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MECANISMOS CELULARES Y MOLECULARES RELACIONADOS A LA EFICIENCIA DE UTILIZACIÓN DE LA ENERGÍA EN BOVINOS

por

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RESUMEN

El objetivo del trabajo fue incrementar el conocimiento de componentes de los mecanismos de partición de la energía (la masa y composición de los órganos, así como de la densidad, función mitocondrial y marcadores de estrés oxidativo hepáticos) y su relación con la eficiencia productiva, en dos modelos bovinos. Modelo 1: Novillos con fenotipos divergentes para consumo residual de alimento (**RFI**) y Modelo 2: Vacas de cría multíparas puras y cruzas F1 (**CR**) pastoreando distintas ofertas de forraje de campo natural. Los novillos de alta eficiencia (bajo RFI) tuvieron una mayor eficiencia en el metabolismo de los nutrientes hepáticos, que se asoció fuertemente con una mayor densidad y funcionamiento mitocondrial hepática (principalmente del complejo II), mayor capacidad antioxidante y, por lo tanto menor estrés oxidativo. La disminución del estrés oxidativo hepático reduciría los requisitos de mantenimiento debido a un menor recambio de proteínas y lípidos y una mayor eficiencia en el uso de la energía. En vacas de cría, los animales pastoreando alta oferta de forraje (**HI**) y CR serían más eficientes en el uso de la energía, mayor rendimiento carnicero y menor relación entre el peso total de órganos y peso de la canal. Además, las vacas HI tuvieron mayor densidad mitocondrial hepática. Sin embargo, las vacas CR presentaron un aumento en los marcadores de daño oxidativo a nivel hepático, lo que podría deberse a una mayor actividad metabólica de este órgano. En los novillos con diferentes RFI la eficiencia energética se ha asociado con mejor función mitocondrial y menor estrés oxidativo hepático sin embargo, esta asociación no es tan clara en el sistema vaca-ternero. Otros factores, como el tamaño de las vísceras, y su actividad metabólica, el consumo de materia seca, masa y composición corporal presentarían mayor asociación con los mecanismos relacionados a demandas de energía de mantenimiento y a la eficiencia energética del sistema vaca-ternero.

Palabras Clave: bioenergética, estrés oxidativo, mitocondrias, RFI

CELL AND MOLECULAR MECHANISMS RELATED TO THE EFFICIENCY OF ENERGY USE IN BOVINOS

SUMMARY

The objective of the work was to increase the knowledge of components of the energy partition mechanisms (the mass and composition of the organs, as well as the density, mitochondrial function and markers of hepatic oxidative stress) and their relationship with productive efficiency, in two bovine models. Model 1: Steers with divergent phenotypes for residual food consumption (**RFI**) and Model 2: Pure and F1 crosses (**CR**) multiparous cows grazing different herbage allowances of native pastures. The steers of high efficiency (low RFI) had a greater efficiency in the metabolism of the hepatic nutrients, which was strongly associated with a greater density and mitochondrial liver function (mainly of complex II), greater antioxidant capacity and, therefore, lower oxidative stress. The reduction of hepatic oxidative stress would reduce the maintenance requirements due to a lower protein and lipid turnover and a greater efficiency in the use of energy. In cows, the animals grazing high herbage allowances (**HI**) and CR would be more efficient in the use of energy, greater performance and lower ratio between the total weight of organs and carcass weight. In addition, HI cows had greater hepatic mitochondrial density. However, CR cows showed an increase in markers of oxidative damage at the liver level, which could be due to a greater metabolic activity of this organ. In steers with different RFI, energy efficiency has been associated with better mitochondrial function and lower hepatic oxidative stress, however, this association is not as clear in the cow-calf system. Other factors, such as the visceral mass and their metabolic activity, the dry matter intake, body mass and composition would be more associated with the mechanisms related to maintenance energy demands and the energy efficiency of the cow-calf system.

Keywords: bioenergetics, mitochondria, oxidative stress, RFI

1. <u>INTRODUCCIÓN</u>

1.1. PLANTEO DEL PROBLEMA

La producción de carne basada en ecosistemas pastoriles resulta en importantes aportes a la economía nacional (primer rubro en importancia en las exportaciones, totalizando 1767 millones de dólares en venta de carne y animales en pie ejercicio 2016-2017; DIEA, 2018). La cría de bovinos de carne (primer eslabón de la cadena cárnica) en Uruguay involucra 7,6 millones de cabezas y 7,7 millones de hectáreas, que significan el 51% de las hectáreas de pastoreo con bovinos de carne y ovinos y el 54% de los productores agropecuarios. Por otra parte, la recría y engorde involucran 4,3 millones de cabezas y 6,7 millones de hectáreas, que representan el 41% de las hectáreas de pastoreo con bovinos de carne y ovinos y el 27% de los productores (DIEA, 2018).

El sistema ganadero agro-exportador continúa siendo competitivo pero, los altos niveles de transferencias hacia otros sectores de la economía (agricultura, forestación) y el peso de algunas ineficiencias relativas a los resultados obtenidos, particularmente en el sistema criador (65% destete, 75 kg carne/ha; DIEA, 2018; IPA 2018), comprometen la sostenibilidad de los productores y sus empresas, limitan la expansión exportadora del complejo cárnico uruguayo y muestran que no debe darse por sentado que este nivel de competitividad es infinito (Lanfranco *et al.*, 2012). El incremento en la producción por unidad de superficie, con reducción o mantenimiento de los costos unitarios de producción, permite una mejora en la eficiencia de producción y constituye una estrategia central para mantener la competitividad de la producción de bovinos de carne.

Los costos asociados con la alimentación representan alrededor 65% de los costos totales de la producción de carne en Uruguay (IPA, 2018) y Estados Unidos (Anderson *et al.*, 2005). Por lo tanto, la mejora de la eficiencia alimenticia (principalmente en el uso de la energía por parte de los animales) constituye un objetivo importante para los productores ganaderos. Se ha demostrado, en estudios de simulación conducidos por Fox *et al.*,

(2004), que el impacto en los beneficios por aumentar 10% en novillos la eficiencia en el uso de la energía es muy superior que el impacto en los beneficios de aumentar 10% la tasa de ganancia (43 vs 18%). Esto podría tener un mayor impacto en el sector criador, ya que, en el ciclo de cría vacuna, más del 70% de los costos energéticos son debidos al mantenimiento de los vientres (Ferrell y Jenkins, 1985).

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 (Ritchie, 2000). Sin embargo, se estima que los costos energéticos para mantenimiento representan ~65 % de los costos totales requeridos por un rodeo, con considerable variación entre los animales e independientemente de su peso vivo (**PV**; Montaño-Bermudez *et al.*, 1990, Parnell *et al.*, 1994). De esta manera, el aumento de la eficiencia en el uso de la energía conllevaría al aumento de la productividad animal con iguales o menores costos de mantenimiento.

El mantenimiento es el estado fisiológico en cual no hay cambio neto en la energía corporal o cuando el balance de energía es cero (Baldwin, 1995). O sea la energía de mantenimiento de un animal es la fracción de la energía consumida necesaria para mantener el equilibrio energético del animal (NRC, 2000) y comprende a la energía destinada a mantener constantes los tejidos corporales y actividades vitales básicas. Según NASEM (2016), la estimación de la energía requerida para el mantenimiento incluye: la energía requerida para el metabolismo basal, la termorregulación y la actividad voluntaria del animal, en confinamiento y en un ambiente no estresante. Para animales en pastoreo se le debe sumar la energía necesaria para el pastoreo, la rumia y la búsqueda del alimento. El metabolismo basal representa entre el 60-80 % del costo energético de mantenimiento dependiendo de la actividad realizada por el animal. Se ha reportado que los mecanismos biológicos que explican la eficiencia en el uso de la energía están muy relacionados con procesos que determinan las necesidades de energía de mantenimiento, fundamentalmente del

metabolismo basal (Richardson y Herd, 2004). Rolfe y Brand (1997) estimaron que ~90% de la producción de energía y ~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. Variaciones en la eficiencia de producción de energía (mitocondria) y/o la utilización de la misma por parte de los tejidos puede contribuir a explicar a las diferencias fenotípicas observadas entre los animales (Herd y Arthur, 2009).

En resumen, la mejora de la eficiencia en el uso de la energía, incluso en un bajo porcentaje, no solo representaría importantes beneficios económicos para el productor sino también tendría beneficios a nivel social y ambiental (Ahola y Hill, 2012). Sin embargo, la investigación en esta temática en ganado de carne es muy reciente y limitada a nivel internacional y prácticamente no existente en nuestro país. Este trabajo de tesis de doctorado busca continuar profundizando sobre la identificación de mecanismos celulares y moleculares relacionados a la eficiencia de utilización de la energía en bovinos. La información generada permitirá una mejor comprensión de los sistemas ganaderos buscando optimizar la eficiencia en el uso de los recursos y contribuir a mejorar la sostenibilidad de los sistemas pastoriles, disminuyendo el impacto ambiental (heces, emisión de gases de efecto invernadero; Steinfeld *et al.*, 2006; Dini *et al.*, 2019).

1.2. PRODUCTIVIDAD Y EFICIENCIA

La productividad de los animales se encuentra asociada a su balance energético, determinado por las diferencias entre el consumo y los requerimientos de energía (mantenimiento y producción). El mejoramiento genético en ganado de carne se ha centrado fundamentalmente en características de crecimiento y habilidad materna que aumentan los "outputs" o salidas en un sistema de producción, pero que están estrechamente ligadas al uso creciente de alimento. Asimismo, la selección de reproductores por crecimiento acelerado y mayor PV ha generado un

incremento del tamaño adulto promedio de las diferentes razas y de sus requerimientos de mantenimiento, con una menor eficiencia en uso de los recursos alimenticios y por lo tanto mayores costos de producción (Evans, 2001). Esta menor eficiencia también se ve reflejada en los indicadores reproductivos de los rodeos de cría, traducido en el estancado porcentaje de destete, en promedio de ~65% (promedio anual; DIEA, 2018) y también podría aumentar el potencial de los problemas ambientales.

De forma básica se pueden definir a la eficiencia energética como: entrada de energía (consumo) dividida por salida de energía en productos (producción). Tradicionalmente se utilizó la eficiencia de conversión (kg de alimento consumido/kg de ganancia) o la eficiencia de producción (kg de ganancia/kg de alimento consumido) para medir la eficiencia de los animales en crecimiento. Sin embargo, estas medidas están muy influenciadas por el tamaño y ganancia de peso de los animales. Como alternativa, el consumo de alimento (o energía) residual o neto (**RFI** en inglés) se ha utilizado como una característica de eficiencia independiente de tamaño corporal (Koch *et al*., 1963), surgiendo como una importante caracteristica por la cual seleccionar los animales más eficientes.

El RFI es una medida de eficiencia alimenticia que se define como la diferencia entre el consumo observado y el consumo estimado para cada individuo (en base a las exigencias de mantenimiento y crecimiento; Koch *et al*., 1963). Esta medida es utilizada para identificar los animales que se desvían de su consumo esperado clasificándolos como de alta eficiencia alimenticia (RFI negativo) o de baja eficiencia alimenticia (RFI positivo). El RFI se ha convertido en una medida muy aceptada para evaluar la eficiencia animal, sin embargo, la clasificación por RFI tiene una utilidad limitada en la identificación de ganado energéticamente eficiente en pastoreo, debido a nuestra incapacidad para medir con precisión la ingesta de animales en estas condiciones o sistemas de producción.

La eficiencia energética en vacas de cría se define como salidas de energía a la producción de terneros (número de terneros y/o peso (o energía

retenida) de terneros destetados) e insumos relevantes: número de vacas entoradas o consumo de materia seca/energía (Jenkins y Ferrell, 1994; Scholljegerdes y Summers, 2016). No se han reportado diferencias entre entre animales clasificados en alta, media y/o baja RFI para el peso de la cría al nacer o al destete por vaca entorada así como tampoco en las tasas de preñez de vacas o de vaquillonas (Arthur *et al.*, 2005; Donoghue *et al.*, 2010). Además, debido al hecho de que la eficiencia del sistema vaca-ternero probablemente esté influenciada por múltiples factores, es razonable suponer que las métricas múltiples serán más precisas que cualquier marcador individual.

1.3. EFICIENCIA EN EL USO DE LA ENERGÍA, LAS VÍSCERAS Y MITOCONDRIAS

En general, los requerimientos de mantenimiento pueden ser clasificados como funciones de servicio (funcionamiento del corazón, riñón, hígado, sistema nervioso, etc.), y el mantenimiento celular (renovación proteica y lipídica y transporte de iones), representando este último del 40-60% de gasto energético correspondiente al metabolismo basal (Baldwin *et al.*, 1995). Los requerimientos de mantenimiento son estimados por los distintos sistemas de alimentación (NRC, CSIRO) como una constante del peso metabólico ($PV^{0.75}$), sin embargo está reportado que los mismos varían no solo en función de la actividad y conducta de pastoreo (Brosh *et al.*, 2006; Scarlato *et al.*, 2011) sino también debido al consumo de materia seca (Freetly et al., 1995), la masa y composición corporal (Ferrell y Jenkins, 1985; Casal *et al.*, 2017), el tamaño de vísceras del tracto gastrointestinal (TGI) y otros órganos (Baldwin *et al.*, 2004; Casal *et al.*, 2014) y la actividad metabólica de los mismos (Herd y Arthur, 2009).

Si bien el músculo representa ~50% del cuerpo, los órganos vinculados al funcionamiento de los sistemas digestivo, respiratorio, circulatorio y actividades de excreción constituyen una menor proporción del peso del cuerpo (15-25%), no obstante, representan más del 50% de los

costos de mantenimiento (Baldwin *et al.*, 2004; Ferrell, 1988; Seal y Reynolds, 1993). Particularmente, las vísceras del TGI e hígado que representan del ~13% del PV, son responsables de ~40% del total de consumo de oxígeno de los animales (Baldwin *et al.*, 2004). Existe considerable evidencia que indica que la masa total de órganos difiere entre genotipos, entre estados fisiológicos y entre planos nutricionales y que estas diferencias pueden traducirse en variaciones en las necesidades de energía de mantenimiento (Baldwin, 1995; Baldwin *et al.*, 2004; Casal *et al.*, 2014). En particular, el hígado representa ~3% del PV y ~8% del recambio de proteínas corporales diario (Lobley *et al.*, 1980). Además, este órgano puede considerarse como el primer regulador e integrador del estatus metabólico de los animales y tiene una gran influencia en el perfil y aporte de nutrientes absorbidos por el TGI que podrán ser usados en los procesos productivos (síntesis de reservas corporales, tejido fetal o producción de leche). Esto determina que el hígado y los órganos del TGI sean centrales en el metabolismo energético y en su impacto sobre la eficiencia alimenticia.

Herd *et al.* (2004) estimaron que un tercio de la variación biológica en RFI en bovinos de carne en crecimiento, podría ser explicado por diferencias en la digestión, incremento de calor, la composición de la ganancia y la actividad, y postuló que los dos tercios restantes de la variación en RFI podría estar relacionada con diferencias en los gastos energéticos asociados con los procesos biológicos como el recambio proteico, bombeo de iones y metabolismo mitocondrial. Es así que, sobre la base de la importancia de las mitocondrias en la producción de energía celular, ha sido propuesto que las diferencias en la función mitocondrial pueden contribuir a las diferencias observadas entre los animales en su expresión de la eficiencia alimenticia. Elsasser *et al.* (2008) indicaron que una actividad mitocondrial alterada (o disfunción) puede tener impactos críticos sobre el estrés oxidativo, que podría resultar en cambios en el equilibrio metabólico y la eficiencia en el uso de nutrientes. Rolfe y Brand (1997) sugieren que la función mitocondrial puede aportar datos de la variación entre animales en los requerimientos de

energía de mantenimiento. Sin embargo, todavía falta mucho para conocer sobre los mecanismos celulares/moleculares que controlan las diferencias en eficiencia alimenticia.

1.4. EFICIENCIA EN EL USO DE LA ENERGÍA, FUNCIÓN MITOCONDRIAL Y ESTRÉS OXIDATIVO

En las mitocondrias se produce ~90% de la energía celular, son el organelo fundamental en el metabolismo intermediario (catabolismo y anabolismo), por lo tanto están integradas en numerosas funciones de diferentes redes metabólicas y señalización con otros compartimentos celulares. El ciclo del ácido cítrico (o del ácido tricarboxílico) es el eje central del metabolismo energético celular siendo la vía final común para la oxidación de las moléculas energéticas (carbohidratos, lípidos y proteínas) y es fuente de precursores para la biosíntesis de otras moléculas Todos los compuestos energéticos acaban siendo metabolizados hasta acetil-CoA o a algún componente del ciclo del ácido cítrico. Cada vuelta de esta ruta cíclica incluye una serie de reacciones de óxido-reducción que conducen a la oxidación de un grupo acetilo hasta dos moléculas de dióxido de carbono.

La función específica del ciclo del ácido 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 oxígeno 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, la coenzima Q y el citocromo *c*; complejo I (NADH-ubiquinona reductasa), complejo II (succinato-ubiquinona reductasa), complejo III (ubiquinol-citocromo *c* reductasa), complejo IV, (citocromooxidasa) y complejo V (ATP sintasa). Los electrones se transportan desde el complejo I y el II a la coenzima Q. Desde la coenzima Q a través del complejo III (ciclo Q) son transferidos al

citocromo *c* que luego los cede al complejo IV donde se produce la reducción tetravalente del oxígeno a agua. Los complejos que forman parte de la cadena respiratoria se agrupan en estructuras funcionales o supercomplejos formados por la aglomeración de los complejos I, III y IV en la membrana de las mitocondrias en diferentes configuraciones. A través de los complejos I, III y IV ocurre el transporte de electrones, que se acopla al bombeo de protones hacia el espacio intermenbrana, generando un gradiente de protones a través de la membrana mitocondrial interna. Los protones luego fluyen a través de la ATP sintasa hacia la matriz mitocondrial y la disipación de este gradiente de protones genera la energía necesaria para la síntesis de ATP acoplada al transporte de electrones (Berg y Tymoczko, 2008). Estos complejo no son entidades separadas, sino que están formados por subunidades multiproteicas, 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.

Las mitocondrias son reconocidas como fuentes principales de especies reactivas de oxígeno (**ROS**) y especies reactivas de nitrógeno (**RNS**) en la mayoría de los tipos de células. Estas ROS-RNS provocan la oxidación o nitración de biomoléculas que pueden ser parte de un sistema se señalización o formar parte de mecanismos de defensa celular, sin embargo tambien pueden ser agentes que provocan daño celular (Turrens, 2003; Bottje y Carstens, 2009; Valez *et al*., 2012). El estrés oxidativo se produce cuando la producción de ROS-RNS y otros oxidantes exceden la capacidad del sistema biológico (capacidad antioxidante) para desintoxicar los intermedios reactivos o para reparar el daño resultante (Chirase *et al*., 2004; Radi, 2018). Neutralizar el oxidante y/o degradar y reemplazar las biomoléculas dañadas por la oxidación (proteínas oxidadas, lípidos, ADN y carbohidratos) o células que contienen las biomoléculas dañadas es energéticamente costoso, por lo tanto, el estrés oxidativo puede aumentar el gasto de energía disminuyendo la eficiencia energética (Mehlhase y Grune,

2002; Bottje y Carstens, 2009). El estrés oxidativo de las biomoléculas y los organelos celulares no solo eleva el gasto de energía celular sino que también puede afectar negativamente a otros procesos celulares como la alteración de enzimas, proteínas y lípidos de vías metabólicas involucradas en eventos de señalización celular o disfunción de orgánulos (Turrens, 2003; Bottje y Carstens, 2009; Radi, 2018).

Los antioxidantes, sustancias que retrasan o inhiben la oxidación de un sustrato a pesar de sus menores concentraciones en relación con el sustrato oxidable, se integran en un sistema antioxidante multifacético responsable de mitigar la acción del oxidante y prevenir el estrés oxidativo (Gutteridge, 1995). Los mecanismos antioxidantes suelen actuar de forma coordinada, ejercen su función en localizaciones subcelulares concretas, y se agrupan en dos sistemas de defensa antioxidante: sistema enzimático y sistema no enzimático. El sistema antioxidante enzimático está integrado por tres enzimas principales que trabajan en cadena para desactivar selectivamente a los oxidantes: superóxido dismutasa (**SOD**), catalasa, glutatión peroxidasa (**GPX**). A su vez, otras tres enzimas, glutatión reductasa, glutatión-S-transferasa y gama-glutamilcisteinil sintetasa (o glutamato cisteína ligasa), sin ser estrictamente enzimas antioxidantes, colaboran indirectamente con la glutatión peroxidasa ya que contribuyen a regular el pool intracelular de glutatión reducido, uno de los principales antioxidantes celulares no enzimáticos. La SOD convierte el superóxido en peróxido de hidrógeno (**H_2O_2**), y la GPX y peroxirredoxinas (**PRDX**) junto con otras enzimas son responsables de reducir el H_2O_2, el peroxinitrito, los hidroperóxidos de ácidos grasos libres y otros peróxidos a compuestos menos tóxicos (Quijano *et al.*, 2016; Radi, 2018).

El sistema antioxidante no enzimático está integrado por una serie de sustancias que, aun estando presentes a bajas concentraciones, en presencia de compuestos oxidables (ADN, proteínas o lípidos), se oxidan antes que éstos, y retrasan, inhiben, amortiguan o previenen su oxidación. El sistema antioxidante no enzimático incluye una larga serie de compuestos

de bajo peso molecular, siendo los más importantes el glutatión reducido, la vitamina E y la vitamina C. Además, los flavonoides, ácidos fenólicos, ácido α-lipóico, ácido úrico, bilirrubina, algunos azúcares y aminoácidos, coenzima Q o ubiquinona y varios derivados de ésta y la melatonina, también forman parte de los antioxidantes no enzimáticos. El manganeso, selenio, cobre, hierro y otros minerales, al ser parte del sitio activo de las enzimas antioxidantes, juegan un papel importante en la defensa mediada por enzimas, sin ser verdaderos antioxidantes. El glutatión reducido y el grupo de enzimas encargadas del reciclaje del glutatión constituyen el centro del sistema de defensa antioxidante. La reducción del glutatión oxidado se realiza en el citosol y luego es exportado a la mitocondria, la glutamato cisteína ligasa es la enzima limitante en la síntesis de glutatión reducido (Surai *et al.*, 2019; Ran *et al.*, 2004; Kidd, 1997).

En este marco, se define estrés oxidativo como el desbalance entre la formación de oxidantes y el sistema antioxidante, donde una elevada formación de oxidantes y/o menor protección del sistema antioxidante, llevan a un inevitable 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. El glutatión reducido es importante en la protección de la oxidación de las proteínas (Bolaños *et al.*, 1996). La relación entre glutatión oxidado y reducido se ha usado como un indicador de estrés oxidativo (Kidd, 1997). En homogenizados de tejidos de pollos (músculo, intestino, corazón, hígado), los animales de alto RFI presentaron mayor presencia de carbonilos proteicos (modificaciones post-traduccionales ya sea por reacción directa con oxidantes o por reacción con productos de la peroxidación lipídica) en comparación a los de bajo RFI, lo cual apoya las observaciones anteriores que afirman que los animales menos eficientes presentan mayor estrés oxidativo (Iqbal *et al.*, 2004; 2005; Ojano-Dirain *et al.*, 2007). En bovinos,

Sandelin *et al.* (2005) observó que novillos de alto RFI presentaban mayor cantidad de carbonilos proteicos en muestras de músculo.

En resumen, la función mitocondrial y el estrés oxidativo a nivel hepático tiene un impacto significativo en metabolismo energético celular y por lo tanto pueden afectar la eficiencia biológica de producción. Es así que, una mayor comprensión de los factores asociados a la eficiencia de uso de la energía permitirá generar manejos/tecnologías que colaboren a mejorar la eficiencia global (productiva, económica y ambiental) del sistema de producción de carne vacuna.

1.5. HIPÓTESIS Y OBJETIVOS

1.5.1. Hipótesis

Cambios en la masa de órganos y vísceras afectan la partición energética (mantenimiento vs. producción) de los animales determinando diferencias en el comportamiento productivo. A su vez, cambios en el metabolismo energético celular y un aumento del estrés oxidativo a nivel hepático afectan la eficiencia de uso de la energía en vacas de cría o novillos en crecimiento. Por lo tanto, Animales mas eficientes vs. menos eficientes en el uso de la energía, presentarán menor masa de órganos en proporción a la masa corporal, mejor metabolismo energético celular (mayor función mitocondrial y menor estrés oxidativo) a nivel hepático

1.5.2. Objetivo general

Profundizar en algunos mecanismos relacionados con la eficiencia en el uso de la energía en dos modelos bovinos. Modelo 1: Novillos con fenotipos divergentes para RFI y Modelo 2: Vacas de cría multíparas puras [**PU**; Hereford (**H**) y Angus (**A**)] y cruzas F1 [**CR**; H-A y A-H] pastoreando distintas ofertas de forraje de campo natural.

1.5.3. Objetivos específicos:

1- Determinar la densidad y función mitocondrial y marcadores de estrés oxidativo hepáticos en novillos con fenotipos divergentes para RFI.

2- Evaluar el efecto de la oferta de forraje y genotipo de los animales sobre la masa y composición de los órganos, así como sobre la densidad y función mitocondrial y marcadores de estrés oxidativo hepáticos en vacas de cría pastoreando campo natural.

1.6. ESTRUCTURA GENERAL DE LA TESIS

Consiste en cuatro artículos científicos que constituyen la estructura central de la tesis y un capítulo final de discusión general y conclusiones globales.

El artículo titulado *"Hepatic mitochondrial function in Hereford steers with divergent residual feed intake phenotypes",* fue publicado en la revista Journal of Animal Science y constituye el segundo capítulo de esta tesis. Este trabajo tuvo como objetivo determinar la densidad y la función mitocondrial hepática, en términos de respiración, expresión de genes y proteínas y actividad enzimática de las proteínas del complejo respiratorio mitocondrial, en novillos con fenotipos divergentes para consumo residual de alimento. Se demostró que la respiración basal y la frecuencia respiratoria máxima fueron mayores para los novillos de bajo RFI cuando se utilizaron sustratos del complejo II (succinato). Sin embargo, cuando se usaron sustratos del complejo I (glutamato/malato), la capacidad respiratoria máxima tendió a ser mayor para los novillos de bajo RFI frente a los de alto RFI. Los novillos de bajo RFI presentaron mayor densidad de mitocondrias en el tejido hepático que los novillos de alto RFI. La expresión hepática de genes que codifican proteínas de la cadena respiratoria mitocondrial fue mayor en los novillos de bajo RFI que en los de alto RFI. La expresión de la proteína hepática SDHA tendió a ser mayor, mientras que las actividades enzimáticas de succinato deshidrogenasa y de NADH deshidrogenasa fueron mayores para los novillos de bajo RFI. Se concluyó que los novillos

de alta eficiencia (bajo RFI) probablemente tuvieron una mayor eficiencia en el metabolismo de los nutrientes a nivel hepático, lo que estuvo fuertemente asociado a mayor densidad y funcionamiento mitocondrial, principalmente del complejo II.

