Facultad de Veterinaria
Universidad de Zaragoza

MECANISMOS FISIOLÓGICOS A TRAVÉS DE LOS QUE LA CRIANZA DEL TERNERO ACTÚA SOBRE LA REPRODUCCIÓN EN LA VACA NODRIZA

TESIS DOCTORAL

Memoria presentada por **Javier Álvarez Rodríguez** para optar al grado de Doctor con mención Europea por la Universidad de Zaragoza

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Esta tesis doctoral se ha realizado en el marco del proyecto *"Optimización de la eficiencia reproductiva de los sistemas de explotación de vacas nodrizas en el marco de los objetivos de extensificación de la Comisión Europea"* (INIA RTA 2005-00231-00-00). Investigadora responsable: Albina Sanz.

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1) Álvarez-Rodríguez J., Palacio J., Casasús I., Revilla R., Sanz A. (2009). Performance and nursing behaviour of beef cows with different types of calf management. *Animal* 3 (6): 871-878.

2) Álvarez-Rodríguez J., Palacio J., Sanz A. (*aceptado*). Effects of nursing frequency and parity on the productive, metabolic and reproductive parameters of beef cows. *Livestock Science* (ref. LIVSCI-D-09-1895).

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5) Álvarez-Rodríguez J., Sanz A. (2009). Physiological and behavioural responses of cows from two beef breeds submitted to different suckling *strategies. Applied Animal Behaviour Science* 120: 39-48.

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CERTIFICACIÓN DEL DIRECTOR DE TESIS

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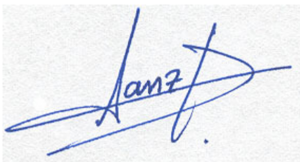
INFORMA

Que la memoria titulada ***"Mecanismos fisiológicos a través de los que la crianza del ternero actúa sobre la reproducción en la vaca nodriza"***, elaborada por D. **Javier ÁLVAREZ RODRÍGUEZ**, ha sido realizada bajo mi dirección, se ajusta al proyecto de tesis inicialmente presentado y cumple los requisitos exigidos por la legislación vigente para optar al grado de Doctor con mención europea por la Universidad de Zaragoza.

Zaragoza, a 19 de mayo de 2009.



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DIMORFIPA
DIPARTIMENTO DI MORFOFISIOLOGIA VETERINARIA
E PRODUZIONI ANIMALI

TO WHOM IT MAY CONCERN

Object: Evaluation of the PhD thesis presented by Javier Álvarez Rodríguez

Title:
'Mecanismos fisiológicos a través de los que la crianza del ternero actúa sobre la reproducción en la vaca nodriza'

This doctoral work aims at studying several physiological mechanisms by which the suckling calf may modulate the reproductive function in beef cows managed under different nursing systems in moderate nutritional conditions.

In general, the argument developed by the Candidate is of great importance in the field of animal reproduction, from both the points of view of basic and applied research.

The thesis starts with a background section that analyses the state of the art until the present; the data collected are always up to date, showing that the Author has conducted an accurate bibliographical research. The following section is devoted to describe briefly the synopsis of the experimental designs as well as results of the experiments.

The results and discussion of the thesis have been spread in 6 peer reviewed papers from scientific journals included in the 'Agriculture, Dairy and Animal Science' subject area. The experimental designs, animal measurements, blood assays and statistical analyses have been accurately conducted and described in all the manuscripts.

All the above mentioned works have been published on highly impacted, peer-reviewed journals, which guarantee the quality and the goodness of the different papers as a whole. As a consequence, my feeling is that I can only confirm the positive evaluation which resulted in paper publication.

As for other points, the organization of the manuscript is fine, the paper is well structured and easy to read; the single aims of the thesis are clear and well explained, the results obtained are interesting.

Overall, I believe that this thesis gives a very good contribution to the knowledge of the influence of the suckling calf on the productive and reproductive physiology of two beef cattle breeds widely spread in Spain.

On the basis of all the above observations, I strongly recommend Dr. Javier Alvarez Rodriguez thesis to be favourably evaluated by the commission to obtain the European mention degree.

I am willing to answer to any other question regarding this assessment.

May 25, 2009

Prof. Carlo Tamanini

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PARECER

TESE DE DOUTORAMENTO

"MECANISMOS FISIOLÓGICOS A TRAVÉS DE LOS QUE LA CRIANZA DEL TERNERO ACTÚA SOBRE LA REPRODUCCIÓN EN LA VACA NODRIZA"

DE Javier Álvarez Rodríguez

O presente trabalho possui objectivos claros e de grande interesse prático para a tomada de decisões por parte dos criadores de bovinos de aptidão cárnica. Ele centrou-se no ponto fulcral da criação deste tipo de animais, ou seja, como obter um produto de qualidade hoje, sem pôr em causa, simultaneamente, produções futuras. Avalia correcta e profundamente os efeitos da aplicação de diferentes sistemas de aleitamento sobre diversos parâmetros metabólicos, comportamentais e reprodutivos. Fá-lo ao longo de várias lactações. Não descura factores essenciais como: o genótipo, a idade, o número de lactações, o anestro pós-parto e as relações mãe-cria. Desta forma, alicerça correctamente o seu trabalho, enriquece-o e valoriza decisivamente as suas conclusões.

O volume de dados recolhidos e analisados neste trabalho é muito elevado. As metodologias de trabalho e de análise estatística são, sem dúvida alguma, as actualmente mais correctas, conferindo aos resultados alcançados um elevado nível de confiança. A discussão e as conclusões encontradas são consentâneas com as metodologias utilizadas. Não surpreende pois o elevado número de artigos já publicados, aceites e em avaliação para possível publicação em revistas científicas de referência mundial.

O empenho do candidato, assim como a quantidade e a qualidade do trabalho por ele desenvolvido, de que somos pessoalmente testemunhas, são dignas de uma referência muito especial.

Pelos motivos anteriormente referidos, recomenda-se que o trabalho intitulado "Mecanismos fisiológicos a través de los que la crianza del ternero actúa sobre la reproducción en la vaca Nodriza", apresentado pelo candidato **Javier Álvarez Rodríguez**, seja aceite para a obtenção do Grau Europeu de Doutor.

Bragança, 1 de Junho de 2009

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LISTA DE ABREVIATURAS

ADF	Acid-detergent fibre
ADG	Average daily gain
ADL	Acid-detergent lignin
ADLIB	Acceso libre del ternero a la madre
AGNE	Ácidos grasos no esterificados
AISL	Aislamiento visual, táctil y olfativo del ternero entre períodos de amamantamiento
app	Anestro post-parto
BCS	Body condition score
BW	Body-weight
CC	Condición corporal
CCp	Condición corporal al parto
CP	Crude protein
CV	Coeficient of variation
DM	Dry matter
ECM	Energy-corrected milk
EM	Energía metabolizable
EOP	Péptidos opioides endógenos
g	gramo
GH	Growth hormone
GHR	Growth-hormone receptor
GMD	Ganancia media diaria
GnRH	Gonadotropin-releasing hormone
h	Hora
IGF-I	Insulin-like growth factor-I
kg	kilogramo
l	litro
LH	Luteinizing hormone
LW	Live-weight
M	Mantenimiento
m	metro
ME	Metabolizable energy
mg	miligramo
min	minuto
MJ	Megajoule
ml	mililitro
mmol	Milimol
N	Nitrogen
NDF	Neutral-detergent fibre
NEFA	Non-esterified fatty acids
ng	Nanogramo
Nov	Novillas
ns/NS	not significant
P4	Progesterone
PA	Parda de Montaña
PI	Pirenaica
PLE	Producción de leche estándar
pp	Post-parto
PRL	Prolactina
PV	Peso vivo
PVp	Peso vivo al parto
RESTR	Acceso restringido del ternero a la madre a un período de amamantamiento al día de 30 minutos cada uno
RESTR1	Acceso restringido del ternero a la madre a un período de amamantamiento al día de 30 minutos cada uno
RESTR2	Acceso restringido del ternero a la madre a dos períodos de amamantamiento al día de 30 minutos cada uno
SD	Standard deviation
SE	Standard error
T3	Triyodotironina
TMR	Total mixed ration
vs.	Versus

Introducción general

1. Introducción general

El sector vacuno español es, dentro de las producciones ganaderas, el segundo en importancia económica, por detrás del sector porcino, representando aproximadamente el 19% de la producción final ganadera (MARM, 2009).

La estructura productiva presenta una serie de características específicas que permite diferenciar dos subsectores: el de las **vacas nodrizas** y el de cebo. España ocupa el segundo lugar en la Unión Europea en cuanto a censo de vacas nodrizas por detrás de Francia, con un 15% del total. Geográficamente, las vacas madres se localizan en las zonas montañosas del norte peninsular y en el suroeste español. Cinco Comunidades Autónomas concentran el 80% del censo de vacas nodrizas: Castilla y León, Extremadura, Andalucía, Galicia y Asturias (MARM, 2009).

La **reproducción** es uno de los principales factores limitantes de la eficiencia productiva en el vacuno de carne. Después del parto, la vaca sufre un periodo de inactividad sexual, denominado **anestro post-parto**, en el que se reajustan los mecanismos hormonales desencadenados para el mantenimiento de la gestación, y que consta de dos fases (Nett, 1987): La primera (días 15-30 post-parto) se caracteriza por el aumento de la síntesis de LH hipofisaria, y es independiente del estímulo del amamantamiento o de factores ambientales. En la segunda fase (días 30-150 post-parto) aumenta la liberación pulsátil de LH hasta que alcanza la amplitud y frecuencia de pulsos suficientes para estimular el crecimiento folicular, la producción de estradiol y, finalmente, la ovulación. Esta segunda fase condicionaría la duración del anestro post-parto, estando sujeta a la acción de factores ambientales y de manejo, entre los que destacarían la nutrición y el amamantamiento.

Diversos ensayos han indicado que la falta de ovulación que se produce durante el post-parto se debería a una insuficiente pulsatilidad de LH para estimular el crecimiento folicular y la maduración final (Williams, 1990; Hoffman *et al.*, 1996) y/o a la incapacidad del folículo dominante de responder a una adecuada frecuencia pulsátil de LH (Diskin *et al.*, 1999; Sinclair *et al.*, 2002), más que a una falta de desarrollo folicular (Sanz *et al.*, 2001a y b; Sinclair *et al.*; 2002; Sanz *et al.*, 2004b).

Por tanto, el reinicio de la ciclicidad después del parto va a depender fundamentalmente de que aumente la secreción de GnRH hipotalámica, estimulando así la liberación pulsátil de LH y/o la normalización de la función ovárica.

El primer factor que determina la duración del anestro post-parto es la nutrición pre-parto, mientras que la nutrición post-parto y el amamantamiento del ternero ejercerían un papel modulador del primer factor cuando el nivel de alimentación pre-parto no ha sido excesivamente limitante (Short *et al.*, 1990; Revilla, 1997; Sanz, 2000; Sanz *et al.*, 2004a). Dicho periodo no debería exceder de 80-85 días para lograr el objetivo de un ternero por vaca y año.

La base de la explotación de los rebaños de vacas nodrizas suele estar condicionada por el aprovechamiento de recursos pastorales, cuya disponibilidad presenta una marcada estacionalidad a lo largo del año en las condiciones mediterráneas. En este contexto, la vaca está sometida a diferentes niveles de alimentación, desencadenando importantes variaciones en su peso vivo y en sus reservas corporales (Casasús *et al.*, 2002), que pueden poner en peligro la consecución del objetivo de producción anual.

La paulatina adaptación de las explotaciones a condiciones cada vez más extensivas y multifuncionales impuestas por las Políticas Agrarias de la UE ha permitido la reducción de los elevados costes de alimentación invernal (García-Martínez *et al.*, 2009). Sin embargo, impide en muchos casos la aplicación del manejo del ternero utilizado en numerosas zonas de montaña, como el Pirineo, que consistía en mantener al ternero separado de su madre permitiéndole lactar durante uno o dos cortos periodos diarios (Revilla, 1987). Estas condiciones extensivas conducen a situaciones de subnutrición tanto antes como después del parto, así como a una permanencia continua del ternero con la madre, cuyas repercusiones pueden ser muy negativas en el rendimiento reproductivo de los rebaños de vacas nodrizas.

El efecto negativo de la crianza sobre la función reproductiva depende de forma importante de la **frecuencia, intensidad y duración de la lactación**, es decir, de si el ternero tiene acceso libre o restringido a su madre, así como del número de terneros criados y de la duración de la lactación. En general, la reducción en la frecuencia y/o intensidad y/o duración de la crianza se ha asociado a un reinicio de la ciclicidad ovárica más temprano.

Diversos estudios han caracterizado desde un punto de vista productivo y reproductivo algunas **razas** de ganado vacuno existentes en el Pirineo oscense (Blasco, 1991; San Juan, 1993; Villalba *et al.*, 2000; Casasús *et al.*, 2002; Sanz *et al.*, 2003; Sanz *et al.*, 2004a; Casasús *et al.*, 2004; Blanco *et al.*, 2009a y b), detectando algunas diferencias entre genotipos, que han sido más o menos marcadas en los distintos ensayos. La comparación racial se ha realizado entre animales de raza Parda de Montaña (antigua Parda Alpina), caracterizada por su aptitud mixta carne-leche, y la Pirenaica, distinguida por su adaptación a las condiciones de montaña y su conformación cárnica.

En las condiciones moderadas de alimentación que permiten los sistemas extensivos y manteniendo los terneros en acceso restringido, las razas Parda de Montaña y Pirenaica presentan una duración del anestro post-parto similar (Revilla, 1997; Sanz *et al.*, 1999). Sin embargo, la raza Parda de Montaña se ha mostrado más sensible que la Pirenaica a la permanencia continua del ternero, presentando un aumento en el número de ondas foliculares previas a la primera ovulación y evidentemente un retraso importante en la reactivación ovárica post-parto en relación con la Pirenaica (Sanz *et al.*, 2003).

En estos trabajos no se han evidenciado diferencias raciales en la dinámica folicular ovárica (tasa de crecimiento o diámetro máximo del folículo dominante) atribuibles al manejo del ternero. Sin embargo, la permanencia continua del ternero no provocó un retraso en la reactivación ovárica post-parto en vacas de raza Parda de Montaña cuando la condición corporal al parto fue elevada (Sanz *et al.*, 1996). ¿Podrían tener estas razas un umbral nutricional diferente a partir del cual la crianza influiría en el control de la reproducción?. ¿Existe algún indicador metabólico que pudiera influir a nivel sistémico o local sobre la capacidad ovulatoria de los folículos dominantes?. Algunos autores (Hoffman *et al.*, 1996; Stagg *et al.*, 1998; Rabiee y Lean, 2000; Sinclair *et al.*, 2002; Wettemann *et al.*, 2003; Diskin *et al.*, 2003; Flores *et al.*, 2008) han implicado determinadas señales metabólicas como componentes específicos en el control reproductivo (Figura 1), pero no se ha estudiado su influencia en los sistemas de manejo aplicados a estos genotipos.

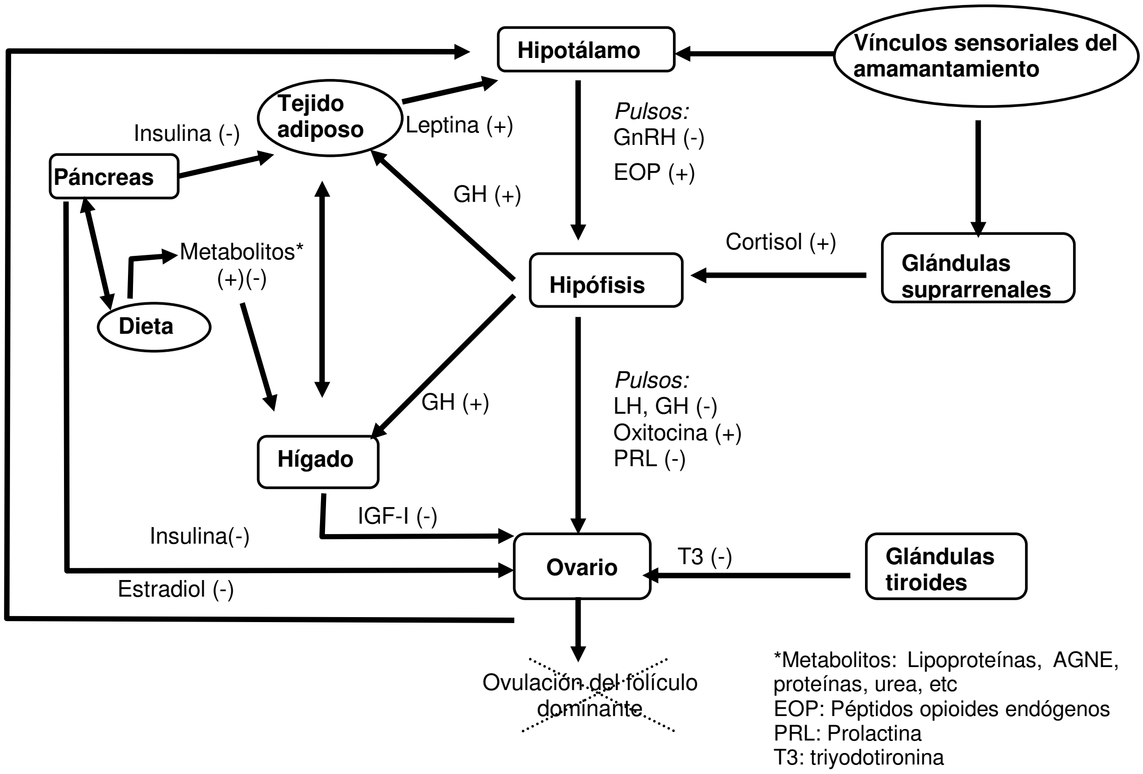

Figura 1. Factores que actúan sobre el eje hipotálamo-hipófisis-ovario durante el período anovulatorio post-parto

Así mismo, diversos trabajos (Acosta *et al.*, 1983; Shively y Williams, 1989; Short *et al.*, 1990; Williams, 1990; Zalesky *et al.*, 1990; Lamb *et al.*, 1999) han señalado que la restricción del acceso del ternero a la madre a dos o tres periodos diarios prolonga la duración del anestro post-parto, en comparación con un sistema de acceso restringido a un sólo periodo diario. Sin embargo, no existen estudios comparativos en las condiciones españolas sobre la respuesta reproductiva de la vaca nodriza cuando es sometida a uno, dos o más periodos de amamantamiento al día.

Los vínculos sensoriales entre la madre y su cría parecen influir sobre el eje hipotálamo-hipófisis-ovario. Se ha confirmado que la identificación maternal y la selectividad por el propio ternero influyen en la respuesta de dicho eje al estímulo de amamantamiento (Williams y Griffith, 1995). Varios autores han determinado que la glándula mamaria, así como su inervación no parecen presentar un papel fundamental en la mediación del efecto de la crianza sobre la secreción de gonadotropinas, debiendo de existir algún otro tipo de interacción vaca-ternero, aparte del amamantamiento, que es el responsable de la supresión del eje hipotálamo-hipófisis (Williams *et al.*, 1993; Viker *et al.*, 1993; Stevenson *et al.*, 1994).

La crianza es capaz de inducir periodos de aciclia a través de complejas interacciones sensoriales, de comportamiento y espaciales entre la vaca y su propio ternero (Williams *et al.*, 1996). En este sentido, Griffith y Williams (1996) consideran necesarios los componentes visuales u olfativos para que la crianza prolongue el anestro post-parto. En ese trabajo, se demostró que la vaca es capaz de reconocer a su ternero mediante la vista o el olfato (Griffith y Williams, 1996), y sólo la eliminación de ambos sentidos impediría el reconocimiento del ternero, anulando la inhibición que ejerce el amamantamiento sobre la secreción de LH (Stagg *et al.*, 1998). En vacas privadas del sentido del olfato una vez establecida la unión madre-hijo, éstas fueron capaces de reconocer visualmente a su ternero; les permitieron amamantar de nuevo, y se mantuvo la inhibición en la frecuencia de LH inducida por la crianza (Griffith y Williams, 1994).

Por el contrario, algunos autores mantienen que los estímulos visuales, auditivos y olfativos, así como el contacto del ternero con la cabeza y el cuello de la madre no son capaces de suprimir la función reproductiva, siendo necesario que el ternero estimule la zona inguinal (en vacas mastectomizadas) o exista el amamantamiento real (Viker *et al.*, 1993; Lamb *et al.*, 1999). Dicha mediación entre la crianza y la función reproductiva en el ganado vacuno podría ejercerse a través de los péptidos opioides endógenos a nivel hipotálamo-hipofisario (Whisnant *et al.*, 1986; Malven *et al.*, 1986; Zalesky *et al.*, 1990; Williams *et al.*, 1996), mediante la acción estimuladora de la oxitocina hipofisaria sobre la interacción materno-filial (Uvnäs-Moberg *et al.*, 2001) o la elevación de cortisol producida por la presencia del ternero (Hoffman *et al.*, 1996), más que a través de una disfunción del crecimiento folicular (Sanz *et al.*, 2001a y b).

La atenuación de los vínculos sensoriales podría llevarse a cabo a través del aislamiento visual, táctil, olfativo y/o auditivo del ternero durante los períodos entre amamantamientos. Así, diversos trabajos han señalado un efecto positivo del aislamiento del ternero a 60 m de sus madres sobre la duración del anestro post-parto (Diskin *et al.*, 1995; Mackey *et al.*, 1997a y b; Stagg *et al.*, 1998). Ante estos resultados, estudios posteriores trabajaron con la hipótesis de que la restricción y el aislamiento del ternero (a 15 m de las madres) realizados cuando se detectaba el primer folículo dominante (alrededor del día 30 post-parto), desencadenaría la ovulación, independientemente del nivel alimenticio de los animales. Esta manipulación del ternero sólo indujo la ovulación en las vacas que recibieron un nivel alto de alimentación (pre y post-parto) (Sanz *et al.*, 1997; Diskin *et al.*, 1999; Sinclair *et al.*, 1999), que a su vez presentaron concentraciones plasmáticas de insulina más elevadas que las vacas con un plano de alimentación bajo (Sinclair *et al.*, 2002).

En sistemas extensivos, el destete temporal del ternero a través de tablillas nasales o por separación completa transitoria a los 2 meses después del parto podrían ofrecer una alternativa de bajo coste para acortar la duración del anestro en vacuno de carne, aunque la respuesta estaría condicionada por el nivel de reservas corporales de las vacas en el momento de la aplicación de dicho manejo (Alberio *et al.*, 1984). En este sentido, se requieren períodos de restricción del amamantamiento con una duración superior a 6 días para conseguir la ovulación en vacas con condición corporal baja (Quintans *et al.*, 2004), especialmente en el caso de hembras primíparas (Quintans *et al.*, 2009a). Así mismo, se ha sugerido que la respuesta ovárica a la restricción del amamantamiento temporal con y sin presencia del ternero podría estar mediada por la concentración sanguínea de insulina (Quintans *et al.*, 2009b).

Objetivos / OBJECTIVES

2. Objetivos / OBJECTIVES

Esta tesis doctoral se planteó para profundizar en los mecanismos fisiológicos a través de los que la crianza del ternero puede actuar sobre la reproducción en la vaca nodriza, cuando es sometida a distintos sistemas de amamantamiento en condiciones moderadas de alimentación pre- y post-parto. Se evaluaron tres sistemas de crianza: acceso restringido del ternero a la madre a un período al día (RESTR1), dos períodos al día (RESTR2), o acceso libre (ADLIB). Se trabajó con dos razas con distinto origen-aptitud, la Parda de Montaña y la Pirenaica, con el fin de establecer pautas de manejo que puedan favorecer el reinicio de la actividad ovárica post-parto. Los objetivos de las publicaciones incluidas en esta memoria son:

1. Estudiar la influencia del sistema de amamantamiento (RESTR1, RESTR2, ADLIB) sobre los resultados productivos y el comportamiento epimelético de vacas de raza Parda de Montaña.

2. Evaluar los efectos del sistema de amamantamiento del ternero (RESTR1, RESTR2, ADLIB) y de la edad sobre los metabolitos de glucocorticoides fecales después del parto y los resultados productivos, metabólicos y reproductivos de vacas de raza Parda de Montaña.

3. Valorar los efectos del sistema de crianza (RESTR1 *vs.* ADLIB) y de la raza (Parda de Montaña *vs.* Pirenaica) sobre la función metabólica y la actividad luteal de vacas nodrizas.

4. Analizar el efecto de la raza sobre el comportamiento epimelético de vacas nodrizas en distintos sistemas de amamantamiento (RESTR1 *vs.* ADLIB).

5. Estudiar los efectos del aislamiento visual, táctil y olfativo del ternero en un sistema con dos períodos de amamantamiento al día sobre parámetros metabólicos, etológicos y reproductivos de vacas nodrizas.

6. Determinar la influencia del sistema de amamantamiento del ternero (RESTR1, RESTR2, ADLIB) y de la raza sobre la secreción de la hormona luteinizante y hormona del crecimiento en vacas nodrizas al final del primer mes de lactación.

OBJECTIVES

This doctoral thesis was designed to study several physiological mechanisms by which the suckling calf may module the reproductive function of beef cows, when they are managed under different nursing systems in moderate pre- and post-partum feeding conditions. From the day after calving, three nursing systems were studied: once a day restricted access of calves to dams (RESTR1), twice a day restricted access (RESTR2) and *ad libitum* access (ADLIB). Two beef cattle breeds with different origin-purpose, Parda de Montaña and Pirenaica, were used with the aim of establishing management strategies that enhance an early post-partum ovarian cyclicity. The objectives of the manuscripts included in this thesis were:

1. To study the influence of the type of calf management (RESTR1, RESTR2, ADLIB) on the productive performance and suckling behaviour of Parda de Montaña beef cows.

2. To evaluate the effects of the type of calf management (RESTR1, RESTR2, ADLIB) and dam age on faecal glucocorticoid metabolites after calving as well as on productive, metabolic and reproductive traits of Parda de Montaña beef cows.

3. To assess the effects of the type of calf management (RESTR1 *vs.* ADLIB) and breed (Parda de Montaña *vs.* Pirenaica) on the metabolic and luteal function of beef cows.

4. To analyse the effect of breed on the epimeletic behaviour of beef cows under different types of calf management (RESTR1 *vs.* ADLIB).

5. To study the effects of visual, tactile and olfactory isolation with calves in a twice-daily nursing system on metabolic, ethologic and reproductive parameters of beef cows.

6. To analyse the influence of the type of calf management (RESTR1, RESTR2, ADLIB) and breed on the secretion of luteinising hormone and growth hormone of beef cows at the end of the first month of lactation.

Presentación de las publicaciones

3. Presentación de las publicaciones

En esta memoria se han analizado los efectos del sistema de amamantamiento del ternero (RESTR1, RESTR2, RESTR2 con aislamiento visual, táctil y olfativo, ADLIB), de la edad (primíparas y multíparas) y de la raza (Parda de Montaña y Pirenaica) sobre distintos parámetros productivos, metabólicos, etológicos y reproductivos en la vaca nodriza. En todos los ensayos, la separación diaria del ternero se aplicó desde el día siguiente al parto y se prolongó durante, al menos, 100 días post-parto. Los experimentos se realizaron en dos épocas de parto: otoño e invierno.

En la Tabla 1 se resumen los resultados zootécnicos de los ensayos realizados. El ensayo A ha dado lugar a la publicación 1, el ensayo B a la publicación 2, el ensayo C a las publicaciones 3 y 4, el ensayo D a la publicación 5, y a partir de los ensayos B y D se ha generado la publicación 6. En todos los casos se trabajó con una única dieta integral, cuya cantidad se adaptó para conseguir distintos niveles de alimentación. El nivel de alimentación pre-parto se ajustó para conseguir una nota de condición corporal al parto intermedia en la escala de Lowman *et al.* (1976).

En el vacuno de carne, las revisiones más recientes sobre el tema apuntan a que el retraso en la primera ovulación después del parto parece ser debido a la insuficiente maduración de los folículos ováricos en crecimiento, más que a la falta de desarrollo folicular (Wettemann *et al.*, 2003; Montiel y Ahuja, 2005; Crowe, 2008; Sinclair, 2008). En nuestras condiciones de trabajo, la nutrición pre- y post-parto son los efectos con mayor influencia sobre el desarrollo folicular y luteal (Sanz, 2000; Sanz *et al.*, 2001a y b), mientras que la presencia continua del ternero ejercería un papel modulador en condiciones moderadas de alimentación (Sanz *et al.*, 2004a).

Tabla 1. Síntesis de los resultados zootécnicos obtenidos en los ensayos de la presente memoria

Ensayo (época de parto)	n	PV vaca parto (kg)	CC vaca parto	PV ternero nacimiento (kg)	EM pp (MJ/d)	Nivel pp	Raza-Tipo acceso ternero	GMD 3 meses pp		PLE (kg/d)	App (d)
								Vaca (%PVp)	Ternero (kg/d)		
A (otoño)	12	568	2,56	42,8	80	0,75 x M	PA-RESTR1	-1,3	0,68	7,0	65
	11	566	2,55	41,5			PA-RESTR2	-3,0	0,76	6,2	89
	11	565	2,61	40,4			PA–ADLIB	-5,5	0,90	8,9	69
B (otoño)	15	562	2,57	40,6	100	1 x M	PA-RESTR1	+0,6	0,67	7,8	46
	14	556	2,57	42,6			PA-RESTR2	-1,8	0,92	8,5	52
	17	558	2,57	41,0			PA–ADLIB	-2,1	0,84	8,9	58
	17	495	2,49	36,1			Nov-PA-ADLIB	-0,2	0,86	7,6	79
C (invierno)	22	549	2,48	41,3	100	1 x M	PA	-2,3	0,79	8,4	69
	28	590	2,69	40,1	90		PI	-1,5	0,73	7,2	73
	23	564	2,59	40,3	100-90		RESTR1	+0,2	0,70	7,3	56
	27	575	2,58	41,1			ADLIB	-3,9	0,82	8,3	87
D (invierno)	24	556	2,54	41,8	100	1 x M	PA-RESTR2	-0,5	0,93	8,9	52
	27	579	2,58	40,4	90		PI–RESTR2	+0,2	0,80	8,3	47
	26	560	2,56	40,7	100-90		RESTR2	-0,3	0,86	8,9	48
	25	574	2,56	41,5			AISL–RESTR2	0,0	0,87	8,2	51

n = número de animales, PV = peso vivo, CC = condición corporal (escala de 1 a 5, Lowman *et al.*, 1976), EM = energía metabolizable, pp = post-parto, M = mantenimiento, PA = Parda de Montaña, Nov=Novillas, PI = Pirenaica, RESTR1 = un período de amamantamiento al día (30 min), RESTR2 = dos períodos de amamantamiento al día (2 x 30 min), ADLIB = acceso libre, AISL = aislamiento visual, táctil y olfativo, PVp = peso vivo al parto, PLE = producción de leche estandarizada por contenido energético, App = duración del anestro post-parto.

En el ensayo A, el nivel de alimentación post-parto (0,75 x Mantenimiento) ejerció un efecto más marcado sobre los resultados reproductivos de las vacas que el sistema de amamantamiento del ternero (RESTR1, RESTR2, ADLIB). Por este motivo, en el resto de ensayos (B, C y D) las vacas se sometieron a un nivel de alimentación que garantizó el 100% de sus necesidades teóricas de mantenimiento y producción de leche.

En la Figura 1 se representa la duración del período de anestro post-parto y la duración del amamantamiento en los distintos ensayos. Los períodos de anestro post-parto más largos se observaron en las vacas con mayor intensidad de amamantamiento. Los períodos de anestro post-parto más cortos se observaron en las vacas con acceso restringido a su ternero.

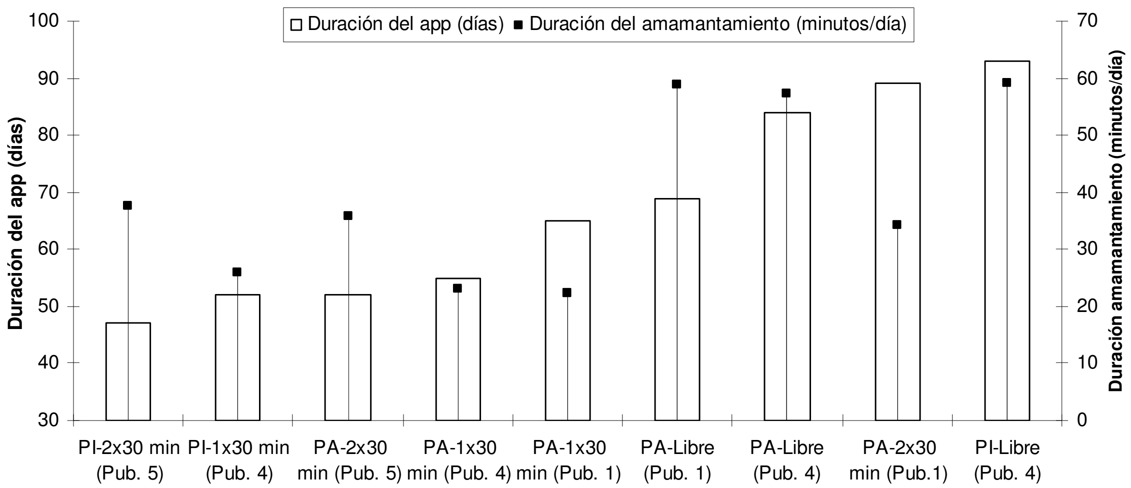

Figura 1. Duración del anestro post-parto (app, días) y duración del amamantamiento (minutos/día) en los distintas publicaciones

Por el contrario, la ganancia media diaria de los terneros sí mostró una mayor relación con la duración del amamantamiento (Figura 2). Los mayores ritmos de crecimiento se observaron en la raza Parda de Montaña en régimen de dos períodos de amamantamiento al día y en crianza libre, mientras que los menores se registraron cuando ambas razas se explotaron en un régimen de un período de amamantamiento al día.

