influence heifer progeny. *Journal of Animal Science* **88,** 4094-101.

Gaines JA, Richardson GV, McClure WH, Vogt DW, Carter, RC (1967) Heterosis from crosses among British breeds of beef cattle: carcass characteristics. *Journal of Animal Science* **26**, 1217-25.

Gutiérrez V, Espasandin AC, Astessiano AL, Casal A, López-Mazz C, Carriquiry M (2013) Calf foetal and early life nutrition on grazing conditions: metabolic and endocrine profiles and body composition during the

growing phase. *Journal of Animal Physiology and Animal Nutrition* **97**,720-31

Hammond AC, Rumsey TS, Haaland GL (1988) Prediction of empty body components in steers by urea dilution. *Journal of Animal Science* **66**, 354–60.

Hammond AC, Waldo DR, Rumsey TS (1990) Prediction of body composition in Holstein steers using urea space. *Journal of Dairy Science* **73,** 3141–45.

Hansard SL, Butler WO, Comar CL (1953) Blood volume of farm animals. *Journal of Animal Science* **12,** :402-13.

Haydock K, Shaw N, (1975). The comparative yield method for estimating dry matter yield of pasture. *Australian Journal of ExperimentalAgriculture and Animal Husbandry* **15**, 663–70.

Houghton PL, Lemenager RP, Horstman LA, Hendrix KS, Moss G. (1990a) Effects of body composition, pre- and postpartum energy level and early weaning on reproductive performance of beef cows and preweaning calf gain. *Journal of Animal Science.* **68,** 1438-46.

Houghton PL, Lemenager RP, Hendrix KS, Moss GE, Stewart TS (1990b). Effects of body composition, pre- and postpartum energy intake and stage of production of energy utilization by beef cows. *Journal of Animal Science* **68,** 1447-56.

Jenkins TG, Ferrell CL (1994) Productivity through weaning of nine breeds of cattle under varying feed availabilities: 1: Initial evaluation. *Journal of Animal Science* **72**, 2787-97.

Kock SW, Preston RL (1979) Estimation of bovine carcass composition by the urea dilution technique. *Journal of Animal Science* **48,** 319-27.

Laporta J, Rosa GJ, Naya H, Carriquiry M (2014) Liver functional genomics in beef cows on grazing systems: novel genes and pathways revealed. *Physiological genomics*. 15; 46(4):138-47.

Laurenz JC, Byers FM, Schelling GT, Greene LW (1992) Periodic changes in body composition and in priorities for tissue storage and retrieval in mature beef cows *Journal of Animal Science* **70**, 1950-56.

Long CR, Gregory KE (1975) Heterosis and management effects in carcass characters of Angus, Hereford and reciprocal cross cattle. *Journal of animal science* **41**, 1572-80.

Long CR (1980) Crossbreeding for beef production: Experimental results. *Journal of Animal Science* **51**,1197-223.

Marshall DM (1994). Breed differences and genetic parameters for body composition traits in beef cattle. *Journal of animal science* **72**, 2745-55.

Mott GO (1960) Grazing pressure and the measurement of pasture production. In: *Proceedings 8th International Grassland Congress.* Reading, UK. pp 606-611.

NRC. National Research Council (1996). Nutrient Requirements of Beef Cattle. 6th rev. ed. National Academy Press, Washington, D.C.

NRC. National Research Council (2000). Nutrient Requirements of Dairy Cattle. 7th rev. ed. National Academy Press, Washington, D.C.

Quintans G, Banchero G, Carriquiry M, López C, Baldi F (2010) Effect of body condition and suckling restriction with and without presence of the calf on cow and calf performance. *Animal Production Science* **50,** 931–38.

Reid JT, Wellington GH, Dunn HO (1955). Some relationships among the major chemical components of the bovine body and their application to nutritional investigations. *Journal of Dairy Science*, **38**, 1344-59.

Robinson JJ (1986) Changes in body composition during pregnancy and lactation. *Proceedings of the Nutrition Society* **45**, 71-80.

Rohweder DW (1994) Estimating forage hay quality. In: *Proceedings National Alfalfa Hay Quality Testing Workshop*. National Hay Quality Committee, Chicago, IL. pp 31-37.

Rule DC, Arnold RN, Hentges EJ, Beitz DC (1986) Evaluation of urea dilution as a technique for estimating body composition of beef steers in vivo: Validation of published equation and comparison with chemical composition. *Journal of Animal Science* **63**,1935-48

Scarlato S, Faber A, Do Carmo M, Soca P (2011) Foraging behaviour of beef cows grazing native pasture: I. Effect of breed and herbage allowance on

grazing and ruminating time. In Proceedings of the 9th International Rangeland Congress. Eds. Feldman SR, Oliva GE, Sacido MB. IX International Rangeland Congress. Rosario, Argentina. p 657.

Soca P, Carriquiry M, Keisler DH, Claramunt M, Do Carmo M, Olivera-Muzante J, Rodríguez M, Meikle A (2013) Reproductive and productive response to suckling restriction and dietary flushing in primiparous grazing beef cows. *Animal Production Science* **53**, 283–91.

Sollenberger LE, Moore JE, Allen VG, Pedreira CGS (2005) Reporting Forage Allowance in Grazing Experiments. *Crop Science*. **45**, 896-900.

Tesseraud S, Métayer S, Duchêne S, Bigot K, Grizard J, Dupont J (2007). Regulation of protein metabolism by insulin: value of different approaches and animal models. *Domestic animal endocrinology* **33**, 123-42.

Thompson WR, Meiske JC, Goodrich RD, Rust JR, Byers FM (1983) Influence of body composition on energy requirements of beef cows during winter. *Journal of Animal Science* **56,** 1241-52.

van der Drift SG, van Hulzen KJ, Teweldemedhn TG, Jorritsma R, Nielen M, Heuven HC (2012) Genetic and nongenetic variation in plasma and milk β-hydroxybutyrate and milk acetone concentrations of early-lactation dairy cows. *Journal of Dairy Science* **95**, 6781-87

Vizcarra JA, Ibañez W, Orcasberro R (1986) Repetibilidad y reproductibilidad de dos escalas para estimar la condición corporal de vacas Hereford. *Investigaciones. Agronomicas* **7**, 45-47.

Wells RS, Preston RL (1998) Effect of repeated urea dilution measurement on feedlot performance and consistency of estimated body composition in steers of different breed types. *Journal of Animal Science* **76**, 2799– 804.

3. VISCERAL ORGAN MASS, CELLULARITY INDEXES AND EXPRESSION OF GENES ENCODING FOR MITOCHONDRIAL RESPIRATORY CHAIN PROTEINS IN PURE AND CROSSBRED MATURE BEEF COWS GRAZING DIFFERENT FORAGE ALLOWANCES OF NATIVE PASTURES.

A. Casal[a*], M. Veyga[a], A.L. Astessiano[a], A.C. Espasandin[b], A.I. Trujillo[a], P. Soca[b], M. Carriquiry[a]

[a]Department of Animal Production and Pastures, School of Agronomy, Universidad de la República (UdelaR). Av. E. Garzón 780, 12900, Montevideo, Uruguay.
[b]Department of Animal Production and Pastures, EEMAC School of Agronomy, Universidad de la República (UdelaR). Ruta 3 km 363, 60000, Paysandu, Uruguay.

[*]Corresponding author: Tel: +598 2357-2344 addresses: Av. E. Garzón 780, 12900, Montevideo, Uruguay. E-mail address: alcas@adinet.com.uy (A. Casal).

3.1. ABSTRACT

Visceral tissues impact on the partitioning of metabolizable energy between maintenance and production. The aim of this study was to evaluate long-term effects of two herbage allowances of native pastures on mass, composition and expression of mitochondrial respiratory protein encoding genes on gastrointestinal tract (GIT) organs and liver in pure (Angus and Hereford, PU) and crossbred (reciprocal F1, CR) beef cows. Mature beef cows (n = 32) were used in a complete randomized block design with a factorial arrangement of herbage allowance (2.5 vs. 4 kg DM/d; LO vs. HI) and cow genotype (PU vs. CR). The experiment was conducted during three years and at the end of the third year, cows were slaughtered at 190 ± 10 d postpartum, and GIT organs and liver were dissected, weighed, and samples collected and immediately frozen. Reticulum-rumen (P = 0.02) and total intestine (P = 0.02) absolute mass (kg) was greater in HI than LO cows and greater in CR than PU cows, while reticulum-rumen (P = 0.02) and liver (P < 0.01) mass were greater in HI than LO cows. Abomasum protein content was greater (P < 0.01), while omasum

protein content tended (P = 0.10) to be greater for HI-PU than LO-PU cows. The reticulum-rumen and abomasum lipid contents tended to be less (P < 0.10) in HI than LO cows. Except for the large intestine and liver, concentration of DNA and protein:DNA ratio did not differ in GIT viscera of different cow groups. The protein:DNA ratio of the large intestine was greater (P = 0.03) in HI-CR than LO-CR cows, while the hepatic protein:DNA ratio was less (P = 0.04) in HI-CR than LO-CR cows while not differing of HI-PU and LO-PU cows that presented intermediate values. The small intestine expression of *NDUFB8* and *COX5B* mRNA were greater (P < 0.05) and *NDUFS4* mRNA tended (P = 0.06) to be greater in HI than LO cows. The expression of *UQCRC1* mRNA was greater (P = 0.04) and *SDHA* mRNA tended to be greater (P = 0.08) in CR than PU cows. Hepatic *NDUFB8*, *NDUFS4* and *COX5B* mRNA was greater (P < 0.05) in LO-CR than HI-CR and LO-PU cows being intermediate in HI-PU cows. The *CYC1* mRNA was greater (P = 0.05) in LO than HI cows. These results suggest that CR cows would have greater plasticity in order to adapt their visceral mass and gene expression to sparse environments.

Key words: beef cattle, gastrointestinal, liver, mRNA, rangelands, viscera.

3.2. INTRODUCTION

Gastrointestinal tract (**GIT**) viscera and liver comprise 10 to 13% of whole-body tissue mass, but account for 45 to 50% of whole-body heat energy (Seal and Reynolds, 1993) and are an important source of variation for maintenance requirements within a breeding herd (DiCostanzo et al., 1991) and among breeds and breed crosses (Jenkins et al., 1991). Thus, visceral mass can contribute to determine differences in cow-calf productivity efficiency (Ferrell and Jenkins, 1985).

Several experiments have determined a positive relationship between feed intake and total and individual GIT organ and liver mass in ruminants (reviewed by Ortigues and Doreau, 1995). Recently, Meyer et al. (2012) reported nutrient restriction during mid-gestation affected maternal visceral mass changes during gestation which may partially explain differences observed in calf performance.

However, to our knowledge there are no studies that evaluate long-term effect of nutrition (various restriction-refeeding cycles) on viscera weight and cellularity of rangeland beef cows.

Mitochondria produce 90% of cellular energy (90% of oxygen consumption) and are integrated into numerous functional, metabolic, and signaling networks with other cellular compartments (Rolfe and Brand, 1997). Thus, this organelle is an appropriate place to study variation in maintenance energy requirements that may contribute to the phenotypic differences observed among animals (Herd and Arthur, 2009). Despite its potential significance, information available in this area for cattle is scarce.

Previous reports indicated grazing intensity of native pastures, controlled through herbage allowances, can affect energy intake (Chapman et al., 2007) and modified forage as well as animal BW and production (Soares et al., 2003; Gutierrez et al., 2013; Laporta et al., 2014). In addition, research (Morris et al., 1987; Espasandin et al., 2010) evaluating reciprocal crossbreeding between Angus (**A**) and Hereford (**H**) cattle in rangelands conditions, have estimated a crossbred F1 superiority (heterosis) for cow-calf system productivity between 15 and 30% over the purebred mean and it has been indicated that this difference could depend on herbage allowance of native grasslands through the gestation-lactation cycle (Espasandin et al., 2012). Thus, our hypothesis was that control of grazing intensity through management of herbage allowance (**HA**) of native rangelands, would affect, in the long-term, visceral mass, composition, and mitochondrial function of the GIT organs and liver of mature beef cows. The different HA could impact differently in pure than crossbred beef cows, explaining – at least partially – differences in productivity between pure and crossbred cows in both environments. Our objective was to evaluate the long-term effect of two HA of native pastures on mass, composition and expression of genes encoding for mitochondrial respiratory complex proteins on GIT organs and liver in pure (A and H) and the reciprocal crossbred (F1) beef cows which could explain, at least in part, differences in productivity between environments and genotypes.

3.3. MATERIALS AND METHODS

Animal procedures were approved by the Animal Experimentation Committee of Universidad de la República.

Location, animals and experimental design

The experiment was conducted on 95 ha of native grasslands (Campos biome) located at the Professor Bernardo Rosengurtt Experimental Station (School of Agronomy, UdelaR, Uruguay; 32°S, 54°W) from June 2007 to May 2010. Native pastures were dominated by summer-growing C4 grasses (*Poaceae*), with few C3 grasses associated with the winter cycle. The main forb families included *Asteraceae, Fabaceae, Rubiaceae* and *Umbelliferae*.

Thirty-two multiparous cows (4 to 5 year-old), were used in a randomized block design with two replications (block 1: sandy loam soil, 60 ha and block 2: clay loam soil, 35 ha) and four plots in each block to which a 2 x 2 factorial arrangement of HA and cow genotype (CG) was allocated.

Herbage allowance the ratio between forage mass and stocking rate (kg of dry matter (DM) per kg of body weight (BW) (Sollenberger et al., 2005)) represented 4 and 2.5 kg DM/kg BW of annual mean (HI and LO, respectively) and varied with season of the year (Table 1). Herbage mass (kg DM/ha) was estimated monthly by the comparative yield method (Haydock and Shaw, 1975). A continuous stocking method was applied throughout the year, thus herbage allowance in each plot was adjusted monthly by the "put-and-take method" (Mott, 1960). Experimental cows were maintained in the plot throughout the experiment, and 'put-and-take' cows of similar genotype and physiological status to the experimental ones were added or removed based on the herbage mass available to adjust forage allowance and thus stocking rate. Herbage samples were collected monthly and composite by season for chemical analyses (AOAC, 2000) and metabolizable energy (ME) was estimated according to Reid et al. (1991) as [-0.027 + 0.0428 x (88.9 - 0.779 x acid detergent fiber)] x 0.82. Herbage allowance treatments determined differences in forage mass and height throughout the year but did not differ in chemical composition (Table 1).

Spring-calved purebred (PU; A and H) and reciprocal crossbred F1 (CR; AxH and HxA) cows belonged to a group of experimental animals generated as part of a diallel crossbreeding experiment carried out for 10 years at the Experimental Station

(Espasandin et al., 2010). Eight experimental cows were evaluated per treatment (HI-CR, HI-PU, LO-CR, and LO-PU; n = 4 for each individual genotype: H and A for PU or HxA and AxH for CR). Cows grazed on the same HA (HI or LO) since June 2007, and gestated and lactated one calf every year from 2007 to 2010.

Table 1. Herbage allowances (HA), forage mass, height and chemical composition, by season, during the three experimental years.

