

**UNIVERSIDAD DE LA REPÚBLICA
FACULTAD DE AGRONOMÍA**

**LA CONDICIÓN CORPORAL AL PARTO AFECTA LAS RESPUESTAS
REPRODUCTIVA Y METABÓLICA AL DESTETE TEMPORARIO Y
FLUSHING EN VACAS PRIMÍPARAS.**

por

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TABLA DE CONTENIDO

	Página
<u>PÁGINA DE APROBACIÓN</u>	II
<u>DEDICATORIA</u>	III
<u>AGRADECIMIENTOS</u>	IV
<u>TABLA DE CONTENIDO</u>	V
<u>RESUMEN</u>	VII
<u>SUMMARY</u>	VIII
 1. INTRODUCCIÓN	 1
1.1. MARCO TEORICO.....	7
1.1.1. Descripción del sistema criador del Uruguay y preguntas que orientaron la investigación para mejorar su productividad	7
1.1.2. La reproducción y el balance de energía de vacas de cría	9
1.1.2.1. Partición de energía en gestación tardía y lactancia: énfasis en el control endócrino.....	10
1.1.2.2. Balance energético y reproducción: énfasis en la regulación endocrino metabólica de los procesos reproductivos.....	11
1.1.3 Marco teórico relación nutrición-reproducción en vacas de cría de nuestro grupo de investigación: antecedentes y propuesta de la presente tesis	13
2 . ARTÍCULO I	21
Reproductive and productive response to suckling restriction and dietary flushing in primiparous grazing beef cows	21
2.1 Summary	21
2.2 Introduction.....	22
2.3 Material &Methods	24
2.3.1 Location.....	24
2.3.2. Experimental design.....	25
2.3.3. Pasture quantity, quality and weather parameters.....	26
2.3.4 Animal measurements.....	27
2.3.5. Statistical analyses	28
2.4 Results.....	29
2.4.1. Body condition score	29
2.4.2. Postpartum anoestrus and probability of cyclicity	30
2.4.3. Early and total pregnancy.....	35
2.4.5. Calf weight.....	36
2.5. Discussion	37
2.7. Conclusions	40
2.8. References	40
3. ARTICULO II.....	46

Metabolic and endocrine profiles of primiparous beef cows grazing native grassland.	
1. Relationships between body condition score at calving and metabolic profiles during the transition period	46
3.1. Summary	46
3.2 Introduction	47
3.3. Materials and methods	48
3.3.1. Experimental site, animals and treatments	48
3.3.2. Data and sample collection	49
3.3.3. Metabolite and hormone analyses	50
3.3.4. Statistical analyses	51
3.4. Results	51
3.4.1. Body condition score	51
3.4.2. Metabolic and hormone profiles	52
3.5. Discussion	56
3.6. Conclusions	59
3.7. References	59
4. ARTICULO III	64
Metabolic and endocrine profiles of primiparous beef cows grazing native grassland.	
2. Effects of body condition score at calving, type of suckling restriction and flushing on plasmatic and productive parameters	64
4.1. Summary	64
4.2. Introduction	65
4.3. Materials and methods	67
4.3.1. Location and pasture description	67
4.3.2. Experimental design	68
4.3.3. Animal measurements	69
4.3.4. Blood sampling and metabolite and hormone determinations	69
4.3.5. Statistical analyses	70
4.4. Results	70
4.4.1. Body condition score	71
4.4.2. Plasma cholesterol and hormone determination	71
4.4.3. Calf weight and milk production	73
4.4.4. Reproductive responses	74
4.5. Discussion	75
4.6. Conclusions	78
4.7. References	78
5. DISCUSIÓN	83
5.1. Integración de estos resultados a la propuesta de investigación a largo plazo para mejorar la producción, consumo y eficiencia de uso del campo nativo por la cría vacuna en Uruguay	92
6. CONCLUSIONES	95
7. BIBLIOGRAFÍA	96

RESUMEN

La hipótesis de esta tesis fue que la combinación del tipo de destete temporario y suministro de afrechillo de arroz entero (AA) como suplemento energético durante corto período de tiempo (flushing) interactúa con la condición corporal (BCS) al parto para mejorar la eficiencia reproductiva de vacas Hereford primíparas sobre campo natural, asociándose esto a la endocrinología metabólica. El DT comenzó a los 61 ± 10 días posparto y consistió en la aplicación de tablillas nasales a los terneros durante 12 días (TS) o 5 días de aislamiento del par vaca-ternero, seguido de TS durante 7 días cuando los terneros fueron reunidos con sus madres (IS). Al finalizar el DT, se inició el entore y se ofreció o no 2 kg/día de AA durante 22 días. En el Artículo I se observó que los pesos al destete y la ganancia diaria de peso no fueron afectados por los tratamientos. Se demostró que la BCS al parto redujo el anestro y mejoró la preñez temprana y tardía, y que el Flushing mejoró un 40% la preñez temprana. En el Artículo II se observó que los perfiles de BCS, NEFA, urea, e IGF -I fueron afectados por la BCS al parto. Las vacas de baja BCS presentaron un mayor aumento de NEFA que comenzó antes, un retraso en la recuperación de BCS y menores concentraciones de IGF-I e insulina respecto las moderadas. En el artículo III, el tipo de DT no afectó las hormonas metabólicas y metabolitos, pero la concentración de IGF-I aumentó y la de BHB disminuyó durante el DT. El flushing aumentó la concentración de colesterol en ambos grupos de BCS, mientras que las concentraciones de insulina y el IGF -I se incrementaron durante el flushing sólo en vacas de moderada BCS. La adiponectina disminuyó en vacas de baja BCS. Las vacas de moderada BCS tuvieron un anestro más corto y una mejor preñez temprana. La BCS al parto afectó la evolución y las respuestas de los perfiles metabólicos y endocrinos al DT y flushing, reflejándose un mejor estado nutricional y plasticidad al balance energético negativo en vacas de BCS moderada. El IS redujo el intervalo parto-retorno a la ciclicidad ovárica y el flushing mejoró un 40% la probabilidad de preñez temprana. Ambos efectos fueron modulados por las reservas corporales (en términos de BCS al parto); diferencias de 0.5 puntos en condición (escala 1 a 8) afectaron el ambiente endocrino metabólico y la eficiencia reproductiva.

Palabras clave: Condición corporal al parto, destete temporario, flushing, respuesta metabólica, campo natural

**BODY CONDITION AFFECTS CALVING REPRODUCTIVE AND
METABOLIC RESPONSES TO SUCKLING MANAGEMENT AND
FLUSHING IN PRIMIPAROUS COWS.**

SUMMARY

The hypothesis of the present thesis was that a combination of type of suckling management and a energy supplement during short periods (flushing) interacts with body condition score (BCS) at calving to improve reproductive efficiency in primiparous beef cows grazing native grassland. Suckling-restriction treatments started at 61 ± 10 days postpartum and consisted of applying nose plates to calves for 12 days (TS) or 5 days of isolation of the calf from the cow followed by applying nose plates to calves for 7 days as calves were reunited with their mothers (IS). When suckling-restriction treatments ended, cows received or not 2 kg/day of whole-rice middling for 22 days and the breeding season started. In Article I, calf weights at weaning and average daily gain were not affected by suckling-restriction or flushing treatments. Increased BCS at calving decreased the length of anestrus and increased early and total pregnancies and flushing increased early pregnancy by 40%. In Article II, cow BCS, NEFA, urea, and IGF-I profiles were affected by the BCS at calving. The low BCS cows presented greater NEFA increase that started earlier during the prepartum period and a delayed BCS recovery and lower IGF-I and insulin concentrations respect moderate BCS cows. In article III, although suckling-restriction did not affect any plasma parameter, IGF-I concentrations increased while BHB decreased during suckling restriction. Flushing increased cholesterol concentration in both BCS groups at calving, while insulin and IGF-I concentrations increased during flushing only in moderate BCS cows. Adiponectin concentration remained declined in low BCS groups. The BCS at calving affected the evolution and the responses of the metabolic and endocrine profiles to suckling-restriction and flushing. The IS reduced the anestrus length and flushing improved early pregnancy by 40 %. Both effects were modulated by body reserves (in terms of BCS

at calving); differences in 0.5 points in BCS (scale 1-8) affected the metabolic/endocrine milieu and reproductive efficiency.

Key Words: BCS at calving, primiparous cows, flushing, suckling management, metabolic response, grazing native grassland.

1. INTRODUCCIÓN

En Uruguay, los ecosistemas pastoriles sobre campo nativo constituyen la base productiva, territorial, social y de competitividad económica de la ganadería de carne (OPYPA, MGAP Anuario estadístico 2013). Durante la última década se incrementó y estabilizó la demanda de la carne vacuna con muy buenos precios internacionales lo que explica la consolidación de una ganadería exportadora (OPYPA, MGAP Anuario estadístico 2013). No obstante, también lo han hecho los costos de producción dado por el incremento en el precio de los principales insumos, en especial los derivados del petróleo. Sumado a esto, se incrementó el precio de la tierra, el valor de la renta ganadera y la competencia entre y dentro de rubros por el recurso campo nativo. Estos cambios interactúan con la variabilidad climática, lo cual justifica el diseño de una investigación que contribuya a incrementar la producción de carne por animal y superficie del campo nativo sin incrementar el empleo de insumos, de los costos de producción y el deterioro de los recursos naturales.

El porcentaje de destete de vacas de cría se relaciona directamente con los ingresos físicos y económicos de los ganaderos que lo llevan a cabo y es determinante de la competitividad de la industria cárnica (Pitroff *et al.*, 2002; Soca *et al.*, 2007). En Uruguay, los indicadores de producción física de la cría vacuna son inferiores al potencial (ej: tasa de destete 64%, OPYPA 2013) y se explican principalmente por los niveles de ingestión de energía durante el ciclo productivo de la vaca de cría (Trujillo *et al.*, 1996; Soca *et al.*, 2007; Astessiano *et al.*, 2012). Esto justificó la búsqueda de alternativas para mejorar la eficiencia de uso del forraje del rodeo de cría, lo cual, fundamentó una propuesta de manejo basada en cambios de la oferta de forraje y en la condición corporal (BCS) conjuntamente con la aplicación del destete temporario al inicio de entore (Soca y Orcasberro, 1992). Su transferencia a nivel comercial confirmó el aumento en la producción de carne y en el ingreso económico sin que fuera necesario reducir la carga animal de los predios ganaderos

(Soca *et al.*, 2007). No obstante, en los sistemas ganaderos, la elevada carga animal y la ausencia de prácticas de manejo de la relación planta-animal, contribuyen a explicar que la mayoría de las vacas logren una BCS al parto inferior a lo recomendada por la investigación nacional (4 y 4.5 para vacas adultas y primíparas respectivamente, Soca y Orcasberro, 1992). Esto explica un largo anestro postparto, ausencia de celos y ovulación y baja probabilidad de preñez del rodeo (Short *et al.*, 1990).

Las vacas primíparas presentan no sólo un peor desempeño reproductivo con respecto a las vacas adultas, sino una superior variación en la respuesta al destete temporario (Grimard *et al.*, 1995; Quintans *et al.*, 2009). La transición desde el estado de preñez avanzada a no preñada- lactante implica importantes cambios metabólicos y endócrinos (Bossis *et al.* 1999). En vacas de carne, este período se caracteriza por presentar un balance energético negativo (BEN), con movilización de reservas, bajas concentraciones de glucosa, colesterol, insulina, factor de crecimiento tipo insulinémico I (IGF-I), elevada concentración de hormona de crecimiento (GH) y de ácidos grasos no esterificados (NEFA) (Lake *et al.*, 2006; Hess *et al.*, 2005; Waterman y Butler, 2010; Astessiano *et al.*, 2012). Estos metabolitos son indicadores de la disponibilidad energética y constituyen señales que impactan en el eje hipotálamo-hipófisis-ovario. Los efectos del BEN sobre la reproducción están asociados a inhibición en la pulsatilidad de GnRH y, por lo tanto, en la frecuencia y amplitud de pulsos de LH (Bossis *et al.*, 1999), y por ende fallas en la ovulación. La recuperación del BEN se refleja en aumentos de la insulina y de IGF-I, que favorecen procesos anabólicos y específicamente estimulan el eje reproductivo (Hess *et al.*, 2005).

En las vacas primíparas, el balance de energía (BE) durante el pre y postparto es más negativo que las adultas debido a que también presentan requerimientos para el proceso de crecimiento (Grimard *et al.*, 1995). Se registran superiores pérdidas de BCS, concentraciones de NEFA y β -hidroxibutirato (BHB), lo cual indica un estado energético más deficiente y catabólico que las multíparas, procesos asociados con

una peor performance reproductiva (Hess *et al.*, 2005). Las relaciones entre la BCS y los cambios en la concentración de metabolitos y hormonas metabólicas durante el pre y postparto en vacas de carne han sido escasamente documentadas a nivel nacional e internacional, especialmente en vacas primíparas (Vizcarra *et al.*, 1998; Lalman *et al.*, 2000; Astessiano *et al.*, 2012).

El destete temporario es una alternativa táctica para mejorar la eficiencia reproductiva, aceptada ampliamente a nivel internacional (Williams, 1990). El destete temporario de 12 días de duración a los 60 días postparto (DPP) redujo la producción de leche y por ende los requerimientos de energía, incrementó los niveles de glucosa e insulina y mejoró el BE (Quintans *et al.*, 2010). Esto incrementa los pulsos de LH y los niveles de IGF-I, lo cual constituye una herramienta táctica eficaz para reducir el intervalo parto-retorno a la ciclicidad ovárica (IPR) (Montiel y Aluja, 2005; Álvarez-Rodríguez *et al.*, 2009; Quintans *et al.*, 2010; Waterman y Butler, 2010). La separación de la vaca-ternero fue más efectiva en reducir el IPR que la sola aplicación de la tablilla nasal (Stevenson *et al.*, 1997). La respuesta metabólica (Bishop *et al.*, 1994), fisiológica (Stagg *et al.*, 1998) y reproductiva (Soca *et al.*, 2007) al destete temporario resultaron afectadas por el balance de energía o BCS de la vaca (Short *et al.*, 1990; Wettemann *et al.*, 2003; Montiel y Aluja, 2005).

La mejora del BEN ha motivado el uso de suplementos basados en suministro de concentrados energéticos durante el pre y postparto utilizando precursores glucogénicos y ácidos grasos (Hawkins *et al.*, 2000; Hess *et al.*, 2005; Mullinick *et al.*, 2008). Ambos enfoques obtuvieron resultados alentadores para mejorar la reproducción y productividad de vacas de cría en ambientes pastoriles (Wettemann *et al.*, 2003 Waterman y Butler, 2010). No obstante, la suplementación durante 45 a 90 días como la de los estudios mencionados, es una práctica de manejo sin sustento económico para la cría vacuna de Uruguay (Soca *et al.*, 2007). La suplementación energética de corta duración (10-20 días) o “flushing” mejoró la tasa ovulatoria de ovinos sin modificar la BCS (Martin *et al.*, 2004). Esto ha sido atribuido a las modificaciones en las concentraciones de glucosa, insulina, IGF-I, leptina y de

algunos aminoácidos lo cual mejoró el BE (Martin *et al.*, 2004). Esto confirmaría la existencia de un “enlace” entre los centros de control de la reproducción y del apetito (Chagas *et al.*, 2007). En vacas de carne, los experimentos que evalúan el efecto de la suplementación de corta duración sobre la eficiencia reproductiva son escasos (Wettemann *et al.*, 1986, Khireddine *et al.*, 1998).

No se han encontrado antecedentes que documenten la respuesta reproductiva en vacas de carne a medidas tácticas conjuntas y secuenciales de corta duración, como el destete temporario y el flushing. Por lo tanto, la hipótesis de la presente tesis es que la combinación del suministro de energía rápidamente disponible, a partir de un suplemento energético rico en ácidos grasos empleados durante cortos períodos, a posteriori de finalizar el destete temporario permitirá mejorar el perfil metabólico-hormonal relacionado con el metabolismo energético-proteico resultando en un mejor desempeño reproductivo de vacas primíparas en pastoreo de campo nativo. Además, el tipo de destete temporario con separación de la vaca y ternero interactuaría con el flushing para mejorar el retorno postparto y la probabilidad de preñez de vacas primíparas en BCS al parto “subóptima (<4.5)” (Soca y Orcasberro, 1992). Por último, hipotetizamos que las respuestas metabólicas y reproductivas a las intervenciones tácticas serán moduladas por las reservas corporales del animal (BCS al parto).

Hipótesis Experimento 1

El empleo secuencial de destete temporario y el flushing mejoran el IPR y la probabilidad de preñez de vacas primíparas en anestro bajo pastoreo de campo nativo. La respuesta reproductiva está afectada por el tipo de destete empleado y la BCS al parto (reflejo de los nutrientes disponibles durante el período gestación).

Hipótesis Experimento 2

El estado metabólico-endócrino durante el pre y postparto está afectado por la BCS al parto. Este ambiente está asociado a la partición de nutrientes hacia lactancia (en términos de peso vivo del ternero) de vacas primíparas en pastoreo de campo nativo.

Hipótesis Experimento 3

Los tratamientos tácticos de destete temporario y el flushing se traducen en cambios diferenciales de señales endocrino-metabólicos que se asocian al éxito reproductivo (IPR y probabilidad de preñez), y son modulados por la condición corporal al parto.

Objetivo general

Contribuir al conocimiento de los mecanismos biológicos por los cuales el destete temporario y el flushing en interacción con la BCS al parto afectan el IPR y la probabilidad de preñez en vacas primíparas en pastoreo de campo nativo.

Objetivos específicos

Estudiar el efecto del destete temporario: (con o sin aislamiento del par vaca-ternero) y flushing sobre el IPR y la probabilidad de preñez de vacas primíparas con distinta BCS al parto durante tres años consecutivos (Experimento 1).

Describir la relación entre los metabolitos y hormonas metabólicas durante el pre y postparto temprano con la BCS al parto de vacas primíparas en pastoreo de campo nativo (Experimento 2).

Relacionar el IPR y la preñez temprana y global con el estado metabólico y la BCS al parto.

Caracterizar la respuesta endocrino-metabólica de vacas primíparas sometidas a destete temporario y flushing relacionándola con la BCS al parto y la respuesta en IPR y preñez temprana y global (Experimento 3).

Esquema general de tesis

Los experimentos llevados a cabo fueron sintetizados en los artículos publicados que se especifican debajo y que en la presente tesis se refieren como Artículos I, II y III:

I. P. Soca, M. Carriquiry, DH. Keisler, M. Claramunt, M. Do Carmo, J. Olivera- Muzante, M. Rodríguez, A. Meikle. 2013 Reproductive and productive responses to suckling-restriction treatments and flushing in primiparous grazing beef cows. *Animal Production Science* 53(4) 283-291. (Experimento 1).

II. P. Soca, M. Carriquiry, M. Claramunt, V. Gestido, A. Meikle. 2013. Metabolic and endocrine profiles of primiparous beef cows grazing native grassland. 1. Relationships between body condition score at calving and metabolic profiles during the transition period. *Animal Production Science* <http://dx.doi.org/10.1071/AN13250>. (Experimento 2).

III P.Soca, M. Carriquiry, M. Claramunt, G. Ruprecht, A. Meikle. 2013. Metabolic and endocrine profiles of primiparous beef cows grazing native grassland. 2. Effects of body condition score at calving, type of suckling restriction and flushing on plasmatic and productive parameters. *Animal Production Science* <http://dx.doi.org/10.1071/AN13251>. (Experimento 3).

1.1. MARCO TEORICO

1.1.1. Descripción del sistema criador del Uruguay y preguntas que orientaron la investigación para mejorar su productividad

En el Uruguay, la cría de bovinos de carne involucra 6.6 millones de vacunos y 83 millones de hectáreas. Del total de empresas especializadas en ganadería de carne el 76 % presenta énfasis en la cría vacuna. El valor de su principal producto (terneros) es del orden de U\$ 30000 millones por año. Durante las últimas décadas, se han destetado en promedio 64 terneros cada 100 vacas entoradas (DIEA, MGAP, 2013), lo cual, constituye una de las principales limitantes para la expansión exportadora del complejo cárnico. Estos niveles contribuyen a explicar los magros resultados físico-económicos prediales, agravan la dependencia del “efecto año” y contribuyen a explicar la pérdida de sostenibilidad ambiental y social del sistema criador de vacunos de carne.

El largo del ciclo biológico y la ineficiencia biológica del proceso, explican que la cría vacuna se lleve a cabo en pastizales nativos (Holeckek *et al.*, 2011). En nuestro país, la producción y composición química del forraje del campo natural presentó variaciones entre y dentro de años (Berretta *et al.*, 2000). Dichas fluctuaciones, que se agravan con el incremento en la variabilidad climática, controlan el consumo de energía y explican el balance de energía (BE) de la vaca, el porcentaje y peso al destete de los terneros y la producción por unidad de superficie (Waterman *et al.*, 2007). En el Uruguay, el patrón de producción de forraje depende de la interacción clima, tipo de suelo y carga animal. La dominancia de especies de ciclo fotosintético C4 (*Paspalum notatum*, *Axonopus affinis*, *Andropogon ternatus*, *Bothriochloa laguroides*, *Paspalum plicatulum*, *Paspalum dilatatum*, *Coelorhachis selloana*, *Panicum milioides*, *Sporobolus indicus*), contribuye a definir un patrón común de producción de forraje con restricciones durante el invierno. La producción

de primavera-verano-otoño resulta primariamente controlada por la precipitación, no obstante, en el Basalto el efecto de la variabilidad climática sobre la producción de forraje se incrementa dado que el 70% de su superficie corresponde a suelos superficiales (Berreta *et al.*, 2000).

Una de las preguntas centrales de la investigación en cría vacuna es: *¿es posible integrar esta variabilidad de nutrientes a un esquema de manejo del consumo de energía por la vaca de cría para maximizar su eficiencia reproductiva?*. Cuando el patrón de producción y variabilidad no son integrados en las decisiones sobre la carga animal y en el manejo de la condición corporal (BCS) del rodeo, la BCS al parto, que refleja el plano de alimentación durante otoño, invierno y parte de la primavera, resulta inferior al “óptimo” recomendado por la investigación nacional (4.5-5 para vacas primíparas, Soca y Orcasberro, 1992). Esto contribuye a explicar el largo período de anestro posparto (superior a 100 días) y el porcentaje de destete promedio del rodeo nacional (64 %, DIEA, MGAP, 2013). Estos registros son inferiores en vacas primíparas las que representan el 20% (800,000 vacas) del stock de vacas del rodeo nacional (DIEA, MGAP, 2013). Dicha categoría constituye un modelo metabólico diferente, con dificultades en modificar la partición de nutrientes y aumentar el consumo de forraje, cuando se las compara con vacas adultas (Blanc *et al.*, 2006).