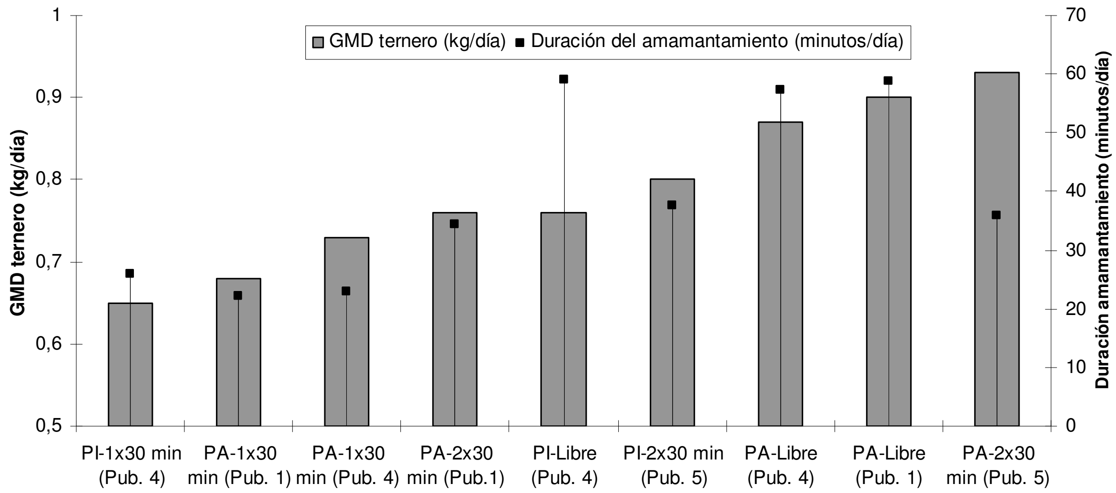

Figura 2. Ganancia media diaria (GMD) de los terneros (kg/día) y duración del amamantamiento (minutos/día) en los distintas publicaciones.

3.1. Resultados productivos y comportamiento durante el amamantamiento en vacas de raza Parda de Montaña con diferentes manejos del ternero

En la publicación 1, se evaluó el efecto de la frecuencia de amamantamiento (RESTR1 *vs.* RESTR2 *vs.* ADLIB) sobre los resultados productivos y reproductivos y el comportamiento durante el amamantamiento de vacas multíparas (n=36) de raza Parda de Montaña con parto en otoño (PVp 566 kg y CCp 2,6) en condiciones moderadas de alimentación (0,75 x M).

Se tomaron muestras de sangre quincenales para analizar la concentración de ácidos grasos no esterificados (AGNE) y dos muestras de sangre semanales para analizar la concentración de progesterona periférica. Se registró el comportamiento epimelético entre vaca y ternero en los días 30 y 66 de lactación.

Se observó que las pérdidas de peso de las vacas durante los 3 primeros meses de lactación fueron mayores en ADLIB que en RESTR1 y RESTR2 (-5 vs. -1 y -3% del peso vivo al parto, respectivamente). Esta respuesta productiva se tradujo en mayores ganancias medias diarias en los terneros del lote ADLIB (0,90 vs. 0,68 y 0,76 kg/día, respectivamente; $P<0,05$). El tipo de acceso del ternero afectó a la concentración plasmática media de AGNE en las vacas durante la lactación, siendo inferior en RESTR1 (0,18 mmol/l) que en RESTR2 y ADLIB (0,29 y 0,25 mmol/l, respectivamente; P<0,05).

La mayor frecuencia de amamantamientos se observó en las vacas de ADLIB, lo que llevó a una mayor duración diaria de esta actividad en dicho lote (58,8 minutos) que en RESTR1 y RESTR2 (22,2 y 34,3 minutos, respectivamente). La proporción de vacas que reiniciaron su actividad cíclica ovárica en los 3 meses siguientes al parto fue similar en los tres tratamientos (58, 46 y 55% en RESTR1, RESTR2 y ADLIB, respectivamente; P>0,10).

3.2. Efectos del sistema del amamantamiento y de la edad sobre la función metabólica y reproductiva de vacas de raza Parda de Montaña

En la publicación 2, se evaluaron los efectos del sistema de amamantamiento (en vacas multíparas) y de la edad sobre la concentración de metabolitos de glucocorticoides fecales después del parto, así como sobre la respuesta productiva, metabólica y reproductiva de vacas nodrizas (n=64) de raza Parda de Montaña con parto en otoño, en condiciones de alimentación de mantenimiento (1 x M). Las vacas multíparas (n=46, PVp 559 kg y CCp 2,57) se sometieron a uno (RESTR1) o dos períodos de amamantamiento al día de 30 minutos cada uno (RESTR2) o a crianza libre (ADLIB), desde el día siguiente al parto. Las vacas primíparas (n=18, PVp 494 kg y CCp 2,49) se sometieron a crianza libre (ADLIB).

Se tomaron muestras de sangre quincenales para analizar la concentración de metabolitos sanguíneos (triglicéridos, colesterol, AGNE, β-hidroxibutirato y urea) y el factor de crecimiento similar a la insulina-I (IGF-I) y dos muestras de sangre semanales para analizar la concentración de progesterona periférica. Se registró la actividad de las vacas como indicador de estro por medio de collares con radiofrecuencia.

La concentración de glucocorticoides en las heces durante los 3 primeros días post-parto no se vio afectada por el manejo del ternero o la edad de la vaca (P>0,10), pero fue superior a las 12 horas que a 48 y 72 horas post-parto (24,2 vs. 13,1 y 10,4 ng/g, respectivamente; P<0,05).

Las ganancias medias de las vacas fueron superiores en el lote de un amamantamiento al día que en dos y libre (0,04 vs. -0,11 y -0,13 kg/día, respectivamente; P<0,05). Sin embargo, la producción de leche y el crecimiento del ternero fueron menores en uno que en doble y libre amamantamiento (7,4 vs. 8,6 y 9,1 kg/día, 0,67 vs. 0,92 y 0,84 kg/día, respectivamente; P<0,05). Estos caracteres no difirieron entre multíparas y primíparas (9,1 vs. 8,0 kg/día, 0,84 vs. 0,86 kg/día, respectivamente; P>0,10).

La concentración sanguínea de triglicéridos y urea no se vio afectada por el manejo del ternero o la edad de la vaca (promedio 0,22 y 4,59 mmol/l, respectivamente; P>0,10). La concentración de colesterol y factor de crecimiento similar a la insulina-I (IGF-I) no difirió entre sistemas de amamantamiento (promedio 2,77 mmol/l y 60,9 ng/ml, respectivamente; P>0,10), pero su concentración fue inferior en vacas multíparas que en primíparas (2,56 vs. 3,08 mmol/l, y 54,7 vs. 71,8 ng/ml, respectivamente; P<0,05).

La concentración sanguínea de AGNE fue inferior en el lote de un amamantamiento al día que en doble y libre crianza en las semanas 7 y 9 de lactación (0,34 vs. 0,15 mmol/l, respectivamente; P<0,05). Además, los lotes sometidos a uno y dos amamantamientos al día mostraron una concentración menor de AGNE que el de crianza libre en la semana 11 de lactación (0,26 vs. 0,5 mmol/l, respectivamente; P<0,05). La concentración sanguínea de AGNE fue superior en las vacas multíparas que en las primíparas en la semana 1 y después de la semana 7 de lactación (media 0,38 vs. 0,19 mmol/l, respectivamente; P<0,05). La concentración media de β-hidroxibutirato fue inferior en las vacas de uno y doble amamantamiento que en crianza libre (0,23 vs. 0,26 mmol/l, respectivamente; P<0,05).

El sistema de amamantamiento del ternero no afectó significativamente el intervalo desde el parto hasta la primera ovulación (46, 52 y 58 días, en uno, doble y libre, respectivamente; P>0,10) o hasta el primer estro (45, 44 y 56 días, respectivamente; P>0,10). Sin embargo, las vacas multíparas mostraron un intervalo hasta la primera ovulación más corto que las primíparas (58 vs. 79 días, respectivamente; P<0,05).

En conclusión, la restricción del amamantamiento y la edad no desencadenaron una respuesta adrenal en las vacas después del parto. Desde un punto de vista productivo y metabólico, las vacas sometidas a doble amamantamiento al día se comportaron de forma más parecida a las vacas en crianza libre que a las de un amamantamiento al día. Esta respuesta no provocó diferencias remarcables en los parámetros reproductivos de las vacas. En el régimen de crianza libre, las vacas mostraron distintas características metabólicas a las novillas, pero éstas no pudieron relacionarse con el retraso observado en el comienzo de la ciclicidad en las vacas primíparas.

3.3. Efectos del sistema de amamantamiento y de la raza sobre la función metabólica y luteal en vacas nodrizas con parto en invierno

En la publicación 3, se evaluó el efecto del sistema de amamantamiento (RESTR1 vs. ADLIB) y de la raza (Parda de Montaña vs. Pirenaica) sobre la función metabólica y reproductiva de vacas multíparas (n=50) con parto en invierno (PVp 549 y 590 kg, CCp 2,48 y 2,69, en Parda de Montaña y Pirenaica, respectivamente), sometidas a una dieta de mantenimiento (1 x M).

Se tomaron muestras de sangre quincenales para analizar la concentración de metabolitos sanguíneos (ácidos grasos no esterificados (AGNE), β-hidroxibutirato, proteínas totales y urea) y el factor de crecimiento similar a la insulina-I (IGF-I) y dos muestras de sangre semanales para analizar la concentración de progesterona periférica.

Las vacas sometidas a RESTR1 al día mantuvieron peso durante los 3 primeros meses post-parto, mientras que las de ADLIB perdieron entorno a un 4% de su peso vivo inicial (0,01 vs. -0,25 kg/día, respectivamente; $P<0,001$). Ambos genotipos mostraron ganancias similares de peso durante ese período (-0,14 vs. -0,10 kg/día, en Parda y Pirenaica, respectivamente; $P>0,10$). Las ganancias diarias del ternero fueron menores en RESTR1 que en ADLIB (0,70 vs. 0,82 kg/día, respectivamente; $P<0,05$), pero similares entre razas (0,79 vs. 0,73 kg/día, respectivamente; $P>0,10$). La producción de leche y lactosa fue inferior en las vacas de RESTR1 que en las de ADLIB (6,8 vs. 8,4 kg/día y 0,32 vs. 0,40 kg/día, respectivamente; P<0,05). La producción de leche y de proteína fue superior en la raza Parda de Montaña que en la Pirenaica (8,4 vs. 6,8 kg/día y 0,39 vs. 0,33 kg/día, respectivamente; P<0,05).

La concentración sérica de AGNE, proteínas totales y urea fue mayor en las vacas de raza Pirenaica en ADLIB que en el resto (0,49 vs. 0,25 mmol/l; 82,3 vs. 77,6 g/l; 5,69 vs. 5,32 mmol/l, respectivamente, P<0,05). Las vacas de raza Pirenaica mostraron mayores valores de AGNE que las de raza Parda de Montaña en la primera semana post-parto (0,52 vs. 0,23 mmol/l, P<0,001). La concentración de IGF-I no se vio afectada por la frecuencia de amamantamiento, la raza o su interacción (promedio 59,5 ng/ml; P>0,10). El tipo de acceso del ternero pero no la raza afectó al intervalo desde el parto a la primera ovulación, que fue inferior en un amamantamiento al día que en libre (56 vs. 87 días, respectivamente; P<0,001).

En conclusión, la crianza libre mejoró la producción de leche y la ganancia de la cría a expensas de retrasar el reinicio de la ciclicidad de las vacas después del parto. La respuesta de los metabolitos sanguíneos indicadores del estado nutricional al manejo del ternero difirió entre genotipos, pero ninguno de ellos influyó sobre la concentración endocrina de IGF-I en estas razas.

3.4. Comportamiento maternal en dos razas de vacuno de carne en condiciones de un amamantamiento al día o crianza libre

En la publicación 4, se evaluó el efecto de la raza (Parda de Montaña vs. Pirenaica) sobre el vínculo materno-filial y se valoró su posible influencia sobre la duración del anestro post-parto, en vacas multíparas (n=24) con parto en invierno (PVp 544 y 588 kg, CCp 2,51 y 2,62, en Parda de Montaña y Pirenaica, respectivamente), sometidas a RESTR1 y ADLIB.

Se tomaron dos muestras de sangre semanales para analizar la concentración de progesterona periférica y se realizaron controles de comportamiento en las semanas 3, 8 y 13 de lactación. Los resultados se compararon dentro de cada sistema de manejo del ternero.

Ambas razas amamantaron a su cría durante un tiempo similar, tanto en RESTR1 (23,0 vs. 25,9 minutos/día, en Parda de Montaña y Pirenaica, respectivamente; P>0,10), como en ADLIB (57,2 vs. 59,0 minutos/día, respectivamente; P>0,10). Ambas razas reiniciaron su actividad cíclica ovárica de forma similar (70 y 73 días post-parto, respectivamente; P>0,10), aunque el acceso libre al ternero retrasó marcadamente la primera ovulación post-parto (89 vs. 54 días; P<0,001).

La única evidencia de distinto comportamiento maternal entre estos genotipos fue la mayor duración del amamantamiento de terneros ajenos en condiciones de crianza libre y un menor comportamiento agresivo hacia otros animales en la raza Parda de Montaña comparado con la Pirenaica.

3.5. Respuesta fisiológica y comportamental en dos razas de vacuno de carne sometidas a distintas estrategias de amamantamiento

En el ensayo 5, se evaluó el efecto del aislamiento visual, táctil y olfativo en un sistema de RESTR2 y de la raza sobre los resultados productivos, metabólicos, etológicos y reproductivos de vacas multíparas (n=52) con parto en invierno (PVp 556 y 579 kg, CCp 2,54 y 2,58, en Parda de Montaña y Pirenaica, respectivamente), con una dieta de mantenimiento (1 x M).

Se tomaron muestras de sangre de las vacas para analizar metabolitos sanguíneos (triglicéridos, colesterol, AGNE, β-hidroxibutirato y urea) y progesterona a distintos intervalos. Se monitorizó la actividad de las vacas a través de collares con radiofrecuencia como indicador de estro. Se registró el comportamiento de la pareja vaca-ternero durante el amamantamiento en las semanas 4, 9 y 15 de lactación.

Las vacas de ambos tratamientos (con y sin aislamiento) y razas mostraron similares ganancias de peso durante los 3 primeros meses post-parto (promedio -0,02 kg/día; P>0,10). La producción de leche y la ganancia de los terneros no se vieron afectadas por el aislamiento aplicado al ternero (promedio 8,2 kg/día y 0,87 kg/día; P>0,10) pero fueron superiores en la raza Parda de Montaña que en la Pirenaica (8,7 vs. 7,6 kg/día, 0,93 vs. 0,80 kg/día, respectivamente; P<0,05).

Los triglicéridos plasmáticos y la urea no se vieron afectados por el aislamiento del ternero o la raza (promedio 0,20 y 4,18 mmol/l, respectivamente; P>0,10). El colesterol plasmático incrementó a partir de la semana 6 de lactación en las vacas de raza Parda de Montaña (2,42 vs. 2,94 mmol/l, P<0,05), mientras que dicho ascenso se retrasó hasta la semana 7 en las de raza Pirenaica (2,48 vs. 2,90 mmol/l, P<0,05). Los niveles plasmáticos de AGNE fueron mayores en las vacas de raza Parda de Montaña con contacto visual, táctil y olfativo con sus terneros que en el resto (0,43 vs. 0,32 mmol/l, respectivamente; P<0,05). La concentración de β-hidroxibutirato no se vio afectada por el manejo del ternero o la raza (promedio 0,19 mmol/l, P>0,10).

Durante los períodos de amamantamiento, las vacas con contacto visual, táctil y olfativo con sus terneros tardaron menos tiempo en aproximarse hasta ellos que las que no tenían contacto entre estos períodos (0,1 vs. 0,6 minutos, respectivamente; P<0,05). Las vacas de ambos tratamientos amamantaron a sus terneros y se mantuvieron cercanos a ellos durante un tiempo similar en los períodos de amamantamiento (promedio 18,4 y 20,8 minutos/período, respectivamente; P>0,10). Las vacas de raza Parda de Montaña dedicaron mayor tiempo a lamer a su ternero que las de raza Pirenaica (1,5 vs. 0,2 minutos/período, respectivamente; P<0,001).

El aislamiento del ternero y la raza no afectaron al intervalo hasta la primera ovulación o el primer estro en estas vacas (50 y 49 días post-parto, respectivamente; P>0,10).

En conclusión, el aislamiento visual, táctil y olfativo en sistemas de doble amamantamiento no desencadenó diferentes características maternales o reproductivas en estas razas y únicamente una ligera mayor movilización de sustratos adiposos corporales en la raza Parda de Montaña comparado con la Pirenaica.

3.6. Secreción de hormona luteinizante (LH) y hormona del crecimiento (GH) en vacas nodrizas al principio de lactación

En la publicación 6, se estudiaron los efectos de la frecuencia de amamantamiento y de la raza sobre la liberación episódica de hormona luteinizante (LH) y la hormona del crecimiento (GH) en vacas multíparas (n=28) con un nivel de alimentación de mantenimiento (1 x M). Los efectos del sistema de amamantamiento (RESTR1, RESTR2 y ADLIB) se evaluaron en la raza Parda de Montaña. El efecto de la raza (Parda de Montaña vs. Pirenaica) se analizó en el régimen de RESTR2.

Se tomaron dos muestras de sangre por semana durante 100 días post-parto para establecer la duración del anestro post-parto a través de los niveles plasmáticos de progesterona. En el día 32 de lactación se tomaron muestras seriadas de sangre durante 8 horas a intervalos de 15 minutos para analizar la concentración plasmática de LH y GH.

La duración del anestro post-parto fue superior en las vacas de raza Parda de Montaña en ADLIB que en RESTR1 (79 vs. 55 días, respectivamente; P=0,05), mientras este período no difirió de los anteriores en RESTR2 (62 días; P>0,05). Las vacas sometidas a RESTR1 mostraron una tendencia a tener picos de LH más estrechos que las de ADLIB (107 vs. 191 minutos, respectivamente; P=0,09).

No se observaron diferencias en la duración del anestro entre las vacas de raza Parda de Montaña y Pirenaica con un sistema de RESTR2 (62 vs. 52 días; P>0,05). Las vacas de raza Parda de Montaña en RESTR2 tuvieron picos de LH ligeramente más anchos que sus homólogas de raza Pirenaica (148 vs. 81 minutos, respectivamente; P=0,07), así como una mayor área de dichos picos (31,5 vs. 12,5 ng/mL x minutos, respectivamente; P<0,05).

No se observaron diferencias entre sistemas de amamantamientos o razas en ninguna de las medidas de secreción de GH (P>0,05).

No se encontró correlación entre la frecuencia de pulsos de LH y GH (P>0,10). El área debajo de la curva y el valor medio de LH se correlacionaron negativamente con la duración del anestro post-parto, mientras que lo contrario ocurrió entre estas medidas de GH y la duración de dicho período ($P<0,05$). Las variables que mejor permitieron la discriminación entre los sistemas de amamantamiento y la raza fueron la frecuencia de pulsos de LH en las 8 horas de muestreo y la duración del anestro post-parto.

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5. Publicaciones

5.1. Performance and nursing behaviour of beef cows with different types of calf management

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Performance and nursing behaviour of beef cows with different types of calf management

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The aim of this experiment was to evaluate the effect of calf management during lactation on dam performance and nursing behaviour. Thirty-six multiparous beef cows (aged 7.5 ± 0.52 years) of Parda de Montaña breed (mean live-weight (LW) at calving 566 ± 9.3 kg) with a moderate body condition (mean 2.6 ± 0.02 on a 1 to 5 scale), were assigned to three nursing frequencies from the day after parturition: Once-daily restricted nursing during a 30 min period at 0800 h (RESTR1), twice-daily restricted nursing during two 30 min periods at 0800 and 1530 h (RESTR2) and ad libitum *nursing (ADLIB). Cows were fed throughout the experiment 80 MJ/day of metabolizable energy. Cow–calf behaviour recordings were performed on days 30 and 66 of lactation in each treatment. Blood samples were collected fortnightly to analyse non-esterified fatty acids (NEFA) by an enzymatic colorimetric method, and twice weekly to determine progesterone concentrations by radioimmunoassay. Cow LW losses ($P < 0.05$), fat-corrected milk yield ($P < 0.05$) and calf average daily gain ($P < 0.01$) during lactation were higher in ADLIB. Nursing frequency affected the mean serum concentrations of NEFA, being lower in RESTR1 (0.18 mmol/l; $P < 0.05$) than in RESTR2 and ADLIB cows (0.29 and 0.25 mmol/l, respectively). The highest occurrence of nursing bouts was observed in ADLIB, leading to greater nursing duration per day (not statistically compared) in this treatment (58.8 ± 5.84 min) than in their restricted nursing counterparts (22.2 ± 1.42 and 34.3 ± 1.16 min, in RESTR1 and RESTR2, respectively). The nursing system did not affect the proportion of cows cycling within 3 months* post partum *(58%, 46% and 55% in RESTR1, RESTR2 and ADLIB, respectively; $P > 0.05$). The majority of cows in all treatments showed a short oestrus cycle after first ovulation (75%, 100% and 83%; $P > 0.05$). In conclusion, the* post-partum *luteal function in cows managed under continuous access to calves was similar to restricted nursing frequencies, despite the greater suckling intensity of the formers. Restricting calf presence does not enhance the resumption of ovarian activity when suckler cows are managed in moderate nutritional conditions.*

Keywords: nursing, lactation, NEFA, suckling frequency, anoestrus

Introduction

Nutrient intake, body energy reserves and suckling are major regulators of reproductive performance in beef cows (Wettemann *et al.*, 2003). Body condition score (BCS) at calving and secondarily *post-partum* feeding level, assessed through live-weight (LW) changes during lactation, together with suckling frequency, are high explanatory factors affecting the length of the *post-partum* interval of suckler cows managed in harsh mountain areas (Sanz *et al.*, 2004a).

The traditional management in the Spanish Pyrenees takes into account a grazing season in which cattle graze on high mountain ranges (1500 to 2200 m) during summer and on permanent and forest pastures (900 to 2200 m) during spring and autumn. The duration of the winter housing period depends on the organisation of calving seasons, which are normally grouped from early autumn to late spring. Calf handling throughout the indoor period used to restrict suckling to once or twice daily in short intervals (Sanz *et al.*, 2003). However, suckler cattle systems are currently involved in an extensification process, which forces farmers to aim at new herd management strategies in accordance with the subsidies of the Common Agricultural Policy of the EU (D'Hour *et al.*, 1998).

The use of herbaceous and shrub vegetation by beef cattle during autumn and winter has been proposed as an alternative practice (Casasús *et al.*, 2007), though these sward surfaces do not meet animal requirements throughout

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lactation and result in cow-weight losses and low calf gains (Casasús *et al.*, 2006). Underfeeding beef cows leads to raised concentrations of their circulating non-esterified fatty acids (NEFA) (Easdon *et al.*, 1985; Grimard *et al.*, 1995; Ponter *et al.*, 2000) owing to adipose tissue mobilization. These metabolites have also been suggested as potential metabolic signals to the hypothalamic–pituitary axis in the *post-partum* period (Stevenson *et al.*, 1997).

In addition, the continuous access of calves to dams (*ad libitum* suckling), which is enhanced under grazing stocking, has been associated with a delay in the resumption of *post-partum* ovarian activity (Short *et al.*, 1990; Williams *et al.*, 1996), especially in moderate nutritional conditions (Diskin *et al.*, 1992; Sinclair *et al.*, 2002; Sanz *et al.*, 2004b). We hypothesize that calf management practices influence nursing behaviour, thereby, the cows that establish a more intense mother–young bonding during lactation attain a longer interval to reach first *post-partum* ovulation.

The aims of this study were to compare, under the same moderate nutritional conditions, the animal performance and nursing behaviour of beef cows managed according to three different suckling frequencies.

Material and methods

Animals and management

The experiment was carried out at La Garcipollera Research Station, in the mountain area of the southern Pyrenees (North-eastern Spain, 42°37′N, 0°30′W, 945 m a.s.l.).

Thirty-six multiparous autumn-calving cows (aged 7.5 ± 0.52 years) of Parda de Montaña breed (selected from old Brown Swiss for beef purpose) were housed in a loose-housing system after summer grazing on mountain pastures, and randomly assigned to three nursing frequencies from the day after parturition according to their calving date (average, 25 October ± 3.2 days), LW (mean, 566 ± 9.3 kg), BCS (mean, 2.6 ± 0.02) and calf sex (19 females and 17 males). Treatments were: Once-daily restricted nursing during a 30 min period at 0800 h (RESTR1), twice-daily restricted nursing during two 30 min periods at 0800 and 1530 h (RESTR2) and *ad libitum* nursing (ADLIB). In both restricted-nursing treatments, calves remained in groups in cubicles ($5 \times 5\ m^2$) adjacent to their dams' resting areas, with no visual or olfactory isolation. Cows and calves were housed on straw-bedded pens (one pen per treatment).

Cows were group-fed throughout the experiment, a diet containing around 80 MJ metabolizable energy (ME)/cow per day that met close to 75% of the energy recommendation (winter conditions simulation) for a 565-kg beef cow producing about 9 kg of milk with no change in LW during lactation (Agricultural Research Council (ARC), 1980). Diet consisted of 10 kg (as-fed basis) of a total mixed ration (Table 1) offered once daily at 0900 h. Group-allocation was used to allow better expression of behaviours. The biggest cows might have eaten slightly more than the smallest due to hierarchy, but requirements are proportional to LW, thereby needing more feed for the biggest than the smallest. There were no feed refusals over the experimental period. Water was supplied *ad libitum* in longitudinal troughs and mineral block supplements were placed on the feeders to allow free consumption. Calves did not receive any feed supplement other than milk throughout lactation and they had access to water during suckling periods (RESTR1 and RESTR2) or continuously (ADLIB).

Table 1 *Chemical composition of the total mixed ration used in the experiment (g/kg)*[†]

Dry matter (DM) (g/kg)	890
Crude fat (g/kg DM)	11
Ash (g/kg DM)	78
Crude protein (g/kg DM)	104
Neutral-detergent fibre (g/kg DM)	532
Acid-detergent fibre (g/kg DM)	295

Feedstuffs (g/kg fresh-weight basis): barley straw (470), barley grains (126), dehydrated alfalfa (100), beet molasses (80), citric pulp pellets (72), maize gluten meal (54), soybean meal (38), rape meal (38), alfalfa pellets (12) and vitamin and mineral supplement (10). Vitamin and mineral supplement contained per kg (fresh-weight basis) Ca 107 g, P 85 g, Cl 156 g, Mg 9 g, Na 102 g, S 20 g, Fe 4 g, Zn 12 g, vitamin A 12 000 IU, vitamin D_3 1200 IU, vitamin E (α-tocopherols 91%) 53 mg and Cu 20 mg.
[†]Analyses from weekly feed samples pooled on fortnight intervals.

One bull of the same breed was introduced into each treatment pen on day 52 ± 5.2 *post partum* (3 months after the first theoretical calving date) during a 3-month breeding season. Cows were weaned on day 106 ± 5.6 *post partum*. Data from two cow–calf pairs, one in RESTR2 and one in ADLIB, were excluded before the end of the experiment because of calf death. Necropsies revealed that calves died from digestive complications not attributed to the study. The care and use of animals followed the European guidelines (European Union Directive No. 86/609/CEE, 1986).

Measurements

Cows and calves were weighed before morning feeding within 24 h after calving and thereafter at weekly intervals during the first 3 months *post partum*. Cow BCS was assessed at calving on a 1 to 5 scale (Lowman *et al.*, 1976) by two trained technicians. Calf average daily gain (ADG) was calculated by linear regression of weights against time.

Milk yield and composition was measured on day 61 ± 2.2 *post partum* in eight cows per treatment. Milk yield was estimated by the oxytocin technique proposed by Le Du *et al.* (1979) and machine milking (0900 and 1500 h). Individual milk samples were taken for fat and protein analysis using an infrared technique (Milkoscan 4000; Fosselectric, Ltd, Hillerød, Denmark). Production of 4% fat-corrected milk was calculated.

Behaviour recordings were performed in two sub-groups of four cow–calf pairs (a total of eight pairs) balanced for calf sex, formed out of the 12 cows per treatment, on days 30 ± 0.7 and 66 ± 0.8 of lactation. Dams nursed their calves in a barn (4.5 m × 7.5 m) close to their resting area. Each sub-group was brought into the recording barn (which was a sub-part of the main barn) according to their calving date, after which the calves were allowed to enter. Cow–calf pairs from restricted-nursing systems (RESTR1 and

RESTR2) were kept in the recording barn for 30 min. The ADLIB sub-group was brought into the barn 24 h prior to sampling in order to assure acclimatisation and they were kept in it for the following 24 h-recording period.

In RESTR1 and RESTR2, every cow–calf pair was randomly allocated each day to one observer (a total of four observers), and additional video recordings were used as back up for occasional uncertain observations. In the ADLIB treatment, the observations were performed through scan-sampling video recording at 5-min intervals. The duration of nursing bouts was extrapolated for the 5 min separating two successive scans.

The pen was artificially illuminated at night (200 lx), allowing monitoring of the whole circadian cycle (24 h). Every cow–calf pair had coloured neck collars to aid identification. A single observer, who was also involved in recording the restricted-nursing treatments, viewed all the video recordings of the *ad libitum* treatment.

The number and duration of filial and non-filial nursing bouts were recorded, and the nursing duration per day was calculated as the sum of nursing time per bout in each observation period (30 min, 2×30 min or 24 h, respectively). A nursing bout started when the calf took a teat in its mouth and ended when the last teat was left and the calf moved away from the udder (Das *et al*., 2001). In RESTR1 and RESTR2, the following nursing bout was considered after a period of other activities of at least 1 min from the previous bout. The circadian nursing pattern in ADLIB treatment was analysed as the mean time that a calf devoted to suckle every hour, divided by the total nursing duration per day.

Blood sampling and assays

NEFA and progesterone concentrations were determined through blood samples (5 ml) collected fortnightly (NEFA) and twice weekly (progesterone), before morning feeding, by tail vessel puncture, into plain glass and heparinized tubes, respectively. Samples were centrifuged at $3000 \times \mathbf{g}$ for 15 min at 4°C. Serum and plasma were harvested and stored at −20°C until analysis.

Serum NEFA were analysed using a commercial kit (Randox Laboratories Ltd., Crumlin, Co. Antrim, UK). Samples were assayed in duplicate. Medium (0.58 mmol/l) and high (1.31 mmol/l) commercial reference serum samples (bovine precision serum; Randox Laboratories Ltd, Co. Antrim, UK) were used to evaluate the accuracy of the analysis. The mean intra- and inter-assay coefficients of variation were 6.0% and 8.9% for medium concentration sample, and 4.4% and 6.3% for the high concentration sample, respectively.

Plasma progesterone concentrations were measured using a solid-phase radioimmunoassay commercial kit (Coat-A-Count progesterone kit®; Diagnostic Products Corporation, Los Angeles, CA, USA) validated in our laboratory. Low (0.8 nmol/l), medium (2.6 nmol/l) and high (12.6 nmol/l) concentration samples were used to estimate the mean intra-assay coefficients of variation (CV) (13.3%, 8.5% and 6.0%) and inter-assay CV (20.0%, 10.8% and 10.9%). The sensitivity averaged 0.1 nmol/l. The onset of luteal activity after calving was considered when progesterone levels were >1.6 nmol/ml. If cows had not ovulated before weaning, the interval to first *post-partum* ovulation was regarded as this date and all experimental procedures were terminated. Short oestrus cycles (8 to 12 days) after first ovulation were considered when progesterone rises lasted for a single sampling.

Statistical analyses

Normal distribution of data was tested with the Shapiro–Wilk test before further analysis with SAS statistical software (version 9.1; SAS Institute Inc., Cary, NC, USA). Data from calf LW at birth and daily gains, interval to first *post-partum* ovulation and plasma progesterone concentrations of cows during the first luteal phase were tested with analysis of variance (ANOVA) (PROC GLM), according to the model:

$$y_{ij} = \mu + \alpha_i + \varepsilon_{ij},$$

where y_{ij} is the dependent variable, μ is the overall mean, α_i is the treatment effect and ε_{ij} is the residual error. The preliminary model concerning calf performance data contained the fixed effect of calf sex and the interaction calf sex × treatment, but they were omitted from the final analysis because they were not significant ($P > 0.05$).

The cow LW evolution and the serum concentrations of NEFA (previously log-transformed to achieve normal distribution) were tested with repeated measures ANOVA (PROC MIXED), by means of the following mixed model:

$$y_{ijk} = \mu + \alpha_i + d_j + \beta_k + (\alpha\beta)_{ik} + \varepsilon_{ijk},$$

where y_{ijk} is the dependent variable, μ is the overall mean, α_i is the treatment effect, d_j is the animal effect j, β_k is the week of lactation effect and ε_{ijk} is the residual error.

Differences between proportions were analysed using the Fisher exact test (PROC FREQ).

The different sampling methodology for behavioural traits did not allow comparing the nursing activities statistically. Total nursing duration, bout durations and bout frequency were only compared between morning and afternoon nursing periods within the RESTR2 treatment. Normal distribution of data could not be verified and thus means were compared with the Kruskal–Wallis non-parametric test (PROC NPAR1WAY).

Data are reported as least-square means (if normally distributed) or means and their associated standard errors. Differences with a $P < 0.05$ were considered significant.

Results

Animal performance

All treatments lost around 5% of their LW at calving until the start of the breeding period (average 52 ± 5.2 days *post partum*) (Figure 1). However, by the end of the third month *post partum*, RESTR1 and RESTR2 had nearly recovered their initial LW (loss of around 1% and 3%, respectively), while ADLIB maintained the same magnitude of weight loss than during the previous period (around 5% of their initial LW).

Likewise, cows nursing *ad libitum* showed a higher ($P < 0.05$) fat-corrected milk yield (8.9 ± 0.72 kg/day) than their restricted nursing counterparts (7.0 ± 0.72 and 6.2 ± 0.77 kg/day in RESTR1 and RESTR2, respectively). The mean milk fat and milk protein content in all cows was 3.7 ± 0.1% and 3.2 ± 0.1%, respectively.

Calf LW at birth was similar across treatments but their ADG during the first 3 months of lactation was higher in ADLIB than in restricted-nursing groups (Table 2; $P < 0.05$).

