UNIVERSIDAD DE LA REPÚBLICA
FACULTAD DE AGRONOMÍA

UTILIZACIÓN DE LA GLICERINA CRUDA DERIVADA DE LA INDUSTRIA DEL BIODIESEL PARA LA SUPLEMENTACIÓN DE VACAS DE CRÍA

por

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

MONTEVIDEO
URUGUAY
OCTUBRE 2014

Tesis aprobada por el tribunal integrado por Ing. Agr. (MSc) María de Jesús Marichal, Ing. Agr. (PhD) Fabio Montossi e Ing. Agr. (MSc) Ana Laura Astessiano, el 10 de octubre de 2014. Autor: Ing. Agr. Juan Manuel Clariget. Directora: Dra M.V. (PhD) Raquel Pérez Clariget, Co-directora: Ing. Agr. (MSc) María de los Ángeles Bruni.

AGRADECIMIENTOS

A mi familia y amigos por su apoyo incondicional y comprensión durante esta etapa.

A mi tutora Dr. M.V. (PhD) Raquel Pérez y a mi cotutora, Ing. Agr. (MSc) María de los Ángeles Bruni por su permanente guía y compromiso con este trabajo. Por todos los consejos vertidos para mi formación como investigador y como persona.

A todos los integrantes del tribunal de defensa de seminarios y defensa de tesis Ing. Agr. (PhD) Mariana Carriquiry, Ing. Agr. (PhD) Pablo Soca, Ing. Agr. (MSc) Ana Inés Trujillo, Ing Agr. (Msc) Ana Laura Astessiano, Dr. M. V. (PhD) Jorge Gil, Ing. Agr. (PhD) Fabio Montossi e Ing. Agr. (MSc) María de Jesús Marichal y por las correcciones y aportes brindados para el análisis de este trabajo.

A la Ing. Agr. (PhD) Graciela Quintans y la Dr. M.V. (PhD) Carolina Viñoles por sus revisiones y comentarios de los artículos en ingles.

A la Agencia Nacional de Investigación e Innovación por otórgame una beca de Maestría (POS_2011_1_3522) y financiarme parte del proyecto de Maestría (FMV_3_2011_1_6713), sin los cuales no hubiera podido continuar con mi capacitación.

Al Instituto Nacional de Investigación Agropecuaria por brindarme el tiempo necesario para terminar la redacción de la tesis.

A la Estación Experimental “Bernardo Rosengurtt” por la posibilidad de realizar el trabajo de campo de esta tesis en sus instalaciones y a todo el personal quienes de una forma u otra colaboraron en la realización de la misma y especialmente al Director de la Estación Ing. Agr. Carlos Mantero.

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RESUMEN

Con el objetivo de aportar información sobre el uso de la glicerina cruda (GC) como suplemento energético en ganado de carne pastoreando campo natural (CN) tres experimentos fueron realizados. En el primero el objetivo fue evaluar el impacto de la inclusión de GC (550 mL/vaca/día) asociada o no al afrechillo de arroz (AA; 1 kg de MS/vaca/día) sobre el consumo, parámetros ruminales y variables plasmáticas. En el segundo y tercero el objetivo fue evaluar el efecto de una suplementación en vacas primíparas con AA+GC (1 kg de MS/vaca/día + 550 mL/vaca/día) durante 21 días pre-entore (segundo); o una suplementación 52 días pre-parto o 21 días pre-entore y su posible interacción (tercero), sobre el peso vivo (PV) de las vacas y terneros, la condición corporal (CC), la composición y producción de leche (PL), perfiles hormonales y metabólicos, actividad ovárica, tasa de preñez y funcionamiento hepático. En el primero la suplementación con GC no afectó la tasa de degradabilidad de la MS del forraje, la concentración N-NH_3 ruminal ni de B-hydroxibutirato (BHB) plasmática. Por otro lado la suplementación con AA+GC logró los mayores consumos de energía y las mayores concentraciones de glucosa e insulina plasmática. En el segundo experimento la suplementación con AA+GC no mostró daños hepáticos y mejoró el balance energético reflejado en incrementos plasmáticos de glucosa, insulina y colesterol y disminuciones de ácidos grasos no esterificados (AGNE), permitiendo una mayor PL y PV al destete de los terneros, pero sin tener efecto significativo en las variables reproductivas. En el tercer experimento la suplementación con AA+GC en el pre-parto o en el pre-entore mejoraron el balance energético reflejado en incrementos plasmáticos de colesterol, glucosa o insulina y disminuciones de BHB, AGNE y urea, permitiendo una mejor CC al parto (suplementación pre-parto) o un incremento en la PL (suplementación pre-entore), pero solo la suplementación pre-parto mostró una mejora en el comportamiento reproductivo. No hubo beneficio por la suplementación en ambos períodos. Estos resultados muestran que es posible la utilización de la GC como suplemento de vacas de carne pastoreando CN y que su administración junto con AA logra mejorar el desempeño productivo y/o reproductivo de las vacas.

Palabras clave: glicerol, metabolismo, rumen, toxicidad

UTILIZATION OF CRUDE GLYCERIN DERIVED FROM BIODIESEL INDUSTRY FOR BEEF COWS SUPPLEMENTATION

SUMMARY

With the aim to provide information on the use of crude glycerin (CG) as an energy supplement in beef cattle grazing natural grass (NG), three experiments were performed. In first experiment, the aim was evaluate the impact of inclusion CG (550 mL/cow/day) associated or not with rice bran (RB, 1 kg of DM/cow/day) on intake, ruminal parameters and plasma variables. In second and third experiment, the aim was evaluate the effect of supplementation in primiparous cows with RB + CG (1 kg DM/cow/day + 550 mL/cow/day) for 21 days pre-mating (second); and 52 days pre-partum or 21 days pre-mating and possible interactions (third) on the body weight of cows and calves, body condition score (BCS), composition and milk production (MP), hormonal and metabolic profiles, ovarian activity, pregnancy rate and liver function. In first experiment, CG supplementation did not affect rate of degradability of forage DM, ruminal N-NH_3 concentration or B-hydroxibutirate (BHB) plasma concentration. Moreover, RB+CG supplementation showed the most energy intake and the highest plasma concentrations of glucose and insulin. In second experiment, RB+CG supplementation showed no liver damage and improved energy balance reflected on increases in plasma concentration of glucose, insulin and decreases in cholesterol and non-esterified fatty acids (NEFA), allowing greater MP and weaning weight of calves, but without significant effect on reproductive variables. In third experiment, RB+CG supplementation in pre-partum or pre-mating improved energy balance reflected on increases in plasma concentration of cholesterol, glucose or insulin and decreases in BHB, NEFA and urea, allowing better BCS at calving (pre-partum supplementation) or increase in MP (pre-mating supplementation), but only pre-partum supplementation improved reproductive performance. No benefit for supplementation in both periods was found. Results shown that is possible the use of CG as a supplement to beef cows grazing NG and the administration with RB improve the productive and/or reproductive performance.

Keywords: glycerol, metabolism, rumen, toxicity

1. INTRODUCCIÓN

La exportación de carne bovina es el segundo rubro exportador de Uruguay, con un ingreso que supera los 1.400 millones de dólares anuales (DIEA, 2013). Sin embargo, la eficiencia reproductiva de los rodeos de cría del país está lejos de su potencial biológico. El porcentaje de destete nacional en los últimos 30 años ronda en 64% y presenta marcadas oscilaciones entre años (DIEA, 2013). Por ejemplo, la seca de 2008-2009 determinó 13 puntos porcentuales menos de preñez con la consecuente disminución de terneros (DIEA, 2009). Este bajo porcentaje del destete compromete el complejo exportador cárnico.

La cría en Uruguay se realiza a cielo abierto sobre campo natural constituyendo una fortaleza ambiental del país, pero la producción de pasturas es estacional presentando una crisis invernal en disponibilidad y calidad (Bermudez y Ayala, 2005; Formoso, 2005; Carámbula, 1991). En particular, la baja producción invernal de forraje, coincide con el momento en el que las vacas se encuentran en gestación avanzada o inicio de lactancia y determina un período de balance energético negativo. Esto se ve reflejado en un pobre estado nutricional de las vacas al parto e inicio del entore, determinando un largo período de anestro pos-parto (92 días en promedio en vacas adultas y mayores a 120 días en vacas primíparas; Quintans *et al*., 2004; Quintans y Vázquez, 2002; Lalman *et al*., 2000) y una baja probabilidad de preñez (Orcasberro, 1991).

Tanto durante el pre-parto como en el pos-parto, las demandas fetales y de lactación incrementan los requerimientos de energía y proteína de la madre (Davis *et al*., 1994), comprometiendo su estatus metabólico. El estatus metabólico, nutrientes y energía disponibles para la vaca en determinado momento, depende de la utilización de energía, de las reservas corporales y de la cantidad y calidad de alimento que el animal ingiere (Blache *et al*., 2006). Metabolitos y hormonas como: glucosa, insulina, ácidos grasos no esterificados (AGNE), colesterol, B-hydroxibutirato

(BHB) y urea han sido propuestos como señales que actúan en la interacción nutrición-reproducción (Hess *et al*., 2005; Webb *et al*., 2004; Bossis *et al*., 2000).

La capacidad de la madre para adaptarse y sobrellevar el balance energético negativo provocado por el crecimiento fetal y posterior lactancia, depende de la capacidad de los mecanismos endócrinos y metabólicos de mantener la homeostasis (Chilliard *et al*., 1998). Estos mecanismos se caracterizan por el aumento de la gluconeogénesis hepática, la disminución de la utilización de glucosa por parte de los tejidos periféricos, la movilización de AGNE del tejido adiposo y el aumento de la utilización de los mismos por parte de los tejidos periféricos (Bell, 1995). Durante este período, también se produce catabolismo proteico que aporta aminoácidos a la gluconeogénesis hepática ya sea para el feto o para la producción de leche, siendo posible también su destino para la síntesis de proteína de la leche (Lucy, 2008). Estos cambios se reflejan en modificaciones de las concentraciones plasmáticas de metabolitos: disminución de la glucosa y aumento de los AGNE (Astessiano, 2010; Gestido *et al*., 2008; Meikle *et al*., 2004) y de hormonas metabólicas como la insulina, cuyas concentraciones disminuyen (Scarsi, 2012; Meikle *et al*, 2004). Estas adaptaciones permiten aumentar la disponibilidad de glucosa y aminoácidos para el feto durante el pre-parto y la glándula mamaria durante el pos-parto-lactancia temprana. En nuestro sistema criador, ambas situaciones, pre y pos-parto-lactancia temprana, coinciden con una disminución de la disponibilidad y/o calidad de forraje lo que agrava la situación (Astessiano, 2010; Gestido *et al*., 2008).

La vaquillona de primer parto es la categoría del rodeo más exigido metabólicamente, ya que a las demandas del feto y lactancia temprana, se suman las de su propio crecimiento, por lo que el reinicio de la actividad cíclica pos-parto se ve comprometida (Short *et al*., 1990). Es así, que la duración del anestro pos-parto es dependiente del balance energético negativo (Chagas *et al*., 2006; Meikle *et al*., 2004; Lucy, 2003). Una de las principales causas del prolongado anestro pos parto, entonces, es la nutrición a la que se suma, el efecto del amamantamiento/presencia del ternero (Hoffman *et al*., 1996; Williams, 1990).

Teniendo en cuenta que el balance energético pre y pos-parto influyen el comportamiento productivo y reproductivo de las vacas de cría (Hess *et al*., 2005) se ha planteado la suplementación pre-parto como posible alternativa para levantar esta restricción. Para algunos autores, la nutrición pre-parto, que se refleja en la CC al parto, es el factor más importante en la determinación de la duración del anestro pos-parto en vacas primíparas de carne (Lalman *et al*., 1997; Perry *et al*., 1991). Sin embargo, los resultados de suplementaciones proteicas o energéticas sobre las variables reproductivas arrojan resultados disímiles (Radunz *et al*., 2010; Staples *et al*., 1998). La suplementación proteica pre-parto (90 días antes de parto) parece no influir la tasa de preñez en el pos-parto (Larson *et al*., 2008; Stalker *et al*., 2006), sin embargo, ésta aumenta cuando la suplementación es energética (Bellows *et al*., 2001). También existen trabajos que reportan no observar diferencias en la duración del anestro pos-parto o tasa de preñez utilizando suplementos energéticos durante el pre-parto (Small *et al*., 2004; Alexander *et al*., 2002). En Uruguay, Scarsi (2012) suplementando vaquillonas de carne que pastoreaban campo natural durante los últimos 35-40 días de gestación con 4,5 kg de un alimento compuesto por 68% de grano de sorgo, 32% de alimento proteico comercial (88% materia seca, 18% de proteína) observó que la suplementación aumentó el peso de las hembras al parto y durante el pos-parto temprano (56 días), pero no se encontraron diferencias ni en producción ni calidad de leche ni en tasa de preñez comparado con las vacas no suplementadas.

Por otra parte, la nutrición pos-parto puede compensar, al menos parcialmente, el efecto negativo de una restricción nutricional pre-parto dependiendo del nivel de restricción a la cual estuvieron sometidas las hembras (Ciccioli *et al*, 2003; Lalman *et al*., 1997; Perry *et al*., 1991). Aún más, Perry *et al*. (1991) plantearon que la alimentación pre-parto determinaría cuando la ovulación se produce en el pos-parto, pero la alimentación pos-parto determinaría si esta se producirá o no. En Uruguay, se ha trabajado con suplementaciones cortas (menos de un mes) ya sea con pasturas mejoradas o utilizando afrechillo de arroz antes del entore con resultados que

incrementan la tasa de preñez temprana y total (Soca *et al*., 2013; Pérez-Clariget *et al*., 2007), sin embargo estos resultados no siempre son consistentes.

El afrechillo de arroz, subproducto de uno de los principales cultivos del país (DIEA, 2013), es considerado un alimento energético con buen aporte proteico (14% de proteína cruda), presenta una buena palatabilidad para los animales y está disponible en el mercado nacional. Constituye así una muy buena alternativa para utilizar en suplementaciones en rodeos de cría.

En años recientes, Uruguay ha comenzado a producir biocombustibles: etanol y biodiesel. El biodiesel es una mezcla de los ésteres metílicos producto de una transesterificación. Cuando se mezclan triglicéridos con metanol y un catalizador se obtienen el éster metílico y glicerina en el orden del 10% del biodiesel elaborado (Larosa, 2001). La glicerina cruda disponible en Uruguay, más que un subproducto de interés comercial, en la actualidad representa un residuo de difícil gestión ya que debe ser quemado en hornos de cementeras para alcanzar altas temperaturas que impidan la generación de acroleína (compuesto tóxico), sin generar ganancias y constituyendo un riesgo ambiental.

La glicerina cruda está compuesta fundamentalmente por glicerol y por una proporción de metanol que es dependiente del proceso industrial. El glicerol (propano-1,2,3-triol), compuesto orgánico de tres átomos de carbono, pertenece a la familia de los alcoholes; es líquido a temperatura ambiente (25ºC), higroscópico, inodoro, incoloro, viscoso, de sabor dulce y altamente soluble en agua (IUPAC, 1993). Es reconocido como un ingrediente seguro para la alimentación animal (GRAS) por la legislación de EEUU (Code of Federal Regulations, 2004) y está registrado como aditivo a los alimentos en la Unión Europea (Schröder y Südekum, 1999). Desde el punto de vista biológico, el glicerol es un componente estructural de los triglicéridos y los fosfolípidos animales y vegetales y un compuesto normal del metabolismo de los rumiantes. Se encuentra tanto en la sangre como en las células. Las fuentes de glicerol para el organismo pueden ser la lipólisis del tejido adiposo, la

hidrólisis de los triglicéridos de las lipoproteínas de la sangre y la dieta (Machado *et al*., 2009).

El glicerol suministrado a un rumiante, llega al rumen donde tiene tres destinos: la fermentación, la absorción o continuar sin ser atacado por los micro-organismos del rumen. El glicerol es rápidamente fermentado en el rumen (Rémond *et al*., 1993) y la producción de ácidos grasos volátiles (AGV) aumenta tanto *in vitro* (Ferraro *et al*., 2009; Trabue *et al*., 2007) como *in vivo* (Mach *et al*., 2009; Wang *et al*., 2009a; Rémond *et al*., 1993). La producción de ácido propiónico y la tasa de producción propiónico: acético aumentan (Ferraro *et al*., 2009; Wang *et al*., 2009a; Trabue *et al*., 2007; Rémond *et al*., 1993), pudiendo disminuir la producción de acetato (Ferraro *et al*., 2009; Trabue *et al*., 2007; Rémond *et al*., 1993) o mantenerse (Wang *et al*., 2009a). También puede aumentar la producción de butirato (Ferraro *et al*., 2009; Wang *et al*., 2009a; Rémond *et al*., 1993) y se ha observado que cuando ésta aumenta, también aumenta la concentración plasmática de BHB (Rémond *et al*., 1993). El propionato, producido por la fermentación ruminal, es el principal sustrato para la gluconeogénesis hepática; vacas lecheras de alta producción llegan a obtener entre 50 y 60% del total de glucosa requerida de esa fuente (Lomax y Baird, 1983).

Una porción del glicerol que entra al rumen escapa a la fermentación y es absorbido directamente (Rémond *et al*., 1993). Llega al hígado por la vena porta donde es convertido en glicerol fosfato, reacción catalizada por la enzima glicerol quinasa, luego convertido a dihidroxiacetona fosfato y ésta entra en la vía gluconeogénica cuando es convertido a gliceraldehído-3-fosfato (Lin *et al*., 1977). Por lo que, ya sea porque aumenta el propiónico ruminal o porque es absorbido directamente, el destino final del glicerol administrado es convertirse en glucosa en el hígado a través de la gluconeogénesis. De ahí su potencial uso en la alimentación animal como elemento gluconeogénico.

El eje hipotálamo-hipofisario-gonadal tiene un rol dominante en la regulación de la reproducción y requiere, para su correcto funcionamiento, de la integración de

señales periféricas que indican el estatus fisiológico y nutricional de la vaca e identifica a la misma como “pronta para ciclar y concebir” y llevar adelante una gestación.

La glucosa es uno de los más importantes sustratos metabólicos para una adecuada función reproductiva en vacas de carne (Short y Adams, 1988). Sin embargo, la homeostasis de la glucosa está bajo un fuerte control endócrino que determina que su concentración permanezca más o menos constante, por lo que se ha propuesto que su rol como mediadora entre la nutrición y la reproducción es más permisivo que causal (Hess *et al*., 2005). La hormona insulina, por su parte, juega un rol clave en la homeostasis de la glucosa. Esta hormona promueve la captación celular de glucosa y su oxidación y puede disminuir la gluconeogénesis hepática en rumiantes (Brockman y Laaveld, 1986). Existen evidencias de que la insulina es una de las señales que transfieren información del estatus energético del animal al sistema reproductivo (Blache *et al*., 2006). Se ha observado que la insulina estimula la función ovárica incrementando la producción de hormonas esteroideas y la proliferación celular (Wettemann y Bossis, 2000). También se han encontrado asociaciones entre las concentraciones de insulina plasmática y las respuestas reproductivas, siendo la primera responsable de la mayor variación en el intervalo parto-primera ovulación (Sinclair, 2008). Estos antecedentes nos llevan a plantear que el agregado de glicerina al suplemento de vacas de cría podría tener un efecto benéfico sobre el comportamiento reproductivo. También, es posible suponer que la producción de leche pudiera aumentar con la suplementación de glicerol. Trabajos realizados en vacas lecheras en nuestro país (Echeverría *et al*., 2010) así como a nivel internacional (Wang *et al*, 2009a; Chung *et al*., 2007; Bodarski *et al*., 2005) avalan este planteo.