El artículo, titulado *"Differential hepatic oxidative status in steers with divergent residual feed intake phenotype"* fue publicado en la revista Animal Journal y constituye el tercer capítulo de esta tesis. Este trabajo tuvo como objetivo determinar los marcadores de estrés oxidativo de lípidos y proteínas y la expresión de genes y proteínas, así como la actividad de las enzimas antioxidantes en el hígado de novillos con fenotipos divergentes para consumo residual de alimento. Se demostró que las sustancias reactivas al ácido tiobarbitúrico y los carbonilos proteicos hepáticos fueron mayores y 4-hydroxynonenal aductos proteicos hepático tendieron a ser mayores para los bovinos con alto que con bajo RFI. La expresión génica hepática de algunas enzimas antioxidantes fue mayor y la expresión de la proteína hepática y la actividad enzimática de la superóxido dismutasa de manganeso y la actividad enzimática de la glutatión peroxidasa tendieron a ser mayores para los novillos con bajo que alto RFI. En conclusión, la alta eficiencia de alimentación de los novillos con bajo RFI se relacionó con un mejor equilibrio oxidativo hepático: mayor capacidad antioxidante hepática cerca del sitio de producción de oxidantes y por lo tanto, menor estrés oxidativo hepático, en comparación a los de alto RFI. La disminución del estrés oxidativo hepático reduciría los requisitos de mantenimiento debido a una menor recambio de proteínas y lípidos y una mayor eficiencia en el uso de la energía.

El artículo, titulado *"Masa y composición de la canal y de los órganos en vacas de carne puras y cruza pastoreando diferentes ofertas de forraje de campo natural"*, fue publicado en la revista Veterinaria (Montevideo) y constituye el cuarto capítulo de esta tesis. Este trabajo tuvo como objetivo evaluar el efecto de la oferta de forraje y genotipo de los animales sobre la

masa y composición de la canal y órganos en vacas de cría pastoreando campo natural [alta (HI) vs baja (LO)]. Demostramos que las vacas en HI presentaron mayor peso absoluto de la masa total de órganos que las vacas en LO, mientras que la masa relativa al peso de la canal del total de órganos fue mayor en las vacas PU que CR. Ademas, la masa absoluta y relativa de la canal fue mayor en las vacas en HI que en las vacas en LO, y tendió a ser mayor en vacas PU que CR. Se concluyó que las vacas en vacas en HI y vacas CR presentaron mayor peso de canal y mayor rendimiento carnicero y un menor peso relativo de los órganos respecto al peso de la canal, contribuyendo a explicar las mejoras en la eficiencia en el uso de la energía para producción, dado la menor relación entre masa total de órganos y canal.

El artículo, titulado *"Mechanisms related with energy efficiency on the liver of pure and crossbred beef cows grazing different herbage allowances of grasslands",* se encuentra en formato borrador y será enviado a revisión a la revista Animal Production Science. Este artículo constituye el quinto capítulo de esta tesis y tuvo como objetivo evaluar el efecto de la oferta de forraje [alta (HI) vs baja (LO)] y genotipo de los animales [puras (PU) vs cruzas (CR)] sobre la densidad mitocondrial hepática y función en términos de expresión génica y proteica y actividad enzimática del complejo respiratorio mitocondrial, proteínas antioxidantes, y marcadores de estrés oxidativo en vacas de cría pastoreando campo natural. La densidad de mitocondrias fue mayor en vacas pastoreando HI vs LO, pero no se vieron afectadas por el genotipo de la vaca o su interacción. La expresión génica hepática de las proteínas de la cadena respiratoria mitocondrial no se vio afectada por la oferta de forraje, el genotipo de la vaca o su interacción. A nivel plasmático los niveles de pro-oxidantes fueron mayores y la capacidad antioxidante tendió a ser mayor en vacas CR que en vacas PU, mientras que el índice de estrés oxidativo se vio afectado por la interacción entre oferta de forraje y genotipo de la vaca, siendo mayor para las vacas LO-CR que para

vacas HI-CR, mientras que las vacas HI-PU y LO-PU presentaron valores intermedios. A nivel hepatico, las concentraciones de especies reactivas al ácido tiobarbitúrico y carbonilos proteicos no se vieron afectadas por la oferta de forraje, el genotipo de la vaca o su interacción, pero la expresión de los aductos proteicos del 4-hidroxinonenal tendieron a ser mayores para vacas CR que para vacas PU y a su vez presentó una tendencia a estar afectado por la interacción entre oferta de forraje y genotipo de la vaca siendo mayor en vacas LO-CR que en vacas LO-PU, mientras que no presento diferencias para vacas HI-CR y HI-PU. Las actividades enzimáticas a nivel hepático de superóxido dismutasa y glutatión peroxidasa no se vieron afectadas por la oferta de forraje, el genotipo de la vaca o su interacción. En conclusión, la mayor eficiencia observada, se asoció con una mayor densidad mitocondrial hepática, sin diferencias en la función de las mitocondrias, esto podría estar asociado a una mayor actividad metabólica asociada con una mayor ingesta de energía y productividad para las vacas en HI vs. LO. Sin embargo, al contrario de lo que se esperaba, la mayor eficacia para CR que las vacas PU se asoció con un aumento del daño oxidativo hepático, lo que probablemente reflejaría una mayor actividad metabólica de este órgano en las vacas CR asociada con una mayor ingesta de energía y productividad.

2. HEPATIC MITOCHONDRIAL FUNCTION IN HEREFORD STEERS WITH DIVERGENT RESIDUAL FEED INTAKE PHENOTYPES

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Hepatic mitochondrial function in Hereford steers with divergent residual feed intake phenotypes[1]

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ABSTRACT: Variations in phenotypic expression of feed efficiency could be associated with differences or inefficiencies in mitochondria function due to its impact on energy expenditure. The aim of this study was to determine hepatic mitochondrial density and function in terms of respiration, gene and protein expression, and enzyme activity of mitochondrial respiratory complex proteins, in steers of divergent residual feed intake (**RFI**) phenotypes. Hereford steers ($n = 111$ and $n = 122$ for year 1 and 2, respectively) were evaluated in postweaning 70 d standard test for RFI. Forty-six steers exhibiting the greatest ($n = 9$ and 16 for year 1 and 2; high-RFI) and the lowest ($n = 9$ and 12 for year 1 and 2; low-RFI) RFI values were selected for this study. After the test, steers were managed together until slaughter under grazing conditions until they reached the slaughter body weight. At slaughter, hepatic samples (biopsies) were obtained. Tissue respiration was evaluated using high-resolution respirometry methods. Data were analyzed using a mixed model that included RFI group as fixed effect and slaughter date and year as a random effect using PROC MIXED of SAS. RFI and dry matter intake were different ($P < 0.001$) between low and high-RFI groups of year 1 and year 2. Basal respiration and maximum respiratory rate were greater ($P \leq 0.04$) for low than high-RFI steers when complex II substrates (succinate) were supplied. However, when Complex I substrates (glutamate/malate) were used maximum respiratory capacity tended to be greater ($P < 0.09$) for low vs. high-RFI steers. Low-RFI steers presented greater mitochondria density markers (greater ($P < 0.05$) citrate synthase (**CS**) activity and tended ($P \leq 0.08$) to have greater CS mRNA and mtDNA:nDNA ratio) than high-RFI steers. Hepatic expression $SDHA$, $UQCRC1$, and $CYC1$ mRNA was greater ($P \leq 0.02$) and expression of $NDUFA4$, $NDUFA13$, $SDHD$, $UQCRH$, and $ATP5E$ mRNA tended ($P \leq 0.10$) to be greater in low than high-RFI steers. Hepatic SDHA protein expression tended ($P < 0.08$) to be greater while succinate dehydrogenase activity was greater ($P = 0.04$) and NADH dehydrogenase activity was greater ($P = 0.03$) for low than high-RFI steers. High-efficiency steers (low-RFI) probably had greater efficiency in hepatic nutrient metabolism, which was strongly associated with greater hepatic mitochondrial density and functioning, mainly of mitochondrial complex II.

Key words: beef cattle, feed efficiency, liver, mitochondria, oxygen consumption

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INTRODUCTION

Residual feed intake (**RFI**), the difference between an animal's actual dry matter intake (**DMI**) and its expected DMI based on the requirements for maintenance and growth over a specified period is a useful measure of feed efficiency (Koch et al., 1963). Variations in phenotypic expression of feed efficiency could be associated to differences or inefficiencies in mitochondria function (Harper et al., 2002) due to its impact on energy expenditure (Rolfe and Brand, 1997).

Studies on mitochondrial function and phenotypic expression of feed efficiency in beef cattle are scarce. Reduced activity but increased expression of some proteins of respiratory complexes was reported in muscle and lymphocytes of high-efficiency steers when compared with low-efficiency steers (Sandelin et al., 2005; Ramos and Kerley, 2013). In addition, research showed greater muscle or hepatic mitochondrial respiration rates in low than high RFI steers, without differing in mitochondrial function or protein leak kinetics (Kolath et al., 2006; Lancaster et al., 2014). However, previous research has focused on isolated mitochondria in which several functional properties can be lost (Kuznetsov et al., 2008). Indeed, high-resolution respirometry studies in biopsies have been indicated as the most physiologically relevant approach to study intracellular energy metabolism (Gneiger et al., 2008). Particularly, mitochondria function in the liver, a central organ of metabolism that represents ~25% of whole body oxygen consumption (Baldwin et al., 2004), could be key for identifying pathways affecting cattle feed efficiency.

Thus, our hypothesis was that efficiency of hepatic mitochondrial function would be improved in low RFI when compared to high RFI animals. Our objective was to determine hepatic mitochondrial density and function in terms of respiration, gene and protein expression, and enzyme activity of mitochondrial respiratory complex proteins, in steers whit divergent RFI phenotypes.

MATERIALS AND METHODS

Animals, RFI Test, and Tissue Sampling

The RFI tests were conducted at the Kiyú Test Station (San José, Uruguay; 32°S, 56°W) of the Uruguayan Hereford Breed Association. Animal procedures were approved by the Animal Experimentation Committees of Universidad de la República (Uruguay). Animals used in this study were part of a larger project with the aim of building a training population of 1,000 animals for genomic selection of RFI in the Uruguayan Hereford breed (Navajas et al., 2014).

Hereford steers ($n = 111$ and $n = 122$ for year 1 and 2, respectively) were evaluated in postweaning tests for RFI. Steers came from 10 breeders and were sired by 24 pedigree bulls, confirmed by SNP paternity test before the RFI evaluation (data not shown). Steers entered the test with 290 ± 10 d of age, weighed 226 ± 33 kg, and had electronic tags for measuring individual feed intake with the GrowSafe system (Model 6000 GrowSafe Systems Ltd., Airdrie AB, Canada). The 70-d test started after 21 d of acclimatization to the feeding system and diet by the animals. All steers had ad libitum access to a total mixed ration (Table 1) and water. During the tests, animals were weighed fortnightly. Ultrasound measurements of subcutaneous back fat were taken by certified technicians. The RFI value for each animal was calculated for the test period as the difference (or residual) between the actual feed intake and the expected feed intake based on its body weight (**BW**) and average daily gain (Koch et al., 1963). Forty-six steers exhibiting the greatest ($n = 9$ and 16 for year 1 and 2; high-RFI) and the lowest ($n = 9$ and 12 for year 1 and 2; low-RFI) RFI values were selected for this study.

After the test, steers were managed together until slaughter in grazing conditions until they reached the slaughter BW (326 or 400 d; 500 ± 15 kg). During the summer, steers grazed on a sorghum pasture (*Sorghum vulgare;* herbage mass and allowance of 4,782 kg dry matter (**DM**)/ha and 9.5 ± 2.6 kg DM/d for year 1 and 5,294 kg DM/ha and 15.0 ± 2.5 kg DM/d for year 2; Table 1) without concentrate supplementation or supplemented with sorghum silage when pasture availability was limiting. During the fall-winter period, steers grazed on an oat pasture (*Avena sativa;* herbage mass and allowance of 1,735 kg DM/ha and 4.5 ± 2.3 kg DM/d for year 1 and 2,099 kg DM/ha and 7.5 ± 2.1 kg DM/d for year 2; Table 1) and were supplemented with 5.4 kg DM/animal of corn grain (Table 1). During the period that the animals consumed the forage diet, initial (final RFI test

weight), final (slaughter), and average daily gain did not differ between low and high RFI steers and their weight was not significantly different either (Table 2).

Steers were slaughtered in a commercial abattoir (Breeders and Packers Uruguay S.A; Durazno, Uruguay). Prior to transport, steer BW were recorded. Steers were stunned with a captive bolt gun, exsanguinated, and carcass was weighed (Table 2). The liver was dissected, separated from surrounding connective tissue and liver samples from the caudal lobe were obtained ~20 min after the animal was killed. For respiration measurements, liver samples (~25 mg) were obtained by duplicate using a 14-gauge biopsy needle (Tru-Core-II Automatic Biopsy Instrument; Angiotech,

Table 1. Ingredients and chemical composition of total mixed ration, pastures and corn grain provided to steers

Component	TMR[1]		Sorghum grass		Oats grass		Corn grain	
	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
Ingredients, % DM								
Sorghum silage	55	38	...	...	...	...	...	...
Barley silage	21	35	...	...	...	...	...	...
Corn grain	21	20	...	...	...	...	...	...
Vitamin and mineral mix	3	7	...	...	...	...	...	...
Chemical composition[2]								
DM, %	47.5	54.2	23.2	16.1	18.1	18.3	89.6	89.3
CP, % DM	12.3	13.5	8.1	9.8	22.5	22.5	9.1	9.8
NDF, %DM	39.8	42.8	65.2	62.3	50.5	48.3	10.3	10.9
ADF, %DM	26.3	27.8	40.7	41.5	30.5	30.2	3.2	3.6
ME (Mcal/kgDM)[3]	2.96	2.48	1.98	1.96	2.26	2.25	2.51	2.63

ADF = acid detergent fiber; CP = crude protein; DM = dry matter; NDF = neutral detergent fiber.

[1]Total Mixed Ration offered during the RFI test.

[2]Estimated by AOAC (2000).

[3]ME = Metabolizable energy of pastures estimated: ME = $[-0.027 + 0.0428 \times (88.9 - 0.779 \times$ acid detergent fiber)] $\times 0.82$ according to (Reid et al., 1991).

Table 2. Steer performances according to RFI phenotype

Item	Year 1				Year 2				Pooled data			
	Low-RFI (n = 9)	High-RFI (n = 9)	SE	P-value	Low-RFI (n = 12)	High-RFI (n = 16)	SE	P-value	Low-RFI (n = 21)	High-RFI (n = 25)	SE	P-value
RFI, kg DM/d	-0.69	0.90	0.62	<0.01	-0.79	0.62	0.70	<0.01	-0.75	0.72	0.66	<0.01
Feed intake, kg DM/d	9.2	10.6	0.53	<0.01	9.0	10.5	0.33	<0.01	9.0	10.4	0.31	<0.01
RFI test ADG, kg/d[1]	1.61	1.52	0.84	ns	1.41	1.46	0.65	ns	1.51	1.48	0.53	Ns
RFI test initial weight, kg	226.2	218.7	16.62	ns	243.8	246.5	21.61	ns	225.8	224.2	16.11	Ns
RFI test final weight, kg	364.7	354.4	19.89	ns	342.9	349.3	25.13	ns	351.4	343.4	14.72	Ns
Sorghum ADG, kg/d	0.92	0.93	0.51	ns	0.95	0.96	0.49	ns	0.91	0.94	0.48	Ns
Oat ADG, kg/d	0.84	0.81	0.48	ns	0.82	0.83	0.51	ns	0.83	0.82	0.53	Ns
Slaughter weight, kg	520.6	508.7	11.45	ns	505.1	506.1	14.01	ns	510.4	508.7	7.43	Ns
Carcass weight, kg	277.5	274.1	5.11	ns	260.7	263.4	4.12	ns	267.9	267.1	3.44	Ns
Liver weight, kg	5.31	5.79	0.25	0.06	5.62	5.81	0.22	0.10	5.56	5.74	0.18	0.09
Liver relative weight, g/kg EBW[2]	11.5	11.8	0.60	ns	13.08	13.21	0.28	ns	12.29	12.65	0.45	Ns

DM = dry matter; RFI = residual feed intake.

[1]ADG = average daily gain.

[2]EBW = empty body weight calculated according to NRC, 2000.

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Lausanne, Switzerland), immersed immediately in 1 mL of modified University of Wisconsin medium [20 mM histidine, 20 mM succinate, 3 mM glutathione, 1 µM leupeptin, 2 mM glutamate, 2 mM malate, 2 mM ATP, 0.5 mM ethylene glycol tetraacetic acid (EGTA), 3 mM $MgCl_2.6H_2O$, 60 mM 3-(N-morpholino)propanesulfonic acid (MOPS), 20 mM taurine, 10 mM KH_2PO_4, 20 mM N-(2-hydroxyethyl)piperazine-N'-2-ethanesulfonic acid (HEPES), 110 mM sucrose, 1 g/L BSA, and 10% dimethylsulfoxide, pH 7.4] in cryotubes and frozen in a stepwise freezing procedure (Garcia-Roche et al., 2018). For gene, protein expression and enzymes activity analyses, a liver sample (10 g) was snap-frozen in liquid nitrogen. Both sets of samples were stored at −80 °C until analyses.

High-resolution respirometry assays were performed in all samples ($n = 46$, steers of year 1 and 2) while hepatic DNA, RNA and protein contents, quantitative real-time qPCR, western blot, and enzyme activity assays were only determined in year 1 animals ($n = 18$) when differences between high and low-RFI steers were the greatest.

Oxygen Consumption Rate Measurements

The evaluation of mitochondrial function was analyzed by studying the oxygen consumption in liver frozen samples (Garcia-Roche et al., 2018). Briefly, samples were measured in 2.4 mL chambers using high-resolution respirometry in an Oroboros Oxygraph 2-k (Oroboros Instruments, Innsbruck, Austria) at 37 °C. Chambers were previously calibrated with MIR05 mitochondrial respiration medium (0.5 mM EGTA, 3 mM $MgCl_2.6H_2O$, 60 mM MOPS, 20 mM taurine, 10 mM KH_2PO_4, 20 mM HEPES, 110 mM sucrose, 1g/L BSA, pH 7.1) with a calculated saturated oxygen concentration of 190 nmol O_2 per mL at 100 kPa barometric pressure. Weight-specific oxygen flux (pmol $O_2 \cdot min^{-1} \cdot mg^{-1}$ wet weight) was calculated using the DatLab 4 analysis software (Oroboros Instruments). A substrate-inhibitor titration protocol was utilized (Fig. 1). The assay was initiated with the addition cytochrome c titration (1 to 3.5 µM; controlling outer mitochondrial membrane integrity), before to addition of complex specific substrates: 10 mM glutamate and 5 mM malate for complex I or 20 mM succinate for complex II, followed by ADP (1 to 4 mM). Oligomycin (2 µM) was added to inhibit ATPase activity and carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP; 1 to 4 µM; controlling outer mitochondrial membrane integrity) was added to uncouple oxidative
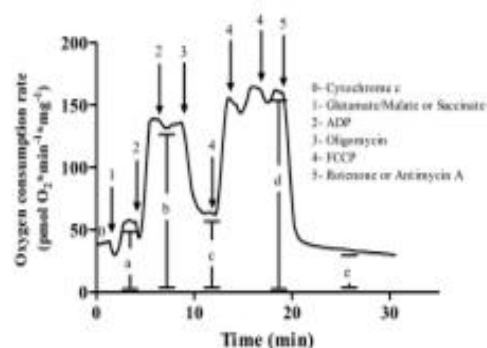

Figure 1. Representative high-resolution respirometry experiment and titration protocol. Respiration rates were measured at 37 °C and substrate-inhibitor titration protocol was utilized. Oxygen consumption rates were measured after the sequential addition of Cytochrome c (1 to 3.5 µM), addition of complex specific substrates (10 mM Glutamate and 5 mM Malate for complex I or 20 mM Succinate for complex II), ADP (1 to 4 mM), Oligomycin (2 µM), FCCP (1 to 4 µM) and 1 mM of Rotenone to inhibit complex I or Antimycin A 1 mM to inhibit complex II. Respiratory parameters and indices: Non-mitochondrial respiration (rate e) is substrate from other values: State 4 respiration (rate a); State 3 respiration after the addition of ADP that resembles the basal respiration of the tissue at saturating concentrations of substrates and ADP (rate b); Oligomycin-resistant respiration (rate c); Maximum respiratory rate (rate d). ATP-linked respiration (rate b-rate b). Spare respiratory capacity (rate d-rate b); Coupling efficiency ((rate b-rate c)/rate b) [adapted to Garcia-Roche et al., 2018; Brand and Nicholls, 2011).

phosphorylation. Finally, 1 mM rotenone was added to inhibit complex I or antimycin A 1 mM to inhibit complex III and consequently complex II. Titration curves were done for ADP and FCCP in order to reach saturating concentrations for each reagent. Different respiratory parameters and indices were obtained from oxygen consumption rate measurements using the DatLab 4 analysis software (Oroboros Instruments).

Hepatic DNA, RNA, and Protein Concentrations

The high-salt protocol procedure was used to determine genomic DNA concentrations (Sunnucks and Hales, 1996) and total RNA was isolated using TRIzol (Invitrogen, Life Technologies, Carlsbad, CA, USA) starting from 50 mg of tissue for each analysis. Concentrations of genomic DNA and total RNA were determined by measuring absorbance at 260 nm (NanoDrop ND-1000 Spectrophotometer; Nanodrop Technologies Inc., Wilmington, DE, USA), and purity of all DNA and RNA isolates were assessed from 260/280 nm and 260/230 nm absorbance ratios. The Coomassie brilliant blue G (Bradford) assay was used to determine protein concentration of liver homogenates (300 mg of tissue; Polytron; Brinkmann, Westbury,

NY) in 10 volumes of modified MRM buffer (5 mM KH$_2$PO$_4$, 1 mM EGTA, 5 mM MOPS, 300 mM sucrose, pH 7.4) Hepatic DNA, RNA, and protein contents were calculated by multiplying liver weight by DNA, RNA, and protein concentrations, respectively. Tissue concentrations of protein and nucleic acids were used to estimate cellularity indices according to Casal et al. (2014), relative cell number (hyperplasia; DNA concentration), cell size (hypertrophy; protein:DNA), or protein synthesis capacity (RNA concentration and RNA:protein ratio). Isolated DNA and RNA were used for real time qPCR analyses and, protein homogenates for mitochondrial extraction and enzymes activity analyses.

Quantitative Real-time qPCR

Isolated RNA was precipitated with lithium chloride, DNase-treatedwith a DNA-Free kit (Applied Biosystems Ambion, Austin, TX, USA). Integrity was assessed by electrophoresis in 1% agarose gel. Isolated RNA was stored at −80 °C until analyzed. The SuperScript III Transcriptase (Invitrogen), with random hexamers and 2 µg of total RNA as a template, was used to conduct the reverse transcription. The cDNA was stored at −20 °C until its use. Primers (Supplementary Table S1) to specifically amplify cDNA of 12 target genes of the mitochondrial respiratory complex and citrate synthase and 3 internal control genes: β-actin (*ACTB*), hypoxanthine phosphoribosyltransferase (*HPRT1*) and ribosomal protein L19 (*RPL19*), were obtained from literature or specifically designed using the Primer3 website (http://bioinfo.ut.ee/primer3-0.4.0/) based on bovine nucleotide sequences available from NCBI (http://www.ncbi.nlm.nih.gov/). 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). The genes selected for this study were selected based on previous results from a microarray analysis (Laporta et al., 2014). Mitochondrial respiratory complex candidate genes selected were: NADH:ubiquinone oxidoreductase subunit C1 (*NDUFC1*, complex I); NADH:ubiquinone oxidoreductase subunit A4 (*NDUFA4*, complex I); NADH:ubiquinone oxidoreductase subunit A13 (*NDUFA13*, complex I); succinate dehydrogenase complex, subunit A, flavoprotein (Fp) (*SDHA*, complex II); succinate dehydrogenase complex, subunit D (*SDHD*, complex II); ubiquinol-cytochrome c reductase core

protein I (*UQCRC1*, complex III); ubiquinol-cytochrome c reductase hinge protein (*UQCRH*, complex III); cytochrome c oxidase assembly factor (*COX19*, complex IV); cytochrome c-1 (*CYC1*, complex IV); ATP synthase, H$^+$ transporting, mitochondrial F1 complex, epsilon subunit (*ATP5E*, complex V); ATP synthase, H+ transporting, mitochondrial F1 complex, O subunit (*ATP5O*, complex V); and citrate synthase (*CS*).

Real-time qPCR reactions were performed according to Casal et al. (2014) in a total volume of 15 µL using KAPA SYBR FAST Universal 2X qPCR Master Mix (Kapa Biosystems, Inc. Woburn, MA, USA) using the following standard amplification conditions: 3 min at 95 °C and 40 cycles of 3 s at 95 °C, 40 s at 60 °C, and 10 s at 72 °C in a Rotor-Gene 6000 (Corbett Life Sciences, Sidney, Australia). 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 samples analyzed in triplicate to be used as the basis for the comparative expression results (exogenous control) and duplicate tubes of water (nontemplate control). Gene expression was measured by relative quantification (Pflaffl, 2009) to the endogenous control and normalized to the geometric mean expression of internal control genes (*ACTB, HPRT1,* and *RPL19*). 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 0.343, 0.379, and 0.285 for *ACTB, HPRT1,* and *RPL19*, respectively. Amplification efficiencies of 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 interassay CV for all genes were less than 1.4% and 4.2%, respectively.

In addition, mitochondrial DNA (**mtDNA**) and nuclear DNA (**nDNA**) ratio (**mtDNA:nDNA ratio**) was used to estimate tissue mitochondrial density (Guo et al., 2009). The real-time qPCR was carried out in separate tubes for mtDNA and nDNA amplification using PCR primers (Supplementary Table S1) for detecting the mitochondrial encoded cytochrome c oxidase I (*mt-CO1*, mitochondrial gene) and NADH:ubiquinone oxidoreductase core subunit V1 (*NDUFV1*, nuclear gene), respectively and real-time qPCR reactions were conducted as described above. The cycle number (Ct) at which the fluorescent signal of a given reaction crossed the threshold value was used as basis for quantification

of mtDNA and nDNA copy numbers. The difference between cycle numbers measured at the threshold (designated ß) was used to calculate the relative ratio of mtDNA:nDNA using the equation: $2^ß$.

Western Blot Analysis

Total protein extracts of hepatic samples were obtained from homogenates 10% (wt/vol) in ice-cold RIPA buffer (with protease inhibitors) [50 mM Tris, 150 mM NaCl, 2 mM EDTA, 2 mM EGTA, 1% Triton X-100 and 0.1% Sodium dodecyl sulfate (SDS)] and stored at −80 °C until its use. Protein samples (30 to 40 µg) were separated on a 12% SDS-polyacrylamide gel and transferred to nitrocellulose membrane overnight at 20 mV and 4 °C. The immunochemical detection of ATPsynthase subunit alpha (ATP5A), succinate dehydrogenase subunit A (SDHA) or β-actin (ACTB) proteins were performed using specific mouse monoclonal anti-ATP5A, anti-SDHA, or anti-ACT antibodies (Abcam, Cambridge, UK). The membranes were blocked for 1 h at room temperature in TBS buffer (50 mM Tris-HCL, 150 mM NaCl, pH 7.4) with 0.6% (vol/vol) of Tween-20 and 5% (wt/vol) of milk. Next, membranes were incubated with the mouse monoclonal anti-ATP5A and anti-SDHA antibodies (1:2,000) and mouse monoclonal anti-ACTB (1:3,000) in the same blocking buffer (without milk) for 1 h at room temperature with gentle agitation. Subsequently, membranes were washed twice for 10 min and 3 times for 5 min in TBS plus 0.6% Tween-20. Then, membranes were incubated with anti-mouse IR Dye 680CW (Li-COR, Lincoln, NE, USA) at 1:15,000 for 1 h in TBS plus 0.6% Tween-20. The membranes were washed four times for 15 min, and signals were detected using the ODYSSEY CLx Infrared Imaging System (Li-COR) and analyzed using ODYSSEY CLx and ImageJ-software. Density of the area of each lane was measured. The relative levels of ATP5A and SDHA were normalized with ACTB density and normalized values for high-RFI were expressed as a percentage relative to the low-RFI group.