The proportion of cows cycling before the start of the breeding period and within the first 3 months of lactation, as well as the proportion of cows showing a short oestrus cycle after first ovulation were similar across nursing systems (Table 2; $P > 0.05$). The interval to first *post-partum* ovulation was not affected by the nursing frequency (average 74 ± 9.2 days; $P > 0.05$). The amount of plasma progesterone during the luteal phase of a short cycle was 29% of that produced during the luteal phase of a normal cycle (3.8 ± 0.35 *v.* 13.0 ± 1.21 nmol/ml; $P < 0.001$).

The profile of serum NEFA throughout lactation in each nursing system is shown in Figure 2. The mean serum concentrations of NEFA were different among treatments ($P < 0.01$), being lower ($P < 0.05$) in RESTR1 (0.18 mmol/l) than in RESTR2 and ADLIB cows (0.29 and 0.25 mmol/l, respectively). These metabolites were affected by time after calving ($P < 0.001$), with higher values ($P < 0.05$) during the first 5 weeks *post partum* (0.33, 0.32 and 0.27 mmol/l on week 1, 3 and 5 of lactation) than subsequently (average 0.20 mmol/l), regardless of treatment.

Nursing behaviour

Descriptive results of nursing behaviour are shown in Table 3. As expected, the highest occurrence of nursing bouts was observed in ADLIB, leading to greater nursing duration per day (not statistically compared) in this treatment (58.8 ± 5.84 min) than in their restricted-nursing counterparts (22.2 ± 1.42 and 34.3 ± 1.16 min, in RESTR1 and RESTR2, respectively).

Cows nursing twice daily (RESTR2), showed a higher duration of nursing throughout the morning (18.5 ± 0.87) than during the afternoon meal (15.8 ± 1.01), although this difference was only a tendency ($P < 0.10$). The number of bouts (2.3 ± 0.24 *v.* 2.3 ± 0.28) and duration of nursing per session (9.6 ± 1.10 *v.* 8.2 ± 0.97 min), as well as the time spent nursing other calves (1.2 ± 0.37 *v.* 0.7 ± 0.40 min), were similar across morning and afternoon periods ($P > 0.05$).

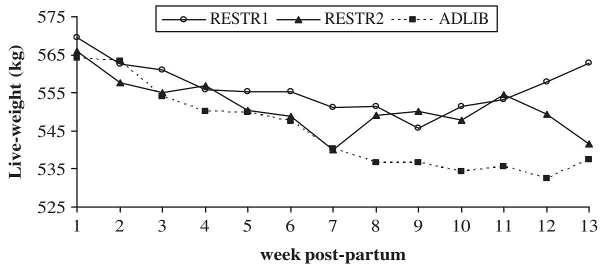

Figure 1 Live-weight evolution in beef cows nursing once daily (RESTR1), twice daily (RESTR2) or *ad libitum* (ADLIB) during lactation.

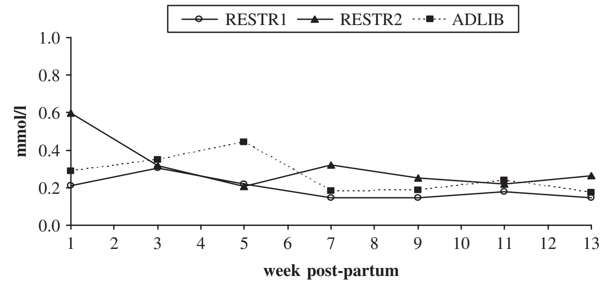

Figure 2 Profile of serum non-esterified fatty acids in beef cows nursing once daily (RESTR1), twice daily (RESTR2) or *ad libitum* (ADLIB) during lactation (back transformed values of least-square means from the analysis of log values).

Table 2 *Productive and reproductive performance of Parda de Montaña beef cows managed under restricted nursing once daily (RESTR1), twice daily (RESTR2) or* ad libitum *(ADLIB)*

	RESTR1	RESTR2	ADLIB	s.e.	*P*-value
Calving date	24 October	18 October	29 October	5.6	ns
LW at calving (kg)	568	566	565	16.6	ns
BCS at calving	2.56	2.55	2.61	0.039	ns
Days *post partum* at the start of breeding period	50	56	50	5.2	ns
Proportion of cows with onset of luteal activity before the start of breeding period	5/12	2/11	4/11	–	ns
Proportion of cows with onset of luteal activity within 3 months *post partum*	7/12	5/11	6/11	–	ns
Proportion of cows with a short cycle after first ovulation‡	6/8	7/7	5/6	–	ns
Calf sex ratio#	6/12	6/11	4/11	–	ns
Calf LW at birth (kg)	42.8	41.5	40.4	1.43	ns
Calf ADG in 3 months of lactation (kg)	0.68^a	0.76^a	0.90^b	0.047	**

LW = live weight; BCS = body condition score; ADG = average daily gain.
‡Only cows that resumed second ovulation prior to weaning were included.
#Male to female ratio within treatment.
** $P < 0.01$, ns = not significant ($P > 0.05$).
Within a row, least-square means with different superscripts are significantly different ($P < 0.05$).

Table 3 *Nursing activities in Parda de Montaña cows with total available time for nursing being 30 min (RESTR1), 2 × 30 min (RESTR2) and 24 h (*ad libitum *(ADLIB))*†

	RESTR1	RESTR2	ADLIB
Nursing duration per bout (min)	12.2 ± 2.21	8.9 ± 0.72	9.2 ± 0.55
Nursing bouts per day (number)	2.4 ± 0.25	4.6 ± 0.32	6.4 ± 0.52
Non-filial nursing duration per day (min)	2.4 ± 0.98	1.0 ± 0.27	8.8 ± 1.96

†Mean ± standard error; treatments were not statistically compared due to different sampling methodology.

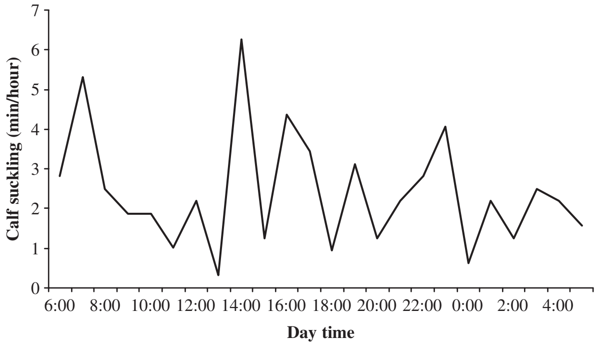

Figure 3 Circadian nursing pattern in cows kept indoors in continuous access with their offspring during lactation.

When cows nursed *ad libitum*, four suckling peaks were detected (Figure 3). The first coincided with sunrise between 0600 and 0700 h (9% total nursing time), the second just after noon between 1300 and 1400 h (11% total nursing time), the third in the afternoon between 1500 and 1600 h (7% total nursing time) and the last between 2200 and 2300 h (7% total nursing time). The remaining nursing activity (66%) was evenly distributed throughout the rest of the day (54% of nursing time occurred between 0600 and 1800 h).

Discussion

Nursing frequency affected cow and calf gains during the first 3 months *post partum* in moderately fed Parda de Montaña cows. Even though actual feed consumption was not measured, all cows were fed the same ration, leading to a higher milk production in dams nursing *ad libitum* and consequently more important weight losses within 3 months *post partum* in this treatment than in restricted-nursing groups.

Previous studies conducted in the same conditions did not find significant differences in animal performance between twice daily and *ad libitum* nursing (Sanz, 2000) nor once daily and *ad libitum* nursing (Sanz *et al*., 2003) in this breed. However, in the latter works, the level of feeding was above maintenance (100% and 110% of the theoretical energy requirements, respectively) and therefore, a different distribution of dietary energy to support nutrient demands may have arisen.

A long-term energy restriction in lactating dairy cows (20% of their energy requirements) has been reported to produce a decrease of 2.2 kg fat-corrected milk yield (Coulon and Rémond, 1991), through a mechanism of metabolic adaptation in cows suffering negative energy balance, in order to reduce energy expenditures and maintain homoeostasis. Notwithstanding, in our experiment, the lower level of production was only proved in cows managed under restricted nursing, since cows nursing *ad libitum* achieved milk yield and thereby calf growth rates similar to well-fed animals (Sanz, 2000; Sanz *et al*., 2003). These results would suggest that differences across nursing frequencies, regarding animal performance, become more marked under moderate *post-partum* feeding levels than in situations in which cow requirements are met. Cows managed under continuous access to calves may divert a higher proportion of their energy intake to promote milk yield instead of assuring LW stability.

The greater serum concentrations of NEFA in cows nursing twice daily and *ad libitum* compared to the most restricting management (once-daily nursing) was not linked with the evolution of LW in these treatments. Cow LW gains reflect energy balance (Petit and Micol, 1981), and according to the earlier work, once- and twice-daily nursing groups may have adapted their productive performance to achieve a nearly zero-energy balance within 3 months *post partum*, although they resumed ovulation in a similar way to cows nursing *ad libitum*. Cows nursing twice daily showed higher serum concentrations of NEFA but had similar performance to their counterparts nursing once daily. However, the NEFA mobilisation in cows nursing *ad libitum* did not allow coping with nutrient demands and these dams lost LW.

In that sense, Marongiu *et al*. (2002) reported lower plasma NEFA soon after *ad libitum* nursing restriction to once-daily nursing (25 to 50 *post partum*) in beef Sarda and Charolais × Sarda cows, which was attributed to an improvement in cows' energy balance.

The serum concentrations of NEFA were relatively high until week 5 of lactation and dropped smoothly afterwards. This probably highlighted the activation of lipolytic pathways during the early *post-partum* period as well as a greatly increased adrenergic stimulation of lipolysis at this time (Bell, 1995). The NEFA stabilization with advancing lactation may have been partly due to the falling LW, which resulted in lower maintenance requirements, as found by Easdon *et al*. (1985), Grimard *et al*. (1995) and Ponter *et al*. (2000) in suckled beef cows with similar level of feeding to the present experiment. In addition, the absorption of fatty acids in our case is probably low because the most abundant feedstuffs of the mixed ration (barley straw and dehydrated alfalfa, 570 g/kg) contain little crude fat content and rather mostly acetogenic nutrients (hemicellulose and cellulose). On the whole-day video recordings (ADLIB cow–calf pairs), we also observed that the time devoted to eat straw bed was over two-fold the time used to consume the actual feed provided (224 ± 16 *v.* 93 ± 9 min/day, unpublished data). Even considering that intake rate was probably higher when cows ate the mixed ration (121 ± 19 g dry matter/min) and the greater fill value of cereal straw compared to the current total dry mixed ration, straw consumption might have supplied some energy to overcome their moderate feeding level. However, the low quality of this forage did not help in improving reproductive response in any treatment.

The nursing activity was influenced by calf raising system. Cow–calf pairs allocated 74%, 57% and 4% of the time spent together to suckle in RESTR1, RESTR2 and ADLIB, respectively. In case of restricted-nursing treatments, this proportion is high compared to other studies conducting similar managements (36% of the time, Das *et al*., 2001). This longer nursing period could correspond to the lack of alternative feeding herein, since pasture and concentrate consumption increased with calves' age in that work (Das *et al*., 1999). In contrast, cows nursing *ad libitum* displayed similar nursing behaviour to other studies in which calves were raised exclusively on milk in confinement (3% to 4% of the day, Lewandrowski and Hurnik, 1983; 5% of the day, Williams *et al*., 1984). These nursing patterns were linked to ADGs of calves in each treatment and support the idea that descriptive information of suckling behaviour provides practical value to assess the maternal effects on calves' performance (Paranhos da Costa *et al*., 2006).

However, re-establishment of cyclic ovarian activity was unrelated with nursing management, in line with other authors who found no relationships across oestrus and nursing behaviour (Lewandrowski and Hurnik, 1983; Odde *et al*., 1985; Day *et al*., 1987). These results are in agreement with the experiments of Williams *et al*. (1993), who showed that suckling-mediated anovulation was independent from mammary somatosensory cues but probably dependent upon visual and olfactory cues.

The non-filial nursing accounted for 11%, 3% and 15% of filial nursing time in RESTR1, RESTR2 and ADLIB, respectively. It has been suggested that this activity is merely non-nutritious when it lasts for a short period (<1 min) (Das *et al*., 2000), but may indicate a problem of milk availability when it is accompanied by a high rate of butting (de Passillé, 2001). Nevertheless, the number of butts towards a cow udder was similar in dams nursing once and twice daily (Álvarez *et al*., 2006). In our experiment, we also found that there was no evidence that the maternal–offspring bonding could be impaired in the most restricted-nursing management because the number of cow licks to calves per day was significantly higher in RESTR1 than in RESTR2 (55.5 *v.* 22.5, respectively).

With regard to circadian variation of nursing activity in ADLIB, our results might reflect a metabolic need of the calves to obtain milk at intervals not longer than 7 to 8 h. The existence of a high incidence of nursing during the nocturnal period (nearly 50%) is similar to some previous studies in free-ranging (Somerville and Lowman, 1979) and enclosure-kept beef cows (Lewandrowski and Hurnik, 1983), but could be partially induced by the light used for night-monitoring since other authors reported more suckles during daylight (Odde *et al*., 1985). The occurrence of nursing peaks at sunrise (between 0500 and 0700 h) and around midday (between 1200 and 1400 h) seems to be consistent in all the afore-mentioned studies, regardless of housing conditions.

Restricted nursing, once daily for approximately 30 min, has been reported to reduce the interval to first *post-partum* ovulation in beef cows (Randel, 1981; Reeves and Gaskins, 1981) but not twice-daily nursing (LaVoie *et al*., 1981; Lamb *et al*., 1999), when compared with a control group (*ad libitum* nursing). Other authors have found no differences between once- and twice-daily nursing, but both systems were useful to shorten the interval to first ovulation compared to *ad libitum* nursing (Diskin *et al*., 1992). Our results do not support the latter experiments since the higher nursing intensity of cows with continuous access to calves did not seem to delay the onset of luteal activity, when compared with restricted-nursing frequencies in moderate nutritional conditions. Hence, we hypothesize that *post-partum* feeding level and nursing were confounded factors affecting the resumption of the ovarian activity and thus, most of the experiments may not be comparable with the present conditions because they worked with cows, which calved in good condition and were fed diets that exceeded requirements during lactation.

Energy restriction both before and during the early *post-partum* period, delays the onset of oestrus cycles and ovulation by suppressing LH pulse frequency (Schillo *et al*., 1992). In primiparous and multiparous Brahman cows (*Bos indicus*) fed 90% of their energy requirements, the ovarian response to restricted once-daily nursing has been proved inconsistent (Browning *et al*., 1994), in a similar way to our results, whereas increasing *post-partum* feeding level enhanced the onset of luteal activity in their case. Likewise, in primiparous Angus cows, LH release in response to calf removal was weak when energy intake was below requirements (Whisnant *et al*., 1985).

Sinclair *et al*. (2002) observed that cows with moderate BCS (below 2.5) displayed a poor ovarian response, when restricted nursing was imposed shortly after selection of the first dominant follicle to emerge after day 21 *post partum*, in a multi-centric study including Simmental, Sarda, Brown Swiss and Hereford $\times$ Holstein genotypes. In a parallel way, earlier experiments in Parda de Montaña cows confirmed that calf restriction from day 45 *post partum* compared to *ad libitum* suckling was a useless practice to induce ovulation, when cows showed a BCS at calving of 2.0 and were fed 75% of their energy requirements during lactation (Sanz *et al*., 2004b).

The proportion of dams cycling within the first 3 months *post partum* was poor in the three treatments. Such situation would impair their subsequent reproductive performance, especially if a short breeding season wished to be applied, and also compromise their target 12-month calving interval in almost half the cows. In addition, this outcome would suggest that the level of *post-partum* dietary energy may exert a deeper influence than the effect of nursing frequency on the re-establishment of ovarian activity in Parda de Montaña cows.

We found no differences across nursing frequencies regarding the incidence of short oestrus cycles (8 to 12 days) after the first ovulation, which have been observed more frequently in cows nursing twice daily and *ad libitum* than in non-nursing cows (LaVoie *et al*., 1981). Short cycles are probably caused by an early release of prostaglandin F2α (PGF2α) from the uterine endometrium which results in premature luteolysis (Zollers *et al*., 1991), which involves nursing-induced oxytocin release (Troxel *et al*., 1984). Another hypothesis has suggested that transient increases in progesterone, occurring before the first oestrus after calving, may prepare the reproductive tract for rebreeding so as to provide a more desirable uterine environment (Werth *et al*., 1996). With regard to this issue, we observed that plasma progesterone concentrations in the first short cycle after calving were remarkably lower than in subsequent normal cycles, which was in agreement with previous reports (Wells *et al*., 1986).

In conclusion, the *post-partum* luteal function in cows managed under continuous access to calves was similar to once- and twice-daily nursing systems, despite the greater suckling intensity of the formers. Restricting calf presence rarely enhances the resumption of ovarian activity when suckler cows are managed in moderate nutritional conditions.

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5.2. Effects of nursing frequency and parity on the productive, metabolic and reproductive parameters of beef cows

Effects of nursing frequency and parity on the productive, metabolic and reproductive parameters of beef cows

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Abstract

Nursing restriction and parity might be stressful factors influencing the challenging post-partum period. This experiment aimed to evaluate the effects of nursing frequency (in multiparous dams) and parity on faecal glucocorticoid metabolites after calving in beef cows (n=64). Also, the role of these effects on productive, metabolic and reproductive function was assessed. From the day after calving, forty-six Parda de Montaña cows were assigned at random to three nursing frequencies: once-daily nursing (1 x 30 min at 0800 h) (**RESTR1**), twice-daily nursing (2 x 30 min at 0800 and 1530 h) (**RESTR2**) and *ad libitum* nursing (**ADLIBC**). Eighteen heifers were maintained with free access to their calves as in the ADLIB cow's group (**ADLIBH**). Data were analysed through analysis of variance and survival analysis.

The faecal glucocorticoid metabolites during the first three days post-partum were not affected by either calf management or parity (P>0.10) but they were greater at 12 h post-partum than in subsequent samplings (P<0.05). Cow average daily gain (ADG) was greater in RESTR1 than in RESTR2 and ADLIBC (P<0.05). However, milk yield and calf ADG was lower in RESTR1 than in the rest (P<0.05). These traits did not differ between parities (P>0.10). Serum triglycerides and urea were not affected by calf management or parity (P>0.10). Peripheral cholesterol and IGF-I did not differ among suckling systems (P>0.10), but their mean concentration was lower in ADLIBC than in ADLIBH (P<0.05). Serum NEFA was lower in RESTR1 than in RESTR2 and ADLIBC on week 7 and 9 of lactation (P<0.05). Both RESTR1 and RESTR2 treatments showed lower serum NEFA than ADLIBC on week 11 post-partum (P<0.05). Serum NEFA were greater in ADLIBC than in ADLIBH on week 1 and after week 7 of lactation (P<0.05). Serum β-hydroxybutyrate was lower in RESTR1 and RESTR2 than in ADLIBC (P<0.05). Calf management did not affect significantly the interval to first post-partum ovulation or oestrus (P>0.10) but ADLIBC had shorter post-partum intervals to first ovulation than ADLIBH (P<0.05). In conclusion, calf restriction and parity were factors that did not affect cow's welfare after parturition. Twice-daily and *ad libitum* suckled cows showed a similar productive and metabolic behaviour which differ from their once-daily suckled counterparts. This response did not trigger remarkable differences in the reproductive parameters of cows. *Ad libitum* suckled cows had different metabolic traits compared to heifers, as well as a shorter duration of the post-partum anoestrus than *ad libitum* suckled first-calf dams.

Key words: cattle, calf management, blood metabolites, IGF-I, anoestrus, parity

Introduction

Suckling is one of the main effects delaying the resumption of post-partum ovarian cyclicity in beef cows, when nutrient intake and body reserves are not limiting factors (reviewed by Short et al., 1990; Williams, 1990; Wettemann et al., 2003). The mechanism of inhibition is regulated by the hypothalamic-pituitary-ovarian axis and appears to require both tactile stimulation to the inguinal area and the establishment and maintenance of a maternal bond (Williams and Griffith, 1995). Interactions between suckling and nutrition may arise in moderate nutritional conditions, and restriction of calf access has provided an effective means of overcoming the nutritional inhibition of pulsatile LH release in post-partum beef cows (Sinclair et al., 2008). The physiological pathways by which the hypothalamic-pituitary-ovarian axis detects the nutritional status of the animal are complex, and involve several blood metabolites and hormones, such as the GH-IGF-I system (Diskin et al., 2003).

There is no much information concerning the carry-over effects of daily temporary separation on beef cow's welfare, but it is known that the stimulation of the hypothalamic-pituitary-adrenal axis may impair the hypothalamic-pituitary-gonad axis (Dobson and Smith, 2000).

Parity influences the resumption of ovarian cyclicity after calving (Short et al., 1990; Blasco, 1991; Sanz et al., 2004), but the potential mechanisms involved in this physiological response are not fully understood. Accordingly, we have hypothesized that primiparous cows have lower coping ability to peripartal metabolic adaptations than multiparous cows, and certain blood metabolites and endocrine IGF-I might act as possible signals linking their post-partum nutritional status and luteal function.

The aim of this experiment was to evaluate the effect of nursing frequency (in multiparous dams) and parity on faecal glucocorticoid metabolites after calving, as well as on productive, metabolic and reproductive function of beef cows.

Material and methods

Animals and experimental design

Sixty-four autumn-calving Parda de Montaña cows (selected from old Brown Swiss for beef purpose), 46 multiparous (aged 5.7±2.4 year, mean±standard deviation) and 18 heifers (aged 3.0±0.2 year), were selected from a herd at 'la Garcipollera' Research Station (North-eastern Spain, 42°37′N, 0°30′W, 945 m a.s.l., average mean temperature 10.2±0.2 º C and annual rainfall 1059±68 mm throughout the period 1999-2006). The care and use of animals followed the European guidelines (European Union Directive No. 86/609/CEE 1986).

Replacement heifers were born in the autumn season (average birth date 28-october±28 days, birth body-weight (BW) 39.7±6.0 kg) and weaned at 3.5 months of age (BW 133±24 kg). Their average daily gain (ADG) during lactation was 0.89 kg/day. After weaning, they were maintained on permanent pastures until the autumn with intermittent cereal supplementation according to animal needs. The growing heifers were housed until the following spring season. Throughout this period they were fed alfalfa hay and/or a forage-based total mixed ration *ad libitum*. Heifers were bred by natural service at 27 months of age (BW 481±30 kg), when they had reached 85% of their expected mature weight. Pregnancy was diagnosed by ultrasonography 45 days after bull removal. Then, heifers were turned out with the adult herd to mountain ranges at 31 months of age. Their ADG from weaning to turning out was 0.43 kg/day.

All the cows grazed on permanent mountain pastures during spring and summer (mid to late pregnancy) and they were housed in early autumn (last week prior to parturition). Dams gained BW during spring-summer grazing (21 week). The initial and final BW at that time was 516±58 and 579±59 kg in multiparous cows and 489±30 and 520±38 kg in heifers, respectively (maternal weight plus conceptus). At housing, body condition score (BCS) was 2.59±0.19 and 2.44±0.13 in multiparous cows and heifers, respectively (1 to 5 scale, Lowman et al., 1976).

Indoors, multiparous cows were group-fed 10 kg/head per day (as-fed basis) of a total dry mixed ration (TMR) (Table 1) during 6 week, to meet energy and protein requirements for maintenance and pregnancy. Heifers were fed the same amount of the TMR throughout this period to account for their additional growth requirements. The latter were allocated in a different pen to avoid hierarchical behaviours. Diets were established in accordance with NRC recommendations for beef cattle (NRC, 2000) and they were planned to achieve a moderate body condition at calving in all cows (around 2.5).

The day after parturition, multiparous cows were assigned to three nursing frequencies according to their calving date (4 november±26 days), BW (559±59 kg), BCS (2.57±0.18) and calf sex (27 females/19 males). Treatments were: once-daily nursing during a 30 min-period at 0800 h (**RESTR1**), twice-daily nursing during two 30 min-periods at 0800 and 1530 h (**RESTR2**) and *ad libitum* nursing (**ADLIBC**).

Heifers (calving date: 21 october±24 days, BW 494±32 kg, BCS: 2.49±0.12 and calf sex ratio: 11 females/7 males) were maintained with free access to their calves (**ADLIBH**) as in the ADLIB multiparous cow group to compare the parity effect.

In both restricted nursing treatments, calves remained in groups in cubicles nearby their dams resting areas, with no visual or olfactory isolation. Cow-calf pairs remained indoors throughout lactation in a loose-housing system with straw-bedded pens (one pen per treatment).

After parturition, cows were group-fed a diet containing around 100 MJ metabolizable energy (ME) per cow per day which met energy and protein requirements for a 560-kg beef cow producing about 9 kg of energy-corrected milk at peak yield (NRC, 2000). This diet also met energy and protein requirements for a 500-kg beef heifer producing the same amount of milk as multiparous cows with a 0.4-kg/day target growth rate. The ration was 13 kg (as-fed basis) of the afore-mentioned TMR (Table 1) offered once-daily at 09:00 h. There were no feed refusals (or eventually negligible) throughout the experimental period. Water was supplied *ad libitum* in longitudinal troughs and mineral block supplements were placed on the feeders to allow free consumption. Calves did not receive any feed supplement other than milk throughout the first three months of lactation. One bull of proven fertility from the same breed was introduced into each treatment pen on week 8 post-partum during a 13-week breeding season. Cows were weaned on week 16±4 post-partum.

Table 1. Chemical composition of the total mixed ration used in the experiment [1]

	Mean	SD
Dry matter (DM), g/kg	892	11
Ether extract, g/kg DM	14	4
Ash, g/kg DM	69	10
Crude Protein (CP), g/kg DM	96	19
Neutral-detergent Fibre (NDF), g/kg DM	523	69
Acid-detergent Fibre (ADF), g/kg DM	287	43
Acid-detergent lignin (ADL), g/kg DM	45	9
Insoluble protein in the NDF, g/kg DM	25	6

[1] Feedstuffs (g/kg as-fed basis): barley straw (470), barley grains (126), dehydrated alfalfa (100), beet molasses (80), citric pulp pellets (72), maize gluten meal (54), soybean meal (38), rape meal (38), alfalfa pellets (12) and vitamin and mineral supplement (10). Vitamin and mineral supplement contained per kg (as-fed basis): Ca 107 g, P 85 g, Cl 156 g, Mg 9 g, Na 102 g, S 20 g, Fe 4 g, Zn 12 g, vitamin A 12000 IU/kg, vitamin D3 1200 IU/kg, vitamin E (α-tocopherols 91%) 53 mg/kg, Cu 20 mg/kg.

Measurements and blood sampling

Fresh faecal samples were collected directly from the rectal ampulla of the cows within 12, 48 and 72 h after calving for determination of glucocorticoid metabolites. Fresh faecal samples were immediately frozen at -20 º C before being freeze-dried. Cows and calves were weighed within 12 h after calving and before morning feeding at weekly intervals during the first three month post-partum (period which determine the target one year calving interval). Body-weight at calving was considered as the mean between BW within 12-h post-partum and the one registered the week after. The balance had an accuracy of ±0.5 kg. Cow and calf ADG throughout the experimental period was calculated by linear regression of BW against time. Cow BCS was assessed at calving and at the end of the first three months post-partum.

Milk yield and composition was measured on weeks 2, 7 and 13 of lactation in a sub-sample of 6 cows per treatment by the oxytocin and machine milking technique (Le Du et al., 1979). On the day of milking, calves were removed and cows were administered an intramuscular injection of oxytocin (40 IU, Gineamin, Laboratorios Maymó, Barcelona) 5 min before milking to facilitate milk letdown. Cows were milked by using a portable milking machine. Milk collected from the initial milking was subsequently discarded. Cows were kept separated from their calves for 6 h, and then milked a second time. Milk weight was recorded after this milking, and individual 40 ml aliquots were retained for fat, protein and lactose analysis through infrared (Milkoscan 4000^{TM}, Fosselectric, Ltd., Hillerod, Denmark). Final milk weight was multiplied by four to provide an estimate of 24-h milk production. The production of energy-corrected milk (ECM) was calculated through the equation: ECM= milk production (kg) x ((0.38 x (milk fat (%) + 0.24 x (milk protein (%) + 0.78)/3.14).

During the post-partum period, blood samples (5 ml) were collected twice weekly before morning feeding, by tail vessel puncture into heparinized tubes. In addition, blood samples were withdrawn fortnightly with the same procedure into plain glass tubes and allowed to clot. Samples were centrifugated at 3000 x *g* for 15 min at 4 º C. Serum was used to determine blood metabolites (fortnightly). Plasma aliquots were prepared to analyze IGF-I (fortnightly) and progesterone (twice weekly) and stored at -20 º C until analysis.

The first post-partum oestrus was monitored through an automated activity sensor (Alfa Laval Agri, Tumba, Sweden) placed on cow's neck collar. The activity meter system measures activity and created a motion pattern for every cow carrying an activity tag. The activity pattern was continuously updated through calculations by a Kalman filter. A Kalman filter is a mathematical formula comparing and predicting hour by hour data to create an activity pattern for each cow. The latest sending of data always has the greatest impact on the calculation while old date loose importance as days pass. This enabled the process to follow changes in the cow behavioural pattern. As the day progressed, activity varied as the cow was carrying out her daily routines. The actual activity level was therefore not what detected heat. Data from the activity meters was evaluated hour by hour continuously and compared with data from the previous six hours. If data during this time diverged enough from the usual pattern the processor reported unusual high activity for that particular cow. Unusual high activity was displayed within three degrees of certainty shown with one, two or three plusses (Larsson, 2007).

Chemical analyses

Feed samples were kept at weekly intervals and pooled on fortnight intervals. Samples were dried at 60 ºC until constant weight and mill-ground (1 mm screen). DM, ash, ether extract and CP (N x 6.25) contents were determined according to the AOAC methods (AOAC, 1999). NDF, ADF and ADL analyses were carried out according to the sequential procedure of van Soest et al. (1991). Neutral-detergent insoluble protein was determined by N analysis of NDF residues. All values were corrected for ash-free content.

Assays

Faecal sample extraction was performed according to slight modifications to the procedure of Morrow et al. (2002). To carry out the extraction, approximately 0.5 g of well-mixed powdered freeze-dried faeces was suspended in 80% methanol (5 ml) and vortexed at 2500 x *g* for 30 min. After centrifugation, the supernatant was diluted 1:10 in phosphate buffered saline (pH=7.4). Faecal glucocorticoid metabolite concentrations were analysed in duplicate with a commercial ^{125}I radioimmunoassay kit (ICN double-antibody glucocorticoid kit; MP Biomedicals, LLC, NY, USA). The mean intra- and inter-assay CV were 7.1% and 7.2%, respectively. The results were expressed as ng glucocorticoid metabolite (ICN GC) per g dry faeces.

Serum concentration of triglycerides (enzymatic-colorimetric method), cholesterol (enzymatic-colorimetric method), urea (kinetic UV test) and β-hydroxybutyrate (enzimatic-colorimetric method) were determined with an automatic analyzer (GernonStar, RAL/TRANSASIA, Dabhel, India). Reagents for triglycerides, cholesterol and urea were provided by the analyzer manufacturer (RAL, Barcelona, Spain) and those for β-hydroxybutyrate were supplied by Randox (Randox Laboratories Ltd., Crumlin, Co. Antrim, UK). The mean intra- and inter-assay CV for the afore-mentioned metabolites were <5.4% and <5.8%, respectively. Serum NEFA were analysed in duplicate using a commercial kit (Randox Laboratories Ltd., Crumlin, Co. Antrim, UK). Commercial reference serum samples (bovine precision serum, Randox Laboratories Ltd., Crumlin, Co. Antrim, UK) were used to evaluate the accuracy of the analysis. The mean intra- and inter-assay CV were 5.1% and 7.4%, respectively.

Plasma IGF-I was analysed through solid-phase enzyme-labelled chemiluminiscent immunometric assay (Immulite ®, Siemens Medical Solutions Diagnostics Limited, Llanberis, Gwynedd, UK). Bovine plasma samples previously analysed through a commercial EIA kit (Blanco et al., 2008) were re-assayed to evaluate the accuracy of the analysis. A regression analysis was performed to compare the results obtained with both methods (r=0.96). The mean intra- and inter-assay CV were 3.1% and 12.0%, respectively.

Plasma progesterone was measured using a solid-phase RIA commercial kit (Coat-A-Count P4 kit®, Diagnostic Products Corporation, Los Angeles, CA, USA). The mean intra- and inter-assay CV were 8.0% and 10.4%, respectively. The sensitivity averaged 0.03 ng/ml. The interval to first ovulation was regard at the interval between calving and the first rise in peripheral progesterone. The onset of normal luteal activity after calving was considered when progesterone levels were ≥1 ng/ml in at least 3 or more consecutive samples. However, first short oestrus cycles (8 to 14 days) prior to the second ovulation were considered when progesterone rises >0.5 ng/ml were detected in 2 or less consecutive samples. This peripheral progesterone concentration confirmed the disappearance of the dominant follicle when ovulation was also determined by ultrasonography (Quintans et al., 2004). Dates of increased activity (detected through the automated device) were crossed with dates of progesterone rises in order to verify that they were caused by oestrus. If cows had not ovulated before weaning, the interval to first ovulation was considered as this date and all experimental procedures were terminated.

Statistical analyses

Data were analysed using the SAS statistical software (SAS Institute Inc., Cary, NC, USA). Nursing restriction and parity effects were analysed in separate models. Cow-calf productive and reproductive performance were analysed with a general linear model (GLM procedure):

$$y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$$

where $y_{ij} =$ dependent variable, $\mu =$ overall mean, $\alpha_i =$ suckling restriction (*i*=RESTR1, RESTR2, ADLIBC) or parity effect (*i*=ADLIBC, ADLIBH) and $\varepsilon_{ij} =$ residual error.

In the previous model, week of lactation was included as fixed effect when analysing milk yield and composition. Calf sex was included in the model as fixed effect when analysing BW at birth and ADG during the first three months of lactation.