La producción de propionato en el rumen es mayor en los animales consumiendo concentrado que en los que consumen forraje. Por lo que se ha planteado que la suplementación con glicerol podría tener un efecto aún más benéfico sobre la disponibilidad de energía en animales en pastoreo (Drouillard,

2008). Por otra parte, los resultados del impacto de la administración de glicerol sobre la digestibilidad de la fibra es motivo de discusión. Existen reportes en los que la administración de glicerol no afectó la digestibilidad de la fibra (Hess *et al*., 2008; Hippen *et al*., 2008; Schröder y Südekum, 1999). Sin embargo, Wang *et al*. (2009b) han planteado que la administración de glicerol podría aumentar la digestibilidad de la fibra hasta ciertos rangos, mientras que Donkin *et al*. (2009) reportaron que la digestibilidad de la fibra disminuyó con el agregado de glicerol en la dieta. Considerando que el sistema criador está basado en el pastoreo de campo natural, parece de gran importancia estudiar el posible impacto que puede tener la suplementación con glicerina cruda sobre la producción de propionato en el rumen y la digestibilidad del forraje.

La mayor parte de la literatura internacional está referida al uso del glicerol en vacas lecheras de alta producción en el periodo de transición (Carvalho *et al*., 2011; Osborne *et al*., 2009; Wang *et al*., 2009b; Chung *et al*., 2007; Ogborn, 2006; Bodarski *et al*., 2005; De Frain *et al*., 2004; Goff y Horst, 2001). También se ha utilizado como integrante de la dieta en el engorde de ganado bovino (Mach *et al*., 2009; Parson *et al*., 2009) y de ovinos (Gunn *et al*., 2010), en terneros (Gunn *et al*., 2011) y en vaquillonas de re-emplazo (Moriel *et al*., 2010). Sin embargo, los trabajos realizados en ganado de carne utilizan animales estabulados (feed-lot), condiciones muy disímiles de las de pastoreo de campo natural del sistema criador nacional. La información disponible sobre el uso del glicerol o la glicerina cruda en la alimentación de rumiantes no es abundante, y en forma especial, existe una carencia marcada sobre su uso en vacas de carne en pastoreo.

Considerando el valor que este producto representa en la alimentación de los rumiantes (Donkin *et al*., 2009; Chung *et al*., 2007; De Frain *et al*., 2004), y la disponibilidad de glicerina cruda en nuestro mercado, nos proponemos estudiar los efectos de la utilización de la glicerina cruda en la suplementación animal en las condiciones de pastoreo características de la cría en nuestro país.

Esta tesis se plantea transformar estas dos debilidades actuales: la gestión de la glicerina cruda y los bajos porcentajes de destete y terneros livianos de las vacas de segundo entore, en una alternativa para ambas industrias, la del biodiesel y la de carne vacuna, utilizando el residuo de la primera en alimentación para el cuello de botella de la última. Siendo la glicerina cruda un líquido a temperatura ambiente, lo que dificulta su administración en animales en pastoreo, se plantea su asociación con afrechillo de arroz, un subproducto de uno de los principales cultivos agrícolas del país que además, tiene un interesante nivel de proteína.

La hipótesis del presente trabajo fue, que la suplementación con afrechillo de arroz y glicerina cruda, promovería una mejora en el balance energético de las vaquillonas preñadas y/o paridas que se vería reflejado en aumento de la producción de leche, cambios en su composición, aumento de las ganancias de peso de los terneros e incrementos en el porcentaje de preñez como consecuencia, al menos en parte, de modificaciones en la dinámica ruminal.

El objetivo general del proyecto es aportar información sobre el uso de la glicerina cruda derivada de la industria del biodiesel como integrante de un suplemento energético brindado en momentos estratégicos (pre-parto y/o pre-entore) en vacas de cría pastoreo campo natural.

Esta tesis contiene tres artículos científicos, los cuales serán los capítulos dos, tres y cuatro de la tesis, respectivamente:

- ***Suplementación con glicerina cruda y afrechillo de arroz a vacas de carne pastoreando campo natural***, este artículo será enviado a la revista Agrociencia Uruguay.

- ***Supplementation with a mixture of whole rice bran and crude glycerin on metabolic responses and performance of primiparous beef cows***, este artículo será enviado a la Revista Brasileira de Zootecnía.

- ***Strategic supplementation with a mixture of whole rice bran and crude glycerin in primiparous beef cows: metabolic, productive and reproductive responses***, este artículo será enviado a la revista Livestock Science.

En el quinto capítulo de esta tesis se presenta una discusión general de los resultados obtenidos en los tres trabajos y conclusiones globales.

2. SUPLEMENTACIÓN CON GLICERINA CRUDA Y AFRECHILLO DE ARROZ A VACAS DE CARNE PASTOREANDO CAMPO NATURAL

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2.1. RESUMEN

Con el objetivo de evaluar los efectos de la suplementación con glicerina cruda (GC) y afrechillo arroz (AA), sobre la dinámica ruminal, la cinética de degradación de la MS, el consumo de forraje y variables plasmáticas en vacas de carne pastoreando campo natural, cuatro vacas fistuladas en el rumen fueron asignadas a un cuadrado latino 4×4 con un período adicional. Los tratamientos evaluados fueron: suplementación con GC (550 mL/vaca/día), suplementación con AA (1kg de MS/vaca/día), suplementación con GC+AA (550 mL/vaca/día + 1kg de MS/vaca/día) y sin suplementación (CON). La asignación diaria de forraje para todos los tratamientos fue 10% del peso vivo. La GC asociada o no al AA disminuyó el pH ruminal en las primeras 6 horas pos-suplementación, pero no afectó ningún parámetro de degradabilidad de la MS del forraje. Comparada con el CON la suplementación con GC disminuyó el consumo de forraje pero no afectó el consumo total de MS ni de energía metabolizable (EM). El consumo de forraje ni el consumo total fueron diferentes entre la suplementación con GC+AA y AA, pero el consumo de EM fue mayor para GC+AA en comparación con el resto. La suplementación con GC+AA aumentó la concentración plasmática de glucosa e insulina sin afectar la de

B-hydroxibutirato. La suplementación con GC+AA parece ser la forma más adecuada de suplementación; a los aportes nutricionales del AA se suma el que es un excelente vehículo para dar a nivel de campo la GC, siendo esta mezcla la que logró los mejores resultados.

Palabras claves: rumen, metabolismo, glicerol, energía

2.2. SUMMARY

Supplementation of beef cows with crude glycerin associated or not with rice bran grazing natural grass

To study the effects of supplementation with crude glycerin (GC) associated or not with whole rice bran (AA) on ruminal dynamics, kinetics of DM degradation, forage intake and plasma variables in beef cows grazing natural grass, four rumen fistulated cows were allocated to a Latin square 4×4 with an additional period. The treatments were: supplementation with GC (550 mL/cow/day), supplementation with AA (1kg DM/cow/day), supplementation with GC+AA (550 mL/cow/day + 1kg DM/cow/day) and without supplementation (CON). All treatments were with daily herbage allowance of 10% body weight. GC associated or not to AA decreased ruminal pH on the first 6 hours pos-supplementation, but did not affect any parameters of degradability of forage DM. GC supplementation decreased forage intake but did not affect total DM intake and metabolizable energy (ME) compared with CON. GC+AA and AA supplementation did not differ on intake of forage and total DM, but the ME intake was higher for GC+AA compared to other treatments. GC+AA supplementation increased plasma concentration of glucose and insulin without change on B-hydroxibutirate. GC+AA seems to be the most appropriate way of supplementation, in addition to the nutritional contributions that give the AA, this serves as a vehicle on field level to give the GC, being this mixture that achieved the best results.

Key words: rumen, metabolism, glycerol, energy

2.3. INTRODUCCIÓN

La producción de biodiesel, pone a disponibilidad de la alimentación animal, glicerina cruda en grandes cantidades, la cual se produce en el orden del 10% del biodiesel elaborado (Larosa, 2001). La glicerina cruda contiene glicerol, agua, lípidos, cenizas y metanol (Schröder y Südekum, 1999). El glicerol, componente principal de la glicerina cruda, es reconocido como un ingrediente seguro para la alimentación animal por la legislación de EEUU (Code of Federal Regulations, 2004) y de Europa (Alexander *et al*., 2010).

El glicerol llega al rumen donde tiene tres posibles destinos: la fermentación, la absorción o continuar sin ser atacado por los micro-organismos (Krehbiel, 2008). El glicerol incrementa la producción de ácidos grasos volátiles (AGV) tanto *in vivo* (Rémond *et al*., 1993; Mach *et al*., 2009) como *in vitro* (Trabue *et al*., 2007; Ferraro *et al*., 2009; Bruni *et al.*, 2013); fundamentalmente aumenta la producción de ácido propiónico y de ácido butírico (Ferraro *et al*., 2009; Wang *et al*., 2009a). Cuando la producción de ácido butírico se incrementa se observa un aumento de la concentración plasmática de B-hydroxibutirato (BHB; Rémond *et al*., 1993). Tanto el propionato producido por la fermentación del glicerol y el glicerol absorbido como tal en el rumen, llegan al hígado donde son sustratos de la neoglucogénesis. El control de la gluconeogénesis tiene un componente endocrino en el que se destaca la hormona insulina responsable de la homeostasis de la glucosa. Esta hormona promueve la captación celular de glucosa y su oxidación y puede disminuir la gluconeogénesis hepática en rumiantes (Brockman y Laarveld, 1986). La mayor parte de la literatura internacional está referida al uso del glicerol en vacas lecheras (Chung *et al*., 2007; Wang *et al*., 2009b; Carvalho *et al*., 2011). También se ha utilizado como integrante de la dieta en el engorde de ganado bovino (Mach *et al*., 2009; Parson *et al*., 2009) y de ovinos (Gunn *et al*., 2010), en terneros (Gunn *et al*., 2011) y en vaquillonas de re-emplazo (Moriel *et al*., 2010). Sin embargo, son escasos los reportes sobre su utilización en ganado de cría en pastoreo.

Existen reportes sobre aumentos del consumo (Bordarsky et al., 2005), disminución (De Fraín et al., 2004) o ausencia de cambios observables (Donkin et al, 2009) cuando se agregó glicerina cruda a la dieta de vacas lecheras. Por un lado, no se han observado cambios en la digestibilidad de la materia seca (MS) y de la fibra detergente neutra (FDN) cuando el glicerol se incorporó a la dieta de ovinos (Schröder y Südekum, 1999) o bovinos (Donkin *et al*., 2009). Sin embargo, Wang *et al*. (2009b) han planteado que la administración de glicerol podría aumentar la tasa de degradabilidad de la MS y todos los parámetros de degradabilidad de la FDN, mientras que Shin *et al*. (2012) sugieren que la digestibilidad de la primera no se vería modificada pero la digestibilidad de la fibra se vería disminuida.

El afrechillo de arroz, subproducto de uno de los principales cultivos de la región, es considerado un alimento energético con buen aporte proteico (14% de proteína cruda; Wang *et al*., 2012). El mismo presenta una buena palatabilidad para los animales y está disponible en el mercado regional. Constituye así una alternativa para utilizar en suplementaciones en rodeos de cría ya sea mezclado o no a la glicerina cruda.

La hipótesis planteada es que la glicerina cruda administrada a vacas adultas pastoreando campo natural modificaría la dinámica ruminal, incrementando la concentración plasmática de glucosa, insulina y BHB, sin afectar ni el consumo de forraje ni la degradabilidad de la MS del forraje tanto, cuando se la administra sola o cuando se la agrega al afrechillo de arroz. El objetivo del presente trabajo fue evaluar los efectos de la suplementación con glicerina cruda asociada o no al afrechillo de arroz, sobre la dinámica ruminal estimada a través del pH y la concentración de N-NH_3, la cinética de degradación de la MS, el consumo de forraje y la concentración plasmática de glucosa, insulina y BHB en vacas de carne pastoreando campo natural.

2.4. MATERIALES Y MÉTODOS

El protocolo experimental fue aprobado por la Comisión de Ética en el Uso de Animales (CEUA). El experimento se llevó a cabo en la Estación Experimental "Bernardo Rosengurtt", Facultad de Agronomía, Cerro Largo, UdelaR (Latitud 32˚21'.20 S, Longitud 54˚26'.32 O), entre mayo y setiembre 2013.

2.4.1. Animales, tratamiento y diseño experimental

Se utilizaron 4 vacas Angus y Angus x Hereford, vacías, fistuladas en el rumen, con más de 6 unidades de condición corporal (CC; escala: 1-8 unidades; Vizcarra *et al.*, 1986) y 604 ± 43 kg de peso vivo (PV), las cuales se asignaron a un cuadrado latino 4×4 con un período adicional (5 períodos en total: P1–P5). Los cuatro tratamientos evaluados fueron: suplementación con glicerina cruda (550 mL/vaca/día; GC); suplementación con afrechillo de arroz (1kg de MS/vaca/día; AA); suplementación con glicerina cruda (550 mL/vaca/día) + afrechillo de arroz (1 kg MS/vaca/día; GC+AA) y sin suplementación (CON). Las vacas pastorearon durante todo el experimento sobre campo natural. Cada periodo de suplementación abarcó 18 días: 12 días de adaptación a la dieta (Días 1 al 12) y 6 días de muestreos (Días 13 al 18). La composición química del afrechillo de arroz y de las pasturas ofrecidas (Cuadro 1) fue evaluada a través de análisis químico [%MS, %Extracto Etéreo (AOAC, 1990; N.167.03, N. 954.02, respectivamente), %Cenizas, %Proteína Cruda (AOAC, 2007; N.942.05, N.984.13; respectivamente), %Fibra Detergente Neutra y Ácida de la materia orgánica con tecnología ANKOM de forma secuencial (Van Soest *et al*, 1991)]. La composición de la glicerina cruda fue: 3% agua (AOCS, 2009; Ea8-58), 6% cenizas (AOCS, 1973; Ea 2-38), 77% glicerol (AOCS, 2012; Ea 6-51), 13% materia grasa (AOAC, 1980; 14.019) y su contenido de metanol fue aportado por ALUR (1%). La energía metabolizable (EM) aportada por la glicerina cruda y por el afrechillo de arroz entero fue tomada de las tablas FEDNA (Fundación Española para el Desarrollo de la Nutrición Animal), la energía de las pasturas fue

estimada a partir de la digestibilidad *in vitro* de la MS utilizando la ecuación de Mahanna (1990).

Cuadro 1. Composición química del afrechillo de arroz entero (AA) y las pasturas ofrecidas en cada período (P1-P5).

	AA	**P1**	**P2**	**P3**	**P4**	**P5**
MS (%)	88	39	29	38	43	39
C (%MS)	11	10	12	15	17	18
PC (%MS)	14	7	6	7	6	6
aFDNmo (%MS)	24	68	67	62	62	61
FDAmo (%MS)	9	31	32	28	29	31
EE (%MS)	17	2	2	2	1	1

MS: materia seca; C: ceniza; PC: proteína cruda; aFDN: fibra detergente neutra de la materia orgánica; FDAmo: fibra detergente ácida de la materia orgánica; EE: extracto etéreo.

2.4.2. Pasturas y asignación de forraje

Durante todo el experimento las vacas pastorearon en conjunto campo natural con una asignación de forraje de 10% PV. La disponibilidad del forraje fue determinada por el método de doble muestreo (Haydock y Shaw, 1975) a través de un cuadrado de 50 cm x 50 cm, con 5 puntos en la escala y dos repeticiones, cortando el forraje al ras del suelo. La altura del mismo, se registró como la altura de la última hoja que tocara la regla. Por apreciación visual del cuadrado de muestreo se estimó la relación verde/seco (Rel V/S; Cuadro 2). Los animales se pesaban al inicio de cada período y en base a la disponibilidad y el peso de los animales se determinaba el área a pastorear para ese período.

Cuadro 2. Atributos de las pasturas.

	Disponibilidad		Altura		Rel. V/S
	Kg MS/ha	ee	cm	ee	
P1	2600	± 697	13	± 1	93/7
P2	2324	± 710	11	± 1	93/7
P3	4148	± 886	13	± 1	90/10
P4	2685	± 978	9	± 1	84/16
P5	1810	± 634	7	± 1	88/12

2.4.3. Muestreos y determinaciones

Se determinó la degradabilidad *in situ* de la pastura y el consumo de forraje durante los Días 13, 14 y 15 de cada periodo experimental. Para estimar la degradabilidad *in situ* de la MS de la pastura se utilizó la técnica *in situ* de Mehrez y Ørskov (1977). Se colocaron 20 bolsas ANKOM (20 cm × 10 cm; tamaño promedio de poros: 50 micrones) por vaca en cada período experimental en las que se colocaron 4 g de MS de una muestra representativa de forraje utilizando la técnica de hand-clipping (Weir y Torell, 1959). El forraje fue previamente picado para lograr un tamaño de 5 mm. Las bolsas se introdujeron en el saco ventral del rumen todas juntas a la hora 0 (7:00 AM) y se extrajeron por duplicado en 10 horarios (0, 2, 4, 8, 12, 16, 24, 36, 48 y 72 hs) de incubación en el rumen. Cada bolsa fue lavada bajo corriente de agua fría hasta obtener un líquido incoloro. Luego se secaron en estufa a 60ºC durante 48 hs y se pesaron para obtener el residuo no degradado. Para cada vaca, período y tratamiento se utilizó el siguiente modelo (Ørskov y McDonald, 1979):

$Di = a + b\ (1 - e^{-c\ t})$

Di: Degradabilidad al tiempo ti (%)

a: Fracción soluble (%)

b: Fracción potencialmente degradable (%)

c: Tasa de degradación (%/h)

t: Tiempo en horas

Con los parámetros de desaparición (a, b y c) y asumiendo una tasa de pasaje (Kp) de 2%/h y 4%/h se estimó la degradabilidad efectiva (DE) aplicando la siguiente ecuación: DE=a+[bc/(c+Kp)].

El consumo de materia seca del forraje (CMSf) se determinó con la técnica de doble marcador (Penning, 2004). Para esto se empleó Óxido de Cromo (Cr_2O_3) como marcador externo y la Fibra Detergente Ácido indigestible (FDAi) como indicador interno. Dos veces por día se dosificaron 7,5 g de Cr_2O_3 por un periodo de 10 días: 7 días para estabilizar el marcador y 3 días para recolección de heces. A partir del día 7 se recolectaron (2 veces por día) muestras de heces directamente desde el recto. Posteriormente fueron secadas en estufa de aire forzado a 60°C hasta peso constante y molidas en molino con malla 1 mm. Luego por animal y periodo de muestreo se determinó la concentración de Cr por espectrofotometría de absorción atómica (Silva y Queiroz, 2006). Se tomaron muestras del forraje ofrecido (hand-clipping) en cada periodo de determinación del consumo. Las muestras fueron secadas en estufa de aire forzado a 60ºC hasta peso constante. Las muestras de forraje, suplementos y heces recolectadas fueron molidas con malla de 2 mm y se sometieron a una prueba de digestibilidad *in situ* durante 144 hs por duplicado. Una vez finalizado el procedimiento de incubación *in situ*, se retiraron los residuos correspondientes a cada muestra y se conservaron hasta la realización del análisis de la FDAi (Van Soest *et al*, 1991). Finalmente para estimar el CMSf se utilizó la siguiente ecuación:

CMSf (kg/vaca/d) = ([FDAih]*H/0.8 - [FDAis]*CMSs)/[FDAif]

CMSf: Consumo de materia seca del forraje

FDAih: Porcentaje de FDAi en las heces

0,8: Recuperación de la FDAi en las heces, según Sunvold y Cochran, 1991

FDAis: Porcentaje de FDAi en el suplemento

CMSs: Consumo de materia seca del suplemento

FDAif: Porcentaje de FDAi en el forraje

A las: 0, 0:30, 1:30, 3, 6, 12 y 24 hs de comenzada la suplementación (07:00 AM) de los Días 17 y 18 se tomaron muestras de sangre de la vena yugular utilizando tubos heparinizados para la determinación de la concentración plasmática de glucosa, insulina y BHB. Luego de extraídas, las muestras fueron centrifugada a 1529 g durante 15 minutos y el plasma almacenado a -20 ºC. La insulina se determinó por análisis inmunoradiomético (IRMA) en fase sólida (Diasource, Bruselas, Bélgica). Los coeficientes de variación intraensayo para el control bajo (23 uUI/mL) y alto (86,5 uUI/mL) fueron 7,5 y 6,9%, respectivamente y el límite de detección fue de 0,52 uUI/mL. La concentración de BHB y glucosa se determinaron por espectrofotometría utilizando kits comerciales (D-3 Hydroxibutyrate, Randox, Reino Unido y Glucose-Glucose oxidasa/Peroxidasa, ByoSistem S.A, Barcelona, España) con coeficientes de variación intraensayo de 6,5 y 7,7%, respectivamente.