Enzyme Activity

Enzyme activity assays were adapted from Spinazzi et al. (2012) and Janssen et al. (2007). Assays were performed in duplicate at 37 °C using a final volume of 500 µL in a UV-2401 PC spectrophotometer (Shimadzu Corporation, Tokyo, Japan). Mitochondria from liver protein homogenates were isolated by differential centrifugation according to Cassina and Radi (1996). Enzyme activities were expressed as mU per mg of mitochondrial or homogenate protein.

Citrate synthase activity: Since citrate synthase is present only in mitochondria, it is commonly used as an indirect marker of tissue mitochondrial density. The assay was performed at 412 nm following the reduction of 0.1 mM 5,50'-dithiobis(2-nitrobenzoic acid) in the presence of 30 to 80 µg of liver homogenate protein, 0.2 mM acetyl-CoA in a medium with 100 mM Tris–HCl, pH 7.5, and 0.1% Triton X-100. The reaction was incubated 5 min at 37 °C, and the absorbance change determined 5 min before and after the addition of 0.25 mM oxalacetic acid and an extinction coefficient of 13.6 $mM^{-1}cm^{-1}$ was used to quantify enzyme activity.

Nicotinamide adenine dinucleotide (NADH) ubiquinone oxidoreductase activity: The assay was performed at 600 nm following the reduction of 2,6-dichlorophenolindophenol (DCPIP) by decylubiquinone in the presence of 80 to120 µg of mitochondrial protein, 60 µM DCPIP, 10 µM antimycin, 3.5 mg/mL bovine serum albumin and 70 µM decylubiquinone in a 25 mM phosphate buffer, pH 7.2. The reaction was initiated by the addition of 200 µM NADH. The decrease in the optical density at 600 nm measured 5 min before and after the addition of 2 µM rotenone and the extinction coefficient of 21 $mM^{-1}cm^{-1}$ wereused to quantify enzyme activity.

Succinate dehydrogenase (SDH) activity: The assay was performed at 600 nm following the reduction of DCPIP by decylubiquinone in the presence of 80 to 120 µg of mitochondrial protein, 50 µM DCPIP, 1 mM potassium cyanide, 5 µM rotenone and 50 µM decylubiquinone in a 25 mM phosphate buffer, pH 7.2. The absorbance change determined 5 min before and after addition of 10 mM succinate and an extinction coefficient of 21 $mM^{-1}cm^{-1}$ was used to quantify the enzyme activity.

Statistical Analyses

Data were analyzed using the SAS System program (SAS Institute Inc., Cary, NC, USA). The UNIVARIATE analyses were performed on all variables to identify outliers and inconsistencies and to verify normality of residuals. Respiratory data were analyzed using the MIXED procedure with a model that included RFI group as fixed effect and slaughter date and year as a random effect. Liver weight (fixed) and breeders and sires data (random) were included in the model and when covariance parameter estimates were zero or close to zero it was

removed from the model. All the other data were analyzed using the MIXED procedure with a model that included RFI group as fixed effect and slaughter date as a random effect. Correlation coefficients were estimated using the CORR procedure. Mean separation was performed using the Tukey test, and differences were considered significant at $P \leq 0.05$ and a trend when $0.05 < P \leq 0.10$. Data are presented as least square means ± pooled standard errors.

RESULTS

RFI Test

RFI and DMI were different ($P < 0.001$) between low and high-RFI groups of year 1, year 2 and when pooled data were analyzed together (Table 2). However, RFI groups neither differed in the initial and final weights of the RFI test, average daily gain, nor in preslaughter and carcass weights (Table 2).

Hepatic Oxygen Consumption Rate

A representative oxygen consumption assay is shown in Fig. 1. Basal respiration (state 3) was greater ($P = 0.03$) for low than high-RFI steers when complex II substrates were supplied but not when complex I substrates were added (Table 3). Maximum respiratory rate was greater ($P = 0.01$) with complex II substrates and tended ($P = 0.09$)

to be greater with complex I substrates for low than high-RFI steers (Table 3). The ATP-linked respiratory, spare respiratory capacity, and coupling efficiency did not differ between RFI groups (Table 3). RFI values showed a moderate and negative correlation with maximum respiratory rate of complex II ($r = -0.33$, $P = 0.05$, $n = 17$).

Liver Mass, Cellularity Indices, and Mitochondrial Density

High-RFI steers tended ($P < 0.06$) to have greater absolute liver weight than low-RFI steers. Hepatic protein concentration tended ($P < 0.08$) to be greater for low than high-RFI steers but DNA and RNA concentrations as well as protein:DNA and RNA:protein ratios did not differ between RFI groups. Low-RFI steers presented greater ($P < 0.05$) CS activity and tended ($P \leq 0.08$) to have greater *CS* mRNA and mtDNA:nDNA ratio than high-RFI steers (Table 4).

Hepatic Gene Expression of Mitochondrial Respiratory Chain Proteins

Hepatic expression *SDHA, UQCRC1*, and *CYC1* mRNA was greater ($P \leq 0.02$) and expression of *NDUFA4, NDUFA13, SDHD, UQCRH*, and *ATP5E* mRNA tended ($P \leq 0.10$) to be greater in low than high-RFI steers (Table 5). However, *NDUFC1, COX19*, and *ATP5O* mRNA did not differ between RFI groups (Table 5). RFI values showed a moderate to high and negative correlation

Table 3. Hepatic respiratory parameters (pmol O_2 min^{-1}·mg^{-1}) and indices of steers differing in RFI phenotype

	RFI group			
Item	Low ($n = 21$)	High ($n = 25$)	SE	P-value
Complex I[1]				
Respiratory parameters and indices[2]				
Basal respiration (State 3)	6.23	6.32	0.57	ns
Maximum respiratory rate	8.08	6.34	0.71	0.09
ATP-linked respiration	3.42	3.56	0.73	ns
Spare respiratory capacity	1.06	0.95	0.19	ns
Coupling efficiency	0.56	0.47	0.05	ns
Complex II[1]				
Respiratory parameters and indices[2]				
Basal respiration (State 3)	31.46	23.71	3.58	0.03
Maximum respiratory rate	38.66	28.70	3.71	0.01
ATP-linked respiration	5.68	5.72	0.65	ns
Spare respiratory capacity	4.75	6.23	1.61	ns
Coupling efficiency	0.22	0.21	0.06	ns

[1]Mitochondrial Complex I NADH ubiquinone oxido reductase or Complex II Succinate dehydrogenase.

[2]Respiratory parameters and indices according to Figure 1.

Table 4. Hepatic tissue characterization according to RFI phenotype

	RFI group			
Item	Low ($n = 9$)	High ($n = 9$)	SE	P-value
Tissue cellularity indices				
Protein, mg/g tissue	203.6	184.4	7.2	0.08
RNA, µg/mg tissue	1.43	1.30	0.12	ns
DNA, µg/mg tissue	1.66	1.36	0.26	ns
Protein:DNA ratio	122.5	135.5	28.2	ns
RNA:Protein ratio	0.007	0.007	0.001	ns
Mitochondrial density indices				
Citrate synthase activity, mU/mg protein	48	43	2	0.05
mRNA Citrate synthase, AU	1.57	0.96	0.55	0.08
mtDNA/nDNA ratio, AU[1]	148.1	89.7	29.8	0.08

RFI = residual feed intake.

[1]mtDNA/nDNA ratio = mitochondrial DNA (mtDNA) and nuclear DNA (nDNA) ratio

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coefficients with hepatic *CS*, *SDHA*, and *CYC1* mRNA (r = −0.59, −0.51, and −0.50, respectively, $P \le 0.04$, n = 17).

Hepatic Mitochondrial Respiratory Chain Protein Expression and Enzyme Activity

Hepatic SDHA protein expression tended ($P < 0.08$) to be greater while SDH activity was greater ($P = 0.04$) for low than high-RFI steers (Figs. 2 and 3). RFI values and SDHA protein expression were higher and negatively correlated (r = −0.77, $P < 0.01$, n = 17). In addition, NADH dehydrogenase activity was also greater ($P = 0.03$) for low than high-RFI steers (Fig. 3). Hepatic ATP5A protein expression did not differ between RFI groups (data not shown).

DISCUSSION

This study determined hepatic mitochondrial density and function in steers with divergent RFI phenotypes. Results showed that high-feed efficiency steers (low-RFI) would have a better efficiency of nutrient utilization associated with greater hepatic mitochondrial density and better mitochondrial functioning (greater gene and protein expression and enzyme activity) than low-feed efficient steers (high-RFI). Herein, indicators of liver mitochondrial function are discussed in the context of beef cattle feed efficiency.

In agreement with previous reports, low-RFI steers achieved similar body and carcass weight and had a similar growth rate with a reduced DMI than high-RFI steers (Kolath et al., 2006; Castro Bulle et al., 2007; Lancaster et al., 2014). Individual

Table 5. Hepatic gene expression of mitochondrial respiratory complex (Cx) proteins of steers differing in RFI phenotype

| Gene[2] | Complex | RFI group | | SE | P-value |
		Low (n = 9)	High (n = 9)		
NDUFC1	CxI	0.85	0.91	0.35	ns
NDUFA4	CxI	0.93	0.69	0.20	0.06
NDUFA13	CxI	1.18	0.91	0.14	0.09
SDHA	CxII	1.26	0.84	0.19	0.02
SDHD	CxII	1.01	0.62	0.19	0.06
UQCRH	CxIII	1.28	0.96	0.23	0.07
UQCRC1	CxIII	1.02	0.59	0.30	0.02
CYC1	CxIV	1.05	0.83	0.17	<0.01
COX19	CxIV	0.91	0.99	0.10	ns
ATP5E	CxV	1.06	0.85	0.33	0.10
ATP5O	CxV	0.98	1.03	0.27	ns

RFI = residual feed intake.

[1]Genes: NADH:ubiquinone oxidoreductase subunit C1 (*NDUFC1*), NADH:ubiquinone oxidoreductase subunit A4 (*NDUFA4*), NADH:ubiquinone oxidoreductase subunit A13 (*NDUFA13*), succinate dehydrogenase complex, subunit A, flavoprotein (Fp) (*SDHA*), succinate dehydrogenase complex, subunit D (*SDHD*), ubiquinol-cytochrome c reductase core protein I (*UQCRC1*), ubiquinol-cytochrome c reductase hinge protein (*UQCRH*), cytochrome c oxidase assembly factor (*COX19*); cytochrome c-1 (*CYC1*), ATP synthase, H+ transporting, mitochondrial F1 complex, epsilon subunit (*ATP5E*); ATP synthase, H+ transporting, mitochondrial F1 complex, O subunit (*ATP5O*).
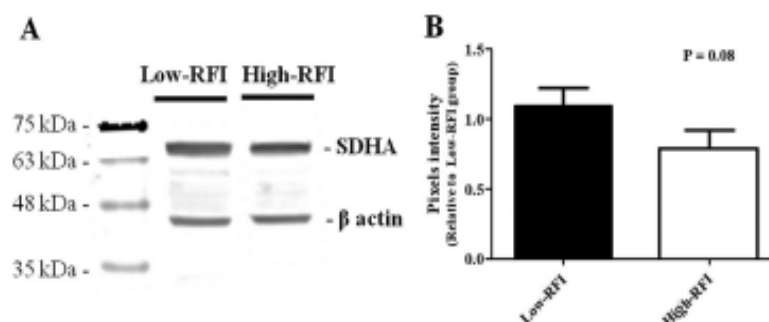

Figure 2. Western blot analysis with monoclonal anti-SDHA antibody in liver (A). Means ± SE of pixels intensity expressed with proportion of low-RFI animals (B) according to steer RFI phenotype (n = 9 and 9 for low and high-RFI steers, respectively).
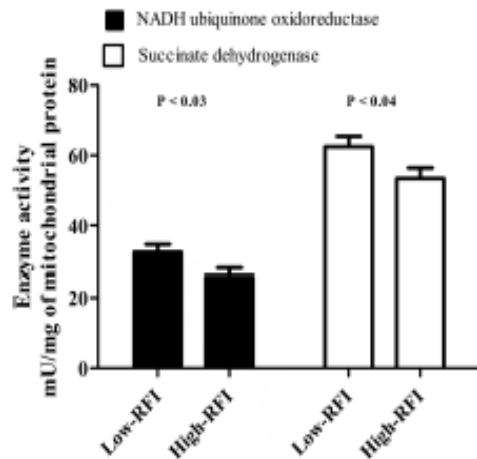
Figure 3. Hepatic enzyme activity (mU/mg of mitochondrial protein) of NADH dehydrogenase (■) and Succinate dehydrogenase (□) of steers differing in RFI phenotypes. Each bar represents the mean ± SE of 18 observations (n = 9 and 9 for low and high-RFI steers, respectively).

variations in feed efficiency resulted from differences in feed intake and, consequently, would impact on the metabolic workload of visceral organs, especially the liver (Montanholi et al., 2017). Indeed, high-RFI steers tended to present greater liver mass compared to low-RFI steers. However, this trend disappeared when liver mass was expressed as relative to empty body weight (g/kg EBW) which would indicate that the increase in liver mass associated with DMI, was a compensatory mechanism for metabolic nutrient processing.

Lancaster et al. (2014) and Montanholi et al. (2017) observed no differences in liver biometrics when efficient and inefficient steers were compared, suggesting gravimetric assessments of organ function do not capture structural and functional changes such as those due to variation in feed efficiency. However, in agreement with the trend for a greater liver protein content for low vs. high-RFI steers, these latter authors (Montanholi et al., 2017) reported larger hepatocytes and hepatocyte nuclei in efficient than inefficient steers. These results would suggest an increased hepatic metabolic rate with improved feed efficiency.

The present study, to our knowledge, is the first work that compared mitochondrial respiration of low vs. high RFI steers using high-resolution respirometry in cryopreserved hepatic biopsies. Low-RFI steers exhibited a greater basal respiration (state 3 respirations after addition of ADP; maximum ADP-stimulated respiration) and

maximum respiratory rate when provided succinate (complex II) as respiratory substrates with respect to high-RFI steers. The greater oxygen consumption recorded in low vs. high-RFI steers at the same level of substrates reflected a greater capacity to obtain ATP per unit of substrate. Similar to our results, greater basal respiration was also reported in muscle and hepatic isolated mitochondria for low than high-RFI steers and heifers (Kolath et al., 2006; Lancaster et al., 2014).

The rate of energy use by an organism—the metabolic rate—is directly proportional to the rate of oxygen consumption. Therefore, rate of oxygen consumption can be measured and used to infer metabolic rate indirectly (Brand and Nicholls, 2011). Maximum respiratory rate is induced by the uncoupler (FCCP) and represents maximum activity of electron transport and substrate oxidation achievable by the cells (Brand and Nicholls, 2011), being, when decreased, a strong indicator of potential mitochondrial dysfunction (Hill et al., 2012). The greater feed efficiency of low than high-RFI steers would be related to greater availability of respiratory chains to cope with their metabolic demands in the former ones and could be considered as an indirect indicator of mitochondrial density. Indeed, a greater amount of available respiratory chains activity in low than high-RFI steers may be the result of a greater number of mitochondria per unit of hepatic weight in the former ones as indicated by differences in mitochondrial content biomarkers (CS activity and mRNA, mtDNA:nDNA ratio; Larsen et al., 2012). These differences between low and high-RFI steers could be explained by a greater proton or electron leak, uncoupling proteins or protein modifications (oxidation, nitration, acetylation; Bottje and Carstens, 2009; Cimen et al., 2010). Thus, low-RFI steers would have a greater reserve capacity to protect cells, among other stresses, from energy/substrate insufficiency and oxidative stress than high-RFI steers.

In addition, in the present study, stronger evidence on differences on maximum respiratory rate between low and high-RFI steers was observed when substrates for complex II than for the complex I was supplied. Complex II is the only enzyme complex component of the electron transport chain that not only catalyzes a tricarboxylic acid (TCA) cycle reaction (oxidation of succinate to fumarate with the reduction of ubiquinone to ubiquinol by coupling the two reactions together) but it is also a key step in the use of ruminal propionate for neoglucogenesis. These characteristics confer this enzyme complex an unique position in the metabolism to serve as a

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regulated source of the maximum and spare respiratory capacity that would remain unused under normal conditions, but becomes manifest when oxygen consumption is uncoupled from ATP production, or if there is an increase in energy demand (Pfleger et al., 2015). Moreover, it has been suggested (Guaras et al., 2016; Scialò et al., 2017) that the increase in the activity of complex II in low-RFI animals, could lead to an increase in the flow of electrons from the oxidation of $FADH_2$. This could cause reverse electron transport that fosters separation of Complex I and III supercomplex and a greater passage of the electrons coming from the $FADH_2$ to the rest of the respiratory chain. Thus, low-RFI could optimize nutrient utilization when compared to high-RFI steers.

Contrary to our results, Lancaster et al. (2014) did not find differences in maximum respiratory rate between low and high-RFI steers fed high concentrate (trial 1) or a more fibrous diet (trial 2) when hepatic mitochondria were provided with substrates for any of the two complexes. Similarly, Acetoze et al. (2015) did not report differences in hepatic respiration rates or proton leak of steers descent of bulls of divergent lines of RFI. Moment of tissue collection related to RFI measure could explain differences between results from this vs. previous reported studies. Phenotypic correlations between RFI measured during growing and finishing periods were positive and moderate to high. (0.50 and 0.85 using high- or medium-concentrate diets; Arthur et al., 2001; Kelly et al., 2010; McGee et al., 2014) and others (Durunna et al., 2011; Russell et al. 2016) reported relative classification of a steer as highly or lowly feed efficient was repeatable between growing and finishing phases even if steers changed of diet. Moreover, positive and moderate correlations between RFI values derived from forage- and grain-based diets in heifers were reported (Trujillo et al., 2013; Cassady et al. 2016). Thus, it could be expected that more efficient young animals when fed one diet type were also more efficient when the diet is changed, however, reranking may occur and could contribute to explain some of the differences between our and other studies findings. Methodological differences could also explain, at least partially, differences between studies, as in our study measurements were performed in a tissue sample vs. isolated mitochondria and in a high-resolution equipment vs. Clark-type oxygen electrode. The isolation process could decrease mitochondrial integrity and mitochondria isolated from cells and tissues are essentially stripped from their intracellular locales (Salabei et al., 2014). The use of high-resolution equipment allowed us a more precise titration of the FCCP, critical to determine uncontrolled respiration (Brand and Nicholls, 2011). Thus, greater basal respiration and maximum respiratory rate associated with greater mitochondria content would indicate an improved bioenergetics capacity in feed efficient animals as they are important quantitative indicators of oxidative capacity (Lee and Wei, 2005) and metabolic activity (Weikard and Kühn, 2016).

The greater estimated mitochondrial respiration for low than high-RFI steers was associated with greater expression of various genes encoding for respiratory chain protein complexes in the liver of low-RFI steers (*NDUFA4* and *NDUFA13*) for complex I, *SDHA* and *SDHD* for complex II, *UQCRH* and *UQCRC1* for complex III, *CYC1* for complex IV and *ATP5E* for complex V). Similar to our results, Iqbal et al. (2005) reported that only a few protein respiratory chain subunits were differentially expressed between broilers with low and high feed efficiency in liver mitochondria, being most of the subunits expressed at similar levels between groups. Mitochondrial complexes are not separate entities, but assembled multiprotein subunits, which have a great structural and functional dependence of the individual subunits as the alteration of one of them can cause partial or global disturbance of the whole complex functionality. Connor et al. (2010) studied differential hepatic gene expression in feed restricted steers using microarrays and reported up regulation of nearly 30 genes participating in mitochondrial electron transport, including some of the ones evaluated in the present study (complex II: *SDHA*, complex IV: *CYC1*, complex V: *ATP5O*) in steers exhibiting high vs. low feed efficiency during the re-alimentation period. In contrast, other hepatic transcriptome studies—using total RNA sequencing or microarray—did not report differently expressed genes related to electron transport chain or oxidative phosphorylation when high vs. low feed efficiency cattle were compared (Chen et al., 2011; Alexandre et al., 2015; Tizioto et al., 2015; Zarek et al., 2017). Differences between methodologies (qRT-PCR vs. RNA-seq or microarrays) or breeds (*Bos Taurus* vs. *Bos indicus*) may explain contradictory results. Interestingly, these latter studies reported, differences in expression of genes related to mitochondrial biogenesis, oxidative stress, transport of fatty acids to the mitochondrion for β-oxidation, antioxidant enzymes and uncoupling proteins (Chen et al., 2011; Alexandre et al., 2015; Tizioto et al., 2015; Zarek et al., 2017). In addition, although Kelly et al. (2011) reported no differences between

low and high RFI-steers in muscle gene expression of complex I, II, or III proteins, in agreement with our results, they observed a negative correlation between RFI values and *COXII* (complex IV) gene expression. Results suggest that the differences in respiration parameters between steers of low or high-RFI would be associated with changes at the transcription level, by affecting rates of mRNA synthesis and/or degradation, of some proteins of the mitochondrial respiratory chain complexes.

In agreement with the greater *SDHA* mRNA expression, we detected an increased protein expression of SDHA in low than high-RFI steers. As already mentioned, SDH is an enzyme complex (complex II) that participates in both the TCA cycle and the respiratory chain and constitutes one of the fundamental steps in the utilization of propionate (generated at ruminal level) for hepatic gluconeogenesis. Therefore, this could result in an increase in the performance of hepatic gluconeogenesis in low-RFI steers. In addition, in agreement with Sharifabadi et al. (2012), hepatic activity of both, NADH dehydrogenase (complex I) and SDH (complex II) was between 15% and 20% less in high than low-RFI steers, which would indicate complex I and complex II of respiratory chain complex activities may be compromised in low-efficiency animal phenotypes. Electrons enter the respiratory chain from NADH or $FADH_2$ at complex I and complex II respectively, transferred through coenzyme Q to complex III, and shuttled by cytochrome *c* from complex III to complex IV. We postulated that a greater activity of complex I and complex II would result in a greater proton gradient—that can finally generate ATP—generated per unit of time.

CONCLUSIONS

In summary, high-feed efficiency steers (low-RFI) probably had greater efficiency in hepatic nutrient metabolism, which was strongly associated with greater hepatic mitochondrial density and function, mainly of mitochondrial complex II. Further research is needed to identify the mechanism involved in these different physiological processes between animals with low and high efficiency.

SUPPLEMENTARY DATA

Supplementary data are available at *Journal of Animal Science* online.

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Table S1. Primers used for real time qPCR quantification.

Gene[1]	Accession#[2]		Primer sequence[3]	Length (bp)	Efficiency
ACTB	BT030480	F	CTCTTCCAGCCTTCCTTCCT	178	1.02
		R	GGGCAGTGATCTCTTTCTGC		
ATP5E	NM_001143741	F	GACATCATGGTGGCGTACTG	211	1.09
		R	CCACAGCTTCACCTTGAACA		
ATP5O	NM_174244.1	F	CCTCTCACGTCCAACCTGAT	131	1.11
		R	GCAGTGGTAACTGTGCATGG		
COX19	NM_001109966	F	CATACGGACATCCCTTTGCT	171	1.17
		R	GCTTCGGCAACTCCTTACTG		
CS	NM_001044721.1	F	AGCCAAGATACCTGTTCCT	217	1.14
		R	TGTGCTGGAAGAAACGATTG		
CYC1	NM_001038090	F	CCAGGTAGCCAAGGATGTGT	227	1.14
		R	GACCCTGAAGCTCAGGACAG		
HPRT1	XM_580802	F	TGGAGAAGGTGTTTATTCCTC	105	1.03
		R	CACAGAGGGCCACAATGTGA		
NDUFA4	NM_175820	F	TGCGGCTTAGCTTTTCTCTC	152	1.12
		R	GCGTGACATACAGTGCTGCT		
NDUFA13	NM_176672	F	TCGACTACAAGCGGAACCTT	233	1.19
		R	AGTTGCCTCCTCCTCCAAGT		
NDUFC1	NM_174564	F	GGTTCCCGAGTGTCTCTTCA	242	1.18
		R	CGTGAATCCAGAGGAACTGC		
RPL19	NM_001040516.1	F	CCCCAATGAGACCAATGAAATC	156	1.09
		R	CAGCCCATCTTTGATCAGCTT		
SDHA	NM_174178.2	F	ACATGCAGAAGTCGATGCAG	155	1.01
		R	GGTCTCCACCAGGTCAGTGT		
SDHD	NM_174179.2	F	TTTGGCTAGGATGGATGGAG	92	1.02
		R	ACTGAACAGAGGGGGAGGTT		
UQCRC1	NM_174629.2	F	CAGTCTTCCCAGCCTACCTG	105	1.10
		R	AGCCAGATGCTCCACAAAGT		
UQCRH	NM_001034745	F	CTGGTGTGGCTAAGGGGATA	232	0.98
		R	GGACTCAACACAAGCAGCAA		
NDUFV1	Genomic DNA	F	GTTCTTCTTAGGTTCTCACGTGG	251	
		R	TGAGAATTACTGACGTGACCTCT		
mt-CO1	Mitochondrial DNA	F	TCTTCCCACAACACTTTCTAGGA	198	
		R	TGTCGTGGTTAAGTCTACAGTCA		

[1]ß-actin (*ACTB*), ATP synthase, H+ transporting, mitochondrial F1 complex, epsilon subunit (*ATP5E*); ATP synthase, H+ transporting, mitochondrial F1 complex, O subunit (*ATP5O*), cytochrome c oxidase assembly factor (COX19); citrate syntase (*CS*), cytochrome c-1 (*CYC1*), hypoxanthine phosphoribosyl transferase (*HPRT1*); NADH:ubiquinone oxidoreductase subunit A4

(*NDUFA4*), NADH:ubiquinone oxidoreductase subunit A13 (*NDUFA13*), NADH:ubiquinone oxidoreductase subunit C1 (*NDUFC1*), ribosomal protein L19 (*RPL19*),succinate dehydrogenase complex, subunit A, flavoprotein (Fp) (*SDHA*), succinate dehydrogenase complex, subunit D (*SDHD*), ubiquinol-cytochrome c reductase core protein I (*UQCRC1*), ubiquinol-cytochrome c reductase hinge protein (*UQCRH*). Mitochondrially encoded cytochrome c oxidase I (*mt-CO1*, mitochondrial gene) and NADH:ubiquinoneoxidoreductase core subunit V1 (*NDUFV1*, nuclear gene).

[2]Gene bank sequences.

[3]F = foreword; R = reverse

3. DIFFERENTIAL HEPATIC OXIDATIVE STATUS IN STEERS WITH DIVERGENT RESIDUAL FEED INTAKE PHENOTYPE

animal

Differential hepatic oxidative status in steers with divergent residual feed intake phenotype

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Oxidative stress occurs when oxidant production exceeds the antioxidant capacity to detoxify the reactive intermediates or to repair the resulting damage. Feed efficiency has been associated with mitochondrial function due to its impact on cell energy metabolism. However, mitochondria are also recognized as a major source of oxidants. The aim of this study was to determine lipid and protein oxidative stress markers, and gene and protein expression as well as activity of antioxidant enzymes in the liver of steers of divergent residual feed intake (RFI) phenotypes. Hereford steers (n = 111) were evaluated in post-weaning 70 days standard test for RFI. Eighteen steers exhibiting the greatest (n = 9; high-RFI) and the lowest (n = 9; low-RFI) RFI values were selected for this study. After the test, steers were managed together under grazing conditions until slaughter when they reached the slaughter body weight. At slaughter, hepatic samples were obtained, were snap-frozen in liquid nitrogen and stored at −80°C until analyses. Hepatic thiobarbituric acid reactive species and protein carbonyls were greater (P = 0.05) and hepatic 4-hydroxynonenal protein adducts tended (P = 0.10) to be greater for high- than low-RFI steers. Hepatic gene expression glutathione peroxidase 4, glutamate–cysteine ligase catalytic subunit and peroxiredoxin 5 mRNA was greater (P ≤ 0.05) and glutathione peroxidase 3 mRNA tended (P = 0.10) to be greater in low- than high-RFI steers. Hepatic protein expression and enzyme activity of manganese superoxide dismutase and glutathione peroxidase enzyme activity tended (P ≤ 0.10) to be greater for low- than high-RFI steers. High-efficiency steers (low-RFI) probably had better hepatic oxidative status which was strongly associated with greater antioxidant ability near to the oxidant production site and, therefore, reduced oxidative stress of the liver. Decreased hepatic oxidative stress would reduce maintenance requirements due to a lower protein and lipid turnover and better efficiency in the use of energy.