	Season[1]				
	Fall	**Winter**	**Spring**	**Summer**	**se**
HA (kg DM/kgBW)[2]					
HI	5	3	4	4	
LO	3	3	2	2	
Forage mass (kg DM/ha)					
HI	2556^{a}	1459^{bc}	1892^{b}	2382^{a}	160
LO	1422^{bc}	971^{d}	1587^{b}	1371^{c}	160
Height (cm)					
HI	5.4^{ab}	4.1^{c}	5.0^{b}	6.2^{a}	0.3
LO	3.4^{c}	2.9^{c}	3.7^{c}	3.6^{c}	0.3
	Chemical composition				
DM, %					
HI	53.1^{a}	55.0^{a}	41.7^{b}	53.9^{a}	2.5
LO	53.6^{a}	52.5^{a}	38.9^{b}	46.6^{a}	2.5
CP, % of DM					
HI	7.5^{b}	8.3^{b}	10.4^{a}	8.4^{b}	0.5
LO	7.0^{b}	9.0^{b}	10.6^{a}	8.7^{b}	0.5
ADF, % of DM					
HI	42.6^{a}	39.9^{a}	35.5^{b}	41.2^{a}	1.6
LO	42.7^{a}	43.0^{a}	36.5^{b}	39.2^{a}	1.6
ME, MJ/kg DM[3]					
HI	8.4^{b}	8.7^{b}	9.3^{a}	8.6^{b}	0.2
LO	8.4^{b}	8.4^{b}	9.1^{a}	8.7^{b}	0.2

[1]Fall (April, May, and June), Winter (July, August, and September), Spring (October, November, and December), Summer (January, February and March).[2]HA:Herbage allowance treatments. Mean annual allowance represented 4 and 2.5 kg DM/kg BW (HI and LO, respectively) and changed seasonally. [3]Estimated according to Rohweder, (1984). [a,b] Means with different literals differed with $P \leq 0.05$.

At the beginning of the experiment (June 2007) cow BW was 447 ± 58 kg and body condition score (BCS) (scale 1 to 8; Vizcarra et al., 1986) was 4.1± 0.5. Milk yield and milk composition were individually measured by direct milking previous injection with oxytocin at 15 days post-partum and from 30 days to weaning (142 ± 15 days) in 30-day intervals and presented by Gutierrez et al. (2013). On

average, during the three experimental years, BCS and BW were greater for HI than LO the differences being more evident during the last gestation-lactation cycle (2009 – 2010, Figure 1). In addition, during the last gestation-lactation cycle (2009-2010) milk energy output was 22% greater ($P < 0.05$), calf BW and ADG at weaning were 10% greater ($P < 0.05$) in HI than LO and CR than PU cows (Gutierrez et al., 2013) and commencement of luteal activity was 59 and 34 earlier ($P < 0.05$) for HI than LO and for CR than PU cows, respectively (Laporta et al., 2014). Estimated ME intake (MEI) during the three experimental years, was calculated based on cow requirements (Macoon et al., 2003; Smit et al., 2005) according National Research Council equations (NRC, 2000) using individual cow for maintenance ($BW^{0.75}$), gestation (calf birth weight) or lactation (milk yield and composition), and retained energy (changes in protein and fat tissues based on changes in BW and BCS). Estimated MEI was greater for HI than LO cows (73.8 vs. 66.7 ± 3.9 MJ/d) and for CR than PU cows (73.7 vs. 67.8 ± 3.9 MJ/d).

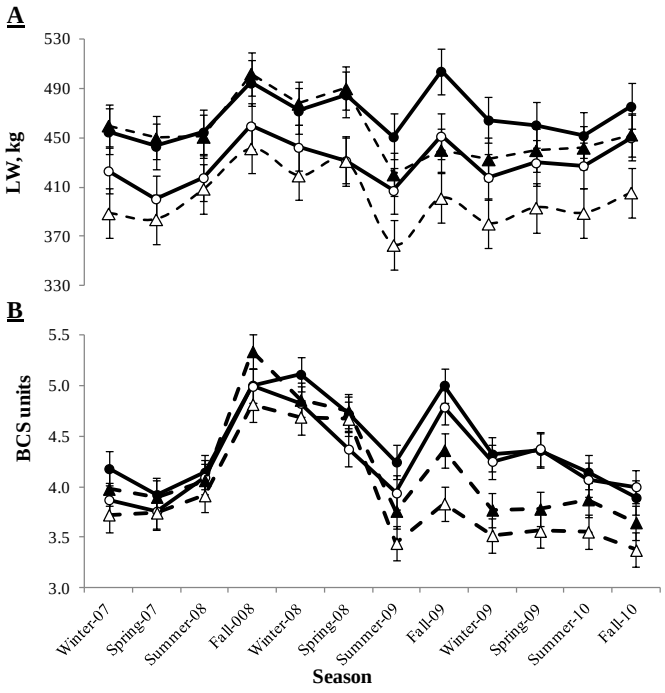

Figure 1. Cow BW (A) and BCS (B) throughout the three experimental years (2007-2010) in in purebred (Hereford and Aberdeen Angus; triangles) and crossbred (F1; squares) beef cows grazing high (solid symbols and lines) and low (open symbols and dashed lines) herbage allowances (2 vs. 4 kg dry matter (DM)/kgBW of annual mean, respectively) of native grasslands [n = 32, 8 cows per treatment]. Data are presented as lsmeans ± standard error.

Data and sample collection

At the end of the third experimental year (May 2010), at 190 ± 10 d postpartum (45 d after calf weaning), cows were slaughtered in a commercial abattoir (PUL S.A; Cerro Largo, Uruguay; 10 km from the Experimental Station). Prior to transport, cow BW and BCS were recorded. Cows were stunned with a captive bolt gun, exsanguinated, the organs and the carcass were weighed and GIT organs and liver were dissected. Forestomachs (reticulum-rumen, omasum and abomasum), and small and large intestines were separated from connective and adipose tissue, flushed with warm water to remove all digest, blotted and weighed. The liver was separated from surrounding connective tissue and weighed. The omental/mesenteric fat was weighed. Samples (100 to 200 g) including all tissue layers of reticulum-rumen (from dorsal sac), omasum and abomasum (at small curvatures), small (at central jejunum) and large (at central colon) intestines and liver (caudal lobe) were collected and immediately frozen at -20 ºC for chemical composition analysis. Additionally, samples (20 g) of small intestine and liver were snap-frozen in liquid nitrogen and then stored at -80 °C for analysis of gene expression and cellularity indexes. Measurements of gene expression and cellularity indexes have been previously determined in samples obtained from slaughtered animals (Burrin, et al., 1992; Connor et al., 2010). All organs were weighed, processed, and frozen within 35 min of exsanguination. Cow empty BW (EBW) was estimated as carcass weight plus total offal weight (Hersom et al., 2004).

Chemical composition and DNA analyses

Samples were ground in liquid nitrogen and analyzed for moisture, fat (ether extract), protein (Kjeldahl nitrogen) according to AOAC (2000). Isolation of DNA was performed using the high salt protocol (Sunnucks and Hales, 1996). Concentration of DNA was determined by measuring absorbance at 260 nm (NanoDrop ND-1000 Spectrophotometer; Nanodrop Technnologies Inc., Wilmington, DE, USA), and purity and integrity of all DNA isolates were assessed from 260/280 nm (>1.8) and 260/230 nm (>1.9) absorbance ratios.

Quantitative real time PCR

Total RNA from hepatic and small intestine samples 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), and purity and integrity of all RNA isolates were assessed from 260/280 nm (>1.8) and 260/230 nm (>1.9) absorbance ratios and by electrophoresis in 1 % agarose gel. Isolated RNA was stored at -80 °C until analyzed. The SuperScript III Transcriptase (Invitrogen), with random hexamers and 1 µg of total RNA as a template, was used to conduct the reverse transcription. The cDNA was stored at -20 ºC until its use.

Primers (Supplementary Table S1) to specifically amplify cDNA of three internal control genes: *β-actin (**ACTB**), hypoxanthine phosphoribosyltransferase (**HPRT1**)* and *ribosomal protein S9 (**RPS9**)* and nine target genes of the mitochondrial respiratory complex were obtained from literature or specifically designed using the Primer3 website (http://frodo.wi.mit.edu/primer3/) based on bovine nucleotide sequences available from NCBI (http://www.ncbi.nlm.nih.gov/). The mitochondrial respiratory chain protein genes analyzed included: *NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 8 (**NDUFB8**,* complex I*), NADH dehydrogenase (ubiquinone) Fe-S protein 4 (**NDUFS4**,* complex I*), succinate dehydrogenase complex, subunit A, flavoprotein (Fp) (**SDHA**,* complex II*), succinate dehydrogenase complex, subunit D, integral membrane protein (**SDHD**,* complex II*), ubiquinol-cytochrome c reductase core protein I (**UQCRC1**,* complex III*), cytochrome c oxidase subunit Vb (**COX5B**,* complex IV*); cytochrome c-1 (**CYC1**,* complex IV), *ATP synthase subunit beta (**ATP5B**,* complex V*); and ATP synthase subunit O* (***ATP5O***, complex V). Before use, primer product size (as estimated by 1% agarose gel separation) and sequence (Macrogen Inc., Seoul, Korea) were determined to ensure that primers produced the desired amplicons (data not shown).

Real time PCR reactions were performed in a total volume of 15 µL using KAPA SYBR FAST Universal 2X qPCR Master Mix (Kapa Biosystems, inc. Woburn, MA, USA) according to Astessiano et al. (2012) using the following

standard amplification conditions: 3 min at 95ºC and 40 cycles of 3 s at 95ºC, 40 s at 60ºC, and 20 s at 72ºC in a LineGeneK thermocycler (Bioer Technology CO., LTD, China). Dissociation curves were run on all samples to detect primer dimers, contamination, or presence of other amplicons. Each run included a pool of total RNA from bovine liver or small intestine samples analyzed in triplicate to be used as the basis for the comparative expression results (exogenous control) and duplicate tubes of water (non-template control). Gene expression was measured by relative quantification (Pfaffl, 2004) to the exogenous control and normalized to the geometric mean expression of internal control genes (*β-actin, HPRT1,* and *RPS9).* Expression stability of 3 selected housekeeping genes was evaluated using the MS-Excel add-in Normfinder (MDL, Aarhus, Denmark). The stability values obtained with Normfinder they were 0.341, 0.380, and 0.287 in liver, and 0.368, 0.296 and 0.141 in small intestine for *β-actin, HPRT1,* and *RPS9,* respectively. Amplification efficiencies or target and endogenous control genes were estimated by linear regression of a dilution cDNA curve (n = 5 dilutions, from 100 to 6.25 ng/tube (Supplementary Table S1). The intra and inter-assay CV for all genes were less than 1.2% and 3.7%, respectively.

Calculations and Statistical analyses

Tissue concentrations of protein and nucleic acids were used as indicators to estimate relative cell number (hyperplasia; DNA concentration), cell size (hypertrophy; protein:DNA ratio) or protein synthesis capacity (RNA concentration and RNA:protein ratio) (Burrin et al., 1992; Sainz and Bentley, 1997; Nozière et al., 1999).

Data were analyzed using the SAS Systems program (SAS 9.0V; SAS Inst., Cary, NC, USA). Univariate analyses were performed on all variables to verify normality of residual. Cow BW and BCS, individual absolute (kg) and relative (g/kg EBW) mass, chemical composition and cellularity indexes of the GIT organs and liver as well as small intestine and hepatic gene expression were analyzed as a mixed model using the MIXED procedure. The model included effects of HA, CG and their interaction as fixed effects and block as a random effect. The block was used as the

experimental unit to evaluate HA effect and cow as the experimental unit to evaluate CG and the HA by CG interaction. The interaction between HA and block was included in the model as a random effect but as covariance parameter estimates were zero or close to zero it was removed from the model. Mean separation was performed using the Tukey test, and differences were considered significant at $P \leq 0.05$ and a trend when $0.05 < P \leq 0.10$. Results were presented as least square means ± pooled standard error.

3.4.RESULTS

Cow BCS, EBW and visceral mass

During the last 50 d before slaughter (from calf weaning in March 2010 to slaughter in May 2010) estimated ME intake was greater in HI than LO cows (52.5 vs. 49.3 ± 1.23 MJ/d) and for CR than PU cows (52.0 vs. 49.3 ± 1.23 MJ/d). During this period of time, cow BCS and BW did not vary for any group (-0.30, -0.11, 0.33, 0.33 ± 0.30 units of BCS change and -2.9, -2.7, -10.4, -11.9 ± 6.7 kg of BW change for LO-CR, LO-PU, HI-CR and HI-PU, respectively). Cow BCS at slaughter was not affected by HA treatment, CG or their interaction (4.0, 3.8, 3.8, 3.8 ± 0.1 units for LO-CR, LO-PU, HI-CR and HI-PU, respectively). Cow EBW at slaughter tended (P = 0.07) to be greater for HI than LO cows and CR than PU cows. (Table 2).

The mass of the reticulum-rumen was greater ($P = 0.02$) for HI than LO cows and greater for CR than PU cows. However, there were no effects of HA treatment, CG or their interaction in omasal and abomasal mass. Total intestinal mass was greater ($P = 0.02$) in HI than LO cows as the mass of both small and large intestines tended to be greater ($P < 0.10$) in HI than LO cows, but it was not affected by CG. Liver mass tended ($P = 0.09$) to be greater in HI than LO cows and it was greater ($P < 0.01$) in CR than in PU cows. The omental/mesenteric fat mass was affected ($P = 0.05$) by the interaction between HA treatment and CG as it was greater ($P < 0.05$) for LO-CR than HI-CR cows, while HI-PU and LO-PU cows presented intermediate values (Table 2).

Purebred cows tended ($P = 0.10$) to present a greater relative mass of stomach complex as expressed on an EBW basis than CR cows, mainly due to a trend ($P =$

0.09) to greater relative omasal mass in the former ones. Relative mass of liver tended (P = 0.07) to be greater in CR than PU cows. However, neither total intestine, nor omental/mesenteric fat relative mass were affected by HA treatment, CG or their interaction (Table 2).

Table 2. Effects of herbage allowance (HA) and cow genotype (CG) and their interaction (HAxCG) on gastrointestinal tract viscera and liver absolute (kg) and relative (g/kgEBW) weights.