Faecal glucocorticoid metabolites, blood metabolites and endocrine IGF-I of the cows were analysed with a repeated measurements mixed linear model (MIXED procedure):

$$y_{ijk} = \mu + \alpha_i + d_j + \beta_k + (\alpha\beta)_{ik} + \varepsilon_{ijk}$$

where y_{ijk} = dependent variable, μ = overall mean, α_i = suckling restriction or parity effect, d_j = animal effect j, β_k = day or week of lactation effect and ε_{ijk} = residual error.

An unstructured covariance matrix within the animal random effect was fitted. Multiple comparisons among treatments were performed by the Tukey's method. Levene's test was conducted to test equality of variances across groups in the data concerning reproductive parameters.

The level of significance was set at *P*=0.05. Probabilities between 0.06-0.10 were considered to show trends. The interactions are commented in the text only when they approached statistical significance (P<0.10).

Differences between proportions were analysed using the Fisher exact test of the FREQ procedure. Survival analysis utilizing the LIFETEST procedure was used to evaluate the effects of nursing frequency and parity on the interval from calving to first ovulation. The survival analysis was a regression of the number of anoestrous cows at weekly intervals over the first three months post-partum (censored observations). The Wilcoxon test was used to examine the differences between the survival curves.

Data from one heifer and its young were removed from the statistical analysis because the offspring had a traumatism in its leg, which begged individual allocation and veterinary treatment.

Results

Faecal glucocorticoid metabolites after calving

Faecal glucocorticoid concentrations during the first three days post-partum were not affected by either calf restriction or parity (Table 2; P>0.10). However, the concentration was greater at 12 h post-partum (24.2 ng/g), than in subsequent samplings, regardless of treatment or parity (13.1 and 10.4 ng/g, at 48 and 72 h after calving, respectively; P<0.05).

The percentage of cows having greater faecal glucocorticoid concentrations at 48 h and 72 h after calving than on the first sampling did not differ among types of calf management or parities (average 36.7% and 24.1%, on 48 h and 72 h post-partum, respectively; P>0.10).

Table 2. Faecal glucocorticoid metabolite concentrations during three days after calving in beef cows and heifers with different types of calf management (RESTR1= once-daily for 30 min, RESTR2= twice-daily for 2 x 30 min, ADLIB=*ad libitum*) (Least square means±SD)

	Cows			Heifers	*P*-value †	
	RESTR1	RESTR2	ADLIBC	ADLIBH	T	P
n	15	14	17	17	-	-
12 h post-partum, ng/g dry faeces	21.2^{a}±15.9	20.2^{a}±18.9	26.8^{a}±35.3	24.6^{a}±24.0		
48 h post-partum, ng/g dry faeces [1]	11.3^{b}±10.6	12.6^{b}±11.1	11.6^{b}±8.0	16.8^{b}±19.9	0.68	0.60
72 h post-partum, ng/g dry faeces	8.7^{b}±7.6	6.9^{b}±4.4	10.0^{b}±7.0	14.6^{b}±12.8		

† T, Treatment, P, Parity. Within a column, means without a common superscript letter differ (*P*<0.05) over time ($^{a,\ b}$).

[1] Suckling restriction in RESTR1 and RESTR2 was induced approximately 24 h after parturition.

Productive performance

The results concerning changes of BW and BCS over time are shown in Table 3. BW and BCS at calving did not differ across multiparous cows (P>0.10), but cow ADG throughout the first three months post-partum were greater in RESTR1 than in RESTR2 and ADLIBC (P<0.05). BW but not BCS at calving was greater in multiparous cows nursing *ad libitum* than in heifers with the same calf management (P<0.05). The change of BW throughout lactation did not differ between parities (P>0.10), but BCS at the end of the third month post-partum was greater in cows (ADLIBH) than in heifers (ADLIBH) (P<0.05).

Calf BW at birth was similar across treatments (P>0.10), but calf ADG throughout the first three months of lactation were lower in RESTR1 than in RESTR2 and ADLIBC groups (P<0.05). There was a tendency for interaction between cow parity and calf sex in the offspring BW at birth (P=0.07). Female calves born from heifers were lighter (32.7 kg) than the rest of calves (40.5 kg averaged; P<0.05). Nevertheless, parity did not affect calf gains throughout the first three months of lactation (P>0.10).

Table 3. Productive performance of beef cows and heifers with different types of calf management (RESTR1= once-daily for 30 min, RESTR2= twice-daily for 2 x 30 min, ADLIB=*ad libitum*) (Least square means±SD)

	Cows			Heifers	*P*-value †	
	RESTR1	RESTR2	ADLIBC	ADLIBH	T	P
Cows						
n	15	14	17	17	-	-
BW at calving, kg	562±65	556±50	558^{x}±64	495^{y}±32	0.96	<0.001
ADG during first 3 months post-partum, kg/day	0.04^{a}±0.20	-0.11^{b}±0.16	-0.13^{b}±0.25	-0.01±0.28	0.05	0.18
BCS at calving	2.57±0.16	2.57±0.12	2.57±0.23	2.49±0.12	0.99	0.22
BCS end of third month post-partum	2.68^{a}±0.21	2.56^{ab}±0.16	$2.55^{b,x}$±0.16	2.45^{y}±0.10	0.09	0.03
Calves						
Male to female ratio	6:9	6:8	7:10	7:10	1.00	1.00
BW at birth, kg [1]	40.6±6.8	42.6±6.3	41.0^{x}±5.4	36.1^{y}±5.9	0.68	0.01
ADG during first 3 months of lactation, kg/day	0.67^{b}±0.16	0.92^{a}±0.22	0.84^{a}±0.24	0.86±0.22	0.009	0.80

† T, Treatment, P, Parity. Within a row, means without a common superscript letter differ (*P*<0.05) within treatments ($^{a,\ b}$) or parity ($^{x,\ y}$).

[1] Tendency for cow parity x calf sex interaction (P=0.07).

Milk yield was lower in RESTR1 than in RESTR2 and ADLIBC (Table 4; P<0.05), but differences were diluted when milk production was adjusted for energy-standard content (P>0.10). The milk fat content was greater in RESTR1 than in RESTR2 and ADLIBC (P<0.05), but it was similar in *ad libitum* cows and *ad libitum* heifers (P>0.10). The milk protein and lactose contents did not vary across suckling systems or parities (P>0.10). The energy-corrected milk yield was greater on weeks 2 and 7 than on week 13 of lactation (9.0, 9.0 vs. 7.3 kg, respectively; P<0.05).

Table 4. Milk production and composition of beef cows and heifers with different types of calf management (RESTR1= once-daily for 30 min, RESTR2= twice-daily for 2 x 30 min, ADLIB=*ad libitum*) (Least square means±SD)

	Cows			Heifers	*P*-value †	
	RESTR1	RESTR2	ADLIBC	ADLIBH	T	P
n	6	6	6	6	-	-
Milk yield, kg	7.4^{b}±1.6	8.6^{ab}±1.8	9.1^{a}±2.7	8.0±1.5	0.05	0.16
Energy-corrected milk, kg	7.8±1.8	8.5±2.2	8.9±3.1	7.6±1.7	0.40	0.13
Milk composition						
Milk fat, %	4.20^{a}±0.80	3.58^{b}±0.51	3.66^{b}±0.66	3.54±0.81	0.01	0.86
Milk protein, %	3.90±0.46	3.74±0.46	3.67±0.32	3.47±0.55	0.24	0.21
Milk lactose, %	4.71±0.26	4.87±0.34	4.66±0.39	4.47±0.63	0.21	0.30

† T, Treatment, P, Parity. Within a row, means without a common superscript letter differ (*P*<0.05) within treatments ($^{a,\ b}$) or parity ($^{x,\ y}$).

Blood metabolites and endocrine IGF-I

The profile of serum lipoproteins in the cows throughout the first three months of lactation is shown in Figure 1. Serum triglycerides were not affected either by suckling restriction or parity (P>0.10). However, they were greater from week 1 through week 7 of lactation than afterwards (0.26 vs. 0.16 mmol/l, respectively; P<0.05). Serum cholesterol did not differ across suckling systems (P>0.10), but its mean concentration throughout lactation was lower in ADLIBC than in ADLIBH (2.56 vs. 3.08 mmol/l, respectively; P<0.05). Serum cholesterol increased from week 7 of lactation onwards in both parity groups (mean before and after that week: 2.39 vs. 2.82 mmol/l in ADLIBC, 2.56 vs. 3.07 mmol/l in ADLIBH; P<0.05).

Serum NEFA concentration were affected by nursing frequency and week post-partum interaction (P<0.05; Figure 2). Multiparous cows from RESTR1 had lower NEFA than their RESTR2 and ADLIBC counterparts on weeks 7 and 9 of lactation (P<0.05) whereas both RESTR1 and RESTR2 treatments showed lower serum NEFA than ADLIBC on week 11 post-partum (P<0.05).

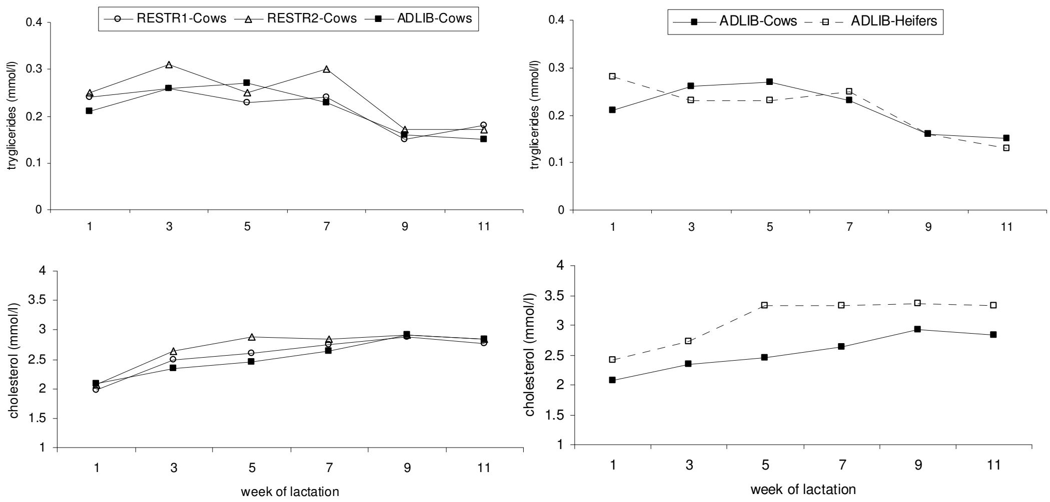

Figure 1. Serum concentration of triglycerides (SE= 0.03 mmol/l) and cholesterol (SE= 0.18 mmol/l) during the post-partum period in beef cows and heifers with different types of calf management (RESTR1= once-daily for 30 min, RESTR2= twice-daily for 2 x 30 min, ADLIB=ad libitum). [Serum triglycerides were not affected either by suckling restriction or parity (P>0.10), but they were affected by week of lactation (P<0.05). Serum cholesterol did not differ across suckling systems (P>0.10), but it was affected by parity and week of lactation (P<0.05)].

Also, the evolution of serum NEFA throughout the post-partum period varied according to parity (P<0.05), being greater in ADLIBC than in ADLIBH the concentration of these metabolites on week 1 and after week 7 of lactation (P<0.05).

Nursing frequency affected the mean serum concentration of β-hydroxybutyrate, which was lower in RESTR1 and RESTR2 than in ADLIBC (0.22 and 0.23 vs. 0.26 mmol/l, respectively, P<0.05; Figure 2). Nevertheless, the mean serum concentration of β-hydroxybutyrate remained steady throughout lactation (P>0.10). Parity did not affect the concentration of serum β-hydroxybutyrate (P>0.10).

The serum concentration of urea was not affected by either calf management or parity (P>0.10). The concentration of this protein metabolite was greater on weeks 5 and 7 than in the rest of lactation (5.07 vs. 4.46 mmol/l, respectively; P<0.05).

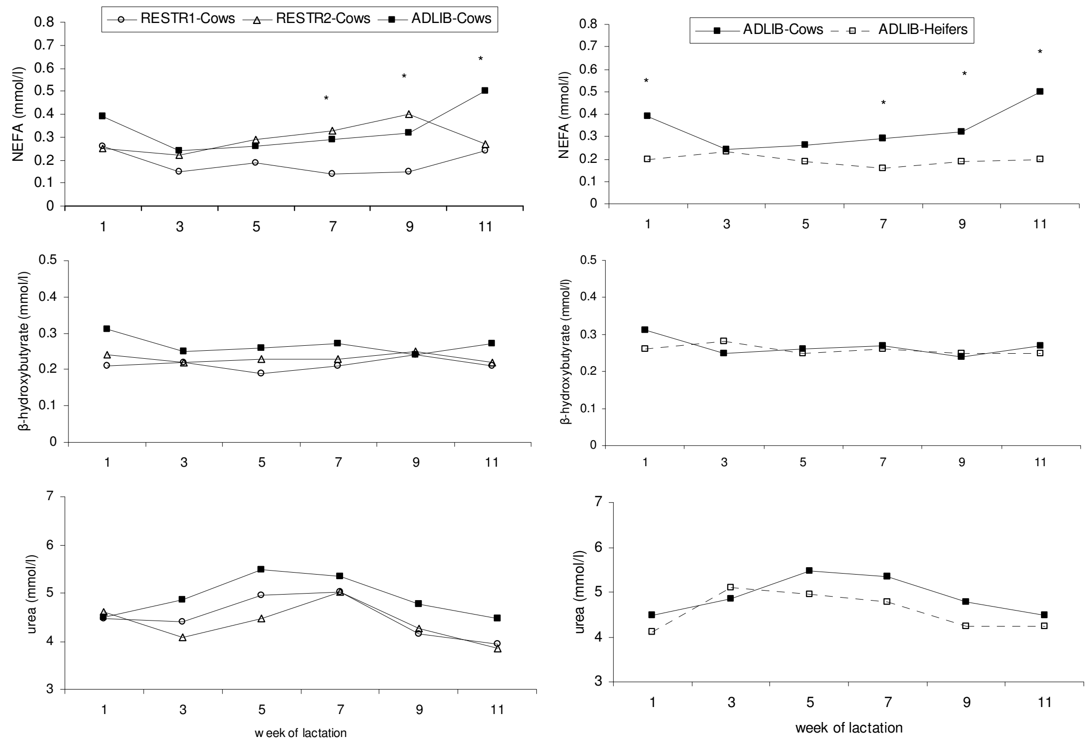

Figure 2. Serum concentration of non-esterified fatty acids (NEFA) (SE= 0.06 mmol/l), β-hydroxybutyrate (SE= 0.02 mmol/l) and urea (SE= 0.36 mmol/l) during the post-partum period in beef cows and heifers with different types of calf management (RESTR1= once-daily for 30 min, RESTR2= twice-daily for 2 x 30 min, ADLIB=ad libitum). [There was an interaction between nursing frequency and week post-partum on NEFA (*P<0.05). Nursing frequency but not parity affected β-hydroxybutyrate (P<0.05), and this remained steady throughout lactation (P>0.10). Urea was not affected by either calf management or parity (P>0.10), but it was affected by week of lactation (P<0.05)].

The mean plasma concentration of IGF-I did not differ among suckling systems or week of lactation (P>0.10; Figure 3). However, ADLIBC had lower IGF-I than ADLIBH throughout the post-partum period (54.7 vs. 71.8 ng/ml, respectively; P<0.05).

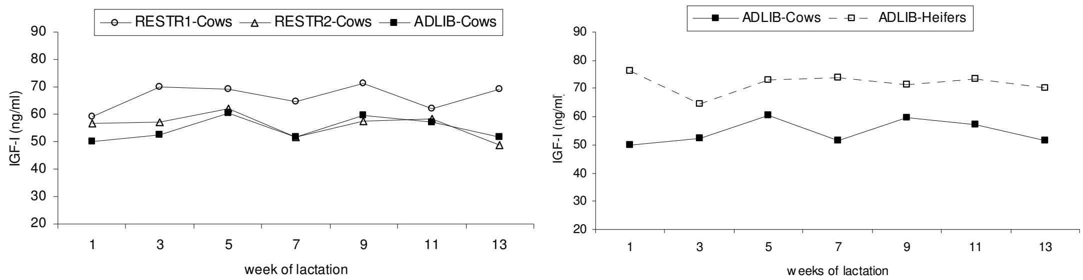

Figure 3. Plasma concentration of IGF-I (SE= 6.8 ng/ml) during the post-partum period in beef cows and heifers with different types of calf management (RESTR1= once-daily for 30 min, RESTR2= twice-daily for 2 x 30 min, ADLIB=ad libitum) [Endocrine IGF-I did not differ among nursing systems or week of lactation (P>0.10), but parity affected this hormone (P<0.05)].

Resumption of ovarian cyclicity

Suckling restriction did not affect the interval to first post-partum ovulation or oestrus in multiparous cows (Table 5; P>0.10). In contrast, cows had shorter post-partum intervals to first ovulation than heifers (P<0.05). There were no differences across suckling treatments or parity groups in the variances of interval from calving to first ovulation (P>0.10). The equality of variances of this parameter in the cows with first oestrus detected was verified across suckling treatments (P>0.10) but it was not observed across both parity groups (P=0.05). In addition, the variances of the interval from calving to first oestrus did not vary across suckling treatments (P>0.10) but they differ across parities (P<0.05).

Thirty-five out of 63 cows (55.6%) had a short oestrus cycle after first ovulation, irrespective of treatment and parity (P>0.10). According to the used automatic activity recorder, 38 out to 49 cows (77.6%) showed behavioural signs of oestrus before the first two-three ovulations post-partum. The remaining 14 cows did not ovulate throughout the experimental period, being evenly distributed across groups (P>0.10). Out of the cows displaying oestrus, only 34.2% (13/38) showed increased activity before the first post-partum ovulation, regardless of treatment or parity (P>0.10). A transient increase in progesterone without behavioural signs of oestrus prior to the second ovulation was detected in 20 out of 38 cows (52.6%). Normal length of the first oestrus cycle after calving without behavioural signs of oestrus was observed in 5 out of 38 cows (13.2%).

Table 5. Reproductive performance of beef cows and heifers with different types of calf management (RESTR1= once-daily for 30 min, RESTR2= twice-daily for 2 x 30 min, ADLIB=*ad libitum*) (Least square means±SD)

	Cows			Heifers	*P*-value †	
	RESTR1	RESTR2	ADLIBC	ADLIBH	T	P
n	15	14	17	17	-	-
Interval to first post-partum ovulation, days	46±21	52±26	58^{y}±26	79^{x}±34	0.40	0.05
First oestrus cycle duration, days	14±7	14±7	14±5	10±4	0.98	0.10
Interval to first post-partum ovulation in cows with first oestrus detected, days [1]	35±13	40±18	49^{y}±20	74^{x}±34	0.24	0.07
Interval to first post-partum oestrus, days	45±20	44±16	56±17	73±32	0.32	0.17

† T, Treatment, P, Parity. Within a row, means without a common superscript letter differ (*P*<0.05) within treatments ($^{a, b}$) or parity ($^{x, y}$).

[1] First oestrus was expressed or detected in 77.6% of the cows, regardless of treatment and parity (P>0.10).

The ovarian resumption pattern based on the analysis of survival curves over lactation did not differ among types of calf access (Figure 4; P>0.10). There was a tendency for different ovarian resumption pattern between parities (P=0.07), with a greater proportion of cows than heifers being cyclic at breeding (8 week after calving) and at the end of the third month post-partum.

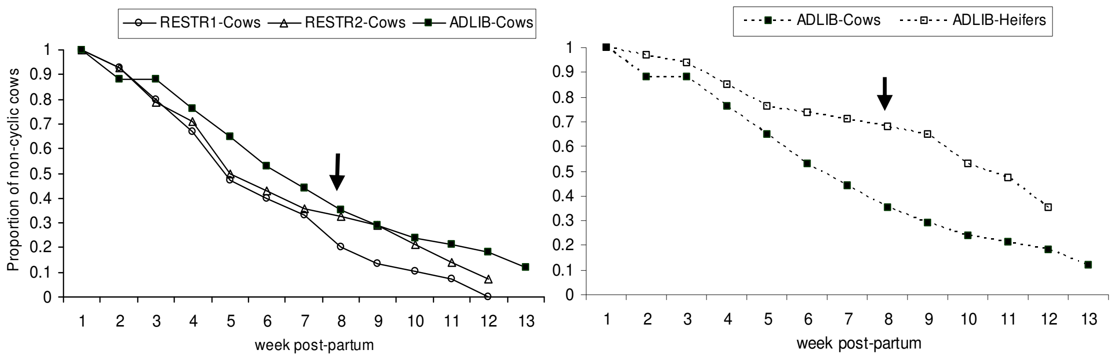

Figure 4. Survival curves for the proportion of non-cyclic cows and heifers during the first three mo post-partum when conducting different types of calf management (RESTR1= once-daily for 30 min, RESTR2= twice-daily for 2 x 30 min, ADLIB=ad libitum) (arrows denote date of bull introduction). [The ovarian resumption pattern did not differ among types of calf access (P>0.10), but there was a tendency for different ovarian resumption pattern between parities (P=0.07)]

Discussion

In the present experiment, there was no effect of calf management or parity on the concentration of faecal glucocorticoid metabolites after calving. However, they declined in the second and third day compared to the level on day of parturition. Glucocorticoids (or their metabolites) have been suggested as potential indicators of physiological stress (Möstl and Palme, 2002). As their excretion metabolites are detected in faeces, they represent a non-invasive technique to monitor adrenocortical activity (Möstl et al., 2002).

Considering that the concentration of glucocorticoid metabolites in a ruminant faecal sample reflects the cortisol production about 10-12 h earlier (Möstl and Palme, 2002), the result from approximately 12 h post-partum may be triggered by the foetal-induced increase of maternal cortisol around parturition (Patel et al., 1996). The subsequent suckling restriction did not play an adrenal response in once-daily and twice-daily suckling cows because otherwise it would be expected that cortisol had been metabolised into faecal derivatives.

Peripheral cortisol was reduced in dual purpose cows when they were allowed to nurse their calves after mother-young visual contact during milking (Hernández et al., 2006). In the current study, the continuous visual and olfactory contact with calves' pen might have attenuated the stressful response after separation. In fact, glucocorticoids may produce energy mobilisation during short-term stress (Raynaert et al., 1976), but herein the three suckling systems had similar circulating

NEFA at the first week post-partum. Despite the fact that multiparous cows nursing *ad libitum* had similar faecal glucocorticoid metabolites to primiparous cows with the same management, cows showed greater serum NEFA than heifers at the first week of lactation.

Multiparous cows nursing once-daily had greater BW gains and BCS at the end of the third month of lactation than the rest of groups. Conversely, their milk yield was the lowest and their milk fat content was the greatest. Nevertheless, the rest of milk nutrients did not differ across treatments, being the energy-standard milk yield similar among them. This slight autocrine control of the mammary gland might be mediated by the protein feedback inhibitor of lactation, which matches at a local level immediate supply to immediate demand (Flint and Knight, 1997), and this may explain the differences in calf growth among groups.

Parity influenced BW at calving. Heifers had reached around 88% of their expected mature weight (560 kg) by the time they calve. Both multiparous and primiparous cows nursing *ad libitum* lost BW throughout lactation, but multiparous tended to maintain better body condition than primiparous cows. Accordingly, Freetly et al. (2006) demonstrated that overall efficiency of energy retention in young beef cows was similar to that of dairy cows, even though their demands for milk production were lower. Milk yield and composition was similar in cows and heifers when both had free access to their calves, in line with their parallel calf ADG. Thus, milk yield and calf growth rate of primiparous cows was offset to the detriment of body condition recovery of first-calf dams.

Among the blood biochemical constituents studied, suckling restriction only affected the serum concentration of NEFA throughout the post-partum period and the mean β-hydroxybutyrate levels. Once-daily nursing cows showed the lowest NEFA values during the second and third month of lactation, in agreement with the greatest ADG and BCS registered in this lot at the end of the experimental period. Circulating levels of NEFA are a reliable index of the magnitude of adipose tissue mobilization (Bell, 1995). In beef cows, the greatest nutritional demands arise by the time the peak of milk yield is attained, which may have occurred herein during the second month post-partum.

Heifers nursing *ad libitum* showed greater mean peripheral cholesterol and IGF-I and lower NEFA than cows with the same calf management. This trend was not observed by Meikle et al. (2004) in Holstein cattle managed under a pasture-based feeding system. Considering that blood cholesterol is a constituent of cell membranes (Bruss, 1997) and IGF-I is positively related to bone growth and muscle development (Connor et al., 2000), we suggest that the post-partum feeding level in the present work may allow better match with growth demands than the one attained in certain grazing conditions. By contrast, although blood cholesterol is a precursor of steroid hormones (Grummer and Carroll, 1988), the greater peripheral levels of this substrate in primiparous cows did not advance their onset of ovarian cyclicity nor improved the proportion of females in oestrus at first ovulation.

In multiparous cows, serum concentration of triglycerides decreased as lactation progressed, whereas that of cholesterol increased concomitantly. The nadir when triglycerides dropped and cholesterol rose significantly was at week 7 post-partum, irrespective of the suckling system. Bastidas et al. (1990) reported that serum cholesterol was lower while serum NEFA was greater in Brahman cows that were fed to loose BW than in those that were fed to maintain BW during lactation. The response of lipoproteins in the current experiment may indicate an increased lipogenic activity together with decreased energy requirements from late second month of lactation onwards.

Plasma IGF-I concentration was not significantly affected by the suckling restriction, but the profile of this metabolic hormone in once-daily suckling cows was numerically different from their twice-daily and *ad libitum* suckling contemporaries at several points of the post-partum period. In a recent review, Velázquez et al. (2008) suggested that endocrine IGF-I acts as a monitoring signal that allows reproductive events to occur when nutritional conditions for successful reproduction are reached. Accordingly, this trait may be linked with the slight differences (although not significant) among cows in the interval to first post-partum ovulation. Nevertheless, it must be pointed out that peripheral IGF-I during lactation was rather steady. Beef compared to dairy cattle may not uncouple their somatotropic axis after calving due to the inactivation of the growth hormone-receptor (GHR) expression (Lucy, 2008). The few loss of liver GHR induces more IGF-I production in response to GH in beef genotypes. The failure to uncouple this axis leads to a relatively anabolic endocrine state throughout lactation.

In the current work, the interval to first post-partum ovulation in multiparous cows (52 days averaged) was shorter than in an earlier experiment (74 days) conducting the same managements under more moderate post-partum feeding levels (100 vs. 80 MJ ME/day) (Álvarez-Rodríguez et al., 2009). However, the post-partum interval herein was longer than the average for the autumn-calving group identified by cluster analysis (31 days) in these mountain conditions (Sanz et al., 2004). The resumption of ovarian cyclicity in this experiment was probably constrained by lower pre-partum gains than usual during the grazing period. Collectively, calf restriction during lactation did not advance significantly the resumption of ovarian cyclicity in any restricted suckling system, thereby not justifying the increase of labour associated with daily calf moving and housing to their pen. However, the proportion of non-cyclic cows at breeding was numerically lower in once-daily than in twice-daily and *ad libitum* nursing (20 vs. 33 and 35%, respectively).

The lack of reproductive differences across nursing treatments might have been caused by the partial contact between cows and calves in restricted suckling groups. The fact that cows could still see, hear and even touch their calves probably prevented any major treatment differences in both stress and reproductive responses. In this regard, strict calf isolation is recommended to benefit from the implementation of restricted suckling (Stagg et al., 1998; Marongiu et al., 2002).

The interval to first post-partum ovulation was shorter in cows than in heifers when both had free access to their calves. Grimard et al. (1992) reported that reproductive performance was poorer in primiparous than in multiparous suckled beef cows due to their lower stocks of glucose precursors (amino acids and glycerol) than fully grown cows. Also, Sinclair et al. (1998) suggested that mechanisms controlling the anabolic processes governing maternal growth are antagonistic towards the mechanisms controlling reproduction. These authors found that the catabolism of lean tissue rather than fat tissue during the early post-partum period impaired reproductive performance in young cows. Nevertheless, we did not detect differences in peripheral urea (a by-product of protein catabolism) between adult cows and heifers.

There was a tendency for longer first oestrus cycle duration in multiparous than in primiparous cows, although this trait was not associated with a different proportion of short oestrus cycles between parities. Indeed, Sartori et al. (2004) reported an average oestrus cycle length of 23.0 and 20.8 days for cows and heifers, respectively.

When patterns of circulating progesterone were compared with dates of first oestrus after calving, we observed that only 34.2% of the cows expressed oestrus at first post-partum ovulation. This fact coincides with the observations of Mackey et al. (2000), who noted while performing daily ovarian scanning that the same afore-mentioned proportion of cows had clinical changes in the reproductive tract (such as increased mucus and uterine tone) associated with oestrus, regardless of suckling system.

Furthermore, first oestrus before an increase in progesterone was not expressed or not detected in 22.4% of the cows. These values are close to another experiment in winter-calving cows of the same herd (Álvarez-Rodríguez and Sanz, 2009) and highlight a potential failure to elicit oestrus expression in nearly one fourth of the cows. Oestrogen, specifically oestradiol-17ß, is the primary signal to the hypothalamus that induces oestrus, but only in the absence of progesterone (Vailes et al., 1992). Several ovarian function abnormalities (as the development of luteinised follicles) which lead to temporal or persistent low oestrogen and/or high progesterone in blood might explain partly the lack of increase in the activity patterns of this proportion of cows.

In those situations that calf management does not allow overcoming anovulatory condition, several pharmacological therapies might be taken into account. Currently, the treatment of anoestrus cows can be performed through progesterone to ensure that the first ovulation is associated with expression of oestrus and a normally functioning luteal phase. Also, the use of eCG may accompany progesterone treatment in cows that are in deep anovulatory anoestrus to ensure ovulation (Crowe, 2008).

The ovarian resumption pattern tended to differ between cows and heifers nursing *ad libitum*, with lower proportion of cyclic cows at breeding in the latter group. The feeding level in *ad libitum* suckled heifers was not enough to guarantee their target ADG gains. According to the prediction equation obtained by Agabriel and Petit (1987), primiparous cows in this experiment may have achieved an average voluntary intake capacity of 14.8 kg (13.2 kg DM) while the amount of TMR supplied was 13 kg (11.6 kg DM). Therefore, if heifers were fed *ad libitum*, they may have eaten 12.1% more feed to overcome their lower ability to retain energy, but their post-partum adaptation constraint, if any, did not impair their metabolic homeostasis.

It is drawn that emphasis on heifer's diet should be made not only during lactation but also during last trimester of pregnancy (Engelken, 2008). In fact, BW gains throughout the pre-partum grazing period were two-fold greater in multiparous than in primiparous cows. In addition, early weaning cannot be ruled out as a consistently good strategy to induce ovulation in post-partum heifers (Quintans et al., 2009). However, these authors suggest not removing calves younger than 60 days old to avoid a decline in the offspring performance.

In conclusion, suckling restriction and parity were factors that did not affect cow's welfare after parturition. Twice-daily and *ad libitum* suckled cows showed a similar productive and metabolic behaviour which differ from their once-daily suckled counterparts. This response did not trigger remarkable differences in their reproductive parameters. *Ad libitum* suckled cows had different metabolic traits compared to heifers, as well as a shorter duration of the post-partum anovulatory period than *ad libitum* suckled first-calf dams.

Implications

In conditions of post-partum level of feeding set at maintenance, we hypothesize the reproductive performance might mirror the pre-partum gains throughout the previous grazing season rather than reflect the influence of type of calf management. The current reproductive parameters might indicate that mother-young bonding was poorly attenuated because nursing restriction was not linked to complete calf isolation between suckling periods.

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5.3. Metabolic and luteal function in winter-calving Spanish beef cows as affected by calf management and breed

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ORIGINAL ARTICLE

Metabolic and luteal function in winter-calving Spanish beef cows as affected by calf management and breed

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Summary

This experiment aimed at evaluating the effect of calf management and breed on the metabolic and luteal function of post-partum beef cows fed at maintenance. Fifty multiparous cows, 22 Parda de Montaña (PA) and 28 Pirenaica (PI), were assigned to either suckling once-daily for 30 min (RESTR) or *ad libitum* (ADLIB) from the day after calving. Blood samples were collected to analyse metabolites [non-esterified fatty acids (NEFA), β-hydroxybutyrate, total protein and urea)], insulin-like growth factor-I (IGF-I) and progesterone (P4) at different intervals. Cows from RESTR maintained their live-weight (LW) over the first 3 months post-partum, whereas ADLIB cows lost nearly 4% LW. Both genotypes showed similar LW gains during this period ($p > 0.10$). Calf daily gains were lower in RESTR than in ADLIB treatment ($p < 0.05$), but similar across breeds ($p > 0.10$). Milk and lactose production were lower in RESTR cows than in ADLIB ($p < 0.05$). Milk and protein yield were greater in PA than in PI breed ($p < 0.05$). Serum NEFA, total protein and urea were higher in PI cows suckling ADLIB than in the rest ($p < 0.05$). Cows from PI breed had greater NEFA values than PA ones on the first week post-partum ($p < 0.001$). Circulating IGF-I was not affected by suckling frequency, breed nor their interaction ($p > 0.10$). Suckling frequency, but not breed, affected the interval from calving to first ovulation ($p < 0.001$), being shorter in RESTR than in ADLIB cows. In conclusion, the *ad libitum* suckling practice improved cow milk yield and offspring gain compared to once-daily suckling for 30 min from the day after calving, at the expense of impairing the onset of cyclicity. The effect of calf management was confounded with breed on the studied blood biochemical constituents, but any of these metabolites influenced the role of endocrine IGF-I in these genotypes.

Introduction

Suckler cattle farms on South-European mountain areas have increased throughout last decades their herd dimension and forage land area, thereby achieving greater use of pastures. This situation has produced some management changes within farming systems, especially over the duration of the housing period and the calf suckling strategy during lactation.

The traditional calf management system used to take into account one or two restricted suckling periods during morning and/or evening short sessions in the barn. However, at present calves are maintained continuously with dams both in and outdoors. Suckling stimulus has been shown to be the most important factor contributing to extended post-partum acyclicity when nutrition is not a limiting factor (Short et al., 1990; Williams, 1990; Stagg et al., 1998), involving more than mammary-somatosensory pathways (Williams et al., 1993).