En base a estos antecedentes, la cría vacuna en sistemas pastoriles con campo nativo debería de contar con tecnologías de bajo costo, con alto valor agregado de conocimiento de los procesos biológicos involucrados en la nutrición energética, de manera de mejorar el intervalo parto-retorno (IPR) y probabilidad de preñez de vacas primíparas en BCS “subóptima” e “independizar” el resultado reproductivo de la variabilidad ambiental. Dicho enfoque, permitirá reducir el riesgo económico-financiero que provoca la variabilidad climática y aumentar la sostenibilidad del sistema. En base a estos antecedentes, la Facultad de Agronomía, Universidad de la República (FAGRO-UDELAR) a lo largo de dos décadas se propuso investigar alternativas de manejo para mejorar la probabilidad de preñez, concentrar los

servicios, “atenuar” el efecto año y aumentar los ingresos económicos sin modificar la carga animal empleada por la ganadería comercial. En la presente revisión se describen y comparan con la literatura internacional, algunos de los principales aportes que fundamentan la importancia de estudiar el efecto de la BCS al parto y las intervenciones con destete temporario y flushing sobre el estado metabólico y productividad de vacas de cría primíparas.

1.1.2. La reproducción y el balance de energía de vacas de cría

En un entorno de variabilidad climática y en la producción de forraje, el estado metabólico de la vaca de cría es uno de los principales factores que controla la partición de nutrientes y la eficiencia de uso de la energía (Blanc *et al.*, 2006). Para invertir la escasa cantidad de energía que se requiere para iniciar los eventos reproductivos (IPR, concepción), la vaca de cría debe integrar una gran cantidad de información procedente de múltiples órganos a diversas escalas de tiempo (Waterman y Butler, 2010). La cantidad de forraje modula el consumo, el BE y los productos finales de la fermentación; estos procesos se reflejan en cambios metabólicos que señalizan el eje reproductivo (Hess *et al.*, 2005).

Los modelos conceptuales sobre la relación nutrición-reproducción de vacas de cría (Hess *et al.*, 2005; Waterman y Butler, 2010) permiten analizar la respuesta en la concentración de metabolitos, hormonas metabólicas, expresión génica, parámetros reproductivos y productivos de vacas de cría (Waterman *et al.*, 2003, Ciccioli *et al.*, 2003, Lake *et al.*, 2006; Mullinick *et al.*, 2008). En un sistema complejo, como la vaca de carne en pastoreo de campo nativo, las señales que relacionan el BE con el metabolismo y eje reproductivo integran diversos niveles jerárquicos del animal y las respuestas emergen a diversas escalas de tiempo (en escalas de horas al año, o incluso una generación). Esto debe ser considerado, para entender, como las vacas de cría redistribuyen la energía disponible, ya sea de reservas o de ingestión de energía, de acuerdo con su historia metabólica (Blache *et al.*, 2007). En nuestro sistema de producción de cría a campo nativo, la gestación y

lactancia transcurren durante el período de menor aporte de forraje (invierno-inicio de primavera), lo cual explica el BEN. Esto justifica revisar y cuantificar el modelo de partición de energía de la vaca de cría durante el pre y postparto, su relación con el estado metabólico, el BE y la respuesta reproductiva.

1.1.2.1. Partición de energía en gestación tardía y lactancia: énfasis en el control endócrino

Durante los últimos dos meses de gestación, como parte del control homeorético, y con el objetivo de mantener la gestación, tiene lugar una fina coordinación metabólica entre los diferentes tejidos y órganos (Bauman y Currie, 1980). La transición desde preñez avanzada a no preñada lactante implica importantes cambios metabólicos y endócrinos, ya que se deben movilizar reservas para satisfacer los requerimientos de nutrientes. Los ácidos grasos de cadena larga no pueden atravesar la placenta, por tanto, los lípidos movilizados por la vaca en BEN, no pueden ser utilizados como combustible fetal (Bell 1995, Robinson *et al.*, 1999). En caso de subnutrición moderada, la vaca aumenta el catabolismo lipídico y la pérdida de BCS, para obtener energía y reduce el consumo periférico de glucosa, dejándola disponible para el feto. Al inicio de la lactancia, la homeorhesis se manifiesta al priorizar el flujo de nutrientes hacia la glándula mamaria, sobre la base de una reorganización hormonal, que cambia las rutas metabólicas para mantener las nuevas prioridades fisiológicas a expensas de otras funciones (Bauman y Currie, 1980). Esta reorganización metabólica depende, aunque no exclusivamente, de la insulina, del eje somatotrófico (GH-IGF-I) y de la prolactina. Al comienzo de la lactación los niveles de prolactina y de GH resultaron elevados (Waterman y Butler, 2010). La GH estimula la síntesis de IGF-I en el hígado pero la respuesta puede estar inhibida por un BEN (Meikle *et al.*, 2004; Lake *et al.*, 2006). En condiciones de subnutrición, los receptores de GH a nivel hepático disminuyen y la concentración de IGF-I baja, aunque la GH sea elevada (García-García, 2012). La reducción en el número de receptores de GH en el tejido hepático se debe a la baja concentración de insulina circulante en condiciones de BEN (Thissen *et al.*, 1994).

El efecto lipolítico de la GH y el limitado efecto antilipolítico y lipogénico de la insulina, dada su baja concentración, provoca una intensa lipomovilización a nivel del tejido adiposo (Rémesy *et al.*, 1986). La concentración plasmática de NEFA es indicativa de movilización de tejido adiposo para hacer frente a un BEN (Richards *et al.*, 1989), asociado a aumento en la demanda de nutrientes, a nutrición energética inadecuada, o a ambos (Lake *et al.*, 2006). Durante la primera semana postparto, la concentración de NEFA presentó una correlación negativa con insulina y positiva con la GH (Bossis *et al.*, 1999; Lake *et al.*, 2006). En este contexto, la insulina, a pesar de su baja concentración, manifiesta su efecto lipogénico en la glándula mamaria, favoreciendo la captación de aminoácidos y de glucosa (Rémesy *et al.*, 1986). En este período, la gluconeogénesis hepática aumenta y se produce un descenso en la utilización de glucosa en los tejidos periféricos (Rémesy *et al.*, 1986).

La mejora en el BE y/o el aumento de consumo postparto, incrementó la glucosa, insulina e IGF-I (Lalman *et al.*, 2000; Webb *et al.*, 2004). La concentración de IGF-I está positivamente asociada a la BCS y ambos resultan estimadores del BE durante el pre y postparto en vacas de carne (Lalman *et al.*, 2000, Hess *et al.*, 2005; Wateman y Butler, 2010).

1.1.2.2. Balance energético y reproducción: énfasis en la regulación endocrino metabólica de los procesos reproductivos

Las bajas concentraciones de glucosa, insulina e IGF-I conjuntamente con elevadas concentraciones de GH y NEFA, son indicadores de la disponibilidad energética y brindan señales que le informan al eje hipotálamo-hipófisis-ovario (Hess *et al.*, 2005). Estos cambios en las hormonas metabólicas y metabolitos están asociados a la inhibición en la pulsatilidad de GnRH que disminuye la secreción de LH (Bossis *et al.*, 1999). Cuando se reduce el consumo, por lo general lo hace la digestibilidad de la materia seca, la producción de propionato y de glucosa (Gunter *et al.*, 1995). La glucosa es el principal combustible metabólico usado por el sistema nervioso central y su baja disponibilidad reduce la liberación hipotalámica de GnRH (Keisler y Lucy 1996). El hipotálamo parece detectar la cantidad de glucosa

mediante un mecanismo de umbral (Dhuyvetter y Caton 1996). Se ha postulado que el BEN se asocia con un aumento de sensibilidad a los efectos del feedback negativo causado por los estrógenos y baja la frecuencia y amplitud de la secreción de LH (Williams 1990; Keisler y Lucy 1996).

En vacas primíparas amamantando, la frecuencia en los pulsos de LH presentó una correlación negativa con la concentración de NEFA en plasma (Grimard *et al.*, 1995). Durante el período parto/nadir de balance de energía, las condiciones metabólicas desfavorables constituyen una barrera para el crecimiento de los folículos y la ovulación. Los folículos dominantes que se desarrollan luego del nadir de energía tienen mayor tamaño promedio, superior producción de estradiol y, en general, presentan ovulación: 24 vs 75% folículos pre y post nadir de BE respectivamente (Beam y Butler, 1997). El retraso del reinicio de la ciclicidad en vacas de carne lactantes con BEN podría explicarse por la reducción en la liberación pulsátil de GnRH y de la respuesta de la adenohipófisis a esta última que determinan una inadecuada secreción de LH (Lalman *et al.*, 2000). En vacas de carne primíparas, las concentraciones de glucosa durante el entore fueron mayores en aquellas que tuvieron actividad luteal comparadas con las que no la tuvieron (Vizcarra *et al.*, 1998).

Luego del nadir de energía, el BE comienza a recuperarse, lo que se asocia con aumentos de insulina e IGF-I que favorecen procesos anabólicos (Webb *et al.*, 2004; Hess *et al.*, 2005). La insulina combinada con la glucosa puede estimular la liberación de GnRH (Hess *et al.*, 2005). A nivel ovárico, la insulina estimula la proliferación celular y la esteroidogénesis (Wettemann *et al.*, 2003), mientras que a nivel hepático facilita la producción de IGF-I (Keisler y Lucy 1996; Webb *et al.*, 2004). La concentración de IGF-I se asoció con la duración del anestro postparto y con la capacidad de reiniciar la ciclicidad ovárica postparto (Roberts *et al.*, 1998; Lake *et al.*, 2006), ya que afecta la liberación de LH (Bossis *et al.*, 1999). Es decir, tanto los aumentos de insulina e IGF-I estimulan un correcto desarrollo folicular, con aumento en la concentración de estrógenos y consecuente incremento en la pulsatilidad de LH, liberación del pico preovulatorio de LH y ovulación (Sinclair *et al.*, 2002).

En síntesis el correcto funcionamiento del eje hipotálamo-hipófisis-ovárico requiere la integración de señales periféricas (metabolitos y hormonas) que le indican el estatus nutricional de la vaca e identifican a la misma como pronta para concebir y llevar adelante una gestación. Existen pocos trabajos en rumiantes que se ha focalizado en los efectos nutricionales vía directa o indirecta sobre el tracto reproductivo y el embrión (Abecia *et al.*, 2006). Una vez que la primera limitante reproductiva es superada (la primera ovulación posparto), el ambiente endócrino periférico y local (del tracto reproductivo) debe ser el adecuado para estimular el desarrollo embrionario y mantener la preñez. Si bien existen numerosos estudios que reportan la señalización entre el BE e IPR (Sinclair *et al.*, 2002), existen escasos trabajos que estudien el ambiente endócrino y su relación con la preñez temprana en vacas de cría.

Resultan escasos y recientes los trabajos nacionales sobre cambios en los niveles de hormonas metabólicas y metabolitos que “señalizan” el ambiente variable en vacas de cría primíparas con diversa BCS al parto (Astessiano *et al.*, 2012; Carriquiry *et al.*, 2013). Esto justifica estudios que integren la respuesta productiva y metabólica a cambios de corto y largo plazo en el BE y la BCS al parto de vacas primíparas en pastoreo de campo nativo.

1.1.3 Marco teórico relación nutrición-reproducción en vacas de cría de nuestro grupo de investigación: antecedentes y propuesta de la presente tesis

En sistemas de producción de cría vacuna que no toman decisiones (ej: destete definitivo de los terneros y diagnóstico de gestación a las vacas en Marzo, cambios en la oferta de forraje en base a BCS), la variabilidad en la producción y concentración de nutrientes del campo natural, se traduce como el “efecto año” y se expresa directamente en la cantidad y peso de terneros, producción de carne por unidad de superficie y oscilaciones en el ingreso económico (Pereira y Soca, 1999). La asociación estimada en base a análisis de series temporales de información (13 años) de la producción anual de forraje de campo natural expresado como carga animal segura (número de vacas /ha que permitiría obtener 80% de destete) y la tasa

de destete estimada por la Declaración Jurada (DICOSE) del siguiente año resultó significativa ($r^2 = 0,6$; $P \leq 0,01$) (Soca *et al.*, 2007).

La carga animal empleada por los predios ganaderos familiares (0,9-1 vacas/ha) (DIEA, 2013) en 9 de 13 años resultó superior a la capacidad de carga animal segura promedio (0,65 vacas/ha). Solo tres de los 13 años, en los que el clima fue favorable y por ende existió una buena producción de forraje, tuvieron una capacidad de carga coincidente con la carga animal efectivamente empleada en los sistemas ganaderos criadores y esto se acompañó con registros superiores de tasa de destete. Estos trabajos muestran que la principal debilidad de la eficiencia reproductiva es la insuficiente ingestión de energía de las vacas atribuido al manejo tradicional del campo natural. Estas restricciones en el porcentaje de destete y su directa asociación con los niveles de ingestión y utilización de la energía consumida, ha sido señalada como la principal limitante de la reproducción de rodeos de cría en países que realizan ganadería pastoril (Short *et al.*, 1990; Pitroff *et al.*, 2002; Montiel y Aluja, 2005; Hess *et al.*, 2005; Blanc *et al.*, 2006; Crowe, 2008; McGowan y Holroyd, 2008; Burns *et al.*, 2010).

La investigación de FAGRO - UDELAR permitió plantear un modelo de manejo para sostener elevados indicadores reproductivos, integrar la variabilidad en la producción de forraje y ajustar la capacidad de carga animal de los sistemas comerciales de cría vacuna (Figura 1).

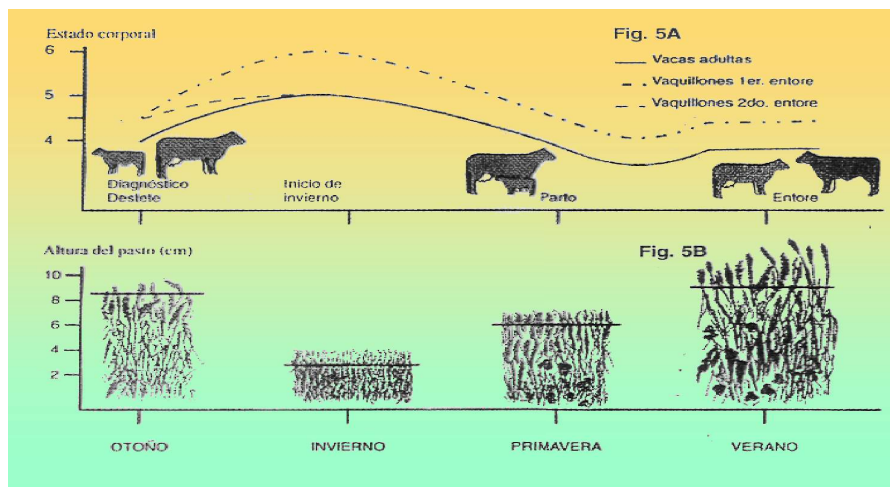


Figura 1 Propuesta de cambio del balance energético del rodeo de cría en pastoreo de campo natural (Soca y Orcasberro, 1992).

Este modelo postuló que - en base al manejo del animal (disposición de vacas y vaquillonas en lotes diferentes, destete temporario a inicio de entore, diagnóstico de preñez y destete definitivo de los terneros en Marzo) y de la relación planta animal (asignación de forraje en base a BCS y altura de pasto) - se puede mejorar el consumo de energía en el ciclo productivo de la vaca de cría. La aplicación de estos conceptos, ha permitido, mejorar el desempeño reproductivo, ingreso neto y la eficiencia de uso del campo nativo destinado a la ganadería de cría (Soca *et al.*, 2007). Debido a los atributos de la pastura y al ciclo productivo, el otoño, constituye la única estación del año en que una vaca sin ternero (con requerimientos de energía asociados únicamente a la gestación temprana), es capaz de alcanzar un consumo de energía superior al necesario para mantenimiento. Esto permite que las vacas preñadas, sin el ternero al pie, ganen peso y BCS bajo pastoreo de forraje con una altura superior a 7 cm (Figura 1). Dicha propuesta, derivó de una secuencia de experimentos y análisis de registros llevados a cabo en las Estaciones Experimentales de la FAGRO, UDELAR (EEBM, EEMAC, EEFA).

Se ha documentado extensamente que el amamantamiento actúa de forma negativa sobre el IPR (Williams, 1990). Con la hipótesis de que el amamantamiento era una de las principales limitantes para el IPR, se incluyó el destete temporario con la tablilla nasal que interrumpe temporalmente el amamantamiento y es una medida de muy bajo costo. En Uruguay, los ensayos de destete temporario en base a la aplicación de tablillas nasales a los terneros durante 13 días comenzaron en la estación de cría de 1983 (Quintans y Salta, 1984). Durante 5 años, el destete temporario provocó una mejora de 20% en el porcentaje de preñez y redujo el intervalo parto-concepción de vacas multíparas y primíparas Aberdeen Angus y Hereford (Casas y Mezquita, 1991). Parte de esa mejora puede explicarse por la reducción de 19% en la producción de leche de las vacas (3,9 vs 4,8 litros por vaca/día; Soca *et al.*, 1992).

Trabajos internacionales mostraron que la respuesta reproductiva al destete temporario se vería afectada por el BCS (Williams, 1990). Esto fundamentó, la hipótesis que el destete temporario y la BCS al parto interactúan sobre el porcentaje de preñez. Esta hipótesis fue confirmada, dado que el destete temporario acortó el intervalo parto-celo, mejoró el porcentaje de preñez, pero además las vacas multíparas con BCS al parto igual 3,5 fueron las que mejor respondieron al destete temporario (Soca *et al.*, 1992; Echenagusía *et al.*, 1994). A pesar de la importante respuesta diferencial al destete temporario acorde a las reservas corporales, son escasos los antecedentes que evalúan en forma conjunta la interacción entre la BCS al parto y el destete temporario e indagan en los mecanismos biológicos que podrían explicar comportamientos diferenciales (Bishop *et al.*, 1994; Staggs *et al.*, 1998).

El análisis de los registros permitió cuantificar las relaciones entre variables que describen el estado del sistema “*cría vacuna en campo natural*” (Orcasberro *et al.*, 1992) y fue empleado para diseñar experimentos de pastoreo, donde se evaluó el efecto de cambios en la oferta de forraje durante otoño-invierno-primavera y el destete temporario a inicio de entore, sobre la performance reproductiva de vacas de cría (Soca *et al.*, 2007). En los experimentos se empleó un diseño completamente al azar con un arreglo factorial de tratamientos donde se investigaron los efectos de 1) oferta de forraje en otoño (EEBM), invierno (EEMAC) y primavera (EEFAS) y 2) empleo del destete temporario en base a aplicación de tablillas nasales a los terneros durante 14 días a inicio del entore. También se estudió la función de respuesta entre la altura del campo natural durante gestación media-avanzada (invierno) y la BCS a inicio de invierno sobre los niveles de BCS al parto (Trujillo *et al.*, 1996). Si bien se postuló la hipótesis de que es posible trasladar reservas corporales desde el otoño a la primavera, las mejoras en BCS en otoño no se mantuvieron al parto (Orcasberro *et al.*, 1990). Durante el invierno, con restricciones en la altura del campo natural, las vacas con mejor BCS al fin de otoño perdieron más BCS. No obstante, las vacas con mejor BCS en otoño mejoraron el porcentaje de preñez cuando fueron sometidas a destete temporario al inicio de entore respecto de las vacas con ternero al pie (83 vs 45% de preñez), mientras que las de baja BCS presentaron una baja tasa de preñez (30%) y no respondieron al destete temporario (Orcasberro *et al.*, 1990). Esto

evidencia, que la respuesta de las vacas al destete temporario depende de la historia nutricional a la que fue sometida varios meses atrás (220 días después de finalizados los tratamientos de otoño).

Estos efectos de la nutrición en el tiempo sobre la reproducción en el tiempo han sido llamados más recientemente “memoria metabólica” (Blanc *et al.*, 2006). Es decir, existiría una “memoria” nutricional de lo que sucede en la etapa temprana de la gestación (otoño en nuestro sistema), que se expresa en la respuesta al control del amamantamiento durante el anestro posparto, en el siguiente entore (finales de primavera-verano). La función de respuesta entre la altura del campo natural durante gestación media-avanzada (invierno), la BCS a inicio de invierno y la respuesta de BCS al parto, permitió postular una estrategia de manejo de la BCS y del campo natural durante el otoño-invierno para la obtención de la BCS al parto “óptima” (Soca y Orcasberro, 1992; Trujillo *et al.*, 1996) (Figura 1).

Controlar el consumo de energía a través del manejo de la BCS y altura del forraje permitió mejorar la eficiencia reproductiva, el ingreso económico y plantear como hipótesis, que de aplicarse en forma sostenida a lo largo de varios años, se “optimizaría” la eficiencia de uso del campo natural destinado a la cría vacuna. De esa manera estamos frente a una propuesta que también preserva la condición del recurso natural, generando un sistema productivo económico y sostenible ecológicamente. Dicha propuesta fue sometida a validación durante un ciclo productivo del rodeo de cría en 9 predios ganaderos del centro del país y confirmó, que aún parcialmente aplicada, mejoró el resultado físico-económico predial (Soca *et al.*, 2007). El análisis económico de la ruta de cambio técnico de la ganadería nacional a través de modelos econométricos, PLAN G (Pereira y Soca, 1999) permitió poner en manos de sector productivo y decisores de políticas públicas una propuesta para modificar la productividad de 6 millones de hectáreas ganaderas de bajo costo y fácil aplicación.