	Treatment[1]					*P*-value		
Item	HI-PU	LO-PU	HI-CR	LO-CR	SE	HA	CG	HAxCG
No. of animals	8	8	8	8				
EBW, kg[2]	349.5^{xy}	319.7^{y}	362.4^{x}	349.6^{xy}	13.4	0.07	0.07	0.44
Absolute organ mass, kg								
Reticulum-rumen	9.8^{abx}	8.6^{by}	10.4^{a}	9.8^{abx}	0.4	0.02	0.02	0.41
Omasum	7.8	7.0	7.1	6.5	0.6	0.29	0.32	0.94
Abomasum	3.9	4.3	4.1	3.7	0.3	0.96	0.42	0.21
Small intestine	3.0	2.6	3.4	2.7	0.3	0.09	0.40	0.68
Large intestine	3.9^{xy}	3.4^{y}	4.1^{x}	3.7^{xy}	0.3	0.06	0.35	0.82
Total stomachs	21.4	20.1	21.5	19.8	0.7	0.06	0.87	0.81
Total intestine	7.8^{xy}	6.8^{y}	8.45^{x}	7.2^{xy}	0.5	0.02	0.22	0.87
Liver	4.4^{abx}	3.9^{by}	4.9^{a}	4.7^{ax}	0.2	0.09	<0.01	0.37
Omental/mesenteric fat	2.7^{xy}	2.4^{xy}	2.3^{y}	2.7^{x}	0.3	0.61	0.95	0.05
Relative organ mass, g/kg EBW								
Reticulum-rumen	27.6	26.8	28.5	27.3	0.7	0.15	0.32	0.78
Omasum	22.1^{x}	22.4^{x}	19.5^{y}	19.1^{y}	1.7	0.95	0.09	0.84
Abomasum	11.3	13.4	11.5	10.5	1.0	0.56	0.20	0.12
Small intestine	8.7	8.1	9.4	7.7	0.7	0.14	0.89	0.48
Large intestine	13.6	13.4	13.8	12.8	0.6	0.36	0.81	0.52
Total stomachs	61.2^{x}	62.9^{x}	59.5^{y}	57.1^{y}	2.4	0.87	0.10	0.34
Total intestine	22.3	21.5	23.2	20.5	1.0	0.11	0.96	0.37
Liver	12.5^{y}	12.3^{y}	12.9^{x}	13.4^{x}	0.4	0.73	0.07	0.42
Omental/mesenteric fat	8.1	7.8	7.1	7.9	0.6	0.70	0.51	0.33

[1]Treatment : purebred and crossbred beef cows grazing high and low in average herbage allowances of native pastures: High-crossbred cows (HI-CR); High-purebred cows (HI-PU); Low-crossbred cows (LO-CR); Low-purebred cows (LO-PU). [2]EBW = Empty body weight.[a,b] Means with different literals differed with $P \leq 0.05$; [x,y] Means with different literals differed with $0.05 < P \leq 0.10$.

Visceral chemical composition and cellularity indexes

The protein content of the abomasum was affected ($P < 0.01$) and the protein content of the omasum tended to be affected ($P = 0.10$) by the interaction between HA treatment and CG as they were greater in HI-PU than LO-PU cows (Table 3). The lipid content of the reticulum-rumen and abomasum tended to be less ($P < 0.10$) in HI than LO cows. The protein and lipid contents of the omasum, small and large intestines, and liver were not affected by HA treatment, CG or their interaction (Table 3).

Except for the large intestine and liver, concentration of DNA and protein:DNA ratio did not differ in the GIT organs of the different cow groups. Large intestine DNA tended to be less ($P = 0.06$) in HI than LO cows (Table 3). The protein:DNA ratio of the large intestine was greater ($P = 0.03$) in HI than LO cows and was affected by the interaction between HA treatment and CG ($P = 0.02$) as it was greater ($P < 0.05$) in HI-CR than LO-CR cows while not differing between HI-PU and LO-PU cows (Table 3). Although hepatic DNA concentration did not differ among cow groups, the protein:DNA ratio was less ($P = 0.01$) in HI than LO cows and was also affected by the interaction between HA treatment and CG ($P = 0.04$) as it was less ($P < 0.05$) in HI-CR than LO-CR cows but did not differ between HI-PU and LO-PU cows which presented intermediate values (Table 3).

The small intestine RNA concentration and RNA:protein ratio were affected by the interaction between HA treatment and CG ($P < 0.01$) being greater ($P < 0.05$) in the LO-CR than in the other three cow groups (Table 3). The hepatic RNA and RNA:protein ratio tended ($P = 0.10$) to be affected by the interaction between HA treatment and CG since was greater ($P < 0.05$) in HI-CR than HI-PU and intermediate in LO (CR and PU) cows (Table 3).

Table 3. Effects of herbage allowance (HA) and cow genotype (CG) and their interaction (HAxCG) on gastrointestinal tract viscera and liver chemical composition and cellularity.

	Treatment[1]					*P*-value		
Item	HI-PU	LO-PU	HI-CR	LO-CR	SE	HA	CG	HAxCG
No. of animals	8	8	8	8				
Water, g/kg tissue								
Reticulum - rumen	850.8	851.2	853.5	844.2	4.7	0.39	0.65	0.31
Omasum	822.5	810.6	813.1	807.9	6.4	0.21	0.33	0.59
Abomasum	844.5	847.2	841.1	848.9	5.7	0.23	0.73	0.55
Small intestine	839.5	845.6	842.8	845.6	3.2	0.20	0.59	0.61
Large intestine	727.6	736.7	737.7	736.6	6.1	0.54	0.41	0.40
Liver	831.8^{x}	818.7^{y}	829.6^{xy}	827.2^{xy}	4.2	0.08	0.44	0.20
Lipids, g/kg tissue								
Reticulum - rumen	9.3^{y}	18.5^{x}	10.0^{y}	12.0^{xy}	0.3	0.09	0.32	0.22
Omasum	6.6	12.1	10.6	11.8	0.2	0.12	0.34	0.26
Abomasum	40.7^{y}	63.8^{x}	56.1^{xy}	59.4^{x}	0.7	0.08	0.42	0.14
Small intestine	14.1	19.6	18.8	13.8	0.5	0.95	0.88	0.16
Large intestine	20.5	15.1	13.9	15.6	0.4	0.65	0.37	0.29
Liver	66.2	59.1	69.7	58.1	0.8	0.11	0.82	0.68
Protein, g/kg tissue								
Reticulum - rumen	150.0	154.9	153.2	153.3	5.1	0.60	0.86	0.57
Omasum	136.2	130.2	130.7	137.6	3.9	0.91	0.80	0.10
Abomasum	129.0^{a}	115.5^{b}	119.3^{ab}	126.1^{a}	3.2	0.30	0.88	<0.01
Small intestine	136.2	128.8	133.2	133.3	2.6	0.18	0.77	0.13
Large intestine	132.6	131.8	136.9	131.9	2.8	0.34	0.43	0.46
Liver	190.6	189.8	181.6	191.7	4.1	0.24	0.34	0.14
DNA, µg/mg tissue								
Reticulum - rumen	0.54	0.5	0.44	0.47	0.07	0.88	0.35	0.61
Omasum	0.45	0.51	0.52	0.53	0.08	0.70	0.58	0.76
Abomasum	0.90	0.93	0.96	0.76	0.14	0.57	0.69	0.40
Small intestine	0.71	0.78	0.85	0.67	0.11	0.60	0.92	0.19
Large intestine	0.60	0.66	0.62	0.88	0.08	0.06	0.13	0.19
Liver	1.34	1.44	1.20	1.13	0.20	0.90	0.28	0.66
Protein:DNA								
Reticulum - rumen	284.6	329.3	337.6	312.5	42.4	0.82	0.67	0.41
Omasum	322.9	297	267.3	303.5	49.4	0.92	0.61	0.51
Abomasum	155.1	133.5	136.9	224.1	39.3	0.44	0.35	0.17
Small intestine	243.2	209.2	209.3	238.7	38.5	0.91	0.91	0.15
Large intestine	204.7^{abx}	206.7^{abx}	237.6^{a}	133.6^{by}	21.4	0.03	0.34	0.02
Liver	138.3^{ab}	154.0^{ab}	104.2^{b}	185.9^{a}	37.0	0.01	0.96	0.04
RNA, µg/mg tissue								
Small intestine	0.98^{b}	1.02^{b}	1.10^{b}	2.16^{a}	0.16	<0.01	<0.01	<0.01
Liver	0.64^{y}	0.77^{xy}	0.90^{x}	0.68^{xy}	0.10	0.71	0.43	0.10
RNA:Protein								
Small intestine	0.007^{b}	0.008^{b}	0.008^{b}	0.016^{a}	0.001	<0.01	<0.01	<0.01
Liver	0.003^{y}	0.004^{xy}	0.005^{x}	0.004^{xy}	0.0005	0.66	0.45	0.10

[1]Treatment : purebred and crossbred beef cows grazing high and low in average herbage allowances of native pastures: High-crossbred cows (HI-CR); High-purebred cows (HI-PU); Low-crossbred cows (LO-CR); Low-purebred cows (LO-PU).[a,b] Means with different literals differed with $P \leq 0.05$; [x,y] Means with different literals differed with $0.05 < P \leq 0.10$.

Gene expression of mitochondrial respiratory chain proteins in small intestine and liver

The small intestine expression of *NDUFB8* and *COX5B* mRNA was greater ($P < 0.04$) in HI than LO cows and a similar trend ($P = 0.06$) was observed in *NDUFS4* mRNA (Table 4). The expression of *UQCRC1* mRNA was greater ($P = 0.04$) and *SDHA* mRNA tended ($P = 0.08$) to be greater in CR than PU cows (Table 4). The mRNA expression of *SDHD, CYC1, ATP5B* and *ATP5O* in the small intestine was not affected by HA treatment, CG or their interaction (Table 4).

Table 4. Effects of herbage allowance (HA) and cow genotype (CG) and their interaction (HAxCG) on small intestine of gene expression encoding mitochondrial complex proteins.

Gene[1]	Complex[2]	Treatment[3]					*P*-value		
		HI-PU	LO-PU	HI-CR	LO-CR	SE	HA	CG	HAxCG
NDUFB8	I	2.13^{a}	1.24^{b}	1.71^{ab}	1.48^{b}	0.21	0.04	0.68	0.13
NDUFS4	I	1.42^{x}	0.99^{xy}	1.30^{x}	0.94^{y}	0.17	0.06	0.62	0.83
SDHA	II	1.03^{xy}	0.75^{y}	1.40^{x}	1.29^{xy}	0.27	0.50	0.08	0.72
SDHD	II	1.71	1.41	2.29	1.92	0.40	0.48	0.20	0.92
UQCRC1	III	1.15^{y}	1.26^{xy}	1.90^{x}	1.50^{xy}	0.21	0.56	0.04	0.25
COX5B	IV	1.24^{a}	0.91^{ab}	1.22^{a}	0.77^{b}	0.13	0.02	0.54	0.63
CYC1	IV	1.30	1.21	1.71	1.50	0.22	0.57	0.14	0.80
ATP5B	V	1.66	1.28	1.59	1.79	0.24	0.73	0.33	0.19
ATP5O	V	1.83	1.29	2.13	2.07	0.33	0.43	0.13	0.47

[1]*NDUFB8 = NADH dehydrogenase (ubiquinone) 1 beta subcomplex. 8; NDUFS4 = NADH dehydrogenase (ubiquinone) Fe-S protein 4; SDHA = succinate dehydrogenase complex. subunit A. flavoprotein (Fp); SDHD = succinate dehydrogenase complex. subunit D. integral membrane protein; UQCRC1 = ubiquinol-cytochrome c reductase core protein I; COX5B = cytochrome c oxidase subunit Vb; CYC1 = cytochrome c-1; ATP5B = ATP synthase subunit beta; ATP5O = ATP synthase subunit O.* [2]Mitochondrial respiratory complex number belonging protein codified by the gene.[3]Treatment : purebred and crossbred beef cows grazing high and low in average herbage allowances of native pastures: High-crossbred cows (HI-CR); High-purebred cows (HI-PU); Low-crossbred cows (LO-CR); Low-purebred cows (LO-PU). [a,b] Means with different literals differed with $P \leq 0.05$; [x,y] Means with different literals differed with $0.05 < P \leq 0.10$.

Hepatic expression of *NDUFB8*, *NDUFS4* and *COX5B* mRNA was affected by the interaction between HA treatment and CG ($P < 0.05$) due to greater ($P < 0.05$) expression in LO-CR than HI-CR and LO-PU cows and intermediate in HI-PU cows (Table 5). The expression of *CYC1* mRNA was greater ($P = 0.05$) and *ATP5B* mRNA tended ($P = 0.10$) to be greater in LO than HI cows (Table 5). The mRNA expression of *SDHA, SDHD, UQCRC1* and *ATP5O* in the liver was not affected by HA treatment, CG or their interaction (Table 5).

Table 5. Effects of herbage allowance (HA) and cow genotype (CG) and their interaction (HAxCG) on hepatic gene expression encoding mitochondrial complex proteins.

Gene[1]	Complex[2]	Treatment[3]					*P*-value		
		HI-PU	LO-PU	HI-CR	LO-CR	SE	HA	CG	HAxCG
NDUFB8	I	0.91^{ab}	0.69^{b}	0.63^{b}	1.09^{a}	0.12	0.40	0.62	0.01
NDUFS4	I	1.27^{ab}	0.58^{b}	0.82^{b}	1.50^{a}	0.20	0.97	0.27	<0.01
SDHA	II	0.69	0.39	0.74	0.75	0.17	0.43	0.23	0.32
SDHD	II	1.16	2.12	1.97	2.32	0.55	0.22	0.27	0.50
UQCRC1	III	0.76	0.47	0.66	0.96	0.20	0.98	0.35	0.17
COX5B	IV	1.39^{ab}	0.79^{b}	0.93^{ab}	1.42^{a}	0.24	0.85	0.73	0.04
CYC1	IV	0.96^{ab}	1.36^{a}	0.54^{b}	1.31^{a}	0.39	0.05	0.34	0.44
ATP5B	V	1.02^{xy}	1.84^{x}	0.69^{y}	1.23^{x}	0.52	0.10	0.19	0.69
ATP5O	V	0.96	0.58	0.68	0.64	0.17	0.31	0.51	0.31

[1]*NDUFB8 = NADH dehydrogenase (ubiquinone) 1 beta subcomplex. 8; NDUFS4 = NADH dehydrogenase (ubiquinone) Fe-S protein 4; SDHA = succinate dehydrogenase complex. subunit A. flavoprotein (Fp); SDHD = succinate dehydrogenase complex. subunit D. integral membrane protein; UQCRC1 = ubiquinol-cytochrome c reductase core protein I; COX5B = cytochrome c oxidase subunit Vb; CYC1 = cytochrome c-1; ATP5B = ATP synthase subunit beta; ATP5O = ATP synthase subunit O.* [2]Mitochondrial respiratory complex number belonging protein codified by the gene.[3]Treatment : purebred and crossbred beef cows grazing high and low in average herbage allowances of native pastures: High-crossbred cows (HI-CR); High-purebred cows (HI-PU); Low-crossbred cows (LO-CR); Low-purebred cows (LO-PU). [a,b] Means with different literals differed with $P \leq 0.05$; [x,y] Means with different literals differed with $0.05 < P \leq 0.10$.

3.5. DISCUSSION

This study evaluated long-term effects of nutrition, on weight, cellularity and gene expression of GIT organs and liver of rangeland pure and crossbred beef cows through controlling grazing intensity (herbage allowance) during the gestation-lactation cycle. Results have shown that GIT organ and/or liver weight and characteristics (cellularity indexes, expression of gene encoding for mitochondrial respiratory chain proteins) were responsive to differences in HA (high vs. low) and CG (pure vs. crossbred beef cows).