Parda de Montaña (PA) and Pirenaica (PI) are two suckler cattle breeds widely spread throughout northern Spain. The former comes from the selection for beef and mothering abilities from the old Brown Swiss, which was introduced in the country two centuries ago as a dual-purpose breed (milk-beef). The latter is an autochthonous hardy breed from the mountain area of Pyrenees, which was utilized in the past as a triple-purpose breed (work-milk-beef) and currently is used for beef production. Despite having relatively similar mature weight, the live-weight (LW) evolution during the grazing season is different between breeds (Casasús et al., 2002), mainly due to lower gains of PA compared to PI cows. Furthermore, milk potential and intake capacity throughout the post-partum period are greater in PA cows so that their higher milk yield can be supported (Casasús et al., 2004).

Even though some traits of the phenotypic performance have been already studied, little is known about the metabolic and endocrine signals triggering specific mechanisms of adaptive response when cows are challenged with different suckling managements. Several authors have suggested that chronic and acute alterations in blood substrates and metabolic hormones might link the neural effects of suckling to the control of the GnRH pulse generator (Williams and Griffith, 1995), signalling the hypothalamic-pituitary-ovarian axis as to the metabolic status of the animal (Diskin et al., 2003; Wettemann et al., 2003).

Thereby, we hypothesized that the profile of certain blood metabolites related with energy and protein catabolic pathways [non-esterified fatty acids (NEFA), β-hydroxybutyrate, total protein, urea)], together with the endocrine regulator insulin-like growth factor-I (IGF-I), might be a useful tool to monitor the nutrient prioritization of dams in the short term. The aim of this study was to assess the effects of suckling frequency (once vs. *ad libitum*) and breed (PA vs. PI) on the metabolic and reproductive function of winter-calving Spanish beef cows.

Material and methods

Animals and experimental design

Fifty multiparous winter-calving cows (aged 8.5 ± 3.4 years, mean ± SD), 22 Parda de Montaña (PA) and 28 Pirenaica (PI), were selected from the herd of 'la Garcipollera' Research Station (North-eastern Spain, 42°37′N, 0°30′W, 945 m a.s.l., average mean temperature 10.2 ± 0.2 °C and annual rainfall 1059 ± 68 mm throughout the period 1999–2006). Cows grazed on mountain pastures during early autumn (mid pregnancy) and they were housed in late autumn (last 3 months of pregnancy).

Dams from both breeds lost LW during the autumn grazing (10 weeks). The initial and final LW on that moment was 548 ± 50 and 522 ± 57 kg in PA and 598 ± 75 and 589 ± 76 kg in PI, respectively (maternal weight plus conceptus). At housing, body condition score (BCS) was 2.38 ± 0.19 and 2.58 ± 0.20 in PA and PI, respectively (1–5 scale, Lowman et al., 1976). Indoors, cows were group-fed 10 kg (as-fed basis) of a total dry mixed ration (Table 1) during 10 weeks to meet energy and protein requirements for maintenance and pregnancy (NRC 2000). This feeding level was planned to achieve a moderate body condition at calving (around 2.5).

The day after parturition (mean 18 February ± 18.2 days), cows were randomly assigned, within breed, to two suckling frequencies: Once-daily restricted suckling during a 30 min-period at 08:00 h (RESTR) and *ad libitum* suckling (ADLIB). Treatments were balanced according to cow LW and BCS, calf LW at birth and calf sex.

Cow-calf pairs remained indoors throughout lactation in a loose-housing system with straw-bedded pens. In the restricted suckling treatment, calves remained in groups in fenced cubicles adjacent to dams' resting areas with no visual or olfactory isolation.

Cows after parturition were group-fed the same total mixed ration once-daily at 09:00 h (13 kg for

Table 1 Chemical composition of the total mixed ration used in the experiment*

Dry matter (DM) (g/kg)	905
Ether extract (g/kg DM)	12
Ash (g/kg DM)	77
Crude protein (g/kg DM)	92
Neutral-detergent fibre (g/kg DM)	554
Acid-detergent fibre (g/kg DM)	312

*Feedstuffs (g/kg fresh-weight basis): barley straw (470), barley grains (126), dehydrated alfalfa (100), beet molasses (80), citric pulp pellets (72), maize gluten meal (54), soybean meal (38), rape meal (38), alfalfa pellets (12) and vitamin and mineral supplement (10). Vitamin and mineral supplement contained per kg (fresh-weight basis): Ca 107 g, P 85 g, Cl 156 g, Mg 9 g, Na 102 g, S 20 g, Fe 4 g, Zn 12 g, vitamin A 12000 IU/kg, vitamin D3 1200 IU/kg, vitamin E (α-tocopherols 91%) 53 mg/kg, Cu 20 mg/kg.

PA, 12 kg for PI, as fed basis; Table 1). The diet met maintenance requirements for energy and protein in a 550- or 590-kg beef cow producing approximately 9 kg or 7.5 kg of energy-corrected milk (ECM), in PA and PI, respectively (NRC 2000). There were no feed refusals (or eventually negligible) throughout the experiment. Cows were supplied water and mineral supplements *ad libitum*. Calves did not receive any feed supplement other than milk throughout the first 3 months of lactation. One bull of proven fertility from the same breed was introduced into each treatment pen on week 8 post-partum (3 months after the first theoretical calving date) during a 9-week breeding season.

The care and use of animals followed the European guidelines (European Union Directive No. 86/609/CEE 1986).

Measurements

Cows and calves were weighed before morning feeding within 24 h after calving and thereafter at weekly intervals during the first 3 months post-partum. Cow and calf LW variation throughout this period was calculated by linear regression of LW against time. LW at calving was considered as the mean between LW within 24-h post-partum and the one registered the week after. BCS was assessed at calving and at the end of the first 3 months post-partum.

Milk yield and composition was measured on week 3 of lactation in all the cows of the experiment and on week 7 and 12 in a sub-sample of seven cows per treatment. On the day of milking, calves were removed and cows were administered an intramuscular injection of oxytocin (40 UI, Gineamin, Laboratorios, Maymó, Barcelona) 5 min before milking to facilitate milk letdown. Cows were milked by using a portable milking machine. Milk collected from the initial milking was subsequently discarded. Cows were kept separated from their calves for 6 h, and then milked a second time. Milk weight was recorded after this milking, and individual 40 ml aliquots were retained for fat, protein and lactose analysis through infrared (Milkoscan 4000™; Foss-electric Ltd., Hillerod, Denmark). Final milk weight was multiplied by four to provide an estimate of 24-h milk production. Daily (24 h) milk constituent excretion (kg/day) was calculated by multiplying constituent concentration by daily milk production. The production of ECM was calculated through the equation: $\mathrm{ECM} = \text{milk production (kg)} \times [(0.38 \times (\text{milk fat (\%)}) + 0.24 \times (\text{milk protein (\%)}) + 0.78)/3.14)]$.

Chemical analyses

Feed samples were kept at weekly intervals and composited over fortnight intervals. Samples were dried at 60 °C until constant weight and mill-ground (1 mm screen). Dry matter, ash, ether extract and protein ($N \times 6.25$) contents were determined according to the AOAC methods (AOAC, 1999). Neutral-detergent fibre and acid-detergent fibre analyses were carried out by following the sequential procedure of van Soest et al. (1991).

Blood sampling and assays

Blood samples (5 ml) were collected twice weekly before morning feeding by tail vessel puncture into heparinized tubes during the post-partum period. In addition, blood samples were withdrawn fortnightly with the same procedure into plain glass tubes and allowed to clot. Samples were centrifugated at $3000 \times g$ for 15 min at 4 °C. Serum was used to determine blood metabolites (fortnightly). Plasma aliquots were prepared to analyse IGF-I (fortnightly) and P4 (twice weekly). Serum and plasma were harvested and stored at −20 °C until analysis.

Serum concentration of β-hydroxybutyrate (enzymatic method), total protein (Biuret method) and urea (Glutamate dehydrogenase method) were determined with an automatic analyzer (Olympus AU400; Olympus, Hamburg, Germany). Reagents for β-hydroxybutyrate were supplied by Randox (Randox Laboratories Ltd., Crumlin Co., Antrim, UK) and those for total protein and urea were provided by the analyzer manufacturer (Olympus®; Olympus System Reagent, Clare, Ireland). The mean intra- and inter-assay coefficients of variation (CV) for the afore-mentioned metabolites were <3.7% and <9.6%, respectively. Serum NEFA were analysed in duplicate using a commercial kit (Randox Laboratories Ltd.). Commercial reference serum samples (bovine precision serum, Randox Laboratories Ltd.) were used to evaluate the accuracy of the analysis. The mean intra- and inter-assay CV were <5.1% and <7.4%, respectively.

Plasma IGF-I was analysed through solid-phase enzyme-labelled chemiluminiscent immunometric assay (Immulite®; Siemens Medical Solutions Diagnostics Limited, Llanberis, Gwynedd, UK). Bovine plasma samples previously analysed through a commercial enzyme immunoassay kit (Blanco et al., 2008) were re-assayed to evaluate the accuracy of the analysis. A regression analysis was performed to compare the results obtained with both methods

($r = 0.96$). The mean intra- and inter-assay CV were <3.1% and <12.0%, respectively.

Plasma P4 was measured using a solid-phase radioimmunoassay commercial kit (Coat-A-Count P4 kit®; Diagnostic Products Corporation, Los Angeles, CA, USA). The mean intra- and inter-assay CV were <8.0% and <10.4%, respectively. The sensitivity averaged 0.03 ng/ml. The onset of luteal activity after calving was considered when P4 levels were >0.5 ng/ml for short cycles and >1 ng/ml for normal cycles. If cows had not ovulated prior to the end of fourth month post-partum, the interval to first ovulation after calving was regarded as this date and all experimental procedures were terminated. P4 rises lasting for a single sampling were considered short oestrus cycles (8–12 days) after first ovulation.

Statistical analyses

Data were analysed using the SAS statistical software (SAS Institute Inc., Cary, NC, USA). Cow-calf LW, BCS and reproductive performance of cows were analysed according to the general linear model procedure:

$$y_{ij} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ij}$$

where: y_{ij}, dependent variable, μ, overall mean, α_i, suckling treatment effect, β_j, breed effect and ε_{ijk}, residual error.

Calf sex was included as fixed effect in the previous model when analysing birth weight and daily gains during the first 3 months of lactation.

Cow milk production and composition, blood metabolites and IGF-I hormone were analysed by means of the following mixed model (Mixed procedure):

$$y_{ijkl} = \mu + \alpha_i + d_j + \beta_k + \delta_l + (\alpha\beta)_{ik} + (\alpha\delta)_{il} + (\beta\delta)_{kl} + \varepsilon_{ijklf}$$

where: y_{ijk}, dependent variable, μ, overall mean, α_i, suckling treatment effect, d_j, animal random effect j, β_k, breed effect, δ_l, week of lactation effect and ε_{ijk}, residual error.

Data are reported as least square means and their associated SE. The level of significance was set at 0.05. Levels of significance between 0.06–0.10, were considered to show trends. The interactions were commented in the text only when they approached statistical significance ($p < 0.10$).

Differences between proportions were analysed using the Fisher exact (FREQ procedure). Survival analysis utilizing the LIFETEST procedure was used to evaluate the effects of suckling frequency and breed on the interval to first ovulation. The survival analysis was a regression of the number of anoestrous cows at weekly intervals over the first 3 months post-partum (censored observations). The Wilcoxon test was used to examine the differences between the survival curves.

Results

Productive performance

Cows from both suckling managements had similar LW and BCS at calving ($p > 0.10$; Table 2), but those from RESTR maintained their LW over the first 3 months post-partum, whereas ADLIB cows lost nearly 4% of their initial LW throughout this period. There was a trend towards higher body condition in

Table 2 Productive performance of beef cows and calves managed under once-daily (RESTR1) or *ad libitum* (ADLIB) suckling systems in Parda de Montaña (PA) and Pirenaica (PI) breeds

	Suckling (S)		Breed (B)			p-value†	
	RESTR1	ADLIB	PA	PI	SE	S	B
Cows							
LW at calving (kg)	564	575	549	590	14	NS	0.06
Daily LW variation during first 3 months post-partum (kg)	0.01	−0.25	−0.14	−0.10	0.04	***	NS
BCS at calving	2.59	2.58	2.48	2.69	0.04	NS	**
BCS end of third month post-partum	2.70	2.56	2.60	2.66	0.05	0.06	NS
Calves							
Male to female ratio	9/14	13/14	9/13	13/15	–	NS	NS
LW at birth (kg)‡	40.3	41.1	41.3	40.1	1.1	NS	NS
Daily gain during first 3 months of lactation (kg)	0.70	0.82	0.79	0.73	0.03	*	NS

LW, live-weight; BCS, body condition score.
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, NS, not significant ($p > 0.10$).
†S × B, not significant ($p > 0.10$), SE, standard error.
‡Calf sex was a significant effect ($p < 0.05$).

RESTR than in ADLIB cows at the end of the first three months of lactation (p = 0.06).

Cows of PA breed tended to weigh less than their PI counterparts at calving (p = 0.06), but both genotypes showed similar LW gains during the first 3 months post-partum (p > 0.10).

Calves from both treatments and breeds weighed similar at birth (p > 0.10), but males were heavier than females on that moment (42.6 vs. 38.9 kg; p < 0.05). Calf daily gains during the first 3 months of lactation were lower in RESTR than in ADLIB treatment (p < 0.05), but similar across breeds (p > 0.10).

Milk yield and composition are shown in Table 3. Milk and lactose production were lower in RESTR cows than in ADLIB (p < 0.05). Milk and protein yield were greater in PA than in PI breed (p < 0.05). However, differences were smaller when milk production was adjusted for energy-standard content. Milk fat and lactose content were lower in PA than in PI breed (p < 0.05), but milk protein content was not affected by genotype (p > 0.10).

Milk yield was higher throughout the first and second month of lactation than in the third one (8.2 and 7.6 vs. 7.0 kg/day; p < 0.05); ECM production was greater throughout the first month post-partum than onwards (8.6 vs. 7.6 and 7.2 kg/day, respectively; p < 0.05).

Blood metabolites

Serum NEFA, total protein and urea were affected by the interaction between suckling frequency and breed (p < 0.05; Fig. 1), the concentration of all these metabolites being higher in blood of PI cows suckling ADLIB than in the rest.

Table 3 Milk production and composition of beef cows suckling once-daily (RESTR1) or *ad libitum* (ADLIB) from Parda de Montaña (PA) and Pirenaica (PI) breeds

	Suckling (S)		Breed (B)			p-value†	
	RESTR1	ADLIB	PA	PI	SE	S	B
Milk production (kg)							
Milk yield	6.8	8.4	8.4	6.8	0.4	*	**
Fat yield	0.31	0.33	0.34	0.31	0.02	NS	NS
Protein yield	0.24	0.28	0.28	0.24	0.01	0.07	*
Lactose yield	0.32	0.40	0.39	0.33	0.02	**	0.06
ECM	7.3	8.3	8.4	7.2	0.5	NS	0.08
Milk composition (%)							
Fat content	4.52	3.99	4.03	4.48	0.15	*	*
Protein content	3.56	3.31	3.39	3.49	0.07	*	NS
Lactose content	4.67	4.82	4.59	4.90	0.05	*	***

ECM, energy-corrected milk yield.
*p < 0.05, **p < 0.01, ***p < 0.001, NS, not significant (p > 0.10).
†S × B, not significant (p > 0.10), SE, standard error.

Serum NEFA was not influenced by the interaction between suckling treatment and week of lactation (p > 0.10), but PI cows had greater NEFA values than PA cows on the first week post-partum (p < 0.001; Fig. 2). Serum β-hydroxybutyrate was higher in cows suckling ADLIB than in RESTR on week 3 of lactation (p < 0.01), but had similar values elsewhere. There was no breed effect on this blood ketone (p > 0.10).

Serum total protein and urea did not differ across suckling frequencies or breed with advancing lactation (p > 0.10; Fig. 2). Serum urea was greater on weeks 7 and 9 post-partum than in the rest (5.94 and 5.79 vs. 5.23 mmol/l, respectively; p < 0.05).

Endocrine IGF-I

Circulating IGF-I was not affected by suckling frequency, breed nor their interaction (p > 0.10). Likewise, this hormone did not differ across suckling managements or breed with advancing lactation (p > 0.10; Fig. 3). IGF-I was lower from week 3 to 7 post-partum than during the rest of the post-partum period (averaged 55.4 vs. 62.5 ng/ml; p < 0.05).

Reproductive performance

Suckling frequency affected the interval from calving to first ovulation, being shorter in RESTR than in ADLIB cows (56 vs. 87 days, p < 0.001). However, there was no breed effect on this parameter (69 vs. 73 days, in PA and PI, respectively; p > 0.10). The proportion of cows with a short oestrus cycle after first ovulation did not differ across treatments (13/23 vs. 18/27 in RESTR and ADLIB, respectively; p > 0.10) or breeds (13/22 vs. 18/28, in PA and PI, respectively; p > 0.10).

The ovarian resumption pattern differed across suckling managements (p < 0.001; Fig. 4), leading to higher proportion of cyclic cows at breeding (8 weeks after calving) and at the end of the first 3 months post-partum in RESTR than in ADLIB treatment. Nevertheless, both breeds had similar ovarian resumption pattern throughout lactation (p > 0.10).

Discussion

The suckling system influenced more than genotype the cow-calf gains during early and mid lactation. This trait was linked with different milk production

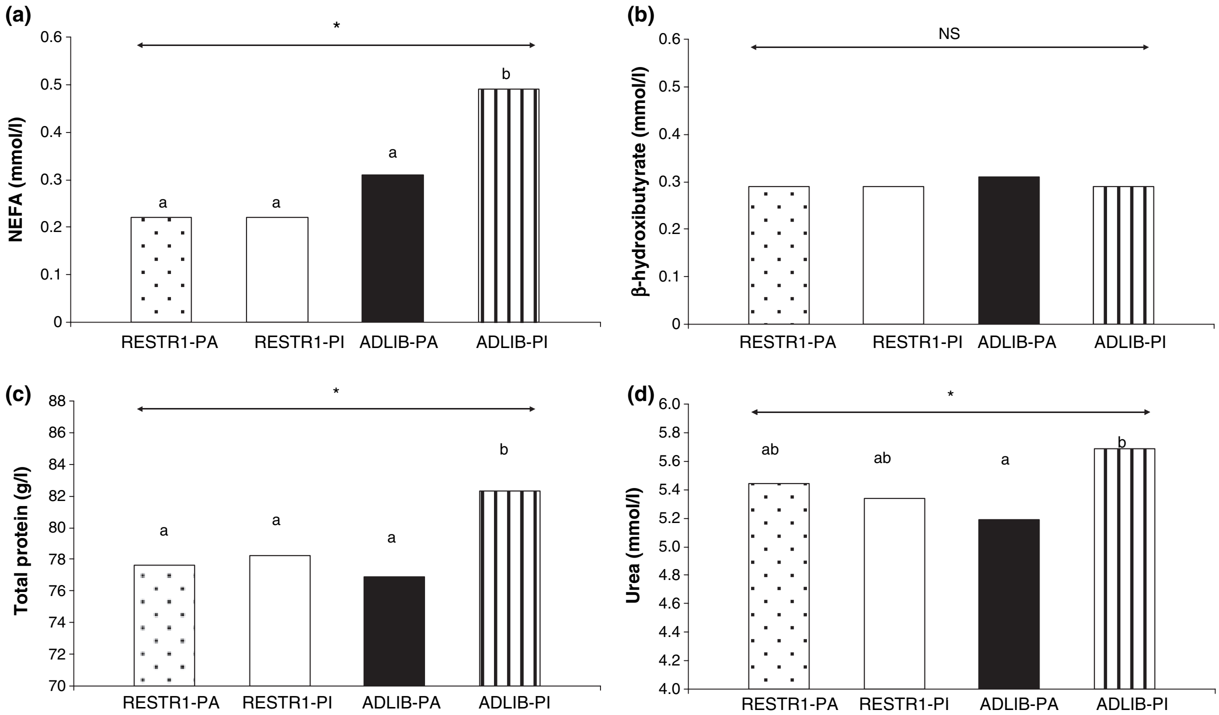

Fig. 1 Serum concentrations of non-esterified fatty acids (NEFA) (a), β-hydroxibutyrate (b), total protein (c) and urea (d) in beef cows suckling once-daily (RESTR1) or *ad libitum* (ADLIB) from the Parda de Montaña (PA) and Pirenaica (PI) breeds (average from sampling week 1–13 post-partum). Within each metabolite, different superscript denote statistical differences ($p < 0.05$); *$p < 0.05$; NS, not significant ($p > 0.05$).

but similar energy-standardized milk yield in cows with different calf management. In an evaluation of energy requirements in lactating beef cows, Petit and Micol (1981) reported that cows with a zero energy-balance (calculated) had a significant weight loss of 0.12 kg/day while no change in LW was obtained with a metabolizable energy intake 16.2 MJ above the theoretical energy requirements. In the present experiment, *ad libitum* suckled cows may have more difficulties to overcome a feasible negative energy balance compared to cows suckling once-daily. In addition, yields of milk protein and lactose were higher in cows kept always together with their calves than in their restricted suckled counterparts. This output trait may involve a significant mobilization of body water and labile body protein, which are required for milk protein and glucose synthesis (Bauman and Currie, 1980).

Regarding breed effect, earlier works have proven the greater ability of Pirenaica cattle to recover LW and body condition compared to suckled Brown Swiss cows (currently Parda de Montaña). This characteristic was observed when diets were set according to their milk potential during the housing period (early and mid lactation) (Sanz et al., 2003) as well as throughout grazing on high mountain ranges and forest pastures (late lactation to mid pregnancy) (Casasús et al., 2002). In the current experiment, the breed effect on cow productive performance was less severe than in the afore-mentioned studies but yet detectable. Milk fat and lactose content were greater in Pirenaica than in Parda de Montaña breed, which might be caused by a more marked udder filling due to lower mammary capacity in the hardy breed (Pirenaica) compared to the old dual-purpose breed (Parda de Montaña).

There was an interaction between suckling system and breed in most of the analysed blood substrates. *Ad libitum* suckled cows from the Pirenaica breed showed greater serum NEFA than the rest of lots on the first week post-partum and overall the first 3 months of lactation. However, the mobilization of NEFA from adipose tissue was not associated with concomitant increases in their oxidative metabolite (β-hydroxybutyrate). Elevated blood NEFA could be the result of increased adrenergic stimulation of lipolysis by catecholamines during and soon after parturition (Bell, 1995). In addition, peripheral NEFA have been suggested as indirect criteria for stress evaluation (García-Belenguer and Mormède,

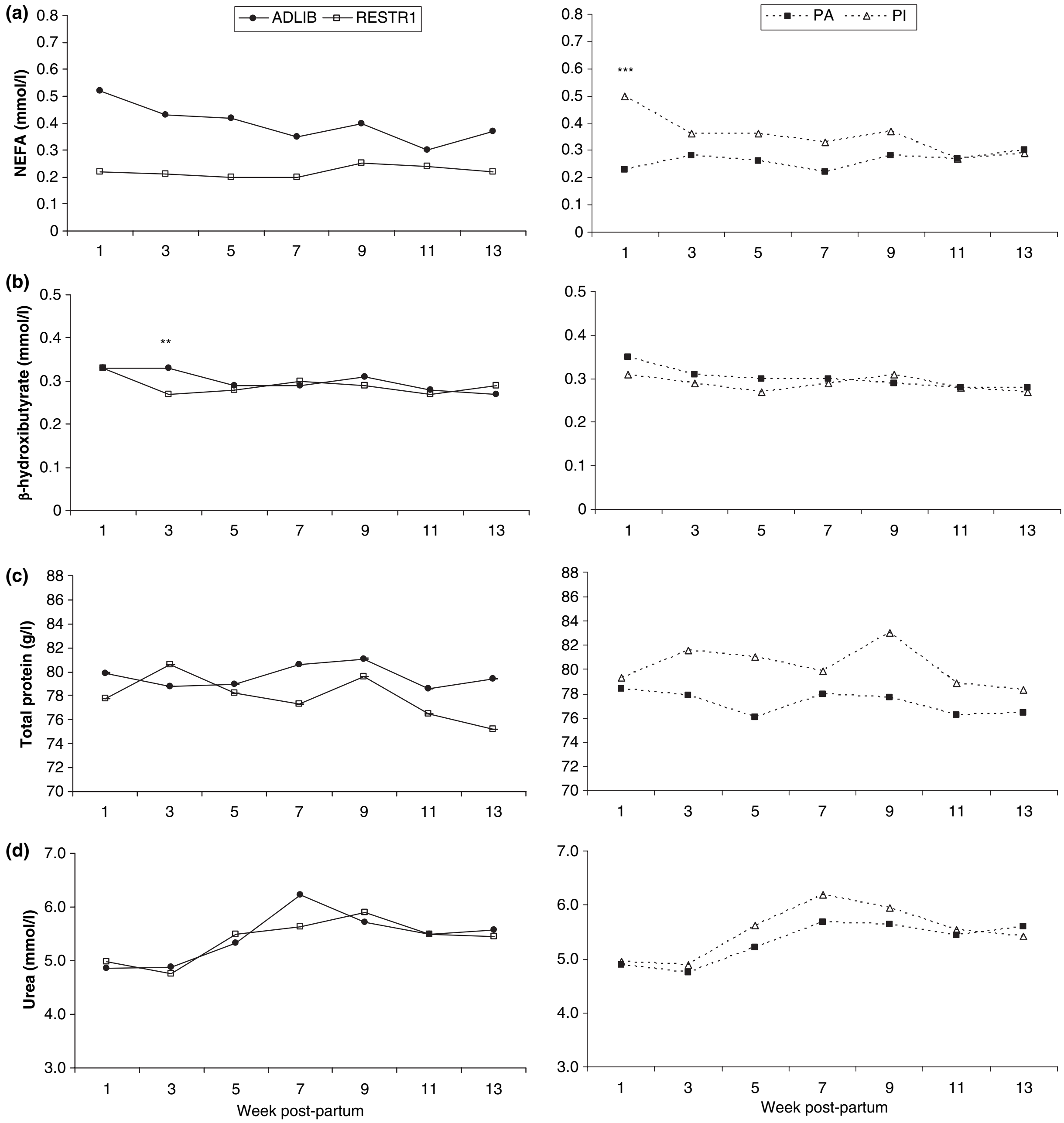

Fig. 2 Serum concentrations of non-esterified fatty acids (NEFA) (a), β-hydroxibutyrate (b), total protein (c) and urea (d) in beef cows suckling once-daily (RESTR1) or *ad libitum* (ADLIB) in Parda de Montaña (PA) and Pirenaica (PI) breeds. **p < 0.01; ***p < 0.001.

1993). Previous works have demonstrated that Pirenaica cattle had higher basal NEFA levels (San Juan, 1993) and the latter are more reactive against management practices than Parda de Montaña ones (García-Belenguer et al., 1996; Palacio et al., 1997). Therefore, we hypothesized that Pirenaica cattle might protect more strongly their offspring when managed under continuous access to calves, since their greater blood NEFA were not accompanied with higher LW losses or impaired re-establishment of luteal activity.

Ad libitum suckled cows from Pirenaica breed had also greater serum total protein than the rest of treatments and they showed greater serum urea than Parda de Montaña cows suckling *ad libitum*. The lower levels of blood protein and urea in the latter group may reflect slightly lower peripheral protein availability in response to higher milk

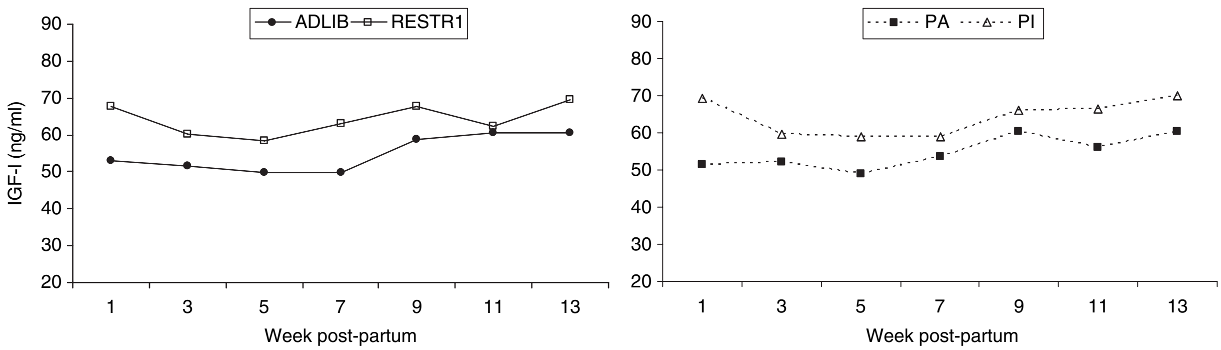

Fig. 3 Plasma concentration of insulin-like growth factor-I (IGF-I) in beef cows suckling once-daily (RESTR1) or *ad libitum* (ADLIB) in Parda de Montaña (PA) and Pirenaica (PI) breeds.

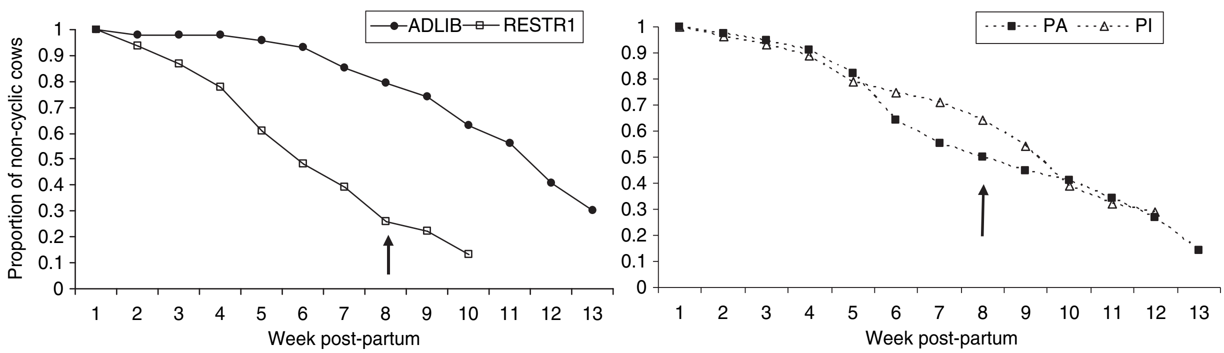

Fig. 4 Survival curves for the proportion of non-cyclic cows during the first trimester post-partum when suckling once-daily (RESTR1) or *ad libitum* (ADLIB) and in Parda de Montaña (PA) and Pirenaica (PI) breeds (arrows denote date of bull introduction).

protein output (Kaneko, 1997). However, these biochemical constituents did not reflect either any sign of undernutrition or trigger a delayed resumption of the ovarian activity after calving in this treatment. Collectively, the lower NEFA, total protein and urea in *ad libitum* suckled Parda de Montaña cows compared to their Pirenaica counterparts, suggest that lipid metabolism and protein synthesis may be different between these breeds when they are challenged with continuous calf presence. Nonetheless, the studied blood substrates were below the thresholds identifying limited adaptive performance (Hachenberg et al., 2007) or under-nourishment (Agenäs et al., 2006) in all treatments.

The metabolic regulator IGF-I was not affected by suckling system or breed, but it showed different trend throughout lactation. The greater values on the first week post-partum possibly reflected the homeorhetic mechanism which redirects energy utilization to support lactation (Bell, 1995). The lower levels of plasma IGF-I on the first and second month post-partum may have been modulated by the greater level of milk production during this time. The greater plasma IGF-I after week 7 of lactation was parallel to the mean resumption of luteal activity in cows suckling once-daily but not in those dams suckling *ad libitum*, regardless of breed. As plasma concentration of this hormone did not differ across suckling frequencies, the four-week delay in the first ovulation after calving in *ad libitum* suckling cows might be related to direct inhibiting effects of maternal-offspring cues (via endogenous opioid peptides) on luteinizing hormone secretion and its receptors within the ovary (Saleri et al., 1998), which may impair the subsequent first post-partum ovulation.

Restricting suckling once-daily from the day after parturition shortened the interval to first post-partum ovulation compared to *ad libitum* suckling, but exerted similar effect on the observed frequency of short oestrus cycles after first ovulation. The effect of calf management on the length of post-partum anovulation interacts strongly with pre- and post-partum feeding level (Sanz et al., 2004), thereby not enabling detailed comparison among literature reviews (Montiel and Ahuja, 2005). Overall, the benefits of once-daily suckling seem more emphasized in similar conditions to the current study, which were characterized by feeding levels planned to achieve a moderate body condition at calving (around 2.5 on a 1–5 scale) and to meet maintenance requirements during early and mid lactation. In extensive production systems run on harsh environments, this diet could be supplied during the winter housing period, which is the unique part of the year in which farmers are able to control rations and take part on the herd feeding decisions. Restricted suckling allowed nearly all cows to ovulate prior to the end of the first 3 months of lactation. This trait is useful to maximize the opportunities of pregnancy success during short mating periods, as to concentrate breeding prior to summer grazing on communal lands located on high mountain ranges.

In conclusion, the *ad libitum* suckling practice improved cow milk yield and offspring gain compared to once-daily suckling for 30 min from the day after calving, at the expense of impairing the onset of cyclicity. The effect of calf management was confounded with breed on the studied blood biochemical constituents, but any of these metabolites influenced the role of endocrine IGF-I in these genotypes.

Acknowledgements

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5.4. Does breed affect nursing and reproductive behaviour in beef cattle?

Does breed affect nursing and reproductive behaviour in beef cattle?

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Abstract

This experiment was designed to assess the role of genetic differences in mother-young bonding and the resumption of post-partum ovarian cyclicity in beef cows with different types of calf management. Twenty-four multiparous winter-calving cows, 12 Parda de Montaña (**PA**) and 12 Pirenaica (**PI**), were randomly assigned to once-daily restricted nursing during 30 min (**RESTR**) or *ad libitum* nursing (**ADLIB**). Cow-calf behaviour was recorded at weeks 3, 8 and 13 of lactation. Results were compared within suckling system. Twice-weekly blood samples were drawn throughout lactation to analyse progesterone as indicator of ovulation.