No obstante, la respuesta de las vacas primíparas ante la aplicación de estas pautas de manejo, resultó inferior y con superior coeficiente de variación que las obtenidas en vacas adultas. Este problema obligó a retomar la investigación-validación de tecnología en base al planteo de la siembra y utilización de campo

natural mejorado con *Lotus subbiflorus* cv *el Rincón* (LR) para mejorar en forma estable la eficiencia reproductiva de vacas primíparas (Pereira y Soca, 1999). En base a registros colectados sobre experiencias de validación predial y empleo del PLAN G para la estimación del resultado económico, se concluyó que la aplicación de la Propuesta de Manejo del Rodeo (Figura 1) y el empleo de un área no superior a 5 % del total de la superficie de LR, permitirían mejorar un 50% el ingreso neto de la ganadería tradicional (Soca *et al.*, 2007). Una vez más, una ruta de cambio técnico para la cría vacuna sencilla, de bajo costo y con probado impacto económico predial.

Propuesta de la presente tesis; Tipo de destete temporario (con o sin aislamiento del par vaca-ternero) y Flushing

Dada la relevancia de la respuesta reproductiva al destete temporario en diferentes experimentos (Soca *et al.*, 1992), este tratamiento se incluyó en los experimentos subsiguientes como control. Dado que la literatura internacional confirmó que la presencia del propio ternero aumentó la duración del IPR, mientras que las vacas que fueron amamantadas por terneros que no son propios y/o cuyos terneros fueron destetados presentaron mayor frecuencia de pulsos de LH y una reducción en el IPR (Silveira *et al.*, 1993; Hoffman *et al.*, 1996; Stevenson *et al.*, 1997), se investigó en la presente tesis el efecto del tipo de destete temporario (con o sin aislamiento del par vaca-ternero) sobre el IPR y su interacción con la BCS al parto. Se incluyó además la determinación de variables endocrino-metabólicas asociadas a BE e IPR en vacas primíparas.

Por otro lado, experimentos que han buscado mejorar la eficiencia reproductiva de vacas de cría incrementando la disponibilidad de nutrientes en base al empleo de la suplementación con precursores gluconeogénicos, grasa y proteína de sobrepaso han sido exitosos (Williams y Stanko, 1990, Mullinicks *et al.*, 2007). Sin embargo, en estos trabajos se emplea suplemento por un período prolongado de tiempo (superior a 45 días), siendo económicamente no viables en nuestras condiciones de producción. En ovinos, cambios de corta duración en la nutrición energética (flushing) conducen a modificaciones tanto en la concentración sanguínea

de glucosa, insulina, leptina y de algunos aminoácidos, así como del fluido cerebro espinal (Martín *et al.*, 2004 Scaramuzzi *et al.*, 2006; Viñoles *et al.*, 2006). Los experimentos de flushing en ganado de carne son escasos (Wetteman *et al.*, 1986 Kheredine *et al.*, 1996;).

Estos antecedentes llevaron a nuestro grupo a proponer que la interacción entre el destete temporario y el suministro de energía rápidamente disponible durante cortos períodos de tiempo (no mayor a 20-25 días) permitiría mejorar el desempeño reproductivo de vacas primíparas en pobre BCS al parto y sería un paso del cambio técnico relacionado con la propuesta planteada anteriormente. Para mejorar la probabilidad de preñez temprana, se procedió a la elección de medidas de bajo costo (no superior a 4-6 U\$S /vaca) de carácter *táctico*, que pudieran ser aplicadas a vacas primíparas en BCS al parto “subóptima” en la generalidad de los predios ganaderos del Uruguay (Figura 2).

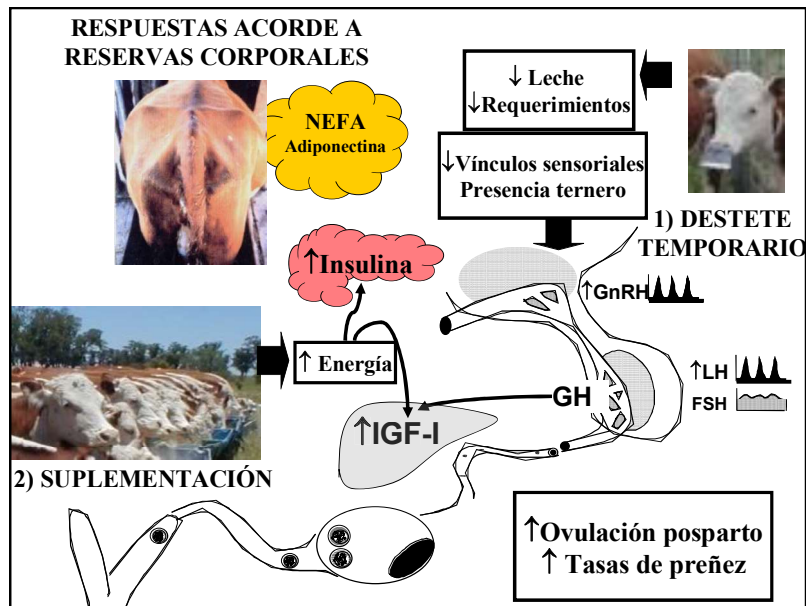


Figura 2. Modelo conceptual empleado en el diseño y análisis de los experimentos del presente trabajo (Artículos I, II y III).

El primer objetivo, lo constituye la salida del anestro para el cual es necesario provocar cambios en las señales (fisiológicas, metabólicas y productivas) que explican su alargamiento y mantienen reducida la probabilidad de preñez. El modelo

de investigación postuló que la reducción en la producción de leche (Soca *et al.*, 1992) y en los requerimientos de mantenimiento y el incremento de la IGF-I que provocaría el destete temporario (Staggs *et al.*, 1998) constituye una poderosa señal para reiniciar los ciclos ováricos luego del parto. Se postuló que el momento de aplicación del destete temporario debería ser posterior al nadir de BE (55 DPP), donde ocurre un cambio en la partición de nutrientes que favorece los procesos anabólicos. Dicho período, también coincide con la edad que los terneros deben de tener cuando se aplicó el destete temporario de manera de no provocar efectos detrimentales en el peso del ternero al destete (Soca *et al.*, 1992).

Si bien se postuló que los cambios endócrino-metabólicos provocados por el destete temporario podían mejorar la probabilidad de ovulación - y por lo tanto, la probabilidad de preñez - en vacas primíparas con BCS “subóptima”, tomando en cuenta los antecedentes se consideró que estas señales no serían suficientes para alcanzar tasas de preñez aceptables. Esto justificó el empleo de suplemento energético (2 kg afrechillo de arroz/vaca/día) de corto plazo (22 días), una vez finalizado el destete temporario. El afrechillo de arroz resultó un suplemento energético con grasa derivado de la industria del arroz con buena disponibilidad en la mayoría de las regiones del país y con un costo por unidad de energía aceptable. Por lo tanto, se testó la hipótesis de que el empleo de una fuente de energía rápidamente disponible de ser suministrado luego del nadir de energía y a posteriori de la reducción en la producción de leche provocada por el destete temporario, mejoraría el estatus energético que no sería destinado a mejorar la producción de leche sino a las funciones reproductivas. El ambiente endócrino-metabólico provocado por las intervenciones tácticas a su vez interactuaría con el nivel basal generado por las reservas corporales (memoria metabólica) generando respuestas reproductivas diferenciales acordes a la BCS al parto.

2. ARTÍCULO I

Reproductive and productive response to suckling restriction and dietary flushing in primiparous grazing beef cows

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2.1 Summary

The objectives of the present experiment were to analyze the reproductive and productive responses to suckling-restriction treatments and flushing in primiparous grazing beef cows. During 3 years, 153 primiparous anoestrus cows were assigned randomly to one of four treatments in a 2 by 2 factorial arrangement of suckling-management treatments and flushing. Suckling-restriction treatments started at 61 ± 10 days postpartum and consisted of applying nose plates to calves for 12 days (i.e. TS treatment) or 5 days of isolation of the calf from the cow followed by applying nose plates to calves for 7 days as calves were reunited with their mothers (i.e. IS treatment). Nutritional treatments (flushing v. control) started at the beginning of the breeding season, immediately after the suckling-restriction treatments were finished (73 ± 10 days postpartum), with cows receiving or not receiving 2 kg/day of whole-rice middling for 22 days. Cow body condition score (BCS) was recorded every 20 days from calving until 120 days postpartum. Duration of postpartum anoestrus

(PPA) and probability of cyclicity were estimated by plasma progesterone concentrations analyzed in weekly samples. Pregnant cows were determined by ultrasound 42 days after bull introduction (early pregnancy; EP) and 30 days after the end of the breeding season (total pregnancy; TP). BCS at calving and changes in BCS from calving to the day of BCS nadir (Δ BCS) varied among years depending on forage availability and weather conditions. Increased cow BCS at calving decreased PPA ($b = -41$ days, $P < 0.0001$) and, in interaction with Δ BCS, increased EP ($P < 0.008$) and TP ($P < 0.003$). Calf weights at weaning and average daily gain were not affected by suckling-restriction or flushing treatments. Isolated temporary suckling control reduced PPA by 11 days when compared with temporary suckling control ($P < 0.004$). Flushing increased EP by 40%, which was also affected by BCS at calving and was greater in cows that gained, than in those that maintained or lost BCS. We conclude that flushing was useful in improving early pregnancy rates of primiparous beef cows with 'suboptimal' body condition (lower than 4.5) at calving and grazing native pasture.

Additional keywords: body condition at calving, probability of cycling and pregnancy.

2.2 Introduction

In beef-production systems, weaning percentage dramatically affects producer income and success in the beef industry (Pitroff *et al.*, 2002). Short *et al.* (1990) reported that one of the main variables affecting weaning percentage is the duration of postpartum anoestrus. Shortening the postpartum anoestrus interval, particularly in operations with short breeding seasons, increases the cow's probability of pregnancy success (Hess *et al.*, 2005). Moreover, the length of the postpartum anoestrus interval is known to be affected by the cow's energy status, which is a function of both the actual DM intake and body energy reserves (Short *et al.*, 1990).

In grazing beef-production systems, high stocking rates and variability in forage resources dictate the cows' energy intake and body condition score (BCS) at calving and breeding, which along with the stressors associated with suckling are known to prolong postpartum anoestrus and decrease probability of ovulation, conception and

maintenance of pregnancy (Short *et al.*, 1990; Williams 1990; Hess *et al.*, 2005). Compelling evidence exists to support the use of suckling management in beef cows to stimulate the re-initiation of ovarian cyclicity and thus shorten postpartum anoestrus interval (Williams 1990; Stagg *et al.* 1998; Alvarez-Rodríguez *et al.* 2009; Quintans *et al.* 2009). Stevenson *et al.* (1997) reported that the effect of short-term calf removal was most effective when a complete calf–cow isolation occurred. A small number of reports have provided more detail on the type of suckling management (e.g. complete calf–cow isolation v. restricted suckling with calves remaining with their dams for a period of 2 weeks), and their application to primiparous beef cows (Alvarez-Rodríguez *et al.*, 2010; Quintans *et al.*, 2010). Moreover, few reports exist that have examined the functional relationships between BCS at calving and BCS changes after calving, in relation to suckling management and probability of re-initiation of ovarian cyclicity and pregnancy in primiparous grazing beef cows (Wettemann *et al.*, 2003). Since technology of suckling restriction to improve reproductive performance in beef cows has been widely adopted by the beef industry, the first aim of the present study was to test whether an isolated temporary suckling control (5 days) followed by 7 days of temporary suckling with nose plates shortened postpartum anoestrus when compared with temporary suckling control with nose plates (12 days) as well as their interaction with BCS at calving and BCS postpartum evolution.

Nutrition of the dam before and after calving interacts with suckling in modifying postpartum anoestrus interval and subsequent pregnancy rates (Lalman *et al.* 2000; Sinclair *et al.*, 2002). Short-term energy supplementation, referred to as flushing, has long been known to be beneficial in sheep reproduction (Nottle *et al.*, 1997). However, in beef cattle the use of supplementation treatments for 45–90 days to improve pregnancy rates has yielded variable or null results (Wettemann *et al.* 1986). In grazing beef-production systems of South America, such medium to long-term supplementation is not economically profitable (Soca *et al.*, 2007). Wettemann *et al.* (1986) and Khireddine *et al.* (1998) demonstrated improvement in follicular growth and size, luteal function and pregnancy retention when a short period of supplementation was applied. An additional variable affecting postpartum anoestrus

interval is the parity of the dam. Primiparous cows are affected more by both negative energy balance and suckling than are multiparous cows (Grimard *et al.*, 1995). Rae *et al.* (1993) reported that the postpartum anoestrus interval was longer and reproductive efficiency lower in primiparous than multiparous beef cows. Collectively, it is the population of postpartum primiparous-cow herds that is most difficult to manage and these cows represent the greatest economic losses due to reproductive failure in beef cattle grazing systems in South America (Soca *et al.*, 2007; Quintans *et al.*, 2009).

The objectives of the present experiment were to analyze the effects of the management tools, namely, suckling-management treatments and flushing, on reproduction and production parameters of primiparous cows grazing native South American pastures with different BCS at calving during three consecutive years. We hypothesized that a suckling-restriction treatment with the isolation of the cow–calf pair may improve the restoration of ovarian cyclicity. Moreover, we proposed that a 12-day suckling restriction, followed by a short-term increase in energy supplementation (i.e. flushing) would improve overall reproductive performance (e.g. restoration of ovarian cyclicity and pregnancy rates), on the basis that the suckling-restriction treatments would aid redistribution of energy to other processes (reproductive) than lactation (Alvarez-Rodríguez *et al.*, 2010) and that a subsequent extra energy input would continue to stimulate these processes even when the cow–calf pair was reunited. This hypothesis therefore needed an experimental design whereby (1) all treatments included suckling restriction, with one involving also isolation of the cow–calf pair, (2) suckling-restriction treatments and flushing were separate events over time, e.g. flushing was applied after suckling-restriction treatments were finished, and (3) the duration of energy supplementation was not longer than 22 days (flushing).

2.3 Material & Methods

2.3.1 Location

The experiment was carried out at the Experimental Station of the Faculty of Agronomy, (Salto, Uruguay; 31°24'S, 54°19'W) during spring and summer of three consecutive years (Y1, Y2, Y3). Cows and calves grazed native pasture on basaltic soils (Altamirano *et al.*, 1976; Berretta *et al.*, 2000). The most frequent pasture species found were *Paspalum notatum* (19%), *Axonopus affinis* (11%), *Bothriochloa laguroides* (6%), *Paspalum dilatatum* (6%), *Coelorachis selloana* (6%), *Stipa setigera* (6%), *Piptochaetium montevidensis* (5%) and *Desmodium incanum* (4%).

2.3.2. Experimental design

All experimental procedures were approved by the Animal Experimentation Committee, University of Uruguay. To evaluate the effect of suckling-management treatments and flushing, primiparous (3-year-old) Hereford cows ($n = 153$; Y1 = 50, Y2 = 50 and Y3 = 53) with normal calving and BCS at calving of 3.7 ± 0.4 (visual scale 1–8; Vizcarra *et al.*, 1986) and 365 ± 15 kg of bodyweight (BW) were used. Cows and their calves were randomly assigned, during three consecutive years, according to calving date, BCS at calving, and calf sex to a factorial arrangement of suckling-restriction treatments and flushing. At the beginning of suckling-restriction treatments (61 ± 10 days postpartum), all cows were in anoestrus as confirmed from two consecutive weekly plasma samples, with progesterone concentration being <1 ng/mL.

The suckling-restriction treatments consisted of (1) temporary suckling control (TS) by applying plastic nose plates (Walmur Ltda, Montevideo, Uruguay) to calves for 12 days without separation of the cow–calf pair (Quintans *et al.*, 2010), or (2) isolated temporary suckling control (IS) which consisted of complete isolation (>5000 -m distance) of the cow–calf pair for 5 days, followed by application of nose plates to calves for 7 days after the cow–calf pair was reunited. During the 5 days of complete separation in the IS treatment, calves did not have visual or auditory contact with mothers and remained in corrals with hay, supplement (89% DM, 18% crude protein(CP), and 12.54 MJ/kg DM of metabolisable energy) and water ad libitum. The temporary suckling-restriction treatments were concluded on 73 ± 10 days postpartum and cows were then assigned to nutritional treatments consisting of

(1) flushing group, in which cows were offered 2 kg/animal. day of whole rice bran (86.5% DM, 13.5% CP, 44% neutral detergent fiber, 13.5% ether extract) for 22 days or (2) control group which received no supplementation. During flushing, both groups grazed different plots of the same pasture and a trained observer verified supplement ingestion. On the same day as the suckling treatments were initiated, two bulls with proven fertility (McGowan *et al.*, 1995) were placed with the cows for 83 ± 1.7 days.

2.3.3. Pasture quantity, quality and weather parameters

Forage quantity was determined by the double-sampling method (Haydock and Shaw 1975; Coates and Penning 2000) during the phases of production while cows were gestating, at calving, and during the breeding period. Forage height was determined as described previously (Soca *et al.*, 2007). In addition, representative pasture forage samples were collected dried in a forced-air oven at 60°C, and ground in a 1-mm-mesh Wiley® Mini Mill, 115V, 60Hz. Samples were analyzed in the Laboratory of Animal Nutrition (School of Agronomy, Montevideo, Uruguay) for DM, CP, neutral detergent fiber and ash (AOAC 2000; Goering and Van Soest 1970). Monthly and seasonal records of precipitation and frost days during the experimental period were recorded (Table 1).

Table 1. Weather and pasture description from late gestation to the end of breeding season in experimental Years 1, 2 and 3

Parameter	Year 1		Year 2		Year 3	
Number of cows	50		50		53	
Calving season	26	Aug.–2	22	Aug.–3	21	Aug.–12
		Oct.		Oct.		Oct.
Breeding season	1	Dec.–28	24	Nov.–22	23	Dec.–29
		Feb.		Feb.		Feb.
Precipitation						
Winter (mm)	240 $\pm$ 10		150 $\pm$ 20		180 $\pm$ 13	
Spring (mm)	354 $\pm$ 12		245 $\pm$ 35		339 $\pm$ 23	
Summer (mm)	132 $\pm$ 12		45 $\pm$ 20		223 $\pm$ 25	
Frost days	19		35		32	
Herbage quantity (kg DM/ha)						
Late gestation	300 $\pm$ 180		800 $\pm$ 130		1200 $\pm$ 200	

Calving season	500 ± 150	300 ± 200	800 ± 300
Breeding season	1000 ± 200	700 ± 400	1600 ± 600
Herbage height (cm)			
Late gestation	1.5 ± 0.5	3 ± 1	5 ± 1.5
Calving season	n.a.	2.0 ± 0.8	4.5 ± 0.4
Breeding season	5.0 ± 0.6	4.0 ± 0.7	8.2 ± 0.5
Herbage chemical composition			
Crude protein (% DM)	11.3 ± 1.1	9.0 ± 2	13.0 ± 0.5
Neutral detergent fibre (% DM)	50 ± 4	48 ± 3	46 ± 5

Precipitation shows accumulated precipitation in Spring (September, October and November) and in Summer (December, January and February). Values of herbage chemical composition are the mean of monthly sampling. n.a. = non-available

2.3.4 Animal measurements

Cow BCS (visual scale 1–8; Vizcarra et al. 1986) was determined from calving to 20 days before definite weaning (120 ± 10 days postpartum) by one trained observer. Changes in BCS between calving and the day of BCS nadir and between calving and the start of the breeding (Δ BCS) were estimated. Number of cows for low (3), medium (3.5) and high (>4) BCS at calving were 45 (23, 7 and 15 for Y1, Y2 and Y3), 60 (18, 20 and 22 for Y1, Y2 and Y3) and 48 (9, 23 and 16 for Y1, Y2 and Y3).

Blood sampling and progesterone determination

Blood samples were collected from the jugular vein weekly with vacutainer tubes containing lithium heparin, from 30 to 120 days postpartum, refrigerated at 5°C until centrifuged (2000g, 15 min) within 2 h post-sampling and plasma aliquots were stored at –20°C until progesterone analyses.

The duration of postpartum anoestrus (PPA) was defined as the interval from calving to the day of the first of two consecutive weekly plasma samples with progesterone >1 ng/mL. Pregnancy diagnoses were performed at 42 days (early pregnancy) after the initiation of breeding period and confirmed at 30 days (total pregnancy) after bull withdrawal, using a linear array real time ultrasound scanner with 5 MHz rectal transducers (Aloka SSD-500, Hitachi Aloka Medical Ltd, Tokyo, Japan). Identification of a sharply demarcated black area with an ecogenic picture of an embryo within the uterus and/or observation of the heart beat was used as criterion

for pregnancy (sensitivity 94.8%, specificity 95.3%, positive predictive value 97.7% and negative predictive value 89.8%; Taverne *et al.*, 1985).

Calves were weighed at birth, at 73 ± 10 days of age, and at definitive weaning at 120 ± 10 days of age without fasting, and average daily gain (ADG) was calculated.. Plasma was assayed in the Laboratory of Nuclear Techniques (Veterinary Faculty, Montevideo, Uruguay). Progesterone concentrations were determined by a direct solid-phase radioimmunoassay (RIA) using DPC kits (Diagnostic Product Co., Los Angeles, CA, USA). The RIA had a sensitivity of 0.1 ng/mL. Intra-assay coefficients of variation for low (0.8 ng/mL), medium (2.2 ng/mL) and high (8 ng/mL) controls were 6.6%, 6.4% and 6.3%, respectively. The inter-assay coefficients of variations for the same controls were 9.8, 6.4 and 6.1% respectively.

2.3.5. Statistical analyses

Weather and pasture attributes were summarize as means $\pm$ standard deviation. All data were analyzed in a completely randomized design using the SAS Systems programs (SAS Institute Inc., Cary, NC, USA; SAS, 2006; Littell *et al.*, 1996). Cow BCS from calving to 120 ± 10 days postpartum was analyzed by repeated-measures using the MIXED procedure, with days postpartum as the repeated effect and first-order autoregressive as the covariance structure. The model included suckling-restriction treatments, flushing, days postpartum, and all first- and second-order interactions as fixed effects, year as random effect, and BCS at calving as covariate. The Kenward–Rogers procedure was used to adjust the denominator degrees of freedom. Initial analyses included all possible interactions, but only significant ($P < 0.05$) components were presented in results. In addition, changes in BCS were fitted to appropriate order-polynomial curves and tested for heterogeneity of regression by year (Snedecor and Cochran 1968). Days at nadir of BCS were estimated from modelling coefficients.