Implications

This study demonstrated that hepatic oxidative stress differed in steers with different residual feed intake phenotype. Results showed that high feed efficient steers (low-residual feed intake) had less abundance of lipid and protein oxidative stress markers in the liver associated with greater hepatic gene and protein expression as well as activity of antioxidant enzymes than low feed efficient steers (high-residual feed intake). Decreased hepatic oxidative stress would reduce maintenance requirements due to a lower protein and lipid turnover and better efficiency in the use of energy.

Introduction

The residual feed intake (RFI) is a measure of feed efficiency that can be considered to be a predictive measure of energy

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use (Koch *et al.*, 1963; Herd and Arthur, 2009). Phenotypic differences in feed efficiency have been associated to mitochondria function due to its impact on cell energy metabolism (Iqbal *et al.*, 2005; Casal *et al.*, 2018). In addition to its role in energy metabolism, mitochondria are recognized as major sources of reactive oxygen species (**ROS**) and reactive nitrogen species (**RNS**) in most of cell types (Turrens, 2003; Bottje and Carstens, 2009; Valez *et al.*, 2012). Oxidative stress occurs when ROS/RNS production and other oxidants exceed the biological system's ability (antioxidant capacity) to detoxify the reactive intermediates or to repair the resulting damage (Chirase *et al.*, 2004; Radi, 2018). The cellular response to damage usually involves a component that senses a stress and transmits the information as a cellular signal to transcription factors. Transcription factors then induce or regulate the expression of genes encoding cytoprotective enzymes and proteins (Kobayashi *et al.*, 2004).

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Antioxidants, substances that delay or inhibit oxidation of a substrate despite their lesser concentrations relative to the oxidizable substrate, are integrated in a multi-faceted anti-oxidant system responsible for mitigating oxidant pressure and preventing oxidative stress (Gutteridge, 1995). Neutralizing the oxidant and/or degrading and replacing the oxidatively damaged biomolecules (oxidized proteins, lipids, DNA, and carbohydrates) or cells containing the damaged biomolecules are energetically costly, thus, oxidative stress can increase energy expenditure decreasing energy efficiency (Mehlhase and Grune, 2002; Bottje and Carstens, 2009). Oxidative stress of biomolecules and cell organelles not only elevates cellular energy expenditure but may also negatively affect other cellular processes as alteration of enzymes, proteins, and lipids from metabolic pathways involved in cell signaling events or organelle dysfunction (Turrens, 2003; Bottje and Carstens, 2009; Radi, 2018).

Studies on oxidative stress, antioxidant capacity, and phenotypic expression of feed efficiency in beef cattle have been scarce and contradictory. Sandelin *et al.* (2005) reported greater protein oxidation in muscle of low *v.* high feed efficient steers while Kolath *et al.* (2006) reported that mito-chondrial ROS production was reduced in muscle of high (low efficient) *v.* low-RFI steers. However, recent research showed less plasma or hepatic mitochondrial protein and/or lipid oxidation in high than low-RFI steers (Russell *et al.*, 2016; Zulkifli, 2016). In addition, Kidrick *et al.* (2016) did not report differences in serum total antioxidant capacity between heifers of different phenotypic RFI, although Russell *et al.* (2016) reported reduced activity of antioxidant enzymes in red blood cells for high than low feed efficiency animals.

Due to the central role of the liver in energy and protein metabolism (Baldwin *et al.*, 2004), hepatic oxidative status could explain, at least partially, phenotypic differences in feed efficiency in cattle. Thus, our hypothesis was that hepatic oxidative stress would be reduced in low compared to high-RFI steers due to an increased activity of the antioxidant system. Our objective was to determine lipid and protein oxidative stress markers and mRNA and protein expression as well as activity of antioxidant enzymes in the liver of steers of divergent RFI phenotypes.

Materials and methods

Animals, residual feed intake test and tissue sampling
A detailed description of the experiment has been previously reported (Casal *et al.*, 2018). Briefly, Hereford steers ($n = 111$; 290 ± 10 days old, weighed 226 ± 33 kg at the beginning of the test) were evaluated in post-weaning standard tests for RFI. The 70 days test started after 21 days of acclimatization to the diet and the feeding GrowSafe system (Model 6000 GrowSafe Systems Ltd., Airdrie AB, Canada). All steers had *ad libitum* access to a total mixed ration (Table 1) and water. During the tests, animals were weighed fortnightly. The RFI value for each animal was calculated for the test period according Koch *et al.* (1963).

Table 1 *Ingredients and chemical composition of total mixed ration, pastures, sorghum silage and corn grain provided to steers*

Component	TMR	Sorghum grass	Oats grass	Sorghum silage	Corn grain
TMR ingredients, % DM					
Sorghum silage	55	...	...	...	...
Barley silage	21	...	...	...	...
Corn grain	21	...	...	...	...
Vitamin and mineral mix	3	...	...	...	...
Pastures					
Herbage mass, kg DM/ha	...	4782	1735	...	...
Herbage allowances, kg DM/d	...	9.5	4.5	...	...
Chemical composition[1]					
DM, %	47.5	23.2	18.1	35.5	89.6
CP, % DM	12.3	8.1	22.5	5.4	9.1
NDF, % DM	39.8	65.2	50.5	66.0	10.3
ADF, % DM	26.3	40.7	30.5	42.4	3.2
ME (MJ/kg DM)	12.4	8.3	9.5	7.9	13.0

TMR = total mixed ration offered during the residual feed intake test; ME = metabolizable energy of pastures estimated ME = [−0.027 + 0.0428 × (88.9 − 0.779 × ADF)] × 0.82 expressed in MJ according to (Reid *et al.*, 1991).
[1] Estimated by AOAC (2000) and Van Soest *et al.* (1991).

Eighteen steers (from year 1 of RFI evaluation) exhibiting the greatest ($n = 9$; high-RFI steers with RFI values 1.5 SD above the mean) and the lowest ($n = 9$; low-RFI steers with RFI values 1.5 SD below the mean) RFI values were selected for this study.

After the RFI-test, steers were managed together until reaching the slaughter body weight (326 days; 500 ± 15 kg) under grazing conditions (grazing of *Sorghum vulgare* supplemented with sorghum silage when pasture availability was limiting during the summer and grazing of *Avena sativa* supplemented with 5.4 kg dry matter/animal of corn grain during fall and winter). Steers were slaughtered in a commercial abattoir (Breeders and Packers Uruguay S.A., Durazno, Uruguay) and prior to transport, pre-slaughter body weight was recorded. At slaughter, the liver was dissected, separated from surrounding connective tissue and liver samples from the caudal lobe were obtained ~20 min after the animal was killed. Liver samples (10 g) were snap-frozen in liquid nitrogen and stored at −80°C until analyses. Residual feed intake and dry matter intake were different ($P < 0.001$) between low- and high-RFI groups. However, RFI groups neither differed ($P \geq 0.28$) in the initial and final weights of the RFI test, average daily gain, nor in pre-slaughter and carcass weights (Table 2 from Casal *et al.*, 2018).

Determination of lipid and protein oxidation markers
Frozen liver samples (300 mg) were homogenized with an extraction buffer (1.5 ml; 150 m*M* KCl, 20 m*M* EDTA, and 300 m*M* butyrate hydroxytoluene) using a handheld

Table 2 *Steer performances according to residual feed intake (RFI) phenotype (From Casal et al., 2018. Copyright with permission from Oxford University Press)*

	RFI group[1]			
Item	Low ($n=9$)	High ($n=9$)	SEM	*P*-value
RFI, kg DM/d	−0.69	0.90	0.62	<0.01
Feed intake, kg DM/d	9.2	10.6	0.53	<0.01
Average daily gain, kg/d	1.61	1.52	0.84	ns
Initial RFI test weight, kg	226.2	218.7	16.62	ns
Final RFI test weight, kg	364.7	354.4	19.89	ns
Slaughter weight, kg	520.6	508.7	11.45	ns
Carcass weight, kg	277.5	274.1	5.11	ns
Liver weight, kg	5.31	5.79	0.25	0.06

ns = not significant.
[1] RFI group: Steers were classified into two RFI groups: Low-RFI steers with RFI values 1.5 SD below the mean and high-RFI steers with RFI values 1.5 SD above the mean.

Table 3 *Hepatic oxidative stress markers of steers differing in residual feed intake (RFI) phenotype*

	RFI group[1]			
Item	Low ($n=9$)	High ($n=9$)	SEM	*P*-value
Oxidative stress markers				
TBARS, μg MDA/mg protein	15.7	17.4	0.12	0.05
Protein carbonyl, nmol DNPH/mg protein	0.57	0.65	0.02	0.05

TBARS = thiobarbituric acid reactive species expressed as concentration of malondialdehyde (MDA); DNPH = dinitrophenylhydrazine.
[1] RFI group low *v.* high (−0.62 *v.* 0.90 kg DM/d, respectively).

Pro200 homogenizer (Pro Scientific Inc., Oxford, CT, USA) at 10 000 r.p.m. during 1 min and the Bradford (Coomassie) assay was used to determine protein concentration of liver homogenates.

The thiobarbituric acid reactive species (**TBARS**) procedure for the determination of lipid oxidation was adapted from Gatellier *et al.* (2004) using a Varioskan Flash microplate reader (Thermo Electron Corp., Louisville, CO, USA). The concentration of malondialdehyde (**MDA**) was calculated using the molar extinction coefficient of the MDA (156 000 mol/cm; adjusted for the path length of the solution in the well). Results were expressed as μg MDA/mg of protein. The protein carbonyl assay, to evaluate protein oxidation, was performed according to Gatellier *et al.* (2004). The concentration of dinitrophenylhydrazine (**DNPH**) was calculated using the DNPH molar extinction coefficient (22 000 mol/cm; adjusted for the path length of the solution in the well). Results were expressed as nmol of DNPH/mg of protein. Details are given in Supplementary Material S1.

Quantitative real time qPCR

Total RNA isolated, synthesis of cDNA by reverse transcription, and primer design were performed according to Casal *et al.* (2018). Primers (Supplementary Table S1) to specifically amplify cDNA of eight target genes of antioxidant enzymes and three internal control genes (β-actin (*ACTB*); hypoxanthine phosphoribosyltransferase (*HPRT1*) and ribosomal protein L19 (*RPL19*)) were obtained from literature or specifically designed. Antioxidant enzymes candidate genes selected were: glutathione peroxidase 1 (*GPX1*); glutathione peroxidase 3 (*GPX3*); glutathione peroxidase 4 (*GPX4*); glutamate–cysteine ligase catalytic subunit (*GCLC*); copper zinc superoxide dismutase (CuZnSOD) soluble (*SOD1*); manganese superoxide dismutase (MnSOD) mitochondrial (*SOD2*); peroxiredoxin 3 (*PRDX3*); and peroxiredoxin 5 (*PRDX5*).

Real time qPCR reactions were performed according to Casal *et al.* (2018) in a total volume of 15 μl using a

Rotor-Gene 6000 (Corbett Life Sciences, Sidney, Australia). Gene expression was measured by relative quantification (Pflaffl, 2009) to the endogenous control and normalized to the geometric mean expression of internal control genes. Expression stability of three selected housekeeping genes was evaluated using the MS Excel add-in Normfinder (MDL, Aarhus, Denmark). The stability values obtained with Normfinder were 0.343, 0.379, and 0.285 for *ACTB, HPRT1,* and *RPL19,* respectively. Amplification efficiencies of 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 1.3 and 4.4%, respectively.

Western blot analysis

Total protein extracts in ice-cold RIPA buffer and electrophoresis were performed according to Casal *et al.* (2018). The immunochemical detection of 4-hydroxynonenal protein adduct (**4-HNE**), MnSOD, or β-actin proteins were performed using highly specific rabbit polyclonal anti-4-HNE antibodies (1 : 1000) mouse monoclonal anti-MnSOD (1 : 2000) and mouse monoclonal anti-β-actin (1 : 3000) (Abcam, Cambridge, UK). Membranes were incubated with anti-mouse IR Dye 680CW (Li-COR, Lincoln, NE, USA) at 1 : 15 000 or anti-rabbit IR Dye 800CW (Li-COR) at 1 : 20 000 and signals were detected using the Odyssey CLx Infrared Imaging System (Li-COR) and analyzed using Odyssey CLx and ImageJ software. Density of the area of each lane was measured. The relative levels of 4-HNE and MnSOD were normalized with β-actin density and normalized values for high-RFI were expressed as a percentage relative to the low-RFI group.

Hepatic antioxidant enzyme activity

The hepatic enzyme activities of glutathione peroxidase (**GPX**) and total superoxide dismutase (**SOD**) were determined spectrophotometrically using commercial kits (Ransel for GPX and Ransod for SOD; Randox Laboratories, Antrim, UK) in a Varioskan Flash microplate reader (Thermo) according to Reglero *et al.* (2009). Details are given
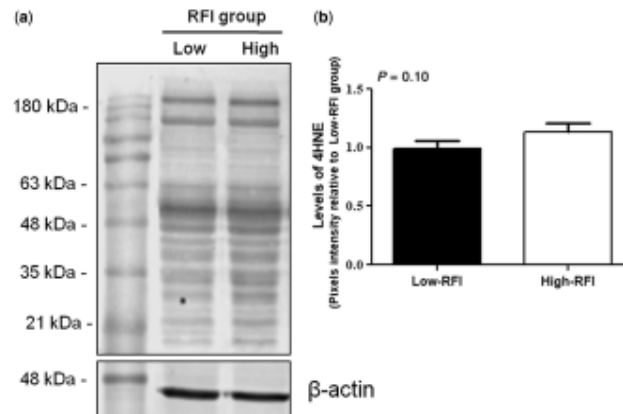
Figure 1 Representative western blot analysis of 4-hydroxynonenal protein adduct (4-HNE) in liver (a) according to steer RFI phenotype. Data were normalized with β-actin density and normalized values for high-RFI were expressed as a percentage relative to the low-RFI group. Means ± SEM of pixels (b) expressed according to steer RFI phenotype ($n = 9$ and 9 for low- and high-RFI steers, respectively). RFI = residual feed intake.

in Supplementary Material S2. The intra-assay CV was less than 8.4% and enzyme activities were expressed relative to mg of protein in the tissue homogenate.

Statistical analyses

Data were analyzed using the SAS System program (SAS 9.0V; SAS Institute Inc., Cary, NC, USA). UNIVARIATE analyses were performed on all variables to identify outliers and inconsistencies and to verify normality of residuals. All data were analyzed using the MIXED procedure with a model that included RFI group as fixed effect and slaughter date as a random effect. Mean separation was performed using the Tukey test, and differences were considered significant at $P \leq 0.05$ and a trend when $0.05 < P \leq 0.10$. Data are presented as least square means ± pooled standard errors.

Results

Hepatic lipid and protein oxidation

Hepatic TBARS concentration was greater ($P = 0.05$; Table 3) and 4-HNE expression tended to be greater ($P = 0.10$; Figure 1 and Supplementary Figure S1) for high- than low-RFI steers. Protein carbonyls in liver homogenates were greater ($P = 0.05$) for high- than low-RFI steers (Table 3).

Hepatic gene expression of antioxidant enzymes

Hepatic expression of antioxidant enzymes GPX4, GCLC and PRDX5 mRNA was greater ($P \leq 0.05$) and expression of GPX3 mRNA tended ($P = 0.10$) to be greater for low- than high-RFI steers (Table 4). However, GPX1, SOD1, SOD2, and PRDX3 mRNA expression did not differ between RFI groups (Table 4). Residual feed intake values and GPX4 mRNA were negative and highly correlated ($r = -0.66$, $P < 0.01$, $n = 18$).

Hepatic activity and protein expression of antioxidant enzyme

Hepatic activity of GPX tended ($P = 0.10$) to be greater in low- than high-RFI steers (Table 5). Activity of SOD and CuZnSOD in the liver did not differ between RFI groups. However, both activity (Table 5) and protein expression of MnSOD tended ($P = 0.09$) to be greater in low- than high-RFI steers (Figure 2 and Supplementary Figure S2). Hepatic GPX activity was highly and negative correlated to 4-HNE expression ($r = -0.61$, $P < 0.02$, $n = 18$).

Discussion

In agreement with previous reports, low-RFI steers had reduced dry matter intake with similar growth rate, body (and carcass) weight than high-RFI steers (Casal et al., 2018). Durunna et al. (2011) report that the relative classification of a steer as highly or lowly feed efficient was repeatable between growing and finishing phases whether steers changed the diet. Moreover, positive and moderate correlations between RFI values derived from forage- and grain-based diets in heifers were reported by Trujillo et al. (2013) and Cassady et al. (2016).

High-RFI steers exhibited greater hepatic protein carbonyls, TBARS, and 4-HNE levels than low-RFI steers. Protein and lipid oxidation can be used as an indicator of oxidative stress. Protein carbonyls, such as TBARS and 4-HNE, reflect not only damaged molecules that must be degraded but also act in a toxic manner, causing further oxidative stress inside and outside of cells by oxidizing proteins and DNA (Del Rio et al., 2005; Ayala et al., 2014). Protein oxidation and repair would represent an energetic drain, decreasing energetic efficiency as oxidized proteins are no longer usable and are marked for degradation via proteolytic ATP-dependent ubiquitin system

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Table 4 *Hepatic gene expression of antioxidant enzymes of steers differing in residual feed intake (RFI) phenotype*

| Gene | RFI group[1] | | SEM | P-value |
	Low (n = 9)	High (n = 9)		
GCLC	1.16	0.81	0.15	0.05
GPX1	0.94	1.01	0.34	0.62
GPX3	0.21	0.41	0.13	0.10
GPX4	1.29	0.62	0.23	<0.01
PRDX3	1.12	0.74	0.21	0.18
PRDX5	1.04	0.70	0.12	0.02
SOD1	0.95	0.90	0.28	0.71
SOD2	1.00	1.05	0.34	0.82

GCLC = glutamate–cysteine ligase catalytic subunit; GPX1 = glutathione peroxidase 1; GPX3 = glutathione peroxidase 3; GPX4 = glutathione peroxidase 4; PRDX3 = peroxiredoxin 3; PRDX5 = peroxiredoxin 5; SOD1 = copper zinc superoxide dismutase 1, soluble; SOD2 = manganese superoxide dismutase 2, mitochondrial.
[1] RFI group low v. high (−0.62 v. 0.90 kg DM/d, respectively).

Table 5 *Hepatic antioxidant enzyme activity of steers differing in residual feed intake (RFI) phenotype*

| Item | RFI group[1] | | SEM | P-value |
	Low (n = 9)	High (n = 9)		
Antioxidant enzyme activity				
GPX activity, U/mg protein	284.2	255.8	10.9	0.10
SOD total activity, U/mg protein	18.7	16.5	1.9	0.15
MnSOD activity, U/mg protein	13.4	11.2	1.0	0.09
CuZnSOD activity, U/mg protein	5.3	4.6	1.4	0.20

GPX = glutathione peroxidase; SOD = superoxide dismutase; CuZnSOD = copper zinc superoxide dismutase; MnSOD = manganese superoxide dismutase.
[1] RFI group low v. high (−0.62 v. 0.90 kg DM/d, respectively).

process (Mehlhase and Grune, 2002). Similar to our results, Sandelin *et al.* (2005) reported greater protein carbonyl content in muscle samples of lowly feed efficiency steers when compared to highly efficient steers. Conversely, studies in plasma and liver isolated mitochondria of cattle, reported greater protein carbonyl content in low than high feed efficiency animals (Russell *et al.*, 2016; Zulkifli, 2016). Xu *et al.* (2014), who studied the relationship between oxidative stress and physiology in mice, reported differences between liver and serum concentrations of TBARS and protein carbonyls, the discrepancy between our and previous studies may reflect tissue differences and/or even dietary differences.

Mitochondria are major sites of intracellular oxidants production, first reported by Boveris and Chance (1973). Eleven different sites of oxidant formation associated with substrate catabolism and the electron transport chain can be found, mainly in complexes I and III (see review Brand, 2016). Greater oxygen consumptions should result in more ROS/RNS production which could increase the content of oxidized proteins or lipids in this subcellular fraction compared to determinations in whole tissue. On the other hand, ROS is a normal

product of oxygen metabolism and result from a variety of intracellular mechanisms mainly related to the NADPH oxidase complexes which are found in most cell membranes (mitochondria, peroxisomes, and endoplasmic reticulum; Murphy, 2009; Brand, 2016). Therefore, the level of oxidant production is not only based on the inefficiency of mitochondria, but also other sources of oxidants may have greater relevance depending on the sample or tissue that is analyzed.

The greater hepatic oxygen consumption recorded by high-resolution respirometry in low- *v.* high-RFI steers at the same level of mitochondrial complex substrates (glutamate/malate or succinate) reflected a greater capacity to obtain ATP per unit of substrate (Casal *et al.*, 2018). The increased metabolic rate could potentially cause increased oxidant production and result in oxidative stress (Speakman, 2008). However, ROS/RNS are not simply generated in direct proportion to oxygen consumption, and furthermore, animals can potentially upregulate a variety of antioxidant defenses in response to increased oxidant production, repairing oxidative stress, and limiting its subsequent impact. The cellular response to damage as oxidative stress is controlled by the coordinated function of multicellular regulatory factors. These processes must be tightly regulated and precisely coordinated in order to sustain cellular homeostasis. Recently, a system regulated by the nuclear factor (erythroid-derived 2)-like 2 (Nrf2 or NFE2L2)-Kelch-like ECH-associated protein 1(Keap1) or Nrf2-Keap1 pathway has been proposed as one of the most important multiple-stress response systems. Indeed, transcription factor *NFE2L2* is a major regulator of genes encoding antioxidant proteins in response to oxidative stress (Kobayashi *et al.*, 2004).

Mitigation and prevention of oxidative stress occurred by various antioxidant (enzymatic and no enzymatic) systems (Turrens, 2003). The enzymatic antioxidant system constitutes the first defense against ROS/RNS; SOD convert the superoxide to hydrogen peroxide (H_2O_2), and GPX and PRDX together with other enzymes are responsible for reducing H_2O_2, peroxynitrite, free fatty acid hydroperoxides, and other peroxides to less toxic compounds (Quijano *et al.*, 2016; Radi, 2018). Greater antioxidant status in low-RFI steers could reduce the oxidized protein and lipid turnover which could impact the efficiency in the use of energy.

The SOD family presented three forms, each one encoded by a different gene, cytosolic CuZnSOD (SOD1), mitochondrial MnSOD (SOD2), and extracellular CuZnSOD (SOD3), in addition, it is reported that CuZnSOD is an important antioxidant in the intermembrane space of mitochondria (Sheng *et al.*, 2014). In the present study, no differences were found between RFI groups neither in hepatic gene expression of *SOD1* or *SOD2* mRNA nor in total SOD or CuZnSOD enzyme activity. However, protein expression and enzyme activity of MnSOD, which are localized in the mitochondrial matrix and act as the primary defense against superoxide produced (Epperly et al., 2002), tended to be greater (+13%) in low- than high-RFI steers. Zulkifli (2016), in a study of quantitative trait loci using single nucleotide polymorphism markers for RFI, proposed SOD2 as a candidate gene related to mitochondrial function that might affect RFI due to its
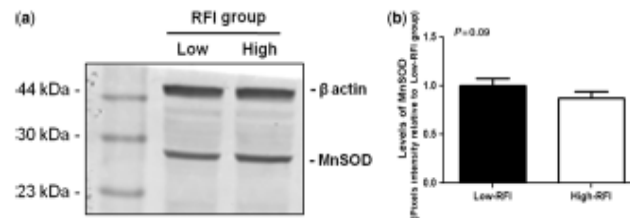
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Figure 2 Representative western blot analysis of manganese superoxide dismutase (MnSOD) in according to steer RFI phenotype (a). Data were normalized with β-actin density and normalized values for high-RFI were expressed as a percentage relative to the low-RFI group. Means ± SEM of pixels (b) expressed according to steer RFI phenotype (n = 9 and 9 for low- and high-RFI steers, respectively). RFI = residual feed intake.

importance in the local regulation of the level of oxidative stress. Similar to our study, Alexandre et al. (2015), using total RNA sequencing, did not report differences in the expression of *SOD2* in animals of different RFI phenotype. Thus, changes in MnSOD protein expression and activity in low-RFI steers appear to be not associated to changes in gene expression, which could indicate possible post-translational modifications.

Glutathione peroxidases comprise an enzyme family including several isoenzymes (GPX 1 to 8, humans) encoded by different genes which vary in cellular location and substrate specificity, at the expense of glutathione (Toppo et al., 2009). In the present study, low-RFI had greater GPX activity and greater liver mRNA expression of *GPX3*, *GPX4*, and *GCLC* mRNA than high-RFI steers. The glutathione is synthesized in the cytosol and imported into the mitochondria; glutamate–cysteine ligase is the rate limiting enzyme in glutathione synthesis (Bolaños et al., 1996). The higher gene expression of the catalytic unit of glutamate–cysteine ligase (*GCLC*) indicates that low-RFI steers could be able to synthesize more glutathione or synthesized it at a higher rate than high-RFI steers. The GPX4 is one of the main cellular defenses against oxidative stress to cell membranes and serves to scavenge lipid hydroperoxides, increased *GPX4* mRNA could protect lipid peroxide products (Ran et al., 2004). In agreement with these previous finding, we detected a negative correlation between GPX activity and 4-HNE levels in low-RFI steers. In contrast to our work, Kidrick et al. (2016) reported that serum GPX activity and serum total antioxidant capacity were not correlated with RFI in grazing heifers. Additionally, Russell et al. (2016) reported in red blood cell lysate that not only GPX activity but also total SOD and MnSOD activities were increased in the lowly v. the highly efficient roughage-grown steers while no differences in SOD activity was detected in corn-grown fed steers differing in feed efficiency. Thus, disagreement between our and the latter studies may reflect tissue differences and/or even dietary differences or animal growing phases (Xu et al., 2014).

Peroxiredoxins comprise other family enzymes that participate in the detoxification of different peroxides. Among them, PRDX3 (exclusively located in mitochondria) and PRDX5 (found in various compartments in the cell, including peroxisomes and mitochondria) reduce most of H_2O_2 and peroxynitrite produced in the mitochondria (Cox et al., 2010). We detected greater expression of *PRDX5*

mRNA in low- than high-RFI steers which is in line with the greater antioxidant ability near to oxidants production site. In this sense, Banmeyer et al. (2005) reported that overexpression of PRDX5 was shown to protect from H_2O_2-mediated mitochondrial DNA damage. In addition, given the multiple subcellular localization, the increase in expression of PRDX5, without changes in the expression of PRDX3, could indicate better protection against the extra-mitochondria oxidants produced.

The trend to a greater hepatic activity of the enzyme and the genetic and protein expression of components of the anti-oxidant system (GPX, GPX4, GCLC, MnSOD, and PRDX5) combined with a lower content of hepatic oxidation markers of lipids and proteins (TBARS, 4-HNE, and protein carbonyl) suggest that low-RFI animals would have a 'more coordinated' antioxidant system in the liver. Kong et al. (2016), in a proteomic study of broiler breast muscle tissue, report that the activation of *NFE2L2* led to an increase in the expression of some of the enzymes of the antioxidant system (GPX, SOD, and PRDX). Therefore, it is possible that the increase in apparent antioxidant capacity in the liver of low-RFI animals would be due to greater expression or activity of *NFE2L2*; however, this requires future research.