Absolute and relative visceral mass

Reticulum-rumen, total intestine (small and large) and liver absolute weights (kg) were less or tended to be less in LO than HI cows in agreement with their reduced estimated ME intake during both, the three experimental years (June 2007 to March 2010) and during the last 50 days of experiment (March to May 2010). Absolute reticulum-rumen, small or large intestine or liver weights decreased with reduced DM intake in growing steers (McLeod et al., 2007; Wang et al., 2009) and in feed-

restricted (60 to 70% of energy requirements) mature ewes (Scheaffer et al., 2004) and beef cows (Meyer et al., 2012) during mid to late gestation.

Kozloski et al. (2001) suggested that decreased DMI leads to a decreased GIT size due to the decreased metabolic activity of the epithelial cells and decreased contractile activity of muscle cells of the GIT. Absolute ruminal and intestinal weights seem to be influenced by dietary fiber intake as it increased in growing steers and lambs when increasing intake of a forage-based but not concentrate-based diet (Sainz and Bentley, 1997; McLeod et al., 2000). In addition, liver weight appears to respond to dietary energy-yielding nutrients and amino acids and thus overall metabolic workload (Sainz and Bentley, 1997; McLeod et al., 2000; 2007).

Absolute reticulum-rumen and liver weights (kg) were also greater in CR than PU cows. Ferrell and Jenkins (1985) reported that differences in maintenance energy requirements among genotypes of beef cows were associated with the potential of milk production, which determined differences in the mass of the liver and GIT organs. Similarly, Baldwin et al. (2004) reported that liver size was greater in dairy cows with higher milk production potential. Indeed, in the present study, milk production during the last lactation (2009-2010; 140 d) was greater in CR than PU cows (Gutiérrez et al., 2013) associated with a greater estimated ME intake (Laporta et al., 2014). The greater milk production and energy intake of a forage-based fiber of CR cows could have lead to an increased activity of the reticulum-rumen (contractile and absorptive), then to a greater mass of these organs.

Contrary to previous results (Ortigues and Doreau, 1995; Hersom et al., 2004), in the current experiment, differences in absolute reticulum-rumen, and/or intestine weights disappeared when expressed as a proportion of EBW (g/kg EBW), suggesting that the mass of these organs followed BW. Meyer et al. (2012) reported that after re-feeding (above requirements) during late gestation, beef cows that were restricted during mid-gestation presented similar absolute and proportional ruminal and liver weight that control cows. On the other hand, Carlson et al. (2009) found that ewe lambs that were nutrient restricted during mid-gestation then fed according to their requirements during late gestation had less absolute ruminal and liver weight but greater proportional liver weight than control ewes near term. In the present

experiment, all cows were fed at maintenance or above maintenance requirements during the last lactation (Laporta et al., 2014) and were fed to maintain BW and BCS during the last 50 d before slaughter (from calf weaning in March 2010 to slaughter in May 2010), which could have allowed cows to regain organ mass.

However, proportional weight of the stomach complex and omasum tended to be less in CR than PU cows, although the latter ones presented lower estimated energy intake and milk production. This difference due to CG was more evident in LO grazing cows, and could suggest a greater ability of CR cows to adapt their maintenance requirements to sparse nutritional environments. In contrast, liver proportional weight tended to be greater in CR than PU cows which reflected the need of nutrient processing (i.e. gluconeogenesis, oxidation, fatty acid) for milk production as previously reported in beef and dairy cows (Ferrell and Jenkins, 1985; Baldwin et al., 2004).

Visceral chemical composition and celluarity indexes

The chemical composition in terms of protein and lipid contents showed minor variations. Protein content in omasum and abomasum was greater for HI-PU than LO-PU cows while lipid concentration in the tissue structure of the reticulum-rumen and abomasum was less for HI-PU than LO-PU cows. It has been reported that dairy cows and goats in early lactation lost lipids from the GIT, but gained (when food intake was increased), or at least did not lose (when feed intake remained constant), proteins in the GIT (Ortigues and Doreau, 1995). In contrast, Drouillard et al. (1991) observed that severe undernutrition increased the proportion of lipids in the viscera of growing lambs, especially when dietary protein was limiting. Therefore, these results would suggest that when forage mass and height increased in spring and cows are able to maintain or regain body reserves lost during the negative energy balance of winter-gestation (Laporta et al., 2014), the GIT would regain mass through protein accretion in HI-PU or lipid accretion in LO-PU cows.

Differences in protein and lipid content in GIT organs were not evident between CR cows in different HA treatment. However, omental/mesenteric fat absolute weight (kg) was greater for LO-CR than HI-CR which is consistent with

reports that showed increased abdominal fat deposition when restricted animals were re-fed (Lim et al., 1996; Duarte et al., 2012). Taken together, these results would indicate that long-term dynamics of protein and lipid loss and accretion in organs or around organs of the GIT differed between PU and CR cows, depending on HA treatment.

The DNA concentration has been used to estimate the number of organ cells (hyperplasia), while the concentration of RNA was used as an indicator of the ability of protein synthesis and protein:DNA and RNA:protein ratios have been used to estimate the cell size (hypertrophy) and ribosomal capacity (indicating the *in vivo* rate of synthesis of fractional tissue protein), respectively (Burrin et al., 1992; Sainz and Bentley, 1997; Nozière et al., 1999). No differences among cow groups were found in DNA, protein and protein:DNA ratio in the reticulum-rumen, therefore, the greater reticulum-rumen size in HI than LO cows was due to increased cellularity as previously have been shown in growing steers (Sainz and Bentley, 1997; Wang et al., 2009) and dairy cows (Baldwin et al., 2004) with increased DM intake and days in milk.

However, LO-CR cows had greater DNA content and reduced protein:DNA ratio in the large intestine when compared with the other groups. Sainz and Bentley (1997) indicated that the intestines maintained constant cell sizes and became larger or smaller in response to nutrition due to changes in cell number. Results of the present study would indicate that reduced hypertrophy (but not hyperplasia) was the main mechanism involved in nutritional effects on reducing large intestinal mass in LO-CR cows but not in LO-PU cows, suggesting differential adaptation mechanisms of these genotypes in severe restricted environments. Hersom et al. (2004) reported greater protein synthetic capacity in the duodenum of growing steers grazing a wheat pasture to maintain a low rate of BW gain vs. to achieve a high rate of BW gain. In agreement with these authors, LO-CR cows showed increased protein synthetic capacity (RNA) and a fractional synthesis rate of tissue protein (RNA:protein) when compared with LO-PU in the small intestine. This could be a strategy of the former to compensate for the smaller size registered in both LO-CR and LO-PU cows (when

compared with HI cows) in order to be able to maintain the workload of this organ associated to a greater intake in LO-CR than LO-PU cows.

Although differences among groups in hepatic DNA concentration were not verified in this study, the greater protein:DNA ratio in the liver of LO-CR than HI-CR cows indicated that the greater absolute (and relative) liver size of CR than PU cows was achieved mainly by increasing cell size (hypertrophy) in LO-CR cows while both hyperplasia and hypertrophy explained the increased liver size of HI-CR cows. This could suggest a differential mechanism of LO-CR cows to increase liver mass, in order to increase its workload capacity to meet its physiological demands (i.e. gluconeogenesis for milk production), sparing the energy cost of mitosis. Greater RNA content and RNA:protein ratio was determined in the liver of HI-CR than HI-PU indicating a higher hepatic fractional synthesis and protein turnover, thus, a greater metabolic activity per unit of mass. This was probably due to an increased intake and nutrient absorption (Sainz and Bentley, 1997) in the former when herbage mass and height allowed it.

Gene expression of mitochondrial respiratory chain proteins in small intestine and liver

On the basis of the importance of mitochondria in cellular energy production, Rolfe and Brand (1997) have hypothesized that differences in the mitochondrial functional activity may contribute to the phenotypic differences observed between animals. However, despite its potential significance, information available in this area for cattle is scarce and has been generated in growing steers while no information is available for beef cows in rangelands. The greater expression of various genes encoding for respiratory chain protein complexes in the small intestine of HI than LO cows (*NDUFB8*, *COX5B* and *NDUFS4* mRNA) and of CR than PU cows (*UQCRC1* and *SDHA* mRNA) could be associated with the greater estimated energy intake in these cows which, if translated to protein, could boast a higher metabolic activity. *In vivo* oxygen consumption of viscera rises with increasing intake of dietary energy (Burrin et al., 1989; Reynolds et al., 1992; Freetly and Ferrell, 1995). Indeed, increased oxygen consumption of small intestine not only has

been observed due to greater mass but also to greater weight-specific oxygen consumption (Hersom et al., 2004).

In contrast to what was observed in the small intestine, in the liver an increased expression of genes encoding for proteins of mitochondrial respiratory complexes, particularly of genes associated to complex I, was determined in LO-CR cows compared to other groups. Together with the greater protein:DNA ratio, this increase in gene expression could indicate a higher metabolic activity of cells, particularly related to protein synthesis which in turn, could suggest differential adaptation of CR vs. PU cows in more restrictive environments.

Mitochondrial complexes are not separate entities, but assembled multiproteic subunits, which have a great structural and functional dependence of the individual subunits, wherein the alteration of one of them can cause partial or global disturbance of the whole complex functionality. The electron transport chain is also a recognized site of production of reactive oxygen species (**ROS**), mainly complexes I and III (Bottje and Carstens, 2009). Elevated ROS involve high susceptibility of the various cellular components to oxidative damage. This increased expression of genes encoding proteins of complex I and III in the small intestine of HI and CR cows and the complexes I and by the LO-CR in liver could indicate a reduction in ROS production, therefore less severe oxidative damage with impacts on metabolic balance and efficiency in the use of nutrients. Indeed, increased expression of several proteins (or genes encoding for these proteins) of these respiratory complexes in various tissues in high vs. low feed efficiency chickens (Bottje et al., 2002; Ojano-Dirain et al., 2007) or steers (Connor et al., 2009). These results would suggest that a substantial proportion of the greater productivity of HI vs. LO cows as well as LO-CR vs. LO-PU cows (Gutierrez et al., 2013; Espasandin et al., 2012) could be due differences in the regulation of gene expression of mitochondrial respiratory complexes in GIT organs or liver. The differential regulation of genes encoding for mitochondrial respiratory chain proteins between the small intestine and liver may reflect that the energy expenditure pattern in an organ is associated with its specific metabolism. However, further research is needed in this area.

3.6. CONCLUSION

Control of grazing intensity of native grasslands through changes in HA treatment, affected in the long-term consequences of nutrition, related to GIT organ mass, cellularity, or gene expression of proteins thought to be important in regulating energy utilization and efficiency, and these effects differed between PU and CR breed cows. Results suggest that CR cows had a greater plasticity in order to adapt their visceral mass, cellularity and or gene expression to sparse environments than PU cows.

3.7. ACKNOWLEDGMENTS

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3.8. REFERENCES

AOAC., 2000. Official methods of analysis. 17th ed. Assoc. Off. Anal. Chem., Arlington, VA.

Astessiano, A.L., Pérez-Clariget, R., Quintans, G., Soca, P., Carriquiry, M., 2012. 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. J. Anim. Physiol. Anim. Nutr. 535–544.

Baldwin, R.L., McLeod, K.R., Capuco, A.V., 2004. Visceral Tissue Growth and Proliferation During the Bovine Lactation Cycle. J. Dairy. Sci. 87, 2977–2986.

Bottje, W., Iqbal, M., Tang, Z. X., Cawthon, D., Okimoto, R., Wing, T., Cooper, M., 2002. Association of mitochondrial function with feed efficiency within a single genetic line of male broilers. Poult. Sci. 81:546–555.

Bottje, W.G., Carstens, G.E., 2009. Association of mitochondrial function and feed efficiency in poultry and livestock species. J. Anim. Sci. 87, E48–63.

Burrin, D. G., Ferrell, C. L., Eisemann, J. H., Britton, R. A., Nienaber, J. A., 1989. Effect of level of nutrition on splanchnic blood flow and oxygen consumption in sheep. Br. J. Nutr. 62, 23-34.

Burrin, D.G., Britton, R.A., Ferrell, C.L., Bauer, M.L., 1992. Level of nutrition and visceral organ protein synthetic capacity and nucleic acid content in sheep. J. Anim. Sci. 70, 1137–1145.

Carlson, D.B., Reed, J.J., Borowicz, P.P., Taylor, J.B., Reynolds, L.P., Neville, T.L., Redmer, D.A., Vonnahme, K.A., Caton, J.S., 2009. Effects of dietary selenium supply and timing of nutrient restriction during gestation on maternal growth and body composition of pregnant adolescent ewes. J. Anim. Sci. 87, 669–680.

Chapman, D.F., Parsons, A.J., Cosgrove, G.P., Barker, D.J., Marotti, D.M., Venning, K.J., Rutter, S.M., Hill, J., Thompson, A.N., 2007. Impacts of spatial patterns in pasture on animal grazing behavior, intake, and performance. Crop. Sci. 47, 399–415.

Connor, E.E., Kahl, S., Elsasser, T.H., Parker, J.S., Li, R.W., Van Tassell, C.P., Baldwin, R.L., Barao, S.M., 2009. Enhanced mitochondrial complex gene function and reduced liver size may mediate improved feed efficiency of beef cattle during compensatory growth. Funct. Integr. Genomics 10, 39–51.

Connor, E.E, Li, R.W, Baldwin VI, R. L., Li, C., 2010. Gene expression in the digestive tissues of ruminants and their relationships with feeding and digestive processes. Animal, 4:7, pp 993–1007.

DiCostanzo, A., Meiske, J.C., Plegge, S.D., 1991. Characterization of energetically efficient and inefficient beef cows. J. Anim. Sci. 69, 1337–1348.

Drouillard, J.S., Klopfenstein, T.J., Britton, R.A., Bauer, M.L., Gramlich, S.M., Wester, T.J., Ferrell, C.L., 1991. Growth, body composition, and visceral organ mass and metabolism in lambs during and after metabolizable protein or net energy restrictions. J. Anim. Sci.69, 3357–3375.

Duarte F.O., Sene-Fiorese M., Cheik N.C., Maria A.S., de Aquino A.E. Jr, Oishi J.C., Rossi E.A., Garcia de Oliveira Duarte A.C., Dâmaso A.R., 2012., Food restriction and refeeding induces changes in lipid pathways and fat deposition in the adipose and hepatic tissues in rats with diet-induced obesity. Exp. Physiol. 97(7), 882-94.

Espasandin, A.C., Ciria, M., Franco, J.B., Pereyra, F., Gimeno, D., 2010., Heterosis and productive and reproductive performance in Angus, Hereford and F1 reciprocal crossed cows on grazing systems of Uruguay Internacional , XXVI World Buiatrics Congress 2010 , Santiago de Chile , 2010.

Espasandin A.C., Do Carmo M., López-Mazz C.R., Carriquiry M., Soca P., 2012. Heterosis of productivity rates in the breeding cycle of pure and crossbred Hereford and Angus cattle grazing native pastures at low and high allowances. J. Anim. Sci. 90 (Suppl. 3), 531.

Ferrell, C.L., Jenkins, T.G., 1985. Cow type and the nutritional environment: nutritional aspects. J. Anim. Sci. 61, 725–741.

Freetly, H. C., Ferrell, C. L., 1995. Plane of nutrition effects on net flux of oxygen and glucose across splanchnic tissues of ewes. In: C. Wenk and M. Boessinger (Ed.) Proc. 12th Symp. Energy Metabolism of Farm Animals. Eur. Assoc. Anim. Prod. Publ. 58:72.