The duration of PPA was analyzed using the MIXED procedure with a model that included suckling-restriction treatments, flushing, and the first-order interactions as fixed effects, year as random effect and BCS at calving and Δ BCS as covariates. Probability of cycling, and early and total pregnancy (number of cows pregnant/total

cows) were fitted using the GLIMMIX procedure, with the binomial distribution and the logit as the link function specified. Suckling-restriction treatments, flushing and the first-order interactions as fixed effects, year as random effect and the covariates BCS at calving and Δ BCS were included in the model. The interactions between BCS at calving or Δ BCS and treatment effects were analyzed by slope heterogeneity. Calf weight and calf ADG were analyzed with the MIXED procedure, utilizing a model that included suckling-restriction treatments, flushing, their interactions, and calf age and sex as fixed effects and year as a random effect. Least-squares means were compared using the Tukey–Kramer test. Data are expressed as lsmeans $\pm$ standard error.

2.4 Results

2.4.1. Body condition score

Cow BCS was affected by days postpartum ($P < 0.001$), but there was no effect ($P > 0.32$) of suckling-restriction treatments, flushing, or their interaction. Changes in BCS fitted a quadratic model ($P < 0.001$) for all experimental years (Fig. 1).

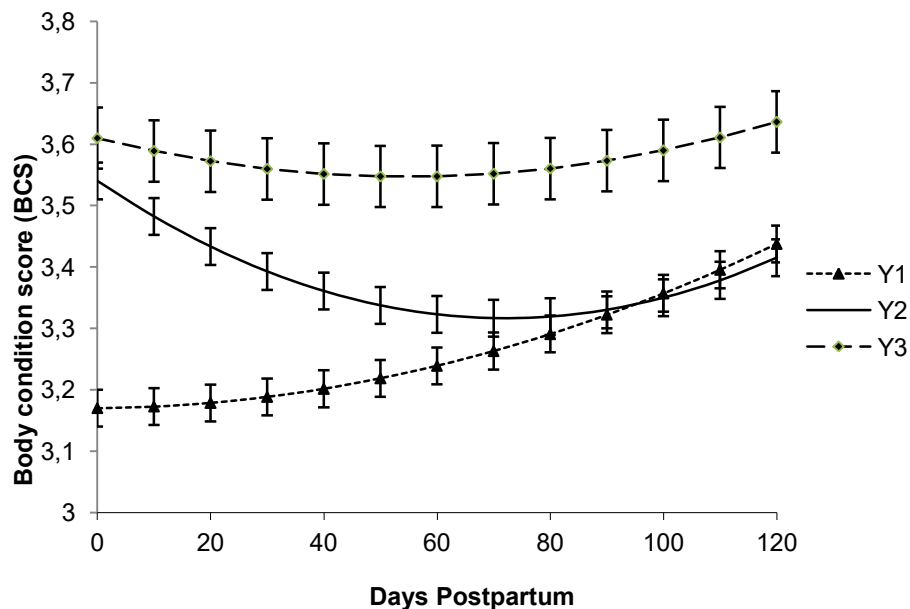


Fig. 1. Changes in body condition score (BCS) during the postcalving days in three experimental years Y1 (▲), Y2 (—) and Y3 (□). Regression coefficient $\pm$ s.e of estimation for Y1 (▲) = $3.17 (0.053) + 0.000093 (0.0013)$ days postpartum + $0.000018 (6.46E-6)$ days postpartum², for Y2 (—) = $3.54 (0.053) - 0.0063 (0.00067)$ days postpartum + $0.000043 (3.8E-6)$ days postpartum² and Y3 (□) = $3.65 (0.058) - 0.0023 (0.0012)$ days postpartum + $0.000021 (5.4E-6)$ days postpartum².

Cow BCS at calving was lower ($P < 0.001$) in year Y1, than in Y2 or Y3, but there was no difference between the latter two years (3.4 v. 3.9 v. 3.8 ± 0.3 units, respectively). Linear and quadratic coefficients were different ($P < 0.003$) between Y1 and Y2 and between Y2 and Y3, but not ($P > 0.18$) between Y1 and Y3 (Fig. 1). During Y1, BCS nadir was at calving (3 ± 1.3 days postpartum) and BCS increased thereafter until the end of the breeding period. In contrast, during Y2 and Y3, cow BCS dropped from calving to a subsequent nadir, increasing thereafter until the end of the breeding season (Fig. 1). The postpartum decline in BCS was greater ($P < 0.001$) in Y2 than in Y3, which was also associated with more days to BCS nadir (72 v. 55 ± 1.3 days postpartum, $P < 0.003$) and lower BCS at nadir (3.3 v. 3.6 ± 0.04 units, $P < 0.005$). From BCS nadir to 120 days postpartum, cows in Y1 gained more BCS (i.e. Δ BCS) than those in Y2 and Y3 (0.4 v. and 0.1 ± 0.02 units, respectively, $P < 0.001$) (Fig. 1).

2.4.2. Postpartum anoestrus and probability of cyclicity

Duration of PPA was affected by suckling-restriction treatments ($P < 0.004$) but was not affected ($P > 0.3$) by flushing or its interaction with suckling-restriction treatments (Table 2).

Table 2. Effect of suckling treatment and flushing on reproductive and productive responses in primiparous beef cows

Response			Suckling		Flushing			P-value		
			treatment							
			IS	TS	s.e	F	NF	s.e	ST	F
Estimated value										
PPA (days)			90b	101 ^a	2	94 ^a	98a	5	0.003	0.300
Probability	of	early	0.57a	0.57 ^a	0.01	0.70 ^a	0.30b	0.06	0.840	<0.001
pregnancy										
Probability	of	total	0.92 ^a	0.89a	0.01	0.90 ^a	0.83 ^a	0.03	0.456	0.900
pregnancy										
Calf weight	at	weaning	175 ^a	174a	4	174 ^a	176 ^a	2	0.300	0.420
(kg)										
Calf average	daily	gain	0.68	0.67	0.05	0.62	0.63	0.05	0.370	0.350
(kg/day)										
Mean										
Percentage (number)			50	30 (23)		43	43			
of cows cycling at end of			(37)			(32)	(32)			
suckling										
Early pregnancy rate			50	50 (37)		60	35			
			(37)			(45)	(26)			
Total pregnancy rate			90	88 (67)		94	82			
			(68)			(71)	(62)			

PPA = postpartum anoestrus, S = calf–cow pair isolated for 5 days and suckling control with nose plates for 7 days, TS = temporary suckling control with calf nose plates during 12 days while calves remained with their mothers, F = flushing, NF = control. Interactions between main fixed effects (ST = suckling treatment, F = flushing treatment) were not significant ($P > 0.34$). Calf weaning weight was adjusted at 200 DPP (daypostpartum). Means within the same row followed by the same letter are not significantly different at $P = 0.01$

Duration of PPA was reduced ($P < 0.01$) by 11 days in IS v. TS treatment groups (Table 2). In addition, BCS at calving and Δ BCS were significant ($P < 0.002$); with each unit of increment in BCS at calving or Δ BCS, the duration of PPA was reduced by 41 and 23 days, respectively (Table 3). Table 4 shows the mean values of duration

of PPA according to BCS at calving and the evolution of BCS (loss, maintenance or gain).

Table 3. Covariable coefficients to test effects of body condition score (BCS) at calving and BCS change (Δ BCS) on postpartum anoestrus (PPA) and probabilities of cyclicity, pregnancy and postpartum cyclicity.

Parameter	Intercept	BCS at calving	Δ BCS	BCS at calving $\times$ Δ BCS	BCS at calving $\times$ IS	BCS at calving $\times$ TS
PPA (days)	169 $\pm$ 27	-41 $\pm$ 7	-23 $\pm$ 5	n.s.	n.s.	n.s.
Probability of cyclicity	-19 $\pm$ 2.1	3.9 $\pm$ 0.5	7.3 $\pm$ 2.0	n.s.	3.0 $\pm$ 0.5	4.7 $\pm$ 0.7
Probability of early pregnancy	-11.7 $\pm$ 3.6	3.2 $\pm$ 1.0	14.8 $\pm$ 7.0	-3.1 $\pm$ 1.8	n.s.	n.s.
Probability of total pregnancy	-3.0 $\pm$ 2.9	1.4 $\pm$ 0.8	14.3 $\pm$ 7.1	-3.2 $\pm$ 1.9	n.s.	n.s.

Values are regression-estimate coefficients $\pm$ s.e. of estimation ($P < 0.002$). Interactions between BCS at calving and flushing were not significant ($P > 0.24$) and therefore coefficients were not included in the Table. IS = isolated temporary suckling control, TS = temporary suckling control. n.s. = non-significant

Table 4. Means ($\pm$ s.e.) of body condition at calving (BCS at calving), change in BCS from calving to nadir of BCS (Δ BCS), postpartum anoestrus (PPA) and pregnancy rate for primiparous beef cows with different BCS at calving that lost (L), maintained (M) or gained (G) BCS

Change in BCS from calving to nadir of BCS											
	L		M		G	L		M		G	
BCS at calving means	3.0 $\pm$ 0.1		3.0 $\pm$ 0.1		3.0 $\pm$ 0.2	3.6 $\pm$ 0.2		3.6 $\pm$ 0.2		3.6 $\pm$ 0.1	4.0 $\pm$ 0.2

Δ BCS	-0.37 ± 0.1	0 ± 0.1	0.5 ± 0.1	-0.25 ± 0.1	0 ± 0.1	0.4 ± 0.1	-0.5 ± 0.1	0 ± 0.1	0.3 ± 0.1
PPA (days)	143 ± 12	100 ± 20	104 ± 15	110 ± 22	114 ± 20	92 ± 17	100 ± 17	87 ± 20	83 ± 12
Early pregnancy rate (%)	0	37	70	35	38	81	46	73	81

Percentage of cyclicity was affected by suckling-restriction treatments ($P < 0.004$), and days postpartum ($P < 0.001$), but not by flushing ($P > 0.35$) or the interaction between suckling-restriction treatments and flushing (Table 2). However, there was a significant effect of BCS at calving ($P < 0.001$), Δ BCS ($P < 0.002$), and the interaction between BCS at calving and suckling-restriction treatments ($P < 0.002$; Table 3). Probability of cyclicity increased exponentially with BCS at calving when BCS at calving increased from 3.5 to 4.5 (Fig. 2).

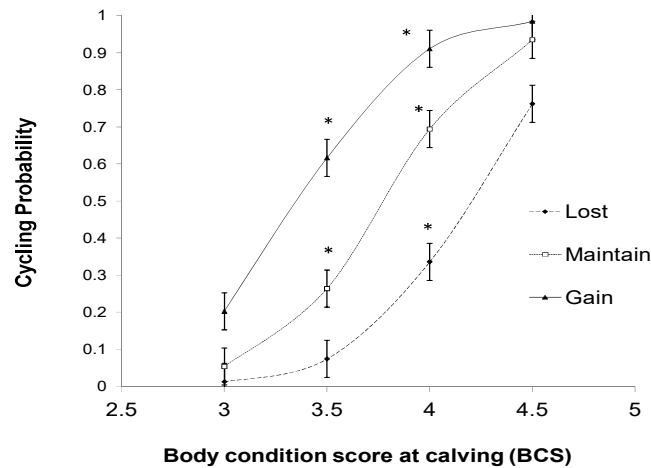


Fig. 2. Effect of body condition score (BCS) at calving and change in BCS (Δ BCS) from calving to start of breeding season on probability of cycling (probability $\pm$ s.e. of estimation). Cows lost ($\square$, $n = 60$), maintain ($\circ$, $n = 58$) or gain ($\blacktriangle$, $n = 35$) BCS. Asterisks denote significant differences at $P = 0.05$.

However, independent of Δ BCS, percentage of cyclicity was low (<0.20) when cows calved with $BCS = 3.0$, and percentage of cyclicity was high (>0.80) when cows

calved with BCS ≤ 4 (Table 4). When BCS at calving was between 3.5 and 4, percentage of cyclicity increased more in cows that gained and maintained BCS during the postpartum interval, than in cows that lost BCS during the postpartum interval (Fig. 2). In addition, percentage of cyclicity was greater in IS- than TS-treated cows when BCS at calving was <4 (Fig. 3a, b), but did not differ when BCS at calving was >4 (Fig. 3c).

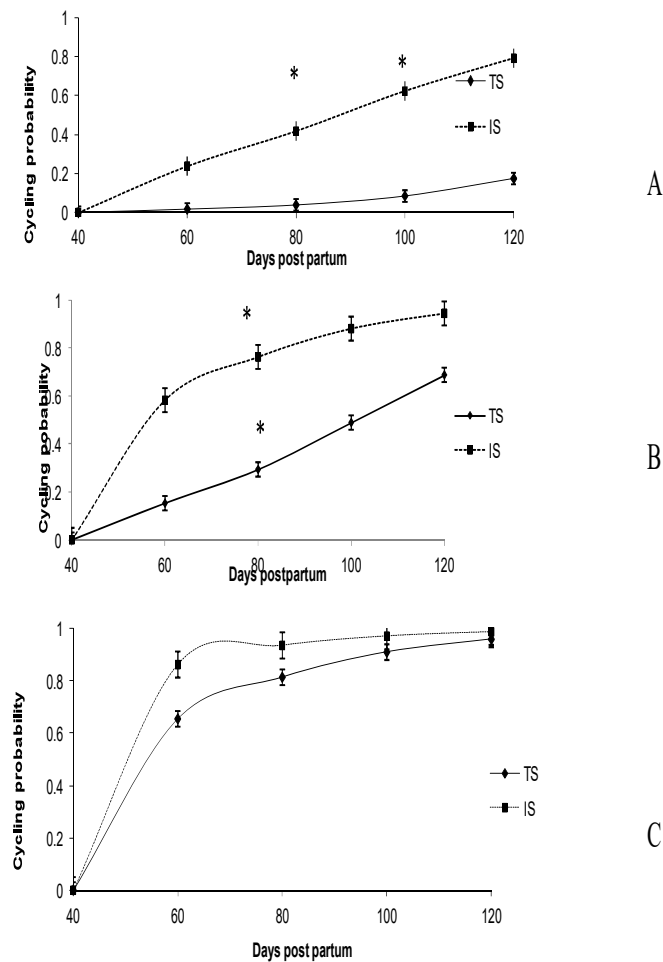


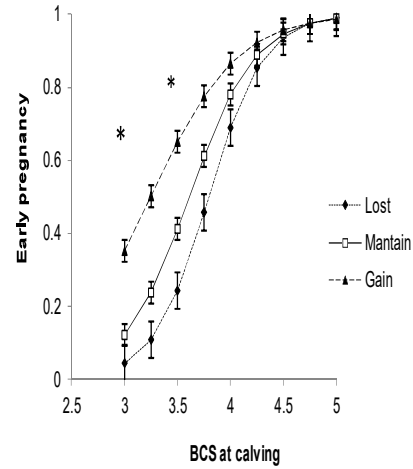
Fig. 3. Effect of days postpartum and suckling management isolated temporary suckling control (IS) (■) and temporary suckling control (TS) (□) on probability of cycling for cows with different BCS at calving: (A) BCS at calving = 3 (n = 45), (B)

BCS at calving = 3.5 (n = 60) and (C) BCS at calving ≤ 4 (n = 48). Values are probability $\pm$ s.e. of estimation. Asterisks denote significant differences at $P = 0.05$.

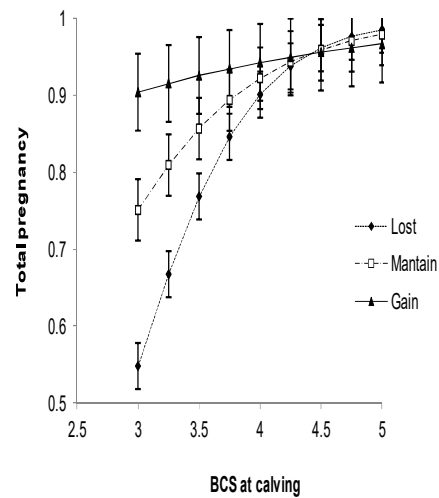
2.4.3. Early and total pregnancy

Probability of early pregnancy was affected by flushing ($P < 0.001$), but there was no effect of suckling-restriction treatments ($P > 0.84$) or its interaction. Flushing increased probability of early pregnancy by 40 percentage points (Table 2). In addition, BCS at calving, Δ BCS, and the interaction between BCS at calving and Δ BCS were significant ($P < 0.002$; Table 3). Probability of early pregnancy increased with BCS at calving and was greater in cows that gained, rather than maintained or lost BCS from calving to the beginning of the breeding season. When BCS at calving was < 4 , the probability of early pregnancy was greater in cows that maintained or gained BCS than in cows that lost BCS. However, when BCS at calving was ≤ 4 , Δ BCS did not affect the probability of early pregnancy (Fig. 4). Mean values of early and total pregnancy rates in cows with different BCS at calving that lost, maintained or gained BCS are shown in Table 4.

Probability of total pregnancy did not differ ($P > 0.90$) due to flushing and suckling-restriction treatments (Table 2), but BCS at calving, Δ BCS (Table 3) and their interaction were significant ($P < 0.002$). Probability of total pregnancy increased with BCS at calving and was greater for cows that gained than for those that maintained or lost BCS. However, when BCS at calving was < 3.5 , the probability of total pregnancy was greater for cows that gained and maintained BCS than for cows that lost BCS from calving to start of the breeding season. In contrast, when BCS was > 3.5 , Δ BCS did not affect the probability of total pregnancy (Fig. 4).



A



B

Fig. 4.Effect of BCS at calving on (A) early and (B) total pregnancy (probability $\pm$ s.e of estimation) for primiparous cows with differences in change in BCS (Δ BCS) from calving to start of breeding season. Cows lost ($\square$, n = 60), maintained ($\square$, n = 58) or gained ($\blacktriangle$, n = 35) BCS. Asterisks denote significant differences at P = 0.05.

2.4.5. Calf weight

Neither flushing nor suckling-restriction treatments, or their interactions, affected (P > 0.30) calf weight at weaning or ADG from birth to weaning.

2.5. Discussion

The present study is the first one to combine different suckling treatments (isolated v. temporary weaning) and a short-term energy supplementation (22 days, flushing) in grazing primiparous beef cows. Isolated temporary weaning decreased postpartum anoestrus interval and flushing increased early pregnancy rates, and BCS at calving modified both reproductive responses.

At the beginning of suckling, all cows were in anoestrus. The changes in metabolic signals (reduction in milk production as well as increased insulin, Quintans *et al.*, 2010) would be similar in both interventions. The 11-day reduction observed in duration of PPA by IS could be explained by the known effects of isolation of the cow–calf pair on the frequency of luteinizing hormone LH pulses (Stevenson *et al.*, 1997; Stagg *et al.*, 1998). Indeed, it has been documented that the presence of the their own calf increased the duration of PPA in cows, while cows that are weaned and/or suckled by alien calves presented a greater LH frequency and a shorter PPA (Silveira *et al.*, 1993; Lamb *et al.*, 1999). Several neuroendocrine mechanisms (e.g. cortisol, oxytocin) have been proposed to explain a differential reproductive behaviour (in terms of postpartum re-initiation of ovarian cyclicity) in cows separated from their calves v. cows that remain with their calves even if they are not suckled (Silveira *et al.*, 1993; Hoffman *et al.*, 1996). As the frequency of LH pulses increases, this promotes follicular growth, thus increasing the probability that the dominant follicle will ovulate and subsequently form a healthy corpus luteum (Stagg *et al.*, 1998; Sinclair *et al.*, 2002; Alvarez-Rodríguez *et al.*, 2010).

Flushing treatment did not modify BCS in the present study, likely due to the short-term exposure and quantity of supplementation, as has been reported by others (Wettemann *et al.*, 1986). Nonetheless, it has been reported that energy supplementation improved energy balance through increased total-energy intake (Scholljegerdes *et al.*, 2008), and/or reduced energy expenditure associated with reduced grazing time (Caton and Dhuyvetter 1997). Although flushing treatment did not alter the duration of PPA, it increased the probability of early pregnancy by 40%. Several long-term (>60 days) supplementation experiments using protein, gluconeogenic precursors or specific fatty acid supplements in beef cows during the

postpartum period have been performed (Lents *et al.*, 2008; Mulliniks *et al.*, 2008). Reports on short-term (<30 days) supplementation in postpartum beef cows are rare. Wettemann *et al.*, (1986) reported that a short-period flushing (28-day treatment with a 4.5-kg supplement (20% CP) with or without cow–calf separation for 48 h, starting 2 weeks after the beginning of the flushing) increased calf weaning weights and reduced the duration of PPA with cow–calf separation, but conception rates and days to conception for cows in thin to moderate body condition were not influenced by flushing or cow–calf separation. Although comparisons among experiments are problematic, differences regarding the effect of flushing on pregnancy rates in Wettemann *et al.* (1986) and in the present study could be explained by the timing of the application of flushing (30 v. 60 days postpartum), length of suckling-restriction treatments (2 v. 12 days), and/or the sequence of treatments (simultaneous events v. sequential exposures in our study to suckling restriction treatments, followed by flushing). Indeed, suckling-restriction treatments may have reduced milk production and increased glucose and insulin concentrations, as has been reported recently (Quintans *et al.*, 2010). Both variables are stimulatory signals for the reproductive axis and act centrally and peripherally to initiate the events for reproductive success. We speculate that the subsequent additive effects of flushing may have improved the already ongoing process of re-initiating cyclicity induced by suckling treatments, thus resulting in greater early pregnancy rates (Khireddine *et al.*, 1998).

The positive effect of flushing on early pregnancy without changes in BCS could be due to a better quality of the oocyte, improved corpus luteum function, and/or lower embryo mortality (Khireddine *et al.*, 1998; Viñoles *et al.*, 2010). Flushing with energy supplementation consisting of 2 kg/head.day of concentrate for 40 days, beginning at 42 ± 3.4 days postpartum, increased fertilisation rate and/or reduced early embryonic death, which could be explained by the positive effect on follicular growth that appeared not to be mediated by LH (Khireddine *et al.*, 1998). In contrast, it has been recently reported that flushing treatment had no effect on the number, health or size of follicles, but improved the quality of the cumulus oocyte complex (Viñoles *et al.*, 2010).