Conclusion

The high feed efficiency of low-RFI steers was related to better hepatic oxidative balance – greater hepatic antioxidant ability near to oxidants production site, and therefore less hepatic oxidative stress – than high-RFI steers. Decreased hepatic oxidative stress would reduce maintenance requirements due to a lower protein and lipid turnover and better efficiency in the use of energy. Further research is needed to identify the specific mechanism involved in these different physiological processes between animals with low and high feed efficiency.

Acknowledgments

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Table 2 from Casal A, Garcia-Roche M, Navajas EA, Cassina A and Carriquiry M 2018. Hepatic mitochondrial function in Hereford steers with divergent residual feed intake phenotypes. Journal of Animal Science 96, 4431–4443, Copyright © 2018 with permission from Oxford University Press.

Declaration of interest

Authors do not have any actual or potential conflict of interest; financial, personal, or other relationships with other people or organizations.

Ethics statement

Animal procedures were approved by the Animal Experimentation Committees of Universidad de la República and National Agricultural Research Institute (Uruguay).

Software and data repository resources

Data is not deposited in an official repository.

Supplementary material

To view supplementary material for this article, please visit https://doi.org/10.1017/S1751731119001332.

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SUPPLEMENTARY MATERIAL

Supplementary Material S1

Determination of lipid and protein oxidation markers

Frozen liver samples (300 mg) were homogenized with an extraction buffer (1.5 mL; 150 mM KCl, 20 mM EDTA and 300 mM butyrate hydroxytoluene) using a hand-held Pro200 homogenizer (Pro Scientific Inc, USA) at 10000 rpm during 1 min and the Bradford (Coomassie) assay was used to determine protein concentration of liver homogenates.

The thiobarbituric acid reactive species (**TBARS**) procedure for the determination of lipid oxidation was adapted from Gatellier*et al.* (2004). Briefly, 500 µL of liver homogenate was centrifuged at 2000 xg for 10 min and 100 µL of the supernatant was incubated with 100 µL of a 2-thiobarbituric acid (**TBA**)-trichloroacetic acid (**TCA**) solution (35 mM TBA and 10% TCA in 125 mMhydrochloric acid; **HCl**) in a boiling water bath for 30 min. After cooling in an ice bath for 5 min and kept at room temperature for 45 min, the pink chromogen was extracted with 400 µL of n-butanol and phase separation by centrifugation at 3000 xg during 10 min. The absorbance of the supernatant was measured at 535 nm using a Varioskan Flash microplate reader (Thermo Electron Corp., Louisville, CO, USA). The concentration of malondialdehyde (**MDA**) was calculated using the molar extinction coefficient of the MDA (156000 mol/cm; adjusted for the path length of the solution in the well). Results were expressed as µg MDA/mg of protein.

The protein carbonyl assay, to evaluate protein oxidation, was performed according to Gatellier*et al.* (2004).Briefly, two aliquots (200 µL each) of liver homogenates were centrifuged at 2000 x g for 10 min and incubated with 200 µL of 2 N HCl (blank) or with 200 µL of 20 mM dinitrophenylhydrazine (**DNPH**) in 2 N HCl, for 1 h at room temperature with regular stirring. After addition of 20% TCA (200 µL), aliquots were incubated at room temperature for 15 min with regular stirring and centrifuged at 2000 x g for 10 min. Pellets were washed three times with 400 µL of ethanol:ethyl acetate (1:1), centrifuging each time, to eliminate traces of DNPH. Pellets were dissolved in 600 µL of 6 M guanidine HCl with 20 mM KH$_2$PO$_4$, pH 2.5, incubated at room temperature for 15 min with regular stirring and centrifugedat 2400 x g for 10 min, the absorbance of the supernatant was measured at 370 nm in a **Varioskan** Flash microplate reader (Thermo), subtracting the blank and the

concentration of DNPH was calculated using the DNPH molar extinction coefficient (22000 mol/cm; adjusted for the path length of the solution in the well). Results were expressed as nmol of DNPH/mg of protein.

Supplementary Material S1

Hepatic antioxidant enzyme activity

The hepatic enzyme activities of glutathione peroxidase (**GPX**) and total superoxide dismutase (**SOD**) were determined spectrophotometrically using commercially kits (Ransel for GPX and Ransod for SOD; Randox Laboratories, Antrim, UK) in a Varioskan Flash microplate reader (Thermo) according to Reglero*et al.* (2009). Briefly, liver (200 mg) was homogenized with 2 mL of ice-cold buffer (1.15% KCl in 10 mM Na-KH$_2$PO$_4$buffer, pH 7.4, with 20 mM EDTA) using a hand-held Pro200 homogenizer (PRO Scientific) at 10000 rpm during 1 min, centrifuged at 2000 *xg* for 10 min and supernatant collected. For GPX activity (Ransel assay, Randox Laboratories), 100 µL of supernatant of liver homogenate were diluted with 200 µL of diluting agent. In the reaction cell at 37°C, 220 µL of a reagent containing 4 mM glutathione, 0.5 U/L glutathione reductase and 0.34 mM NADPH (diluted in 0.05 M phosphate buffer, pH 7.2, with 4.3 mM EDTA) were mixed with 5 µL of diluted sample. Fifteen seconds later, 10 µL of 0.18 mM cumene hydroperoxide was added, and the absorbance was read at 340 nm between 75 and 195 seconds after sample addition. The enzyme activity was calculated by multiplying the increase in the absorbance per min by a factor of 8412.

For SOD activity (Ransod assay, Randox Laboratories), 20 µL supernatant of liver homogenates were diluted with 300 µL of 0.01 M phosphate buffer, pH 7.0. In the reaction cell at 37 °C, 170 µL of mixed substrate (containing 0.05 mM xanthine and 0.025 mM INT was mixed with 5 µL of diluted sample, and 15 s later, 25 µL of 80 U/L of xanthine oxidase was added. The mixed substrate was prepared in 40 mM of N-cyclohexyl-3-aminopropane-sulfonic acid buffer, pH 10.2, with 0.94 mM EDTA. The absorbance was read at 505 nm between 45 and 225 seconds after sample addition and the kinetics of the enzyme activity were calculated based on a calibration curve performed with SOD standards at concentrations ranging from 0.21 to 5.7 UI/mL in 0.01 M phosphate buffer, pH 7. Activity of MnSOD was determined by inhibiting CuZnSOD activity with 3 mM potassium cyanide; CuZnSOD was subsequently determined by subtracting MnSOD activity from total SOD activity.

Supplementary *Table S1* *Primers used for real time qPCR quantification.*

Gene	Accession#[1]		Primer sequence[2]	Length (bp)	Efficiency
ACTB	BT030480	F	CTCTTCCAGCCTTCCTTCCT	178	1.02
		R	GGGCAGTGATCTCTTTCTGC		
GCLC	NM_001083674	F	CACAAATTGGCAGACAATGC	211	1.20
		R	GGCGACCTTCATGTTCTCAT		
GPX1	NM_17407	F	ACATTGAAACCCTGCTGTCC	216	1.17
		R	TCATGAGGAGCTGTGGTCTG		
GPX3	NM_174077	F	TGCAACCAATTTGGAAAACA	224	1.11
		R	TTCATGGGTTCCCAGAAAAG		
GPX4	NM_001346431.1	F	AGCCAGGGAGTAATGCAGAG	203	1.14
		R	CACACAGCCGTTCTTGTCAA		
HPRT1	XM_580802	F	TGGAGAAGGTGTTTATTCCTC	105	1.03
		R	CACAGAGGGCCACAATGTGA		
PRDX3	NM_174432.2	F	CACACCAGAAAAGAGCCACA	210	1.13
		R	CTAGCCATCCATCCACACCT		
PRDX5	NM_174749.2	F	CCTTCTACCTCAGCCTCGAG	245	1.15
		R	CAACCTTAATCGGGGCCATG		
RPL19	NM_001040516.1	F	CCCCAATGAGACCAATGAAATC	156	1.09
		R	CAGCCCATCTTTGATCAGCTT		
SOD1	NM_174615	F	AGAGGCATGTTGGAGACCTG	189	1.14
		R	CAGCGTTGCCAGTCTTTGTA		
SOD2	NM_201527.2	F	CAGGGACGCTTACAGATTGC	212	1.03
		R	CTGACGGTTTACTTGCTGCA		

ACTB = β-actin; *GCLC* =glutamate-cysteine ligase catalyticsubunit; *GPX* =glutathioneperoxidase 1; *GPX3* = glutathioneperoxidase 3; *GPX4* =glutathioneperoxidase 4; *HPRT1* = hypoxanthinephosphoribosyltransferase; *PRDX3* =peroxiredoxin 3; *PRDX5* =peroxiredoxin 5; *RPL19* =ribosomalprotein L19; *SOD1* =copper zinc superoxidedismutase 1, *SOD2* = soluble manganesesuperoxidedismutase 2, mitochondrial

[1]Gene bank sequences. [2]F = foreword; R = reverse.
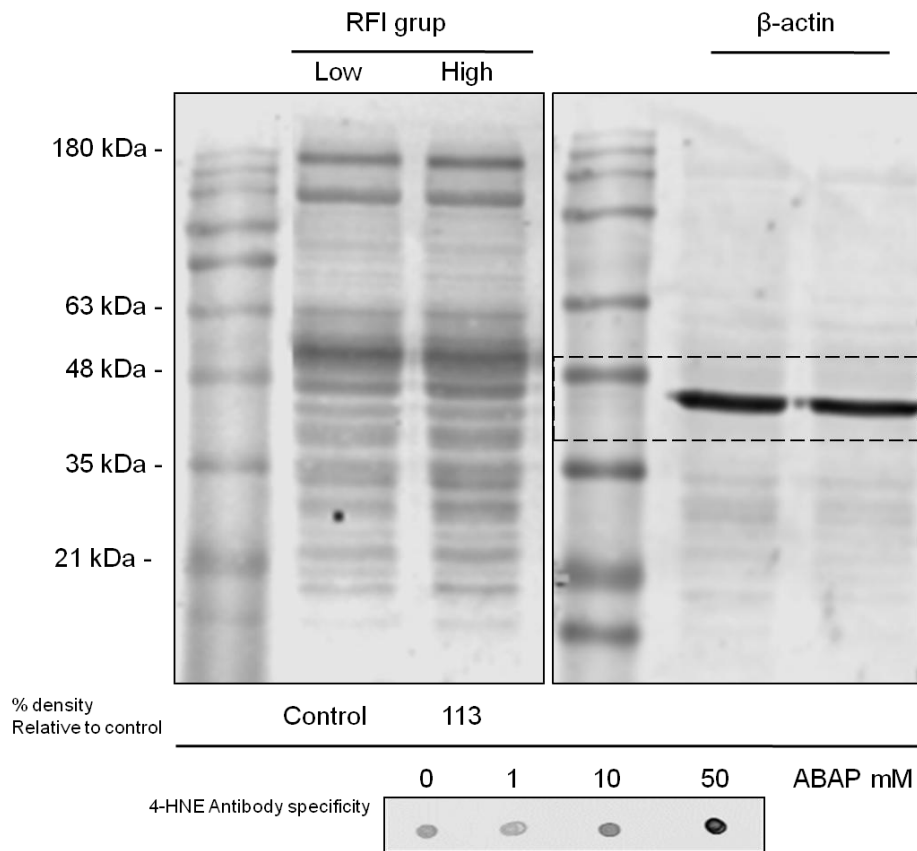
Supplementary Figure S1: Representative western blot analysis of 4-hydroxynonenal protein adduct (4-HNE) in liver. Expressed according to steer residual feed intake (RFI) phenotype. A quantity of 30 μg of proteins were loaded and separated by SDS-PAGE. Immunoblotting was performed with apolyclonal antibody thatrecognized 4-HNE. Data were normalized with β-actin density and normalized values for high-RFI were expressed as a percentage relative to the low-RFI group (Control). Images of 4-HNE (800 nm) and β-actin (680 nm)are from the same membrane.To check the specificity of the anti 4-HNE antibody, a dot blot was performed using liver homogenates incubated with different concentrations of 2,2'-azobis (2-amidinopropane) (ABAP, lipid peroxidation promoter).
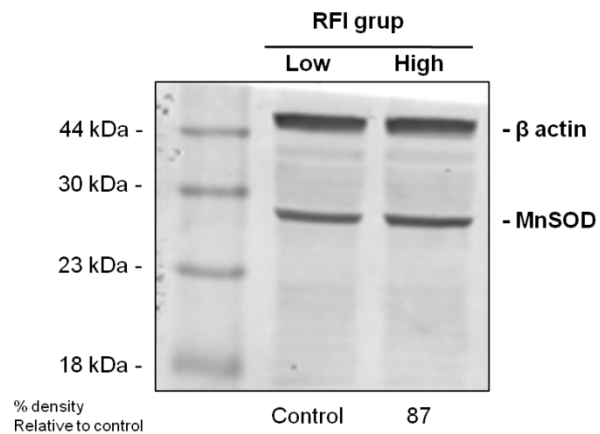
Supplementary Figure S2: Representative western blot analysis of manganese superoxide dismutase (MnSOD) in liver. Expressed according to steer residual feed intake (RFI) phenotype. A quantity of 30 μg of proteins were loaded and separated by SDS-PAGE. Immunoblotting was performed with a monoclonal antibody that recognized MnSOD. Data were normalized with β-actin density and normalized values for high-RFI were expressed as a percentage relative to the low-RFI group (Control). Images of MnSOD and β-actin (both 680 nm) from of the same membrane.

4. MASA Y COMPOSICIÓN DE LA CANAL Y DE LOS ÓRGANOS EN VACAS DE CARNE PURAS Y CRUZA PASTOREANDO DIFERENTES OFERTAS DE FORRAJE DE CAMPO NATURAL.

CASAL, A, SOCA, P , CARRIQUIRY, M. 2017. Veterinaria (Montevideo), 206, 25 – 34.

SMVU

Masa y composición de la canal y de los órganos en vacas de carne puras y cruza pastoreando diferentes ofertas de forraje de campo natural

Mass and composition of carcass and organs in pure and cross breed beef cows grazing different forage allowances of native pastures.

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Resumen

El objetivo fue evaluar el efecto de la oferta de forraje y genotipo de los animales sobre la masa y composición de la canal y órganos en vacas de cría pastoreando campo natural. Se utilizaron 32 vacas adultas en un diseño de bloques completamente al azar con un arreglo factorial de oferta de forraje (4 y 2,5 kg MS/kg PV; AOF vs. BOF) y genotipo de las vacas (puras: Hereford y Aberdeen Angus y sus cruzas recíprocas F1; PU vs. CR). El experimento se realizó durante tres años; al final del tercer año las vacas fueron sacrificadas a 190 ± 10 días postparto, y la canal y los órganos fueron disecados, pesados y se tomaron muestras que fueron inmediatamente congeladas para análisis de composición química. El peso absoluto y relativo de la canal fue mayor ($P \leq 0.02$) en vacas en AOF que en BOF, y tendió a ser mayor ($P = 0.08$) en vacas CR que PU. Las vacas en AOF presentaron mayor rendimiento ($P \leq 0.05$) y mayor proporción de tejidos blandos ($P = 0.03$) en la canal que las vacas en BOF. Las vacas en AOF presentaron mayor ($P = 0.05$) peso absoluto de la masa total de órganos que las vacas en BOF, mientras que la masa relativa al peso de la canal del total de órganos fue mayor ($P = 0.05$) en las vacas PU que CR. Estos resultados en conjunto indicarían que animales pastoreando AOF y CR serían más eficientes en el uso de la energía para producción de carne, dado un mayor rendimiento carnicero y menor relación entre el peso total de órganos y peso de la canal.

Palabras clave: bovinos de carne, canal, órganos, pastoreo.

Summary

The aim was to evaluate the effect of two herbage allowances of native pastures on the mass and carcass and organs composition. Mature beef cows (n = 32) were used in a randomized complete block design with a factorial arrangement of herbage allowance (4 and 2.5 kg DM / kg BW; HI vs. LO) and cow genotype (pure: Hereford and Aberdeen Angus and their reciprocal crosses F1, PU vs. CR). The experiment was conducted during three years; at the end of the third year, cows were slaughtered at 190 ± 10 days postpartum and carcass and organs were dissected, weighed and samples were collected and immediately frozen for chemical composition analysis. The absolute and relative carcass weight was greater ($P \leq 0.02$) for HI than LO cows, and tended to be greater ($P = 0.08$) for CR than PU cows. Cows grazing HI showed greater ($P \leq 0.05$) carcass yield and greater ($P = 0.03$) proportion of soft tissues in the carcass than LO cows. Absolute weight of total organ mass was greater ($P = 0.05$) for HI than LO cows while relative weight of total organ mass (expressed relative to carcass weight) cows was greater ($P = 0.05$) for PU than CR cows. These results would indicate that animals grazing HI and of CR genotype would be more efficient in the use of the energy for beef production, given a greater carcass yield and reduced total organ and carcass weights ratio.

Keywords: beef cattle, carcass, organs, rangelands.

Introducción

Las vacas de carne presentan una importante capacidad de convertir forrajes de baja calidad en proteínas de alta calidad para el consumo humano. No obstante, sólo el 5% del total de energía consumida durante el ciclo de vida es utilizado para la deposición de proteínas (Ritchie, 2000). Las principales razones de la ineficiencia de la producción de carne vacuna, se debería a la relativamente baja y lenta tasa de reproducción y el alto costo energético para su mantenimiento (Ferrell y Jenkins, 1985). Los órganos vinculados al funcionamiento de los sistemas digestivo, respiratorio, circulatorio y actividades de excreción constituyen una menor proporción del peso del cuerpo (15-25%), pero no obstante representan más del 50% de los costos de mantenimiento (Baldwin y col., 2004; Ferrell, 1988; Seal y Reynolds, 1993). Existe considerable evidencia que indica que la masa total de órganos difiere entre genotipos, entre estados fisiológicos y entre planos nutricionales (Baldwin, 1995; Baldwin y col., 2004; Casal y col., 2014; Ferrell, 1988; Jenkins y col., 1986) y que estas diferencias pueden traducirse en variaciones en las necesidades de energía de mantenimiento (Baldwin, 1995; Jenkins y col., 1986).

Considerando el uso global de la energía para los tejidos que la producción ganadera prioriza (ej. canal o musculo esquelético magro) se puede considerar a la energía utilizada por la masa total de los órganos como un "impuesto a la producción" (Reynolds, 2002). El costo de mantenimiento tiene un impacto significativo en la partición de la energía metabolizable mantenimiento y producción, por lo tanto puede afectar la eficiencia biológica y económica de la producción de carne

El control de la intensidad de pastoreo a través del cambio en la oferta de forraje y el uso de genotipos cruza ha permitido mejorar la respuesta productiva y reproductiva de las vacas de cría (Carriquiry y col., 2012). Esta mejor respuesta productiva y reproductiva de las vacas se asocia a un mejor balance energético, esta mejora en el balance de energía 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 (Carriquiry y col., 2012; Casal y col., 2014; 2017; Do Carmo y col., 2016; Soca y col., 2013). 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 mejoraría la comprensión de la eficiencia global del sistema de producción ganadero.

Este trabajo tiene como hipótesis que el control de la intensidad de pastoreo de campo natural, a través del manejo de la oferta de forraje, afecta la masa y composición de la canal así como de los órganos de vacas de cría puras (Hereford y Angus) y cruzas F1. Por lo tanto puede explicar - en parte - las diferencias en el comportamiento productivo-reproductivo entre diferentes ofertas de forraje y genotipos. El objetivo fue evaluar el efecto de dos ofertas de forraje (alta vs. baja) y el genotipo de los animales sobre el peso y composición de la canal y órganos en vacas de cría pastoreando de campo natural.

Materiales y métodos

El protocolo experimental fue aprobado y realizado de acuerdo con las normas de experimentación animal de la Comisión Honoraria de Experimentación Animal (CHEA) de la Universidad de la República.

Diseño experimental

El experimento se llevó a cabo sobre 95 ha de campo natural (bioma Campos) en la Estación Experimental Bernardo Rosengurtt (Facultad de Agronomía, Universidad de la República, Uruguay, 32° S 54° W) desde junio 2007 hasta mayo 2010. Se utilizaron 32 vacas adultas (multíparas, 4 a 5 años) en un diseño de bloques completamente al azar con dos repeticiones en el espacio (según tipo de suelo: bloque 1: 60 ha suelo franco arenoso y bloque 2: 35 ha suelo franco arcilloso; cuatro parcelas en cada bloque) y un arreglo factorial 2 x 2 de oferta de forraje (OF) y genotipo de la vaca [GV, razas puras: Hereford (H) y Aberdeen Angus(A) y sus cruzas recíprocas F1 (HA y AH); PU vs. CR].

La OF, se estimó como la relación entre la masa de forraje y la carga animal (kg de materia seca (MS) por kg de peso vivo (PV); Sollenberger y col., 2005) y representó en el promedio anual 4 y 2,5 kg MS/kg PV (alta y baja oferta respectivamente, AOF vs. BOF) aunque fue variable a lo largo del año (5, 3, 4 y 4 kg MS/kg PV y 3, 3, 2 y 2 kg MS/kg PV para AOF y BOF en otoño, invierno, primavera y verano, respectivamente). La OF se ajustó mensualmente después de medir la disponibilidad de forraje en cada parcela (Haydock y Shaw, 1975) por el método de "put-and-take" (Mott, 1960). Las vacas experimentales se mantuvieron a lo largo del experimento en la misma parcela y en caso de ser necesario se añadieron o eliminaron vacas de similar genotipo y estado fisiológico ("animales volantes") basado en la disponibilidad de forraje. Los tratamientos de OF difirieron en masa de forraje y altura a lo largo del año (2072 vs. 1338 ± 160 kg MS/ha y 5.2 vs. 3.5 ± 0.3 cm de promedio anual para AOF vs. BOF, respectivamente), pero no difirieron (P ≥ 0.27) en su composición química [7.3, 8.6, 10.5 y 8.6 ± 0.5% PC y 42.6, 41.1, 36.2 y 40.2 ± 1.6 % FDA (base seca) en otoño, invierno, primavera y verano, respectivamente].

Las vacas pertenecían a un grupo de animales experimentales generados como parte de un experimento dialélico de cruzamiento que se llevó a cabo durante 10 años en la Estación Experimental (Espasandin y col., 2010). Fueron evaluadas 8 vacas por tratamiento (AOF-CR, AOF-PU, BOF-CR, y BOF-PU; n = 4 para cada genotipo individual: H y A para PU o HA y AH para CR).Al comienzo del experimento (junio 2007) el PV y condición corporal (CC; escala de 1 a 8; Vizcarra y col., 1986) no difirieron (P > 0.20) entre los grupos (447 ± 58 kg y 4,1 ± 0.5 unidades, respectivamente). Las vacas pastorearon en la misma OF (AOF o BOF) y gestaron y lactaron un ternero cada año desde 2007 a 2010.

Muestreo y colecta de datos

Al final del tercer año experimental (mayo 2010), a los 192 ± 10 días postparto (45 días post destete) las vacas fueron sacrificadas en un matadero comercial (PUL SA; Cerro Largo, Uruguay, 40 km de la Estación Experimental). Previo al transporte, se registró el PV y la CC de todas las vacas.

Las vacas fueron aturdidas con una pistola de bala cautiva y luego fueron desangradas. El volumen de sangre se estimó de acuerdo a Hansard y col. (1953). Los animales desangrados se dividieron en cuero, extremidades, cabeza, tracto gastrointestinal (TGI), menudencias (tráquea, pulmones, corazón, diafragma, hígado, riñones), y canal. Los órganos de TGI y las menudencias fueron disecados y pesados individualmente. El peso de cada componente u órgano individual fue registrado y se recogieron muestras representativas que se almacenaron a -20°C para los análisis de composición química. Las muestras (100 a 200 g) fueron colectadas del TGI [retículo-rumen (~20 cm del esfínter pilórico, saco dorsal), omaso y abomaso (~20 cm del esfínter pilórico, curvatura menor), intestino delgado (~5 m unión ileocecal, yeyuno), intestino grueso (~2.5 m unión ileocecal, colon)], vísceras rojas [pulmones (lóbulo apical), tráquea (~20 cm de la laringe), corazón (ventrículo izquierdo), diafragma (zona muscular y fibrosa), riñones (lóbulos caudales) e hígado (lóbulo dorsal). El peso de algunos órganos abdominales y pélvicos (bazo, páncreas, útero, vejiga) no se obtuvieron en su totalidad. El peso de la canal se registró antes y después del procedimiento de dressing. Se colectaron muestras de una sección representativa de la canal (media canal derecha) ubicada entre la 11ª y 13ª costilla (Hedrick, 1983). Esta sección se disecó en los tejidos blandos (tejido muscular, adiposo y conectivo) y el hueso, que se pesaron por separado y una muestra de los mismos se congeló a -20°C para la determinación de su composición química.

Análisis de composición química

Las muestras de todos los órganos/tejidos se molieron en nitrógeno líquido y se analizó el contenido de agua (secado a 105°C en un horno de aire forzado durante 48h), proteína (método Kjeldahl; proteína=nitrógeno x 6,25), lípidos totales (extracto etéreo; en extractor Soxhlet durante 20 h) y minerales (cenizas; incineración en horno mufla a 550°C durante 16h) según normas de la AOAC (2000). La relación proteína:grasa se calculó dividiendo la cantidad de proteína por la cantidad de grasa de los diferentes tejidos.

Cálculos y análisis estadísticos

Luego del sacrificio, se estimó el peso corporal vacío (PCV) como el peso de la canal más el peso total de extremidades, cabeza, cuero, sangre, órganos y vísceras (Hersom y col., 2004b). Los datos fueron analizados utilizando el paquete estadístico SAS Systems Program (SAS 9.0V; SAS Inst, Cary, NC, USA). Se utilizó el procedimiento UNIVARIATE para identificar valores atípicos e inconsistencias y para verificar la normalidad de los residuales. El análisis de los datos se realizó mediante el procedimiento MIXED, usando un modelo mixto que consideraba la OF, el GV y su interacción como efectos fijos y el bloque como un efecto aleatorio. La separación de medias se realizó mediante la prueba de Tukey. Se considera-

ron diferencias significativas, valores de $P \leq 0.05$ y tendencias cuando $0.05 < P \leq 0.10$. Los resultados se presentan como medias de cuadrados mínimos ± error estándar.

Resultados

Peso vivo, peso corporal vacío y condición corporal

En promedio, durante los tres años de experimentación, el PV y CC fueron mayores ($P \leq 0.05$) para vacas que pastorearon AOF vs. BOF y en las vacas CR vs. PU, siendo éstas diferencias más evidentes durante el último ciclo de gestación-lactancia (2009 – 2010, Figura 1).
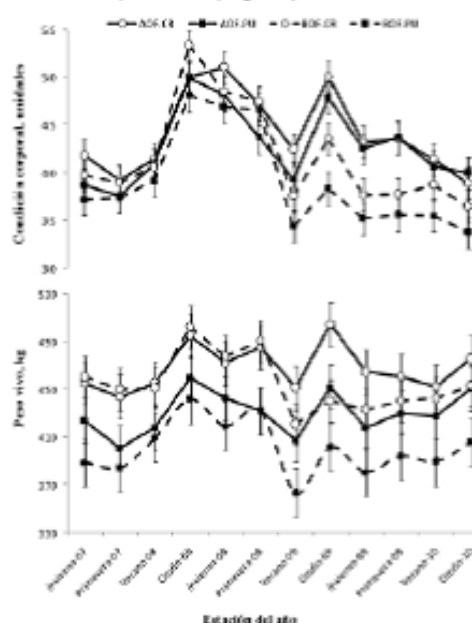

Figura 1: Peso vivo (A) y condición corporal (B) a lo largo de los tres años experimentales (2007-2010) de vacas puras (PU; Hereford y Aberdeen Angus; ■) y cruza recíprocas F1 (CR; ○) pastoreando alta (AOF; —) o baja (BOF; ---) oferta de forraje de campo natural (4 vs. 2.5 kg MS/kg PV, promedio anual). [n=32, 8 vacas por tratamiento]. Los datos se presentan como medias de cuadrados mínimos ± error estándar.