Los Días 17 y 18 también se realizaron extracciones de líquido ruminal, a las: 0, 0:30, 1:30, 3, 6, 9, 12, 16 y 24 de comenzada la suplementación (07:00 AM). Las muestras extraídas fueron filtradas en tela doble de lienzo (quesería) y se determinó el pH en el momento. Posteriormente se tomaron muestras para determinar N-NH_3, las cuales fueron congeladas a -20 ºC hasta su posterior análisis. La determinación de N-NH_3 se realizó por medio de destilación directa con Kjeldahl (Galyean, 1997).

2.4.4. Análisis estadístico

La concentración de pH, N-NH_3, glucosa, BHB, insulina y la desaparición de la MS del forraje, se analizaron con un modelo de medidas repetidas en el tiempo el que incluyó como efecto aleatorio al animal y como efectos fijos al período, tratamiento, hora y la interacción tratamiento por hora. El consumo de materia seca del forraje, materia seca total y energía metabolizable, se analizaron con el mismo modelo pero sin el efecto fijo de la hora y la interacción tratamiento por hora. Los parámetros de la dinámica de desaparición de la MS se analizaron con el modelo no lineal (PROC NLIN) propuesto por Ørskov y MCDonald (1979). Los parámetros se compararon por intervalos de confianza. Los resultados se expresaran en medias

ajustadas ± error estándar (ee) y las diferencias estadísticamente significativas se consideraran con $P \leq 0,05$ y tendencia con $0,05 < P \leq 0,10$.

Modelo:

$Yijkl = b0 + Vi + Pj + Tk + R1 + R2 + R3 + R4 + b1Xijk + eijk + Hl + (TH)kl + eijkl$

b0: intercepto

Vi: efecto animal

Pj: efecto periodo

Tk: efecto tratamiento

R1: efecto residual del tratamiento 1

R2: efecto residual del tratamiento 2

R3: efecto residual del tratamiento 3

R4: efecto residual del tratamiento 4

b1: coeficiente de regression de la covariable Xijk (valor inicial de la variable medida)

eijk: error experimental

Hl: efecto hora de medición

(TH)kl: interacción tratamiento por hora

eijkl: error de la medida repetida

2.5. RESULTADOS

2.5.1. pH y $N\text{-}NH_3$

El tratamiento afectó el pH ruminal (P=0,03); el promedio diario fue mayor (P<0,05) en el CON que cuando se suplementó con AA, mientras que los valores obtenidos cuando la suplementación fue con GC o GC+AA fueron intermedios (CON=6,91±0,03^{a}; GC=6,89±0,03^{ab}; AA=6,79±0,03^{b}; GC+AA=6,82±0,03^{ab}). Se encontró una interacción tratamiento por hora (P=0,04), en efecto, cuando los animales fueron suplementados el pH del rumen fue inferior (P<0,05) comparado

con el CON a la hora: 1,5, 3 y 6, sin que se encontraran diferencias entre las distintas suplementaciones (Figura 1). Los valores de pH en todos los casos fueron mayores a 6,6.

El $N\text{-}NH_3$ no fue afectado por los tratamientos (P=0,30); el promedio diario para los tratamientos CON, GC, AA y GC+AA fue: 10,91±0,99; 9,60±1,00; 9,21±1,01; 9,58±0,99, mg/100mL, respectivamente. Se encontró una interacción tratamiento por hora (P=0,04); el $N\text{-}NH_3$ de los animales suplementados con AA y GC+AA fue mayor (P<0,05) a la hora: 0,5 de terminada la suplementación comparados con el tratamiento CON. A partir de la hora 6 y hasta las 16, el tratamiento CON presentó los mayores (P<0,05) valores de $N\text{-}NH_3$, sin que se encontraran diferencias entre los tratamientos suplementados (Figura 1).

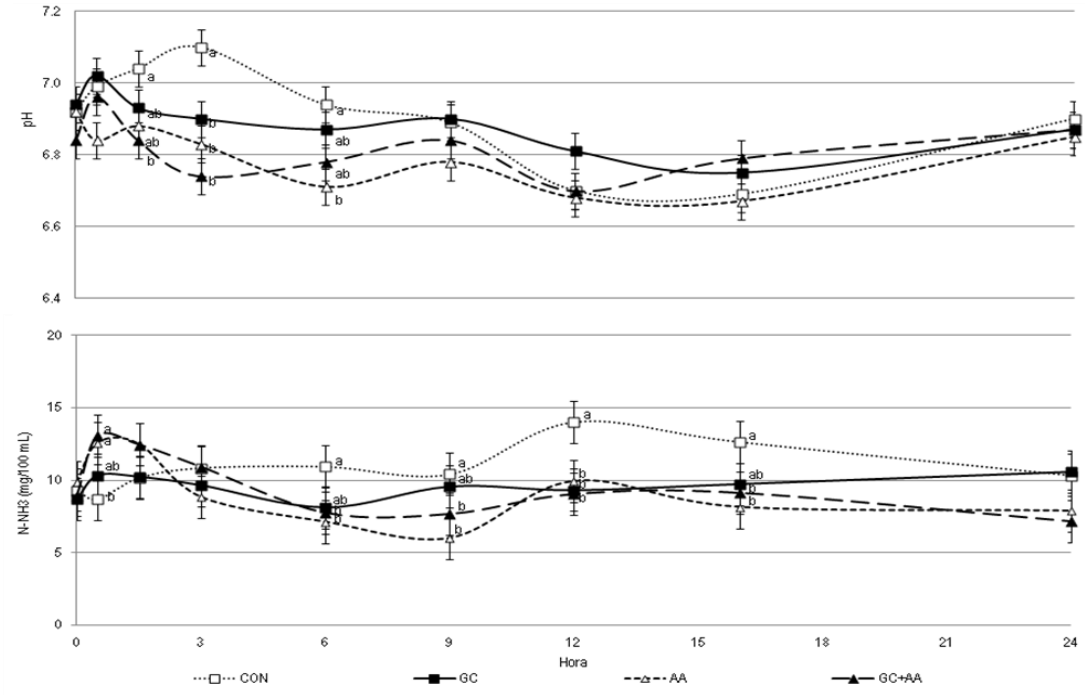

Figura 1. Evolución de pH ruminal y $N\text{-}NH_3$ según tratamiento control (CON), suplementado con 550 mL/día de glicerina cruda (GC), suplementado con 1 kg de MS/día de afrechillo de arroz (AA) o suplementado con 550 mL/día de GC + 1 kg MS/día de AA (GC+AA). Hora 0: inicio de la suplementación (7:00 h).

2.5.2. Parámetros de degradabilidad del forraje y consumo

Los parámetros de degradabilidad de la MS del forraje (a: fracción soluble, b: fracción potencialmente degradable, c: tasa de degradación) no fueron afectados por el tipo de suplemento (P>0,10). La DE tanto al 2%/h como al 4%/h tampoco difirió entre tratamientos (P>0,10; Cuadro 3). La desaparición de la MS del forraje no fue diferente entre tratamientos, (P=0,20), ni se encontró interacción tratamiento por hora (P=0,97). Independientemente de la suplementación, la desaparición de la MS del forraje se fue incrementando en función de la hora (P<0,01). La degradabilidad de la MS del forraje no fue afectada (P>0,10) por la suplementación con GC o con AA, o cuando ambos suplementos fueron brindados juntos (Figura 2).

La suplementación con GC, AA y GC+AA disminuyó (P<0,01) un 21%, 14% y 7% el consumo de forraje en comparación con el CON, respectivamente (Cuadro 3), pero sólo el valor obtenido con la suplementación con GC fue estadísticamente diferente (P<0,05) al observado con el del CON. El consumo de MS total con la suplementación GC y AA fue 15% y 5% menor que el CON, mientras que la suplementación con GC+AA fue 8% mayor; sin embargo ninguna de estas diferencias fueron significativas (Cuadro 3). No se encontraron diferencias en la cantidad de energía metabolizable ingerida entre el CON y la suplementación con GC o AA. El mayor (P<0,05) consumo de energía metabolizable se obtuvo con la suplementación con GC+AA (Cuadro 3).

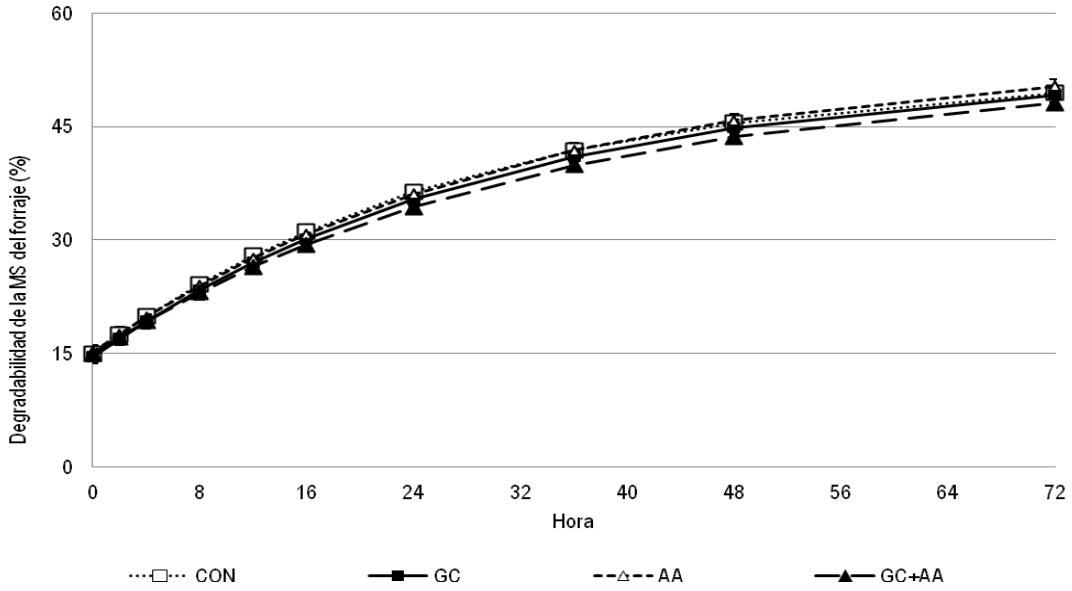

Figura 2. Curva de degradabilidad de la MS del forraje según tratamiento control (CON), suplementado con 550 mL/día de glicerina cruda (GC), suplementado con 1 kg de MS/día de afrechillo de arroz (AA) o suplementado con 550 mL/día de GC + 1 kg MS/día de AA (GC+AA). Hora 0: inicio de la suplementación (7:00 h).

Cuadro 3. Consumo de MS del forraje (CMSf), MS total (CMSt), energía metabolizable (CEM) y parámetros de degradabilidad de la materia seca del forraje según tratamiento control (CON), suplementado con 550 mL/día de glicerina cruda (GC), suplementado con 1 kg de MS/día de afrechillo de arroz (AA) o suplementado con 550 mL/día de GC + 1 kg MS/día de AA (GC+AA).

	Tratamiento				**Ee**	**P-valor**
	CON	**GC**	**AA**	**GC+AA**		
CMSf (kg MS/día)	11,08^{a}	8,78^{b}	9,53^{ab}	10,33^{ab}	0,45	0,03
CMSt (kg MS/día)	11,08^{ab}	9,42^{b}	10,53^{ab}	11,97^{a}	0,45	0,02
CEM (Mcal/día)	18,9^{b}	17,0^{b}	19,0^{b}	22,2^{a}	2,4	<0,01
Fracción soluble (%)	0,150	0,146	0,152	0,149	0,020	>0,10
Fracción potencialmente degradable (%)	0,374	0,381	0,391	0,372	0,049	>0,10
Tasa de degradación (%/h)	0,035	0,033	0,032	0,031	0,010	>0,10

DE 2%/h	0,388	0,383	0,392	0,375	0,050	>0,10
DE 4%/h	0,325	0,318	0,325	0,311	0,041	>0,10

Letras diferentes entre filas indican diferencias significativas (P ≤ 0,05).

2.5.3. B-hydroxibutirato, Glucosa e Insulina

La concentración de BHB no fue afectada por el tratamiento (P=0,78) por la hora (P=0,69) y tampoco se encontró interacción tratamiento por hora (P=0,40). El tratamiento afectó la concentración plasmática diaria de glucosa (P=0,02) e insulina (P<0,01). La suplementación con GC+AA aumentó (P<0,05) 5% la concentración plasmática diaria de glucosa y 24% la de insulina comparado con el CON, mientras que la suplementación con GC o AA presentaron concentraciones intermedias (Cuadro 4). La hora no influyó (P=0,28) la concentración plasmática de glucosa, pero se encontró una tendencia (P=0,09) por la interacción tratamiento por hora (Figura 3). La concentración plasmática de insulina fue influenciada por la hora (P<0,01) y por la interacción tratamiento por hora (P<0,01). A la hora 0,5 y 1,5 la concentración plasmática de insulina fue mayor (P<0,05) cuando se suplementó con GC+AA en comparación con el CON, mientras que la suplementación con GC y AA presentaron concentraciones intermedias (Figura 3).

Cuadro 4. Concentración diaria de B-hydroxibutirato (BHB), glucosa e insulina en plasma, según tratamiento control (CON), suplementado con 550 mL/día de glicerina cruda (GC), suplementado con 1 kg de MS/día de afrechillo de arroz (AA) o suplementado con 550 mL/día de GC + 1 kg MS/día de AA (GC+AA).

	Tratamiento				**ee**	**P-valor**
	CON	**GC**	**AA**	**GC+AA**		
BHB (mmol/L)	0,35	0,34	0,31	0,32	0,03	0,78
Glucosa (mg/dL)	66,1^{b}	66,2^{ab}	68,8^{ab}	69,5^{a}	0,9	0,02
Insulina (uUI/mL)	12,2^{b}	14,0^{ab}	14,4^{a}	15,1^{a}	0,5	< 0,01

Letras diferentes entre filas indican diferencias significativas (P ≤ 0,05).

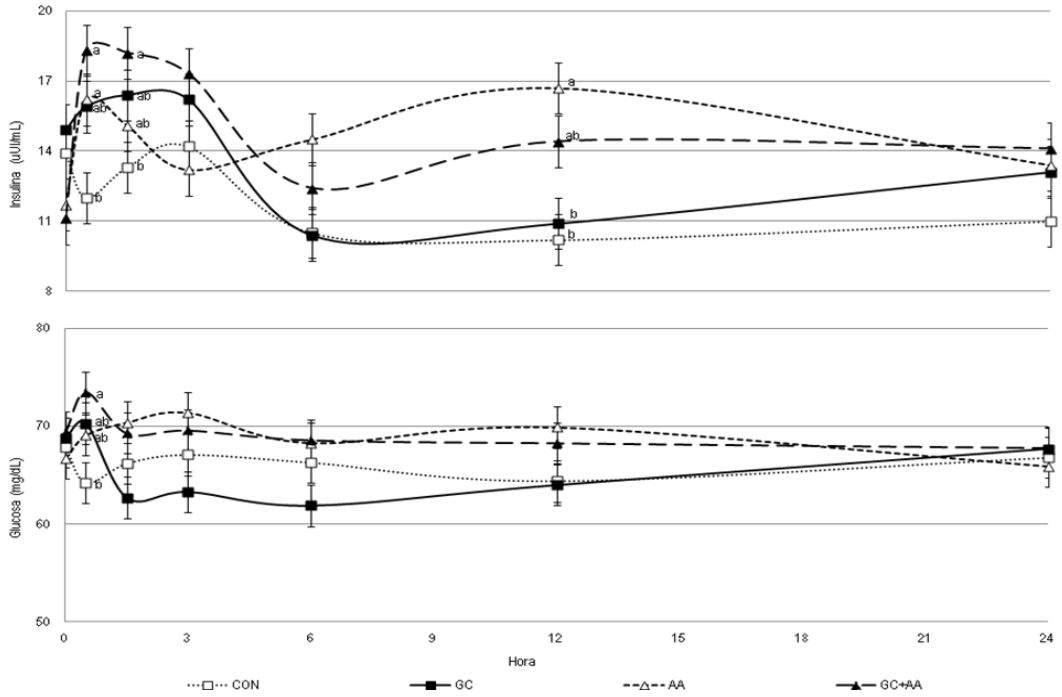

Figura 3. Evolución de la concentración diaria de insulina y glucosa según tratamiento control (CON), suplementado con 550 mL/día de glicerina cruda (GC), suplementado con 1 kg de MS/día de afrechillo de arroz (AA) o suplementado con 550 mL/día de GC + 1 kg MS/día de AA (GC+AA). Hora 0: inicio de la suplementación (7:00 h).

2.6. DISCUSIÓN

Si bien se observó una disminución del pH ruminal con todos los suplementos evaluados en las primeras 6 horas pos-suplementación, los valores registrados se encontraban dentro del rango (6,2 a 7,0) que se considera óptimo para la actividad y multiplicación de microorganismos para los procesos de fermentación de los alimentos, incluyendo la máxima fermentación de los componentes fibrosos del forraje (Calsamiglia y Ferret, 2002). Otros autores (Rémond *et al*., 1993; De Frain *et al*, 2004) también reportaron una disminución del pH ruminal cuando incorporaron glicerol a la dieta dentro de los mismos rangos de valores registrados en el presente trabajo. De Fraín *et al*. (2004) tampoco encontraron cambios en la concentración ruminal de N-NH_3 cuando agregaron glicerol a la dieta. La ausencia de cambios en

$N\text{-}NH_3$ cuando se suplementó con GC está en concordancia con el bajo nivel de PC que presenta la glicerina cruda. Los valores registrados de $N\text{-}NH_3$ en todos los tratamientos (entre 9 y 11 mg/100 mL) están dentro del rango (8-30 mg/100 mL) considerado óptimo para el funcionamiento ruminal. Valores inferiores a 5 mg/100 mL están asociados a dietas con déficit en proteína o con resistencia a la degradación de la misma, mientras que, niveles superiores serían causados por dietas con excesos de proteína o déficit de energía (Mc Donald *et al*., 1986).