Cow BCS at calving was the most important factor affecting the duration of PPA and when paired with postpartum changes in BCS, both variables greatly affected the probability of pregnancy success and maintenance. Indeed, BCS at calving has been widely reported to be a relevant factor on reproductive performance in beef cows (Houghton *et al.*, 1990; Sinclair *et al.*, 2002; Ciccioli *et al.*, 2003; Lents *et al.*, 2008). Collectively, primiparous beef cows grazing native pastures adjusted their reproductive and productive responses to pre- and post-calving energy balance. Indeed, regulation of reproduction by nutritional status relies on the animal's ability to integrate at a given time point, the physiological mechanisms it uses to perceive energy balance and the state of the adipose reserves for making performance decisions (Blanc *et al.*, 2006).

A shorter duration of PPA, associated with greater BCS at calving and a positive Δ BCS, is consistent with the literature (Lalman *et al.* 1997). Indeed, a variety of reproductive variables are linked to PPA, including the frequency and amplitude of LH pulses and the size of the dominant follicle, which are positively associated with BCS at calving (Stagg *et al.*, 1998; Sinclair *et al.*, 2002; Ciccioli *et al.*, 2003). Cows calving with BCS of 3.5–4 and gaining BCS presented greater early or total pregnancy rates than did cows that lost BCS. Moreover, cows with BCS>4 at calving had a high probability of pregnancy rates that were independent of changes in BCS during the postpartum period. Pregnancy rates in the present study were similar to those reported in previous studies where cows calved with higher BCS (4.5, Soca *et al.* 2007). This difference could be attributed to the suckling treatments applied to all cows included in the present study, e.g. reduced milk production and/or a change in the endocrine environment as postulated by others (Quintans *et al.* 2010).

Body condition score at calving was different among years, and this was consistent with the weather and pasture characteristics determined in the present study. Specifically, the year when the cows exhibited the lowest BCS at calving (Year Y1) was congruent with the lowest forage availability during gestation. Indeed, Soca *et al.*, 2007 reported that BCS at calving in beef cows grazing native pastures was positively correlated with BCS in autumn (mid-gestation) and winter (last third of gestation). Similarly, associations among pasture characteristics and changes in

postpartum BCS were observed. The year that cows presented more days after calving without BCS changes (Year Y2) was also the year with the lowest herbage quantity during the calving and breeding seasons.

The finding that short-term supplementation treatments had an important impact on early pregnancy is of significant economic importance since early pregnancy is positively associated with lifetime production (kg of weaned calf per cow and pregnancy), better livestock forage utilization and greater economic result of cow-calf systems (Short *et al.*, 1990). In summary, flushing was a tool to improve early pregnancy of primiparous beef cows calving with 'suboptimal' BCS and submitted to suckling-restriction treatments.

2.7. Conclusions

The present study has re-asserted the relevance of BCS at calving on reproductive performance in primiparous beef cows. Furthermore, isolated temporary weaning reduced the duration of postpartum anoestrus when compared with temporary weaning, but had no effect on pregnancy rates. Short-term flushing with whole-rice bran did not affect duration of postpartum anoestrus but did improve earlier pregnancy rates.

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3. ARTICULO II

Metabolic and endocrine profiles of primiparous beef cows grazing native grassland. 1. Relationships between body condition score at calving and metabolic profiles during the transition period

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3.1. Summary

The body condition score (BCS) at calving has been postulated as the main factor in the interaction of nutrition and reproduction in beef-cow ecosystems. The objective of the present study was to analyze the effect of BCS at calving on endocrine and metabolic profiles during the transition period in primiparous Hereford cows (n = 56) grazing native grassland. Cow BCS was registered and plasma insulin, insulin-like growth factor-I (IGF-I) and metabolites (concentrations of urea, albumin, cholesterol, total protein, non-esterified fatty acids (NEFA)) were determined in plasma from -30 to +45 days postpartum (DPP). The effect of BCS at calving (low ≤ 3.5 and moderate ≥ 4 ; 1–8 visual scale) on BCS evolution and hormone and metabolite concentrations were analyzed using a time repeated-measures analysis. Cow BCS, NEFA, urea and IGF-I profiles were affected ($P < 0.05$) by the BCS at calving and DPP interaction, while insulin tended ($P = 0.06$) to be affected only by BCS at calving. The low-BCS cows presented a smaller loss of BCS during the prepartum than did moderate-BCS cows, and a delayed BCS recovery during the postpartum. The increase in plasma NEFA was greater ($P < 0.05$) and started earlier during the prepartum period in the

low-BCS cows. Protein concentrations increased during prepartum to +30 DPP, while albumin and urea concentrations were maintained until calving and decreased during the postpartum. Urea concentrations were greater ($P < 0.05$) during prepartum in low-BCS cows. Concentrations of IGF-I and insulin were greater ($P < 0.05$) in moderate- than low-BCS cows during prepartum, but did not differ between the groups after calving. No effects were observed in calf weight or milk production at +45 DPP. The association between BCS during prepartum and at calving with the endocrine/metabolic profiles (positive: insulin, IGF-I; negative: NEFA and urea) reflected a better nutritional status and plasticity to negative energy balance on moderate cows. This different endocrine milieu did not affect calf weight and/or milk production. In conclusion, the BCS at calving affected the metabolic and endocrine profile during the transition period of primiparous beef cows grazing native grassland.

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3.2 Introduction

In spring-calving beef cows grazing native pastures, the last months of gestation and calving occur during winter or early spring when forage quantity and quality limit energy intake, which in turn causes a period of negative energy balance (NEB; Astessiano *et al.*, 2012). This NEB is associated with prolonged postpartum anestrus and lower fertility in beef cows (Short *et al.*, 1990; Wettemann *et al.*, 2003; Waterman and Butler 2010). We have shown that a loss of one unit of body condition score (BCS; scale 1–8, Vizcarra *et al.* 1986) from calving to the nadir of BCS prolongs the postpartum anestrus interval (PPI) in 23 days in primiparous beef cows grazing native pastures (Soca *et al.*, 2013). Cow BCS at calving is the single most important factor explaining PPI and pregnancy (Hess *et al.*, 2005; Soca *et al.*, 2013) and is affected by prepartum energy nutrition (Wettemann *et al.*, 2003). Several metabolites reflect a cow's metabolism and can be used objectively, reliably and routinely to assess the nutritional status of cattle (Ndlovu *et al.*, 2007). Moreover, several of these metabolites are the signals by which energy status is relayed to the brain, thereby redirecting nutrient allocation to enhance survival and/or reproductive fitness when nutrient resources are scarce. Such crosstalk among organs

and tissues is finely regulated and involves several hormones such as the growth hormone (GH)–insulin-like growth factor-I (IGF-I) axis and insulin, among others (Waterman and Butler 2010).

In primiparous grazing beef cows, the transition from late gestation to start of lactation is associated with BCS mobilisation, increased blood GH and non-esterified fatty acids (NEFA) and decreased cholesterol, glucose, insulin and IGF-I concentrations (Vizcarra *et al.*, 1998; Ciccioli *et al.*, 2003; Lake *et al.*, 2006; Waterman and Butler 2010). The improvement of energy balance is associated with increased circulating IGF-I and insulin, which promote anabolic processes (Hess *et al.*, 2005). Insulin may also facilitate production of IGF-I by the liver (Robert 2008; Waterman and Butler 2010) and a reciprocal action has been suggested as IGF-I stimulates insulin secretion by the pancreas in other species (Yoshida *et al.*, 2007).

The scientific literature that has evaluated effects of a differential energy balance in terms of body reserves on blood metabolites, IGF-I and insulin concentrations, and their association with reproductive and productive success in primiparous (Vizcarra *et al.*, 1998) and multiparous beef (Lake *et al.*, 2006) cows, is scarce. To our knowledge, there are no reports of the effect of BCS at calving on metabolic and endocrine profiles in beef cows during the transition period. We postulate that the metabolic and endocrine profiles depend – at least in part – on body energy reserves and are associated with productive outcome, and that these profiles might be a useful tool to monitor nutrient prioritisation.

The objectives of the present work were to determine metabolite (i.e. urea, total protein, albumin, cholesterol, NEFA), insulin and IGF-I concentrations during the transition and early lactation periods of primiparous Hereford cows with different BCS at calving, grazing native pastures, and to associate them with calf weight and subsequent milk production.

3.3. Materials and methods

3.3.1. Experimental site, animals and treatments

All animal procedures were approved by the Animal Experimentation Committee (CHEA) of Universidad de la República. The observational study was conducted at

the Experimental Station San Antonio of the School of Agronomy (Salto, Uruguay; 31°24'S, 54°19'W). Fifty-six primiparous Hereford ($LW = 440 \pm 15$ kg, $BCS = 3.9 \pm 0.3$) cows and their calves were used from -30 to $+45 \pm 5$ days postpartum (DPP), corresponding to the period between the end of winter and spring. This experiment was part of an experiment conducted across three consecutive years, and was designed to evaluate effects of type of suckling management and whole-rice middling supplementation on PPI and pregnancy in primiparous cows (Soca *et al.*, 2013).

All cows had normal calving and BCS at the start of the experiment was 4.3 ± 0.4 (Vizcarra *et al.*, 1986). Cows were classified in moderate (≥ 4 ; $n = 27$) and low (≤ 3.5 ; $n = 29$) BCS groups, according to their BCS at calving. The BCS at calving was not due to dietary treatments because all cows were managed together in the same pasture.

Cows and calves grazed native pastures on Basaltic soils (Berretta *et al.*, 2000). The most frequent grassland species found were *Paspalum notatum* (19%), *Axonopus affinis* (11%), *Bothriochloa laguroides* (6%), *Paspalum dilatatum* (6%), *Coelorachis selloana* (6%), *Stipa setigera* (6%), *Piptochaetium montevidensis* (5%) and *Desmodium incanum* (4%).

3.3.2. Data and sample collection

Forage nutritional quantity was determined by the double sampling method (Haydock and Shaw 1975) and forage height was recorded with a rule at the point of greatest forage density (Soca *et al.*, 2007). Herbage samples were dried in a forced-air oven at 60°C, and ground to pass a 1-mm mesh on a Wiley mill. Samples were analyzed in the Laboratory of Animal Nutrition (School of Agronomy, Montevideo, Uruguay) for DM, crude protein (CP), neutral detergent fiber (NDF) and ash (AOAC 2000). Monthly and seasonal records of temperature, precipitation and number of frozen days were collected. During the experiment, the rainfall was lower than, and the number of frozen days exceeded the historical averages (i.e. 40 vs 120 mm and 49 vs 23 days, respectively). This could explain the low forage quantity and height during the experimental period (600 ± 200 kg DM/ha and 3 ± 1 cm) (Soca *et al.*,

2013); although the level of CP (90 ± 2 kg of CP) and NDF (480 ± 30 kg NDF/kg DM) were similar to those of a national report (Berretta *et al.*, 2000).

Body condition score was determined in all animals from -35 to $+45$ DPP every 15 days, by a one trained observer. Date of calving, calf sex and calf weight at calving and at $+45 \pm 5$ DPP were recorded. Blood samples were collected weekly from -30 DPP to calving and twice a week from calving to $+45$ DPP, from the jugular vein into heparinised tubes, and refrigerated at 5°C until centrifuged (2500g, 15 min, within 2 h post-sampling). Plasma aliquots were stored at -20°C until analyses.

Based on BCS at calving and the calving date, six cows from both BCS groups (i.e. moderate and low) were selected for recording milk production at $+45$ DPP by the method of the weight–suckle–weight, with a cow–calf separation of 12 h (Williams *et al.*, 1979).

3.3.3. Metabolite and hormone analyses

Metabolites assayed included NEFA, cholesterol, total protein, albumin and urea. All samples were analyzed in one assay at the Laboratory ‘Dr Miguel C. Rubino’ (DILAVE, Montevideo, Uruguay). Blood biochemistry was analyzed according to the following colorimetric methods: cholesterol – CHOD–PAP; total protein – biuret reaction; albumin – bromocresol green; and urea – urease UV. For these determinations, commercial kits from Wiener Laboratory (Rosario, Argentina) were used and calibrated with control calibrated serum (Wiener Laboratory) on a Vitalab Selectra 2 autoanalyser (Vital Scientific, Dieren, The Netherlands). For quality controls, Lyotrol N (Reference 62373), P (Reference 62373) and internal controls of the Laboratory ‘Dr Miguel C. Rubino’ were used. Concentrations of NEFA were determined by the ACS–ACOD method (Kit NEFA-C, Wako Chemicals, USA). Intra-assay coefficients of variation (CV) were 6.8%, 4.8%, 3.8%, 4.0% and 11% for protein, albumin, urea, cholesterol and NEFA, respectively.

Concentrations of insulin and IGF-I were determined in the Laboratory of Nuclear Techniques (School of Veterinary Sciences, Montevideo, Uruguay) in a single assay. Insulin was determined using an immunoradiometric assay (IRMA) with a commercial kit (Diasource, Brussels, Belgium). The sensitivity of the assay was 2.3

μIU/mL. Intra-assay CVs for Control 1 (19 μIU/mL) and Control 2 (61.6 μIU/mL) were 7.4% and 9.9%, respectively. Concentrations of IGF-I were determined with a commercial kit (IGF1-RIACT Cis Bio International, GIF-SUR-YVETTE CEDEX, France), using an IRMA assay as described by Adrien et al. (2012). The sensitivity of the assay was 0.7 ng/mL and intra-assay CVs for Control 1 (74 ng/mL) and Control 2 (535 ng/mL) were 6.9% and 7.2%, respectively.

3.3.4. Statistical analyses

Cow BCS, metabolite and hormonal concentrations were analysed in a repeated-measures design using a model that included effects of BCS at calving, DPP and their interaction as fixed effects and cow as a random effect (MIXED procedure; SAS Institute Inc., Cary, NC, USA). Data were collected in a sequence of equally spaced points of time and the experimental unit was the subject (cows) (Littell *et al.* 1998). The covariance structure was autoregressive first order and the degrees of freedom were adjusted by the Kenward–Rogers method. The effect of BCS at calving, DPP and calf sex on milk production and calf weight was analysed by lineal model (GLM procedure, SAS Institute Inc.). Tukey–Kramer tests were conducted to analyse differences among groups (at $P = 0.05$). A trend was declared when $0.05 < P \leq 0.10$.

3.4. Results

3.4.1. Body condition score

Cow BCS was affected by BCS at calving, DPP, and their interaction (Table 1). Cow BCS decreased one unit from –30 to +30 DPP (4.30 ± 0.08 vs 3.30 ± 0.06 ; $P < 0.01$) (Fig. 1).

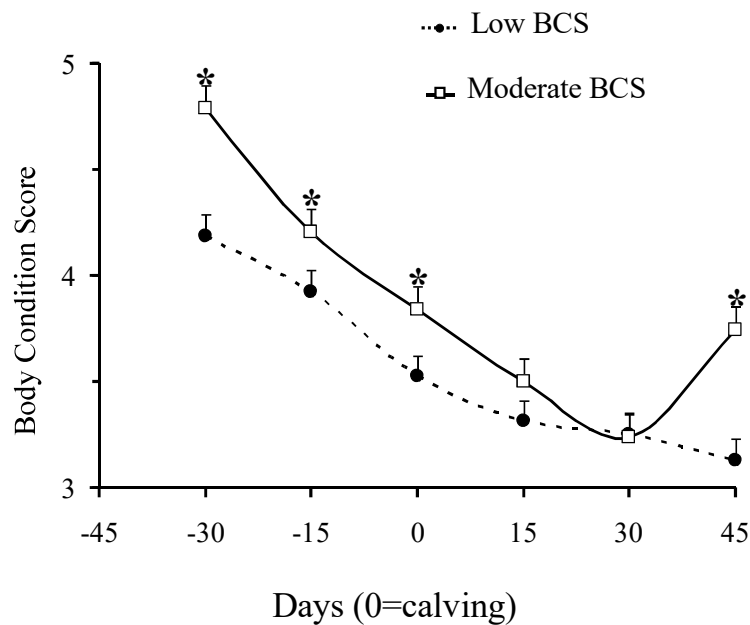


Fig. 1. Evolution of body condition score (BCS) in cows with moderate (≥ 4 , $n = 27$; $\square$) or low (≤ 3.5 , $n = 29$; $\bullet$) BCS at calving from 30 days before to 45 days after calving (least square means $\pm$ s.e. of estimation).

Better BCS was associated with a more pronounced loss of BCS from -30 DPP to nadir of BCS; moderate-BCS cows lost 1.6 units of BCS, while low-BCS cows lost 1 unit of BCS ($P < 0.01$). The moment and magnitude of BCS at nadir did not differ between the groups (Fig. 1); however, BCS increased only in moderate-BCS group at $+45$ DPP.

3.4.2. Metabolic and hormone profiles

Plasma NEFA concentrations were affected by DPP and the interaction between BCS at calving and DPP (Table 1).

Table 1. P-values for body condition score (BCS), metabolites and hormones in primiparous beef cows with moderate (≥ 4 ; $n = 27$) or low (≤ 3.5 , $n = 29$) BCS at calving from 30 days before to 45 days after calving under grazing conditions

Fixed effects were BCS, days relative to calving (days postpartum, DPP) and their interaction

Parameter	BCS at calving	DPP	BCS at calving $\times$ DPP
BCS	0.02	<0.01	0.05
Non-esterified fatty acids	0.15	<0.01	<0.01
Cholesterol	0.12	<0.01	0.22
Insulin	0.06	0.21	0.47
Insulin-like growth factor I	0.05	<0.01	<0.01
Total protein	0.45	<0.01	0.20
Albumin	0.93	<0.01	0.11
Urea	0.25	<0.01	0.01

Concentrations of NEFA were elevated during the prepartum, peaked between -15 DPP and calving (for low-BCS cows) or at $+15$ DPP (for moderate-BCS cows) and decreased thereafter in both groups (Fig. 2A).

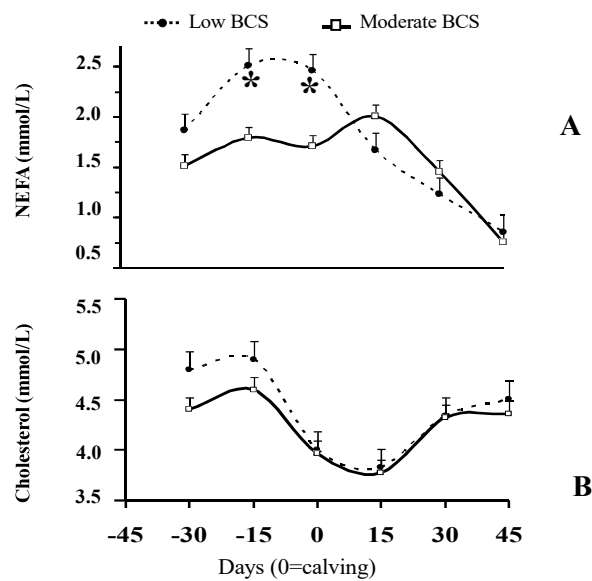


Fig. 2.Concentrations of (A) non-esterified fatty acids (NEFA) and (B) cholesterol in cows with moderate (≥ 4 ; $n = 27$; $\square$) or low (≤ 3.5 , $n = 29$; $\bullet$) body condition score (BCS) at calving from 30 days before to 45 days after calving (least-squares means + s.e. of estimation).

At -15 DPP and calving, NEFA concentrations were greater in low- than in moderate-BCS cows.

Cholesterol concentrations were affected by DPP (Table 1) as they decreased from -15 to +15 DPP and increased thereafter (Fig. 2B).

Insulin concentrations tended ($P = 0.06$) to be greater for moderate-BCS than for low-BCS cows (Fig. 3A).

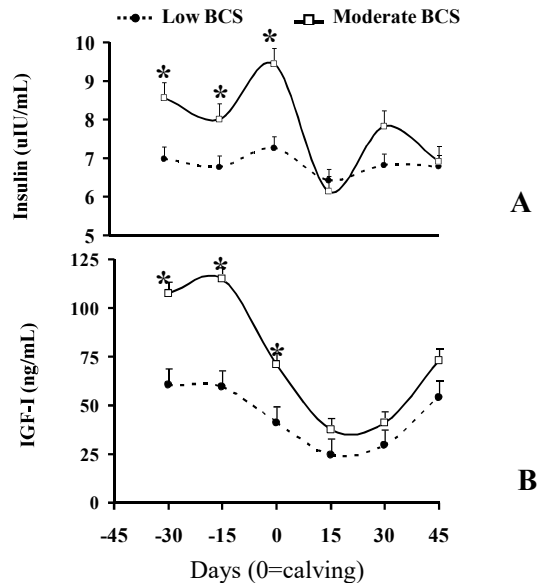


Fig. 3.Concentrations of (A) insulin and (B) insulin-like growth factor I (IGF-I) in cows with moderate (≥ 4 ; $n = 27$; $\square$) or low (≤ 3.5 , $n = 29$; $\bullet$) body condition score (BCS) at calving from 30 days before to 45 days after calving (least-squares means + s.e. of estimation).

Plasma IGF-I was affected by BCS at calving, DPP, and their interaction (Table 1). The difference in IGF-I between the BCS groups was evident only during the prepartum period; moderate-BCS cows had greater IGF-I concentrations than did low-BCS cows (Fig. 3B). Plasma IGF-I concentrations were high at –30 and –15 DPP, decreased until +15 DPP and increased thereafter. While at +45 DPP, low-BCS cows recovered the prepartum IGF-I concentrations during the postpartum period, concentrations did not reach prepartum concentrations in moderate-BCS cows (Fig. 3B).

Total protein, albumin and urea concentrations did not differ between the BCS groups but were affected by DPP, and an interaction between BCS at calving and DPP was found for urea concentrations (Table 1). Total protein increased from –30 to +30 DPP (Fig. 4A).