Gutiérrez, V., Espasandin, A.C., Astessiano, A.L., Casal, A., López-Mazz, C., Carriquiry, M., 2013. Calf foetal and early life nutrition on grazing conditions: metabolic and endocrine profiles and body composition during the growing phase. J. Anim. Physiol. Anim. Nutr. (Berl) 97(4):720-31

Haydock, K., Shaw, N., 1975. The comparative yield method for estimating dry matter yield of pasture. Aust. J. Exp. Agric. 15, 663–670.

Herd, R.M., Arthur, P.F., 2009. Physiological basis for residual feed intake. J. Anim. Sci. 87, E64–E71.

Hersom, M.J., Krehbiel, C.R., Horn, G.W., 2004. Effect of live weight gain of steers during winter grazing: II. Visceral organ mass, cellularity, and oxygen consumption. J. Anim. Sci. 82, 184–197.

Jenkins, T.G., Cundiff, L.V., Ferrell, C.L., 1991. Differences among breed crosses of cattle in the conversion of food energy to calf weight during the preweaning interval. J. Anim. Sci. 69, 2762–2769.

Kozloski, G.V., Rocha, J.B.T., Ciocca, M.L.S., 2001. Visceral metabolism and efficiency of energy use by ruminants. Cienc. Rural. 31, 903–908.

Laporta, J., Rosa, G.J., Naya, H., Carriquiry, M., 2014. Liver functional genomics in beef cows on grazing systems: novel genes and pathways revealed. Physiol Genomics. 15; 46(4):138-47.

Lim, K., Murakami, E., Lee, S., Shimomura, Y., Suzuki, M., 1996. Effects of intermittent food restriction and refeeding on energy efficiency and body fat deposition in sedentary and exercised rats. J. Nutr. Sci. Vitaminol. 42(5), 449-68.

Macoon, B.L., Sollenberger, E., Moore, J.E., Staples, C.R., Fike, J.H., Portier, M., 2003. Comparison of three techniques for estimating the forage intake of lactating dairy cows on pasture. J. Anim. Sci. 81: 2357-2366.

McLeod, K.R., Baldwin, R.L., 2000. Effects of diet forage: concentrate ratio and metabolizable energy intake on visceral organ growth and in vitro oxidative capacity of gut tissues in sheep. J. Anim. Sci. 78, 760–770.

McLeod, K.R., Baldwin, R.L., Solomon, M.B., Baumann, R.G., 2007. Influence of Ruminal and Postruminal Carbohydrate Infusion on Visceral Organ Mass and Adipose Tissue Accretion in Growing Beef Steers. J. Anim. Sci. 85, 2256–2270.

Meyer, A.M., Reed, J.J., Neville, T.L., Taylor, J.B., Reynolds, L.P., Redmer, D.A., Vonnahme, K.A., Caton, J.S., 2012. Effects of nutritional plane and selenium supply during gestation on visceral organ mass and indices of intestinal growth and vascularity in primiparous ewes at parturition and during early lactation. J. Anim. Sci. 90, 2733–2749.

Morris C.A., Baker R.L., Johnson D.L., Carter A.H., Hunter J.C., 1987. Reciprocal rossbreeding of Angus and Hereford cattle. 3. Cow weight, reproduction, maternal performance, and lifetime production. New Zeal. J. Agr. Res. 30, 453–467.

Mott, G.O., 1960. Grazing pressure and the measurement of pasture production. Proceedings 8th International Grassland Congress. Reading, UK. pp 606-611.

Nozière, P., Attaix, D., Bocquier, F., Doreau, M., 1999. Effects of underfeeding and refeeding on weight and cellularity of splanchnic organs in ewes. J. Anim. Sci.77, 2279–2290.

NRC., 2000. Nutrient requirements of beef cattle. 7th rev. ed. Natl. Acad. Press, Washington, DC.

Ojano-Dirain, C.P., Pumford, N.R., Toyomizu, M., Bottje, W.G., 2007. Association of Mitochondrial Function and Feed Efficiency. J. Poult. Sci. 44, 221–237.

Ortigues, I., Doreau, M., 1995. Responses of the splanchnic tissues of ruminants to changes in intake: absorption of digestion end products, tissue mass, metabolic activity and implications to whole animal energy metabolism. Annales De Zootechnie 44, 321–346.

Pfaffl, M.W., 2004. Quantification strategies in real-time PCR. In (Ed. S.A. Bustin) A-Z of Quantitative PCR. International University Line (IUL) La Jolla, CA, USA.pp 89–113

Reid, C. R., Bailey C. M., Judkins M. B., 1991. Metabolizable energy for maintenance of beef-type Bos taurus and Bos indicus x Bos taurus cows in a dry, temperate climate. J Anim Sci 69.7: 2779-2786.

Reynolds, C. K., Lapierre, H., Tyrrell, H. F., Elsasser, T. H., Staples, R. C., Gaudreau, P., Brazeau, P., 1992. Effects of growth hormone-releasing factor and feed intake on energy metabolism in growing beef steers: Net nutrient metabolism by portaldrained viscera and liver. J. Anim. Sci. 70:752.

Rolfe, D.F.S., Brand, M.D., 1997. The Physiological Significance of Mitochondrial Proton Leak in Animal Cells and Tissues. Biosci. Rep. 17, 9–16.

Sainz, R.D., Bentley, B.E., 1997. Visceral Organ Mass and Cellularity in Growth-Restricted and Refed Beef Steers. J. Anim. Sci. 75, 1229–1236.

Scheaffer, A.N., Caton, J.S., Redmer, D.A., Arnold, D.R., Reynolds, L.P., 2004. Effect of dietary restriction, pregn. ancy, and fetal type on intestinal cellularity and vascularity in Columbia and Romanov ewes. J. Anim. Sci. 82, 3024–3033

Seal, C.J., Reynolds, C.K., 1993. Nutritional implications of gastrointestinal and liver metabolism in ruminants. Nutr. Res. Rev. 6, 185–208.

Soares, A.B., Carvalho, P.C.F, Nabinger, C., Frizzo, A., Pinto, C.E., Junior, J.A.F, Semmelmann, C., da Trindade, J., 2003. Effect of changing herbage allowance on primary and secondary production of natural pasture. Proceedings of the 7th International Rangeland Congress, 26 July - 1 August 2003, pp 966-968.

Sollenberger, L.E., Moore, J.E., Allen, V.G., Pedreira, C.G.S., 2005. Reporting Forage Allowance in Grazing Experiments. Crop. Sci. 45, 896.

Smit, H.J., Taweel, H.Z., Tas, B.M., Tamminga, S., Elgersma, A., 2005. Comparison of techniques for estimating herbage intake of grazing dairy cows. J. Dairy. Sci. 88, 1827-36.

Sunnucks, P., Hales, D.F., 1996. Numerous transposed sequences of mitochondrial cytochrome oxidase I-II in aphids of the genus Sitobion (Hemiptera: Aphididae). Mol. Biol. Evol. 13, 510–524.

Vizcarra, J.A., Ibañez, W., Orcasberro, R., 1986. Repetibilidad y reproductibilidad de dos escalas para estimar la condición corporal de vacas Hereford. Inv. Agron. 7(1):45-47.

Wang, Y.J., Wood, K.M., Martin, L., Holligan, S., Kelly, N., McBride, B.W., Fan, M.Z., Swanson, K.C., 2009. Effect of dietary corn silage inclusion on visceral organ mass, cellularity, and the protein expression of ATP synthase, Na^+/K^+ -ATPase, proliferating cell nuclear antigen and ubiquitin in feedlot steers. Can. J. Anim. Sci. 89, 503–512.

4. DISCUSIÓN GENERAL Y CONCLISIONES

El control de la intensidad de pastoreo a través del manejo de la oferta de forraje tuvo como resultado una mejora en el aprovechamiento de la energía por parte del sistema criador. Las vacas que pastorearon AOF y las vacas CR presentaron una mejor respuesta productiva/reproductiva (ie. +19% y +15% de kg de ternero destetado/vaca entorada en AOF vs. BOF y en CR vs. PU, respectivamente; Carriquiry et al., 2012; Soca et al., 2013b). Esta mejora se asoció a un mejor estatus metabólico (ej. disponibilidad de nutrientes y energía para los tejidos; Blache et al., 2007), como resultado de un mayor consumo de energía (*Artículo 2*) y/o un menor gasto de mantenimiento, que se reflejó en mayores reservas corporales en las vacas AOF como en vacas CR (mayor BCS; *Articulo 1*). El presente trabajo identifica el efecto del control de la intensidad de pastoreo del campo nativo, a través del manejo de la oferta de forraje, sobre características asociadas al requerimiento de mantenimiento (composición corporal y de la masa, celularidad, composición y expresión de genes que codifican proteínas mitocondriales en las vísceras del TGI e hígado) de vacas PU y CR, sugiriendo que existen mecanismos diferenciales de adaptación de los distintos genotipos a las distintas ofertas de forraje que podrian explicar las diferencias productivas en la producción global del sistema criador.

4.1. EFECTO DE LA OFERTA DE FORRAJE

Las vacas en AOF presentaron o tendieron a presentar mayor PV y CC que las vacas en BOF, lo cual estuvo asociado a un contenido de grasa tanto en términos absolutos (masa corporal, kg) como relativos (g/kg PCV) (*Artículo 1*). De manera similar, resultados previos (NRC, 1996; Houghton et al., 1990a; 1990b) reportan que una mejora en la alimentación o en la CC de vacas de carne se refleja en un mayor contenido de grasa corporal. Sin embargo, a pesar del mayor contenido de grasa corporal total, al momento del sacrificio (otoño, 45 días postdestete), las vacas en AOF presentaron una menor deposición de grasa asociada a las vísceras del TGI (menor concentración de lípidos del retículo-rumen y abomaso en vacas AOF-PU que en vacas BOF-PU y menor peso de grasa omental/mesentérica (kg) en vacas

AOF-CR que vacas BOF-CR; *Artículo 2*). Esta mayor deposición de grasa abdominal ha sido reportada previamente (Lim et al., 1996; Duarte et al., 2012) durante la re-alimentación de animales previamente restringidos en su consumo de energía (ej. BEN invernal).

Durante la gestación invernal (150-240 días de gestación), tanto las vacas en AOF como vacas en BOF perdieron PV (17-18 ± 5 kg) y CC (0.5 ± 0.1 unidades), con disminución de la masa de agua, proteína y grasa corporal (*Artículo 1*). A su vez, el contenido relativo de agua corporal se incrementó durante este período tanto en vacas en AOF comoen vacas en BOF, indicando una retención de agua a nivel corporal, en detrimento de una mayor pérdida relativa de proteínas y grasa y por lo tanto de un mayor contenido energético por unidad de peso movilizado que retenido (*Artículo 1*). Se ha reportado en varias especies que una de las adaptaciones fisiológicas del organismo materno durante la gestación es una mayor hidratación de los tejidos y un aumento del volumen sanguíneo y liquido extracelular (Foot, 1969; Degan y Young, 1980). Sin embargo, no se evidenciaron cambios en el contenido de proteínas y grasa por unidad de peso retenido (g/kg PCV), indicando que la composición del peso movilizado durante el periodo de balance energético negativo (BEN) de invierno no difirió entre vacas en AOF y vacas en BOF (*Artículo 1*).

A su vez, el mayor PV (y PCV) de las vacas en AOF al sacrificio se acompañó de una mayor masa de retículo-rumen, intestinos (intestino delgado + grueso) e hígado (*Artículo 2*). Investigaciones previas en novillos (McLeod et al., 2007; Wang et al., 2009) y vacas de carne (Meyer et al., 2012) reportan un aumento del tamaño del TGI con el aumento de la ingesta de alimento. Kozloski et al. (2001) sugiere que el aumento de la ingesta conduce a un aumento de la actividad metabólica de las células epiteliales y de la actividad contráctil de las células musculares del TGI, determinando aumento de la masa del TGI. Asimismo, la masa hepática parece responder a la cantidad de nutrientes en la dieta y por lo tanto a la carga de trabajo metabólico general (Sainz y Bentley, 1997; McLeod y Baldwin, 2000; McLeod et al., 2007). Sin embargo, en contraste con resultados previos (Ortigues y Doreau, 1995; Hersom et al., 2004), las diferencias en masa de retículo-

rumen, intestinos e hígado desaparecieron cuando se expresaron como una proporción del PCV (g/kg PCV), lo que sugiere que la masa de estos órganos acompañó las variaciones en el PV de los animales (*Artículo 2*).

En acuerdo con resultados previos en novillos (Sainz y Bentley, 1997) y vacas lecheras (Baldwin et al., 2004), el incremento en la masa de retículo-rumen e intestino delgado en vacas en AOF se debió principalmente a un incremento en la celularidad de los mismos (combinación de hiperplasia e hipertrofia), ya que no se encontraron diferencias en el contenido de ADN ni en la relación proteína:ADN de éstos órganos entre vacas en AOF y vacas en BOF (*Artículo 2*). Sin embargo, el análisis de éstos índices de celularidad en el intestino grueso e hígado muestra una interacción entre la OF y el GG, siendo diferentes en vacas CR y vacas PU en AOF y BOF (ver mas abajo). A su vez, la interacción entre OF y GG, afectó también el contenido de ARN y la relación ARN:proteína del intestino delgado e hígado que indican la capacidad y tasa fraccional de síntesis proteica diferenciándose entre vacas CR y PU y en vacas en AOF y BOF (ver mas abajo) .

Existe evidencia que la expresión de genes (o proteinas) de los complejos mitocondriales, principalmente complejos I y III, en varios tejidos afectan la eficiencia alimenticia de los animales (a mayor expresión, mayor eficiencia; Kolath et al. 2006; Connor et al. 2009). Estos resultados se han asociado a producción de especies reactivas de oxígeno (ROS), que ocurre también en cadena de transporte de electrones (Bottje y Carstens, 2009), cuya elevada producción aumenta la susceptibilidad de los diversos componentes celulares al daño oxidativo. La expresión de genes que codifican para el complejo I de la cadena respiratoria en el intestino delgado fueron mayores en vacas en AOF que en vacas en BOF (ARNm de *NDUFB8 y NDUFS4*). Sin embargo, la expresión de estos genes en hígado fue afectada por la interacción entre OF y GG, difiriendo entre vacas BOF-CR y vacas AOF-CR y entre vacas BOF-CR y vacas BOF-PU (ver debajo).

Tomando estos resultados en su conjunto, la mayor productividad de las vacas pastoreando AOF frente a vacas pastoreando BOF podría, entre otros factores, ser explicado por:

1) las mayores reservas corporales en términos de masa lipídica pero no proteica

que determinaría una mayor disponibilidad de sustratos energéticos en períodos de BEN (Houghton et al., 1990a) sin incrementar el requerimiento de mantenimento (masa proteica total similar entre vacas en AOF y vacas en BOF (Agnew y Yan, 2000); peso relativo de los órganos del TGI e hígado similar entre vacas en AOF y vacas en BOF)

2) la menor deposición de grasa visceral (menor resitencia a la insulina con impactos positivos en productividad, salud y fertilidad; Sinclair, 2010)
3) la mayor expresión de genes que codifican para las proteínas mitocondriales del complejo I en intestino delgado, que permitiría una menor producción de ROS, impactando sobre el equilibrio metabólico y la eficiencia en el uso de los nutrientes (Connor et al., 2009).