La disminución en el consumo observada cuando se suplementó solo con GC es coincidente con los resultados de De Frain *et al*. (2004). Sin embargo, no coincide con trabajos realizados en vacas lecheras (Donkin *et al*., 2009: Wang et al 2009a; Carvalho *et al*., 2011), o ganado de engorde (Mach *et al*., 2009) o vacas primíparas sobre pasturas tropicales (Almeida et al., 2013) donde reportan que la suplementación con glicerol o GC no modificó el consumo total. Tampoco coinciden con los resultados publicados por Fisher et al (1971), Bordaski et al. (2005) y Kass et al (2012) quienes observaron un aumento del consumo en vacas lecheras a las que se les agregó glicerol en la dieta. La falta de consistencia observada sobre el consumo cuando se suplementa con GC o con glicerol pude ser debida, entre otras causas, a la calidad y nivel del glicerol utilizados, a la relación concentrado:forraje de la dieta (Wang et al. 2009a), el nivel de proteína en la dieta o al estatus fisiológico de la hembra (pre o pos-parto; De Frain et al., 2004). Se ha sugerido que la disminución del consumo que puede producir la suplementación con glicerol estaría asociado al poder neoglucogénico (De Frain et al. 2004). Es posible que el aumento de la concentración de ácido propiónico que produce la fermentación ruminal del glicerol (Rémond et al., 1993) actuando en el propio rumen, así como el posterior incremento de la concentración de insulina puedan ser responsables de la disminución del consumo voluntario (Allen, 2000).

Los resultados del presente trabajo avalan la hipótesis que el glicerol actuaría sobre el consumo voluntario a través de señales metabólicas sin modificar los parámetros de degradabilidad de la MS. En efecto, al igual que lo reportado por otros

autores (Shoeder y Sudekum, 1999; Hess *et al.*, 2008; Donkin *et al.*, 2009; Shin *et al.*, 2012; Almeida *et al*., 2013) no se observaron cambios en la degradabilidad con ninguno de los suplementos utilizados.

Goff y Horst (2001) reportaron incrementos de glucosa en el orden de 16% en los primeros 30 minutos luego de brindado el glicerol. El rápido incremento de la concentración de la glucosa estaría asociado a la rápida desaparición en el rumen de los suplementos utilizados. En efecto, más del 80% del glicerol desaparece en las primeras 2 (Kijora *et al*., 1998) o 4 horas (Rémond *et al*., 1993) pos-suplementación. Por otro lado De Frain *et al*. (2004) no encontró diferencia en la concentración de glucosa, BHB o insulina en vacas suplementadas pre-parto y posparto con glicerol en sustitución de concentrado energético, posiblemente esta diferencia se deba a que en el presente trabajo no se sustituyo un suplemento por otro, y a su vez De Frain *et al*. (2004) realizó los sangrados 4 horas después de dar la comida, donde en el presente trabajo a partir de las 3 horas tampoco se encuentran diferencias entre tratamientos.

Donkin *et al.* (2009) aumento la concentración de glucosa sustituyendo en la RTM concentrados energéticos por glicerol, sin embargo Mach *et al*. (2009) no encontró incrementos de este metabolito en toros en terminación pero si incrementó la concentración de insulina y la relación insulina/glucosa realizando el mismo tipo de sustitución. Por lo cual ya sea porque se aumenta la concentración de glucosa o porque aumenta la concentración de insulina lo que ambas variables plasmáticas reflejan es una mejora en el estatus energético provocado por la suplementación.

Wang *et al*. (2009a) encontró en vacas lactando aumentos de glucosa y disminuciones de BHB suplementando con 100, 200 y 300 g de glicerol en la dieta total (RTM) en comparación con las control (sin glicerol). Los sangrados fueron realizados 2 horas después de brindada la comida por lo cual coincide con el presente trabajo donde se observaron incrementos de glucosa a la media hora de finalizada la suplementación. La diferencia encontrada en BHB, Wang *et al.* (2009a) la plantean como positiva ya que lo que buscaban era disminuir la cetosis, la cual se ve reflejada

con menores concentraciones de BHB, en el presente trabajo el no encontrase diferencias en la concentración de BHB parece lógico ya que el mismo podría haber aumentado por efecto de la dieta (dietas con glicerol pueden incrementar la concentración de ácido butírico (Shin *et al*., 2012)) y el mismo se puede ver reflejado en aumentos de BHB plasmático (Rémond *et al*., 1993) o podría haber disminuido dado que el suplemento podría haber cubierto las demandas energéticas y de esta forma presentar un mejor balance energético (Wang *et al*., 2009a). Por lo cual el no verse afectada la concentración de BHB parece razonable.

2.7. CONCLUSIONES

La suplementación con GC no afectó los parámetros de degradabilidad de la MS del forraje, la concentración N-NH_3 ruminal o de BHB plasmática. La suplementación con GC+AA fue la que presentó los mayores consumos de energía y las mayores concentraciones de glucosa e insulina. Estos resultados muestran que es posible la utilización de la GC como suplemento de vacas de carne pastoreando campo natural sin mostrar ningún perjuicio, que su administración junto con el AA es una alternativa viable a nivel de campo y bajo esta forma (GC+AA) fue donde se obtuvieron los mejores resultados biológicos.

2.8. AGRADECIMIENTOS

A la Ing. Agr. (PhD) Graciela Quintans por por las vacas con las cuales se realizó este trabajo. A la ANII por la beca de maestría (POS_2011_1_3522) y proyecto (FMV_3_2011_1_6713) otorgada para realizar y financiar parte de este trabajo. A ALUR por brindarnos la glicerina cruda, a Cousa por realizarnos los análisis químicos de la glicerina cruda y a todo el personal de la EEBR, en particular a Baltasar Martínez y al Ing. Agr. Carlos Mantero.

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3. SUPPLEMENTATION WITH A MIXTURE OF WHOLE RICE BRAN AND CRUDE GLYCERIN ON METABOLIC RESPONSES AND PERFORMANCE OF PRIMIPAROUS BEEF COWS

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Short title: Pre-mating short supplementation in beef cows

3.1. ABSTRACT

To study the effect of a supplement containing whole rice bran and crude glycerin fed for 21 days before mating on metabolic, productive and reproductive responses in primiparous suckling beef cows, 28 Angus, Hereford and their crosses were used. Cows were randomly assigned to a Control group (CON, n=14), grazing on grasslands and Supplemented group (SUP, n=14), grazing grasslands and supplemented daily individually with 1 kg dry matter per cow of whole rice bran + 550 mL per cow of crude glycerin (20% methanol). After 33 days of natural mating, cows that had not expressed estrus were submitted to a fixed-time artificial insemination protocol. Ten days after fixed-time artificial insemination program, bulls were reintroduced for 21 days. Supplementation increased milk yield (SUP: 5.7±0.2 vs CON: 5.0±0.2 kg/day), milk protein content (SUP: 3.1±0.2 vs CON: 2.9±0.2 %), cow (SUP: 379±2 vs CON: 373±2 kg) and calves body weight (SUP: 150±2 vs CON: 142±2 kg). Supplementation improved the energy balance reflected on an increase in plasma concentrations of cholesterol (SUP: 223.2±6.4 vs CON: 202.1±6.4 mg/dL) and glucose (SUP: 72.0±1.2 vs CON: 68.6±1.2 mg/dL) and a decrease in NEFA (SUP: 0.45±0.02 vs CON: 0.56±0.02 mmol/L). The percentage of cows in superficial anestrous was greater in SUP than in CON groups (57 vs 21%, respectively), however no difference in final pregnancy rate was found (SUP: 79 vs

CON: 64%). There was no evidence that the ingestion of crude glycerin with high content of methanol induced clinical or hepatic disorders. These results suggest that supplement provided did not appear toxic, and was able to improved energy balance reflected on achieve an increase in milk yield and calf growth, but had no significant effect on reproductive performance.

Key Words: glycerol, grazing, toxicity

3.2. INTRODUCTION

In extensive pastoral systems for meat production, primiparous suckled cows have the lowest reproductive efficiency and wean the lightest calves, reducing the productivity of the herd (Bellows et al., 1982). The main cause of reproductive failure is the prolonged post-partum anestrus, induced by undernutrition (Short et al., 1990; Hess et al., 2005) and suckling (Williams, 1990). The nutrient supply of grasslands during the winter is insufficient to meet the requirements of the growing fetus in the last third of pregnancy, causing a negative energy balance that continues during early postpartum due to the demand for milk production (Bell, 1995; Astessiano et al., 2013). The negative energy balance is evidenced by a decrease in body condition score (BCS), and endocrine changes, such as an increase in non-esterified fatty acids (NEFA) and a decrease in glucose and insulin, that impact negatively on the follicle growth and ovulation (Wiltbank, 1970; Mulliniks et al., 2011).

Postpartum supplementation can overcome, at least partially, pre-partum undernutrition (Perry et al., 1991; Ciccioli et al., 2003). Short-term supplementations before or during the mating period, associated or not with temporary weaning, are alternatives to increase pregnancy rates in cows with sub-optimal BCS (Pérez-Clariget et al., 2007; Soca et al., 2013). The most frequent ingredient of the supplements used in these studies was whole rice bran, an energy nutrient with 13-18 % of crude protein (CP; Wang et al., 2012).

Biodiesel industry increased the availability of crude glycerin that can be use in ruminant nutrition (Donkin, 2008). The main component of the crude glycerin is glycerol, a powerful neoglucogenic alcohol (Alexander et al., 2010). However, the main disadvantage of crude glycerin is that its methanol content can impair liver function (Schröder and Südekum, 1999).

The hypothesis of this study was that a short-term supplementation before mating with whole rice bran and crude glycerin with high content of methanol improves the energy balance and the performance of primiparous beef cows grazing grasslands without impairs liver function. The aim of this study was to evaluate the effect of supplementation for 21 day with whole rice bran and crude glycerin with high level of methanol on body weight, body condition, milk production, hormonal and metabolic profiles, ovarian activity, pregnancy rate and liver function in primiparous beef cows and the growth of their calves grazing grasslands.

3.3. MATERIALS AND METHODS

The experiment was conducted at the Experimental Station Bernardo Rosengurtt of the School of Agronomy, Universidad de la República (UdelaR), Uruguay (32º S, 54º W) according to the experimental procedures approved by the Animal Experimental Committee of the UdelaR.

Twenty eight pregnant Hereford, Aberdeen Angus and crossbreds heifers (4, 10, 14; respectively) with 424 ± 7 kg of body weight (BW) and 5.1 ± 0.1 units of BCS (scale: 1-8, 1 = very thin, 8 = very fat; Vizcarra et al., 1986) were monitored from 16 ± 1 weeks pre-partum (early winter) until 47 ± 1 days post-partum (DPP), at the beginning of the supplementation period.

At the start of supplementation (Day 0), BW and BCS were 371 ± 7 kg and 3.8 ± 0.1 units, respectively. All the cows were suckling and in deep anestrus confirmed

by the absence of a corpus luteum and the presence of follicles < 9 mm in diameter in the ovaries at two ultrasounds studies 9 days apart (Wiltbank et al., 2002).

Cows were paired based on DPP, BCS, BW, genotype (crosses vs pure) and sex of the calf, and one member of each pair was randomly assigned to one of the following treatments: i) Control group (CON, n = 14): grazing grasslands with no supplementation and, ii) Supplemented group (SUP, n = 14): grazing grasslands and supplemented daily with 1 kg DM per cow of whole rice bran + 550 mL per cow of crude glycerin for 21 days before the mating period. Calves were separated from their mothers on the first 14 days of supplementation, while the cows were supplemented (30 min) to avoid interferences, but remained with visual, auditory and olfactory contact. Whole rice bran and crude glycerin were premixed before individual supplementation. The supplement provided 3.9 Mcal of ME and 152 g of CP. The ME value was estimated using international FEDNA (Fundación Española para el Desarrollo de la Nutrición Animal, 2012) tables.

Chemical composition of whole rice bran and herbage were evaluated on the Laboratory of Animal Nutrition, School of Agronomy, UdelaR [Ether Extract (EE; AOAC, 1990; N. 954.02), Ash and CP (AOAC, 2007; N.942.05, N.984.13; respectively), Neutral and Acid Detergent Fiber (NDF and ADF; Van Soest et al. 1991)]. Chemical composition of crude glycerin was evaluated on the Laboratory of COUSA, Uruguay [Ash (AOCS, 1973; Ea 2-38), Glycerol (AOCS, 2012; Ea 6-51), Water (AOCS, 2009; Ea 8-58), Fat (AOAC, 1980; 14.019), CP (AOAC, 2007; N.984.13)], and content of Methanol was determined by gas chromatography on the Laboratory of the School of Chemistry. Chemical composition of whole rice bran was 874 g kg^{-1} DM, 85 g kg^{-1} Ash, 149 g kg^{-1} CP, 171 g kg^{-1} EE, 185 g kg^{-1} NDF, and 61 g kg^{-1} ADF; and of crude glycerin was 106 g kg^{-1} Water, 85 g kg^{-1} Ash, 289 g kg^{-1} Fat, 311 g kg^{-1} glycerol, 200 g kg^{-1} methanol, and 6 g kg^{-1} CP.

During the last 7 days of the supplementation period, calves of CON and SUP group with 61 ± 1 days of age were separated from their mothers and eye, auditory

and olfactory contacts were prevented. Calves were kept during the temporary weaning with separation in a small paddock and daily supplemented with 0.9 kg DM per animal of alfalfa (*Medicago sativa*) hays bale and 1.1 kg DM per animal of early weaning ration (BIORACIÓN, Melo, Uruguay), containing 180 g kg^{-1} of CP. Free access to water and shade was provided.

The first mating period lasted 33 days and started with 68 ± 1 DPP. The breeding soundness of the bulls was tested two months prior to the beginning of the breeding season. Estrus was detected 3 times per day (7:00, 13:00 and 19:00); a cow was considered in estrus when accept being mounted by the bull. Cows that did not show estrus during this period were subjected to a fixed-time artificial insemination program. The protocol started on 101 ± 1 DPP in the morning, an inert silicone intravaginal device containing 1 g of progesterone (P4; DIB®, Syntex Laboratory, Buenos Aires, Argentina) was placed and 2 mg of Estradiol Benzoate (Syntex Laboratory, Buenos Aires, Argentina) was injected. At the moment of DIB® withdrawal, in the morning of 108 ± 1 DPP, 500 mcg of Cloprostenol (Ciclase D®, Syntex Laboratory, Buenos Aires, Argentina) and 400 IU of Equine Chorionic Gonadotrophin (Novormon®, Sintex Laboratory, Buenos Aires, Argentina) were injected. The following day 1 mg of Estradiol Benzoate were applied. All hormones were injected intramuscularly. The fixed-time artificial insemination was performed 52-56 h after removal DIB®. This protocol of fixed-time artificial insemination was the commercially recommended for beef cows suckled by the Laboratory Syntex S.A. (Buenos Aires, Argentina). Ten days after fixed-time artificial insemination, bulls were reintroduced with the cows for 21 days.

All cows were managed as a single group during all the experiment; they grazed together on the same pens of native grass, with forage availability greater than 2000 kg DM ha^{-1} (minimum: 2121 ± 515, maximum: 6757 ± 969 kg DM ha^{-1}). Every month, cows were weighed and forage availability was determined by the method of double sampling (Haydock and Shaw, 1975) through a square of 50 cm x 50 cm, with five points scale and two replicates, cutting the forage at ground level, and

herbage allowance was estimated. Forage height was determined as described previously (Soca et al., 2007). For visual assessment of the sampling square green/dry ratio was estimated. These determinations were performed prior animals were placed on the pens. Forage height was always greater than 15 cm (minimum: 16 ± 2, maximum: 26 ± 4 cm). Green/dry ratio decreased towards the end of winter and increased in spring from 48/52 in August up to 84/16 in November. The predominant species were *Axonopus* sp, *Paspalum dilatatum*, *Paspalum notatum*, *Paspalum quadrifarium*, *Stipa sp*, *Cynodon dactylon*, *Eryngium horridum*, and *Bothriochloa laguroides*. The average herbage allowance during all the experiment was 24 kg DM (100 kg BW)$^{-1}$ [(minimum: 16, maximum: 37 kg DM (100 kg BW)$^{-1}$]. During supplementation, the cows were in a paddock with a forage availability of 2121 ± 515 kg DM/ha, 16 ± 2 cm sward height and herbage allowance was 21 kg DM (100 kg BW)$^{-1}$. Ten representative samples of herbage were taken and a pool of them was used for chemical composition (418 g kg^{-1} DM, 112 g kg^{-1} Ash, 84 g kg^{-1} CP, 691 g kg^{-1} NDF and 320 g kg^{-1} ADF).

Cow BCS was estimated by two trained technicians using a subjective technique (scale: 1-8, Vizcarra et al., 1986) every 20 days from 16 ± 1 weeks pre-partum until calving and every 14 days from calving until the end of first mating period. The correlation between technicians was 0.91, so for the statistical analysis we used the average of both values. Calves BW were recorded at 47 (start of supplementation or Day 0), 61 (beginning of temporary weaning with separation or Day 14), 68 (end of temporary weaning with separation or Day 21), 82 ± 1 (Day 35) days of age and at definitive weaning (186 ± 1 days of age or Day 139) using an electronic scale (FX15, Iconix, Montevideo, Uruguay).

Milk production was recorded on Days 0, 14, 21 and 35 from the beginning of supplementation, using a portable milking machine according to the method described by Mondragon et al. (1983). In the morning after the cows received their meal, calves were separated and the udder emptied using 20 IU of oxytocin i/m (Neurofisin, Lab Fatro, Uruguay). Seven hours later, cows were milked again using

the same methodology. The total milk was individually weighed on an electronic scale and 24 h production was estimated. On Days 0 and 14 individual samples were taken and milk composition (fat and protein) determined in the laboratory (COLAVECO; Colonia, Uruguay) using absorption of infrared radiation.

Weekly, from Day 0 to Day 49, blood samples were collected by jugular venipuncture in Vacutainer® tubes with heparin (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). Samples were centrifuged within the first hour of collection at 1530 g for 15 min and the plasma collected and stored at -20 °C until processing. To evaluate possible hepatic damage due to the ingestion of the methanol contained in the crude glycerin, cows were daily monitored by a veterinary during the supplementation period, and another blood sample was collected at Day 110 after beginning the supplementation, using tubes without anticoagulant. Samples were immediately centrifuged, the serum frozen and transported to Dirección de Laboratorios Veterinarios (DILAVE, MGAP, Montevideo, Uruguay). The liver function was studied through the concentrations of total protein, albumin, globulin, total bilirubin, aspartate amino transferase (ASAT), alkaline phosphatase (ALP) and gamma-glutamil transpeptidasa (GGT).

From Day -9 to 49 the ovaries were weekly examined by transrectal ultrasonography using a linear bimodal (5.0 to 7.5 MHz) transducer (Ambivision, Digital Notebook B mode, Model AV-3018V, Manufacturer AMBISEA Tecnology Corp., Ltd., China). Ovarian follicles and corpus luteum were identified according to the criteria described by Griffin and Ginther (1992). The size of the largest follicle was used to classify the type of anestrus. Cows with follicles > 8 mm in diameter in two or more occasions without corpus luteum were considered in shallow anestrous and those with follicles ≤ 8 mm in diameter without corpus luteum were considered in deep anestrus. Resumption of ovarian activity was monitored by the concentration of progesterone (P_4), considering that cyclicity was reinitiated if a P_4 concentration ≥ 1 ng/mL was found in two successive samples with one week interval (Meikle et al., 2004) and a corpus luteum was identified in two ultrasound scans with 7 days

interval. Pregnancy was diagnosed by transrectal ultrasonography at 46 and 66 days after fixed-time artificial insemination.