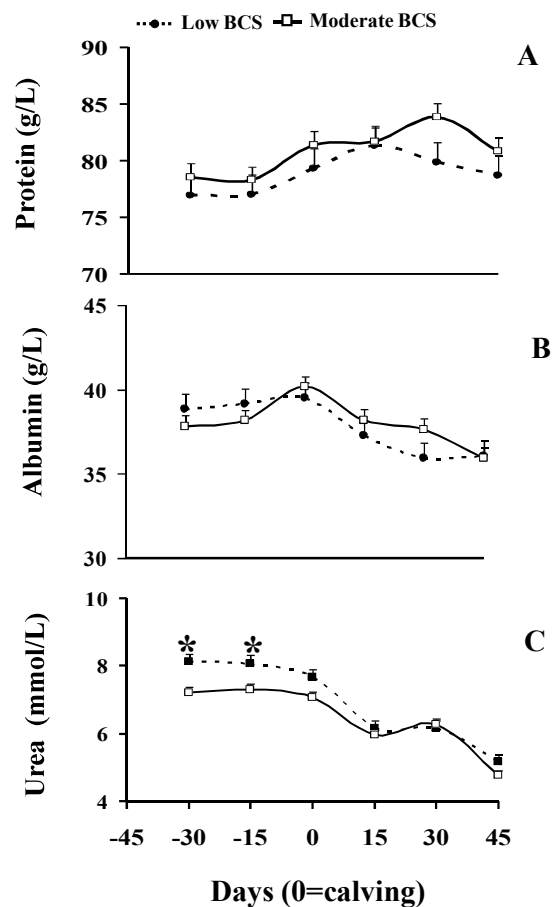


Fig. 4. Concentrations of (A) protein, (B) albumin and (C) urea in cows with moderate (≥ 4 ; $n = 27$; $\square$) or low (≤ 3.5 , $n = 29$; $\bullet$) body condition score (BCS) at calving from 30 days before to 45 days after calving (least-squares means + s.e. of estimation).

Plasma albumin was elevated from -30 DPP to calving and decreased thereafter until $+45$ DPP (Fig. 4B). Concentrations of urea were elevated from -30 DPP to calving and decreased thereafter, reaching the minimum concentration at $+45$ DPP. Moderate-BCS cows presented lower plasma urea than did low-BCS cows only during the precalving period (Fig. 4C).

Calf weight and milk production

The BCS at calving did not affect calf birth weight (35 vs 34 ± 2 kg in moderate- and low-BCS cows, respectively). Milk production and calf weight at $+45$ DPP were not affected by BCS at calving (4.6 vs 4.9 ± 1 L/cow, and 63 vs 61 ± 2 kg for moderate- and low-BCS cows, respectively).

3.5. Discussion

This is the first report characterizing the evolution of BCS, metabolite and metabolic hormones during the transition period of primiparous beef cattle grazing native grassland with different BCS at calving. A rather small difference in BCS at calving (4.1 vs 3.5) affected NEFA, urea, insulin and IGF-I profiles during the transition period, but did not affect calf weight and/or milk production.

The better BCS of moderate BCS cows 1 month before calving was not induced by nutritional treatments. This difference could be due to greater forage intake, reduced grazing cost and/or improved energetic efficiency (Freetly *et al.*, 2005; Blanc *et al.*, 2006; Scarlato *et al.* (2011). Greater postpartum loss of BCS in moderate-BCS cows was expected and has been reported previously (Lake *et al.*, 2006; Astessiano *et al.*, 2012; Soca *et al.*, 2013). Greater BCS was associated with increased bodyweight, weight of internal organs and energy requirements (Lake *et al.*, 2006), which could

explain the more pronounced BCS loss found in moderate-BCS cows. No recovery in BCS was observed in low-BCS cows, which may be associated with a differential nutrient partitioning. Indeed, a differential body composition and, thus, mobilisation during the transition period may be expected in low- vs moderate-BCS cows; low-BCS cows mobilised – in relative terms – more protein than did moderate-BCS cows (Cassady *et al.*, 2009). This protein ‘turnover’ – to maintain glucose homeostasis – is an expensive process and could explain why low-BCS cows did not recuperate BCS after calving (Chagas *et al.*, 2007; Cassady *et al.*, 2009; Waterman and Butler 2010). The more rapid increase in plasma NEFA and the higher concentrations at –15 DPP and at calving in the low-BCS cows may be the result of an earlier start of the NEB and suggest differences in nutrient partitioning (Chagas *et al.*, 2007). Indeed, NEFA profiles suggest that forage intake was not sufficient in the low-BCS cows to maintain gestation and growth requirements. Reduced cholesterol concentrations at the time of calving were found in both groups, which could be associated to reduced DM intake, as has been reported previously (Säköinen 2005; Remppis *et al.*, 2011). Albumin concentrations decreased after calving in both groups. Serum albumin is a very sensitive and early nutritional indicator of protein status because its turnover is only 16 days (Ndlovu *et al.*, 2007). Thus, the albumin decrease observed after calving may be associated with NEFA increase observed 15–30 days earlier, as this process is associated with utilisation of greater proportion of endogenous amino acids as precursors of gluconeogenesis. Higher urea concentrations were observed in low-BCS cows during prepartum, suggesting excessive mobilisation of muscle and/or inadequate ammonia utilisation in the rumen, as reported by Ndlovu *et al.*, (2007). These higher urea concentrations prepartum in the low-BCS cows are consistent with NEFA profiles and suggest greater protein catabolism in these cows (greater NEB).

The greater insulin and IGF-I concentrations at –30 and –15 DPP in moderate-BCS cows appear to provide objective indicators of the better energy status, as has been reported by Hess *et al.* (2005) and Roberts (2008), and can be the result of greater energy stores (BCS) and/or increased forage intake. Both groups had reduced IGF-I concentrations at the time of calving. A metabolic strategy developed in the ruminant

to cope with NEB is the uncoupling of the GH–IGF axis, which results in increased concentrations of GH and decreased concentrations of IGF-I (Lake *et al.* 2006). Decreased IGF-I concentrations reduce the suppressive effect on GH-releasing factor; consequently, GH concentrations increase and insulin resistance is elevated (Chagas *et al.*, 2007). NEB prepartum and at calving increased NEFA and probably GH (Bossis *et al.*, 1999), which could explain the IGF-I reduction in all BCS groups. The lower IGF-I in early lactation could be due to a reduced energy intake, and therefore blood glucose, and is a strategy to cope with increased energy and nutrient demands for milk production (Hess *et al.*, 2005; Waterman and Butler 2010) (e.g. homeorhetical effect). However, at +45 DPP, milk production and calf weight did not differ between the BCS groups. Indeed, BCS at calving in primiparous cows is not correlated with total milk production during lactation, whereas postpartum energy intake has greater relevance in determining milk production and calf weaning weight (Houghton *et al.*, 1990; Vizcarra *et al.*, 1998; Ciccioli *et al.*, 2003).

Increasing IGF-I concentrations postpartum may be associated with a change in nutrient partitioning, due to the change from a catabolic to an anabolic state (Hess *et al.*, 2005; Waterman and Butler 2010). This is associated with the recovery of BCS in moderate-BCS cows, suggesting that they rapidly reoriented metabolism (Chagas *et al.*, 2007; Waterman and Butler 2010). Metabolic responses to changes in energy nutrition might depend on recent metabolism (i.e. nutrition level, dynamic effect) or older (body reserves, static effect) history, a concept called ‘metabolic memory’ (Chilliard *et al.*, 1998; Blanc *et al.*, 2006). We speculate that IGF-I concentrations may integrate the metabolic memory (energy reserves) and the current energy status, while insulin and NEFA concentrations may be more of a reflection of the balance between daily DM intake and energy requirements. Moreover, the decrease in plasma IGF-I at calving could reflect pre- and postpartum energy transactions due to the reduction in DM intake, increased energy requirements, as well as nutrient partitioning between fetus and milk production, and body reserves, which, in turn, control the metabolic state at a different temporal scale.

The higher losses of BCS, the delay in NEFA peak, the pronounced reduction in IGF-I concentrations during prepartum and the earlier BCS recovery postpartum in

moderate-BCS cows suggest a better ability to modify the metabolic internal state in response to NEB. Globally, these changes could be associated with homeoeretical regulation for adaptation to NEB that includes increased glucose synthesis and decreased glucose uptake by dam's peripheral tissues (Robinson *et al.*, 1999; Blanc *et al.* 2006). This differential adaptation to NEB is probably due to differences in body composition (e.g. more fat in moderate cows; Cassady *et al.*, 2009), superior nutritional status (e.g. elevated IGF-I and insulin concentrations; Lalman *et al.*, 2000) and/or grazing behaviour (e.g. reduction in grazing time; Scarlato *et al.*, 2011). Although different metabolic status was observed in moderate- and low-BCS cows, there were no effects in milk production and calf weight.

3.6. Conclusions

The present paper is an observational study and, thus, the results should be considered with precaution; however, a rather small difference in BCS between 1 month before calving and at calving (0.6 points on a 1–8 scale) was associated with differences in metabolic status and temporal changes in BCS and NEFA, urea, insulin and IGF-I concentrations in primiparous grazing beef cows. However, calf birthweight and milk production at 45 DPP were not affected. In conclusion, the BCS at calving affected the metabolic and endocrine profile of primiparous beef cows grazing native grassland.

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4. ARTICULO III

Metabolic and endocrine profiles of primiparous beef cows grazing native grassland. 2. Effects of body condition score at calving, type of suckling restriction and flushing on plasmatic and productive parameters

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4.1. Summary

The objective of the present experiment was to analyse the effect body condition score (BCS) at calving, type of suckling restriction and flushing on metabolic and endocrine profiles, and productive and reproductive responses of primiparous beef cows grazing native grassland. Primiparous beef (n = 56) cows in anoestrus classified by BCS at calving (low ≤ 3.5 and moderate ≥ 4 ; 1–8 visual scale) were assigned randomly to four treatments in a two by two factorial arrangement of type of suckling restriction and flushing. Type of suckling restriction started at 55 ± 10 days postpartum (DPP $\pm$ s.e.m.; Day 0 = initiation of the treatment) and consisted of applying nose plates to calves for 12 days (i.e. TS treatment) or 5 days of isolation of the cow–calf pair, followed by applying nose plates to calves for 7 days as calves were reunited with their mothers (i.e. IS treatment). Immediately after the suckling restriction treatments were finished, the breeding season started, and each cow received (flushing group) or not (control group) 2 kg/day (fresh basis) of whole-rice middling for 22 days. The BCS was superior in moderate-BCS cows through the

experiment. The type of suckling restriction did not affect any plasma parameter, but insulin-like growth factor I (IGF-I) concentrations increased in all cows during suckling restriction. Cholesterol concentration was affected by flushing $\times$ day interaction ($P < 0.05$), while insulin and IGF-I concentrations were affected by the interaction among BCS at calving, flushing and days ($P < 0.03$). Flushing increased cholesterol concentration in both BCS groups at calving, while insulin and IGF-I concentrations increased during flushing only in moderate-BCS cows. Suckling restriction, flushing, and BCS at calving did not affect calf weight or milk production. Moderate BCS cows had a shorter postpartum anestrus interval (PPI) (98 vs 123 DPP; $P < 0.01$). Isolated type of suckling reduced postpartum anestrus interval when compared with TS treatment (97 vs 115 DPP, $P < 0.05$). Early pregnancy rate was greater in flushed than in control cows (0.8 vs 0.55, $P < 0.01$) and in moderate-BCS cows than in low-BCS cows (0.84 vs 0.46, $P < 0.01$). Total pregnancy rate was also greater in flushed and moderate cows and tended to be affected by the interaction between flushing and BCS at calving ($P = 0.06$; flushed cows: moderate = 1 vs low = 0.5, $P < 0.08$; and control cows: moderate = 0.8 vs low = 0.4, $P < 0.09$). These results confirmed the great value of suckling restriction and flushing during post-calving and relevance of BCS at calving as a link between energetic nutrition and metabolic and reproductive processes in primiparous beef cows grazing native grassland.

Additional keywords: body condition at calving, flushing, metabolic response, suckling.

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4.2. Introduction

In grazing cow–calf systems, weaning rate and calf weight at weaning determine the economics of the enterprise and can be partly explained by the postpartum anestrus interval (PPI; Hess *et al.*, 2005). Body condition score (BCS) at calving, as an indicator of maternal energy status, and suckling were the most important factors that controlled PPI, probability of pregnancy and weaning percentage of primiparous beef cows grazing native pastures (Quintans *et al.*, 2010; Soca *et al.*, 2013). The interaction between variability in forage production within and among years, and the

high and constant stocking rate, would explain the inability to achieve an optimum BCS at calving in primiparous beef cows (5 in 1–8 visual scale, Vizcarra *et al.*, 1986; Soca *et al.*, 2013). This suggests that use of tactical interventions based on suckling restriction and/or increased cow energy intake postpartum could redistribute nutrients and energy so as to stimulate initiation of postpartum estrous cycles and thereby improve pregnancy rates (Wettemann *et al.*, 2003; Hess *et al.*, 2005; Waterman and Butler 2010).

Suckling restriction with calf nose plates for 12 days reduced daily milk production, increased concentrations of glucose and insulin and follicle size and reduced PPI (Alvarez-Rodríguez *et al.*, 2010; Quintans *et al.*, 2010). Indeed, insulin and other hormones such as insulin-like growth factor I (IGF-I) are known to stimulate follicular growth (Spicer *et al.*, 2002). Moreover, the isolation of the cow–calf pair has been postulated as a powerful neuroendocrine signal to improve the frequency and amplitude of luteinizing hormone pulses and PPI (Stevenson *et al.*, 1997). However, it is not known whether the type of suckling restriction (i.e. isolated calf–cow pair vs restricted suckling) affects the metabolic hormone concentrations that could explain differential PPI responses (Soca *et al.*, 2013).

Supplementation with fatty acids (Lake *et al.*, 2006) or gluconeogenic specific precursors (Mullinicks *et al.*, 2008) for long periods improved reproductive efficiency and calf weight per cow exposed to breeding. Nevertheless, in grazing beef systems of South America, such medium- to long-term supplementation of beef cows is generally not economically viable (Soca *et al.*, 2007). In beef cows, the effect of short-time energy supplementation (flushing) has been evaluated in a few studies and increases in follicular size, lifespan of corpus luteum, progesterone concentrations and early pregnancy rates have been reported (Khireddine *et al.*, 1998; Soca *et al.*, 2013).

Body condition score at calving has been proposed as an indicator of metabolic memory (Blanc *et al.*, 2006), and has been associated with the length of PPI, conception and maintenance of pregnancy (Hess *et al.*, 2005; Soca *et al.*, 2013). Moreover, the effect of suckling control on IGF-I concentrations and PPI in primiparous beef cows was dependent on BCS at calving (Stagg *et al.*, 1998).

However, we could find no records on metabolite and metabolic hormone profiles associated with interventions based simultaneously on suckling restriction and flushing in primiparous beef cows grazing native pastures. We hypothesise that effects of the type of suckling restriction (i.e. isolated calf–cow pair vs restricted suckling) and flushing on PPI and pregnancy rates in primiparous cows are modulated by BCS at calving, and that the differential biological responses involve changes in metabolites and metabolic hormones.

The objective of the present experiment was to evaluate the type of suckling restriction and flushing with rice middling supplementation during 22 days at 55 ± 10 (days postpartum, DPP), on metabolite and endocrine profiles, and their relationships with PPI, probability of pregnancy rate, milk production and calf weight in primiparous cows of different BCS at calving, grazing native pastures.

4.3. Materials and methods

4.3.1. Location and pasture description

The experiment was carried out at the Experimental Station of the Faculty of Agronomy, Salto, Uruguay ($31^{\circ}23'S$, $57^{\circ}55'W$), during spring and summer in 2005–2006. Cows and calves grazed native pasture on basaltic soils (Berretta *et al.*, 2000). The most frequent pasture species found were *Paspalum notatum* (19%), *Axonopus affinis* (11%), *Bothriochloa laguroides* (6%), *Paspalum dilatatum* (6%), *Coelorachis selloana* (6%), *Stipa setigera* (6%), *Piptochaetium montevidensis* (5%) and *Desmodium incanum* (4%). Forage quantity was determined by the double-sampling method (Haydock and Shaw 1975) at calving, and during the breeding period. Forage height was determined as described previously (Soca *et al.*, 2007). In addition, representative pasture forage samples were collected and dried in a forced-air oven at $60^{\circ}C$, and ground to pass a 1-mm mesh Wiley mill. Forage samples were analysed in the Laboratory of Animal Nutrition (School of Agronomy, Montevideo, Uruguay) for DM, crude protein (CP), neutral detergent fibre (NDF) and ash (AOAC 2000). Forage quantity during late gestation, calving and breeding was 800 ± 130 , 300 ± 200 and 700 ± 400 kgDM/ha and 3 ± 1 , 2.0 ± 0.8 and 4.0 ± 0.7 cm, respectively. Chemical composition of forage was similar to that reported by Berretta *et al.* (2000).

4.3.2. Experimental design

All experimental procedures were approved by the Animal Experimentation Committee (CHEA) of the University of Uruguay (Montevideo). To evaluate effects of type of suckling restriction and flushing, primiparous Hereford cows ($n = 56$) with normal calving were used. Cows had 3.7 ± 0.4 units of BCS (visual scale 1–8, Vizcarra et al. 1986) and 365 ± 15 kg of bodyweight (BW) at calving. Cows were classified in moderate-BCS (≥ 4 ; $n = 27$) and low-BCS (≤ 3.5 ; $n = 29$) groups, according to their BCS at calving. The BCS at calving was not due to pre- or postpartum dietary treatments, because all cows were managed together in the same pasture throughout the experiment. Cows and their calves were randomly assigned, according to calving date, BCS at calving, and calf sex to one of the two groups (i.e. type of suckling restriction and flushing; Soca *et al.*, 2013) in a two by two factorial design. At the beginning of the treatment (Day 0 of the experiment = initiation of the suckling restriction at 55 ± 10 DPP), all cows were in anestrus as confirmed by blood progesterone concentrations.

The type of suckling restriction consisted of (1) temporary suckling control (TS) by applying nose plates to calves for 12 days without separation of the cow–calf pair, or (2) isolated temporary suckling control (IS) which consisted of complete isolation (>5000 -m distance) of the cow–calf pair for 5 days, followed by applying nose plates to calves for 7 days when the cow–calf pair was reunited. During the 5 days of complete separation in the IS treatment, calves did not have visual or auditory contact with their mothers and remained in corrals with hay, supplement based in corn and barley grain (89% DM, 18% CP and 12.54 MJ/kg DM of metabolisable energy) and water ad libitum. The TS treatments concluded at 67 ± 10 DPP, and cows were then assigned to nutritional treatments consisting of (1) flushing group in which cows were offered 2 kg/day (fresh basis) of whole rice bran (86.5% DM, 13.5% CP, 44% NDF and 13.5% ether extract) for 22 days (from Day 12 to Day 34 of the experiment), or (2) control group which received no supplementation. During flushing, both groups grazed different paddocks of the same pastures and the supplement was offered in feeders with individual separations of 100 cm. Individual intake was checked by observing each cow for the entire session of supplementation

(15 min). On the same day, the nutritional treatments were initiated; two bulls per group with proven fertility (McGowan *et al.*, 1995) were placed and rotated with the cows during 83 days.

4.3.3. Animal measurements

Cow BCS (visual scale 1–8, Vizcarra *et al.*, 1986) was determined every 20 days from Day 0 to Day 150 DPP by one trained observer. Changes in BCS between calving and the day of BCS nadir and between calving and the start of the breeding (Δ BCS) were calculated. Six cows of each treatment were selected for recording milk production on Days 0, 12, 22 and 50 by the weigh–suckle–weigh method, with a total separation of the cow–calf pair to 12 h (Williams *et al.* 1979). Pregnancy diagnoses were performed at 42 days (early pregnancy rate) after the introduction of the bulls and were confirmed at 30 days (total pregnancy rate) after bull withdrawal, using a linear array real time ultrasound scanner with 5 MHz rectal transducers (Aloka SSD-500, Hitachi Aloka Medical, Tokyo, Japan). Identification of a sharply demarcated black area with an ecogenic picture of an embryo within the uterus and/or observation of the heart beat were used as criteria for pregnancy (sensitivity of 94.8%, specificity of 95.3%, positive predictive value 97.7% and negative predictive value 89.8%; Taverne *et al.*, 1985).

Calves were weighed at birth, at 73 ± 10 days of age (Day 18 of experiment), and at definitive weaning at 188 ± 10 days of age (Day 123 of experiment) without fasting, and average daily gain (ADG) was calculated.

4.3.4. Blood sampling and metabolite and hormone determinations

Blood samples were collected weekly with vacutainer tubes with lithium heparin from 40 to 100 DPP, refrigerated at 5°C until centrifuged (2000g, 15 min) within 2 h post-sampling and plasma aliquots were stored at –20°C until analyses. All assays were performed in the Laboratory of Nuclear Techniques (Veterinary Faculty, Montevideo, Uruguay).

Progesterone (P4) concentrations were determined by a direct solid-phase radioimmunoassay (RIA) by using a commercial kit (Diagnostic Product Co., Los Angeles, CA, USA). The RIA had a sensitivity of 0.1 ng/mL. Intra-assay CVs for

low (0.8 ng/mL), medium (2.2 ng/mL) and high (8 ng/mL) controls were 6.6%, 6.4% and 6.3%, respectively. The inter-assay CVs for the same controls were 9.8%, 6.4% and 6.1%, respectively. The PPI was defined as the interval from calving to the day of the first of two consecutive weekly blood samples with progesterone ≥ 1 ng/mL.

Cortisol concentrations were determined by a direct solid-phase RIA using a commercial kit (Diagnostic Product Co.). The RIA had a sensitivity of 0.51 $\mu\text{g/dL}$. The intra-assay CVs for low (1.5 $\mu\text{g/dL}$), medium (5.5 $\mu\text{g/dL}$) and high (13.0 $\mu\text{g/dL}$) controls were 13.7%, 9.1% and 8.2%, respectively.

Cholesterol, insulin and IGF-I were determined as described in the companion paper (Soca *et al.*, 2014).

4.3.5. Statistical analyses

All data were analysed as a completely randomised design by using the SAS Systems program (SAS Institute, Cary, NC, USA). Metabolic, endocrine profiles, BCS evolution, milk production and calf weight were analysed by repeated measures using the MIXED procedure, with days as the repeated effect and first-order autoregressive as the covariance structure and the degrees of freedom were adjusted by the Kenward–Rogers method. The model included BCS at calving, type of suckling restriction, flushing, days, and all first-, second- and third-order interactions as fixed effects. For calf-weight analysis, sex was also included as a fixed effect and age as a covariable. Initial analyses included all possible interactions, but only significant ($P < 0.05$) components were retained in the model. Least square means were compared using the Tukey–Kramer test. Probability of early and total pregnancy rate (number of cows pregnant/total cows) was fitted using the GLIMMIX procedure with the binomial distribution and the logit as the link function specified. The effects of BCS at calving, type of suckling restriction and flushing as fixed effects on PPI were analysed by lineal model (GLM procedure, SAS Institute). Variables that better explained the PPI variations were studied using the stepwise REG procedure.