Within each type of calf management, both breeds nursed their calves for a similar total time (23.0 and 57.2 min in PA vs. 25.9 and 59.0 min in PI, when nursing once-daily or *ad libitum*, respectively). Furthermore, they initiated similar ovarian cyclicity after calving (70 vs. 73 d in PA and PI, respectively), although it was shorter in RESTR than in ADLIB (54 vs. 89 d).

Key words: beef cattle, calf management, restricted nursing, post-partum anoestrus

Introduction

Suckling has been shown to play a key inhibitory effect on cattle re-breeding, since cows recognize their own calf through visual and olfactory cues that allow to establish a maternal bond with the young throughout lactation (Williams and Griffith 1995). Hoffman et al. (1996) have demonstrated that cows whose calves remained continually present but were restricted from suckling had shorter post-partum anestrous intervals than cows whose calves were continually present and allowed to suckle *ad libitum*. This cow-calf bond along with mammary stimulation (Lamb et al. 1997) may cause the negatively related effect of suckling stimulus on the resumption of ovarian cyclicity.

Several nursing systems have been used to overcome the suckling effects on reproductive performance (Randel 1981, Galina et al. 2001, Krohn 2001). In the

Spanish Pyrenees, the most usual practice in order to shorten the calving interval were once or twice a day restricted nursing for short periods in the morning and/or afternoon.

Dairy cattle breeds (pure or crossbred) appeared to display less marked maternal behaviour than *Bos indicus* genotypes (Das et al. 2001) or even than *Bos taurus* breeds selected for beef (Le Neindre 1989), although others did not find a significant association *(*Paranhos da Costa et al. 2006). Parda de Montaña (**PA**) and Pirenaica (**PI**) are two suckler cattle breeds widely spread throughout northern Spain. Historically, cattle having PA genetics were selected for beef and mothering abilities from Brown Swiss, whose ancestors were introduced to Spain two centuries ago as a dual-purpose breed (milk-beef). In contrast, PI is a local breed from the mountain area of the Spanish Pyrenees. These cattle have been utilized in the past as a triple-purpose breed (work-milk-beef) but in the last century they have been selected for beef production. Permanently keeping calves with their dams has been more stressful for PA than for PI cows in terms of duration of post-partum anoestrus (Sanz et al. 2003, Sanz et al. 2005). Thus, we hypothesised that PA cows nurse their calves for longer time each day than PI, attaining a more intense relationship with their offspring and this may delay the resumption of their ovarian cyclicity after calving.

Material and methods

Animals and experimental design

Twenty-four multiparous winter-calving cows (aged 8.8±3.3 yr, mean±standard deviation), 12 Parda de Montaña (PA) and 12 Pirenaica (PI), were randomly selected from the herd of 'La Garcipollera' Research Station (North-eastern Spain, 42°37′N, 0°30′W, 945 m a.s.l., average mean temperature 10.2±0.2 º C and annual rainfall 1059±68 mm throughout the period 1999-2006). Both breeds had identical rearing management during their life. Prior to the beginning of the study, cows were allowed to graze in mid pregnancy on mountain pastures (early autumn) and they were housed at the beginning of the last trimester of pregnancy and were provided supplementary feeding (late autumn).

The day after parturition (mean 21 February±16 d) cows were assigned, within breed, to one of two nursing regimens: once-daily restricted nursing during a 30 min-period beginning at 08:00 h (RESTR) and *ad libitum* nursing (ADLIB). Cattle were allotted in four pens (two breeds under two different nursing systems). Treatments were balanced according to cow bodyweight (BW) and body condition score (BCS) (Lowman et al. 1976), calf birth BW and calf sex.

Cow-calf pairs remained indoors in a loose-housing system with straw-bedded pens throughout 4 months of lactation. In RESTR treatment, calves remained in groups in fenced cubicles (5 m x 5 m) adjacent to dams' resting area with no visual, tactile or auditory isolation. In this treatment, cows and calves could not introduce their heads through the fence but they could approach their snouts through it. The fence (1.5 m height) was made on steel barriers placed horizontally with a gap of 15 cm among them.

After parturition cows were fed in groups a total mixed ration once-daily at 09:00 h (13 kg for PA, 12 kg for PI, as-fed basis) composed of 58% forages and 42% grains, by-products and vitamin and mineral supplements (90.5% dry matter, 9.2% crude protein, 55.4% neutral-detergent fibre, 31.2% acid-detergent fibre, on dry matter basis). The diet met maintenance requirements for energy and protein in a 550- or 590-kg beef cow producing about 9 kg or 7.5 kg of energy-corrected milk in PA and PI, respectively (NRC 2000). There were no feed refusals throughout the experiment. Water and mineral block supplements were provided *ad libitum*. Calves did not receive any forage or concentrate supplement throughout lactation and they had access to water during suckling periods (RESTR) or continuously (ADLIB).

The care and use of animals followed the European guidelines (European Union Directive No. 86/609/CEE 1986).

Recording of behaviour activities

Behaviour recordings were obtained in 8 sub-groups composed of 3 cow-calf pairs (a total of 24 pairs) balanced for calf sex, at weeks 3, 8 and 13 of lactation. There were a total of 24 recording days. Dams were allowed to nurse their calves in a barn (4.5 m x 7.5 m) that was located close to their resting area. Each sub-group of cows was brought into the recording barn (which was a sub-part of the main barn) according to their calving date, followed immediately by their calves. The RESTR cow-calf pairs were kept in the recording barn for 30 min. The ADLIB sub-group was brought into the barn 24 h prior to sampling in order to favour acclimatisation and they were kept there for the following 24 hours, during which their nursing behaviour was recorded. The composition of sub-groups remained constant across observing days.

Every cow-calf pair was randomly allocated each day to one trained observer. In RESTR treatment, behaviour activities were continuously live recorded and additional continuous video recordings were used as back up for occasional uncertain observations. In the ADLIB treatment, the observations were performed through video recording using the instantaneous scan sampling technique (Martin and Bateson 1993) with 10-min sampling interval per cow-calf pair. This method involves extrapolation for the 10 min separating two successive scans to calculate the duration of nursing bouts. This interval fits the mean nursing duration recorded during more frequent samplings (Alvarez-Rodríguez et al. 2009). The pen was artificially illuminated at night (200 lx at 5-m height level) allowing the monitorization of the whole circadian cycle (24 h).

Every cow-calf pair had coloured neck collars to aid identification. In RESTR treatment, each observer (a total of 3) was responsible for recording behaviour of one individual cow. In ADLIB treatment, a single observer, who was also involved in recordings of the restricted nursing treatment, viewed all the video recordings. This observer was responsible for recording the activities of 3 cows.

The following events were recorded in both nursing systems: (a) cow nursing its own calf, (b) cow nursing alien calves, (c) cow sniffing and playing with its own calf (non-aggressive head butting), (d), cow licking its own calf, (e), cow licking its own body (self-grooming), (f) cow licking alien calves or other cows, (g) agonistic behaviour of a cow towards its calf or alien animals (head butting and kicking).

Cow sniffing and playing with its own calf was mutually exclusive of cow licking and agonistic behaviour of a cow towards its calf or alien animals. Furthermore, a cow only could lick its own calf, its own body or alien calves/other cows at the same time.

In RESTR, cow sniffing-playing with its own calf, licking and agonistic behaviour were measured as a rate per 30 min-period. In ADLIB, these were measured in minutes of time during 24 h. The bout duration of cow nursing its calf and of cow nursing alien calves were recorded and the nursing duration per day was calculated as the sum of nursing time per bout in each observation period (30 min or 24 h, respectively). A nursing bout started when the calf took a teat in its mouth and ended when the calf moved and stayed away from the udder (Das et al. 2001). In RESTR, the following nursing bout was considered after a period of other activities of at least 1 min from the previous bout. The circadian nursing

pattern in the ADLIB treatment was analysed descriptively as the mean time that a calf devoted to suckle every hour divided by the total nursing duration per day.
In RESTR, the continuous recording methodology allowed to detect certain additional activities including: (a) the latency to first contact of calf with dam's udder, (b) number of butts of calf towards cow's udder, (c) number of cow vocalizations (moos) and (d) total time in close proximity (<1 meter) between mother and young within each suckling session.

Productive measurements

Cows and calves were weighed before morning feeding within 24 h after calving. Thereafter, they were weighed at weekly intervals during the first three months post-partum. Cow and calf bodyweight (BW) gains throughout this period were calculated by linear regression of BW against time. Cow BW at calving was considered as the mean between BW within 24-h post-partum and the one registered the week after. This mean value was more accurate because the first BW post-partum was recorded prior to the expulsion of placenta in some dams. Body condition score (BCS) was assessed at calving and at the end of the first three months post-partum (Lowman et al. 1976).

Blood sampling and assay

Blood samples (5 ml) were collected twice weekly (Monday-Thursday) before morning feeding by tail vessel puncture into heparinised tubes throughout lactation. Cows were blood sampled in a longitudinal sweep where they got used to handling prior to the start of the experimental period. Samples were centrifuged at 3000 x *g* for 15 min at 4 º C. Plasma was harvested and stored at -20 º C until analysis of progesterone.
Peripheral progesterone was measured using a solid-phase radioimmunoassay commercial kit (Coat-A-Count P4 kit®, Diagnostic Products Corporation, Los Angeles, CA, USA). The mean intra- and inter-assay coefficients of variation were <8.0% and <10.4%, respectively. Mean sensitivity was 0.03 ng/ml. The onset of ovarian cyclicity after calving was considered when progesterone levels were ≥1 ng/ml in at least 3 or more consecutive samples. However, first short oestrus cycles (8 to 14 days) prior to the second ovulation were considered when progesterone rises >0.5 ng/ml were detected in 2 or less consecutive

samples. This peripheral progesterone concentration confirmed the disappearance of the dominant follicle when ovulation was also determined by ultrasonography (Sanz et al. 2003; Quintans et al. 2004). If cows had not ovulated prior to the end of fourth month post-partum, the interval to first ovulation after calving was regarded as this date and all experimental procedures were terminated.

Statistical analysis

Data were analysed with SAS statistical software (SAS 2002). The different sampling methodologies for behavioural traits did not allow a valid statistical comparison of nursing activities between suckling systems. Only the breed effect was tested in behaviour analyses. Previously, normal distribution was tested with the Shapiro-Wilk test but it could not be verified. Therefore, means according to breed and week of lactation were compared with the Kruskal-Wallis non-parametric test (PROC NPAR1WAY).

Data from cow-calf productive performance and the interval to first post-partum ovulation were tested with analysis of variance (PROC GLM), by considering breed and nursing system as fixed effects in the model. The model concerning calf birth BW and average daily gain contained also the fixed effect of calf sex.

Differences between breeds concerning the proportion of cows nursing alien calves were analysed using the Fisher exact test of the FREQ procedure.

Data are reported as least square means (if normally distributed) or means and their associated standard errors. Differences with a $P\leq0.05$ were considered significant, and those where $P<0.10$ were discussed as trends.

Results and discussion

Nursing behaviour during once-daily restricted nursing

The time of latency to first contact of calf with dam's udder was similar in both breeds (0.4±0.1 and 0.3±0.1 min, in PA and PI, respectively; $P>0.10$). In addition, there were no statistical differences in the number of suckling bouts within each daily period (1.7±0.2 and 1.8±0.3, respectively; $P>0.10$), the first suckling bout duration (20.7±1.7 vs. 23.6±1.5 min, respectively; $P>0.10$) and the second bout duration within each session (2.0±0.8 vs. 1.8±0.9 min, respectively; $P>0.10$), as well as the total number of butts towards cow's udder within a suckling session in order to stimulate milk ejection (77.5±10.2 vs. 72.3±8.8, respectively; P>0.10). Total nursing duration per daily period did not differ between breeds (Table 1, P>0.10), and accounted for 77% and 86% of the total time spent together within the PA and PI breeds, respectively. This high proportion of time allocated to suckle might be explained by the lack of alternative feeding apart from dam's milk. Pasture and concentrate supplementation for calves may reduce suckling duration per session up to 36% of the time in contact (Das et al. 2001).

The time for cows to allow alien calves to nurse was similar in both breeds (P>0.10), although this was nearly three-fold greater in PA compared to PI breed. The non-filial nursing time accounted for 7% and 3% of the total nursing time in PA and PI, respectively. The number of cows which nursed alien calves did not differ across breeds (50.0 vs. 66.7% in PA and PI, respectively; P>0.10).

Table 1. Behaviour activities of cows during once-daily restricted nursing (RESTR) for 30 min in Parda de Montaña (PA) and Pirenaica (PI) breeds

Continuous sampling (08:00-08:30 hours)	Breed		SE[z]	*P*-value
	PA	PI		
Nursing duration (min)	23.0	25.9	0.8	0.16
Non-filial nursing duration (min)	1.7	0.7	0.5	0.75
Sniffing-playing with own calf (no.)	4.8	4.4	0.9	0.78
Licking own calf (no.)	21.7	98.9	20.4	0.05
Self-grooming (no.)	0.8	1.1	0.5	0.29
Licking alien calves/cows (no.)	0.0	2.0	0.7	0.15
Agonistic behaviour (no.)	0.9	3.7	0.7	0.05

[z]SE = Standard error.

Cow sniffing-playing with its own calf, licking and agonistic behaviour were measured as a rate per 30 min period.

In contrast, cow-calf pairs from the PA breed spent less time in close proximity (<1 meter) throughout the 30-min nursing period than PI cows (24.6±1.3 vs. 27.9±0.8 min, respectively; P<0.05). The PA cows tended to vocalize with a lower rate per 30 min-period (0.4±0.2 vs. 2.3±1.2 times, respectively; P=0.06) and showed less agonistic behaviours towards its calf or other animals than PI dams (Table 1; P=0.05). Evidence shows a phenotypic relationship indicating that both reduced flightiness from humans and strong maternal care are associated with elevated maternal defensive aggression in beef cattle (Turner and Lawrence 2007). Previous studies have revealed a lower reactivity to human presence in PA compared to PI breed (Blanco et al. 2009a), but agonistic encounters towards alien animals were not greatest in PA cows of this study.

The number of suckling bouts per session was lower at week 3 than at weeks 8 and 13 of lactation (1.3±0.1 vs. 2.3±0.4 and 1.8±0.3, respectively; P=0.05). Total nursing time decreased as calves grew from week 3 to 8 and 13 of lactation (27.0±1.0 vs. 23.9±1.5 and 22.5±1.5 min, respectively; P<0.05). During this same interval, the number of butts of calf towards cow's udder declined (96.4±14.5, 72.8±8.8 and 55.5±7.5, respectively; P=0.10). Butting activity might reflect a problem of milk availability (De Passillé 2001). However, in this study the rate of butting was parallel to the decreasing trend of milk production throughout lactation (Álvarez-Rodríguez et al. in press). The reduction of nursing bouts and nursing time might be explained by an increase in the rate of milk intake by calves, since they were not supplemented with any other feed.

The number of licking of cows towards its own body was lower at weeks 3 and 8 than at week 13 of lactation (0.3±0.2 and 0.0±0.0 vs. 2.6±1.3, respectively; P<0.05). The number of agonistic events of cows towards its calf or other animals increased concomitantly (0.3±0.2 vs. 3.9±1.9 and 2.8±1.0, respectively; P<0.01). The rest of observed behaviours in RESTR treatment (latency to first contact with calf, sniffing-playing with own calf, licking own calf or alien calves/cows, number of cow vocalizations, time of cow-calf in close proximity) were not affected by the stage of lactation (P>0.10).

Nursing behaviour during continuous cow-calf access

Under continuous association husbandry of calves with their dams, nursing duration per day was similar in both breeds (Table 2). Cow-calf pairs spent around 1 h per day in suckling (4% of the 24 h-period). This trend is similar to other studies in which calves were raised exclusively on milk in confinement (3 to 4% of the day, Lewandrowski and Hurnik 1983; 5% of the day, Williams et al. 1984; 4% of the day, Álvarez-Rodríguez et al. 2009).

Cows from the PA breed nursed alien calves for longer compared to the PI breed (P<0.05). The non-filial nursing accounted for 19% and 5% of the total nursing time in PA and PI, respectively. The cattle breed producing more milk (PA) allowed more allosuckling to calves (Table 2). According to Vichova and

Bartos (2005), this activity has a compensative function and demonstrates insufficient growth and/or maternal supply. However, the number of cows which nursed alien calves did not differ across breeds (50.0 vs. 33.3% in PA and PI, respectively; P>0.10).

Table 2. Behaviour activities of cows during *ad libitum* nursing in Parda de Montaña (PA) and Pirenaica (PI) breeds

Scan sampling (00:00-23:59 hours)	Breed		SE[z]	*P*-value
	PA	PI		
Nursing duration (min)	57.2	59.0	3.5	0.80
Non-filial nursing duration (min)	10.7	2.8	1.9	0.05
Sniffing-playing duration with own calf (min)	1.3	0.0	0.4	0.11
Licking duration towards own calf (min)	22.0	22.8	3.3	0.71
Self-grooming duration (min)	6.0	5.0	1.2	0.90
Licking duration towards alien calves/cows (min)	1.3	6.7	1.4	0.08
Agonistic behaviour duration (min)	1.3	2.8	0.9	0.26

[z]SE = Standard error.

Cow sniffing-playing with its own calf, licking and agonistic behaviour were measured in minutes of time during 24 hours.

The PI breed doubled the time devoted to agonistic encounters against its calf or other animals compared to PA breed, although this difference was not statistically significant (P>0.10). Yet cows from PI breed showed a tendency for a greater social interaction with other cows and calves (allogrooming duration) than those from PA one (Table 2; P=0.08).

The recorded behaviour activities were not affected by the stage of lactation in the *ad libitum* suckled cows (P>0.10). The steady nursing pattern throughout lactation was not in keeping with the one of restricted nursing system. Probably, calves maintained with free access to their dams spent more non-effective suckling time throughout lactation than calves allowed to nurse only for 30 min daily periods.

The mean nursing patterns in PA and PI breeds throughout the day are described in Figure 1. Four nursing descriptive peaks were detected nearly coincident in both breeds: between 6:00 and 8:00 (21% and 13% of the total nursing time in PA and PI, respectively), between 14:00 and 15:00 (6% of the total nursing time in both breeds), between 17:00 and 19:00 (13% and 12% of the total nursing time, respectively), between 23:00 and 00:00 (6% of the total nursing time in both breeds) and an earlier night-time peak in PA (between 1:00 and 2:00, 9% of the total nursing time) than in PI (between 4:00 and 5:00, 9% of the total nursing time). These results might reflect a metabolic need of the calves to obtain milk at intervals not longer than 7 to 8 hours. Thus, the restricted nursing regimen clearly altered calf's feed requirements, but allowed the advance of dam's re-breeding compared to *ad libitum* nursing.

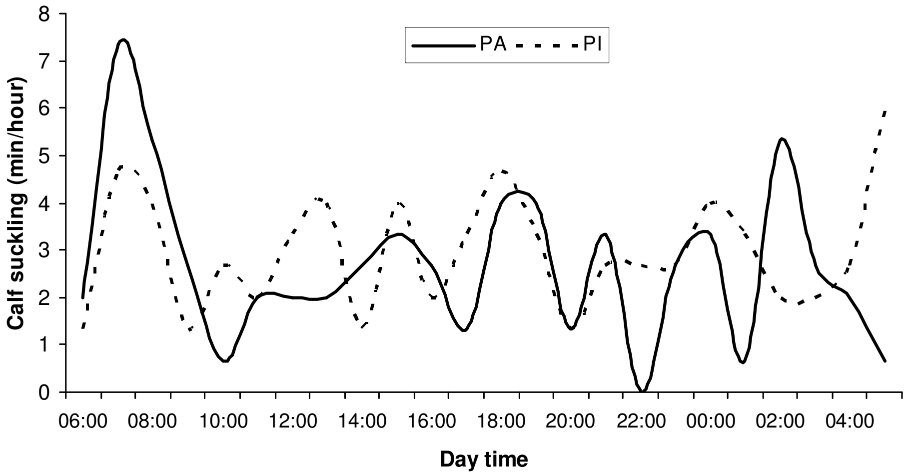

Figure 1. Mean circadian nursing pattern in Parda de Montaña (PA) and Pirenaica (PI) cows having continuous access with their offspring during lactation (each breed was kept in a separate pen indoors).

Around 50% and 58% of the total nursing time occurred between 6:00 and 18:00, in PA and PI, respectively. The existence of a high incidence of nursing during the nocturnal period (nearly 50%) is similar to some previous studies in free-ranging (Somerville and Lowman 1979) and in enclosure kept beef cows (Lewandrowski and Hurnik 1983). The occurrence of a nursing peak at sunrise (between 06:00 and 08:00 h) seems to be consistent in all the afore-mentioned studies, regardless of husbandry practices.

Animal performance

The BW and BCS of PA dams at calving was lower than for PI cows (Table 3), but similar across the two nursing systems (P>0.10). In contrast, cow BW gain and body condition at the end of the first 3 months post-partum was similar between breeds (P>0.10) but greater in the RESTR treatment compared to the

ADLIB treatment (P<0.001). Cows suckled only once during 24 hours gained around 1% of their BW whereas cows suckled *ad libitum* lost around 5% of their BW during this period. The onset of ovarian cyclicity was similar between breeds (P>0.10), but it occurred 5 weeks earlier in the RESTR treatment compared to the ADLIB treatment (P<0.001).

Calf birth BW tended to be greater in PA than in PI cows (Table 3, P=0.06), but this parameter was similar in both nursing systems (P>0.10). There was a tendency for greater calf daily gains in PA than in PI breed (P=0.08), although suckling duration was similar between breeds (P>0.10).

Table 3. Animal performance in Parda de Montaña (PA) and Pirenaica (PI) breeds nursing once-daily (RESTR) for 30 min or *ad libitum* (ADLIB).

	Breed (B)		Suckling (S)		SE[z]	P-value	
	PA	PI	RESTR	ADLIB		B	S
Cows							
Calving bodyweight (BW) (kg)	544	588	565	567	16	0.07	0.91
BW gain during first 3 months post-partum (kg d^{-1})	-0.18	-0.10	0.06	-0.33	0.04	0.24	<0.001
Body condition score (BCS) at calving	2.51	2.62	2.58	2.55	0.03	0.04	0.55
BCS end of third month post-partum	2.58	2.59	2.66	2.51	0.04	0.97	0.03
Interval to first post-partum ovulation (d)	70	73	54	89	6	0.66	<0.001
Calves							
Birth BW (kg)[y]	44.7	40.8	41.5	44.0	1.4	0.06	0.21
BW gain during first 3 months of lactation (kg d^{-1})	0.80	0.71	0.69	0.82	0.03	0.08	0.02

[z] SE = Standard error.

[y] Calf sex only affected calf birth BW, being greater in males than in females (45.0 vs. 40.5 kg; P<0.05).

Pre-weaning growth differences may be due to genetic differences between these two genotypes (Villalba et al. 2000, Casasús et al. 2002, Sanz et al. 2003). The design of this experiment did not allow to test whether differences in rate of growth were due to a limitation in milk supply or in the growth potential in early life. The mean energy-corrected milk yield in the present experiment tended to be greater for the PA than for the PI cows (8.4 vs. 7.2±0.5 kg, P=0.08, reported in Álvarez-Rodríguez et al. in press), but mean feed intake and average daily gain of calves were similar when they were offered the same diet *ad libitum* after weaning (Blanco et al. 2009b). As suckling duration was similar in both breeds regardless of husbandry practice, we theorized that the rate of milk intake might have been greater in PA calves than in those from PI breed to support higher rate of growth prior to weaning. In fact, Le Neindre (1989) suggested that the total suckling time should result from the need for mother–young physical contact instead of from milk production. Accordingly, suckling bout duration is taken rather as indication of calf's needs than of milk intake, as recently discussed in terms of suckling and allosuckling behaviour (Drabkova et al. 2008).

The similar nursing duration in both breeds may explain the lack of differences between genotypes in the interval to first ovulation after calving. However, the initial hypothesis concerning genotype differences in nursing duration did not hold true.

Calves from the ADLIB group grew faster than those from RESTR (P<0.05). The greater rate of growth in calves continuously maintained with their dams was attained by increasing more than two-fold the suckling time compared to once-daily suckling group. However, milk production of dams was only 1 litre greater in the ADLIB treatment than in RESTR treatment (8.3 vs. 7.3±0.5 kg l/day, P>0.10, reported in Álvarez-Rodríguez et al. in press). This free suckling behaviour impaired the onset of ovarian cyclicity in their dams, regardless of breed.

Within each type of calf management, cows from Parda de Montaña and Pirenaica breeds nursed their calves for a similar time. Furthermore, they initiated similar ovarian cyclicity after calving, although it was shorter in cows nursing once-daily than *ad libitum*.

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5.5. Physiological and behavioural responses of cows from two beef breeds submitted to different suckling strategies

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Physiological and behavioural responses of cows from two beef breeds submitted to different suckling strategies

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ABSTRACT

This experiment was designed to study the effects of avoiding calf contact and genotype on the metabolic, behavioural and reproductive traits of beef cows during lactation. Fifty-two multiparous cows, 25 Parda de Montaña (PA) and 27 Pirenaica (PI), fed at maintenance were assigned from the day after calving to twice-daily nursing (2× 30-min sessions at 0800 and 1600 h) either with fence contact with their calves (partial contact, PC) or without visual, tactile and olfactory contact (non-contact, NC) between suckling periods. Blood samples were collected to analyse metabolites (triglycerides, cholesterol, non-esterified fatty acids (NEFA), β-hydroxybutyrate and urea) and progesterone at different intervals. Cow–calf behaviour was monitored on weeks 4, 9 and 15 of lactation. Cow activity at oestrus was recorded through collars. Cows from both treatments and breeds showed similar live-weight gains during the first three months post-partum ($P > 0.10$). Milk yield and calf gains were not affected by treatment ($P > 0.10$) but they were greater in PA than in PI ($P < 0.05$). Plasma triglycerides and urea in the cows were not affected either by calf contact, breed or week post-partum ($P > 0.10$). Plasma cholesterol increased from week 6 post-partum onwards in PA cows ($P < 0.05$) while this rise was delayed to week 7 of lactation in PI breed ($P < 0.05$). Plasma NEFA was greater in blood from PC–PA cows than in the rest of groups ($P < 0.05$), and these metabolites were greater on week 1 and lower on week 11 than in rest of samplings ($P < 0.05$). Plasma β-hydroxybutyrate was not affected by either calf contact or breed ($P > 0.10$), but it was greatest on weeks 1–3 than in the rest of lactation ($P < 0.05$). Cows with PC calves took less time to first contact after they entered the barn than their NC counterparts ($P < 0.05$). Dams from both contact treatments nursed their offspring and remained in close proximity for similar time within suckling periods ($P > 0.10$). PA cows devoted more time than PI ones to lick their young ($P < 0.001$). There were no differences throughout lactation in any of the studied maternal behaviours ($P > 0.10$). Calf contact and breed did not affect the interval to first post-partum ovulation or oestrus in these cows ($P > 0.10$). Under twice-daily nursing conditions, the limitation of visual, tactile and olfactory contact with calves did not trigger different maternal or reproductive traits in these breeds but only a slightly higher mobilisation of body fat substrates in Parda de Montaña compared to Pirenaica.

1. Introduction

Suckling has been proved to be a major effect delaying the resumption of post-partum luteal function in beef cows when nutrition is not a limiting factor (reviewed by Short et al., 1990; Williams, 1990; Wettemann et al., 2003). The mechanism of inhibition is regulated by the hypothalamic–pituitary–ovarian axis and appears to require the establishment of a maternal bond (Williams and Griffith, 1995), rather than the stimulation of mammary somatosensory cues (Williams et al., 2001).

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Once-daily suckling from the day after calving has shortened post-partum anoestrus compared to *ad libitum* suckling when suckler cattle are fed at maintenance on Spanish mountain areas (Álvarez-Rodríguez et al., in press), but this practice has no effect when these cattle are slightly underfed during early and mid-lactation (Álvarez-Rodríguez et al., 2009). Some authors have suggested that restricted calf access without cow–calf contact between suckling periods after day 30 post-partum is even more useful to shorten the interval to first post-partum ovulation (Stagg et al., 1998), although this benefit has been more marked in large-frame crossbred genotypes than in small-frame pure Mediterranean breeds (Marongiu et al., 2002). Nevertheless, some of the afore-mentioned studies conducting once-daily suckling showed impaired calf growth compared to continuous mother–young contact (Marongiu et al., 2002; Álvarez-Rodríguez et al., in press), being this effect overcome by restricting suckling for twice-daily periods (Sanz, 2000).

Parda de Montaña (PA) and Pirenaica (PI) are two suckler cattle breeds widely spread throughout northern Spain. The former comes from the selection for beef and mothering abilities from the old Brown Swiss, which was introduced in the country two centuries ago as a dual-purpose breed (milk-beef). The latter is an autochthonous hardy breed from the mountain area of the Spanish Pyrenees, which was utilized in the past as a triple-purpose breed (work-milk-beef) and is currently used for beef production.

Despite having relatively similar mature weight, the live-weight (LW) gains during the grazing season are different between these breeds (Casasús et al., 2002), mainly due to lower gains of suckled Brown Swiss (currently PA cows) compared to PI cows. Furthermore, milk potential and intake capacity throughout the post-partum period are greater in PA cows so that their higher milk yield can be supported (Casasús et al., 2004). In earlier studies, PA cows have been more sensitive to calf presence than their PI counterparts as far as productive and reproductive performance is concerned (Sanz et al., 2003, 2004).

In this experiment, we hypothesized that the limitation of calf contact between twice-daily suckling periods may reduce the maternal bond and advance the ovarian resumption of dams, being this benefit greater in PA than in PI breed. In addition, we aimed at studying the effects of calf contact on cow energy metabolism during lactation.

2. Materials and methods

2.1. Animals and experimental design

Fifty-two multiparous cows (aged 7.0 ± 3.4 years, mean ± standard deviation), 25 Parda de Montaña (PA) and 27 Pirenaica (PI), were selected from the winter-calving herd of 'la Garcipollera' Research Station (North-eastern Spain), 42°37′N, 0°30′W, 945 m a.s.l., average mean temperature 10.2 ± 0.2 °C and annual rainfall 1059 ± 68 mm throughout the period 1999–2006). Cows grazed on mountain pastures during early autumn (mid-pregnancy) and they were housed in late autumn (last trimester of pregnancy).

Table 1
Chemical composition of the total mixed ration used in the experiment[a].

Dry matter (DM) (g/kg)	884
Ether extract (g/kg DM)	13
Ash (g/kg DM)	70
Crude protein (g/kg DM)	89
Neutral-detergent fibre (g/kg DM)	534
Acid-detergent fibre (g/kg DM)	285
Insoluble protein in the neutral-detergent fibre (g/kg DM)	33

[a] Feedstuffs (g/kg fresh-weight basis): barley straw (470), barley grains (126), dehydrated alfalfa (100), beet molasses (80), citric pulp pellets (72), maize gluten meal (54), soybean meal (38), rape meal (38), alfalfa pellets (12) and vitamin and mineral supplement (10). Vitamin and mineral supplement contained per kg (fresh-weight basis): Ca 107 g, P 85 g, Cl 156 g, Mg 9 g, Na 102 g, S 20 g, Fe 4 g, Zn 12 g, vitamin A 12,000 IU/kg, vitamin D3 1200 IU/kg, vitamin E (α-tocopherols 91%) 53 mg/kg, and Cu 20 mg/kg.

Dams from both breeds gained LW during the autumn grazing (8 weeks). The initial and final LW on that moment was 532 ± 47 and 541 ± 49 kg in PA and 546 ± 58 and 564 ± 62 kg in PI, respectively (maternal weight plus conceptus). At housing, body condition score (BCS) was 2.54 ± 0.10 and 2.61 ± 0.16 in PA and PI, respectively (0–5 scale, Lowman et al., 1976). Indoors, cows were group-fed 10 kg (as-fed basis) of a total dry mixed ration (Table 1) during 13 weeks to meet energy and protein requirements for maintenance and pregnancy (NRC, 2000). This feeding level was planned to achieve a moderate body condition at calving (around 2.5).

The day after parturition (mean 14 February ± 16.6 days) cows were randomly assigned, within breed, to either twice-daily calf access during two 30-min sessions (0800 and 1600 h) with fence contact with their calves (partial contact, PC) or without visual, tactile and olfactory contact (non-contact, NC) between suckling periods. Calves in the PC treatment remained in groups in fenced cubicles (5 m × 5 m) adjacent to dams' resting area with no visual, tactile or auditory isolation. In this treatment, cows and calves could not introduce their heads through the fence but they could approach their snouts through it. The fence (1.5 m height) was made on steel barriers placed horizontally with a gap of 15 cm among them. Calves in the NC treatment were kept 30 m away from dam's resting area in similar conditions to their contemporaries. Cows remained housed throughout lactation in pens (one pen per treatment, 12–14 cows/each), which had a feeding area outdoors (35 m × 5 m) and a straw-bedded area indoors (20 m × 6 m). Treatments were balanced according to cow LW and BCS at calving, calf LW at birth and calf sex.

Cows after parturition were group-fed the same total mixed ration once-daily at 0900 h (13 kg for PA, 12 kg for PI, as-fed basis; Table 1). The diet met maintenance requirements for energy and protein in a 555- or 585-kg beef cow producing about 9 or 8 kg of energy-corrected milk at peak yield, in PA and PI, respectively (NRC, 2000). There were no feed refusals (or eventually negligible) throughout the experiment. Cows were supplied water and mineral supplements *ad libitum*. Calves had free access to water but did not receive any feed supplement other than milk throughout the first three months of lactation. One bull of proven fertility from the same breed was introduced into each treatment pen on week 8 post-partum (3 months after the first theoretical calving date) during a 10-week breeding season.

All procedures were conducted according to the guidelines of the European Union (European Union Directive 86/609/EEC, 1986) on the protection of animals used for experimental and other scientific purposes.