El PV de las vacas al momento de la faena tendió a ser mayor ($P = 0.08$) en las vacas CR que en las vacas PU, mientras que el PCV tendió a ser mayor ($P = 0.07$) en las vacas en AOF que en las de BOF y en las vacas CR que PU. Sin embargo, la CC de las vacas al momento del sacrificio no fue afectada ($P \geq 0.17$) por la OF, el GV o su interacción (Cuadro I).

Canal y su composición

El peso absoluto y relativo de la canal, los tejidos blandos (kg) y rendimiento pre y pos-dressing fueron mayores ($P \leq 0.05$) en las vacas de AOF que en BOF (Cuadro I). El peso de la canal tendió a ser mayor ($P = 0.08$), presentado mayor ($P = 0.04$) proporción de hueso y menor ($P = 0.04$) relación tejidos

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Cuadro I: Peso vivo y peso y composición de la canal de vacas puras y cruza pastoreando diferentes ofertas de forraje sobre campo natural.

Item	AOF-CR	AOF-PU	BOF-CR	BOF-PU	ES	OF	GV	OFxGV
	Tratamientos[1]					Valor-P[2]		
Peso vivo, kg	446.9	419.3	426.2	387.3	20.1	0.17	0.08	0.76
Peso corporal vacío (PCV), kg[3]	362.4	349.5	349.6	319.7	13.1	0.07	0.07	0.44
Peso canal, kg	190.5	182.9	179.2	160.4	7.2	0.01	0.08	0.24
Peso relativo canal, g/kg PCV	534.9	529.3	522.3	509.2	7.3	0.02	0.19	0.58
Composición de la canal								
Huesos, kg	57.8	53.5	58.4	44.7	4.8	0.28	0.04	0.19
Tejidos blandos, kg[4]	133.0	125.6	121.3	110.9	7.4	0.03	0.15	0.80
Rendimiento pre-dressing,%	51.7	52.3	51.2	50.6	0.5	0.04	0.63	0.18
Rendimiento pos-dressing, %	42.7	43.8	42.3	41.7	0.5	0.05	0.64	0.19
Relación tejidos blandos/hueso	2.3[a]	2.3[a]	2.0[b]	2.8[a]	0.2	0.55	0.04	0.05
Composición química de la canal, kg								
Agua	111.2	105.8	104.1	95.7	3.7	0.05	0.12	0.72
Proteína	40.2	38.1	37.3	31.5	2.3	0.01	0.02	0.31
Grasa	21.7	22.1	20.9	18.5	2.0	0.17	0.52	0.38
Relación proteína:grasa	2.4	2.3	2.4	1.9	0.30	0.56	0.21	0.55
Cenizas	18.9	17.4	18.7	16.2	0.91	0.58	0.10	0.68
Composición del tejido blando, g/kg								
Agua	698.4	693.4	692.5	700.8	8.0	0.87	0.96	0.39
Proteína	212.0	199.6	203.0	195.7	5.1	0.22	0.06	0.62
Grasa	80.3	95.0	89.4	98.3	11.2	0.45	0.20	0.88
Relación proteína:grasa	2.3	2.1	2.3	2.0	0.28	0.55	0.21	0.97
Cenizas	12.1	10.8	14.5	12.3	0.06	<0.01	<0.01	0.42

[1]Tratamiento: Vacas de carne puras (Hereford y Aberdeen Angus; PU) o cruzas F1 (CR) pastoreando alta (AOF) o baja (BOF) oferta de forraje (4 vs 2,5 kg MS/kg PV, promedio anual) de campo natural.

[2]OF: Oferta de forraje, GV: Genotipo de la vaca.

[3]Peso corporal vacío: peso de la canal más el peso total de extremidades, cabeza, cuero, sangre, órganos y vísceras (Hersom y col., 2004).

[4]Tejidos blandos de la canal como la sumatoria de tejido muscular, adiposo y conectivo.

[a,b] Diferencia de medias cuando P ≤ 0.05.

[x,y] Diferencia de media cuando 0.05 < P ≤ 0.10

blandos/hueso de la canal en las vacas CR que PU (Cuadro I). Sin embargo, la relación tejidos blandos/hueso de la canal fue afectada por la interacción entre la OF y el GV ya que las vacas BOF-PU presentaron un mayor relación que las vacas BOF-CR, siendo intermedia en las vacas CR y PU que pastorearon AOF (Cuadro I).

La cantidad de agua (kg) de la canal fue mayor (P = 0.05) en vacas que pastorearon AOF que en las que pastorearon BOF, pero la proporción (g/kg) de agua en los tejidos blandos no se vió afectada por la OF, el GV o su interacción (Cuadro I). La cantidad de proteína (kg) de la canal fue mayor (P ≤ 0.02) en vacas de AOF que en BOF y en vacas CR respecto a PU, pero en proporción (g/kg) en los tejidos blandos tendió (P = 0.06) a ser mayor en las vacas CR en comparación con las PU (Cuadro I). La cantidad de grasa en la canal o su proporción en los tejidos blandos, la relación proteína:grasa y la cantidad de ceniza en la canal no fueron afectados (P > 0.10) por la OF, el GV o su

interacción (Cuadro I). La cantidad de cenizas (kg) en la canal tendió a ser mayor (P = 0.10) en las vacas CR que en las PU. Las vacas que pastorearon en BOF y CR presentaron mayor proporción (P ≤ 0.01) de cenizas en los tejidos blandos (g/kg) que las vacas que pastorearon en AOF y PU respectivamente (Cuadro I).

Peso absoluto y relativo a la canal de órganos y/o sección corporal

El peso absoluto (kg) del cuero tendió a ser mayor (P = 0.08) en las vacas que pastorearon en BOF que en las que pastorearon en AOF. En contraste, el peso del TGI+hígado fue mayor (P = 0.02), mientras que los pesos del diafragma y los pulmones tendieron a ser mayores (P ≤ 0.09) en vacas que pastorearon en AOF que en BOF (Cuadro II). El peso de la masa total de órganos (kg) fue mayor (P = 0.05) en las vacas que pastorearon AOF que en las que pastorearon BOF. El peso absoluto del corazón fue mayor (P = 0.01) en vacas CR que en vacas PU. El peso absoluto de extremidades, cabeza, total de vísceras rojas y los

Item	Tratamientos[1]					Valor-P[2]		
	AOF-CR	AOF-PU	BOF-CR	BOF-PU	EE	OF	GV	OF*GV
Peso absoluto de órganos/sección, kg								
Cuero	30.1	30.5	34.3	32.1	1.60	0.08	0.54	0.42
Extremidades	6.3	6.5	6.3	6.9	0.30	0.36	0.11	0.44
Cabeza	14.7	14.1	14.7	13.7	0.50	0.74	0.12	0.68
Tracto gastrointestinal+hígado[3]	34.5	33.5	31.8	31.1	1.06	0.02	0.44	0.93
Vísceras rojas[4]	16.3	16.4	16.1	15.6	0.40	0.24	0.61	0.47
Diafragma	2.3	2.5	2.3	2.1	0.11	0.09	0.86	0.12
Pulmones	4.9	4.7	4.6	4.3	0.18	0.06	0.19	0.59
Corazón	1.6	1.5	1.6	1.4	0.06	0.55	0.01	0.84
Riñones	1.5	1.5	1.6	1.5	0.08	0.90	0.88	0.42
Masa total de los órganos[5]	47.1	46.6	44.6	44.1	1.41	0.05	0.67	0.97
Peso relativo de órganos/sección por kg de canal, g/kg								
Cuero	168.8	169.9	188.8	200.9	11.30	0.01	0.30	0.96
Extremidades	33.0	35.8	35.1	43.8	2.40	0.02	0.01	0.17
Cabeza	78.5	78.4	81.5	81.6	5.60	0.41	0.99	0.98
Tracto gastrointestinal+hígado[3]	175.9	183.7	177.4	191.9	5.65	0.39	0.05	0.53
Vísceras rojas[4]	85.7	89.3	89.6	97.0	3.52	0.06	0.07	0.52
Diafragma	12.1	13.7	12.8	12.8	0.40	0.80	0.04	0.11
Pulmones	25.8	26.2	26.1	25.7	1.20	0.93	0.94	0.69
Corazón	8.3	8.1	8.8	8.9	0.40	0.05	0.90	0.66
Riñones	7.8	8.5	8.7	9.3	0.38	0.02	0.05	0.99
Masa total de órganos[5]	241.1	256.1	249.6	260.7	6.24	0.33	0.05	0.74

[1] Tratamiento: Vacas de carne puras (Hereford y Aberdeen Angus; PU) o cruzas F1 (CR) pastoreando alta (AOF) o baja (BOF) oferta de forraje (4 vs 2,5 kg MS/kg PV, promedio anual) de campo natural

[2] OF: Oferta de forraje, GV: Genotipo de la vaca.

[3] Tracto gastrointestinal+hígado como la sumatoria de Rumen, Omaso, Abomaso, Intestinos e Hígado.

[4] Vísceras rojas como la sumatoria de Corazón, Riñón, Pulmón, Diafragma. [5] Total de órganos como la sumatoria de Tracto gastrointestinal, Vísceras rojas e Hígado.

a,b Diferencia de medias cuando P ≤ 0.05.

x,y Diferencia de media cuando 0.05 < P ≤ 0.10.

riñones no se vieron afectados (P > 0.10) por la OF, el GV o su interacción (Cuadro II).

Los pesos relativos (g/kg respecto a la canal) del cuero, extremidades, corazón y riñones fueron mayores (P ≤ 0.05) en las vacas en BOF que en AOF. El peso relativo del total de las vísceras rojas tendió a ser mayor (P = 0.06) en las vacas en BOF que AOF, y tendió a ser mayor (P = 0.07) en vacas PU que en CR (Cuadro II). A su vez, el peso relativo de las extremidades, TGI+hígado, diafragma y riñones fue mayor (P ≤ 0.05) en las vacas PU que CR (Cuadro II). El peso relativo de la masa total de órganos fue mayor (P = 0.05) en vacas PU que CR. El peso relativo de la cabeza y los pulmones no se vio afectado por la OF, el GV ni su interacción (Cuadro II).

Composición química de los órganos
El contenido de agua del cuero fue mayor (P = 0.01) en las vacas que pastorearon en BOF que en las que pastorearon en AOF,

mientras que en los riñones tendió (P = 0.07) a ser mayor en vacas pastorearon en AOF que en las que pastorearon en BOF. El contenido de agua en la canal, diafragma, TGI+hígado, corazón y pulmones no fue afectada (P > 10) por la OF, el GV o su interacción (Cuadro III).

La relación proteína:grasa del TGI+hígado tendió (P = 0.10) a ser mayor en las vacas que pastorearon en AOF que en vacas que pastorearon en BOF. La relación proteína:grasa del resto de órganos, vísceras y la canal no fue afectada por la OF, el GV o su interacción (Cuadro III).

Discusión

El presente trabajo identificó cambios en el peso y composición de la canal, así como en la masa de varios órganos debido a

Cuadro III: Composición química tisular de vacas de cría puras y cruza pastoreando distintas ofertas sobre campo natural.

Ítem	Tratamientos[1]					Valor-P[2]		
	AOF-CR	AOF-PU	BOF-CR	BOF-PU	EE	OF	GV	OF*GV
Agua, g/kg								
Canal	584.5	578.1	578.8	594.5	7.51	0.57	0.63	0.23
Cuero	704.8	706.6	727.4	719.7	5.75	0.01	0.62	0.43
Tracto gastrointestinal+hígado[3]	824.3	823.3	822.8	820.8	2.12	0.35	0.44	0.82
Diafragma	765.9	704.2	739.2	742.4	8.22	0.19	0.71	0.42
Corazón	770.4	755.2	771.6	771.5	10.71	0.20	0.27	0.28
Pulmones	803.8	795.1	798.5	797.9	4.72	0.78	0.25	0.32
Riñones	803.2	806.9	797.6	796.6	6.15	0.07	0.73	0.56
Relación proteína:grasa								
Canal	2.4	2.3	2.4	1.9	0.30	0.56	0.21	0.55
Cuero	4.7	4.7	5.2	6.4	0.90	0.19	0.50	0.49
Tracto gastrointestinal+hígado[3]	6.3	6.7	5.9	5.2	0.49	0.10	0.71	0.27
Diafragma	3.0	2.1	1.9	2.6	0.60	0.62	0.92	0.19
Corazón	5.2	3.3	4.4	4.2	1.10	0.95	0.12	0.22
Pulmones	2.2	2.1	2.1	2.2	0.20	0.84	0.81	0.60
Riñones	1.8	1.8	1.7	1.7	0.10	0.41	0.75	0.76

[1] Tratamiento: Vacas de carne puras (Hereford y Aberdeen Angus; PU) o cruza F1 (CR) pastoreando alta (AOF) o baja (BOF) oferta de forraje (4 vs 2,5 kg MS/kg PV, promedio anual) de campo natural.

[2] OF: Oferta de forraje, GV: Genotipo de la vaca.

[3] Tracto gastrointestinal+hígado como la sumatoria de Rumen, Omaso, Abomaso, Intestinos e Hígado.

[a,b] Diferencia de medias cuando $P \leq 0.05$.

[x,y] Diferencia de media cuando $0.05 < P \leq 0.10$.

cambios en la OF del campo natural o al GV que sugerirían una mayor eficiencia en el uso de la energía en vacas pastoreando en AOF que en BOF y para vacas CR respecto a PU. Estas diferencias podrían traducirse en variaciones en las necesidades de energía de mantenimiento, lo cual impactaría en la partición de la energía metabolizable entre pérdidas de calor y energía neta de producción y por lo tanto, en la eficiencia biológica y económica de la producción de carne (Reynolds, 2002; Solis y col., 1988).

Efecto de la oferta de forraje

Los menores PCV y peso absoluto y relativo de la canal de vacas que pastorearon en BOF se asociarían a una menor ingesta de energía (o menor ingesta de forraje). Do Carmo y col. (2016) estimaron el consumo de energía utilizando ecuaciones del NRC, donde reportaron un consumo de energía entre 10-12% más bajo en vacas que pastorearon en BOF que en AOF como consecuencia de una inferior masa de forraje. Dicha información resultó coincidente con lo reportado en bovinos para carne por Hersom y col. (2004a) donde los animales mejor alimentados presentaron un peso de canal mayor que los animales más restringidos. Además del mayor peso de la canal (+10%, AOF vs. BOF), los animales que pastorearon en AOF presentaron un mayor rendimiento de faena, mayor peso de tejidos blandos y

mayor contenido proteico en la canal. Sin embargo, no se observaron diferencias en el contenido de grasa. Esto difiere con estudios previos (Houghton y col., 1990a; 1990b; NRC, 1996) donde una mejora en la alimentación y/o en nivel de energía en la dieta de vacas para carne incrementó el contenido de grasa corporal. En el presente trabajo, probablemente no se registraron diferencias en el contenido de grasa debido a que los animales pasaron a lo largo del experimento (3 años) por períodos de restricción y realimentación como se observa en la evolución de PV y CC (Figura 1), y los mismos fueron sacrificados sin un periodo de terminación en un momento de baja acumulación de grasa (Casal y col., 2017).

El mayor PCV al sacrificio de las vacas en AOF se vio asociado, además de un mayor peso de la canal, a que las mismas presentaron o tendieron a presentar un mayor peso absoluto de la masa total de órganos. Investigaciones previas en novillos (McLeod y col., 2007; Wang y col., 2009) y vacas para carne (Meyer y col., 2012) reportan un aumento del tamaño del TGI con el aumento de la ingesta de alimento. En el mismo sentido, Jorge y col. (1997) observaron una reducción del peso de TGI e hígado en bovinos con restricción alimentaria. Las vísceras tienen altas tasas metabólicas (especialmente el hígado) y los órganos del TGI responden a cambios en la ingesta de alimentos. Kozloski y col. (2001) sugiere que el aumento de la ingesta conduce a un

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aumento de la actividad metabólica de las células epiteliales y de la actividad contráctil de las células musculares, determinando un aumento de la masa del TGI.

A pesar que el TGI e hígado constituyen solo el 10-13% del peso del cuerpo, estos tejidos representan más del 50% de los costos energéticos de mantenimiento, y por tanto contribuyen de manera significativa a la variación en la productividad de los animales tanto dentro de un rodeo, así como entre diferentes razas (DiConstanzo y col., 1991; Ferrell y Jenkins, 1985; Jenkins y col., 1991). Las vacas en AOF tendieron a presentar mayor peso de los pulmones y el diafragma, esto podría estar asociado a una mayor demanda de oxígeno a fin de metabolizar (oxidar) la mayor cantidad de nutrientes consumidos. Hentz y col. (2016) reportan en ovinos que a medida que se incrementa el nivel de ingesta de alimento, también aumenta el consumo de oxígeno por parte de la masa total de órganos.

En un estudio realizado por Jenkins y col. (1986) utilizando vacas de diferentes razas y con distintos niveles de alimentación concluyeron que los animales mejor alimentados y con mayor potencial de producción de leche presentaban mayor peso de los órganos del sistema respiratorio y circulatorio (corazón, pulmones y riñones) directamente relacionados a la mayor exigencia metabólica. En términos absolutos, la masa total de órganos acompasó con las variaciones de PV de los animales y fue mayor en vacas que pastorearon AOF. Estos resultados concuerdan con los resultados reportados por Burrin y col. (1992) donde expresan que la masa total de órganos es proporcional al nivel de consumo de alimento y al tamaño corporal de los animales. Contrariamente a lo que se esperaría, las vacas en BOF tendieron a presentar mayor peso del cuero, a pesar de que presentaban un menor tamaño corporal. Estas diferencias en el peso de este tejido estarían asociadas a un mayor contenido de agua en el mismo en vacas en BOF en comparación a las vacas en AOF.

Sin embargo, las vacas en AOF presentaron menor peso relativo a la canal del cuero y extremidades que las que pastorearon en BOF. Ya que no se observaron diferencias en el peso absoluto, el menor peso relativo a la canal del cuero y extremidades estaría relacionado con el mayor peso de canal de las vacas en AOF; una menor proporción de despojos representa un aumento en el rendimiento de la canal. Vacas que pastorearon en BOF tendieron a presentar mayor peso relativo de las vísceras rojas (corazón y riñones principalmente), esto podría estar asociado a la mayor eliminación de urea y otros productos de desecho dado que éstas presentarían una mayor movilización de reservas (grasa y proteínas; Casal y col., 2017). A su vez, los riñones (además del hígado) son tejidos que realizan neoglucogénesis, por lo tanto un aumento de la masa renal podría permitir una mayor suministro de glucosa (Reynolds y col., 2004). Contrariamente a nuestros resultados Wood y col. (2013) no encontraron diferencia en el peso de los órganos expresados como peso relativo a la canal entre vacas bajo distintos niveles de alimentación. Posiblemente, las diferencias en el tipo de dieta y al lago del experimento donde los animales pasaron por periodos de restricción y realimentación podrían estar explicando estas diferencias entre nuestro

trabajo y lo reportado por Wood y col., 2013.

Efecto del genotipo de la vaca

El PV, el PCV y el peso de la canal tendieron a ser mayores en vacas CR que en vacas PU, lo cual concuerda con lo reportado por Cundiff y col. (1974) y Morris y col. (1987), quienes atribuyeron la superioridad en PV de las vacas cruza a diferencias en el tamaño corporal o frame (tamaño del esqueleto). Esta diferencia en tamaño de la canal a favor de las vacas CR se acompañó de una mayor contenido proteico en la misma y en los tejidos blandos y presentaron mayor proporción de huesos. De manera similar, se han reportado efectos de la heterosis sobre el peso de la canal (Long, 1980). Gaines y col. (1967) evaluaron el uso de cruzamientos de razas británicas y reportaron valores de heterosis de 4% para el peso de la canal y para el área del músculo *longissimus dorsi*, pero no observaron diferencias significativas en el contenido de grasa de la canal. Contrariamente a nuestros resultados, Long y Gregory (1975) reportaron efecto de la heterosis no solamente sobre la cantidad de proteína (área del músculo *longissimus dorsi*) sino también sobre la cantidad de grasa en la canal.

Si bien las vacas CR presentaron un mayor peso de canal y contenido proteico en la misma, las vacas PU presentaron mayor relación tejidos blandos/huesos, específicamente las vacas BOF-PU presentaron una mayor proporción de dicha relación que las vacas BOF-CR. Estos resultados podrían ser debido a un mayor tamaño corporal, mayor frame, mayor estructura ósea y por lo tanto, mayor proporción de huesos en relación a la canal en las vacas CR. Esta mayor proporción de huesos se vio reflejado en una tendencia a un mayor contenido de cenizas (minerales) en la canal de las vacas CR. Wheeler y col. (1997) comparando diferentes cruzamientos reportaron valores superiores en el peso de los hueso en cruzas Salers (mayor frame) en comparación con cruzas Angus/Hereford (menor frame).

Los tejidos blandos de la canal de las vacas CR tendieron a presentar mayor contenido de proteínas y presentaron, al igual que las vacas en AOF, mayor contenido de cenizas en los mismos. Williams y col. (1983) reportaron mayor contenido de cenizas en los tejidos blandos de la canal y dentro de estas mayor contenido de zinc, fosforo, magnesio y potasio en estos tejidos en novillos terminados a forraje en comparación a los que fueron terminados con granos. Estos autores sugieren que este mayor contenido de minerales sumado a un mayor porcentaje de tejido magro (proteína) podría tener efectos beneficiosos en las características nutricionales de la carne.

Una mayor carga metabólica asociada a un mayor potencial de producción de leche podría haber llevado a un incremento en las necesidades de transportar nutrientes y en la masa de órganos del sistema circulatorio, lo cual podría explicar el mayor peso absoluto de corazón en vacas CR que en vacas PU. En el presente estudio, la producción de leche durante la última lactancia (2009-2010; 140 d) fue mayor en las vacas de CR que en las de PU (Gutiérrez y col., 2013), asociadas con una mayor ingesta estimada de energía metabolizable (Do Carmo y col. 2016; Laporta y col., 2014). Solís y col. (1988) reportan mayores re-

querimientos de energía para mantenimiento en razas lecheras y sus cruzas vs razas carniceras, esto sería atribuido principalmente a un mayor tamaño de los órganos mas metabólicamente activos (TGI e hígado).

Ferrell y Jenkins (1985) informaron que las diferencias en las necesidades energéticas de mantenimiento entre los genotipos de vacas para carne se asociaron con el potencial de la producción de leche, que determinó las diferencias en la masa del hígado y de los órganos del TGI. Las vacas CR presentaron menor peso relativo a la canal del TGI+hígado lo cual estaría asociado a que estas tendieron a presentar un mayor peso de canal, una menor proporción TGI representaría un aumento en el rendimiento de la canal. Además las vacas CR tendieron a presentar menor peso relativo de las vísceras rojas (espacialmente riñones), diafragma y particularmente de la masa total de órganos que las vacas PU. Particularmente, los costos de mantenimiento varían entre vacas de diferentes genotipos. Estudios realizados por Solís y col. (1988) reportan que a pesar que animales *Bos indicus* y *Bos taurus* presenten un peso de canal y tasa de crecimiento similares, los animales *Bos indicus* y sus cruzas presentaron menores requerimientos energéticos para el mantenimiento, asociado a un menor tamaño del TGI, hígado y corazón y menor cantidad de grasa visceral. Varios trabajos coinciden en que el peso de los órganos difiere entre genotipos, entre estados fisiológicos y entre planos nutricionales (Baldwin, 1995; Jenkins y col., 1986; Solís y col., 1988; Smith y Baldwin, 1974) y que estas diferencias pueden traducirse en variaciones en las necesidades de energía de mantenimiento (Baldwin, 1995; Jenkins y col., 1986; Solís y col., 1988). Por lo tanto, nuestros resultados podrían sugerir que las vacas CR tendrían un menor costo de mantenimiento por unidad de producto que las vacas PU, dado fundamentalmente porque el peso relativo de la masa total de órganos respecto a la canal fue un 9.5% menor en vacas CR. Además, las vacas CR presentaron mayor proporción de grasa corporal, menor deposición de grasa visceral (omental/mesentérica) y mayor movilización de grasa y proteína en periodo de balance energético negativo que las vacas PU (Casal y col., 2014; 2017).

Conclusión

El control de la intensidad de pastoreo de campo natural, a través del manejo de la oferta de forraje y genotipo de los animales afectaron la masa y composición de la canal y órganos. Las vacas en AOF y CR presentaron mayor peso de canal y mayor rendimiento carnicero y un menor peso relativo de los órganos respecto al peso de la canal. Estos resultados contribuyen a explicar las mejoras en la eficiencia en el uso de la energía para producción, dado la menor relación entre masa total de órganos y canal.

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5. MECHANISMS RELATED WITH ENERGY EFFICIENCY ON THE LIVER OF PURE AND CROSSBRED BEEF COWS GRAZING DIFFERENT HERBAGE ALLOWANCES OF GRASSLANDS

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5.1. ABSTRACT

The aim of this study was to evaluate the effect of two herbage allowances (**HA**) of native grasslands (Campos biome) on hepatic mitochondrial density and function and oxidative stress markers of purebred (**PU**) and the reciprocal F1 crossbred (**CR**) beef cows. Mature cows ($n = 32$) were used in a complete randomized block design with a factorial arrangement of HA (2.5 vs. 4 kg dry matter/day; **LO** vs. **HI**) and cow genotype (**CG**; 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. Liver was dissected, weighed, and samples collected and snap-frozen in liquid nitrogen and stored at -80°C until analyses. Cow-calf efficiency (g or kJ of calf/MJ cow metabolizable energy intake) tended ($P = 0.07$) to be greater for HI than LO cows and it was greater ($P = 0.02$) for CR than in PU cows. The hepatic citrate synthase (**CS**) enzyme activity, CS mRNA and mitochondrial DNA:nuclear DNA ratio were greater ($P \leq 0.03$) for HI than LO cows. Plasma

pro-oxidants were greater ($P = 0.02$) and plasma antioxidant capacity tended ($P = 0.07$) to be greater for CR than PU cows. Oxidative stress index was affected ($P = 0.02$) 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. Hepatic thiobarbituric acid reactive species and protein carbonyls concentrations were not affected by HA treatment, CG or their interaction but hepatic 4-hydroxynonenal protein adducts expression tended to be greater ($P = 0.06$) for CR than PU cows and tended ($P = 0.06$) to be affected by the interaction between HA treatment and CG as it was greater ($P < 0.05$) in LO-CR than LO-PU cows while did not differed from HI-CR and HI-PU cows. Neither the hepatic superoxide dismutase and glutathione peroxidase enzyme activity were affected by HA treatment, CG or their interaction. In conclusion, the greater cow-calf efficiency was associated to a greater hepatic mitochondrial density, without differences in mitochondria function, which would probably explain a greater efficiency in hepatic nutrient metabolism for HI vs. LO cows. However, contrary to what was expected the greater efficiency for CR than PU cows was associated to an increased hepatic oxidative damage, which would probably reflect a greater metabolic activity of this organ in CR cows.

Key words: cow-calf system efficiency, energy metabolism, mitochondrial function, oxidative stress

5.2. INTRODUCTION

Beef cow energy efficiency could be defined by an output, such as calf production (number of weaned calves and weight or retained energy of weaned calves) and relevant inputs as required feed/energy intake (Jenkins and Ferrell, 1994; Scholljegerdes and Summers, 2016). Improvement in efficiency of energy utilization of cow-calf systems has the potential to greatly impacton beef production profitability and sustainability, as approximately 65% of the consumed energy for beef production is required by the cow herd,

with maintenance representing ~70% of the required energy (Montaño-Bermudez et al., 1990, Parnell et al., 1994).