4.4. Results

4.4.1. Body condition score

Evolution of BCS was affected by BCS at calving ($P < 0.01$), days ($P < 0.01$) and the interaction between BCS and days ($P < 0.06$), but not by the type of suckling restriction, flushing or their interaction. The BCS of moderate-BCS cows was greater ($P < 0.01$) than that of low-BCS cows throughout the experimental period, with a maximum difference of BCS between groups observed at Day 150 (end of the experimental period; Fig. 1).

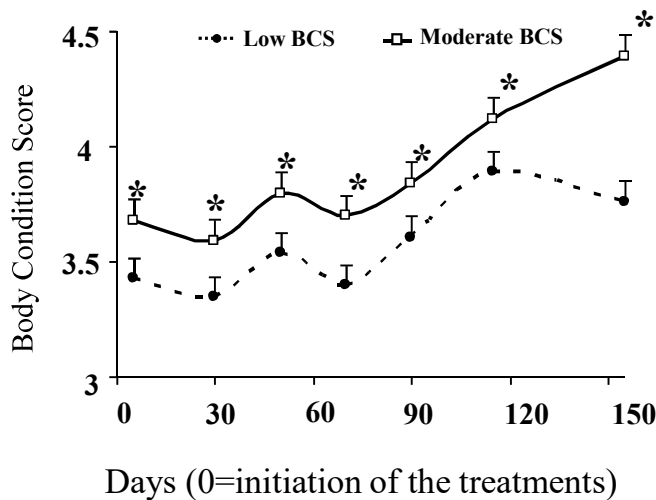


Fig. 1. Evolution of body condition score (BCS) from calving before, during and after suckling-restriction and flushing treatment in cows calving with moderate (□ 4; $n = 27$) or low (● 3.5 $n = 29$) BCS (least square means $\pm$ s.e. of estimation).

4.4.2. Plasma cholesterol and hormone determination

The effects of BCS at calving, type of suckling restriction, flushing and their interaction on the metabolic and endocrine responses are shown in Table 1.

Table 1. F-tests of fixed effects included in the model for metabolites and hormones in primiparous beef cows under grazing conditions.

Parameter	F	S	Days	BCS	BCS × F × days	F × days	BCS × F × days
Cortisol	0.5	0.36	<0.01	0.76	0.48	0.38	0.25

Cholesterol	0.27	0.92	<0.01	0.07	0.94	<0.01	0.93
Insulin	0.28	0.71	<0.01	<0.01	0.03	0.12	0.04
IGF-I	0.07	0.96	<0.01	<0.01	<0.01	0.62	<0.01

Fixed effects were body condition score (BCS) at calving, suckling restriction (S), flushing (F), days and interactions. IGF-I, insulin-like growth factor I

All variables were affected by days and/or the interaction between BCS at calving and days. The type of suckling restriction or its interaction with days did not affect any variable, while the interaction between flushing and days affected cholesterol concentrations and the triple interaction including BCS at calving, flushing and days affected insulin and IGF-I concentrations.

In both BCS groups, cholesterol concentrations increased during flushing (Day 30, 85 DPP) and decreased immediately after ($P < 0.01$) (Fig. 2A, B).

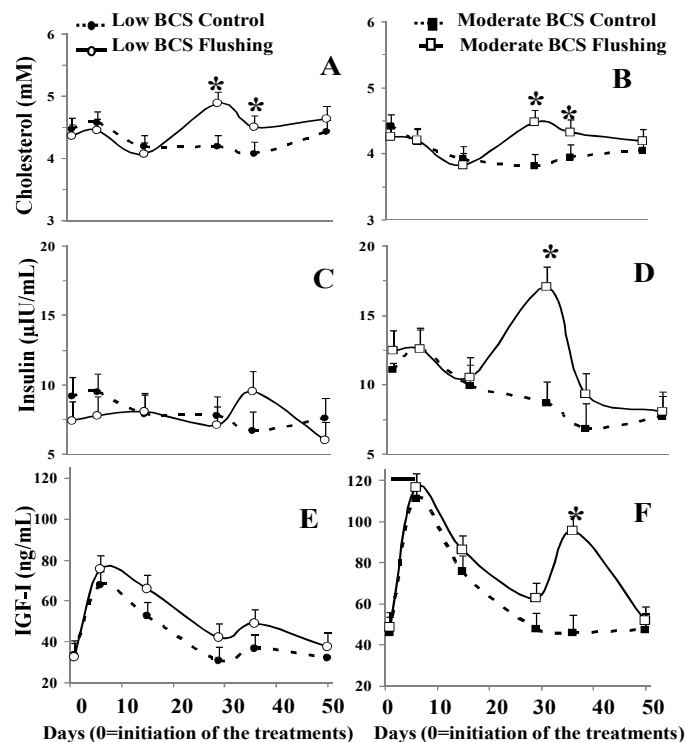


Fig. 2. (A, B) Plasma cholesterol, (C, D) insulin and (E, F) insulin-like growth factor I concentrations before, during and after suckling-restriction and flushing

treatment in cows with moderate (≥ 4 ; $n = 27$, A, C, E) or low (≤ 3.5 , $n = 29$, B, D, F) body condition score at calving (least square means $\pm$ s.e. of estimation).

No difference in cholesterol concentrations between control and flushed groups could be observed 2 weeks after the flushing treatment finished (Day 48).

Insulin concentrations were greater in moderate- than in low-BCS cows (10.6 ± 0.8 vs 7.5 ± 0.8 μ IU/mL, respectively). During flushing, insulin concentrations increased in moderate- but not than in low-BCS cows (Fig. 2C, D). Insulin concentrations decreased immediately after the flushing finished.

Concentrations of IGF-I were greater in moderate- than in low-BCS cows (70 ± 4 vs 46 ± 3 ng/mL, respectively). Plasma IGF-I increased during the suckling restriction, decreasing again after suckling restriction treatments ended (Fig. 2E, F). A 2–3-fold increase in IGF-I concentrations was observed immediately after the flushing treatment ended in moderate-, but not in low-BCS flushed cows (Fig. 2E, F). This IGF-I increase was observed later than the insulin increase in moderate-BCS flushed cows.

Cortisol concentrations decreased from Day 0 to Day 15 (from 2.2 ± 0.13 to 1.5 ± 0.12 μ g/dL) and remained low thereafter until Day 50 (data not shown). No differences among groups were detected.

4.4.3. Calf weight and milk production

Cow BCS at calving, type of suckling restriction, flushing and their interactions did not affect calf weaning weight, average daily gain or cow milk production (Table 2).

Table 2. Effect of suckling restriction (S), flushing (F) and body condition score (BCS) at calving on productive and reproductive responses in primiparous beef cows (estimated values)

Paramete	Effects									P-value		
	S			F			BCS			S	F	BC
	IS	TS	s.e	F	NF	s.e	L	M	s.e			
Productive response												
Milk	4.0	3.8	0.4	3.8	3.7	0.3	3.7	4.0	0.3	0.4	0.3	0.2

Daily	0.5	0.6	0.0	0.4	0.5	0.0	0.5	0.6	0.0	0.3	0.2	0.1
Calf	156	158	4.0	153	161	5.0	151	158	8.0	0.4	0.5	0.1
Reproductive response												
PPI	97	115	5.0	106	106	4.0	94	119	3.0	0.0	0.8	0.0
EP	0.7	0.6	0.0	0.8	0.6	0.0	0.5	0.8	0.0	0.7	0.0	0.0
TP	0.9	0.9	0.0	1.0	0.6	0.0	0.5	1.0	0.0	0.7	0.0	0.0

S, suckling restriction; IS, isolated calf–cow pair for 5 days and suckling control with nose plates for 7 days; TS, temporary suckling control with calf nose plates during 12 days while calves remained with their mothers; F, flushing; NF, control; L, low body condition at calving (≤ 3.5); M, moderate (≥ 4); EP, probability of early pregnancy; TP, probability of total pregnancy; PPI, postpartum anestrus interval. Interactions between main fixed effects (ST = suckling treatment; F = flushing treatment) were not significant ($P > 0.34$)

Milk production was affected by days ($P < 0.01$) as decreased by 33% during suckling restriction (from Day 0 to Day 12, 4.9 ± 0.2 to 3.3 ± 0.2 L/cow.day) and increased again at Days 22 and 50 (4.0 ± 0.1 and 4.9 ± 0.3 L/cow.day, respectively).

4.4.4. Reproductive responses

At the end of the suckling-restriction treatments (Day 12, 67 DPP) and 2 weeks after the flushing (Day 50, 105 DPP), 13% and 43% of total cows were cycling, respectively. Cow BCS at calving, Δ BCS, the interaction among type of suckling restriction and BCS at calving and IGF-I concentration at the initiation of the experiment (Day 0) explained 75% of the variation in PPI. Moderate-BCS cows had reduced PPI when compared with low-BCS cows (Table 2). For every unit of improvement in BCS at calving and Δ BCS, PPI was reduced ($P < 0.01$) by 46 ± 9 and 23 ± 5 days, respectively. The IS reduced PPI when compared with TS (Table 2). Concentrations of IGF-I at Day 0 were greater in cycling than non-cycling cows at Day 50 (45 vs 33 ± 2.5 ng/mL, respectively, $P < 0.01$).

The BCS at calving and flushing affected early and total pregnancy rates but the type of suckling restriction and its interaction with flushing did not affect them (Table 2).

Early and total pregnancy rates were greater in moderate- than low-BCS cows. Early pregnancy rate tended to be greater while total pregnancy rate was greater in flushed than in control cows.

4.5. Discussion

The present study has shown that the interventions used (suckling restriction and flushing) were novel, short-duration and low-cost signals to improve the metabolic status of primiparous anestrous beef cows with suboptimal BCS (<5, scale 1–8, Soca *et al.*, 2013). Suckling restriction, independently of its type (TS or IS) or BCS at calving, reduced milk production and increased IGF-I concentrations. However, BCS at calving modulated plasma insulin and IGF-I increases during or after flushing. Observed changes in the endocrine milieu were consistent with known effects of insulin and IGF-I on ovarian function (Spicer *et al.*, 2002), thereby contributing to the reduction in PPI. Neither suckling restriction nor flushing modified BCS, since it was maintained from Day 0 to Day 90 of the experiment, which could reflect a steady-state in cow energy balance. This could be explained by the difficulty of primiparous beef cows grazing swards with low forage height to increase forage intake and fulfill their requirements. The increase in cow BCS from Day 90 to Day 150 could reflect the improvement in pasture quantity as discussed previously (Soca *et al.*, 2013). The greater BCS in moderate BCS cows was consistent with their greater insulin and IGF-I concentrations throughout the experiment, reflecting their better metabolic status. The upregulation of hormone levels could have been in response to increased body reserves and/or increased DM intake (Blanc *et al.*, 2006; Waterman and Butler 2010; Scarlatto *et al.*, 2011). Moreover, moderate-BCS cows of the present experiment presented greater insulin and IGF-I and lower non-esterified fatty acid concentrations before calving (Soca *et al.*, 2014).

Both types of suckling restriction limited calf suckling and contributed to explain the reduction in milk production, as reported previously (Alvarez-Rodríguez *et al.*, 2010; Quintans *et al.*, 2010). The reduction in milk production was associated with an immediate increase (at least two-fold) in IGF-I concentrations. Indeed, lactation is often accompanied with low IGF-I concentrations (consequence of GH–IGF axis uncoupling, Rhoads *et al.*, 2004), which has been reported as a strategy for nutrient

prioritisation for the mammary gland. Thus, IGF-I increase during suckling restriction is consistent with the reduction in energy requirements and similar findings were reported within 5 days of suckling restriction, with or without calf isolation (Stagg *et al.*, 1998). However, suckling restriction did not affect insulin concentrations in contrast to previous experiments in multiparous cows (Quintans *et al.*, 2010). This could be due to the important restriction in forage quantity (energy intake) in the present study and/or limited ability of the primiparous cow to increase intake (Blanc *et al.*, 2006). This increment in IGF-I without changes in insulin is inconsistent with previous reports in dairy cows (Rhoads *et al.*, 2004), implicating the importance of other mechanisms controlling IGF-I secretion as discussed previously (Waterman and Butler 2010).

The flushing with rice middling increased cholesterol concentrations in both BCS groups, probably due to fat percentage and/or fatty acid composition of this supplement, as reported previously (Williams and Stanko 2000). However, the flushing effect on insulin and IGF-I profiles was dependent on BCS at calving, since no increases were observed in low-BCS cows. The increases found in moderate-BCS flushed cows were similar to the rapid increase in insulin concentrations observed after glucose infusion in beef cows with better BCS (Vieira *et al.*, 2012). Moreover, greater glucose and leptin concentrations and a differential hepatic gene expression of enzymes that regulate energy metabolism in cows with greater BCS have also been postulated to explain, at least partially, the better metabolic status (Vizcarra *et al.*, 1998; León *et al.*, 2004; Astessiano *et al.*, 2012). The differences in the patterns of metabolic response to the flushing treatment in moderate- vs low-BCS cows may reflect the influence of the metabolic memory. For example, it has been shown in sheep that insulin and IGF-I responses to the same nutritional treatment depended on body reserves (Fernández-Foren *et al.*, 2011). This suggests that the pancreas, liver and adipose tissue control harmonically the metabolic flux, as well as regulating the endocrine activity of each other (Yoshida *et al.*, 2007). In addition, greater cow body reserves could have improved feed (forage/supplement) utilisation efficiency and/or reduced grazing costs (by modifying cow grazing strategy) as suggested previously

(Dhuyvetter and Caton 1996), thus, explaining also plasma insulin and IGF-I increases found only in moderate cows.

Milk production and calf weight were not affected by flushing, in contrast with a previous report from our group when improved pastures were used as the flushing feed and no suckling restriction was applied (Astessiano *et al.*, 2012). Although no effect of tactical interventions (suckling restriction and flushing) and BCS at calving was observed on productive parameters, the reproductive indicators were affected by them.

The IS reduced the PPI in 20 days when compared with TS control, as previously reported (Soca *et al.*, 2013). Indeed, Stagg *et al.* (1998) reported that isolated suckling-restricted cows increased follicle size and luteinising hormone pulses when compared with non-isolated suckling-restriction treatment, which, in interaction with greater IGF-I concentrations, promotes ovulation. Even if IGF-I concentrations increased during the suckling-restriction period, neither IGF-I nor cortisol were affected by the type of suckling restriction; thus, other neuroendocrine mechanisms may be involved in the effect of type of suckling on PPI as proposed (Waterman and Butler 2010). The reduction in PPI and greater early and total pregnancy rates in moderate- than in low-BCS cows found is consistent with the concept that the brain integrates both information of body reserves (metabolic memory) and signals from the current energy intake to withstand future environmental challenges to the reproductive cycle (Blanc *et al.*, 2006). However, the BCS of the moderate-BCS cows was also lower than the optimum (Soca *et al.*, 2013), which would further emphasise the importance of suckling restriction and flushing to improve the metabolic status and reproductive performance.

Although the number of animals to evaluate pregnancy responses is limited, flushing improved early and total probability of pregnancy rates, as previously reported in a larger number of animals (Soca *et al.*, 2013). We did not find any report of short-term flushing on pregnancy rates, but long-term supplementation with gluconeogenic precursors and fat improved pregnancy rates (Hess *et al.*, 2005). Nutritional strategies that increase P4 concentrations in cattle before or after breeding have been positively associated with pregnancy rates, given that P4 is required for proper

establishment and maintenance of pregnancy (Khireddine *et al.*, 1998; Cooke *et al.*, 2012). This is consistent with the greater concentrations of the P4 precursor – cholesterol – found in flushed cows, regardless of their BCS at calving. However, 100% vs 50% of moderate- and low-BCS cows, respectively, were pregnant at the end of the breeding season, which cannot be explained by cholesterol concentrations. The insulin and IGF-I increases found only in moderate-BCS cows could explain this better fertility in flushed cows (Soca *et al.* 2013), as has been previously reported (Sinclair *et al.*, 2002). Indeed, insulin and IGF-I are involved in embryo growth and implantation (Cooke *et al.*, 2012).

4.6. Conclusions

Our data suggest that in primiparous beef heifers grazing native pastures, body energy reserves significantly modulate reproductive responses to the effects of suckling restriction and flushing on nutrient availability and metabolic/endocrine status.

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5. DISCUSIÓN

Este es el primer estudio que incorpora herramientas tácticas secuenciales (destete temporario y flushing) para evaluar el estado metabólico, la performance productiva y reproductiva de vacas de carne primíparas en pastoreo de campo nativo. Las reservas corporales (BCS al parto) de las vacas fue el elemento que afectó en mayor medida la endocrinología metabólica y las respuestas reproductivas a los tratamientos efectuados.

Las diferencias relativamente pequeñas (0.5 puntos en una escala 1-8, Vizcarra *et al.*, 1986) se asociaron con diferencias de 30% a 50% en probabilidad de preñez temprana y tardía, respectivamente (Artículos I y III). Las distintas BCS al parto no fueron inducidas nutricionalmente y podrían deberse a diferencias individuales en la eficiencia de uso de la energía, en el consumo de forraje y/o en los costos de pastoreo (Freetly *et al.*, 2005; Blanc *et al.*, 2006). En los 3 años consecutivos en los que se realizó este trabajo de tesis, la BCS al parto fue consistente con las condiciones climáticas y atributos de la pastura (Artículo I). En nuestro país, se ha reportado que la BCS al parto es el resultado de la BCS durante el otoño y la cantidad de forraje asignado durante otoño-invierno (Trujillo *et al.*, 1996). De manera similar, la cantidad de forraje afectó los cambios de BCS desde el parto al inicio del entore (Δ BCS) (Artículo I). Tanto los efectos estáticos (BCS al parto) como los dinámicos (Δ BCS) de la nutrición afectaron el IPR, la probabilidad de preñez temprana y global (Artículo I). El efecto de la BCS al parto reflejaría cambios en la nutrición energética de corto plazo y/o en la interacción genética-ambiental en el ambiente materno durante la concepción-gestación y la recría de ese grupo de animales (Funston y Summer, 2013).

Colectivamente, las vacas primíparas bajo pastoreo de campo nativo ajustaron sus respuestas metabólicas y reproductivas en base a la nutrición energética pre y postparto. El grupo de baja BCS al parto (≤ 3.5) perdió menos BCS durante el preparto respecto el grupo de moderada BCS al parto (≥ 4), y no recuperó la BCS en el postparto (Figura 3, Artículos II y III). Estos hallazgos conjuntamente con una anticipada movilización grasa (aumento de NEFA, Artículo II) sugieren un peor BE.

Esto es consistente con las menores concentraciones de insulina e IGF-I y las mayores concentraciones de urea (Artículo II) que indicarían un mayor catabolismo proteico -en términos relativos -en estos animales, pudiendo esto explicar porque las vacas de baja BCS al parto no recuperaron BCS durante el postparto (Cassady *et al.*, 2009). El ambiente endócrino-metabólico y la evolución de la BCS acorde al grado de reservas corporales al parto reflejan una partición de nutrientes diferencial. Por otro lado, la disminución pronunciada en la concentración de IGF-I, la menor concentración preparto de NEFA y la recuperación posparto de la BCS observadas en las vacas de BCS al parto moderada (Artículo II), sugieren una mayor plasticidad metabólica.

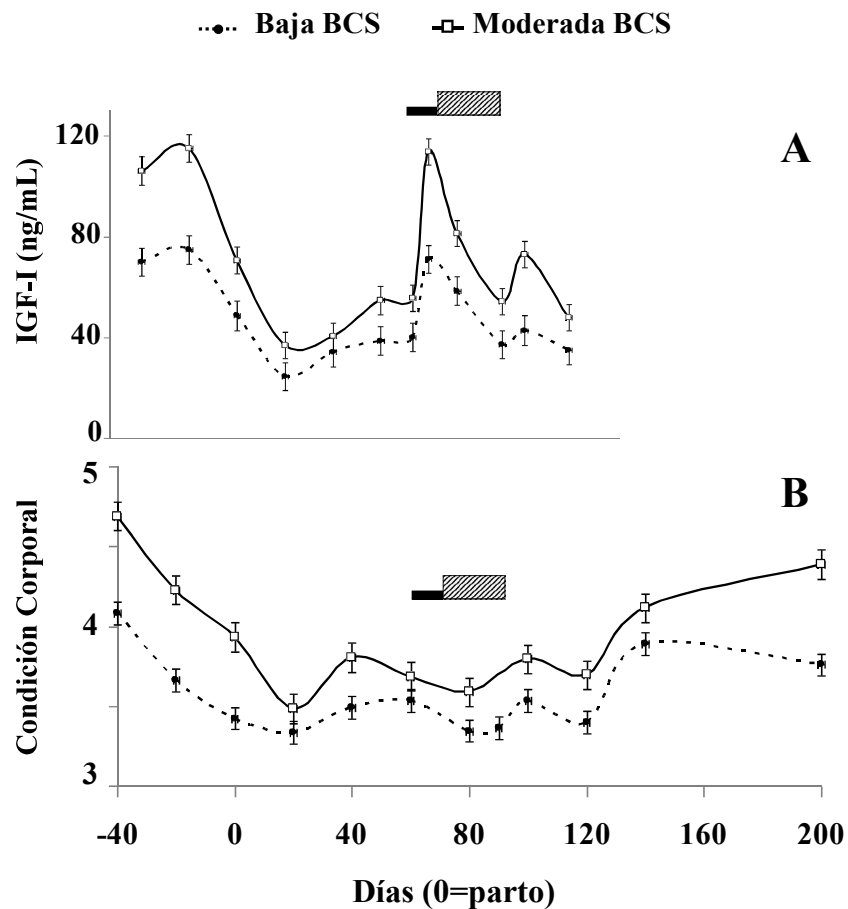


Figura 3. Evolución de la IGF-I (A) y condición corporal (B) en vacas de baja o moderada condición corporal al parto (promedio de mínimos

cuadrados $\pm$ error estándar). La intervención del destete temporario está identificada con una línea negra y el flushing con línea rayada.