4.2. EFECTO DEL GENOTIPO DE LA VACA DE CRÍA (HETEROSIS) Y SU INTERACCIÓN CON LA OFERTA DE FORRAJE

Las vacas CR presentaron mayor PV y CC que las vacas PU, asociados a una mayor masa corporal de agua, proteínas y grasa en términos absolutos y a un mayor contenido relativo de agua y proteínas (pero no de lípidos) en las vacas CR en comparación con vacas PU (*Artículo 1*). En acuerdo con el mayor contenido proteico de las vacas CR, se han reportado efectos de la heterosis sobre el peso de la canal (Espasandin et al., 2006), la proporción muscular y el área del musculo *longissimus* (Long, 1980; Marshall, 1994) entre razas británicas puras y sus cruzas recíprocas F1.

Durante la gestación invernal (150-240 días de gestación), tanto las vacas CR como vacas PU perdieron PV y CC disminuyendo la masa corporal de agua, lípidos y proteína corporal. Sin embargo, la composición por unidad de peso retenido (o movilizado) durante la gestación invernal difirió entre vacas CR y vacas PU (*Artículo 1*). Si bien la cantidad de agua por unidad de peso se incrementó durante este período en todas las vacas, las vacas PU movilizaron fundamentalmente grasa mientras que las vacas CR movilizaron grasa y proteína en relación a su composición corporal. La movilización de grasa en vacas gestantes cuando el consumo no cumple con sus requerimientos es una estrategia de los animales para cubir el déficit de energía. Aunque el feto no es capaz de utilizar los sustratos lipídicos movilizados,

éstos aseguran una menor utilización de glucosa (y aminoácidos) por parte de los tejidos maternales, lo que permite que se prioricen estos nutrientes para el crecimiento fetal (Bell, 1995). Esta movilización de lípidos durante el BEN invernal ocurre tanto en vacas CR y vacas PU, sin embargo, el punto diferencial entre ambos GG es la movilización de masa proteica que ocurre fundamentalmente en las CR y que indicaría una adaptación diferencial entre GG. van der Drift, et al. (2012) demostraron en vacas lecheras, que el grado de movilización proteica durante el BEN durante la transición está directamente asociado al espesor muscular. Entonces, con estos resultados, las vacas CR presentaron mayor masa proteica a los 150 días de gestación (comienzo del invierno). Los mecanismos involucrados en la movilización proteica no están tan profundamente estudiados pero se ha sugerido que bajas concentraciones de insulina, así como deficiencias de aminoácidos específicos favorecería la movilización proteica (Tesseraud et al., 2007, van del Drift et al., 2012). En este experimento, las vacas CR durante los días 150 a 240 de gestación presentaron una mayor caída en las concentraciones de insulina (Laporta et al., 2014) durante el periodo de invierno cuando la concentración de proteína cruda del fooajr en baja. Esta movilización de proteínas puede impactar posteriormente en la salud animal (ie. reducir cetogénesis por aumento de precursores neoglucogénicos) y a su vez reducir los costos de mantenimiento ya que se ha indicado que el costo de metabolismo basal depende más de la masa proteica corporal que del PV o peso metabólico (Agnew y Yan, 2000).

El mayor PV (y PCV) de las vacas CR que vacas PU al sacrificio se acompañó de una mayor masa de retículo - rumen e hígado (kg) (*Artículo 2*) Se ha informado que diferencias en los requerimientos de energía de mantenimiento entre genotipos de vacas de carne (Ferrell y Jenkins, 1985) y vacas de leche (Baldwin et al., 2004) se asociaron al potencial de producción de leche, que determina diferencias en la masa de los órganos TGI e hígado. En efecto, en el presente experimento, la producción de leche durante la última lactación (120 d) fue mayor en vacas CR que PU (Gutiérrez et al., 2013). Sin embargo, cuando los pesos de éstos órganos se expresaron como proporción del PCV (peso relativo; g/kg PCV), el peso relativo del complejo estomacal tendió a ser menor en vacas CR que vacas PU

debido a una tendencia de las primeras a presentar menor peso relativo del omaso pero el peso relativo del hígado tendió a ser mayor en vacas CR que vacas PU (*Artículo 2*). Es así que el peso relativo del total de órganos del TGI e hígado no difirió entre vacas CR y vacas PU, compensándose el mayor peso relativo del hígado con un menor peso relativo de omaso en vacas CR. Esto sugeriría una mayor capacidad de vacas CR para adaptar sus requerimientos de mantenimiento para ambientes nutricionales diversos.

De manera similar a los ocurrido en vacas pastoreando AOF, el incremento en la masa de retículo-rumen e intestino delgado en vacas CR se debió principalmente a un incremento en la celularidad de lo mismo (combinación de hiperplasia e hipertrofia), ya que no se encontraron diferencias en el contenido de ADN ni en la relación proteína:ADN de estos órganos entre vacas en AOF y vacas en BOF (*Artículo 2*). Asimismo la disminución de la masa relativa del omaso, estuvo explicada por una disminución tanto en la hiperplasia como en la hiptertrofia. Sin embargo, el contenido de ADN fue mayor y la relación proteína:ADN fue menor en el intestino grueso de las vacas BOF-CR en comparación con los otros grupos. Sainz y Bentley (1997) indicaron que los intestinos mantienen constantes el tamaño de las células y varían su masa en respuesta a la nutrición debido a los cambios en el número de células. Los resultados del presente estudio indicarían que la menor hipertrofia (pero no hiperplasia) fue el principal mecanismo implicado en los efectos de una nutrición restringida sobre la reducción de la masa intestinal en las vacas BOF-CR pero no en las vacas BOF-PU, lo que sugiere mecanismos de adaptación diferenciales de las vacas CR y vacas PU en ambientes con alimentación restringida.

A su vez, si bien no se evidenciaron cambios en la concentración hepática de ADN, la mayor relación proteína:ADN en el hígado de vacas BOF-CR que vacas AOF-CR indicaría que el mayor tamaño del hígado de vacas CR sería principalmente debido a un aumento del tamaño de las células (hipertrofia) en vacas BOF-CR, mientras que en vacas AOF-CR estaría dado por mayor hiperplasia e hipertrofia. Esto podría sugerir un mecanismo diferencial de vacas BOF-CR para aumentar la masa del hígado, con el fin de aumentar su capacidad de carga de trabajo para satisfacer sus demandas fisiológicas (ie. la gluconeogénesis para la producción de la leche),

ahorrando el costo de energía de la mitosis. Asimismo, el contenido de ARN y la relación ARN:proteína que indican la capacidad y tasa fraccional de síntesis proteica en el intestino delgado fue mayor en vacas BOF-CR que vacas AOF-CR y que en vacas BOF-PU. Esto indicaría una estrategia de las vacas CR en BOF (pero no de las PU) para compensar, en términos de síntesis proteica, la reducción en el tamaño de este órgano frente a las vacas en AOF. A su vez, el mayor contenido de ARN y ARN:proteína en el hígado de vacas AOF-CR que en vacas AOF-PU, indicaría una mayor síntesis hepática y recambio de proteínas, por lo tanto, una mayor actividad metabólica por unidad de masa asociado probablemente a la mayor ingesta y absorción de nutrientes (Sainz y Bentley, 1997) así como al mayor trabajo metabólico (ie. gluconeogénesis) debido a la mayor producción de leche (Gutiérrez et al., 2013).

La expresión de genes que codifican para proteínas del complejo I de la cadena respiratoria no difirió en el intestino delgado de vacas CR y vacas PU pero las vacas CR presentaron una mayor expresión de ARNm de *UQCRC1* (complejo III) que las vacas PU, lo que podría estar asociado con una mayor eficiencia alimenticia de las primeras. En contraste, en el hígado la expresión de genes que codifican para las proteínas del complejo I de la cadena respiratoria fue mayor en vacas BOF-CR que en vacas AOF-CR y que en vacas BOF-PU, lo que sugeriría una reducción en la producción de ROS, por lo tanto, un menor daño oxidativo, y un posible aumento en la eficiencia en el uso de los nutrientes.

Tomando estos resultados en su conjunto, la mayor productividad de las vacas CR frente a las vacas PU podría, entre otros factores, ser explicada por:

1) las mayores reservas corporales, en términos tanto de masa lipídica como proteica, que permitiría en períodos de BEN una movilización mayor de la masa proteica que junto con la movilización de grasa permitirían aumentar la disponibilidad de sustratos no solo para ser usados como fuente de energía sino también como precursores glucogénicos (Houghton et al., 1990a; 1990b; van der Drift et al., 2012). Esta movilización de la masa proteica durante el BEN les permitiría a las vacas CR reducir requerimiento de mantenimento (masa proteica total similar entre vacas CR y vacas PU al fin del invierno,

Agnew y Yan, 2000) y contar con ventajas metabólicas (van der Drift et al., 2012)

2) la capacidad de compensar el mayor peso relativo del hígado (asociado a una mayor producción de leche; Montaño-Bermudez et al., 1990) con un menor peso relativo de omaso en vacas CR, determinando que el peso relativo del total de órganos del TGI e hígado no difiera entre vacas CR y vacas PU.
3) la mayor expresión de genes que codifican para las proteínas mitocondriales del complejo III en intestino delgado, que permitiría una menor producción de ROS, impactando sobre el equilibrio metabólico y la eficiencia en el uso de los nutrientes (Sandelin et al., 2005; Connor et al., 2009).

A su vez las vacas CR pastoreando BOF presentaron algunas estrategias diferenciales asociadas a la mayor productividad de estos animales frente a las vacas PU en ambientes restrictivos:

1) un mayor ahorro en el costo energético de mitosis asociado al aumento del tamaño del hígado (debido a la demanda metabólica) frente a las vacas AOF-CR (Sainz y Bentley, 1997)
2) una mayor capacidad de compensación, en términos de síntesis proteica, la reducción en el tamaño del intestino delgado (frente a las vacas en AOF) que las vacas BOF-PU
3) una mayor expresión de genes que codifican para las proteínas del complejo I de la cadena respiratoria en el hígado que en vacas AOF-CR y que en vacas BOF-PU que sugeriría una reducción en la producción de ROS impactando positivamente sobre la eficiencia en el uso de los nutrientes (Connor et al., 2009).

4.3. CONCLUSIONES

El control de la intensidad de pastoreo a través del manejo de la OF del campo nativo tuvo consecuencias en el largo plazo en relación a factores que regulan eficiencia de la utilización de la energía como lo son el contenido de grasa y proteína corporal y su movilización, en el tamaño y masa de los órganos del TGI y/o la expresión de genes que codifican para proteínas de la cadena de transporte mitocondrial. Y estas

diferencias a su vez varían de acuerdo al GG de las vacas, dado que las vacas CR obtuvieron los mejores resultados tanto vacas en AOF y vacas en BOF de forraje. Los resultados sugieren que las vacas CR tienen una mayor plasticidad con el fin de adaptarse a entornos más escasos que las vacas PU.

5. BIBLIOGRAFÍA

Agnew RE, Yan T. 2000. Impact of recent research on energy feeding systems for dairy cattle. Livestock Production Science 66,197-215.

Agnew RE, Yan T, McCaughey WJ, McEvoy JD, Patterson DC, Porter MG, Steen RW. 2005. Relationships between urea dilution measurements and body weight and composition of lactating dairy cows. Journal of Dairy Science 88, 2476-86.

AOAC. 2000. Official methods of analysis. 17th edn. (Association of Official Analytical Chemists: Gaithersburg, MD).

Astessiano AL, Pérez-Clariget R, Quintans G, Soca P, Carriquiry M. 2012. 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. Journal Animal Physiology and Animal Nutrition. 535–544.

Baldwin RL, McLeod KR, Capuco AV. 2004. Visceral Tissue Growth and Proliferation During the Bovine Lactation Cycle. Journal of Dairy Science 87, 2977–2986.

Baldwin RL. 1995. Modeling Ruminant Digestion and Metabolism. London, UK. Chapman and Hall, p. 585.

Baldwin RL, Smith NE, Taylor J, Sharp, M. 1980. Manipulating metabolic parameters to improve growth rate and milk secretion. Journal of Animal Science 51, 1416-28.

Ball AJ, Oddy VH, Thompson JM. 1997. Nutritional manipulation of body composition and efficiency in ruminants. In: Recent Advances in Animal Nutrition in Australia, Armidale, New South Wales, Australia. p 192-208.

Baker RD, Alvarez F, Le Du YLP. 1981. The effect of herbage allowance upon the herbage intake and performance of suckler cows and calves. Grass and Forage Science 36, 189-199.

Barendse W, Reverter A, Bunch RJ, Harrison BE, Barris W, Thomas MB. 2007. A validated whole-genome association study of efficient food conversion in cattle. Genetics, 176, 1893-1905.

Bartle SJ, Kock SW, Preston RL, Wheeler TL G, Davis, W. 1987. Validation of urea dilution to estimate in vivo body composition in cattle. Journal of Animal Science 64, 1024–30.

Basarab JA, Price MA, Aalhus JL, Okine EK, Snelling WM, Lyle KL. 2003. Residual feed intake and body composition in young growing cattle. Canadian Journal of Animal Science 83, 189-204.

Bell, AW. 1995. Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. Journal of Animal Science 73, 2804-19.

Bennett GL, Swiger LA, Preston RL, Cahill VR. 1982. Evaluation of urea space and ultrasonic measurement as selection criteria for beef animal composition. Journal of Animal Science 54, 553–58.

Berg JM, Tymoczko JL. 2008. Ciclo del acido cítrico. In: Bioquimica Cap. 17 p 475-501, Ed. Recerté SA, Barcelona España.

Berretta E, Risso D, Montossi F, Pigurina G. 2000. Campos in Uruguay. In Grassland ecophysiology and grazing ecology. (Eds G Lemaire, J Hogdson, A de Moraes, C Nabinger, PC Carvalho) pp. 377–394. (CAB International: Curitiba Parana, BR).

Berretta EJ, Do Nascimento D. 1991. Glosario Estructurado de Términos sobre Pasturas y Producción Animal. Ed. Puignau J.P. Dialogo XXXII. IICA. Montevideo. p126.

Bishop SC. 1992. Phenotypic and genetic variation in body weight, food intake and energy utilization in Hereford cattle II. Effects of age and length of performance test. Livestock Production Science 30, 19–31.

Blache, D., Chagas, L.M., Martin, G.B. 2007. Nutritional inputs into the reproductive neuroendocrine control system – a multidimensional perspective, In: Juengel, J.I., Murray, J.F., Smith, M.F. (Eds.), Reproduction in Domestic Ruminants VI, Nottingham University Press, Nottingham, UK, p 123-139.

Bottje WG, Carstens GE. 2009. Association of mitochondrial function and feed efficiency in poultry and livestock species. Journal of Animal Science 87, E48–63.