Progesterone concentration was determined in all cows in samples collected on Days 35, 42, 49 from the beginning of supplementation. If a corpus luteum was observed by ultrasonography on any of those days, blood samples collected two weeks before and to two weeks after were also analyzed. The P_4 concentration was determined by solid phase radioimmunoassay using commercial kits (DPC, Diagnostic Products Co. Los Angeles, CA, USA). All samples were analyzed in one assay, with the standard curve and controls in duplicate and the samples in single. The assay sensitivity was 0.12 ng/mL and the intra-assay coefficients of variation for low (0.5 ng/mL), medium (2 ng/mL) and high (8 ng/mL) controls were 3.5, 2.6 and 2.2 %, respectively.

In samples from Days 0, 7, 14, 21 and 28 the concentrations of insulin and metabolites were determine. Glucose, total protein, albumin, urea, cholesterol and non-esterified fatty acids (NEFA) concentrations were determined spectrophotometrically, using commercial kits (Glucose Oxidase/Peroxidase, Biuret, Bromocresol Green; Urease/salicylate; Cholesterol Oxidase/Peroxidase, BioSystems SA, Barcelona, Spain, Wako NEFA-HR (2), Wako Pure Chemical Industries Ltd., Osaka, Japan, respectively), with a sample volume and reagents adjusted to a 96 cells and read in a Multiskan EX (Thermo Scientific, Waltham, Massachusetts, USA). The intra-and inter-assay coefficients of variation for the high and low controls were less than 15 %. Insulin concentration was determined by an immunoradiometric assay (IRMA; Diasource, Brussels, Belgium). All samples were analyzed in one assay, the standard curve and controls in duplicate and the samples in single. The sensitivity of the assay was 1.1 uIU/mL and the intra-assay coefficients of variation for low (24.7 uUI/mL) and high (55.3 uIU/mL) controls were 4.9 and 5.1 %; respectively.

Data were analyzed using the SAS statistical package (SAS Institute Inc., Cary, NC, USA). The experiment was a completely randomized design and the cow was

considered the experimental unit. BCS data were grouped in two different periods: monitoring phase (last third of gestation - beginning of supplementation) and the experimental period (from the beginning of supplementation to the fixed-time artificial insemination). Data of milk production and composition, cows and calf weight, concentrations of metabolites and insulin were analyzed using repeated measures analysis (MIXED procedure) with the date as the repeated factor. The model included the fixed effect of treatment, date and the interaction between the two factors, and cow as the random effect. The model to analyse the data from the monitoring phase included the effects of date, genetic group (pure vs crosses) and to rule out the existence of differences before the initiation of the experimental period, the experimental group was included in the model. The interactions among the main effects were also studied. As there was no effect of genetic group in any of the variables studied, this factor was eliminated from the models. The first measures were used as covariates in the respective analysis. When the main effect was significant, the differences among means were analyzed using the Tukey-Kramer test. Data of reproductive variables were analyzed using generalized model (GENMOD procedure) specifying the binomial distribution with logit transformation of the data (anestrus and pregnancy) or Poisson distribution (interval calving-conception). The model included the effect of treatment. Data of BW of calves were analyzed by repeated measures, but the repeated factor was age and the model included the effects of treatment, age, genotype, maternal genotype, and sex, and birth weight was used as covariate. Correlation coefficients were estimated using de CORR procedure. Data are expressed as mean and standard error of the mean (Mean ± SEM) and considered statistically significant if $P < 0.05$ and trends if $P < 0.10$.

3.4. RESULTS

During the monitoring phase, the BCS of the cows decreased ($P < 0.01$) from 16 ± 1 week's pre-partum (early winter) to 47 ± 1 DPP. Cows lost an average of 1.5 ± 0.1 BCS units throughout this period, which corresponded to a loss of 1.2 ± 0.1 units in the last gestation and 0.3 ± 0.1 units in the postpartum period. The nadir of

BCS was reached in the 4th week postpartum and remained low until the beginning of the supplementation period.

Supplementation did not influence the BCS (SUP: 3.9 ± 0.1 vs CON: 3.9 ± 0.1 units, P = 0.26) and no interaction was found between supplementation and date (P = 0.54). Body weight was affected by supplementation (P = 0.04). Cows from the SUP group were heavier than cows from the CON group (SUP: 379 ± 2 kg vs CON: 373 ± 2 kg; Table 1). Nor the genotype (P = 0.45) neither the interaction supplementation by genotype (P = 0.50) affected BW during this period. Supplementation affected milk production (P = 0.02). Cows of the SUP group (5.7 ± 0.2 kg d^{-1}) produced 14 % more milk than the cows of the CON group (5.0 ± 0.2 kg d^{-1}). The interaction supplementation by date was significant (P = 0.04, Table 1). Supplementation affected calves BW (P < 0.01) and an interaction treatment by date was found (P < 0.01). The calves from dams of the SUP group were heavier (P < 0.05) in Day 14 to 35 (Table 1). From Days 0 to 14 of supplementation, while calves were suckling, they gained 0.26 ± 0.07 kg d^{-1} more than the calves from CON dams (CON: 0.48 ± 0.07 vs SUP: 0.74 ± 0.07 kg d^{-1}; P = 0.01). However, during the temporary weaning with separation from their mothers, daily gain did not differ between groups (P = 0.38), and were lower (P < 0.01) than in the previous period (0.20 ± 0.05 kg d^{-1} for both groups). As it was expected, from Day 0 to Day 35 a positive correlation was found between BW gain of the calves and milk production of their dams (r = 0.34; P < 0.01). At definitive weaning, calves from supplemented dams were in average 8 kg heavier (P = 0.03) than calves from CON dams (Table 1).

Table 1. Body condition scores (BCS), body weight (BW) and milk production of primiparous cows and BW of the calves whose mothers were non-supplemented (CON) or supplemented for 21 days with whole rice bran and crude glycerin (SUP)

	BCS (scale 1-8)		Cows BW (kg)		Milk (kg d^{-1})		Calves BW (kg)	
Day	**CON**	**SUP**	**CON**	**SUP**	**CON**	**SUP**	**CON**	**SUP**
0	3.8±0.1	3.8±0.1	369±3	372±3	6.5±0.3	6.9±0.3	67±2	67±2
14	3.8±0.1	3.9±0.1	373±3^{b}	382±3^{a}	6.8±0.3^{b}	8.1±0.3^{a}	74±2^{b}	78±2^{a}
21	3.9±0.1	3.9±0.1	369±3	374±3	1.5±0.3	1.3±0.3	76±2^{b}	80±2^{a}
35	4.0±0.1	4.0±0.1	381±3	389±3	5.1±0.3^{b}	6.3±0.3^{a}	83±2^{b}	88±2^{a}
139*							142±2^{b}	150±2^{a}

Day 0 = Start of supplementation at 47 ± 1 days postpartum.

*Age at weaning: 186 ± 1.4 days of age.

Means with different letters within rows differ (P < 0.05) (overall means ± SEM).

The supplement did not affect the milk fat content, expressed as a percentage (P = 0.22) or as the total content (P = 0.79). No effect of date (P = 0.13) or the interaction treatment by date (P=0.18) was found. The average fat percentage and total content was 3.0 ± 0.1 % and 216 ± 10 g d^{-1}, respectively. On the contrary, supplementation increased (P < 0.001) the milk protein content (CON: 2.9 ± 0.1 % vs SUP: 3.1 ± 0.1 %), and an interaction treatment by date was found (P < 0.01). In cows from the SUP group the milk protein content increased from Day 0 to 14 (2.9 ± 0.1 to 3.3 ± 0.1 % protein; P < 0.01), while in cows of the CON group it remained unchanged (2.9 ± 0.1 to 2.9 ± 0.1 % protein; P > 0.10).

Supplementation did not affect plasma concentrations of total protein (SUP: 75.6 ± 1.1 vs CON: 76.3 ± 1.0 g/L; P = 0.63), albumin (SUP: 33.0 ± 0.6 vs CON: 32.6 ± 0.6 g/L; P = 0.62) or urea (SUP: 14.4 ± 0.9 vs CON: 15.3 ± 0.9 mg/dL; P = 0.49). There was also no effect of the interaction supplementation by date on albumin (P = 0.69, Fig. 1a) or total protein concentration (P = 0.58, Fig. 1c), however, the

urea concentration was affected by this interaction (P = 0.04). One week after the beginning of supplementation (Day 7), cows in the SUP group had lower urea values (P = 0.01) than cows in the CON group, but these differences disappeared thereafter (Fig. 1b).

Cows in the SUP group had higher plasma glucose concentrations than cows in the CON group (CON: 68.6 ± 1.2 vs SUP: 72.0 ± 1.2 mg/dL; P = 0.03). Glucose concentration increased in SUP cows and differed (P < 0.05) from CON cows on Day 28 (Fig. 1e). The plasma cholesterol concentration was higher (P = 0.03) in SUP (223.2 ± 6.4 mg/dL) than in CON cows (202.1 ± 6.4 mg/dL), and on Day 14 SUP cows had the highest cholesterol concentration and was different from CON cows (P < 0.05; Fig. 1g). Supplementation decreased (P < 0.01) the concentration of NEFA (SUP: 0.45 ± 0.02 vs CON: 0.56 ± 0.02 mmol/L). The concentration of NEFA in the SUP cows decreased (P < 0.05) and remained low from Day 7 to 21 (Fig. 1f).

Concentration of insulin in SUP cows was higher than in CON cows (8.3 ± 0.4 vs 7.0 ± 0.3 uIU/mL). Insulin increased and was higher (P < 0.01) than the CON cows in the first 7 days of supplementation. By the end of the temporary weaning with separation and supplementation periods, insulin concentration tended (P = 0.08, Fig. 1d) to be higher in the SUP than CON cows.

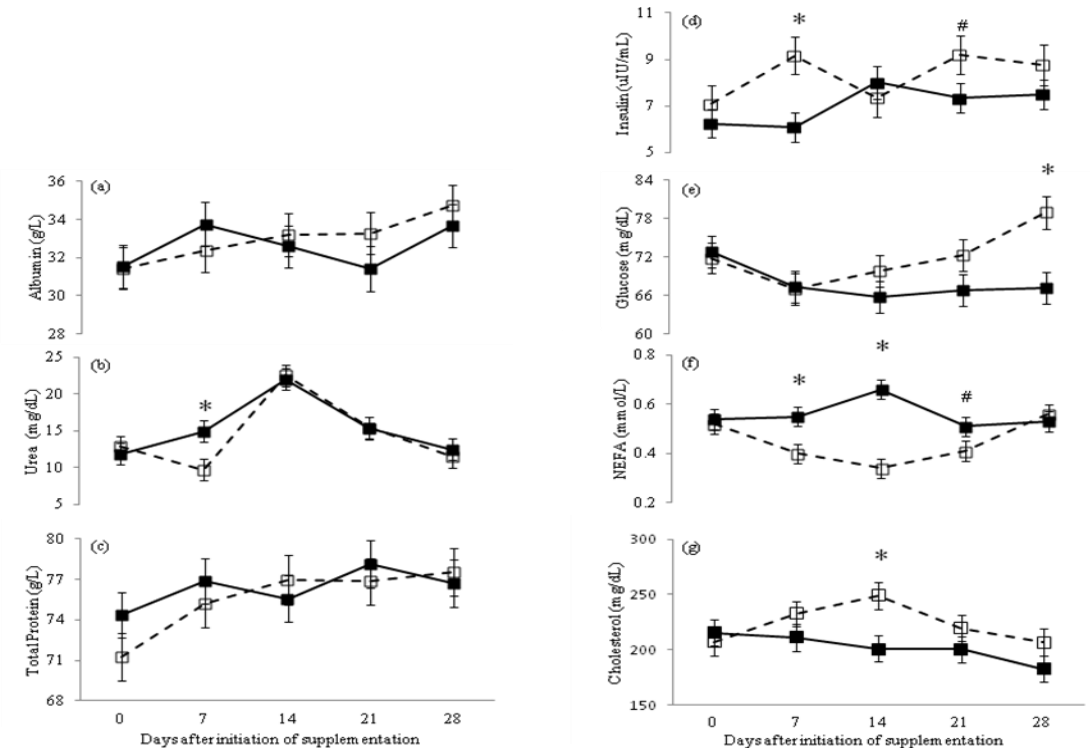

Fig. 1. Concentrations of insulin and metabolites in primiparous cows non-supplemented (■) and supplemented for 21 days with whole rice bran and crude glycerin (□). Day 0 = Start of supplementation, at 47 ± 1.4 days postpartum. Differences between treatments are indicated by * when $P < 0.05$ or $^{\#}$ when $P \leq 0.10$.

Independently of the BCS at calving, all cows were in deep anestrus when the supplementation began. Twenty-one days after the introduction of bulls (Day 42 after the start of supplementation) 36% more ($P = 0.04$) cows of the SUP group were in shallow anestrus than cows of CON group (Table 2).

In the first 33 days of the mating period, only two cows, one of each group were detected in estrus, and both became pregnant. The pregnancy rate after fixed-time artificial insemination in SUP cows was twice than of CON cows, however, this difference was not statistically significant ($P = 0.22$; Table 2). After the second period with the bulls, more cows became pregnant (8/17; 47 %) and no differences between treatments were found ($P = 0.41$). The final pregnancy rate was not different between groups ($P = 0.40$). The type of anestrus on Day 21 of the mating period

influenced overall pregnancy rate (P = 0.03). Indeed, more cows showing shallow anestrus became pregnant (91 %, 10/11) compared with cows showing deep anestrus (53 %; 9/17).

Table 2. Percentage of primiparous cows non-supplemented (CON) and supplemented for 21 days before mating with whole rice bran and crude glycerin (SUP), in shallow anestrus from the start of supplementation until 21 days of mating, pregnancy rates at 33 (early pregnancy), 43 (fixed-time artificial insemination pregnancy) and 74 (total pregnancy) days of mating, and calving - conception interval (days)

	Nutritional treatment		**P-value**
	CON	**SUP**	
Shallow anestrus	21 (3/14)	57 (8/14)	0.04
Early pregnancy	7 (1/14)	7 (1/14)	0.99
Fixed-time artificial insemination pregnancy	23 (3/13)	46 (6/13)	0.22
Total pregnancy	64 (9/14)	79 (11/14)	0.40
Interval calving-conception	108 ± 5	110 ± 5	0.77

Shallow anestrus was defined as the presence of follicles > 8 mm in the absence of corpus luteum in two or more ultrasound studies at 7 days intervals. Cows that were in anestrus in the first 33 days of mating entered at fixed-time artificial insemination program. Ten days after fixed-time artificial insemination cows were naturally rebreed for 21 days. The entire mating period lasted 74 days. Numbers in brackets represent number of cows.

No clinical signs of methanol intoxication were observed during the supplementation period or one week after. Hepatic functionality, evaluated by the concentrations of total protein, albumin, globulin, total bilirubin, ASAT, ALP and GGT performed on all the cows on 110 days after the start of supplementation showed no apparent sign of hepatic damage.

3.5. DISCUSSION

The present experiment showed that supplementation with whole rice bran and crude glycerin with high level of methanol improved the energy balance which is reflected in an increase in plasma concentrations of cholesterol, glucose and insulin and a decrease of NEFA, enabling a transient increase in BW without changes in BCS, increase of milk production with higher protein content and therefore improving the growth rate of the calves without impaired hepatic function. The supplement improved follicle development, since more cows showed follicles > 8 mm in diameter and were in sallow anestrus, although it was insufficient to advance ovulation.

From 16 ± 1 weeks before to 47 ± 1.4 DPP postpartum, cows lost BCS suggesting that nutrients obtained from grasslands were insufficient to support the growing demand of the fetus (Ferrell et al., 1976) and later milk production (Jenkins and Ferrell, 1992; Davis et al., 1994). Often in spring calving grasslands systems, the last third of pregnancy and early post-partum occurs during the winter months, when forage availability and quality are lower than in autumn or spring (Carámbula, 1991). BCS loss during these periods was higher in the pre-partum that early post-partum, this have also been reported by other authors in other countries (Houghton et al., 1990; Perry et al., 1991; Stalker et al., 2006) and in our conditions (Quintans et al., 2010; Scarsi, 2012). The BCS at calving was less than that recommended for obtaining pregnancy probability similar or greater than 70 % (Orcasberro, 1994).

The postpartum supplement had no effect on BCS and stimulated only a transient increase in body weight. These results are consistent with those reported by Astessiano et al. (2013), but do not agree with those published by Astessiano et al. (2012). Possibly the inconsistency is due, at least partially, to the type of supplement used (pasture by Astessiano et al. (2012) vs concentrates by Astessiano et al. (2013) and the present study). Considering that the cows were primiparous, it is conceivable that the energy partitioning followed the priorities described by Short et al. (1990), so

after achieving maintenance requirements, milk production had the highest priority, followed by their own growth and, reproduction activity being the last.

The increase in milk production induced by the supplement has been reported previously in dairy (Reis and Combs, 2000; Bargo et al., 2002), dual purpose (Aguilar-Perez et al., 2009) and beef cows (Perry et al., 1991; Lalman et al., 2000). Also, the increase in milk protein has been reported in dairy cows, and was attributed to a higher energy intake in cows supplemented with concentrates (Dillon et al., 1997; Reis and Combs, 2000; Bargo et al., 2002) and with crude glycerin (Bodarski et al., 2005). Given the physiological state in which the cows were (47 ± 1.4 DPP), all endocrinology is prioritizing the mammary gland (Bauman and Currie, 1980), so it is good time to supplement if the goal is to increase milk production.

Neville (1962) suggested that during the first 60 DPP, milk production and weight gain of calves are linked and this ratio decreases thereafter, due to the increase in the consumption of forage by the calves. The increase in the availability of milk with greater protein content for calves of the SUP cows determined an increase in their daily weight gain. Daily weight gain of the calves decreased during temporary weaning with separation and increased again after calves returned with their dams, giving further support to notion that the development of the calves is milk dependent up to 90 days of age (Grings et al., 2008; Quintans et al., 2010). The daily gains observed after temporary weaning with separation are in agreement with those reported by other authors (Beal et al., 1990). Calves daily gains throughout evaluated period (0.54 and 0.60 kg d^{-1}, CON and SUP groups, respectively) were similar to those reported by Quintans et al. (2010; 0.65 kg/day), and Soca et al. (2014; 0.50 kg/day) working with multiparous and primiparous grazing cows, respectively, with similar BCS than cows in the present work.

The superiority in BW achieved by calves from SUP cows during the supplementation period remained until the date of final weaning, which is in agreement with the results reported by Astessiano (2010), showing that the increase

in milk production was reflected in the increase in weight of calves at weaning and therefore the productivity of primiparous cows.

Beef heifers in anestrus show the high NEFA concentrations, which reflects they negative energy balance (Bossis et al., 2000). It has been observed that the frequency of LH pulses is negatively correlated with the concentration of plasma NEFA in primiparous suckling beef cows (Grimard et al., 1995), as well as an increase in the concentration of NEFA could have a negative effect on the ovarian function (Bossis et al., 1999). In the present work, the plasmatic concentrations of NEFA, that reflects adipose tissue rate of lipolysis (Lucy, 2003), was different between treatments, suggesting a better energy balance in SUP than in CON cows. Moreover, concentration of cholesterol was greater in SUP than in CON cows. Cholesterol concentration increased in supplemented dairy cows probably due to an increase in energy intake (Cavestany et al., 2005), reflecting an improvement in their energy balance. In this study, the rate of lipolysis estimated by the concentration of NEFA, cholesterol and glucose indicate an improvement in the energy balance of the cows as it has been reported previously (Bossis et al., 1999; Lucy, 2003).