Si bien estas diferencias no se observaron en el peso vivo del ternero y la producción de leche de la vaca (Artículos I y II), el grupo de moderada BCS al parto presentó mayor tamaño folicular al día 55 postparto (Moderada = 9 mm vs Baja = 7.5 mm, $P < 0.05$, datos no publicados), lo que es consistente con el mejor BE y mayores concentraciones de insulina e IGF-I (Artículo II). Una mejor BCS ha sido asociada previamente a diámetros foliculares mayores durante el posparto bovino con mayor secreción de estradiol y pulsatilidad de LH (Crowe, 2008). Estos eventos biológicos son los que preceden a una exitosa ovulación, por lo que el mayor desarrollo folicular encontrado en las vacas de BCS al parto moderada es consistente con el impacto de la BCS al parto sobre IPR.

El destete temporario constituyó una poderosa señal metabólica para todas las vacas, sin embargo las concentraciones ni de metabolitos, ni de hormonas fueron afectadas por el tipo de destete. La reducción en la producción de leche que lleva a una disminución en 30% los requerimientos de energía, podrían explicar el descenso en las concentraciones de BHB (Figura 4A, datos no publicados), y de urea (Figura 4B, datos no publicados). Esto está asociado con el aumento inmediato (al menos de dos veces) en las concentraciones de IGF-I (Figura 3A, Artículo III), posiblemente como consecuencia del reacoplamiento del eje somatotrófico (Rhoads *et al.*, 2004). Al finalizar el destete temporario y con el aumento concomitante en la producción de leche, las concentraciones de IGF-I se redujeron, lo cual ha sido reportado como una estrategia de priorización de la partición de nutrientes hacia la glándula mamaria (desacople del eje GH-IGF-I).

Resultados similares fueron reportados dentro de los 5 días de restricción al amamantamiento con o sin separación del par vaca-ternero (Stagg *et al.*, 1998). Sin embargo, el destete temporario, independientemente del tipo, no afectó las concentraciones de insulina (Artículo III) en contraste con los experimentos anteriores en vacas multíparas (Quintans *et al.*, 2010). Estas diferencias podrían

deberse a la importante restricción en la disponibilidad de forraje presente en este trabajo (Artículo III) y/o a una habilidad disminuida de las vacas primíparas para incrementar el consumo de forraje (Blanc *et al.*, 2006). Este incremento en IGF-I sin cambios en la insulina, no es consistente con los reportes en vacas lecheras (Rhoads *et al.*, 2004) y señalaría la importancia de otros mecanismos que controlan la secreción de IGF-I como se indicó anteriormente (Waterman y Butler, 2010).

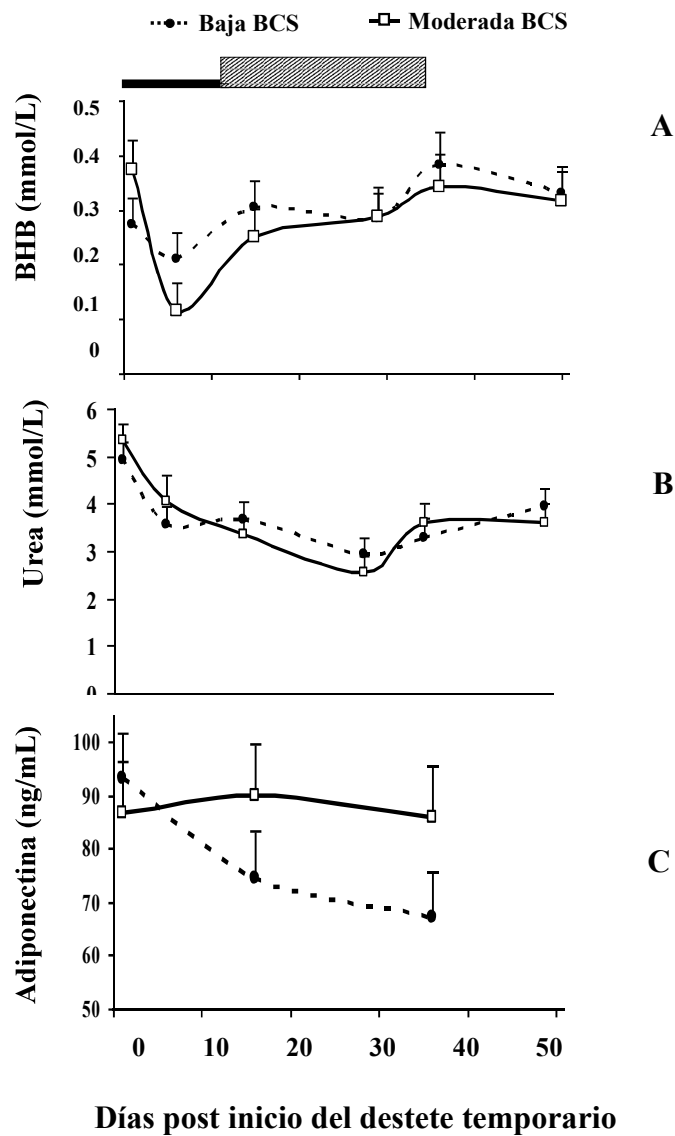


Figura 4. Concentraciones de betahidroxibutirato (BHB, A), urea (B) y adiponectina (C) en vacas en baja o moderada BCS al parto (promedio de mínimos cuadrados $\pm$ error estándar). La intervención del destete temporario está identificado con una línea negra y el flushing con línea rayada.

Si bien la primera evidencia de una proteína altamente expresada por los adipocitos denominada adiponectina es de hace 20 años atrás (Scherer *et al.*, 1995) y de la abundante literatura reciente sobre su rol en el metabolismo y la reproducción, no hemos encontrado reportes de las concentraciones circulantes de adiponectina en vacas de carne. Durante el período de intervención (destete temporario + flushing), las concentraciones de adiponectina no fueron afectadas por el tipo de destete temporario pero si por la BCS al parto, la concentración se mantuvo en las vacas BCS al parto moderada, mientras que disminuyó en las de BCS al parto baja (Figura 4C, datos no publicados). La adiponectina sensibiliza los tejidos periféricos a la insulina, aumentando el consumo de glucosa y la oxidación de ácidos grasos a nivel muscular (Ye *et al.*, 2013; Saremi *et al.*, 2014). La adiponectina también promueve la secreción de insulina estimulada por la glucosa. En nuestro experimento al reducirse los requerimientos de energía (debido al DT), las concentraciones reducidas de adiponectina son consistentes con las menores concentraciones de insulina e IGF-I del grupo de baja BCS al parto (Artículo III) y sugieren que la estrategia metabólica es limitar los procesos anabólicos (consumo de glucosa y ácidos grasos) por los tejidos periféricos y de esa manera contribuir a recuperar la masa básica corporal perdida. La integración hormonal de metabolismo de la vaca de cría acorde a las reservas disponibles (BCS al parto) confirma el modelo conceptual descrito por Blanc *et al.* (2006) en el cual en animales con muy baja disponibilidad estática (BCS) y dinámica (oferta de pastura) en la oferta de nutrientes se prioriza el restablecimiento de la masa básica corporal.

La reducción de 11 días observada en la duración de la IPR por el destete temporario con separación (Figura 5, Artículo I) podría explicarse por los efectos conocidos de aislamiento del par vaca-ternero sobre la frecuencia de pulsos de LH

(Stevenson *et al.*, 1997; Stagg *et al.*, 1998). En efecto, se ha documentado que la presencia del propio ternero aumentó la duración del IPR, mientras que las vacas a las que se les retiró el ternero y/o amamantan terneros que no son propios presentaron mayor frecuencia de pulsos de LH y una reducción en el IPR (Silveira *et al.*, 1993). Si bien en el presente trabajo no se encontraron diferencias endócrino-metabólicas asociadas al tipo de destete, se han propuesto otros mecanismos neuroendocrinos (por ejemplo, el cortisol, la oxitocina) para explicar un comportamiento reproductivo diferencial (en términos de IPR) entre las vacas aisladas vs vacas que permanecen con sus crías, incluso si no son amamantadas (Silveira *et al.*, 1993; Hoffman *et al.*, 1996).

El flushing no modificó la BCS (Artículos I y III), probablemente debido a lo corto del período de intervención. El consumo de energía del suplemento resultó equivalente a un 40% (2 kg afrechillo de arroz, 6 Mcal de EM) de los requerimientos de mantenimiento (13 Mcal EM/día para vaca de 430 kg). Esto explica el aumento en las concentraciones de colesterol en todas las vacas y de insulina e IGF-I en el grupo de moderada BCS al parto (Artículo III). Estos resultados coinciden con reportes de nuestro grupo, donde el colesterol, urea, glucosa e insulina se incrementaron durante la suplementación con afrechillo de arroz mejorando el estado energético, a pesar de no modificar la BCS (Astessiano *et al.*, 2013). Esto indicaría que la ingestión de suplemento aumenta los precursores de propiónico y la tasa de síntesis de glucosa hepática (Reynolds *et al.*, 2003), lo cual promueve cambios en la homeostasis de glucosa (Karcher *et al.*, 2007). El incremento en la concentración de colesterol se explicaría por el porcentaje de grasa y/o composición de ácidos grasos del suplemento (Williams y Stanko, 2000). El aumento de las concentraciones de insulina e IGF-I en vacas con moderada BCS al parto resultó similar al rápido aumento en ambas hormonas reportado en vacas con buen BE sometidas a una infusión de glucosa (Vieira *et al.*, 2012). Las diferencias en los patrones de respuesta endócrina al flushing en moderada vs baja BCS al parto (Artículo III) reflejarían la influencia de la memoria metabólica, lo cual, coincide con lo reportado para ovinos donde los cambios en las concentraciones de insulina e IGF-I en ovejas enfrentadas

al mismo tratamiento nutricional dependió del grado de reservas corporales (Fernández -Foren *et al.*, 2011).

El aumento en las concentraciones de insulina e IGF-I contribuirían a explicar el efecto del flushing en la mejora en la probabilidad de preñez temprana. Dicho efecto se manifestó en las vacas con inferior IPR y que ovularon temprano como consecuencia del mejor BE (Figura 5B). En este caso el incremento del colesterol - precursor de la progesterona (P4)- podría haber contribuido a mejorar la funcionalidad del cuerpo lúteo. Asimismo, los aumentos en las concentraciones de insulina e IGF-I en vacas de moderada BCS al parto (Artículo III) podrían reducir el clearance de P4, como fue demostrado por Vieira *et al.* (2012) en vacas con buen BE sometidas a una infusión de glucosa. Además concentraciones de IGF-I mayores han sido asociadas con mejores tasas de preñez (Kirby *et al.*, 1998) debido a que es un factor regulador del crecimiento embrionario (Wathes *et al.*, 2004).

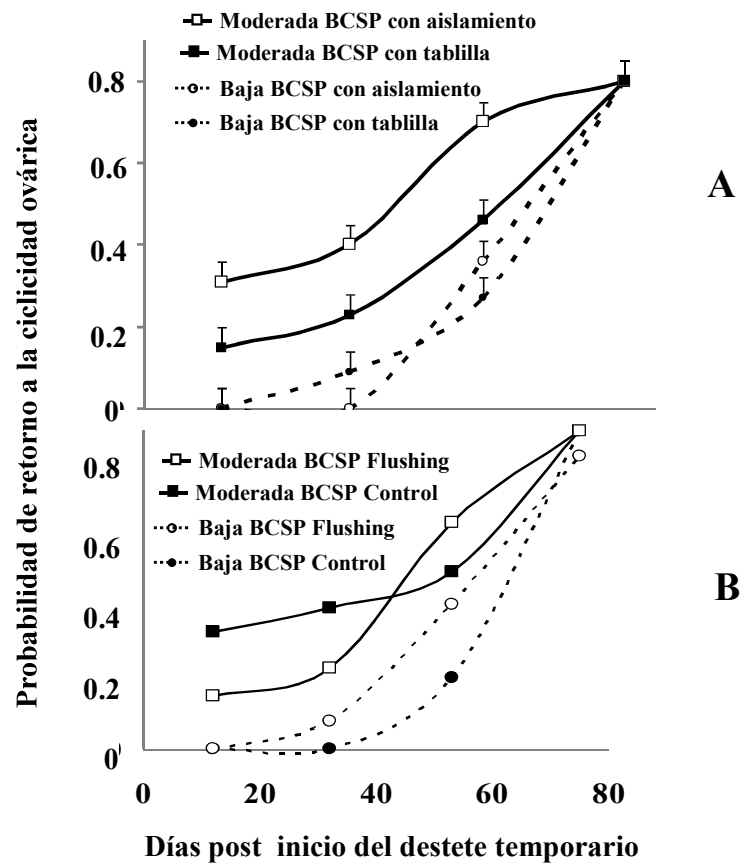


Figura 5. Efecto de la BCS al parto y del tipo de destete temporario (A) y del flushing (B) sobre la probabilidad de retorno a diversos intervalos post destete temporario.

La BCS al parto, la Δ BCS y la concentración de IGF-I a los +45 DPP fueron las variables que mejor explicaron el IPR ($r^2=0.76$, $p<0.0001$). El IPR promedio (102 ± 10 días) fue similar al reportado por Rowe *et al.*, 2008, donde para vacas primíparas con restricciones de BCS al parto fueron requeridas más de 10 ondas foliculares para manifestar la ovulación. En nuestro experimento por cada unidad de aumento en BCS al parto el IPR se redujo en 64 días ($P<0,001$; datos no publicados). Este hallazgo ha sido ampliamente documentado en la literatura de vacas de carne (Houghton *et al.*, 1990; Sinclair *et al.*, 2002; Ciccioli *et al.*, 2003; Hess *et al.*, 2005; Lents *et al.*, 2005). La asociación positiva entre la BCS al parto y las concentraciones de insulina e IGF-I (Artículo III) refleja mejoras del BE y contribuyen a explicar el

incremento en tamaño folicular de vacas de moderada BCS al parto. Estas mejoras podrían haberse asociado con el crecimiento folicular, incremento de los pulsos de LH y consecuente ovulación (Hess *et al.*, 2005). A nivel ovárico, la IGF-I controla positivamente la maduración folicular, la esteirodogénesis, la maduración del oocito y la ovulación, lo cual explicaría su relación con el IPR y confirma su importancia como link entre el BE y la ovulación (Webb *et al.*, 2004; García-García, 2012).

La BSC al parto fue la variable más importante que afectó la probabilidad de preñez (Artículos I y III). La diferencia de 30% en la probabilidad de preñez temprana a favor del grupo moderada BCS al parto podría explicarse por la mejor resiliencia metabólica pre y postparto (recuperan BCS antes en los DPP y en superior magnitud que las de baja BCS al parto), los mayores tamaños foliculares y las mayores concentraciones de hormonas metabólicas durante el destete y el flushing. Estas respuestas fueron favorecidas por el destete temporario en todas las vacas, ya que está ampliamente aceptado que el destete temporario es una herramienta eficaz de estímulo al reducir el IPR (Stagg *et al.*, 1998; Wettemann *et al.*, 2003). Esto fundamenta que en el presente trabajo no se haya planteado un grupo control (sin destete temporario) y que sin embargo, se investigara el efecto del aislamiento del par vaca-ternero. El incremento de la IGF-I durante el destete temporario y el posterior incremento luego del flushing están asociados con el IPR en vacas de BCS al parto moderada y confirma la estrategia propuesta de herramientas tácticas secuenciales (primero control del amamantamiento y luego suministro de energía a corto plazo).

Es posible postular que la relación entre la memoria metabólica (BCS al parto) e IGF-I implicó para estos animales un concepto similar al de “fitness” o adecuación biológica para dejar descendencia empleado por los animales silvestres (Tollefson *et al.*, 2010). Postulamos que cuando las vacas de moderada BCS al parto son sometidas a intervención con destete temporario y flushing se facilitan señales que le informan a la vaca primípara que la reproducción puede llevarse a cabo. No obstante, cuando las reservas caen por debajo de un umbral, como en este caso las

vacas de baja BCS al parto, la vaca prioriza el mantenimiento. Es decir, para este tipo de animal, las señales necesarias para que invierta energía en el proceso reproductivo serían de mayor costo económico (suplementación de largo plazo, destete precoz) y con una significación biológica de escaso valor.

5.1. Integración de estos resultados a la propuesta de investigación a largo plazo para mejorar la producción, consumo y eficiencia de uso del campo nativo por la cría vacuna en Uruguay.

En los trabajos realizados, la BCS al parto moduló la respuesta reproductiva, lo que confirmó, la importancia de cuantificar y controlar a largo plazo la relación entre cantidad de forraje y BE de vacas de cría. Para ello, en ecosistemas pastoriles como la cría vacuna del Uruguay, debe conocerse como se relacionan la producción, consumo y eficiencia de uso del campo nativo (o *Campos*). Dicho flujo de energía es principalmente controlado por la carga animal la cual se expresa a través de los cambios en la oferta de forraje (OF) (kg MS/kg PV). Para *Campos* las OF de 6 y 10 kg de MS/100 kg de PV/día maximizaron la producción por superficie y animal de vacunos en crecimiento (Piaggio, 1994; Maraschin *et al.*, 1997). No obstante, para el Uruguay, no disponíamos de experimentos de largo plazo que estudiaran el efecto de la OF sobre la productividad de la cría vacuna.

Por otra parte, el uso de vacas cruza (Hereford/Angus) puede mejorar la producción física en hasta 30% en su vida útil, sin incrementar los costos de producción (Morris *et al.*, 1987). No se disponía de información nacional que evaluara el efecto de la OF y el genotipo de la vaca sobre la productividad de la cría vacuna en Uruguay. Esto justificó el planteo de un Programa de Investigación orientado a mejorar la producción, consumo y eficiencia de uso del campo natural, en base a vincular la distribución espacial y temporal de los recursos forrajeros, el pastoreo animal, el uso de los nutrientes por las vacas y las respuestas productivas y ambientales que resultan de dichas interacciones. Desde el año 2007 (hasta el presente) se han instalado dos experimentos de pastoreo sobre suelos de Zapallar y Fraile Muerto en el Noreste [120 ha, EEBM], Cerro Largo] y Cuchilla de Haedo-Paso de los Toros e Itapebí-Tres Arboles, del Basalto [90 ha, EEFA, Salto

(EEFAS)] (URUGUAY. MGAP. CONEAT, 1979) de nuestro país. Los experimentos tienen un diseño experimental de bloques al azar con repetición en el espacio y dos tratamientos de oferta de forraje: alta (AOF) y baja (BOF) (10 vs 6 kgMS/100 kgPV/día en promedio, AOF vs BOF; respectivamente) variables a lo largo del año (12,5; 7,5; 10; 10 y 7,5; 7,5; 5 y 5 kgMS/100 kgPV para AOF y BOF en otoño, invierno, primavera y verano, respectivamente). Se utilizaron vacas adultas (múltiparas, n=60) de dos grupos genéticos (puras: Aberdeen Angus y Hereford y cruza: sus respectivas cruza F1, PU vs. CR) (EEBR) y Hereford primíparas (n=40 por año en promedio) que permanecieron en el experimento por un único ciclo productivo de gestación-lactación (EEFAS).

Un cambio de BOF a AOF promedio anual con modificación estacional, permitió incrementar la cantidad de forraje (50%) y en EEER la producción de forraje (30%) sin modificar la carga animal (Soca *et al.*, 2013). La AOF mejoró la respuesta productiva y reproductiva de las vacas de cría y la producción de carne por hectárea (30 y 50% respectivamente) (Do Carmo, 2013; Soca *et al.*, 2013; Carriquiry *et al.*, 2013). Esto se explicó por el mejor BE de las vacas en AOF, reflejado no solo en la BCS sino también en el perfil de hormonas metabólicas (insulina, IGF-I) y de expresión génica en hígado a lo largo del ciclo gestación-lactación (Carriquiry *et al.*, 2013). Esta mejora en el balance de energía de las vacas en AOF podría ser el resultado no sólo de un mayor consumo sino también de una reducción en los costos de pastoreo provocada por reducción de tiempo de pastoreo (Scarlato, 2012, Scarlato *et al.*, 2013) y modificaciones en el patrón de uso del espacio (Scarlato *et al.*, 2012). Los mejores indicadores de AOF podrían explicarse también por el empleo del destete temporario y flushing a todas las vacas, lo cual, confirma la sinergia productiva entre la intervención táctica y estratégica (Claramunt *et al.*, 2013).

Las vacas CR presentaron una superioridad productiva y reproductiva (heterosis) frente a las vacas puras (Angus y Hereford), siendo esta superioridad mayor en ambientes restrictivos (BOF) (Espasandín *et al.*, 2013). Estas respuestas diferenciales probablemente estén asociadas a diferencias en los mecanismos de partición de la energía (Carriquiry *et al.*, 2013), priorizando diferentes funciones ante

cambios ambientales y sugieren una adaptabilidad mayor de las vacas CR a ambientes restrictivos (ej. tamaño órganos) (Casal *et al.*, 2013).

Esta investigación permite postular que no obstante el control climático del patrón anual y estacional de la producción de forraje, el control de la OF (en base a AOF) y el empleo de vacas CR mejoraron la cantidad de energía que se transforma en producto animal, mejoró la producción de forraje, por animal y unidad de superficie de la ganadería de cría sobre campo natural. A su vez la implementación de estos cambios permitiría diseñar sistemas de producción con inferior vulnerabilidad al cambio climático. El modelo de cambio técnico propuesto en base a herramientas tácticas (destete temporario y flushing) y estratégicas (control de la OF) permite mejorar la producción por unidad de superficie, la sostenibilidad de los predios familiares y contribuye a mejorar la competitividad de la cadena cárnica nacional.

6. CONCLUSIONES

Este es el primer reporte que investiga herramientas tácticas secuenciales (tipo de destete temporario y flushing) sobre el estado metabólico, la performance productiva y reproductiva de vacas de carne primíparas en pastoreo de campo nativo. Los cambios en las concentraciones de NEFA, insulina e IGF-I durante la transición entre gestación tardía y postparto temprano y las respuestas endócrinas (IGF-I e insulina) al flushing dependieron de la BCS al parto. El tipo de destete y el flushing no afectaron la BCS de la vaca, la producción de leche o el peso vivo del ternero. El destete temporario con separación redujo en 11 días el intervalo parto-retorno a la ciclicidad ovárica y el flushing mejoró un 40% la probabilidad de preñez temprana. Ambos efectos fueron modulados por las reservas corporales (en términos de BCS al parto); diferencias de 0.5 puntos en BCS (escala 1 a 8) afectaron el ambiente endocrino metabólico y la eficiencia reproductiva.

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