2.2. Measurements

Cows and calves were weighed before morning feeding within 24 h after calving and thereafter at weekly intervals during the first three months post-partum (period determining the target 1 year calving interval). The balance had an accuracy of ±0.5 kg. Cow and calf LW gains throughout this period were calculated by linear regression of LW against time. Live-weight at calving was considered as the mean between LW within 24-h post-partum and the one registered the week after. This mean value was more accurate because the first LW post-partum was recorded prior to the expulsion of placenta in some dams. Cow BCS was assessed at calving and at the end of the first three months post-partum.

Milk yield and composition was measured on week 4 of lactation. On the day of milking, calves were removed and cows were administered an IM injection of oxytocin (40 IU, Gineamin, Laboratorios Maymó, Barcelona) 5 min before milking to facilitate milk letdown. Cows were milked by using a portable milking machine. Milk collected from the initial milking was subsequently discarded. Cows were kept separated from their calves for 6 h, and then milked a second time. Milk weight was recorded after this milking, and individual 40 ml aliquots were retained for fat, protein and lactose analysis through infrared (Milkoscan 4000^{TM}, Fosselectric, Ltd., UK). Final milk weight was multiplied by four to provide an estimate of 24-h milk production. The production of energy-corrected milk (ECM) was calculated through the equation: ECM = milk production (kg) × ((0.38 × (milk fat (%)) + (0.24 × (milk protein (%)) + 0.78)/3.14).

Behaviour recordings were performed in 24 cow–calf pairs (6 per treatment and breed) balanced for calf sex, on weeks 4, 9 and 15 of lactation. Cow–calf pairs were observed through instantaneous sampling (Lehner, 1996) by a single technician at 5-min intervals during the morning and afternoon 30-min suckling periods. The first record was set 1 min after calves enter dams' barn. In a protocol, the following activities were recorded: (a) latency to first contact between the dam and its calf; (b) cow and calf in close proximity (<1 m); (c) cow nursing its calf; (d) cow nursing an alien calf with or without its own; (e) cow licking calf; (f) cow sniffing their own calf; (g) aggressive butting or pushing by the focal cow towards an alien calf or cow (agonistic behaviour); (h) cow standing idling without calf in contact; (i) cow eating on the feeder; (j) cow exploring bed or eating straw bed; (k) cow drinking on the water through.

Some other behaviours as cow licking its own body (self-grooming), cow licking alien calf or other cows (social interaction) or playing between mother and young were initially included in the protocol on the basis of earlier works (Álvarez-Rodríguez et al., 2006), but they were discarded from data analysis because they never occurred at the scan time.

Behaviour recordings of cows nursing their own or alien calves may include one or several suckling bouts. The duration of each activity was extrapolated for the 5 min separating two successive samplings.

The first post-partum oestrus was monitored through an automated activity sensor (Alfa Laval Agri, Tumba, Sweden) placed on cow's neck collar.

2.3. Chemical analyses

Feed samples were kept at weekly intervals and pooled on fortnight intervals. Samples were dried at 60 °C until constant weight and mill-ground (1 mm screen). Dry matter, ash, ether extract and protein ($N \times 6.25$) contents were determined according to the AOAC methods (AOAC, 1999). Neutral-detergent fibre (NDF) and acid-detergent fibre (ADF) analyses were carried out by following the sequential procedure of van Soest et al. (1991). Neutral-detergent insoluble protein was determined by *N* analysis of NDF residues. All values were corrected for ash-free content.

2.4. Blood sampling and assays

Blood samples (5 ml) were collected twice weekly before morning feeding by tail vessel puncture into heparinized tubes during four months post-partum for the analysis of peripheral progesterone. In addition, blood samples were withdrawn weekly (first and second month of lactation) and fortnightly (third month) into EDTA-k_3 tubes for the assay of blood metabolites.

Samples were centrifugated at 3000 × *g* for 15 min at 4 °C. Plasma aliquots were prepared for the assay of blood metabolites and progesterone and stored at −20 °C until analysis.

Plasma concentration of triglycerides (enzymatic-colorimetric method), cholesterol (enzymatic-colorimetric method), urea (kinetic UV test) and β-hydroxybutyrate (enzymatic-colorimetric method) were determined with an automatic analyzer (GernonStar, RAL/TRANSASIA, Dabhel, India). Reagents for triglycerides, cholesterol and urea were provided by the analyzer manufacturer (RAL, Barcelona, Spain) and those for β-hydroxybutyrate were supplied by Randox (Randox Laboratories Ltd., Crumlin, Co. Antrim, UK). The mean intra- and inter-assay coefficients of variation (CV) for the afore-mentioned metabolites were <5.4% and <5.8%, respectively. Plasma NEFA were analysed in duplicate using a commercial kit (Randox Laboratories Ltd., Crumlin, Co. Antrim, UK). Commercial reference serum samples (bovine precision serum, Randox Laboratories Ltd.) were used to evaluate the accuracy of the analysis. The mean intra- and inter-assay CV were <5.1% and <7.4%, respectively.

Plasma progesterone was measured using a solid-phase RIA commercial kit (Coat-A-Count P4 kit®, Diagnostic Products Corporation, Los Angeles, CA, USA). The mean intra- and inter-assay CV were <8.0% and <10.4%, respectively. The sensitivity averaged 0.03 ng/ml. The onset of luteal activity after calving was considered when progesterone levels were >0.5 ng/ml for short cycles and >1 ng/ml for normal cycles. If cows had not ovulated prior to the end of fourth month post-partum, the interval to first ovulation after calving was regarded as this date and all experimental procedures were terminated. Progesterone rises lasting for a single sampling were considered short oestrus cycles (8–12 days) after first ovulation. Dates of increases of activity (detected through the automated device) were crossed with dates of progesterone rises in order to verify that they were caused by oestrus.

2.5. Statistical analyses

Data were analysed using the SAS statistical software (SAS, 2002). Cow–calf productive and reproductive performance in the cows were analysed with a general linear model (GLM procedure):

$$y_{ij} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ij}$$

where y_{ij} = dependent variable, μ = overall mean, α_i = calf contact effect, β_j = breed effect and ε_{ijk} = residual error.

Calf sex was included as fixed effect in the previous model when analysing calf birth weight and daily gains during the first three months of lactation.

Cow blood metabolites were analysed with a repeated measurements mixed linear model (MIXED procedure):

$$y_{ijkl} = \mu + \alpha_i + d_j + \beta_k + \delta_l + (\alpha\beta)_{ik} + (\alpha\delta)_{il}(\beta\delta)_{kl} + \varepsilon_{ijkl}$$

where y_{ijk} = dependent variable, μ = overall mean, α_i = calf contact effect, d_j = animal random effect j, β_k = breed effect, δ_l = week of lactation effect and ε_{ijk} = residual error.

Behavioural data were not normally distributed and therefore they were transformed by a square root transformation. However, normality was not achieved and they were analysed with the Kruskal–Wallis non-parametric test (PROC NPAR1WAY). The tested effects were: calf contact (partial and non-contact), breed (Parda de Montaña and Pirenaica), suckling period (morning and afternoon) and suckling control (weeks 4, 9 and 15 of lactation).

Data are reported as least square means (if normally distributed) or means and their associated standard errors. Multiple comparisons among treatments were performed by the Tukey's method. The level of significance was set at 0.05. Levels of significance between 0.06 and 0.10 were considered to show trends. The interactions are commented in the text only when they approached statistical significance ($P < 0.10$).

Differences between proportions were analysed using the Fisher exact test of the FREQ procedure. Survival analysis utilizing the LIFETEST procedure was used to evaluate the effects of calf isolation and breed on the interval to first ovulation. The survival analysis was a regression of the number of anoestrous cows at weekly intervals over the first three months post-partum (censored observations). The Wilcoxon test was used to examine the differences between the survival curves.

Data from one Parda de Montaña cow–calf pair from the NC treatment were removed from the statistical analysis because the offspring was affected by a chronic pulmonary disease (for causes not attributable to treatment) which depressed significantly its performance.

3. Results

3.1. Productive performance

The cow and calf LW gains during the first three months of lactation are shown in Table 2. Cows from both calf managements and breeds showed similar LW and BCS throughout the experimental period ($P > 0.10$). Milk yield was not affected by treatment ($P > 0.10$) but it was greater in PA than in PI (Table 3; $P < 0.05$). This difference between breeds disappeared when milk production was standardised for energy content ($P > 0.10$). Milk fat content was 0.62% lower in PA than in PI cows ($P < 0.01$). Also, milk lactose content was 0.32% lower in the former than in the latter ($P < 0.01$), whereas milk protein content did not differ significantly between genotypes ($P > 0.10$).

Calves with different degree of contact had similar daily gains during the first three months of lactation ($P > 0.10$) but those from PA breed grew faster than PI ones ($P < 0.01$). Male calves weighed more than their female contemporaries at birth (43.6 ± 0.9 kg vs. 38.7 ± 0.8 kg, respectively; $P < 0.001$) and the former had faster daily gain during the first three months of age than the latter (0.91 ± 0.03 kg/day vs. 0.82 ± 0.03 kg/day, respectively; $P < 0.05$).

Table 2
Productive performance of beef cows and calves managed under twice-daily suckling systems with partial contact (PC) or without visual, tactile and olfactory contact (NC) in Parda de Montaña (PA) and Pirenaica (PI) breeds.

	Calf contact (C)		Breed (B)		S.E.	P-value[†]	
	PC	NC	PA	PI		C	B
Cows							
Live-weight (LW) at calving (kg)	560	574	556	579	10	NS	NS
Gain during first three months post-partum (kg/day)	−0.02	0.00	−0.03	0.01	0.04	NS	NS
Body condition score (BCS) at calving	2.56	2.56	2.54	2.58	0.03	NS	NS
BCS end of third month post-partum	2.54	2.53	2.51	2.56	0.03	NS	NS
Calves							
Male to female ratio	13/26	10/25	10/24	13/27	–	NS	NS
LW at birth (kg)[‡]	40.7	41.5	41.8	40.4	0.9	NS	NS
Gain during first three months of lactation (kg/day)[‡]	0.86	0.87	0.93[a]	0.80[b]	0.03	NS	**

S.E. = standard error; NS = not significant ($P > 0.10$).
[†] C × B = not significant ($P > 0.10$).
[‡] Calf sex was a significant effect ($P < 0.05$).
[**] $P < 0.01$.

Table 3
Milk production and composition (week 4 of lactation) in beef cows managed under twice-daily suckling systems with partial contact (PC) or without visual, tactile and olfactory contact (NC) in Parda de Montaña (PA) and Pirenaica (PI) breeds.

	Calf contact (C)		Breed (B)		S.E.	P-value[†]	
	PC	NC	PA	PI		C	B
Milk yield (kg)	8.5	7.8	8.7[a]	7.6[b]	0.4	NS	*
Energy-corrected milk (kg)	8.9	8.2	8.9	8.3	0.4	NS	NS
Milk fat content (%)	4.41	4.36	4.08[b]	4.70[a]	0.16	NS	**
Milk protein content (%)	3.46	3.48	3.41	3.53	0.05	NS	NS
Milk lactose content (%)	4.94	4.83	4.73[b]	5.05[a]	0.07	NS	**

S.E. = standard error; NS = not significant ($P > 0.10$).
† C × B = not significant ($P > 0.10$).
* $P < 0.05$.
** $P < 0.01$.

3.2. Blood metabolites

The profile of plasma metabolites in the cows throughout the first three months of lactation is shown in Figs. 1 and 2. Plasma triglycerides and urea were not affected either by calf contact, breed, week post-partum or their interaction ($P > 0.10$).

Plasma cholesterol did not differ across calf contact treatments, breed or their interaction ($P > 0.10$), but this blood biochemical constituent showed the lowest concentration on week 1 post-partum in both breeds (1.78 ± 0.08 mmol/l; $P < 0.05$) and intermediate between weeks 2 and 5 of lactation (2.44 ± 0.08 mmol/l). However, PA cows had elevated plasma cholesterol concentration from week 6 of lactation onwards (2.94 ± 0.14 mmol/l; $P < 0.05$) whereas PI had a slightly delayed increase from week 7 onwards (2.90 ± 0.13 mmol/l; $P < 0.05$).

Plasma NEFA showed a tendency to be affected by the interaction between calf contact and breed ($P = 0.08$), being greater the concentration of these metabolites in blood from PA cows with partial calf contact (0.43 ± 0.04 mmol/l) than in the rest of groups (0.32 ± 0.04 mmol/l; $P < 0.05$). There was a week effect on plasma NEFA ($P < 0.001$), with greater values on week 1 (0.49 ± 0.03 mmol/l) and lower on week 11 (0.20 ± 0.05 mmol/l) than in rest of samplings (0.35 ± 0.03 mmol/l; $P < 0.05$).

Plasma β-hydroxybutyrate was not affected either by calf contact, breed or their interaction ($P > 0.10$), but it was greatest on weeks 1–3 (0.21 ± 0.01 mmol/l) than in the rest of lactation (0.18 ± 0.01 mmol/l; $P < 0.05$).

3.3. Behaviour during suckling periods

The activities recorded during suckling periods are shown in Table 4. Cows with adjacent calves (PC) took less time to first contact after they entered the barn than their non-contact (NC) counterparts ($P < 0.05$). In addition, dams from the PC treatment showed a trend to spend longer time

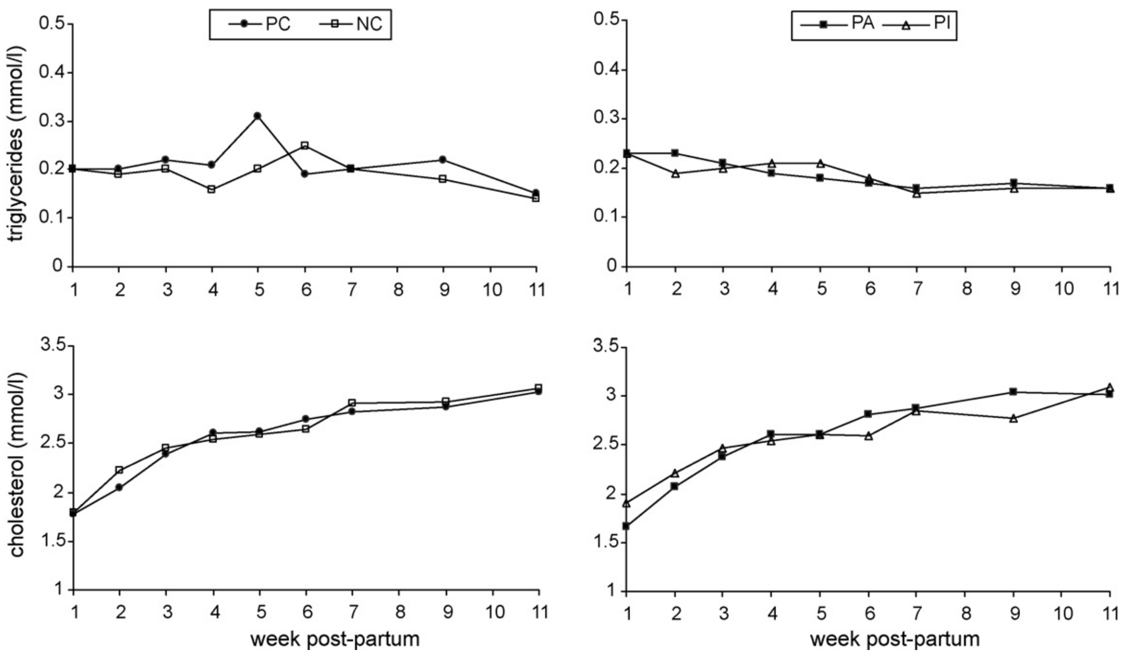

Fig. 1. Plasma concentration of triglycerides (pooled S.E. = 0.03 mmol/l) and cholesterol (pooled S.E. = 0.12 mmol/l) during the post-partum period in Parda de Montaña (PA) and Pirenaica (PI) beef cows managed under twice-daily suckling systems with partial contact (PC) or without visual, tactile and olfactory contact (NC).

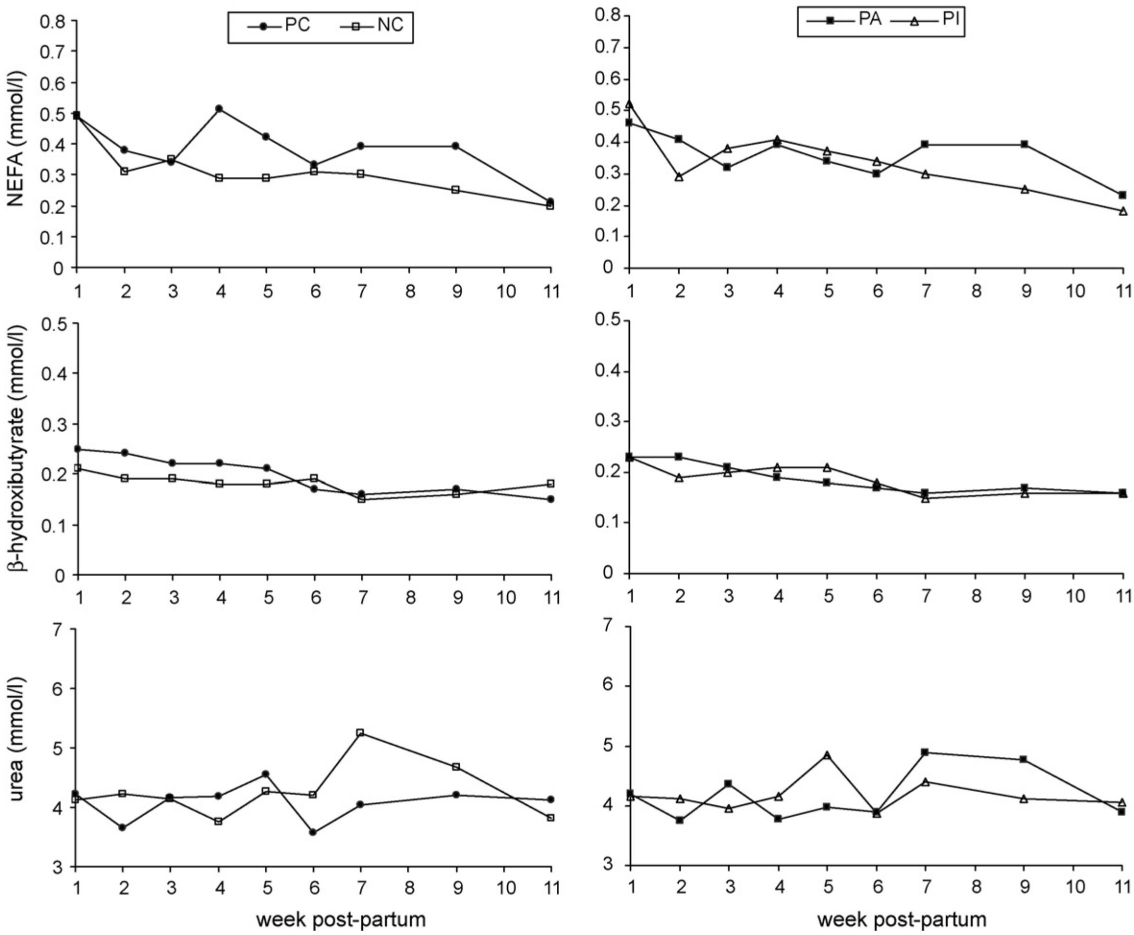

Fig. 2. Plasma concentration of non-esterified fatty acids (NEFA) (pooled S.E. = 0.05 mmol/l), β-hydroxybutyrate (pooled S.E. = 0.02 mmol/l) and urea (pooled S.E. = 0.38 mmol/l) during the post-partum period in Parda de Montaña (PA) and Pirenaica (PI) beef cows managed under twice-daily suckling systems with partial contact (PC) or without visual, tactile and olfactory contact (NC).

standing without their offspring ($P = 0.08$) and shorter period eating the remaining ration on the feeder ($P < 0.001$) than NC ones. Dams from both calf contact treatments nursed their offspring and remained in close proximity for similar time within suckling periods ($P > 0.10$). Calf contact did not affect the mean time nursing alien calves ($P > 0.10$).

Cows from PA breed devoted more time to lick their young than those from PI one ($P < 0.001$). However, both

Table 4
Behaviour activities during suckling periods (2× 30 min at 0800 and 1600 h) in beef cows managed under twice-daily suckling systems with partial contact (PC) or without visual, tactile and olfactory contact (NC) in Parda de Montaña (PA) and Pirenaica (PI) breeds[a].

	Calf contact (C)		Breed (B)		Suckling period (P)		*P*-value†		
	PC	NC	PA	PI	Morning	Afternoon	C	B	P
Latency to first contact (min)	0.1^b	0.6^a	0.2	0.6	0.2	0.6	*	NS	NS
Cow and calf in close proximity (min)	20.8	20.8	21.0	20.6	21.3	20.3	NS	NS	NS
Cow nursing its calf (min)	18.4	18.3	17.9	18.8	19.9^a	16.7^b	NS	NS	***
Cow nursing an alien calf (min)	0.8	1.7	1.0	1.5	1.5	1.0	NS	NS	NS
Cow licking calf (min)	0.9	0.8	1.5^a	0.2^b	0.8	1.0	NS	***	NS
Cow sniffing their own calf (min)	0.1	0.3	0.3	0.1	0.1	0.3	NS	NS	NS
Agonistic behaviour (min)	0.1	0.0	0.0	0.1	0.1	0.1	NS	NS	NS
Cow standing idling without calf (min)	8.2^a	6.6^b	7.4	7.4	7.4	7.4	0.08	NS	NS
Cow eating ration (min)	0.1^b	1.9^a	0.6	1.5	0.6	1.5	***	NS	NS
Cow exploring bed or eating straw bed (min)	0.2	0.2	0.0	0.3	0.0^b	0.4^a	NS	NS	*
Cow drinking (min)	0.0	0.1	0.1	0.0	0.0	0.1	NS	NS	NS

NS = not significant ($P > 0.10$). Within row and effect, means with different superscript differ significantly ($P < 0.05$).
†*$P < 0.05$ and ***$P < 0.001$.
[a] See Section 2.2 for details concerning behavioural variables.

Table 5
Reproductive performance of beef cows managed under twice-daily suckling systems with partial contact (PC) or without visual, tactile and olfactory contact (NC) in Parda de Montaña (PA) and Pirenaica (PI) breeds.

	Calf contact (C)		Breed (B)		S.E.	*P*-value[†]	
	PC	NC	PA	PI		C	B
Interval to first post-partum ovulation (days)	48	51	52	47	5	NS	NS
First oestrus cycle duration (days)	12	13	12	13	1	NS	NS
Interval to first post-partum ovulation in cows with first oestrus detected[a] (days)	43	46	46	42	4	NS	NS
Interval to first post-partum oestrus (days)	49	49	51	48	4	NS	NS
Interval between oestrus and ovulation (days)	4	5	5	4	0.5	NS	NS

S.E. = standard error; NS = not significant ($P > 0.10$).
***$P < 0.001$.
[a] First oestrus was expressed or detected in 70.8% of the cows, regardless of treatment and breed ($P > 0.10$).
[†] C × B = not significant ($P > 0.10$).

genotypes spent similar time in almost all the remaining behaviours ($P > 0.10$).

Overall, cows nursed longer their offspring and spent less time exploring or eating straw bed in the morning than in the afternoon suckling period ($P < 0.05$). There were no differences among recordings on weeks 4, 9 or 15 post-partum in any of the studied behaviours except the time that cows devoted to eat feed within suckling periods, which was higher in the later control (2.3 ± 0.7 min) than in the earlier ones (0.4 ± 0.3 min; $P < 0.01$).

3.4. Reproductive performance

The degree of calf contact, breed and their interaction did not affect the interval to first post-partum ovulation or oestrus in these cows (Table 5; $P > 0.10$). Thirty-four out of 51 cows (66.7%) had a short oestrus cycle after first ovulation, irrespective of treatment and breed ($P > 0.10$). According to the used automatic activity recorder, 34 out to 48 cows (70.8%) showed behavioural signs of oestrus prior to the first two-three ovulations post-partum (the remaining three cows up to 51 did not ovulate throughout the experimental period), but only 32.3% (11/34) showed oestrus before the first post-partum ovulation, regardless of treatment or breed ($P > 0.10$). A transient increase in progesterone without behavioural signs of oestrus prior to the second ovulation was detected in 19 out of 34 cows (55.9%). Normal length of the first oestrus cycle after calving without behavioural signs of oestrus was observed in 4 out of 34 cows (11.8%).

The ovarian resumption pattern based on the analysis of survival curves over lactation did not differ between types of calf access or breeds either (Fig. 3; $P > 0.10$).

4. Discussion

4.1. Productive performance

Cow–calf pairs from the non-contact treatment had a similar productive performance to those which were kept in adjacent pens between suckling periods, which is in agreement with the lack of productive performance differences observed by Stagg et al. (1998) when comparing mother–young pairs with and without contact between once-daily suckling periods.

In contrast, Escrivão et al. (2009) showed that 12 h night-calf separation from 45 days post-partum to the onset of the breeding season reduced calving to breeding interval, it had a positive effect on cow energy balance and improved calf weaning weights in *Bos indicus* beef cattle kept under extensive sub-tropical conditions. As a difference, calves in that study were allowed to graze between suckling periods.

Calves from Parda de Montaña breed had greater gains than those from Pirenaica breed in the first three months of lactation. This effect was not shown in an earlier report on the same winter-calving herd (Álvarez-Rodríguez et al., in press), although the daily gains herein were greater than in that experiment. Pre-weaning growth differences were attributed to genetic background divergences between

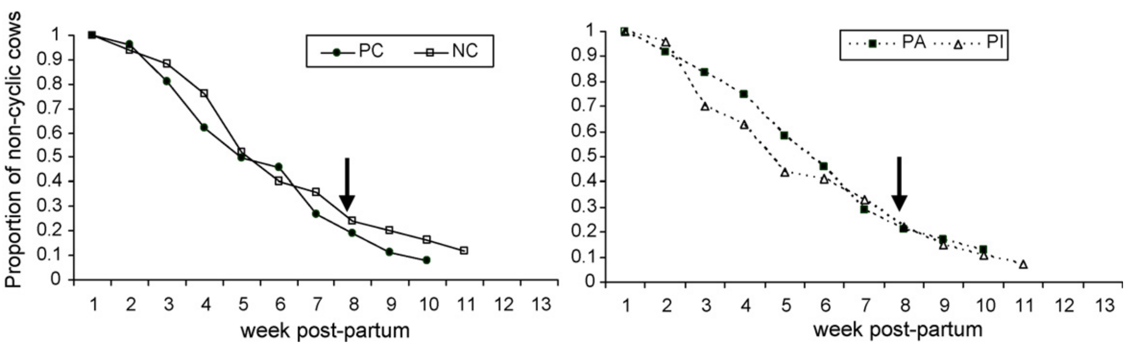

Fig. 3. Survival curves for the proportion of non-cyclic cows during the first three months post-partum when nursing twice-daily with partial contact (PC) or without visual, tactile and olfactory contact (NC) in Parda de Montaña (PA) and Pirenaica (PI) breeds (arrows denote date of bull introduction).

these genotypes (Villalba et al., 2000; Casasús et al., 2002; Sanz et al., 2003), but it is difficult to ascertain whether differences in rate of growth are due to a limitation in milk supply or in the growth potential. Feed intake and average daily gain in these breeds are similar when they are offered the same diet *ad libitum* during the fattening period (Blanco et al., 2009). Thus, as the time spent suckling was also similar in both breeds, we hypothesized that the rate of milk intake might have been greater in calves from Parda de Montaña than in those from Pirenaica breed so that to support higher rate of growth prior to weaning.

4.2. Blood metabolites

There was no effect of calf contact, breed or their interaction on the studied plasma lipoproteins (triglycerides and cholesterol). These molecules transport lipids through blood and they are synthesised in the liver and small intestine. Nearly half of milk fat weight can be derived from plasma triglycerides (Glascock et al., 1966). However, the slightly higher milk yield and lower milk fat content in Parda de Montaña compared to Pirenaica breed did not promote any difference in these metabolites.

Although peripheral cholesterol plays a direct role on ovarian steroidogenesis (Grummer and Carroll, 1988), this substrate did not trigger a significantly different resumption pattern of luteal activity in these breeds.

The concentration of triglycerides into the bloodstream did not vary throughout the experimental period whereas that of cholesterol increased during the same period. Considering only literature using un-supplemented fat diets, the present results are in agreement with those for French beef cows (Guédon et al., 1999) but not with those for Brahman crossbred females (Williams, 1989), since in that study both types of lipids raised until weeks 7–9 post-partum.

Plasma NEFA peaked on the first week of lactation and plasma β-hydroxybutyrate peaked during the first 3 weeks post-partum. The parallel peripheral utilization of both metabolites may highlight increased mobilisation of adipose tissue, associated with oxidative metabolism of NEFA and ketones in the liver (Bell, 1995), but the concentration of these metabolites was below thresholds identifying metabolic stress (Agenäs et al., 2006). Fat metabolism increases in response to cold weather (Tucker et al., 2007). In this study, calving occurred in winter but the herd was allocated in shelter to allow weather protection. Thus, physiological demands of lactation may exert more influence than weather on fatty tissue utilization.

Parda de Montaña showed greater milk yield than Pirenaica cows, although both genotypes had similar BCS throughout the study. This productive response lowered the milk fat content in PA breed, but triggered a slightly greater mobilisation of body fat (as evidenced by plasma NEFA), especially in cows with partial calf contact.

Plasma urea did not differ across calf access treatments, breeds or their interaction. This metabolite is a hepatic by-product of protein catabolism resulting from its diet supply and body utilization (Kaneko, 1997). In addition, blood urea was constant over the post-partum period. Also, the latter was maintained below the threshold which may limit subsequent fertility (around 7 mmol/l; Butler, 1998).

Collectively, the studied blood substrates suggest that the metabolic status of the cows maintained a steady homeostasis during the first three months post-partum and produced nearly undetectable LW and BCS losses during this period. These results reflect a positive energy balance as evidenced by the lower plasma NEFA concentrations by week 11, similar to previous results from Marongiu et al. (2002).

4.3. Behaviour during suckling periods

The maternal–offspring bonding during suckling periods was hardly affected by fence contact. Cows devote to nurse more than half of the time which they remained with their calves. The latency to the first contact between cow and calf after entering in the dams' pen was shorter in partial contact pairs than in non-contact counterparts, but the former cows spent less time with their offspring throughout the suckling session than the latter. Nevertheless, this fact did not affect the suckling duration across treatments. Unexpectedly, cows maintained without contact with calves devoted greater time to consume feed during suckling periods than dams having fence contact with them.

Breed did not influence the activities during suckling either, except the time that cows spent licking their calf, which was greater in Parda de Montaña than in Pirenaica breed. However, this behaviour was not linked to a greater suckling duration, more time of mother–young close to each other or more agonistic encounters towards alien animals. Some reports have pointed out less intense maternal behaviours in dairy breeds compared to typical beef breeds (Le Neindre, 1989) as in *B. indicus* × *Bos taurus* crossbred compared to *B. indicus* cows (Das et al., 2001). The greater tactile contact and allogrooming reduce heart rate and trigger the release of endogenous opioids in mammals (Keverne et al., 1989). Even though the latter effects might have been more pronounced in Parda de Montaña cows compared to Pirenaica dams, overall results do not support a significant effect of genotype on maternal behaviour and therefore on reproductive traits in the present nutritional conditions.

4.4. Reproductive performance

Calves maintained without visual, tactile and olfactory contact with their mothers (30 m separation) and breed did not affect the interval to first post-partum ovulation and oestrus in these cows. However, calf separation (60 m) and once-daily suckling after day 30 of lactation were effective to reduce the post-partum interval (Stagg et al., 1998) although dependent upon genotype (Marongiu et al., 2002). From a behavioural point of view, cows recognize their young mainly through visual and olfactory cues (Le Neindre, 1984). After a maternal bond has been established, deprivation of either visual or olfactory cues maintains continued the inhibition of luteinizing hormone secretion in cows suckled by their own calves (Griffith and Williams, 1996). Accordingly, calf contact limitation did not restrict neurobiological bonding between mother and young but they only limited the physical time in contact. As the twice-daily restricted nursing periods were conducted from 24 to 48 h after parturition, maternal recognition had been set and additional partial separation did not produce any beneficial effect on dam productive or reproductive performance. We separated the cows at this time to assure immunological calf protection through colostrum intake.

When patterns of progesterone in circulation were compared with dates of first oestrus after calving, we observed that only 32.3% of the cows expressed oestrus at first post-partum ovulation. Also, 55.9% of cows showed silent first ovulation by 40–50 days post-partum. Transient increases in progesterone due to short-lived corpus luteum occurring before the first oestrus after calving may prepare the reproductive tract of the cow for rebreeding and may serve to provide a more desirable uterine environment (Werth et al., 1996). It is noteworthy that the first oestrus before an increase in progesterone was not expressed or not detected in 29.2% of the cows. Even though bulls from natural serviced herds use other tools to recognize oestrus other than cow's activity such as body fluids chemo-signals (Sankar and Archunan, 2004), this trait might delay breeding until the third ovulation in this proportion of cows.

5. Conclusions

Under twice-daily nursing conditions, the limitation of visual, tactile and olfactory contact with calves did not trigger different maternal or reproductive traits in these genotypes, but only a slightly higher mobilisation of body fat substrates in Parda de Montaña compared to Pirenaica cows.

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5.6. Luteinizing hormone and growth hormone secretion in early lactating Spanish beef cows

Luteinizing hormone and growth hormone secretion in early lactating Spanish beef cows

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Summary

The episodic release of luteinizing hormone (LH) and growth hormone (GH) was studied in three suckling regimens and two breeds of Spanish suckled cows. Parda de Montaña (PA) cows (n=21) were assigned to once-daily, twice-daily or *ad libitum* suckling. Pirenaica (PI) cows (n=7) were used to evaluate the breed effect in twice-daily suckling. Coccygeal blood samples were collected twice weekly during lactation to determine the interval from calving to first ovulation through peripheral progesterone. On day 32±3 post-partum, jugular blood samples were drawn at 15-min intervals during 8 h to analyse circulating LH and GH.