Higher energy intake increases the size of the follicles and the number of large follicles (diameter > 10 mm) in beef cows (Perry et al., 1991; Aguilar-Perez et al., 2009) and dairy cows (Lucy et al., 1991). This suggests a possible direct effect of nutrition on the ovary, rather than an indirect effect via the hypothalamic-pituitary axis (Khireddine et al., 1998). Taking into account that one of the most important criteria for classify anestrus is the size of the follicle (Wiltbank et al., 2002), it is conceivable the extra energy consumed by SUP cows had a stimulatory effect on folliculogenesis. However either because the temporary weaning with separation failed to stimulate LH pulsatility, or because the supplement does not reach the levels required for this event to occur, the cows remained in anestrus. There is a positive correlation between the concentration of insulin and the reproductive response (Sinclair, 2008) and therefore between insulin concentration and size of follicles (Khireddine et al., 1998). Although the number of SUP cows that become pregnant

doubled the number of cows in the CON group, this difference was not significant, possibly because of the low number of animals and the binomial nature of this variable. However the better results obtained with cows in shallow than deep anestrus after fixed-time artificial insemination reinforces the positive effect of the supplement on the reproductive function (Khireddine et al., 1998).

Cows of SUP group ate 110 g of methanol/day during 21 days. During this period no clinical sings was observed that could be associated with methanol intolerance. Moreover, the concentrations of total protein and albumin did not differ between groups, reflecting that liver synthesis of protein seemed not to be affected. The study of liver function at 110 days after the beginning of supplementation did not show impaired of liver function. These findings are in agreement with those reported by Winsco et al. (2011) who infused directly into the rumen 0 to 210 g of methanol d^{-1}, and did not observed adverse effect on intake, digestion, and ruminal fermentation in steers. These authors suggested that the bovines could tolerate methanol consumption that largely exceeds current recommendation of 150 ppm (United State Pharmacopedia) or 2000 ppm (Europe Pharmacopedia). In agreement, Dasari (2007) and Elam et al. (2008) suggested that maximum recommended levels of methanol should be revised, an issue that requires further research.

3.6. CONCLUSIONS

These results suggest that supplement provided did not appear toxic, and was able to improved energy balance reflected on achieve an increase in milk yield and calf growth, but had no significant effect on reproductive performance.

3.7. ACKNOWLEDGMENTS

To Andrés Pena and Nicolás Spinelli by analysis of crude glycerin, DILAVE by hepatic functional studies, Biogran for providing the glycerin with which this work was conducted and the staff of the EEBR, in particular: Oscar and José Cáceres,

Dorrel Bentancour and Ing. Agr. Carlos Mantero. Special thanks to Dr Carolina Viñoles for the revision of the manuscript. Partially funded by CSIC (PAIE), Universidad de la República, Uruguay. L Román and JM Clariget were holder scholarships is from ANII, Uruguay.

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4. STRATEGIC SUPPLEMENTATION WITH A MIXTURE OF WHOLE RICE BRAN AND CRUDE GLYCERIN IN PRIMIPAROUS BEEF COWS: METABOLIC, PRODUCTIVE AND REPRODUCTIVE RESPONSES

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Short title: Responses of cows to whole rice bran and crude glycerin supplementation.

4.1. ABSTRACT

To study the effect of strategic supplementations on metabolic, productive and reproductive responses in primiparous beef cows, 57 Angus, Hereford and their crosses were used. The experiment was designed as a 2 x 2 factorial with cows receiving 1 kg dry matter/(cow.day) of whole rice bran and 550 mL/(cow.day) of crude glycerin 52 ± 2 before calving and/or 21 days before mating period, resulting in four treatment combinations. On pre-partum supplementation, all cows grazed natural grass with herbage allowance (HA) of 7.5 % of live weight (LW)/day. After calving, cows and their calves were placed in another pen with native pasture with HA of 10 % of LW/day. At 59 ± 2 days of postpartum (DPP) cows were re-raffled and randomized again and pre mating supplementation began. For the last 14 days of the supplementation period, all calves from all groups were fitted a nose plate but

continued having free access to their dams (temporary weaning). At the end of nutritional treatment (80 ± 2 DPP), cows were naturally mated with bulls during 74 days. Cows receiving pre-partum supplement increased concentration of cholesterol, glucose and albumin and decreased concentration of NEFA, BHB and urea. This improvement on energy balance was reflected in greater body condition score (BCS) at calving. On the other hand, pre-mating supplementation, increased cholesterol concentration and decrease concentration of BHB at 7 days of supplementation and decreased concentration of NEFA and increased concentration of insulin at 14 days of supplementation. At end of pre-mating supplementation cows supplemented in both periods, presented the highest concentration of cholesterol. Pre-mating supplementation trend to increase milk production but no effect was observed on BCS, LW and milk composition. None of the supplementation periods affected weaning weight of calves. Pre-partum but not pre-mating supplementation increased total pregnancy rate, but no interaction between treatments was found. These results suggest that under these conditions pre-partum supplementation is more convenient than pre-mating supplementation.

Keywords: beef cattle, energy, glycerol, grassland, grazing

4.2. INTRODUCTION

Primiparous beef cows usually present the lowest pregnancy rates and calves weights at weaning within the herd (Bellows et al., 1982), and in extensive system production it is the main limitation of the cow-calf operation. When native grassland is the only source of nutrients, its variability in quantity and quality among seasons and years (Bermudez and Ayala, 2005; Formoso, 2005), limits the energy intake of the cows. The nutrients supply of native pastures during winter is insufficient to meet the requirements of pregnant cow in the last third of pregnancy (Ferrell et al., 1976), causing a negative energy balance (NEB) (Bell, 1995; Quintans et al., 2010; Astessiano et al., 2012). This NEB continues during early postpartum due to the demand for milk production (Short et al., 1990; Astessiano et al., 2013).

The NEB is evidenced by a decrease in body condition score (BCS) and changes in the concentration of some metabolites and metabolic hormones. For example it was reported an increase in non-esterified fatty acids (NEFA) and beta-hydroxibutirate (BHB) and a decrease in cholesterol, glucose and insulin when cows under similar extensive conditions were in the peripartum period (Reynolds et al., 2003; Wettemann et al., 2003; Quintans et al., 2010; Soca et al., 2014a), and consequently this has a negative impact on the follicle growth and ovulation (Wiltbank, 1970; Sinclair et al., 2002; Mulliniks et al., 2011). The low BCS at calving affects negatively the length of anestrus post-partum, pregnancy rates (Wettemann et al., 2003; Soca et al., 2014a), milk production and calf weight at weaning, especially in primiparous cows (Bellows et al., 1982) because, besides the demand of maintenance and pregnancy/lactation, this category must continue growing (Short et al., 1990).

Pre and postpartum supplementation during 90 days can overcome, at least partially, this NEB (Perry et al., 1991; Lalman et al., 1997). In extensive pastoral systemas of developing countries, concentrates are relatively expensive compared to forage, so its use must be efficient in order to achieve the maximum benefit (Kokkonen et al., 2004; Aguilar-Perez et al., 2009). Short-term supplementation before calving or mating are interesting alternatives to increase productive and reproductive efficiency of beef cows grazing native grass (Pérez-Clariget et al., 2007; Scarsi, 2012; Soca et al., 2013). Moreover, development of the biodiesel industry increased the availability of crude glycerin that can be use in ruminant nutrition as an energetic supplement (Schröder and Südekum, 1999; Donkin, 2008). However, under extensive grazing conditions, the information of the impact of this supplement on productive performance in beef cows is scare. On the other hand, whole rice bran is a very common energy supplement with no negligible values of crude protein (CP, Wang et al., 2012), and more information of its effect on cattle is available (Boucque and Fiems, 1988; Zhao et al., 1996; Scarsi, 2012; Astessiano et al., 2013; Soca et al., 2013).

Therefore, the hypothesis of this study was that a strategic supplementation before calving and before mating with whole rice bran and crude glycerin, improves the energy balance and the productive and reproductive performance of primiparous beef cows grazing native grass. The aim of this study was to evaluate the effect of supplementations 52 days before calving or 21 days before mating and their interaction on metabolic and hormonal profiles, live weight (LW), BCS, milk production and composition, calves weight, ovarian activity, and pregnancy rate in primiparous beef cows grazing native grass.

4.3. MATERIALS AND METHODS

4.3.1. Location, animals, treatments and experimental design

The experiment was conducted at the Experimental Station Bernardo Rosengurtt of the School of Agronomy, Universidad de la República (UdelaR), Uruguay (32º S, 54º W) according to the experimental procedures approved by the Animal Experimental Committee of the UdelaR.

Fifty seven Hereford, Aberdeen Angus and crossbreds pregnant heifers with 4.8 ± 0.1 units of BCS (scale: 1-8, 1 = very thin, 8 = very fat; Vizcarra et al., 1986) and 230 ± 2 days of gestation were used in a 2 x 2 factorial design, in which the factors were supplementation (supplement or not supplement) and period of supplementation (pre-partum or pre-mating). Cows were stratified by BCS and probable calving date and randomly assigned to one of two nutritional treatments. The treatments were: pre-partum supplemented group (S-, n = 29) and pre-partum un-supplemented group (C-, n = 28). Pre-partum supplemented cows were supplemented with 1 kg dry matter (DM)/cow/day of whole rice bran and 550 mL/cow/day of crude glycerin (Alcoholes del Uruguay; ALUR, Uruguay) during the last 52 ± 2 days of gestation. Both groups grazed on native grass with an herbage

allowance (HA) of 7.5% of LW/day until calving. Thereafter, all cows grazed together on native grass with an HA of 10% of LW until the end of the experiment.

At 59 ± 2 days of postpartum (DPP) cows were stratified again by BCS and calving date and, within each previous group (S- and C-),were randomly assigned to: pre-mating supplemented group (-S, n = 28) and pre-mating non-supplemented group (-C, n = 29). Pre-mating supplemented cows were offered 1 kg DM/cow/day of whole rice bran and 550 mL/cow/day of crude glycerin (ALUR, Uruguay) for 21 days before the onset of breeding period. So, 4 treatments were applied to cows: un-supplemented (CC, n = 15), supplemented only in pre-partum (SC, n = 14), supplemented only in pre-mating period (CS, n = 13), and supplemented in pre-partum and pre-mating periods (SS, n = 15).

Whole rice bran and crude glycerin were premixed before individual supplementation. The supplement provided 18.8 MJ of ME and 142 g of CP. The ME values were estimated using international FEDNA (Fundación Española para el Desarrollo de la Nutrición Animal, 2012; Spanish Foundation for the Development of Animal Nutrition) tables. Chemical composition of whole rice bran and herbage were evaluated on the Laboratory of Animal Nutrition, School of Agronomy [Ether Extract (EE; AOAC, 1990), Ash and CP (AOAC, 2007), Neutral and Acid Detergent Fiber (NDF and ADF; Van Soest et al., 1991)]. The composition of the whole rice bran was: 880 g/kg DM, 90 g/kg Ash, 140 g/kg CP, 190 g/kg NDF, 60 g/kg ADF, and 170 g/kg EE. Meanwhile, the compositions of the pre-partum and pre-mating pastures were: 500 and 330 g/kg DM, 230 and 120 g/kg Ash; 70 and 110 g/kg CP, 620 and 560 g/kg NDF, 310 and 270 g/kg ADF, and 20 g/kg EE, respectively. Chemical composition of crude glycerin was evaluated on the Laboratory of COUSA, Uruguay [30 g/kg Water (AOCS, 2009; Ea 8-58); 60 g/kg Ash (AOCS, 1973: Ea 2-38), 770 g/kg Glycerol (AOCS, 2012; Ea 6-51), 130 g/kg Fat (AOAC, 1980; 14.019)] and Methanol (10 g/kg) was provide by ALUR.

Before the pre-mating supplementation began, it was confirmed that all cows had calved normally, were suckling a calf, and were in anestrus determined by the absence of corpus luteum in two ovarian ultrasound study separated by 9 days (Wiltbank et al., 2002).

Seven days after pre-mating supplementation began (66 ± 2 DPP), all calves (n = 57) were fitted a nose plate during 14 days but they continued having free access to their mothers. When supplementation finished, nose plates were removed from the calves, and two bulls, previous andrologically examined, were introduced for 74 days (80 to 154 DPP). During both supplementation periods cows were under veterinary supervision.

4.3.2. Pasture and herbage allowance

Forage availability was determined by the method of double sampling (Haydock and Shaw, 1975) through a square of 50 cm x 50 cm, with five points scale and two replicates, cutting the forage at ground level. These determinations were performed prior animals were placed on the pens. Forage height was determined as described previously by Soca et al. (2007). The mean ± standard error (SE) of the availability and height of pasture for all period (July to December) was: 2272 ± 784 kg DM/ha and 11 ± 2 cm, respectively. In each, occasion the HA was adjusted considering the area of the pen, the pasture availability and LW of the animals.

4.3.3. Data and sample collection

Cows LW and BCS were recorded every 14 days from the beginning of the experiment (- 52 ± 2 DPP) until the start of the pre-mating supplementation (59 ± 2 DPP). Thereafter, LW and BCS, were registered at the beginning of temporary weaning (66 ± 2 DPP), at the end of supplementation (80 ± 2 DPP), and at one week and one month later (87 ± 2, and 117 ± 2 DPP, respectively) using a digital scale (FX15, Iconix, Montevideo, Uruguay). Calves were weighed from birth to 117 ± 2

days of age at the same time as cows. Weaning weight, when calves were 183 ± 2 days of age, was also registered (Figure 1).

Milk production was determined at 7 occasions using a portable milk machine (DYNAMICS ®, Uruguay), on DDP: 8 ± 2, 25 ± 2, 43 ± 2, 58 ± 2 DPP (the day before pre-mating supplementation began), 66 ± 2 (beginning of temporary weaning), 87 ± 2 (one week after the nose plates were removed), 117 ± 2 and (one month later; Figure 1). Milking was carried out according to the method described by Mondragon et al. (1983). In the morning, cows were separated from their calves and the udder emptied using 20 IU of oxytocin i/m (Neurofisin, Lab Fatro, Uruguay); 7 hours later, cows were milked again using the same methodology, and returned with their calves. The total milk was individually weighed on an electronic scale, and 24 h production was estimated. At the same time, one sample of each cow was obtained for chemical analysis. Milk composition (protein, fat and lactose) was determined using absorption of infrared radiation at the Laboratory COLAVECO (Colonia, Uruguay).

From 50 ± 2 DPP until the first month of mating period, ovaries were weekly examined by transrectal ultrasonography using a linear bimodal (5.0 to 7.5 MHz) transducer (Ambivision, Digital Notebook B mode, Model AV-3018V, Manufacturer AMBISEA Tecnology Corp., Ltd., China). Ovarian follicles and corpus luteum were identified according to the criteria described by Griffin and Ginther (1992). The resumption of ovarian activity was determined by the presence of corpus luteum in two successive ultrasound exams.

Estrus was detected twice daily (7:00 am and 7:00 pm) during the first month of mating period (Figure 1); cow was considered in estrus when accepted being mounted by the bull (Alexander et al., 1986). Pregnancy was diagnosed by transrectal ultrasonography at 145 ± 2 and 181 ± 2 DPP to determine the early and total pregnancy rates, respectively.

Weekly blood samples were taken from the coccygeal vein from the beginning of pre-partum supplementation (-52 DPP) until the first two weeks of mating period (94 DPP; Figure 1) using heparinized tubes for determinations of hormones and metabolites concentrations. Samples were centrifuged within the first hour after collection at 1530 g for 15 min, and the plasma collected and stored at - 20 °C until processing.

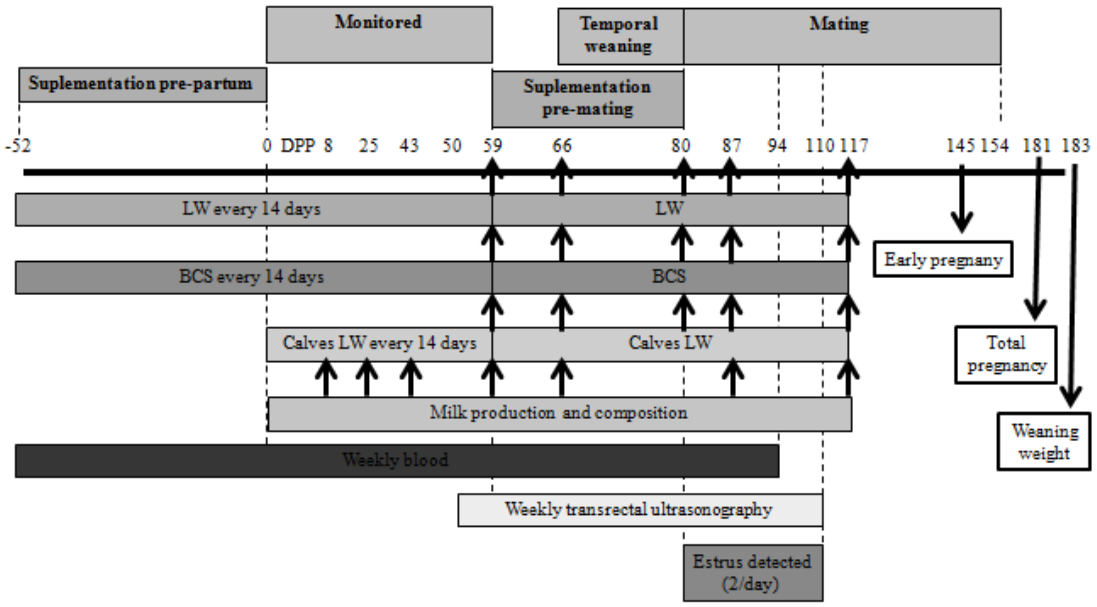

Figure 1: Overall management.

4.3.4. Plasma determinations

Insulin concentration was determined by an immunoradiometric assay (IRMA; Diasource, Brussels, Belgium). All samples were analyzed in one assay, the standard curve and controls in duplicate and the samples in single. The sensitivity of the assay was 0.5 uIU/mL and the intra-assay coefficients of variation for low (22.5 uIU/mL) and high (87.4 uIU/mL) controls were 16.1 and 9.7 %, respectively. Glucose, total protein, albumin, urea, cholesterol, NEFA and BHB concentrations were determined spectrophotometrically, using commercial kits (Glucose Oxidase/Peroxidase, Biuret, Bromocresol Green; Urease/salicylate; Cholesterol Oxidase/Peroxidase, BioSystems SA, Barcelona, Spain, Wako NEFA-HR (2), Wako Pure Chemical Industries Ltd., Osaka, Japan; Ranbut, Randox, Northern Ireland, United Kingdom; respectively),

with a sample volume and reagents adjusted to a 96 cells and read in a Multiskan EX (Thermo Scientific, Waltham, Massachusetts, USA). The intra and inter-assay coefficients of variation for high and low controls were always less than 15 %. All metabolites and hormones were determined at the Laboratory of Nuclear Techniques, School of Veterinary Medicine, Montevideo, Uruguay.

4.3.5. Statistical analysis

The analysis of cow and calf LW, BCS, milk production and composition and metabolites and hormones concentration was carried out in two periods. In Periods 1 (-52 DPP until the first 43 DPP) only pre-partum supplementation was considered in the model and in Period 2 (59 DPP until end of experiment) pre-partum supplementation, pre-mating supplementation and interaction between these factor were analysed. Reproductive variables were analyzed with the second model. Data from LW, BCS, milk production and composition, metabolites and hormones were analyzed using a repeated measures analysis over time using the mixed models (PROC MIXED of SAS), with the date or pre or postpartum days as the repetition factor, and initial values, BCS and DPP as covariates. The first model included the effects of treatments pre-partum, pre or postpartum days and interaction between the two factors as fixed effects and cow as a random effect. The second model included the effects of supplementation (supplement or not supplement), period of supplementation (pre-partum or pre-mating), date and their interactions as fixed effects and cow as a random effect. Reproductive variables were analyzed using a generalized linear model using PROC GENMOD of SAS, with the function natural logarithm link or logit link and indicating gamma distribution (interval partum-conception) or binomial (% cycling, % early and final pregnancy) in the model, respectively. Results were presented as least square means ± pooled standard error and differences were considered statistically significant at $P \leq 0.05$ and trends if $P \leq 0.1$.