Bottje W, Iqbal M, Tang ZX, Cawthon D, Okimoto R, Wing T, Cooper M. 2002. Association of mitochondrial function with feed efficiency within a single genetic line of male broilers. Poultry. Science 81:546–555.

Brosh A. 2007. Heart rate measurements as an index of energy expenditure and energy balance in ruminants: A review. Journal of Animal Science 85, 1213-1227.

Brosh A, Henkin Z, Ungar ED, Dolev A, Orlov A, Yehuda Y, Aharoni Y. 2006. Energy cost of cows' grazing activity: Use of the heart rate method and the global positioning system for direct field estimation. Journal of Animal Science 84,1951-1967.

Brouwer E. 1965. Report of sub-committee on constants and factors. pp 441–443 in Energy Metabolism: Proc. 3rd Symp. European Association of Animal Prodroduction. Publ. No. 11, K. L. Blaxter, ed. Academic Press, London, UK.

Burrin DG, Britton RA, Ferrell CL, Bauer ML. 1992. Level of nutrition and visceral organ protein synthetic capacity and nucleic acid content in sheep. Journal of Animal Science 70, 1137–1145.

Burrin DG, Ferrell CL, Eisemann JH, Britton RA, Nienaber JA. 1989. Effect of level of nutrition on splanchnic blood flow and oxygen consumption in sheep. British Journal of Nutrition 62, 23-34.

Carlson DB, Reed JJ, Borowicz PP, Taylor JB, Reynolds LP, Neville TL, Redmer DA, Vonnahme KA, Caton JS. 2009. Effects of dietary selenium supply and timing of nutrient restriction during gestation on maternal growth and body composition of pregnant adolescent ewes. Journal of Animal Science 87, 669–680.

Carriquiry M, Espasandin AC, Astessiano AL, Casal A, Claramunt M, DoCarmo M, Genro C, Gutiérrez V, Laporta J, Lopez-Mazz C, Meikle A, Olmos F, Perez-Clariget R, Scarlato S, Trujillo AI, Viñoles C, Soca P. 2012. La cría vacuna sobre campo nativo: Un enfoque de investigación jerárquico para mejorar su productividad y sostenibilidad. Veterinaria (Montevideo) 48, 41 - 48

Carstens GE, Johnson DE, Johnson KA, Hotovy SK, Szymanski TJ. 1989. Genetic variation in energy expenditures of monozygous twin beef cattle at 9 and 20 months of age. In Energy metabolism of farm animals: Proceedings 11th Symposium. Lutenen, The Netherlands. EAPP Publication No. 43.

Chapman DF, Parsonsb AJ, Cosgroveb GP, Barkerc DJ, Marottiad DM, Venningae KJ, Rutterf SM, Hilla J, Thompson AN. 2007. Impacts of spatial patterns in pasture on animal grazing behavior, intake, and performance. Crop Science 47, 399–415

Connor EE, Li, RW, Baldwin RL, Li C. 2010. Gene expression in the digestive tissues of ruminants and their relationships with feeding and digestive processes. Animal, 4:7, pp 993–1007.

Connor EE, Kahl S, Elsasser TH, Parker JS, Li RW, van Tassell CP, Baldwin RL, Barao, SM. 2009. Enhanced mitochondrial complex gene function and reduced liver size may mediate improved feed efficiency of beef cattle during compensatory growth. Functional and integrative genomics 10, 39–51.

DiCostanzo A, Meiske JC, Plegge SD. 1991. Characterization of energetically efficient and inefficient beef cows. Journal of Animal Science 69, 1337-48

DiCostanzo A, Meiske JC, Plegge SD, Peters TM, Goodrich RD. 1990. Withinherd variation in energy utilization for maintenance and gain in beef cows. Journal of Animal Science 68, 2156-65

DIEA (Direccion de Estadísticas Agropecuarias – Ministerio de Ganadería Agricultura y Pesca). 2013. Anuario Estadístico Agropecuario 2013 [En línea]. 12 febrero 2014. http://www,mgap,gub,uy/Diea/default,htm

DIEA (Direccion de Estadísticas Agropecuarias – Ministerio de Ganadería Agricultura y Pesca). 2010. Anuario Estadístico Agropecuario 2010 [En línea]. 12 febrero 2014. http://www,mgap,gub,uy/Diea/default,htm

Degan AA, Young BA.1980. Live-weight, total body-water and maternal body-solid changes in pregnant and lactating beef cows. The Journal of Agricultural Science 95, 1-5.

Drouillard JS, Klopfenstein TJ, Britton RA, Bauer ML, Gramlich SM, Wester TJ, Ferrell CL. 1991. Growth, body composition, and visceral organ mass and metabolism in lambs during and after metabolizable protein or net energy restrictions. J. Anim. Sci.69, 3357–3375.

Duarte FO, Sene-Fiorese M, Cheik NC, Maria AS, de Aquino AE Jr, Oishi JC, Rossi EA, Garcia de Oliveira Duarte AC, Dâmaso AR. 2012. Food restriction and refeeding induces changes in lipid pathways and fat deposition in the adipose and hepatic tissues in rats with diet-induced obesity. Experimental physiology 97, 882-94.

Espasandin AC, Do Carmo M, López-Mazz CR, Carriquiry M, Soca P. 2012. Heterosis of productivity rates in the breeding cycle of pure and crossbred Hereford and Angus cattle grazing native pastures at low and high allowances. Journal of Animal Science 90 (Suppl. 3), 531.

Espasandin AC, Ciria M, Franco JB, Pereyra F, Gimeno D. 2010 Heterosis and productive and reproductive performance in Angus, Hereford and F1 reciprocal crossed cows on grazing systems of Uruguay Internacional , XXVI World Buiatrics Congress 2010 , Santiago de Chile , 2010.

Espasandin AC, Franco J, Oliveira G, Bentancour O, Gimeno D, Pereyra F, Rosgberg M. 2006. Impacto productivo y económico del uso del cruzamiento entre las razas Hereford y Angus en el Uruguay. XXXIV Jornadas Uruguayas de Buiatría, Paysandú, Uruguay. p.41-51.

Ferrell CL, Koong LJ, Nienaber JA. 1986. Effect of previous nutrition on body composition and maintenance energy costs of growing labs British Journal of Nutrition. 56, 595-605.

Ferrell CL, Jenkins TG. 1985. Cow type and the nutritional environment: nutritional aspects. Journal of Animal Science 61, 725–41.

Ferrell CL, Jenkins TG. 1984. Energy utilization by mature, nonpregnant, nonlactating cows of different types. Journal of Animal Science 58, 234-43.

Ferrell CL, Garrett WN, Hinman, N. 1976 Growth, development, and composition of the udder and gravid uterus of beef heifers during pregnancy. Journal of Animal Science 42, 1477–89.

Foot JZ. 1969. Body water in ovine pregnancy. Journal of Reproduction and Fertility 9, Suppl. 9-16.

Freetly HC, Nienaber JA, Brown-Brandl T. 2008. Partitioning of energy in pregnant beef cows during nutritionally induced body weight fluctuation. Journal of Animal Science 8, 370-77.

Freetly HC, Ferrell CL, Jenkins TG. 2005. Nutritionally altering weight gain patterns of pregnant heifers and young cows changes the time that feed resources are offered without any differences in production. Journal of Animal Science 83, 916-26.

Freetly HC, Ferrell CL. 1995. Plane of nutrition effects on net flux of oxygen and glucose across splanchnic tissues of ewes. In: C. Wenk and M. Boessinger (Ed.) Proc. 12th Symp. Energy Metabolism of Farm Animals. European Association of Animal Production Publ. 58:72.

Funston RN, Martin JL, Adams DC, Larson DM. 2010. Winter grazing system and supplementation of beef cows during late gestation influence heifer progeny. Journal of Animal Science 88, 4094-101.

Gaines JA, Richardson GV, McClure WH, Vogt DW, Carter, RC. 1967 Heterosis from crosses among British breeds of beef cattle: carcass characteristics. Journal of Animal Science 26, 1217-25.

Gill M, France J, Summers M, McBride BW, Milligan LP. 1989. Simulation of the energy costs associated with protein turnover and Na_, K_-transport in growing lambs. The Journal of nutrition 119, 1287-1299.

Goodrich RD, Byers FM, Schelling GT. 1985. Efficiency of tissue storage and retrieval in mature beef cows. Energy Metabolism of Farm Animals. European Association of Animal Production 32:358.

Gutiérrez V, Espasandin AC, Astessiano AL, Casal A, López-Mazz C, Carriquiry M. 2013. Calf foetal and early life nutrition on grazing conditions: metabolic and endocrine profiles and body composition during the growing phase. Journal of Animal Physiology and Animal Nutrition 97, 720-31.

Hammond AC, Waldo DR, Rumsey TS. 1990. Prediction of body composition in Holstein steers using urea space. Journal of Dairy Science 73, 3141–45.

Hammond AC, Rumsey TS, Haaland GL. 1988. Prediction of empty body components in steers by urea dilution. Journal of Animal Science 66, 354–60.

Hansard SL, Butler W O, Comar CL. 1953. Blood volume of farm animals. Journal of Animal Science. 12, 402-13.

Haydock K, Shaw N. 1975. The comparative yield method for estimating dry matter yield of pasture. Australian Journal of ExperimentalAgriculture and Animal Husbandry 15, 663–70.

Herd RM, Arthur PF. 2009. Physiological basis for residual feed intake. Journal of Animal Science 87, E64–E71.

Herd RM, Oddy VH, Richardson EC. 2004. Biological basis for variation in residual feed intake in beef cattle. 1. Review of potential mechanisms. Animal Production Science, 44, 423-430.

Hersom MJ, Krehbiel CR, Horn GW. 2004. Effect of live weight gain of steers during winter grazing: II. Visceral organ mass, cellularity, and oxygen consumption. Journal of Animal Science 82, 184–197.

Holmes CW, McLean NA, Lockyer KJ. 1978. Changes in the rate of heat production of calves during grazing and eating New Zealand Journal of Agricultural Research, 21, 107-112.

Houghton PL, Lemenager RP, Horstman LA, Hendrix KS, Moss G. 1990a. Effects of body composition, pre- and postpartum energy level and early weaning on reproductive performance of beef cows and preweaning calf gain. Journal of Animal Science 68, 1438-46.

Houghton PL, Lemenager RP, Hendrix KS, Moss GE, Stewart TS. 1990b. Effects of body composition, pre- and postpartum energy intake and stage of production of energy utilization by beef cows. Journal of Animal Science 68, 1447-56.

IPA (Instituto Plan Agropecuario) 2013. Programa de Monitoreo de empresas ganaderas del Plan Agropecuario 2013 [En línea]. 16 febrero 2014.

http://www.planagropecuario.org.uy/uploads/monitoreos/21_Resumen%20del%20ejercicio%202012-2013.pdf

Jenkins TG, Ferrell CL. 1994. Productivity through weaning of nine breeds of cattle under varying feed availabilities: 1: Initial evaluation. Journal of Animal Science 72, 2787-97.

Jenkins TG, Cundiff LV, Ferrell CL. 1991. Differences among breed crosses of cattle in the conversion of food energy to calf weight during the preweaning interval. Journal of Animal Science 69, 2762–2769.

Jenkins TG, Ferrell CL, Cundiff LV. 1986. Relationship of components of the body among mature cows as related to size, lactation potential and possible effects on productivity. Animal Production 43, 245-254

Johnson DE, Ferrell CL, Jenkins TG. 2003. The history of energetic efficiency research: Where have we been and where are we going? Journal of Animal Science 81(E. Suppl. 1):E27–E38.

Klosterman EW, Sanford LG, Parker CF. 1968. Effect of cow size and condition and ration protein content upon maintenance requirements of mature beef cows. Journal of Animal Science 27, 242-246.

Koch RM, Dickerson GE, Cundiff LV, Gregory KE. 1985. Heterosis retained in advanced generations of crosses among Angus and Hereford cattle. Journal of Animal Science 60, 1117-1132.

Koch RM, Swiger LA, Chambers D, Gregory KE. 1963. Efficiency of feed use in beef cattle. Journal of Animal Science 22, 486–494.

Kock SW, Preston RL. 1979. Estimation of bovine carcass composition by the urea dilution technique. Journal of Animal Science 48:319-327.

Kolath WH, Kerley MS, Golden JW, Keisler DH. 2006. The relationship between mitochondrial function and residual feed intake in Angus steers. Journal of Animal Science 84, 861-865.

Kozloski GV, Rocha JBT, Ciocca MLS. 2001. Visceral metabolism and efficiency of energy use by ruminants. Ciencia Rural. 31, 903–908.

Ksiazek A, Konarzewski M, Lapo IB. 2004. Anatomic and energetic correlates of divergent selection for basal metabolic rate in laboratory mice. Physiological and Biochemical Zoology 77, 890- 899.

Laporta J, Rosa GJ, Naya H, Carriquiry M. 2014. Liver functional genomics in beef cows on grazing systems: novel genes and pathways revealed. Physiological genomics. 15; 46(4):138-47.

Laurenz JC, Byers FM, Schelling GT, Greene LW. 1992. Periodic changes in body composition and in priorities for tissue storage and retrieval in mature beef cows Journal of Animal Science 70, 1950-56.

Lim K, Murakami E, Lee S, Shimomura Y, Suzuki M. 1996. Effects of intermittent food restriction and refeeding on energy efficiency and body fat deposition in sedentary and exercised rats. Journal of nutritional science and vitaminology 42, 449-68.

Lobley GE, Milne V, Lovie JM, Reeds PJ, Pennie K. 1980. Whole body and tissue protein synthesis in cattle. British Journal of Nutrition, 43(03), 491-502.

Long CR. 1980. Crossbreeding for beef production: Experimental results. Journal of Animal Science 51, 1197-223.

Long CR, Gregory KE. 1975. Heterosis and management effects in carcass characters of Angus, Hereford and reciprocal cross cattle. Journal of animal science 41, 1572-80.

Macoon, B.L., Sollenberger, E., Moore, J.E., Staples, C.R., Fike, J.H. and Portier, M., 2003. Comparison of three techniques for estimating the forage intake of lactating dairy cows on pasture. J. Anim. Sci. 81: 2357-2366.

Marshall DM. 1994. Breed differences and genetic parameters for body composition traits in beef cattle. Journal of Animal Science 72, 2745-55.

McLeod KR, Baldwin RL, Solomon MB, Baumann RG. 2007. Influence of ruminal and postruminal parbohydrate infusion on visceral organ mass and adipose tissue accretion in growing beef steers. Journal of Animal Science 85, 2256–70.

McLeod KR, Baldwin RL. 2000. Effects of diet forage: concentrate ratio and metabolizable energy intake on visceral organ growth and in vitro oxidative capacity of gut tissues in sheep. Journal of Animal Science 78, 760–770.

Meyer AM, Reed JJ, Neville TL, Taylor JB, Reynolds LP, Redmer DA, Vonnahme KA, Caton JS. 2012. Effects of nutritional plane and selenium supply during gestation on visceral organ mass and indices of intestinal growth and vascularity in primiparous ewes at parturition and during early lactation. Journal of Animal Science 90, 2733–49.

Milligan LP, McBride BW. 1985. Energy Costs of Ion Pumping by Animal Tissues. The Journal of nutrition 115, 1374-82.