Beef production is often conducted in extensive grazing production systems (i.e. *Campos biome*) where grassland nutrient availability varies according with seasonal changes in rainfall and temperature (Berretta et al., 2000) and cow nutrient requirements fluctuate throughout the year due to the pressure of reproduction and lactation. Previous reports indicatedthe simultaneous control of grazing intensity by manipulating herbage allowance (**HA**) and use of crossbred cows increased (additive effects) cow dry mater (**DM**) intake (~10%) and calf weaning (8-17%) which improved (~19%) biological efficiency of beef cow-calf systems (Do Carmo et al., 2016; 2018). The greater efficiency of crossbred cows grazing high HA, was associated tochanges in cow energy partitioning (metabolic-endocrine profiles and body composition; Laporta et al., 2014; Casal et al., 2017), milk production (Gutierrez et al., 2013), and visceral mass, cellularity and activity (Casal et al., 2014).

Energy efficiency in beef cattlehas been associated to mitochondria function (Ramos et al., 2013; Casal et al., 2018). Mitochondria produce 90% of cellular energy and are integrated into numerous functional, metabolic and signaling networks with other cellular compartments (Rolfe and Brand, 1997). Mitochondria are also recognized as a major source of oxidants in the majority of cell types (Turrens, 2003; Bottje and Carstens, 2009). Oxidative stress occurs when production oxidants exceed the antioxidant capacity to repair the resulting damage (Chirase et al., 2004) and elevates cellular energy expenditure, decreasing energy efficiency. Thus, this organelle is an appropriate place to study variation in maintenance energy requirements that may contribute to the phenotypic differences observed among animals (Rolfe and Brand, 1997; Herd and Arthur, 2009). In addition, the liver is a central organ of energy metabolism that represents only ~3% of body weight (**BW**) but represents ~25% of wholebody oxygen consumption and ~8% of daily body protein turnover (Lobley et al., 1980; Baldwin et al., 2004). Therefore,

hepatic mitochondrial function and oxidative stress could play a key role in energy efficiency of beef cows. We have not found studies on hepatic mitochondrial function, oxidative stress and phenotypic expression of energy efficiency in cow-calf production systems.

Thus, our hypothesis was that the improved efficiency of crossbred cows grazing high HA would be associated with an increased hepatic mitochondrial density and function and reduced oxidative stress. The objective of this work was to evaluate the effect of two HA of native grasslands (Campos biome) hepatic mitochondrial density and function in terms of gene and protein expression and enzyme activity of mitochondrial respiratory complex and antioxidant proteins, and oxidative stress markers of purebred (**A**: Aberdeen Angus and **H**; Hereford; **PU**) and the reciprocal F1 crossbred (AxH and HxA; **CR**) mature beef cows.

5.3. MATERIALS AND METHODS

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

Location, animals and experimental design

A detailed description of grazing and cattle management for this experiment has previously been reported (Do Carmo et al., 2018; Casal et al., 2014). Briefly, the experiment was conducted on 95 ha of Campos biome (native pastures were dominated by summer-growing C4 grasses, with few C3 grasses associated with the winter cycle) located at the Professor Bernardo Rosengurtt Experimental Station (Facultad de Agronomia, Universidad de la Republica, Uruguay; 32°S, 54°W) from June 2007 to May 2010.

Multiparous cows (n = 32; 4 to 5 year-old), were used in a randomized block design with two replications and four plots in each block to which a 2 x 2 factorial arrangement of HA and cow genotype (**CG**; PU vs. CR) was allocated.Herbage allowance (the ratio between forage mass and stocking rate; 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 (5, 3, 4 and 4 kg DM/kg BW and 3, 3, 2 y 2 kg DM/kg BW for HI and LO in fall, winter, spring and summer, respectively). 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. Herbage samples were collected monthly and composite by season for chemical analyses (AOAC, 2000; Van Soest et al., 1991) and metabolizable energy (**ME**) was estimated according to Reid et al. (1991). Herbage allowance treatments determined differences in forage mass and height throughout the year (2072 vs. 1338 ± 160 kg DM/ha and 5.2 vs. 3.5 ± 0.3 cm annual average for HI vs. LO, respectively) but did not differ in chemical composition (7.3, 8.6, 10.5 and 8.6 ± 0.5% of crude protein and 42.6, 41.1, 36.2 and 40.2 ± 1.6 % of acid detergent fiber (DM basis) in fall, winter, spring and summer, respectively). 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.

On average, during the three experimental years, body condition score (**BCS**) and BW were greater for HI than LO cows, being the differences more evident during the last gestation-lactation cycle (2009 – 2010, Figure 1). In addition, during the last gestation-lactation cycle cow BW and BCS were greater ($P ≤ 0.01$), milk energy output was 22% greater ($P ≤ 0.05$), calf BW and average daily gain at weaning were 10% greater ($P ≤ 0.05$), and commencement of luteal activity was 59 and 34 days earlier ($P ≤ 0.05$) for HI than LO and CR than PU cows (Gutierrez et al., 2013; Casal et al., 2017; Laporta et al., 2014). Estimated ME intake 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).
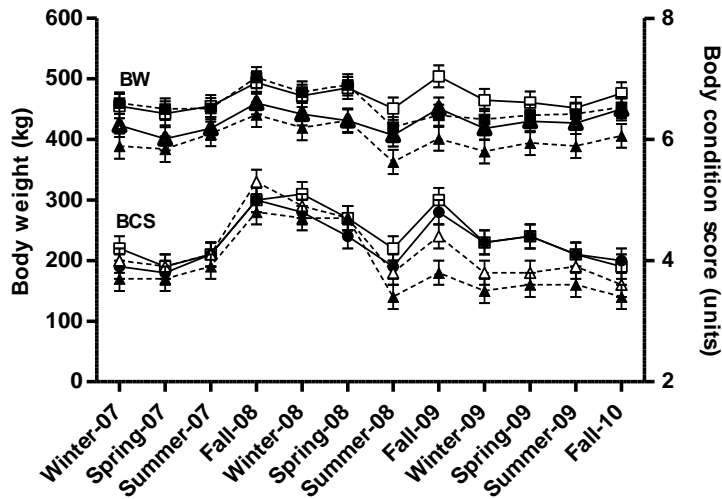
Figure 1: Cow body weight (BW) and body condition score (BCS) throughout the three experimental years (2007-2010) for purebred (Hereford and Aberdeen Angus; triangles) and crossbred (F1; squares) beef cows grazing high (solid symbols and lines) and low (open symbols and dashed lines) herbage allowances (2 vs. 4 kg dry matter/kg BW 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, at 190 ± 10 days postpartum (45 days after calf weaning), cows were slaughtered in a commercial abattoir (PUL S.A; Cerro Largo, Uruguay; 40 km from the Experimental Station). Prior to transport to abattoir, cow BW and BCS were recorded, and blood samples were obtained by jugular venipuncture in tubes with heparin (BD Vacutainer tubes; Becton Dickinson, NJ, USA). Samples were centrifuged (2000 x *g*, 15 min), and plasma was stored at -20ºC for until analyses. At slaughter, the liver was dissected, separated from surrounding connective tissue and liver samples from the caudal lobe were obtained ~20 min after the animal was killed. Liver samples (10 g) were snap-frozen in liquid nitrogen and then stored at -80°C for analysis of protein and gene expression and enzyme activity.

Plasma and hepatic oxidative stress markers

Concentrations of plasma pro-oxidants (**pROS**; using d-ROM Test expressed in arbitrary 'Carratelli Units' (**CarrU**), where 1 CarrU is equivalent to the oxidizing power of 0.08 mg H_2O_2/dL) and plasma antioxidant capacity (**PAC**; OXY-Adsorbent Test expressed as µmol HClO/mL); were determined by colorimetric assays using commercial kits (Diacron International, Grosseto, Italy) according to Abuelo et al. (2013). Both pROS and PAC were measured by micromethods using a Varioskan Flash microplate reader (Thermo Electron Corp., Louisville, CO) and the intra-assay coefficient of variation did not exceed 10%. The oxidative stress index (**OSi**) was calculated as pROS/PAC, expressed as CarrU/µmol HClO/mL (Celi, 2011).

Hepatic thiobarbituric acid reactive species (**TBARS**) expressed as concentration of malondialdehyde (**MDA**) for the determination of lipid oxidation and hepatic protein carbonyl expressed as concentration of dinitrophenylhydrazine (**DNPH**) for the determination of protein oxidation were performed according with Casal et al. (2019).

Quantitative real time qPCR

Synthesis of cDNA by reverse transcription was performed according with Casal et al., (2014). Primers (Supplementary Table S1) to specifically amplify cDNA of target genes and internal control genes: β-actin (**ACTB**), hypoxanthine phosphoribosyltransferase (**HPRT1**) and ribosomal protein L19 (**RPL19**), were obtained from literature or specifically designed using the Primer3 website (http://bioinfo.ut.ee/primer3-0.4.0/) based on bovine nucleotide sequences available from NCBI (http://www.ncbi.nlm.nih.gov/). The genes measured in the present study were selected based on previous results from a microarray analysis (Laporta et al., 2014). Candidate selected genes were: NADH:ubiquinone oxidoreductase subunit C1 (**NDUFC1**); NADH:ubiquinone oxidoreductase subunit A4 (**NDUFA4**); NADH:ubiquinone oxidoreductase subunit A13 (**NDUFA13**); succinate dehydrogenase complex,

subunit A, flavoprotein (Fp) (**SDHA**); succinate dehydrogenase complex, subunit D (**SDHD**); ubiquinol-cytochrome c reductase core protein I (**UQCRC1**); ubiquinol-cytochrome c reductase hinge protein (**UQCRH**); cytochrome c oxidase assembly factor (**COX19**); cytochrome c-1 (**CYC1**); ATP synthase, H$^+$ transporting, mitochondrial F1 complex, epsilon subunit (**ATP5E**);ATP synthase, H$^+$ transporting, mitochondrial F1 complex, O subunit (**ATP5O**), citrate synthase (**CS**), glutathione peroxidase 1 (**GPX1**), glutathione peroxidase 3 (**GPX3**), glutathione peroxidase 4 (**GPX4**), glutamate-cysteine ligase catalytic subunit (**GCLC**), copper zinc superoxide dismutase, soluble (**SOD1**), manganese superoxide dismutase, mitochondrial (**SOD2**), peroxiredoxin 3 (**PRDX3**) and peroxiredoxin 5 (**PRDX5**). 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 qPCR reactions were performed according to Casal et al. (2018) and gene expression was measured by relative quantification (Pflaffl, 2009) to the endogenous control and normalized to the geometric mean expression of internal control genes. 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 0.343, 0.379 and 0.285 for *ACTB, HPRT1,* and *RPL19*, respectively. Amplification efficiencies of 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 coefficient of variation for all genes were less than 1.4 and 4.2%, respectively.

In addition, the mitochondrial DNA:nuclear DNA (**mtDNA:nDNA**) ratio was used to estimate tissue mitochondrial density (Guo et al., 2009). The real-time qPCR was carried out in separate tubes for mtDNA and nDNA amplification using PCR primers (Supplementary Table S1). The cycle number (Ct) at which the fluorescent signal of a given reaction crossed the

threshold value was used as basis for quantification of mtDNA and nDNA copy numbers. The difference between cycle numbers measured at the threshold (designated ß) was used to calculate the relative ratio of mtDNA:nDNA using the equation: $2^{\text{ß}}$.

Hepatic enzyme activity

Citrate synthase, NADH ubiquinone oxidoreductase and succinate dehydrogenase (**SDH**) activity assays were conducted according to Casal et al. (2018). Assays were performed in duplicate at 37°C using a final volume of 500 µL in a UV-2401 PC spectrophotometer (Shimadzu Corporation, Tokyo, Japan). The hepatic activities of glutathione peroxidase (**GPX**) and superoxide dismutase (**SOD**) were determined spectrophotometrically using the commercially kits (Randox Laboratories, Antrim, UK) in a Varioskan Flash microplate reader (Thermo) (Casal et al., 2019). Enzyme activities were expressed as units per mg of mitochondrial or homogenate protein.

Western blot analysis

Total protein extracts in ice-cold RIPA buffer and electrophoresis were performed according with Casal et al. (2018; 2019). The immunochemical detection of 4-hydroxynonenal protein adduct (**4-HNE**), ATP synthase F1 subunit alpha (**ATP5A**), SDHA, SOD2 and ACTB proteins were performed using highly specific mouse monoclonal anti-ATP5A (1:2000), anti-SDHA (1:2000), anti-ACTB (1:3000), anti-SOD2 (1:2000) and rabbit polyclonal anti-4-HNE (1:1000) antibodies (Abcam, Cambridge, United Kingdom). The membranes were incubated with anti-mouse IR Dye 680CW (Li-COR, Lincoln, NE, USA) at 1:15000 or anti-rabbit IR Dye 800CW (Li-COR) at 1:20000. Signals were detected using the ODYSSEY CLx Infrared Imaging System (Li-COR) and analyzed using ODYSSEY CLx and ImageJ-software. Density of the area of each lane was measured. The relative levels of ATP5A, SDHA, SOD2 and 4-HNE were normalized with ACTB density and

normalized values were expressed as a percentage relative to the HI-CR group.

Calculations and Statistical analyses

Estimated cow-calf efficiency during the last gestation-lactation cycle was calculated according National Research Councils for Beef Cattle equations (NASEM, 2016). Cow ME intake was estimated based on individual cow requirements (Macoon et al., 2003; Smit et al., 2005) for maintenance ($BW^{0.75}$), gestation (estimated weight of the gravid uterus adjusted fortheactual calf birth weight (Ferrell et al., 1976) or lactation (milk yield and composition; Gutierrez et al., 2013), and retained energy (changes in protein and fat tissues based on changes in BW and BCS). Estimated calf retained energy was calculated using individual calf BW and body composition (Gutierrez et al., 2013) and 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). UNIVARIATE analyses were performed on all variables to verify normality of residual. All data were analyzed using the MIXED procedure with a 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.

5.4. RESULTS

Total cow ME intake during the gestation-lactation cycle tended ($P = 0.09$) to be greater forHI than LO cows, however, it was was not affected by CG or their interaction (Table 1). Calf retained energy at weaning was greater ($P = 0.01$) for HI than LO cows and for CR than PU cows (Table 1). Cow-calf efficiency tended ($P \leq 0.07$) to be greater for HI than LO cows and it was greater ($P \leq 0.03$) was CR than in PU cows (Table 1).

Hepatic mitochondrial density and function

The hepatic CS enzyme activity, CS mRNA and mtDNA:nDNA ratio were greater ($P \leq 0.03$) for HI than LO cows (Table 2). Neither the hepatic NADH ubiquinone oxidoreductase (Table 2) nor SDHA or ATP5A protein expression (Supplementary Figure S1A, S1B) were affected by HA treatment, CG or their interaction. Hepatic SDH enzyme activity tended ($P = 0.08$) to be greater in CR than PU cows (Table 2). In addition, hepatic expression of *NDUFA4* mRNA tended to be greater ($P = 0.08$) and *CYC1* and *ATP5E* mRNA were greater ($P = 0.05$) for HI than LO cows(Table 2). The expression of *NDUFC1, NDUFA13, SDHA, SDHD, UQCRC1, UQCRH, COX19* and *ATP5O* mRNA in the liver did not change due to HA treatment, CG or their interaction (Table 2). Total cow ME intake during the gestation-lactation cycle showed a moderate to high correlation with hepatic *CS* mRNA and CS activity (r = 0.36 and 0.60, respectively, $P \leq 0.05$, *n* = 32).

Table 1: Effects of herbage allowance (HA) and cow genotype (CG) and their interaction (HAxCG) on cow-calf energy efficiency.

Item	Treatment[1]					P-value[2]	
	HI-PU	HI-CR	LO-PU	LO-CR	SE	HA	CG
Cow total ME intake, MJ[3]	31875	33690	30202	31375	1257	0.09	0.18
Calf retained energy at weaning, MJ	924	1139	855	944	42	0.01	0.01
Cow-calf energy efficiency							
g of calf/ MJ cow ME intake	3.91	4.55	3.97	3.82	0.18	0.07	0.03
kJ of calf/MJ cow ME intake	29.0	33.8	28.3	30.1	1.40	0.07	0.02
MJ of calf/MJ cow ME intake/kg MBW	2.67	3.31	2.51	2.80	0.18	0.06	0.01

[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]The interaction HAxCG was not significant (*P*> 0.17).

[3]Estimated metabolizable energy intake during the gestation-lactation cycle.

Table 2: Effects of herbage allowance (HA) and cow genotype (CG) and their interaction (HAxCG) on hepatic mitochondrial density and function.

Item	Treatment[1]				SE	P-value[4]	
	HI-PU	HI-CR	LO-PU	LO-CR		HA	CG
Mitochondrial density marker							
Citrate synthaseactivity, mU/mg protein	63.9	65.8	34.8	41.1	4.1	<0.01	0.31
Citrate synthases mRNA	0.86	0.88	0.69	0.74	0.04	0.01	0.82
mtDNA:nDNA ratio[2]	265	277	217	225	13	0.03	0.48
Enzyme activity of mitochondrial complex							
NADH ubiquinone oxidoreductase, mU/mg protein	44.0	43.3	44.6	43.4	3.65	0.16	0.60
Succinate dehydrogenase, mU/mg protein	90.0	93.9	88.3	94.1	7.11	0.27	0.08
Gene expression encoding mitochondrial complex proteins[3]							
NDUFA4	1.01	1.02	1.12	1.11	0.04	0.08	0.91
NDUFA13	0.75	0.81	0.83	0.77	0.09	0.29	0.84
NDUFC1	0.81	0.80	0.94	0.93	0.08	0.81	0.95
SDHA	0.68	0.77	0.45	0.77	0.17	0.43	0.23
SDHD	1.26	1.90	2.16	2.22	0.55	0.22	0.27
UQCRC1	0.72	0.64	0.97	1.00	0.29	0.98	0.35
UQCRH	0.86	0.92	1.01	0.93	0.10	0.19	0.98
COX19	0.80	0.77	0.89	0.92	0.09	0.19	0.82
CYC1	1.39	1.33	0.99	0.55	0.39	0.05	0.34
ATP5E	0.79	0.93	0.74	0.88	0.07	0.05	0.18
ATP5O	0.95	0.66	0.59	0.63	0.17	0.31	0.51

[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]mtDNA/nDNA ratio: mitochondrial DNA (mtDNA) and nuclear DNA (nDNA) ratio.
[3]Genes: NADH:ubiquinone oxidoreductase subunit C1 (*NDUFC1*), NADH:ubiquinone oxidoreductase subunit A4 (*NDUFA4*), NADH:ubiquinone oxidoreductase subunit A13 (*NDUFA13*), succinate dehydrogenase complex, subunit A, flavoprotein (Fp) (*SDHA*), succinate dehydrogenase complex, subunit D (*SDHD*), ubiquinol-cytochrome c reductase core protein I (*UQCRC1*), ubiquinol-cytochrome c reductase hinge protein (*UQCRH*), cytochrome c oxidase assembly factor (*COX19*); cytochrome c-1 (*CYC1)*, ATP synthase, H+ transporting, mitochondrial F1 complex, epsilon subunit (*ATP5E*); ATP synthase, H+ transporting, mitochondrial F1 complex, O subunit (*ATP5O).*
[4]The interaction HAxCG was not significant (*P*> 0.16).

Plasma and hepatic oxidative markers and antioxidant function

Plasma pROS was greater (P = 0.02) and PAC tended (P = 0.07) to be greater forCR than PU cows (Table 3). Oxidative stress index was affected (P = 0.02) 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 3). Hepatic TBARS and protein carbonyls concentrations were not affected by HA treatment, CG or their interaction (Table 3) but hepatic 4-HNE expression tended to be greater (P = 0.06) forCR than PU cows and tended to be affected by the interaction between HA treatment and CG (P = 0.06) as it was greater (P < 0.05) in LO-CR than LO-PU cows while did not differed from HI-CR and HI-PU cows (Table 3 and Figure 2). Neither the hepatic GPX and SOD enzyme activity (Table 3) nor SOD2 protein expression (Supplementary Figure S1C) were affected by HA treatment, CG or their interaction. However, the expression of *SOD2* mRNA tended (P = 0.08) to be greater in CR than PU cows (Table 3). The expression of *GLGC, GPX1, GPX3, GPX4, PRDX3, PRDX5* and *SOD1* mRNA in the liver were not affected by HA treatment, CG or their interaction (Table 3).
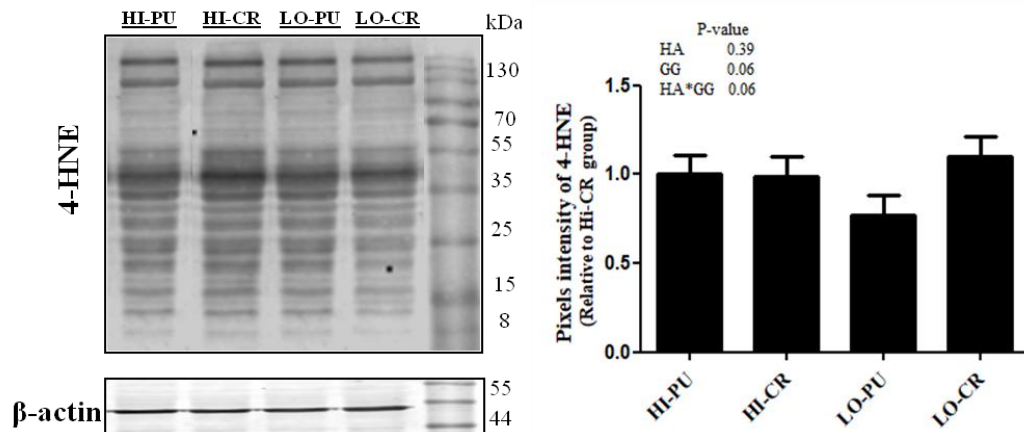
Figure 2: Representative western blot analysis of 4-hydroxynonenal protein adduct (4-HNE) in liver of and means ± SE of pixels expressed according to treatment [purebred and crossbred beef cows grazing high and low in average herbage allowances (2 vs. 4 kg DM/kg BW of annual mean, respectively) of native pastures: High-crossbred cows (HI-CR); High-purebred cows (HI-PU); Low-crossbred cows (LO-CR); Low-purebred cows (LO-PU)]. A quantity of 30 µg of proteins were loaded and separated by SDS-PAGE. Immunoblotting was performed with a polyclonal antibody that recognized 4-HNE. Data were normalized with β-actin density and normalized values were expressed as a percentage relative to the HI-CR group (Control). Means ± SE of pixels expressed according to herbage allowances and cow genotype. [n = 32, 8 cows per treatment].

Table 3: Effects of herbage allowance (HA) and cow genotype (CG) and their interaction (HAxCG) on plasma and hepatic oxidative stress markers and antioxidant enzyme activity.

	Treatment[1]					P-value[6]	
Item	**HI-PU**	**HI-CR**	**LO-PU**	**LO-CR**	**SE**	**HA**	**CG**
Plasma oxidative stress markers[2]							
pROS, CarrU/mL	248.1	235.9	206.8	266.7	13.3	0.71	0.02
PAC, µmol HClO/mL	309.3	317.7	312.7	323.1	8.0	0.27	0.07
OSi, CarrU/µmol HClO/mL	0.81	0.76	0.67	0.83	0.05	0.53	0.55
Hepatic oxidative stress markers[3]							
TBARS, µg MDA/mg protein	16.12	16.7	18.2	16.3	1.6	0.43	0.53
Protein carbonyl, nmol DNPH/mg protein	1.36	1.26	1.39	1.37	0.14	0.94	0.63
4-HNE, pixels intensity	0.99	1.0	0.79	1.1	0.02	0.39	0.06
Hepatic antioxidant enzyme activity[4]							
GPX, U/mg protein	276.5	286.5	274.3	284.0	12.3	0.48	0.65
SOD total, U/mg protein	18.9	19.1	19.2	19.5	2.1	0.35	0.78
Hepatic gene expression of antioxidant enzymes[5]							
GLGC	0.89	0.97	1.04	0.98	0.09	0.36	0.96
GPX1	1.02	1.16	1.08	0.92	0.14	0.49	0.89
GPX3	0.63	0.45	0.61	0.55	0.19	0.78	0.40
GPX4	0.94	1.02	1.01	1.31	0.10	0.17	0.50
PRDX3	0.61	0.69	0.75	0.68	0.08	0.16	0.50
PRDX5	0.99	0.91	1.04	0.99	0.09	0.22	0.18
SOD1	0.92	0.86	0.89	0.76	0.10	0.68	0.76
SOD2	0.81	0.81	0.91	0.83	0.08	0.41	0.08

[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]Plasma pro-oxidants (pROS); Plasma antioxidant capacity (PAC): Oxidative stress index (OSi).

[3]Thiobarbituric acid reactive species (TBARS) expressed as concentration of malondialdehyde (MDA); Dinitrophenylhydrazine (DNPH);4-hydroxynonenal (4-HNE) pixels intensity expressed with proportion of HI-CR animals.[4]Glutathione peroxidase (GPX); Superoxide dismutase (SOD). [5]Genes: glutamate-cysteine ligase catalytic subunit (*GCLC*), glutathione peroxidase 1 (*GPX1*), glutathione peroxidase 3 (*GPX3*), glutathione peroxidase 4 (*GPX4*), peroxiredoxin 3 (*PRDX3*) and peroxiredoxin 5 (*PRDX5*), copper zinc superoxide dismutase 1, soluble (*SOD1*), manganese superoxide dismutase 2, mitochondrial (*SOD2*). [6]The interaction HAxCG was not significant ($P > 0.17$) for all variables, except OSi ($P = 0.02$) and 4-HNE ($P = 0.06$).

5.5. DISCUSSION

This study evaluated effects of nutrition, through controlling grazing intensity, on hepatic mitochondrial density and function and oxidative stress of rangeland pure and crossbred beef cows and their association with energy efficiency in cow-calf systems.

In agreement with their greater cow-calf energy efficiency, estimated ME intake during the gestation-lactation cycle and retained energy of weaned calves were greater or tended to be greater for HI than LO cows. Do Carmo et al. (2016; 2018) estimated energy intake using NRC (2000) equations and reported, as consequence of the reduced forage mass, less energy intake (~11%) in cows grazing low than high HA. Retained energy of weaned calves and cow-calf energy efficiency were also greater in CR than PU cows. Previous research has reported significant heterosis (F1) not only in cow energy intake but also in cow milk production and calf BW at weaning (Do Carmo et al., 2016; Gutierrez et al., 2013). Morris et al. (1987) reported that reciprocal crossbreeding (F1) cows between Angus and Hereford increased calving and weaning rate, kg of calf weaned per cow exposed and cow-calf efficiency when compared to purebred cows. Although ME intake was greater for HI than LO cows, The increased productive response of HI than LO cows was proportionally greater than the differences in ME intake between these groups, therefore HI cows were also more efficient than LO cows in the use of the consumed energy.

Mitochondria are the principal organelle for the production of ATP in the cell and play an essential role in nutrient adaptation. Recently, it has been determined, in steers differing in residual feed intake, that energy efficiency was associated to mitochondria density and function (Lancaster et al., 2014; Casal et al., 2018). However, in the present work, energy efficiency (g or KJ calf retained energy/MJ cow ME intake) did not correlate with mitochondrial density which could be explained by the greater dispersion of the measure of efficiency in calf-cow systems, as it involves several biological functions or process (ie. gestation, lactation, growth) in both, cow

and calf when compared with the RFI measurement.