4.4. RESULTS

4.4.1. Period 1

At the beginning of pre-partum supplementation all cows were on average -52 ± 2 DPP, their LW and BCS were 420 ± 3 kg and 4.8 ± 0.1, respectively. Supplementation during 52 before calving affected ($P < 0.01$) LW and BCS during pre-partum and early postpartum (43 DPP), but no interactions supplementation by days were found (LW: $P = 0.58$; BCS: $P = 0.55$). Pre-partum supplemented cows were heavier ($P < 0.01$) and had greater BCS than pre-partum un-supplemented cows (S-: 396 ± 2 kg, 4.5 ± 0.1 unit vs C-: 388 ± 2 kg, 4.3 ± 0.1 units, LW and BCS, respectively). All cows lost BCS ($P < 0.01$) from day - 52 until one week after calving, and it remained low after 43 DPP. When BCS at calving was analysed alone, pre-partum supplemented cows had greater ($P = 0.03$) BCS than pre-partum un-supplemented cows (S-: 4.5 ± 0.1 vs C-: 4.1 ± 0.1 units). Pre-partum supplementation did not influence ($P = 0.80$) calf birth weight (S-: 35.0 ± 0.7 vs C-: 34.7 ± 0.7 kg), and no calving difficulties were observed.

Milk production in the first 43 DPP was not affected ($P = 0.94$) by pre-partum supplementation (S-: 7.6 ± 0.4 vs C-: 7.6 ± 0.4 kg/day), and no interaction supplementation by DPP was found ($P = 0.99$). Total content of fat, protein and lactose in milk was not different ($P > 0.10$) between cows in both treatment groups. No interactions supplementation by DPP ($P > 0.10$) was found for any of the variables studied. The average of total content of fat, protein and lactose for both groups were: 290 ± 19, 229 ± 11, 383 ± 18 g/day; respectively. Pre-partum supplementation increased ($P = 0.03$) the calf weight (S-: 58.7 ± 1.4 vs C-: 53.9 ± 1.5 kg) during the first 43 DPP but no interaction supplementation by DPP ($P = 0.82$) was found. Calf sex tended ($P = 0.06$) to influence calf weight (Male: 58.2 ± 1.3 vs Female: 54.4 ± 1.6 kg).

Pre-partum supplementation decreased ($P < 0.01$) plasma concentration of NEFA (S-: 1.01 ± 0.05 vs C-: 1.33 ± 0.05 mmol/L) and BHB (S-: 0.54 ± 0.03 vs C-: 0.76 ± 0.03, mmol/L), and interactions supplementation by DPP were found for both variables ($P < 0.01$). Pre-partum supplementation maintained the concentration of both metabolites, meanwhile pre-partum un-supplementation group increased BHB and NEFA plasma concentration during the pre-partum period. After parturition and supplementation, both groups had similar concentrations (Figure 2a and 2b). On the other hand, plasma concentration of cholesterol was greater ($P < 0.01$) in pre-partum supplementation cows (158.0 ± 3.3 mg/dL) than in pre-partum un-supplementation cows (132.0 ± 3.3 mg/dL), and an interaction supplementation by DPP ($P < 0.01$) was found. Indeed, the concentration of cholesterol in pre-partum supplementation cows increased and remained higher ($P < 0.05$) than pre-partum un-supplementation cows until calving, while in the pre-partum un-supplementation group remained without changes ($P > 0.1$; Figure 2e).

Pre-partum supplementation did not affect plasma concentrations of total protein (S-: 73.6 ± 1.1 vs C-: 74.4 ± 1.1 g/L; $P = 0.62$) and there was no interaction between supplementation and DPP ($P = 0.34$; Figure 2h). There was an increase in albumin plasma concentration (S-: 33.0 ± 0.5 vs C-: 31.4 ± 0.5 g/L; $P = 0.01$) and a decrease in urea concentration (S-: 22.4 ± 0.6 vs C-: 27.3 ± 0.6 mmol/L; $P < 0.01$) as a consequence of pre-partum supplementation. There was no interaction between supplementation and DPP ($P = 0.30$) on albumin concentration (Figure 2g). However an interaction ($P < 0.01$) between supplementation and DPP on urea concentration was observed. In fact, urea concentrations were greater ($P < 0.05$) during the three last weeks before calving in pre-partum supplementation cows than in pre-partum un-supplementation cows (Figure 2f). After calving, all these differences disappeared.

Cows in pre-partum supplementation group had greater ($P < 0.01$) plasma concentrations of glucose than cows in pre-partum un-supplementation group (S-: 67.4 ± 0.9 vs. C-: 62.3 ± 0.9 mg/dL), and an interaction supplementation by DPP (P

= 0.01) was found (Figure 2c). However, no effect of supplementation (P = 0.15) nor interaction supplementation by DPP (P = 0.38) influenced plasma concentration of insulin.

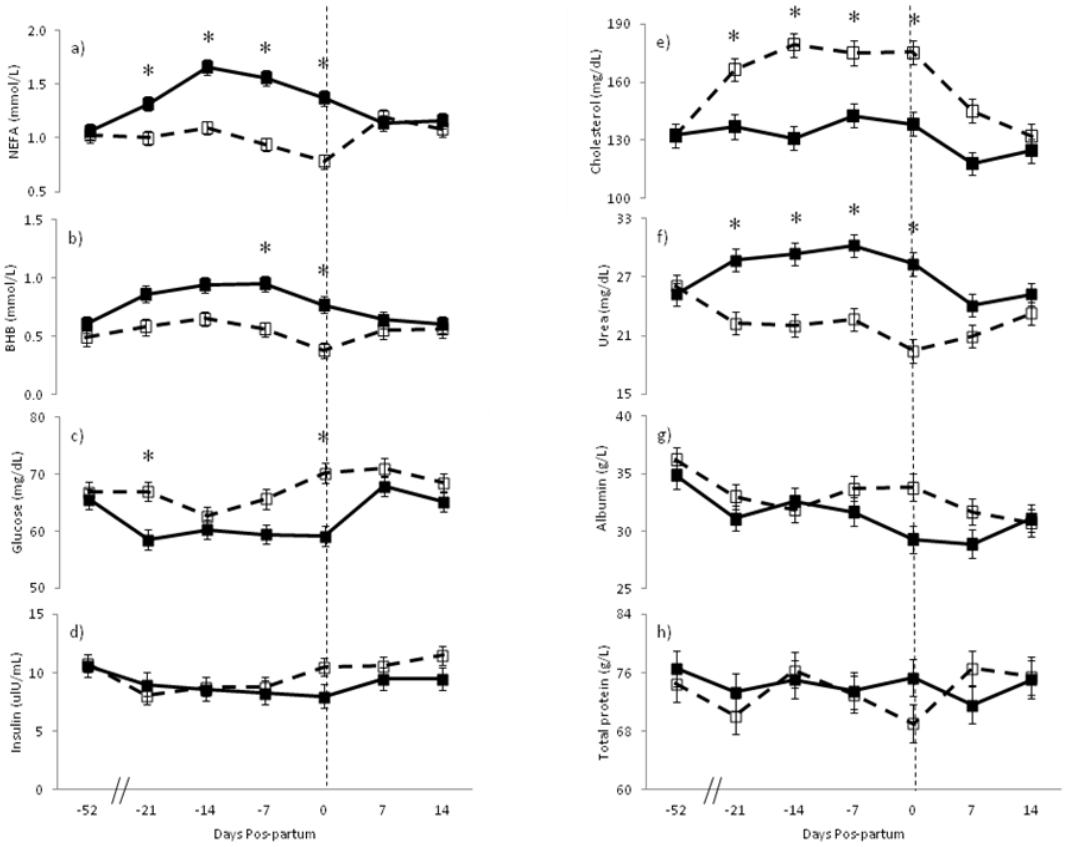

Figure 2: Concentrations of insulin and metabolites in primiparous cows non-supplemented (■) and supplemented for 52 ± 2 days pre-partum with whole rice bran and crude glycerin (□). Day 0 = Partum. Differences between treatments are indicated by * when P ≤ 0.05.

4.4.2. Period 2

At the beginning of pre-mating supplementation all cows were on average 59 ± 2 DPP, their LW and BCS were 383 ± 3 kg and 4.2 ± 0.1, respectively, and were in anoestrus confirmed by the absence of corpus luteum in the ovaries in two consecutive ultrasound examination separated by 9 days.

During pre-mating and mating periods (59 to 117 ± 2 DPP) neither pre-partum nor pre-mating supplementations nor their interaction, influenced (P > 0.1) LW. However, it was observed a pre-partum supplementation tended (P = 0.09) to influence BCS (Table 1). Pre-mating supplementation tended (P = 0.06; Table 1) to increase milk production since supplemented cows produced 8.5% more milk than un-supplemented cows (-S: 6.5 ± 0.3 vs –C: 6.0 ± 0.3 kg/day). No effects of pre-partum or pre-mating supplementations or their interaction were found (P > 0.1) on milk total content of lactose and protein. On the other hand, pre-partum supplementation tended (P = 0.06; Table 1) to increase milk total fat (S-: 222 ± 13 vs C-: 197 ± 13 g/day). Calf weight from 59 to 117 ± 2 days of age was not influenced by any of supplementations periods or their interaction (P > 0.1), therefore calves LW at weaning were no different (P > 0.1; Table 1).

Table 1: Productive performance of primiparous cows non-supplemented (CC), supplemented 52 ± 2 days pre-partum (SC), supplemented 21 days pre-mating (CS) and supplemented 52 ± 2 days pre-partum and 21 days pre-mating (SS) with whole rice bran and crude glycerin.

	Treatments					**p-value**		
	CC	**CS**	**SC**	**SS**	**SE**	**Pre-partum**	**Pre-mating**	**PxP**
LW (kg)	397	393	398	406	5	0.18	0.67	0.19
BCS (scale1-8)	4.2	4.1	4.3	4.3	0.1	0.09	0.17	0.38
Milk production (kg/day)	5.8	6.3	6.1	6.5	0.3	0.42	0.06	0.69
Fat (g/day)	196	197	215	229	13	0.06	0.54	0.60
Protein (g/day)	186	198	191	200	8	0.65	0.19	0.85
Lactose (g/day)	286	312	299	316	13	0.53	0.11	0.75
LW weaning calves* (kg)	157	160	161	161	3	0.56	0.42	0.57

*Age of weaning calves: 183 ± 2 days.

Supplemented cows during pre-partum period had greater (P < 0.01) albumin concentration (31.5 ± 0.5 g/L) than un-supplemented cows (29.6 ± 0.5 g/L) during

pre-mating and two weeks of mating period. Meanwhile, pre-mating supplementation increased (P = 0.04) plasma cholesterol concentration (-S: 162 ± 4 vs. -C: 149 ± 4 mg/dL). Concentration of total protein and cholesterol tended (P ≤ 0.1) to be influence by the interaction between pre-partum and pre-mating supplementations (Table 2).

Table 2: Concentrations of insulin and metabolites in primiparous cows non-supplemented (CC), supplemented 52 ± 2 days pre-partum (SC), supplemented 21 days pre-mating (CS) and supplemented 52 ± 2 days pre-partum and 21 days pre-mating (SS) with whole rice bran and crude glycerin.

	Treatments					**p-value**		
	CC	**CS**	**SC**	**SS**	**SE**	**Pre-partum**	**Pre-mating**	**PxP**
NEFA (mmol/L)	0.38	0.39	0.38	0.40	0.03	0.77	0.38	0.85
BHB (mmol/L)	0.51	0.52	0.53	0.52	0.03	0.78	0.93	0.78
Glucose (mg/dL)	62.5	61.9	63.0	63.8	1.1	0.29	0.95	0.50
Insulin (uIU/mL)	9.7	9.7	9.8	10.5	0.6	0.42	0.57	0.53
Cholesterol (mg/dL)	150^{b}	153^{ab}	148^{b}	172^{a}	6	0.16	0.04	0.09
Urea (mg/dL)	12.3	12.4	12.2	11.4	0.5	0.26	0.50	0.44
Albumin (g/L)	29.0	30.3	31.6	31.3	0.7	<0.01	0.43	0.24
Total protein (g/L)	78.7	75.8	76.1	78.5	1.5	0.99	0.90	0.08

No interaction pre-partum supplementation by date was observed for any of the variables studied during pre-mating and mating periods (59 to 94 ± 2 DPP). On the contrary, interaction pre-mating supplementation by date affected plasma concentration of BHB (P = 0.01), NEFA (P < 0.01), insulin (P = 0.01) and tended (P = 0.06) to influence concentration of urea. Indeed, pre-mating supplementation maintained (P > 0.1) plasma concentration of NEFA, increased (P < 0.05) plasma concentration of insulin and decreased (P < 0.05) concentrations of BHB and urea, compared with un-supplemented cows (Figure 3).

There was only a triple interaction, pre-partum by pre-mating supplementations by DPP (P = 0.03) on plasma concentration of cholesterol. Cows supplemented in both periods had the greatest (P < 0.05) plasma concentration of cholesterol on DPP 80 ± 2 (CC: 156.8^{b} ± 9.6; CS: 156.5^{b} ± 10.3; SC: 153.7^{b} ± 10.0 and SS: 201.0^{a} ± 10.4 mg/dL).

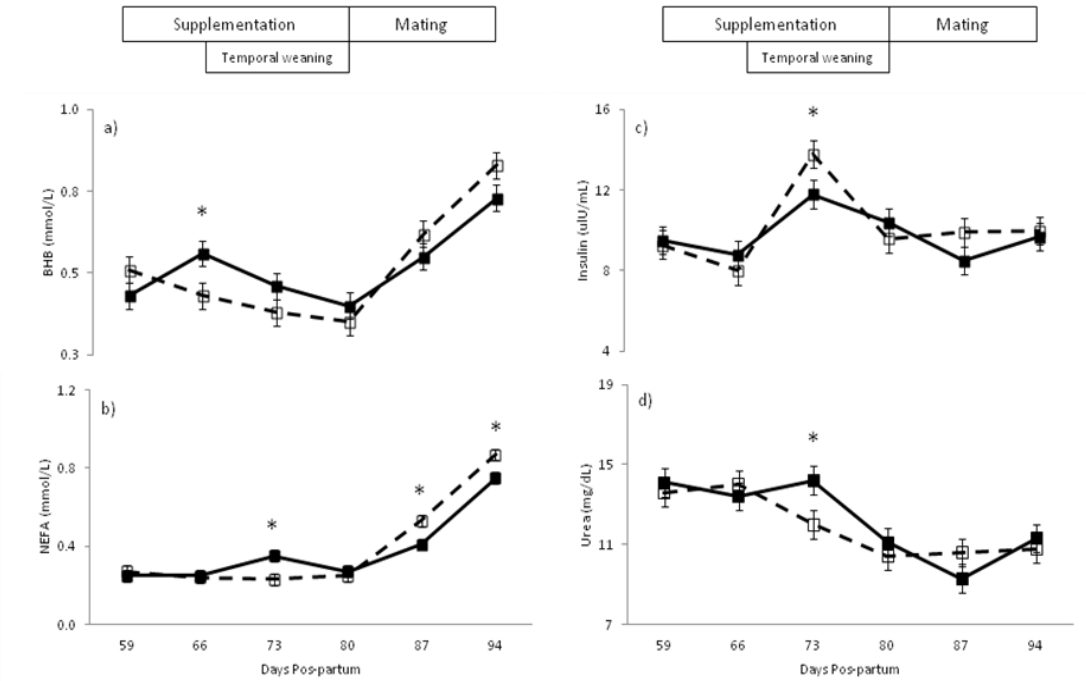

Figure 3: Concentrations of BHB, NEFA, insulin and urea in primiparous cows non-supplemented (■) and supplemented for 21days pre-mating with whole rice bran and crude glycerin (□). Day 0 = Partum. Differences between treatments are indicated by * when P ≤ 0.05.

No interaction (P > 0.10) between pre-partum and pre-mating supplementation on reproductive variables was found. Pre-partum supplementation tended to increase cycling cows at the first month of mating period (P = 0.08), and early pregnancy rate (P = 0.09), and increased (P = 0.02) total pregnancy rate (Table 3). On the other hand, pre-mating supplementation did not affect any of the reproductive variables studied (P > 0.10). The results are shown in Table 3.

Table 3: Cycling cows, early and total pregnancy and interval calving-conception on primiparous cows no-supplemented (C-) or supplemented with whole rice bran and crude glycerin (S-) for 52 days pre-partum or cows no-supplemented (-C) or supplemented with whole rice bran and crude glycerin (-S) for 21 days pre-mating. Interaction between the two factors was not significant (P > 0.10).

	Pre-partum				**Pre-mating**			
	C-	**S-**	**SE**	**p-value**	**-C**	**-S**	**SE**	**p-value**
Cycling (%)*	57	79	9	0.08	67	72	9	0.69
Early pregnancy (%)**	37	61	10	0.09	52	46	10	0.67
Total pregnancy (%)***	61	90	8	0.02	80	77	8	0.82
Interval calving-conception (d)	105	104	4	0.93	100	108	3	0.11

*Cycling was defined by the presence of corpus luteum in two or more ultrasound studies at 7 days intervals until the first month of mating period (110 ± 2 DPP).

**Early pregnancy was considered in the first 35 days of mating period (115 ± 2 DPP).

***Total pregnancy was considered which occurred during the 74 days of mating period (154 ± 2 DPP).

4.5. DISCUSSION

The present experiment showed that pre-partum or pre-mating supplementation with whole rice bran and crude glycerin improved energy balance which was reflected on an increase in plasma concentrations of cholesterol, glucose or insulin and a decrease in BHB, NEFA and urea concentrations, enabling a better BCS at calving (pre-partum supplementation), or an increase in milk production (pre-mating supplementation) but only pre-partum supplementation improve reproductive performance increasing the number of cows that resume ovarian cyclicity at the first month of mating period, and early and total pregnancy rates. However, there was no benefit of supplementation in both periods.

Cows must be fed adequately before and after calving to achieve optimal reproductive and productive performance (Perry et al, 1991). Supplementation before and after calving increases the pregnancy rate to the first service (Soto et al, 2001)

and milk production (Perry et al, 1991) compare with supplementation before or after calving or un-supplemented cows. In the present work, cows were no supplemented after calving until 59 DPP and they received supplement only during 21 days before mating. In both periods, the supplement improved the energy balance, but there was not advantage on productive or reproductive performance or plasma concentration of metabolites, and insulin, except on cholesterol. Cows supplemented in both periods, showed the greatest cholesterol value at the end of pre-mating period supplementation, may be due to an increase in energy intake (Cavestany et al., 2005).