Montaño-Bermudez M, Nielsen MK, Deutscher GH. 1990. Energy requirements for maintenance of crossbred beef cattle with different genetic potential for milk. Journal of Animal Science 68, 2279-88.

Moore SS, Mujibi FD, Sherman EL. 2009. Molecular basis for residual feed intake in beef cattle. Journal of Animal Science, 87, E41-E47.

Morris CA, Baker RL, Johnson DL, Carter AH, Hunter JC. 1987. Reciprocal rossbreeding of Angus and Hereford cattle. 3. Cow weight, reproduction, maternal performance, and lifetime production. New Zealand Journal of Agricultural Research 30, 453–467.

Mott GO. 1960. Grazing pressure and the measurement of pasture production. In: Proceedings 8th International Grassland Congress. Reading, UK. pp 606-611.

Moulton CR. 1923. Age and chemical development in mammals. Journal of Biological Chemistry 57, 79-97

Nicol GB. 1979. Influence of pre and post-calving pasture allowance on hill country beef cow and calf performance. New Zealand Journal of Agricultural Research 22, 417-424.

Nielsen MK. 1995. Genetic variation in feed energy intake: Evidence and questions. Pages 89- 95 in Position Papers for Workshop on beef cattle energetics. Nov 1-3, 1995. Denver Colorado.

Nozière P, Attaix D, Bocquier F, Doreau M. 1999. Effects of underfeeding and refeeding on weight and cellularity of splanchnic organs in ewes. Journal of Animal Science 77, 2279–2290.

NRC. (National Research Council) 2000 Nutrient requirements of dairy cattle. 7th rev. ed. National Academy Press, Washington, D.C.

NRC. (National Research Council) 1996. Nutrient Requirements of Beef Cattle. 6th rev National Academy Press, Washington, D.C.

Ojano-Dirain CP, Pumford NR, Toyomizu M, Bottje WG. 2007. Association of Mitochondrial Function and Feed Efficiency. J. Poult. Sci. 44, 221–237.

Ortigues I, Doreau M. 1995. Responses of the splanchnic tissues of ruminants to changes in intake: absorption of digestion end products, tissue mass, metabolic activity and implications to whole animal energy metabolism. Annales De Zootechnie 44, 321–346.

Panaretto BA, Till AR. 1963. Body composition in vivo. II. The composition of mature goats and its relationship to the antipyrine, tritiated water, and N-acetyl-4-aminoantipyrine spaces. Crop and Pasture Science 14, 926-43.

Pfaffl MW. 2004. Quantification strategies in real-time PCR. In (Ed. S.A. Bustin) A-Z of Quantitative PCR. International University Line (IUL) La Jolla, CA, USA.pp 89–113.

Prins HT, van Langevelde. 2008. Resource ecology, spatial and temporal dynamics of foraging. (Eds) Prins HT, van Langevelde F. Springer. Dordrecht. NL. p 304.

Quintans G, Banchero G, Carriquiry M, López-Mazz C, Baldi F. 2010. Effect of body condition and suckling restriction with and without presence of the calf on cow and calf performance. Animal Production Science 50, 931–38.

Reid JT, Wellington GH, Dunn HO 1955. Some relationships among the major chemical components of the bovine body and their application to nutritional investigations. Journal of Dairy Science, 38, 1344-59.

Reynolds CK, Lapierre H, Tyrrell HF, Elsasser TH, Staples RC, Gaudreau P, Brazeau P. 1992. Effects of growth hormone-releasing factor and feed intake on energy metabolism in growing beef steers: Net nutrient metabolism by portaldrained viscera and liver. Journal of Animal Science 70, 752.

Ritchie HD. 2000. Why is efficiency so important to the beef industry? Feedstuffs February 21: 12–27

Richardson EC, Herd RM. 2004. Biological basis for variation in residual feed intake in beef cattle. 2. Synthesis of results following divergent selection. Animal Production Science, 44, 431-440.

Richardson EC, Herd RM, Oddy VH, Thompson JM, Archer JA, Arthur PF. 2001. Body composition and implications for heat production of Angus steer progeny of parents selected for and against residual feed intake. Animal Production Science, 41, 1065-1072.

Robinson JJ. 1986. Changes in body composition during pregnancy and lactation. Proceedings of the Nutrition Society 45, 71-80.

Rohweder DW. 1994. Estimating forage hay quality. In: Proceedings National Alfalfa Hay Quality Testing Workshop. National Hay Quality Committee, Chicago, IL. pp 31-37.

Rolfe DFS, Brand MD. 1997. The Physiological Significance of Mitochondrial Proton Leak in Animal Cells and Tissues. Bioscience reports 17, 9–16.

Rosengurtt B, Gallinal JP, Bergalli L, Aragone L, Campal E. 1939. Estudios sobre praderas naturales del Uruguay.La variabilidad de la composición de las praderas. Revista de la Asociación de Ingenieros Agrónomos, XI, 28-33.

Rule DC, Arnold RN, Hentges EJ, Beitz DC. 1986. Evaluation of urea dilution as a technique for estimating body composition of beef steers in vivo: Validation of

published equation and comparison with chemical composition. Journal of Animal Science 63, 1935-1948

Sainz RD, Bentley BE. 1997. Visceral Organ Mass and Cellularity in Growth-Restricted and Refed Beef Steers. Journal of Animal Science 75, 1229–1236.

Sandelin BA, Brown Jr AH, Brown MA, Ojano-Dirain C, Baublits RT. 2005. Association of mitochondrial function with feed efficiency in Angus cattle. Arkansas Animal Science Department Report, 535, 118-121.

Scarlato S, Faber A, Do Carmo M, Soca P. 2011. Foraging behaviour of beef cows grazing native pasture: I. Effect of breed and herbage allowance on grazing and ruminating time. In Proceedings of the 9th International Rangeland Congress. Eds. Feldman SR, Oliva GE, Sacido MB. IX International Rangeland Congress. Rosario, Argentina. p 657.

Scheaffer AN, Caton JS, Redmer DA, Arnold DR, Reynolds LP. 2004. Effect of dietary restriction, pregn. ancy, and fetal type on intestinal cellularity and vascularity in Columbia and Romanov ewes. Journal of Animal Science 82, 3024–3033.

Seal CJ, Reynolds CK. 1993. Nutritional implications of gastrointestinal and liver metabolism in ruminants. Nutrition research reviews 6, 185–208.

Sinclair KD. 2010. Declining fertility, insulin resistance and fatty acid metabolism in dairy cows: developmental consequences for the oocyte and pre-implantation embryo. Acta Scientiae Veterinariae, 38(Supl 2), s545-s557.

Smit HJ, Taweel HZ, Tas BM, Tamminga S, Elgersma A. 2005. Comparison of techniques for estimating herbage intake of grazing dairy cows. Journal of Dairy Science 88, 1827-36.

Smith NE, Baldwin RL. 1974. Effects of breed pregnancy, and lactation, on weight of organs and tissues in dairy cattle Journal of Dairy Science 57, 1055–1060.

Soares AB, Carvalho PCF, Nabinger C, Frizzo A, Pinto CE, Junior JAF, Semmelmann C, da Trindade J. 2003. Effect of changing herbage allowance on primary and secondary production of natural pasture. Proceedings of the 7th International Rangeland Congress, 26 July - 1 August 2003, p 966-968.

Soca P, Carriquiry M, Keisler DH, Claramunt M, Do Carmo M, Olivera-Muzante J, Rodríguez M, Meikle A. 2013a. Reproductive and productive response to suckling

restriction and dietary flushing in primiparous grazing beef cows. Animal Production Science 53, 283–91.

Soca P, Carriquiry M, Do Carmo M, Scarlato S, Astessiano AL, Genro C, Claramunt M, Espasandín AC. 2013b. Oferta de forraje del campo natural y resultado productivo de los sistemas de cría vacuna del Uruguay: I producción, uso y conversión del forraje aportado por campo natural. In: Seminario de actualización técnica: cría vacuna, Montevideo. Montevideo: INIA, 2013. p. 97-118. (INIA. Serie técnica, 208). Eds: Graciela Quintans, Antonia Scarsi.

Sollenberger LE, Moore JE, Allen VG, Pedreira CGS. 2005. Reporting Forage Allowance in Grazing Experiments. Crop Science. 45, 896-900.

Sunnucks P, Hales DF. 1996. Numerous transposed sequences of mitochondrial cytochrome oxidase I-II in aphids of the genus Sitobion (Hemiptera: Aphididae). Molecular Biology and Evolution 13, 510–524.

Susenbeth A, Mayer R, Koehler B. 1998. Energy requirement for eating in cattle. Journal of Animal Science 76, 2701-2705,

Tesseraud S, Métayer S, Duchêne S, Bigot K, Grizard J, Dupont J. 2007. Regulation of protein metabolism by insulin: value of different approaches and animal models. Domestic animal endocrinology 33, 123-42.

Thompson WR, Meiske JC, Goodrich RD, Rust JR, Byers FM. 1983. Influence of body composition on energy requirements of beef cows during winter. Journal of Animal Science 56, 1241-52.

van der Drift SG, van Hulzen KJ, Teweldemedhn TG, Jorritsma R, Nielen M, Heuven HC. 2012. Genetic and nongenetic variation in plasma and milk β-hydroxybutyrate and milk acetone concentrations of early-lactation dairy cows. Journal of Dairy Science 95, 6781-87

Vizcarra JA, Ibañez W, Orcasberro R. 1986. Repetibilidad y reproductibilidad de dos escalas para estimar la condición corporal de vacas Hereford. Investigaciones. Agronomicas 7, 45-47.

Wang YJ, Wood KM, Martin L, Holligan S, Kelly N, McBride BW, Fan MZ, Swanson KC. 2009. Effect of dietary corn silage inclusion on visceral organ mass, cellularity, and the protein expression of ATP synthase, Na+/K+ -ATPase, proliferating cell nuclear

antigen and ubiquitin in feedlot steers. Canadian Journal of Animal Science. 89, 503–512.

Webster AJF. 1980. The energetic efficiency of growth. Livestock Production Science 7, 243-252.

Webster AJF, Osuji PO, White F, Ingram JF. 1975. The infuence of food intake on portal blood flow and heat production in the digestive tract of sheep. British Journal of Nutrition. 34, 125-39.

Wells RS, Preston RL. 1998. Effect of repeated urea dilution measurement on feedlot performance and consistency of estimated body composition in steers of different breed types. Journal of Animal Science 76, 2799– 804.

6. ANEXOS

ANEXO 1. *Composición corporal y técnica de dilución de la urea.*

La medición de la composición corporal real se basa en el análisis químico directo de los órganos y tejidos del cuerpo del animal, estos son muy exactos pero no se pueden aplicar en el animal vivo. Por ello se desarrollaron distintos métodos para su estimación indirecta como los métodos de dilución, de ultrasonografía, y mediciones corporales. El método de dilución se basa en dos conceptos fundamentales la composición química del cuerpo del animal está en relación a su contenido de agua (Moulton, 1923) y el agua del cuerpo se puede medir utilizando un marcador químico (óxido de deuterio, agua tritiada o urea entre otros). Es así que, la estimación del contenido de agua corporal puede ser utilizado para predecir la grasa y las proteínas corporales y por consiguiente, la composición corporal (Panaretto y Till, 1963). En particular, la técnica de dilución de la urea (**TDU**; Kock y Preston, 1979) implica la infusión en la sangre de los animales a estudiar, una cantidad conocida de urea, determinando in vivo el espacio urea (**EUL**) definido como el volumen (litros) de líquido necesario para que la solución total de urea se encuentre en el cuerpo a una concentración igual que la determinada en sangre. Partiendo del supuesto que el EUL está relacionado con la proporción de agua en cuerpo vacío, y que el agua en el cuerpo se espera que muestre una fuerte relación positiva con el tejido magro y una relación inversa con la de la grasa corporal, las mediciones del EUL pueden utilizarse como predictores para la estimación de la composición corporal en el ganado. Otra forma de expresión es el espacio urea (**EU**) como porcentaje del PV del animal.

La predicción de la composición corporal mediante esta técnica ha demostrado buenas correlaciones respecto con la composición química real del animal en animales en crecimiento (Hammond et al., 1988; Rule et al., 1986) en vacas de cría (Kock y Preston, 1979) y en vacas de leche (Agnew et al., 2005) especialmente si el EU se combina con datos de PV, condición corporal y otras variables productivas.

ANEXO 2: **Table S1.** Primers used for real time RT-PCR quantification.

Gene[1]	Accession#[2]		Primer sequence[3]	Length(bp)	Amplificacion effficiency
ACTB	BT030480	F	CGTGGC TACAGCTTCA CC	53	2.00
		R	GAA ATCGTCCGTGACATCAA		
ATP5B	NM_175796	F	CCCTCAAGGAGACCATCAAA	112	2.00
		R	GCTTTTGCCACAGCTTCTTC		
ATP50	NM_174244.1	F	CCTCTCACGTCCAACCTGAT	131	2.00
		R	GCAGTGGTAACTGTGCATGG		
COX5B	NM_001034046.2	F	CTGGGCTAGAGAGGGAGGTC	120	1.98
		R	TGGTGATGGAGGGGACTAAA		
CYC1	NM_001038090.2	F	ATGAAGCGGCATAAGTGGTC	85	2.00
		R	AGCAAACAGACACCGGGTAG		
HPRT	XM_580802	F	TGGAGAAGGTGTTTATTCCTCATG	105	1.98
		R	CACAGAGGGCCACAATGTGA		
NDUF8	NM_175826.4	F	CTATCCGAAACTGCCTGACC	250	2.00
		R	AAGCCGAAGAGGTGCTTACA		
NDUFS4	NM_175800.2	F	CGCAATAACATGCAGTCTGG	124	1.89
		R	CCAGGTTGGATAAGGGATCA		
RSP9	NM_001101152.2	F	CCT CGA CCA AGA GCT GAA G	63	1.87
		R	CCT CCA GAC CTC ACG TTT GTT C		
SDHA	NM_174178.2	F	ACATGCAGAAGTCGATGCAG	155	2.00
		R	GGTCTCCACCAGGTCAGTGT		
SDHD	NM_174179.2	F	TTTGGCTAGGATGGATGGAG	92	2.00
		R	ACTGAACAGAGGGGGAGGTT		
UQCRC1	NM_174629.2	F	CAGTCTTCCCAGCCTACCTG	105	1.87
		R	AGCCAGATGCTCCACAAAGT		

[1]*ACTB* = ß-actin; *ATP5B = ATP synthase subunit beta; ATP5O = ATP synthase subunit O; COX5B = cytochrome c oxidase subunit Vb; CYC1 = cytochrome c-1; HPRT1= hypoxanthine phosphoribosyl transferase; NDUFB8 = NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 8; NDUFS4 = NADH dehydrogenase (ubiquinone) Fe-S protein 4; RSP9 = ribosomal protein S9; SDHA = succinate dehydrogenase complex, subunit A, flavoprotein (Fp); SDHD = succinate dehydrogenase complex, subunit D, integral membrane protein; UQCRC1 = ubiquinol-cytochrome c reductase core protein I.* [2]Gene bank sequences. [3]F = foreword; R = reverse.