The greater mitochondrial density (greater *CS* mRNA and enzyme activity and mtDNA:nDNA ratio; Larsen et al., 2012) in liver tissue of HI than LO cows was associated with their greater ME intake during the annual production cycle. This would indicate an improved bioenergetic capacity in cows grazing HI-HA as mitochondrial volume or content are important quantitative indicators of metabolic activity and oxidative capacity (Lee and Wei, 2005; Weikard and Kühn, 2016; Casal et al., 2018). Contrary to our results, Nisoli et al. (2005) reported calorie-restricted mice presented increased mitochondrial density in liver and muscle when compared to non-restricted animals but Hancock et al. (2011) in rats did not report effects of caloric restriction on mitochondrial density. Discrepancy between our and these previous studies may reflect specie differences since in ruminants mitochondria constitutes one of the fundamental steps in the utilization of propionate (generated at ruminal level) for hepatic gluconeogenesis.

Mitochondrial complexes are not separate entities, but assembled multiproteic subunits, which have structural and functional dependence of the individual subunits. The expression of genes encoding for respiratory chain protein complexes in the liver (*CYC1* and *ATP5E*) were greater for HI than LO cows. The *CYC1* mRNA encodes a protein to subunit c of the cytochrome *bc*1 complex, which plays an important role in the mitochondrial respiratory chain by transferring electrons to cytochrome *c* (Schägger et al., 1995) while the *ATP5E* mRNA encodes a protein to complex V (ATP synthase) the universal enzyme that manufactures ATP from ADP and phosphate by using the energy derived from a transmembrane proton-motive gradient (Wang and Oster, 1998). Connor et al. (2009) and Casal et al. (2018) reported greater expression of genes associated with the mitochondrial respiratory chain proteins for high vs. low efficiency steers. These authors indicated that the better efficiency would be associated with changes at the transcription level, by affecting rates of mRNA synthesis and/or degradation, of some proteins of the mitochondrial respiratory chain complexes. However, SDH activity, an

enzyme complex that not only is involved in the respiratory chain but also constitutes one of the fundamental steps in the utilization of propionate (generated at ruminal level) for hepatic gluconeogenesis, tended to be greater for CR than PU cows without differences in neither expression of *SDHA* or *SDHD* mRNA nor SDHA protein expression, suggesting that changes in SDH activity were associated to post-translational modifications.

In addition to its role in energy metabolism, mitochondria are also recognized as a major source of oxidants (reactive oxygen/nitrogen metabolites) in the majority of cell types (Turrens, 2003; Bottje and Carstens, 2009). Oxidative stress occurs when an imbalance between oxidants and antioxidants in which oxidant activity exceeds the antioxidant capacity to detoxify or to repair the resulting damage (Chirase et al., 2004; Radi, 2018). All cows presented PAC values (capacity of a massive dose of hypochlorous acid to oxidize the antioxidant pool; Trotti et al., 2001) lower than 350 umol HClO/mL indicating an impairment of serum antioxidant barrier. Quantification of antioxidant separately does not provide good information of the antioxidant capacity, because the different antioxidants can act synergically to counteract the oxidative offence (Abuelo et al., 2013). Oxidative stress, indicated by OSi (the ratio pROS (oxidants) and PAC (antioxidants); Celi, 2011) was reduced in LO-PU cows (lower pROS and OSi) and increased in LO-CR cows. An increased OSi indicates an increase in oxidants production or decreasein defensive antioxidants (Celi, 2011), which in our study, was probably associated to the greater productive performance thus, metabolic activity of CR than PU cows in restrictive environments (LO-HA). At the hepatic level, although no differences between cow genotypes were reported in other oxidative stress markers or in the main enzymatic antioxidants (enzyme activity or gene expression), expression of 4-HNE tended to be the greatest in LO-CR and the lowest in LO-PU cows. The 4-HNE is a reactive aldehyde originating from the peroxidation of liver microsomal lipids causing covalent modification of macromolecules (Ayala et

al., 2014). Greater OSi and 4-HNE expression in   could be associated to their greater metabolic activity recorded by cellularity index (protein:DNA ratio, RNA:protein ratio; total RNA) when compared to PU cows, especially for LO-CR cows that presented greater hepatic mass, larger and metabolically more active cells (Casal et al., 2014). Several animal studies seem to suggest that oxidative stress biomarkers change toward the same direction in blood and tissues (Argüelles et al., 2004; Celi, 2011; Abuelo et al., 2013; 2014). However, Argüelles et al. (2004) suggested that the increase of oxidative stress just in a particular tissue may not affect plasma biomarkers or increase of oxidative damage produced specifically in the circulatory system, explained the discrepancy between the results found in plasma and liver in the present study. In addition, the greater oxidative stress determined for LO-CR than LO-PU cows, was related with greater hepatic *SOD2* mRNA expression, one of the main antioxidants which is localized in the mitochondrial matrix and acts as the primary defense against superoxide produced in the latter ones.

Although it has been reported in steers of different RFI that energy efficiency was associated with mitochondria function and oxidative stress (Casal et al., 2018; 2019), in cow-calf systems this association was not clear. Both, RFI and energy efficiency of cow-calf systems (g or KJ calf retained energy/MJ cow ME intake) identify the most efficient animals (Koch et al., 1963; Jenkins and Ferrell 1994). However, RFI it is a direct measurement (relationship between observed and expected intake) with a high genetic component and efficiency of the cow-calf system is less direct and includes both, cow and calf efficiency in a single parameter, that although it involves genetic component, is strongly influenced by environmental components. Differences in HA were more associated with differences in the hepatic mitochondrial density, while differences in the liver oxidative status were more related to the genotype of the cows. Other factors such as the dry matter intake (Freetly et al., 1995, Do Carmo et al., 2018), activity and grazing behavior (Brosh et al., 2006, Scarlato et al., 2011), body mass and

composition (Ferrell and Jenkins, 1985; Casal et al., 2017), mass of the gastrointestinal tract and other organs, and their metabolic activity (Baldwin et al., 2004; Casal et al., 2014) present a greater association with the mechanisms that determine maintenance energy requirements and energy efficiency of cow-calf systems.

5.6. CONCLUSION

Relationships between energy efficiency and mitochondrial function and oxidative stress were found in other models such as RFI, however, our results suggest that this association is not as clear in the cow-calf system, although these mechanisms could be associated with energy efficiency, we do not find a relationship as direct as in the case of efficiency measured through RFI. Research in mitochondrial function and oxidative stress in ruminant production, particularly in cow-calf systems, is limited and there is a great deal to be discovered about its role in ruminant health and production. Further research is needed to identify the mechanism involved in the differential physiological processes between animals with low and high energy efficiency.

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5.8. SUPPLEMENTARY MATERIAL

Table S1. Primers used for real time qPCR.

Gene[1]	Accession#[2]		Primer sequence[3]	Length (bp)	Efficiency
ACTB	BT030480	F	CTCTTCCAGCCTTCCTTCCT	178	1.15
		R	GGGCAGTGATCTCTTTCTGC		
ATP5E	NM_001143741	F	GACATCATGGTGGCGTACTG	211	1.18
		R	CCACAGCTTCACCTTGAACA		
ATP5O	NM_174244.1	F	CCTCTCACGTCCAACCTGAT	131	1.06
		R	GCAGTGGTAACTGTGCATGG		
COX19	NM_001109966	F	CATACGGACATCCCTTTGCT	171	1.16
		R	GCTTCGGCAACTCCTTACTG		
CS	NM_001044721.1	F	AGCCAAGATACCTGTTCCT	217	0.99
		R	TGTGCTGGAAGAAACGATTG		
CYC1	NM_001038090	F	CCAGGTAGCCAAGGATGTGT	227	1.10
		R	GACCCTGAAGCTCAGGACAG		
GCLC	NM_001083674	F	CACAAATTGGCAGACAATGC	211	1.11
		R	GGCGACCTTCATGTTCTCAT		
GPX1	NM_17407	F	ACATTGAAACCCTGCTGTCC	216	1.09
		R	TCATGAGGAGCTGTGGTCTG		
GPX3	NM_174077	F	TGCAACCAATTTGGAAAACA	224	1.18
		R	TTCATGGGTTCCCAGAAAAG		
GPX4	NM_001346431.1	F	AGCCAGGGAGTAATGCAGAG	203	0.97
		R	CACACAGCCGTTCTTGTCAA		
HPRT1	XM_580802	F	TGGAGAAGGTGTTTATTCCTC	105	0.99
		R	CACAGAGGGCCACAATGTGA		
NDUFA4	NM_175820	F	TGCGGCTTAGCTTTTCTCTC	152	1.12
		R	GCGTGACATACAGTGCTGCT		
NDUFA13	NM_176672	F	TCGACTACAAGCGGAACCTT	233	1.11
		R	AGTTGCCTCCTCCTCCAAGT		
NDUFC1	NM_174564	F	GGTTCCCGAGTGTCTCTTCA	242	1.07
		R	CGTGAATCCAGAGGAACTGC		
PRDX3	NM_174432.2	F	CACACCAGAAAAGAGCCACA	210	1.16
		R	CTAGCCATCCATCCACACCT		
PRDX5	NM_174749.2	F	CCTTCTACCTCAGCCTCGAG	245	1.08
		R	CAACCTTAATCGGGGCCATG		
RPL19	NM_001040516.1	F	CCCCAATGAGACCAATGAAATC	156	1.14
		R	CAGCCCATCTTTGATCAGCTT		
SDHA	NM_174178.2	F	ACATGCAGAAGTCGATGCAG	155	1.03
		R	GGTCTCCACCAGGTCAGTGT		
SDHD	NM_174179.2	F	TTTGGCTAGGATGGATGGAG	92	1.02
		R	ACTGAACAGAGGGGGAGGTT		
SOD1	NM_174615	F	AGAGGCATGTTGGAGACCTG	189	1.16
		R	CAGCGTTGCCAGTCTTTGTA		
SOD2	NM_201527.2	F	CAGGGACGCTTACAGATTGC	212	1.14
		R	CTGACGGTTTACTTGCTGCA		
UQCRC1	NM_174629.2	F	CAGTCTTCCCAGCCTACCTG	105	0.98

UQCRH	NM_001034745	R	AGCCAGATGCTCCACAAAGT		
		F	CTGGTGTGGCTAAGGGGATA	232	1.10
		R	GGACTCAACACAAGCAGCAA		
NDUFV1	Genomic DNA	F	GTTCTTCTTAGGTTCTCACGTGG	251	
		R	TGAGAATTACTGACGTGACCTCT		
mt-CO1	Mitochondrial DNA	F	TCTTCCCACAACACTTTCTAGGA	198	
		R	TGTCGTGGTTAAGTCTACAGTCA		

[1]Genes: ß-actin (*ACTB*), ATP synthase, H+ transporting, mitochondrial F1 complex, epsilon subunit (*ATP5E*); ATP synthase, H+ transporting, mitochondrial F1 complex, O subunit (*ATP5O*), cytochrome c oxidase assembly factor (*COX19*); citrate syntase (*CS*), cytochrome c-1 (*CYC1*), glutamate-cysteine ligase catalytic subunit (*GCLC*), glutathione peroxidase 1 (*GPX1*), glutathione peroxidase 3 (*GPX3*), glutathione peroxidase 4 (*GPX4*),hypoxanthine phosphoribosyl transferase (*HPRT1*); NADH:ubiquinone oxidoreductase subunit A4 (*NDUFA4*), NADH:ubiquinone oxidoreductase subunit A13 (*NDUFA13*), NADH:ubiquinone oxidoreductase subunit C1 (*NDUFC1*), peroxiredoxin 3 (*PRDX3*) and peroxiredoxin 5 (*PRDX5*),copper zinc superoxide dismutase 1, soluble (*SOD1*), manganese superoxide dismutase 2, mitochondrial (*SOD2*), ribosomal protein L19 (*RPL19*),succinate dehydrogenase complex, subunit A, flavoprotein (Fp) (*SDHA*), succinate dehydrogenase complex, subunit D (*SDHD*), ubiquinol-cytochrome c reductase core protein I (*UQCRC1*), ubiquinol-cytochrome c reductase hinge protein (*UQCRH*). Mitochondrially encoded cytochrome c oxidase I (*mt-CO1*, mitochondrial gene) and NADH:ubiquinoneoxidoreductase core subunit V1 (*NDUFV1*, nuclear gene).

[2]Gene bank sequences.

[3]F = foreword; R = reverse

6. DISCUSIÓN GENERAL Y CONCLUSIONES

La productividad de los animales se encuentra asociada a su balance energético, determinado por las diferencias entre el consumo y los requerimientos de energía (mantenimiento y producción). El costo del mantenimiento tiene un impacto significativo en la partición de la energía metabolizable y, por lo tanto, puede afectar la eficiencia biológica y económica de la producción de bovinos de carne (Johnson *et al.,* 2003). Richardson y Herd (2004) informaron que los mecanismos biológicos que explican el consumo residual de alimento (Koch *et al.,* 1963) están estrechamente relacionados con los mecanismos que determinan los requisitos de energía de mantenimiento (principalmente en el metabolismo basal). Diferencias en los mecanismos de partición de la energía, pueden afectar la eficiencia biológica y económica de la producción de bovinos de carne. El presente trabajo se centra en la comprensión de componentes del mecanismo de partición de la energía y su relación con la eficiencia productiva, en dos modelos bovinos: Novillos con fenotipo divergente para RFI (*Artículos 1 y 2*) y Vacas de cría multíparas pastoreando distintas ofertas de forraje de campo natural (*Artículos 3 y 4*).

6.1. EFICIENCIA ENERGÉTICA Y MASA DE LAS VÍSCERAS

Considerando el uso global de la energía para los tejidos que la producción ganadera prioriza (ej. canal o muscúlo esquelético magro) se puede considerar a la energía utilizada por la masa total de los órganos como un "impuesto a la producción" (Reynods, 2002). Los novillos de bajo RFI alcanzaron un PV y una tasa de crecimiento similar que los novillos de alto RFI pero con menor consumo de MS. De hecho, los novillos con alto RFI tendieron a presentar una mayor masa hepática en comparación con los novillos con bajo RFI. Sin embargo, esta tendencia desapareció cuando la masa hepática se expresó en relación con el peso corporal vacío (*Artículo 1*). Las variaciones individuales en RFI resultaron de las diferencias en la ingesta de alimento y, en consecuencia, afectaron la carga de trabajo

metabólico de los órganos, especialmente el hígado (Montanholi *et al.,* 2017).

En vacas de cría, el control de la intensidad de pastoreo del campo natural, a través del manejo de la oferta de forraje, tuvo como resultado una mejora en la eficiencia energética del sistema vaca-ternero en las vacas que pastorearon HI y en las vacas CR. Esta mejora se asoció a una mayor respuesta productiva (g o MJ ternero/MJ de energía metabolizable consumido, *Artículo 4*) explicada por un mayor consumo de energía, mejor estatus metabólico y menor tamaño de las vísceras del TGI en las vacas que pastorearon HI vs. LO y las vacas CR vs. PU (Do Carmo *et al.,* 2016; 2018; Laporta *et al.,* 2014; Casal *et al.,* 2014).

En acuerdo a lo reportado Hersom *et al.* (2004), las vacas en HI presentaron un mayor rendimiento de faena, mayor peso de la canal (+10%, HI vs. LO), mayor peso de tejidos blandos y mayor contenido proteico en la canal (*Artículo 3*). Asimismo, consistente con Burrin *et al.* (1992), las vacas en HI presentaron mayor peso absoluto de la masa total de órganos que las vacas en LO, estas diferencias desaparecen al expresarlos en términos relativos ya que el tamaño absoluto de los órganos responden al tamaño o *frame* de las vacas (*Artículo 3*).

Por otra parte, la masa del total de órganos relativa al peso de la canal fue mayor en las vacas PU que CR (*Artículo 3*). Por lo tanto, nuestros resultados podrían sugerir que las vacas CR tendrían un menor costo de mantenimiento por unidad de producto que las vacas PU, dado fundamentalmente porque el peso relativo de la masa total de órganos respecto a la canal fue un 9.5% menor en vacas CR. Estudios realizados por Solís *et al.* (1988) reportan que a pesar que animales *Bos indicus* y *Bos taurus* presenten un peso de canal y tasa de crecimiento similares, los animales *Bos indicus* y sus cruzas presentaron menores requerimientos energéticos para el mantenimiento, asociado a un menor tamaño del TGI, hígado y corazón y menor cantidad de grasa visceral.

6.2. EFICIENCIA ENERGÉTICA Y FUNCIÓN MITOCONDRIAL

La tasa de uso de energía por un organismo, la tasa metabólica, es directamente proporcional a la tasa de consumo de oxígeno. Por lo tanto, la tasa de consumo de oxígeno se puede medir y usar para inferir la tasa metabólica indirectamente (Brand y Nicholls, 2011). Mediante respiromentría de alta resolución, se observó que los novillos con bajo RFI mostraron una mayor respiración basal (respiración en estado 3 después de la adición de ADP; respiración máxima estimulada por ADP) y capacidad respiratoria máxima cuando se les proporcionó succinato (complejo II) como sustratos respiratorios (*Artículo 1*). El mayor consumo de oxígeno registrado en novillos de bajo vs. alto RFI en el mismo nivel de sustratos, reflejó una mayor capacidad para obtener ATP por unidad de sustrato. Nuestros resultados no concuerdan con lo reportado en vacas de leche por Garcia-Roche *et al.* (2019) donde observaron un aumento en la respiración basal y capacidad respiratoria máxima en el complejo I pero no en el complejo II, estas diferencias estén posiblemente relacionadas al genotipo de los animales.

La capacidad respiratoria máxima es inducida por el desacoplador (FCCP) y representa la actividad máxima del transporte de electrones y la oxidación del sustrato que pueden lograr las células, se relacionaría con una mayor disponibilidad de cadenas respiratorias para hacer frente a sus demandas metabólicas (Brand y Nicholls, 2011) y podría considerarse como un indicador indirecto de la densidad mitocondrial. Esto último coincide con nuestros resultados donde los novillos de bajo RFI presentaron mayor número de mitocondrias por unidad de peso hepático determinado por medio de biomarcadores de contenido mitocondrial (expresión ARNm y actividad enzimática de citrato sintasa, relación ADNmt:ADNn; Larsen *et al.,* 2012).

El mayor consumo de oxigeno mitocondrial cuando se suministran sustratos del complejo II registrado en novillos de bajo RFI, se vió asociado a mayor expresión de ARNm de *SDHA*, mayor expresión proteica de SDHA y mayor actividad enzimática de la succinato deshidrogenasa (*Artículo 1*). El

complejo II es el único componente de la cadena de transporte de electrones que además cataliza una reacción del ciclo del ácido tricarboxílico mediante el acoplamiento de dos reacciones (oxidación del succinato a fumarato con la reducción de la ubiquinona a la ubiquinol). Estas características confieren a este complejo enzimático una posición única en el metabolismo, es un paso clave en el uso del propionato ruminal para la gluconeogénesis. Además, se ha sugerido (Guaras *et al.,* 2016; Scialo *et al.,* 2017) que el aumento de la actividad del complejo II en animales con bajo RFI podría llevar a un aumento en el flujo de electrones por la oxidación de $FADH_2$. Esto podría causar el transporte de electrones inversos que fomenta la separación del supercomplejo (complejos I y III) y un mayor pasaje de los electrones procedentes del $FADH_2$ al resto de la cadena respiratoria. Por lo tanto, animales con bajo RFI podrían optimizar la utilización de nutrientes en comparación con los novillos con alto RFI.

Si bien en vacas de cría encontramos una diferencia en la densidad de mitocondrias (*Artículo* 4), ésta no se relacionó a una mayor actividad de los complejos mitocondriales como si sucedió en el experimento de los novillos de RFI (*Artículo* 1). Cabe destacar que a diferencia del experimento de los novillos donde la actividad de los complejos se determinó de manera independiente y por respitometría en mitocondrias acopladas, para el caso de las vacas de cría solo fueron determinados en forma individual para cada uno de los complejos de manera independiente, esto pudo haber influído en los distintos resultados obtenidos entre experimentos.

El mayor contenido de mitocondrias en las vacas HI (*Artículo* 4) indicaría una mejor capacidad bioenergética, ya que el volumen o contenido mitocondrial son indicadores cuantitativos importantes de una mayor actividad metabólica y capacidad oxidativa (Lee y Wei, 2005; Weikard y Kühn, 2016). Contrariamente a nuestros resultados, Nisoli *et al.* (2005) informaron en ratones que los animales sometidos a una restricción en la ingesta calórica presentan un aumento significativo en la densidad mitocondrial en el hígado y el músculo en comparación con los animales no

restringidos. Sin embargo, Hancock *et al.* (2011) no encontró efecto de la restricción calórica en la densidad mitocondrial. Las discrepancias entre nuestros estudios y los anteriores puede reflejar diferencias entre especies ya que en los rumiantes la mitocondria constituye uno de los pasos fundamentales en la utilización del propionato (generado a nivel ruminal) para la gluconeogénesis.

6.3. EFICIENCIA ENERGÉTICA Y ESTRÉS OXIDATIVO

Además de su papel en el metabolismo energético, las mitocondrias también son reconocidas como una fuente importante de oxidantes en la mayoría de los tipos de células (Turrens, 2003; Bottje y Carstens, 2009). El estrés oxidativo se produce cuando hay un desequilibrio entre los oxidantes y los antioxidantes para desintoxicar o reparar el daño resultante (Chirase *et al.,* 2004; Radi, 2018).

El aumento de la tasa metabólica podría causar un aumento de la producción de oxidante y provocar un estrés oxidativo (Speakman, 2008). Un mayor consumo de oxígeno en animales con bajo RFI debería dar como resultado una mayor producción de ROS/RNS que podría aumentar el contenido de proteínas o lípidos oxidados. Sin embargo, nuestros resultados indicaron que los novillos de bajo RFI presentaron menor concentración de carbonilos proteicos, TBARS y 4-HNE hepáticos que los novillos de alto RFI (*Artículo 2*). La oxidación, remoción y resintesis de proteínas representaría un drenaje energético, disminuyendo la eficiencia energética ya que las proteínas oxidadas ya no son utilizables y están marcadas para la degradación a través del proceso de sistema dependiente de ATP lo que aumenta las necesidades energéticas para mantenimiento (Mehlhase y Grune, 2002). Por tanto, la oxidación de proteínas y lípidos se puede utilizar como un indicador de estrés oxidativo. De manera similar a nuestros resultados, Sandelin *et al.* (2005) informaron un mayor contenido de carbonilos proteicos en muestras de musculo en novillos de baja eficiencia en comparación con novillos altamente eficientes. Sin embargo resultados

contrarios fueron reportados en estudios realizados en plasma o mitocondrias aisladas de hígado en ganado bovino, mayor contenido de carbonilos proteicos en animales de bajo RFI (Russell *et al.,* 2016; Zulkif, 2016). Las discrepancias entre resultados puede reflejar diferencias debidas al tipo de tejido y/o incluso diferencias en la dieta (Xu *et al.,* 2014).Por lo tanto, el nivel de producción de oxidantes no solo se basa en la ineficiencia de las mitocondrias, sino que también otras fuentes de oxidantes pueden tener mayor relevancia según la muestra o el tejido que se analice.

La mitigación y prevención del estrés oxidativo se realiza mediante diversos sistemas antioxidantes (Turrens, 2003). Los novillos de bajo RFI tendieron a presentar mayor expresión proteica y actividad enzimática hepática de la manganeso SOD (MnSOD) (*Artículo 2*). Las concentraciones más altas de MnSOD aumentan la protección contra el estrés oxidativo, MnSOD se localiza en la matriz mitocondrial y actúa como la defensa principal contra el superóxido producido (Epperly *et al.,* 2002). A su vez, los novillos de bajo RFI presentaban mayor actividad enzimática de GPX y expresión de ARNm de *GPX3*, *GPX4* y *GCLC* (*Artículo 2*). El glutatión reducido y el grupo de enzimas encargadas del reciclaje del glutatión constituyen el centro del sistema de defensa antioxidante frente a los oxidantes.

La reducción del glutatión oxidado se realiza en el citosol y luego es exportado a la mitocondria, glutamato cisteína ligasa es la enzima limitante en la síntesis de glutatión reducido (Bolaños *et al.,* 1996). La mayor expresión de mRNA de la unidad catalítica de la glutamato-cisteína ligasa (*Artículo 2*) indicaría que los novillos con RFI bajo podrían ser capaces de sintetizar más glutatión reducido o sintetizarlo a una tasa mayor que los novillos de alto. En contraste con nuestro trabajo, Kidrick *et al.* (2016) informaron que la actividad de GPX en suero y la capacidad antioxidante total en suero no se correlacionaron con la RFI en vaquillonas en pastoreo. Además, Russell *et al.* (2016) informaron que la actividad de GPX, actividad total de SOD y actividad de MnSOD en lisado de glóbulos rojos aumentaron

en los novillos de alto vs bajo RFI. El desacuerdo entre nuestro estudio y este último puede reflejar diferencias en los tejidos y/o incluso diferencias en la dieta o fases de crecimiento animal (Xu *et al.,* 2014).

En vacas de cría, la concentración de pro-oxidantes en plasma fue mayor en CR que en las vacas PU y se vió afectada por la interacción entre oferta de forraje y genotipo de las vacas y la capacidad antioxidante plasmática tendió a ser mayor en CR que las vacas PU (*Artículo 4*). Sin embargo, la medición separada de oxidantes y antioxidantes dan información limitada respecto al estatus oxidativo (Abuelo *et al.,* 2013). Se ha propuesto al índice de estrés oxidativo (**OSi**; relación oxidantes y antioxidantes) como un indicador del riesgo al daño por estrés oxidativo, por lo tanto, un aumento en el OSi indica el aumento en la producción de oxidantes o disminución de los antioxidantes. El OSi se vio afectado por la interacción entre HA y CG, ya que fue mayor en LO-CR, más bajo en vacas LO-PU e intermedio en las vacas HI-CR y HI-PU (*Artículo 4*). Los resultados sugirieron que el estrés oxidativo probablemente se reduciría en las vacas LO-PU y aumentaría en las vacas LO-CR, esto posiblemente se asociaría a una actividad metabólica de CR que las vacas PU en ambientes restrictivos (LO).

A nivel hepático, a excepción de la expresión de 4-HNE que tendió a ser más alta en CR que en vacas PU, no se reportan diferencias en otros marcadores de estrés oxidativo o en los principales antioxidantes enzimáticos (actividad enzimática o expresión génica) (*Artículo 4*). Una mayor expresión de OSi en plasma y de 4-HNE hepático en vacas CR podría estar asociada a una mayor actividad metabólica de las vacas CR que PU, especialmente las vacas LO-CR que presentaron mayor masa hepática, células más grandes y metabólicamente más activas (Casal *et al.*, 2014). A su vez, las vacas CR tendieron a presentar una mayor expresión génica de MnSOD, uno de los principales antioxidantes que se localiza en la matriz mitocondrial y actúa como la defensa principal contra el superóxido producido (*Artículo 4*). En resumen, las diferencias en las ofertas de forrajes

estuvieron más asociadas a diferencias en el contenido de mitocondrias a nivel hepático, mientras que las diferencias en el estatus oxidativo a nivel del hígado estuvo más relacionado con el genotipo de las vacas.

6.4. CONCLUSIONES

En los novillos de diferentes fenotipos para RFI la eficiencia energética se ha asociado con una mejor función mitocondrial y menor estrés oxidativo hepático (*Artículos 1 y 2*). Sin embargo, esta asociación no es tan clara en el sistema vaca-ternero (*Artículo 4*) y si bien estos mecanismos podrían estar asociados a la eficiencia energética no encontramos una relación tan directa como en el caso de la eficiencia medida a través del RFI. Otros factores, como el tamaño del TGI y otros órganos, y su actividad metabólica (*Artículo 3*), el consumo de MS, masa y composición corporal presentan una mayor asociación con los mecanismos que determinan los requisitos de energía de mantenimiento y la eficiencia energética del sistema vaca-ternero. Se necesitan futuras investigaciones para seguir explorando los mecanismos relacionados a la eficiencia energética del sistema vaca-ternero.

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