In extensive rangeland cow–calf systems, the cow performance depends of the degree to which energy intake obtained from the native pastures satisfies nutrient demand (Kothmann, 1987). Energy intake of pregnant cows in winter does not achieve to cover their demands, therefore suffer a negative energy balance which is reflected in low BCS at calving. This is more dramatic with pregnant heifers because at fetal growth demands add their own growth requirements, therefore they show longer postpartum anestrous than multiparous cows (Bellows et al., 1982; Short et al., 1990; Lalman et al., 2000; Yavas and Walton, 2000). Pre-partum un-supplemented cows should mobilized their body lipid and protein reserves, reflected in an increase of plasma concentration of NEFA, BHB, and urea to cover their demands which is in agreement with others authors reports (Guedon et al., 1999; Holcomb et al., 2001; Reynold et al., 2003; Dann et al., 2005; Quintans et al., 2010; Soca et al, 2013). NEFA and BHB have been suggested as good indicators for recognizing negative energy balance in pre-partum (Lucy, 2003; Whitaker et al., 1999) and early postpartum (Podpečan et al., 2007; Giuliodori et al., 2013; Soca et al., 2014b). NEFA and BHB originate mainly from the catabolism of fatty acids, but BHB also could come from an increase of butyric acid in the rumen caused by the diet (Rémond et al., 1993; De Frain et al., 2004). In the present study, it would be unlikely that the increase in plasma concentration of BHB observed in control group was originated in the rumen from an increase of butyric acid. On the other hand, plasma concentration of urea is directly related to the protein content of the diet and the ratio energy/protein in the rumen (Wittwer, 1996). Decreasing energy intake

affects inversely ruminal ammonia concentration due a reduction of microbial protein synthesis, increasing blood concentration of urea (Wittwer, 1996). Urea may also originate endogenously from desamination of amino acids during protein catabolism (Chimonyo et al., 2002). In the present study, the increase in plasma concentration of urea possibly was due to an increase of muscular catabolism to provide aminoacids to the hepatic neoglucogenic (Hess et al., 2005; Hammond, 2006).

Voluntary intake of cows decreases 1.5% from week 26 of pregnancy until calving (Ingvartsen, 1994), when intake is reduced around 20-30% (Ingvartsen and Andersen, 2000; French, 2006). On the other hand, requirements for maintenance and pregnancy in the last third of gestation increased by 20-30% because fetal demands (Davis et al., 1994). Therefore supplementation in late gestation during winter, when quantity and quality from natural grass are low, seems to be a logically alternative.

The pre-partum supplementation increased the energy intake; supplemented cows were less dependent from body reserves energy, reflected in a decrease in BCS lost and a greater BCS at calving. The increase of energy and/or nutrients intake, contributed to reduce the negative energy balance. Supplementation became less necessary to obtain energy from catabolism; due to the improved plasma concentration of glucose. Therefore catabolic activity decreased, reflected in a decrease of plasma concentration of NEFA, BHB and urea, and anabolic activity increased, indicated by increases in plasma concentration of albumin and cholesterol. Increases in albumin concentration are reflecting increased on protein synthesis which is influenced by nutritional status and decreases when there is a shortage of protein in the diet or malnutrition (Ndlovu et al., 2007). Albumin has been suggested as a good early indicator of nutritional status protein due to its relatively short half-life in plasma (16 days; Agenas et al., 2006).

Pre-partum supplementation during or more than 100 days before calving increase calf birth weight (Corah et al., 1975; Perry et al., 1991; Radunz et al., 2010) and also could induce calving difficulties (Gunn et al., 2014). However, pre-partum supplementation during less than 75 days before calving seems not to influence calf birth weight (Wiley et al., 1991; Bellows et al., 2001; Soto et al., 2001; Alexander et al., 2002; Scarsi, 2012) In agreement with these reports calves weight at calving was not influenced by supplementation, and no difficulties at calving were observed.

It is interest to note that un-supplemented cows showed greater plasma concentration of NEFA, BHB, urea and lower of cholesterol during pre-partum period than pre-mating weeks. It has been postulated that in healthy cows, plasma concentration of urea about 7–15 mg/dL indicates a dietary protein balance. Although the availability of forage was similar in winter (pre-partum) and spring (pre-mating), the chemical composition and herbage allowance were different. Indeed, in agreement with regional reports (Bermudez and Ayala, 2005; Formoso, 2005), spring native pasture had 57% more CP and 11% less NDF than winter pasture. Although, pre-mating supplementation improved energy balance, indicated by a decrease on plasma concentration of NEFA, BHB and urea and an increase of cholesterol (Ndlovu et al., 2007), the impact was smaller than pre-partum supplementation. Indeed, nor changes were observed.

Neither changes on BCS and LW, nor impact on reproductive variables were observed with pre-mating supplementation, only an 8.5 % of increase in milk production that was not reflected in calf's weight was found. It is possible, that not only quality of pasture but also the herbage allowance (7.5 or 10%), physiological status of the cow (late pregnancy or lactating cow) and duration of supplementation periods (52 or 21 days) could explain, at least partially, the different impact between both supplementation. Wettemann et al. (1986), Pérez-Clariget et al. (2007), Astessiano et al. (2013) and Soca et al. (2013) no found differences in LW and BCS supplementing cows less than 30 days on postpartum with energy or protein concentrates, similar result obtained in this study.

Milk production results agree with Wiley et al. (1991) and Lalman et al. (2000) who have reported that nutrition increased during postpartum has greater impact than nutrition increase in the pre-partum on milk production. The trend higher total fat content in milk from cows supplemented pre-partum than cows non-supplemented pre-partum may be due to better BCS at calving (S-: 4.5 ± 0.1 vs C-: 4.1 ± 0 1 units; Pires et al., 2013). Anyway these trends to increased milk production of cows pre-mating supplemented or higher fat content in the milk by the cows pre-partum supplemented, was not reflected in differences on weight weaning of calves at 183 ± 2 days of age (CC = 157, CS = 160, SC = 161, SS = 161 ± 3 kg). Similar results were founded by Soto et al (2001) who neither found differences on weaning weight of the calves, supplemented pre and/or post-partum during 45 days. Quintans et al. (2009) and Scarsi (2012) working with short pre-partum supplementation (less than 40 days) with multiparous and primiparous cows, respectively, no found difference on weaning weight of the calves as well as Astessiano et al. (2013) and Soca et al. (2013) working with short supplementation (less than 22 days) but on pre-mating or mating period, respectively. These results show as well as Neville et al. (1962) that after the first 60 days of age, daily weight gain of calves not depend only on milk production, who showed how the correlation between these factors decreased in the next 60 days (0.74 to 0.63).

Body condition score at calving, which is a consequence of pre-partum nutrition (Wettemann et al., 2003), is the main factor determining the length of postpartum anestrus and the probability of pregnancy (Hess et al., 2005; Montiel y Ahuja, 2005; Soca et al., 2013). Pre-partum supplemented cows had greater BCS at calving and showed better reproductive performance. Indeed, more supplemented cows on pre-partum were cycling at the first month of mating period, thereafter early and total pregnancy rates were greater. Hunter (1991) suggested that pre-partum plane of nutrition affected development of follicles that mature in the subsequent breeding season. It is also possible that pre-partum plane of nutrition affects oocyte quality (Krisher, 2004), size, and steriodogenic capacity of the corpus luteum, and uterine function through mechanisms that cause extended anestrus (Lucy, 2003).

Wiley et al. (1991) found higher percentage of cows cycling (71% vs 31%) when they were fed to maintenance compared when they were fed for weight loss for 75 days prior to calving. Similar results were presented by Soto et al. (2001) who found higher percentage of cows pregnant at first service when they were fed 45 days pre-partum compared to when they were fed 45 days postpartum with 1.5 kg of feed/day. Unlike Scarsi (2012) differences on total pregnancy were founded, this difference may be due to the cows in this study were in better BCS at calving (supplemented: 4.5 vs 4.2 and non-supplemented: 4.1 vs 4.0, present study and Scarsi, 2012; respectively). According Orcasberro (1994) probability of pregnancy based on BCS at calving in primiparous cows is: BCS 4 = 50 % and BCS 4.5 = 75 %, similar or slightly higher results are we obtained in this study: BCS 4.1 = 61% and BCS 4.5 = 90 %). The results obtained with short supplementation before or during mating period agree with reported by Román et al. (2011) and Astessiano et al. (2013) where none differences in early or total pregnancy rate were reported. Unlike the present work, the authors worked with cows with ≤ 50 DPP, so the cows may have to allocate the energy consumed to milk production and no for reproductive function (Short et al., 1990). The present results do not agree with obtained by Carrere et al. (2005), Soca et al. (2005), Do Carmo (2006), Claramount (2007) synthesized by Pérez-Clariget et al. (2007) and the reported by Soca et al. (2013) who observed higher percentages of early and total pregnancy on supplemented animals relative to controls. These studies used animals with similar DPP to the present work (61 vs 59 days, respectively), but unlike BCS at start treatment (3.5 vs. 4.2 units, respectively). Therefore, if the percentages of early and total pregnancy of all control groups of the above works, compared to obtained in the present study, it is observed a lower percentage of early (33 % vs 52 %) and total pregnancy (63% vs 80%) of these authors with respect to this study. Therefore it could be considered that un-supplemented group in our study was in good condition (availability herbage more than 2000 kg DM/ha; herbage allowance 10 % LW, crude protein 110 g/kg), so it might not have been necessary to any measure of tactical management, or just making temporary weaning might have been sufficient to achieve rates higher pregnancy to 75 %.

4.6. CONCLUSION

Both supplementations (pre-partum and pre-mating) improved energy balance, reflected through increases concentrations on cholesterol, glucose or insulin and decreases on NEFA, BHB and urea. No interactions on productive and reproductive responses were found on pre-partum by pre-mating supplementation. Although, none supplementations increase weaning weight of calves, pre-partum supplementation was able to increase total pregnancy rate.

4.7. ACKNOWLEDGEMENTS

To ANII for provided me scholarship POS_2011_1_3522 and FMV_3_2011_1_6713 project which financed part of this work.To ALUR for provided glycerin with which this work was conducted and the staff of EEBR, in particular: Oscar Cáceres, Dorrel Bentancour and Ing. Agr. Carlos Mantero.

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5. DISCUSIÓN GENERAL Y CONCLUSIONES GLOBALES

5.1. DISCUSIÓN GENERAL

La glicerina cruda ya sea sola o asociada al afrechillo de arroz no afectó la tasa de degradabilidad de la MS del forraje, a su vez los valores de PH ruminal nunca fueron inferiores a 6,6 y los de N-NH_3 rondaron entre 5 y 15 mg/100 mL, por lo cual podría pensarse que la degradabilidad de la fibra tampoco fue afectada, ya que valores de pH ruminal entre 6,2 a 7,0 se consideran como ideales para favorecer los procesos de fermentación de los alimentos, incluyendo la máxima fermentación de los componentes fibrosos del forraje (Calsamiglia y Ferret, 2002) y los valores de N-NH_3 están dentro del rango que se considera como óptimos para el funcionamiento ruminal (8–30 mg/100 mL; McDonald *et al*., 1986). De esta forma queda de manifiesto que es posible la suplementación con glicerina cruda sobre campo natural sin afectar la degradabilidad del forraje.

Por otro lado la suplementación con GC+AA en el experimento I no disminuyó el consumo de forraje y fue el que logró las mayores consumos de energía. El mayor consumo de energía durante la suplementación se vio reflejado en los tres experimentos (experimento I, II y III) por las mayores concentraciones plasmáticas de glucosa e insulina, mostrando por lo tanto, el efecto neoglucogénico (Nelson y Cox, 2000) que tiene esta suplementación. Resultados similares han sido reportados por otros autores trabajando con suplementaciones en vacas a pastoreo a nivel internacional (Kokkonen *et al.*, 2004; Lalman *et al.*, 2000) y nacional (Soca *et al*., 2014b; Astessiano, 2013; Scarsi, 2012) o suplementando las mismas con glicerol (Donkin *et al*., 2009; Linke *et al.*, 2004; Goff y Horst, 2001). Aumentos tanto en glucosa como en insulina impactan positivamente en el desarrollo folicular y la ovulación (Mulliniks *et al.*, 2011; Hess *et al.*, 2005; Sinclair *et al.*, 2002; Wiltbank, 1970).

La movilización de lípidos y proteínas tanto en el experimento II como en el III, eran menores para las vacas suplementadas (ya sea en el pre-parto o en el pre-entore) que para las vacas control, esto se ve reflejado en las disminuciones de las concentaciones plasmáticas de AGNE y urea durante la suplementación. Estos metabolitos han sido sugeridos por otros autores como buenos indicadores del balance energético ya sea en el pre-parto (Lucy, 2003; Whitaker *et al.*, 1999) como en el pos-parto temprano (Soca *et al.*, 2014b; Giuliodori *et al.*, 2013; Podpečan *et al.*, 2007). Aumentos de BHB plasmático podrían ser causados por un mayor catabolismo lipídico (Quintans *et al.*, 2010; Reynolds *et al.*, 2003) o podrían originarse por dietas que fermentaran a nivel ruminal hacia ácido butírico como lo es el caso de suplementaciones con glicerol (De Frain *et al.*, 2004; Rémond *et al.*, 1993). En el experimento III las mayores concentraciones plasmáticas de BHB se observaron en el tratamiento control (sin suplementación) y en el experimento I, tanto cuando se suplemento solamente con glicerina cruda o asociada al afrechillo de arroz las concentraciones plasmáticas del mismo no se vieron modificadas. Por lo cual, podría considerarse que la suplementación con glicerina cruda no afectó la fermentación ruminal a favor del ácido butírico y que las mayores concentraciones de BHB en el tratamiento sin suplementación del experimento III se debieron a un mayor catabolismo lipídico.

El colesterol aumentó en los tratamientos suplementados tanto en el experimento II como en el III, reafirmando aún más la mejora en el balance energético de los lotes suplementados, ya que el mismo a sido postulado como reflejo del aumento del consumo de energía (Cavestany *et al.*, 2005; Funston, 2004).

Tanto la proteína total como la albúmina no fueron diferentes en el experimento II entre el tratamiento control y el suplementado, y solamente la albúmina fue diferente en el experiemntos III entre el lote suplementado pre-parto con respecto al control, siendo mayor la concentración de la misma para el lote suplementado. La albúmina por su vida relativamente corta en plasma (16 días; Agenas *et al.*, 2006) a sido sugerida como un indicador temprano del estado proteico

de los bovinos (Ndlovu *et al.*, 2007). El no encontrase diferencias en estos metabolitos entre el grupo suplementado y el control en ambos experimentos, ratifican el resultado del funcional hepático realizado en el experiemento II a los 110 días luego de iniciada la suplementación donde no se encotraron daños hepáticos crónicos causados por la ingesta de metanol en los animales suplementados. La suplementación con glicerina cruda en los tres experimentos sobrepasó los límites de metanol permitidos por la United State Pharmacopedia (150 ppm) y la Europe Pharmacopedia (2000 ppm), sugiriendo tal como lo proponen Elam *et al.* (2008) y Dasari (2007) que los niveles permitidos de metanol en la glicerina cruda para la alimentación de rumiantes deberían ser nuevamente revisados.

Durante el invierno, normalmente la disponibilidad y asignación de forraje (AF) del campo natural es baja (Bermudez y Ayala, 2005; Carámbula, 1991) para lograr un consumo adecuado, y cuando se logra una alta AF generalmente el valor nutritivo del forraje disminuye ya que hay una gran acumulación de restos secos (Quintans y Vaz Martins, 1994). En el experimento II se trabajó con AF muy altas (22% PV) pero con valores nutritivos del forraje bajos y en el experimento III se trabajo con una AF moderada (7,5 % PV) y con valores nutritivos algo superiores. En ninguno de los dos casos se logró cubrir los requerimientos de mantenimiento, costo de cosecha y gestación del último tercio de la vaca (NRC, 1996; Davis et al., 1994; CSIRO, 1990), ya que en ambos trabajos hubo una pérdida de aproximadamente 1 punto de CC en los últimos 60-90 días de gestación. Estos resultados concuerdan con los reportados por Briano (2014), Laporta (2012), Scarsi (2012) y Astessiano (2010) quienes trabajando con niveles de AF entre 7 y 15 % PV tampoco lograron mantener la CC durante esta etapa de fin de gestación. Por lo cual parece lógico y viable la suplementación de las mismas para cubrir parte de dichos requerimiento tratando de no perder CC o ayudar a disminuir la pérdida de la misma, para de esta forma lograr una mejor CCP, factor que se considera como el más importante para lograr una buen comportamiento reproductivo (Soca *et al.*, 2013; Hess *et al.*, 2005; Montiel y Ahuja, 2005; Short *et al.*, 1990).

La suplementación pre-parto no afectó el peso al nacimiento de los terneros ni la dificultad al parto de las vacas primíparas. Estos resultados concuerdan con los encontrados por otros autores a nivel nacional (Scarsi, 2012; Quintans et al., 2009) e internacional (Alexander *et al*., 2002; Bellows *et al*., 2001; Soto *et al*., 2001; Wiley *et al*., 1991) donde la suplementación duró menos de 75 días, pero difierieron de los encontrados por otros autores (Gunn *et al*., 2014; Radunz *et al*., 2010; Perry *et al*., 1991; Corah *et al*., 1975) donde la suplementación duró 100 o más días.

La suplementación pre-entore en el experimento II incrementó la producción de leche y el peso de los terneros, en el experimento III únicamente se observó un incremento en la producción de leche pero sin modificaciones en el peso de los terneros. Posiblemente estas diferencias encontradas se deban a que en el primero se suplementaron 14 días antes de iniciar el destete temporario el cual duró 7 días y en el segundo se suplementaron 7 días y comenzó el destete temporario el cual duró 14 días. Otros autores suplementando un mayor número de días durante el pos parto a vacas de carne, también encontraron incrementos en la producción de leche (Lalman *et al*., 2000; Perry *et al.*, 1991) y en el peso de los terneros (Ciccioli *et al.*, 2003; Spitzer *et al.*, 1995; Perry *et al.*, 1991; Richard *et al.*, 1986), mostrando hacia donde podría haber sido particionada parte de la energía ingerida (Short *et al.*, 1990).

La suplementación pre-entore si bien no encontró diferencias estadísticamente significativas en las variables reproductivas en el experimento II, lo cual posiblemente se deba a la naturaleza de las varibles y el bajo número de animales utilizados, parece haber mejorado su comportamiento reproductivo ya que se encontró un mayor porcentaje de vacas en anestro superficial al primer mes de entore comparando el grupo suplementado con el grupo control. A su vez en la IATF se preñaron el doble de vacas del grupo suplementado en comparación con el control. En el experimento III se observó una mejora en el comportamiento reproductivo evaluado a través del porcentaje de vacas ciclcando al primer mes de entore y en el porcentaje de preñez temprana y final por la suplementación pre-parto pero no por la suplementación en el pre-entore. Posiblemente esto se deba a que las vacas control al

inicio de la suplementación pre-entore se encontraban en una buena CC, situación algo diferente si las comparamos con las del experimento II (4,2 y 3,8 unidades, respectivamente). Por lo cual podría pensarse que en vacas primíparas que están con menos de 6 unidades de CC al inicio del invierno realizarle una suplementación pre-parto de forma estructural durante los últimos 2 meses de gestación para de esa forma tratar de disminuir las pérdidas de CC y luego del parto si la CC antes de iniciar el entore (más de 55 DPP) es sub-optima (3,25-4,00) realizarle una suplementación pre-entore acompañada de un destete temporario para lograr sacarlas del anestro y de esa forma mejorar su perfomance reprodutiva (Soca *et al*., 2013; Pérez-Clariget *et al*., 2007). Para vacas que se encuentren en una mejor CC al momento del entore (> 4,00), no parece favorable una suplementación pre-entore, siempre y cuando la disponibilidad y AF no sea limitante.

5.2. CONCLUSIONES GLOBALES

Estos resultados muestran que es posible la utilización de la glicerina cruda como suplemento de vacas de carne pastoreando campo natural sin mostrar ningún perjuicio, y que su administración junto con el afrechillo de arroz a nivel de campo logra mejorar el balance energético y el desempeño productivo y/o reproductivo de vacas pastoreando campo natural.

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