The interval to first ovulation was greater in PA cows suckling ADLIB than in RESTR1, whereas in RESTR2 it did not differ from the other two treatments. There were no differences between PA and PI cows in the interval to first ovulation. RESTR1 cows showed a tendency to have shorter LH peak widths than ADLIB cows. PA cows showed a tendency to have longer LH peak widths than their PI counterparts. There were no differences across treatments or breeds in any of the GH measures of secretion. The LH release was more affected by breed than by suckling frequency, whereas that of GH was not influenced by any of these parameters. The variables that best allowed discrimination between *ad libitum* and restricted nursing systems were the interval to post-partum first ovulation, LH peak number and the mean GH concentration.

Keywords: cattle, anoestrous, gonadotropins, suckling, luteal function

Introduction

Suckling activity has been proved to be a major effect delaying the resumption of post-partum luteal function in beef cows when nutrition is not a limiting factor (reviewed by Short et al. 1990; Williams 1990; Wettemann et al. 2003). The mechanism of inhibition is regulated by the hypothalamic-pituitary-ovarian axis and appears to require both tactile stimulation to the inguinal area and the establishment and maintenance of a maternal bond (Williams and Griffith 1995).

Achievement of normal oestrous cycles involves the secretion of GnRH by the hypothalamus which further stimulates the release of pituitary gonadotropins (FSH and LH) (Peters and Lamming 1986). In turn, these hormones stimulate estradiol production by the ovarian follicles. At the pituitary level, extended intervals of acyclicity seem to be a consequence of reduced LH pulsatility rather than to a lack of FSH release (Crowe et al. 1998; Stagg et al. 1998). At the ovarian level, although a dominant follicle is selected from the cohort of follicles in the early post-partum period (Hodgen 1982, Ireland et al. 2000), it normally fails to mature properly because of low estradiol synthesis and concomitant prevention of episodic LH surge, ovulation and *corpus luteum* formation (Mihm 1999; Sinclair et al. 2002).

Growth hormone has been suggested to coordinate lipid metabolism by increasing the amount of energy used for milk synthesis (Bines and Hart 1978) and it exerts indirect anabolic actions through circulating insulin-like growth factors (Mol and Rijnberk 1997). This hormone also stimulates ovarian steroidogenesis and folliculogenesis (Hull and Harvey 2000; Kayser et al. 2001) and together with LH, it has been considered as a primary luteotropic hormone which supports *corpus luteum* development and function (Berisha and Schams 2005).

The traditional calf management in suckler cattle farms of the Spanish mountain Pyrenees used to take into account one or two restricted suckling periods during morning and/or evening short sessions in the barn until weaning (Revilla 1987). Conversely, the current most usual suckling system involves the continuous access of calves to dams both in and outdoors, as a consequence of enlarged herd size and decreased labour supply along with extensification policies promoted by the European Union. However, the carry-over effects of every specific suckling system on endocrine mechanisms underlying the length of post-partum anoestrous are not fully understood yet.

Parda de Montaña (PA) and Pirenaica (PI) are two suckler cattle breeds widely spread throughout northern Spain. The former comes from the selection for beef and mothering abilities from the old Brown Swiss, which was introduced in the country two centuries ago as a dual-purpose breed (milk-beef). The latter is an autochthonous hardy breed from the Pyrenees, which was utilized in the past as a triple-purpose breed (work-milk-beef) and is currently used for beef production. Despite the importance of these breeds upon the Spanish local genotypes, the knowledge of their metabolic hormonal profiles and more specifically the pulsatile pattern of secretion of luteotropic hormones have not been described.
The objective of this experiment was to assess the effects of three suckling regimens in one breed and then to examine the influence of the breed in twice-daily suckled cows on the episodic release of LH and GH hormones. In addition, we aimed at establishing, if any, the association of measures of secretion of these hormones with the interval from calving to first ovulation.

Materials and methods

Animals, management and measurements

A total of 28 multiparous cows calving in the autumn-winter season were used (average calving date 15 December±37.5 days, aged 6.9±2.6 years, mean±standard deviation). The animals belonged to the herd of 'La Garcipollera' Research Station (North-eastern Spain, 42°37'N, 0°30'W, 945 m above sea level, mean annual temperature 10.2±0.6 ºC, average annual rainfall 1059±192 mm).
Twenty-one Parda de Montaña cows with their calves were assigned to three suckling frequencies from the day after parturition, being evenly balanced for their live-weight (554±61 kg), body condition score (BCS) at calving (2.53±0.18, 1 to 5 scale; Lowman et al., 1976), calf live-weight at birth (41.3±6.5 kg) and calf sex (9 males and 12 females). Treatments were: once-daily restricted suckling during a 30 min-period at 0800 h (RESTR1), twice-daily restricted suckling during two 30 min-periods at 0800 and 1530 h (RESTR2) and *ad libitum* suckling (ADLIB). Also, seven Pirenaica cows, with similar live-weight (558±69 kg), BCS at calving (2.56±0.20), calf live-weight at birth (38.7±7.0 kg) and calf sex ratio (3

males and 4 females) were allotted identically to the RESTR2 treatment to evaluate the breed effect of twice-daily suckling regimen.

All cows had identical management prior to initiation of treatments. Cow-calf pairs remained indoors throughout lactation in a loose-housing system with straw-bedded pens. In both restricted suckling treatments, calves remained in groups in fenced cubicles adjacent to dams' resting areas with no visual or olfactory isolation.

Cows were group-fed a total mixed ration once-daily at 0900 h (13 kg for PA, 12 kg for PI, as fed basis; 58% forages, 42% grains, by-products and vitamin and mineral supplements; 890 g/kg DM, 90 g CP/kg DM, 526 g NDF/kg DM, 292 g ADF/kg DM). The diet met maintenance requirements for energy and protein in a 560-kg beef cow producing about 9 kg (PA) or 8 kg (PI) of fat-corrected milk with no change in live-weight during lactation (NRC 2000). Cows were supplied water and mineral supplements *ad libitum*.

Cows were weighed before morning feeding within 24 h after calving and thereafter at weekly intervals during the first month post-partum. Cow live-weight variation throughout this period was calculated by linear regression of weights against time.

The care and use of animals followed the European guidelines (European Union Directive No. 86/609/CEE 1986).

Blood sampling

Blood samples (5 mL) were collected twice weekly during 100±26 days post-partum prior to morning feeding by tail vessel puncture into heparinized tubes to analyse the interval from calving to first ovulation (ICOV) through peripheral progesterone (P4) measurement.

On day 32±3 of lactation, cows were brought into tie stalls after morning suckling (RESTR1 and RESTR2) or together with their calves (ADLIB). The procedure for intensive bleed involved the remove of hair from cow's neck with an electrical shaving machine. A topical anaesthetic ointment was spread over the skin (Emla®, AstraZeneca, Madrid, Spain) and it was allowed to set for 30 min. Cows were fitted with indwelling jugular catheters (Cavafix Certo® G14, Braun, Melsungen, Germany) one hour prior to initiation of sampling so that the potential adrenal response of cows was overcome (Alam and Dobson 1986). The catheters were sutured to cow's neck through two stitches. Immediately after insertion, heparin solution (10 IU/mL) was administered into the indwelling catheter to avoid blood clotting between samplings. Then, cows were individually fed their ration in the tie stalls. In the afternoon (1530 h), cows from RESTR2 were allowed to nurse their calves for 30 minutes.

Blood samples (5 mL) were collected with a syringe every 15 min for 8 h into heparinized tubes to characterize LH and GH secretion pattern. Once the last sample was withdrawn, the catheter was removed and an anti-inflammatory gel (Feparil® gel, Madaus, Barcelona, Spain) was spread over the neck area. This sampling methodology had previously been confirmed not to affect feed intake or disturb cow’s welfare (Kadowaka et al. 2003).

During the first month post-partum, LH content in the anterior pituitary is depleted (Nett 1987). Therefore, the sampling date was set after this period so that to account for the re-establishment of LH secretion.

Samples were centrifuged at 3000 x g for 15 min at 4 ºC. Plasma was harvested into aliquots and stored at -20 º C until respective analysis.

Hormone assays

Plasma LH and GH concentrations were assayed by validated homologous double-antibody radioimmunoassays (RIA) (Bono et al. 1983; Baratta et al. 1997). Briefly, bovine LH (H055/H, UCB-Bioproducts S.A., Braine-l'Alleud, Belgique) and bovine somatotropin (supplied by Leo Reichert Jr., Tucker Endocrine Research Institute, LLC; Tucker, GA, USA) were used as labelled ligands and standards. Both hormones were radio-iodinated with ^{125}I according to Salacinski et al. (1981). Rabbit anti-bLH (NIH-LH-B63) and anti-bGH (bGH-489CU) antisera were used at final dilutions of 1:200,000 and 1:10,000, respectively.

Assay sensitivity was 0.11±0.01 and 0.35±0.01 ng/mL for LH and GH, respectively; the intra- and inter-assay coefficients of variation were <6.6 and <11.3% for both assays. All samples from the same cow were analysed in duplicate within the same assay.

Plasma P4 concentrations were measured using a solid-phase radioimmunoassay commercial kit (Coat-A-Count P4 kit®, Diagnostic Products Corporation, Los Angeles, CA, USA) validated in our laboratory. Low (0.25 ng/mL), medium (0.81 ng/mL) and high (3.30 ng/mL) concentration samples were used to estimate the mean intra- (11.3, 8.5 and 6.0%) and inter-assay coefficients of variation (20.0, 10.8 and 10.9 %). The sensitivity averaged 0.03 ng/mL. The onset of luteal activity after calving was considered when P4 levels were >0.5 ng/mL. If cows had not ovulated before 100-120 days post-partum, the ICOV was regarded as this date.

Statistical analyses

A preliminar analysis of variance was performed with the general linear model (GLM) procedure of SAS software (SAS Institute Inc., Cary, NC, USA) to exclude any possible difference across treatments and breeds in the initial cow and calf live-weight and cow BCS. Besides, contingency tables were constructed with the FREQ procedure of SAS to check the calf sex balance across treatments and breeds and to test the proportion of cyclic cows at sampling in each group.

The pulsatile pattern of LH and GH secretion was analysed by cluster analysis (Veldhuis and Johnson 1986) using Pulse_XP, an integrated software package (hormone pulsatility data analysis, Version 2.000, University of Virginia, Charlottesville). A pulse was defined by intra-assay coefficient of variation and by the duration and magnitude of the increase in hormone concentration (Backstom et al. 1982). The considered cluster sizes were two points for the test nadir and one point for the test peak, with a t-statistic of 1.0 in LH or 2.0 in GH for both the upstroke and downstroke.

The analysed parameters were:

1. *Peak number*: Number of significant increases from baseline within the 8-h sampling period.
2. *Width*: Time (in min) between the first significant rise and first significant decline within the overall peak.
3. *Peak amplitude*: Absolute value (in ng/mL) of the maximal concentration attained within the peak.
4. *Increase of mean peak amplitude above baseline*: Proportional increase (in percentage) of the mean peak amplitude above preceding nadir.
5. *Peak area*: The product of the mean peak amplitude that is above the lower of the two flanking nadirs against the peak duration (in ng/mL x minutes).
6. *Area under the concentration data (AUC):* Mean area under the presumed hormone curve (in ng/mL x minutes).
7. *Average data value:* Average mean concentration of the data (in ng/mL).

Differences in the hormone pulsatile pattern and live-weight variation within the first month post-partum were analyzed by means of the GLM procedure of SAS.

Three pre-planned contrasts were performed: the effect of suckling frequency (RESTR1, RESTR2 and ADLIB) in PA breed, the effect of breed (PA and PI) in the RESTR2 suckling system and the effect of ovarian function at sampling (cyclic and anoestrous).

Results are presented as means and standard deviation. Differences between means were compared with a *t*-test.

The level of significance (*P*-value) was set at 0.05. Levels of significance between 0.06 and 0.10, were considered to show trends. Significant Spearman correlation coefficients among measures of secretion of each hormone as well as between these measures and the interval from calving to first ovulation were tested. A canonical discriminant analysis was carried out to classify individuals into treatments and breeds on the basis of the studied quantitative variables.

Results

Six out of 28 cows ovulated prior to hormone sampling date at 32 days of lactation, being evenly distributed across treatments and breeds (Figure 1; $P>0.10$). The interval from calving to first ovulation was longer in PA cows suckling ADLIB than in RESTR1 (79 vs. 55 days; $P=0.05$), whereas in RESTR2 it did not differ from the other two treatments (62 days; $P>0.10$). There were no differences in the interval to first ovulation between PA and PI cows suckling twice-daily (RESTR2) (62 vs. 52 days; $P>0.10$).

Despite the lack of differences among groups in the average initial body condition, cyclic cows on day 32 post-partum were slightly heavier (592±92 vs. 545±49kg; $P=0.09$) and had higher BCS on that moment (2.68±0.12 vs. 2.49±0.17; $P<0.05$) than the anoestrous cows at sampling.

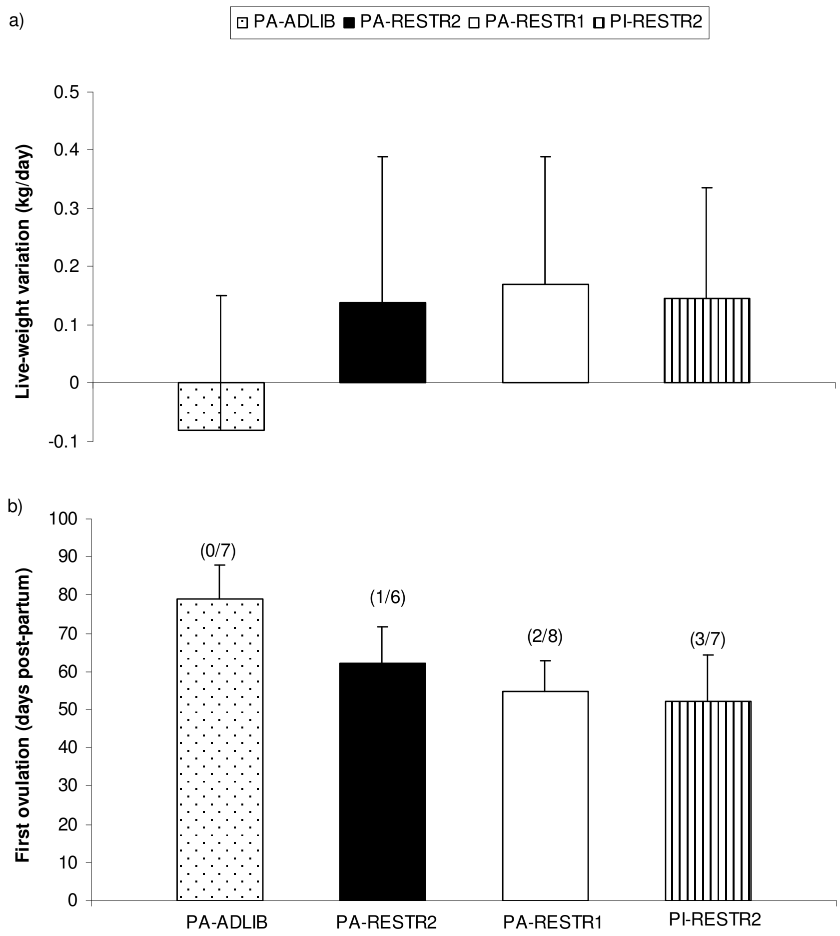

Figure 1. Live-weight variation throughout the first month post-partum (kg/day) (a) and interval to first ovulation (days post-partum) (b) in beef cows managed under different suckling frequencies (ADLIB=*Ad libitum*, RESTR2=Twice-daily, RESTR1=Once-daily, PA=Parda de Montaña, PI=Pirenaica). Proportions within parenthesis are cyclic cows at window bleeding on day 32 of lactation

Luteinizing hormone

The pulsatile pattern of LH secretion in the three suckling regimens (RESTR1, RESTR2 and ADLIB) and both breeds (PA and PI) is shown in Table 1. Some representative examples of LH pulse profiles are depicted in Figure 3.

RESTR1 cows showed a tendency to have shorter LH peak widths than ADLIB ones (P=0.09). However, there were no statistical differences across treatments in any other parameters characterizing LH release (P>0.10).

Table 1. LH parameters on day 32 post-partum in beef cows managed under different suckling frequencies (ADLIB=*Ad libitum*, RESTR2=Twice-daily, RESTR1=Once-daily) (mean ± SD)

	Parda de Montaña (PA)		Pirenaica (PI)	
	ADLIB	RESTR2	RESTR1	RESTR2
Peaks/8 h (number)	1.0 ± 1.0	1.5 ± 1.0 [x]	1.5 ± 0.9	2.6 ± 1.1 [y]
Peak width (min)	191 ± 108 [b]	148 ± 76 [ab,y]	107 ± 61 [a]	81 ± 41 [x]
Peak amplitude (ng/mL)	1.0 ± 0.5	1.1 ± 0.4	1.0 ± 0.6	0.9 ± 0.5
Increase of mean peak amplitude above baseline (%)	164.9 ± 26.7	143.0 ± 30.8 [y]	134.3 ± 41.8	121.2 ± 8.7 [x]
Peak area (ng/mL x min)	46.9 ± 31.8	31.5 ± 18.4 [z]	26.4 ± 30.1	12.5 ± 8.5 [t]
Area under the concentration data (ng/mL x min)	246.1 ± 58.8	296.6 ± 66.0	290.5 ± 72.8	327.0 ± 191.5
Average data value (ng/mL)	0.5 ± 0.1	0.6 ± 0.1	0.6 ± 0.2	0.7 ± 0.4

[a,b] Denote trends (*P*≤0.10) among treatments. [x,y] Denote trends (*P*≤0.10) between breeds. [z,t] Denote differences (*P*≤0.05) between breeds.

Parda de Montaña cows nursing twice-daily showed a tendency to have fewer peaks within the 8-h sampling period (*P*=0.10) and longer peak widths (*P*=0.07) than their counterparts of PI breed. Moreover, although peak amplitude was similar across breeds (*P*>0.10), the increase of the mean peak amplitude above baseline LH tended to be higher in PA cows (*P*=0.10). These differences produced a greater peak area in PA than in PI cows (*P*<0.05). In contrast, the AUC and the mean LH concentration were similar across breeds (*P*>0.10).

Cyclic cows at sampling tended to show a lower increase of the mean peak amplitude above baseline LH than the anoestrous cows (119.7±8.1% vs. 145.0±34.5%, respectively; *P*=0.09), but had similar values as for the remaining parameters (data not shown; *P*>0.10).

Growth hormone

There were no significant differences across treatments or breeds in any of the analysed parameters determining the pulsatile pattern of GH secretion (P>0.10).

The mean GH peak number in these cows throughout 8 h-sampling was 1.5±1.3, with a mean peak width of 80±47 minutes. The mean peak amplitude was 4.3±1.4 ng/mL and the mean peak area was 93.1±64.7 ng/mL x min. The resulting mean AUC was 1283.9±555.0 ng/mL x min whereas the average data value was 2.7±1.2 ng/mL. Some representative examples of GH pulse profiles are shown in Figure 4.

The AUC of GH was smaller in cyclic cows at sampling (day 32 post-partum) than in anoestrous cows on that moment (864.5±293.6 vs. 1398.3±558.2 ng/mL x min, respectively; $P<0.05$). Likewise, mean GH concentration throughout the 8-h sampling was higher in anoestrous than in cyclic cows (2.9±1.2 vs. 1.8±0.6 ng/mL, respectively; $P<0.05$). The rest of parameters were similar ($P>0.10$), regardless the ovarian function of cows on that date.

Correlations between hormone secretory patterns and anoestrous interval

The peak number of GH was positively associated with the LH peak width (r=0.49, P<0.05) and the LH peak area (r=0.52, P<0.01).

The interval from calving to first ovulation was negatively correlated with the peak number (r=-0.40, P<0.05), AUC (r=-0.59, P<0.001) and average data value of LH (r=-0.59, P<0.001). On the contrary, the interval from calving to first ovulation was positively correlated with mean peak amplitude (r=0.51, P<0.05), AUC (r=0.70, P<0.001) and average data value of GH throughout 8-h sampling period (r=0.70, P<0.001).

Discriminant analysis

The canonical discriminant analysis derived canonical variables (Can1 and Can2), which are linear combinations of the studied quantitative variables that summarize between class variation (Figure 2). Function 1 (Can1) accounted for 52.6% of the total variation among suckling systems and breeds and it was mainly determined by the LH peak number (r=0.74). Function 2 (Can 2) accounted for 40.2% of the variance and it was mainly determined by the interval from calving to first ovulation (r=0.49), the LH peak number (r=-0.45) and the GH mean value (r=0.45). Function 3 (not drawn) only accounted for 7.2% of the total variation.

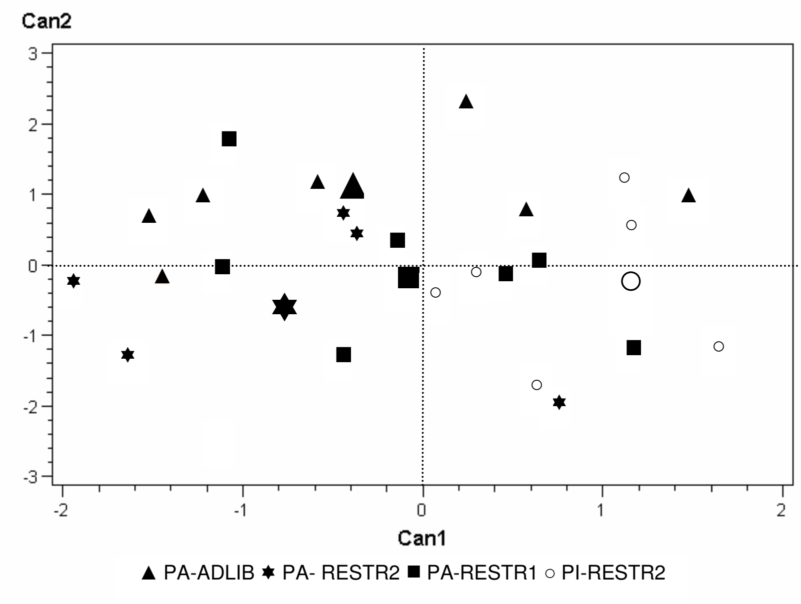

Figure 2. Canonical discriminant analysis among suckling systems and breeds (PA=Parda de Montaña, PI=Pirenaica, ADLIB=*Ad libitum*, RESTR2=Twice-daily, RESTR1=Once-daily) (Can1 accounted for 52.6% of the total variation and Can 2 accounted for 40.2% of the variance).

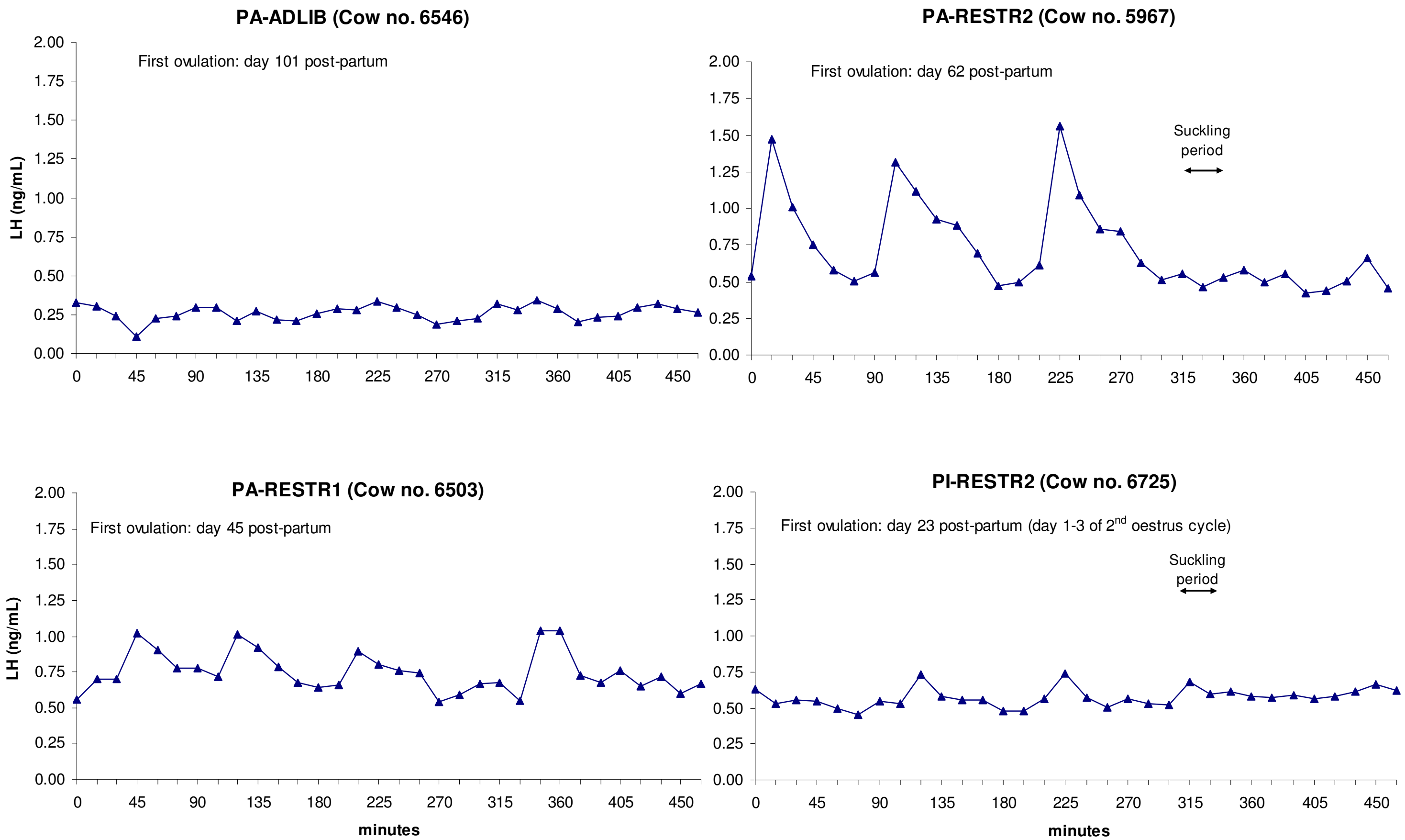

Figure 3. Representative patterns of LH secretion on day 32 post-partum in beef cows managed under different suckling frequencies (RESTR1=Once-daily, RESTR2=Twice-daily, ADLIB=*Ad libitum*, PA=Parda de Montaña, PI=Pirenaica)

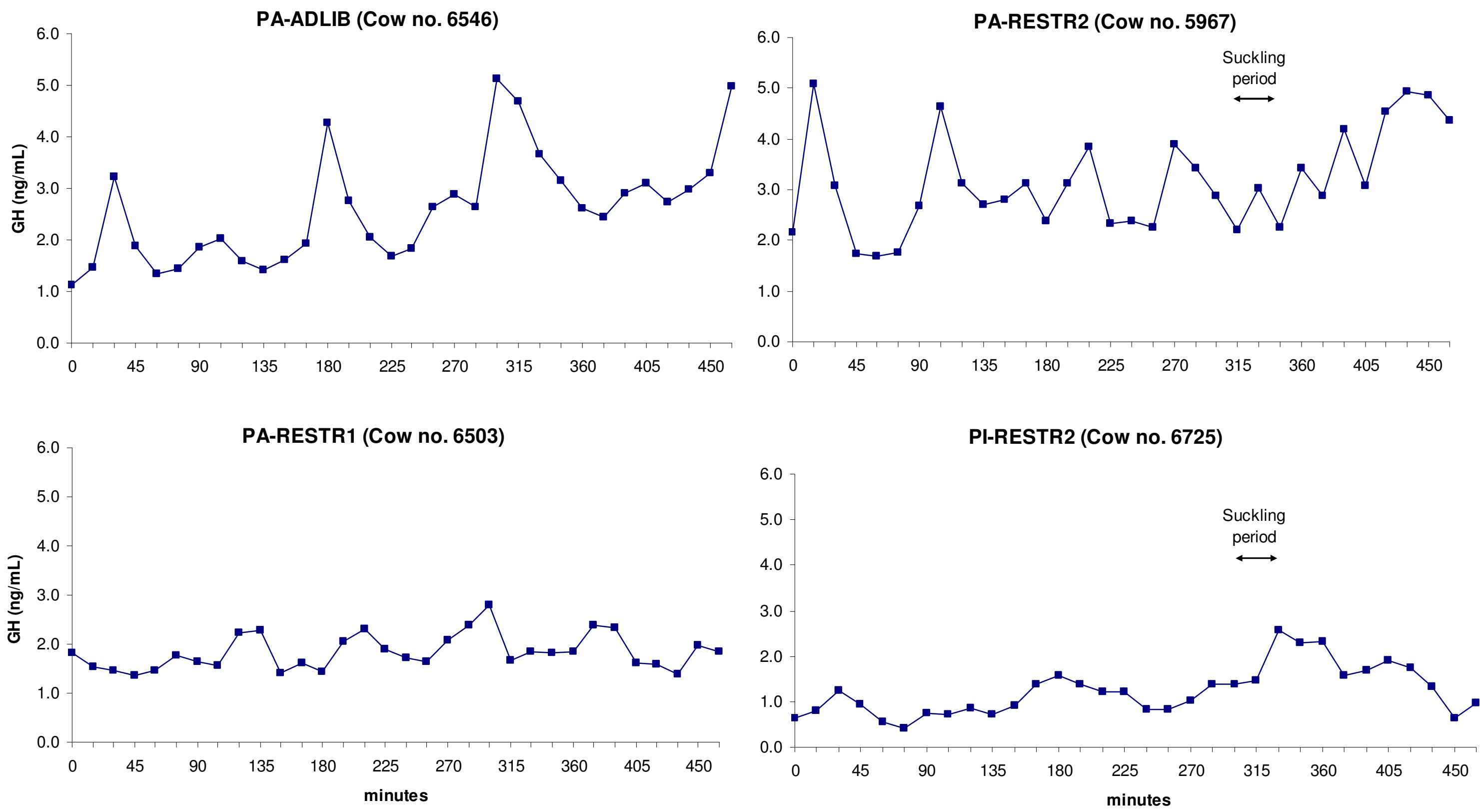

Figure 4. Representative patterns of GH secretion on day 32 post-partum in beef cows managed under different suckling frequencies (ADLIB=*Ad libitum*, RESTR2=Twice-daily, RESTR1=Once-daily, PA=Parda de Montaña, PI=Pirenaica)

Discussion

Luteinizing hormone

To our knowledge, this report is the first examining the GH and LH profiles in Spanish suckler cattle breeds throughout lactation. Although the limited number of cows within treatment might constraint the detection of differences, cows suckling once-daily tended to show shorter peak widths of LH than cows suckling twice-daily or *ad libitum*. In fact, this higher peak frequency was reflected in shorter lengths of post-partum anoestrus in cows from the once-daily suckling treatment. Peters and Lamming (1983) estimated that a pulsatile pattern of LH secretion with a frequency of 0.25 to 1 per h appears to be a prerequisite for the fate of the dominant follicle post-partum, thus exiting in gradually increasing LH concentration before the first LH surge. Accordingly, Rawlings et al. (1980) noted that the maximum magnitude and frequency of LH peaks occurred 10 to 33 days before the initial increase of plasma P4.

At the hypotalamic-hypophyseal level, suckling mediated-anovulation is directly influenced by the endogenous opioid system (Malven et al. 1986; Whisnant et al. 1986). Cows nursing their own calves show stimulated peripheral oxytocin release and increased hypothalamic opioid tone which overall inhibit neuronal stimulation of GnRH secretory neurons (Williams and Griffith 1995). This response might be enhanced when calves are maintained continuously with dams, since time spent suckling, and thus maternal bonding, is nearly two- to three-fold higher than in cows managed under twice- or once-daily suckling (59, 34 and 22 min/day in ADLIB, RESTR2 and RESTR1, respectively; Álvarez-Rodríguez et al. 2009).

At the ovarian level, low LH levels in *ad libitum* suckling cows compared to restricted suckling ones could be due to an increased sensitivity of the hypothalamic-hypophyseal axis to the negative feedback of ovarian steroids (Acosta et al. 1983, Baratta et al. 1994), rather than to a failure of the positive feedback mechanism prior to approaching first post-partum ovulation, which did not occur until late second month of lactation. Inadequate pulses of LH may have caused recurring follicular waves and atresia of the dominant follicle in cows suckling *ad libitum*, as observed by Sanz et al. (2003). However, when calf separation was performed shortly after selection of the dominant follicle of the first follicular wave emerging after day 21 post-partum, it induced significantly greater LH pulse frequency in dams two days after restricting calf access to once-daily suckling (Sinclair et al. 2002).

The area under the concentration data and the average data value of LH were numerically lower in the *ad libitum* suckling cows than in the rest, although there were no differences across suckling systems in these figures. Indeed, lower values of these parameters were significantly correlated with longer post-partum anoestrus. Trout and Malven (1988) reported no differences between anoestrous and cyclic suckled cows in either pituitary receptors for GnRH or secretory patterns of plasma LH, but the former had higher tissue concentration of naloxone (an endogenous opioid receptor antagonist) binding sites in the preoptic area and the basal forebrain.

There were differences between breeds in the pulsatile pattern of LH secretion. Parda de Montaña cows suckling twice-daily tended to show less frequent peaks which had significantly wider area than those from Pirenaica cows. However, these different traits were nearly undetectable on the length of post-partum anoestrus, which was in agreement with the results of Casasús et al. (2002) in the same breeds managed under twice-daily suckling.

LH pulse frequency is controlled by the hypothalamus through the frequency of GnRH pulses, whereas pulse amplitude is controlled primarily by pituitary responsiveness to GnRH (Kadowaka et al. 2003). In this study, as LH pulse amplitude was similar across suckling frequencies and breeds, we hypothesized that the secretion of LH into the bloodstream might be specially mediated by the frequency of GnRH pulses rather than by pituitary responsiveness to the hormone. Likewise, the measure of amplitude of LH on day 32 of lactation does not seem to be linked with the interval to post-partum ovarian resumption. The mean peak amplitude above baseline was declined in Pirenaica breed and in the overall group of cyclic cows at sampling, since their basal concentration of LH was higher than in Parda de Montaña cows and in the group of anoestrous cows, respectively. In the short term, increased response of plasma LH (absolute and proportional) is noticeable when cows are challenged with exogenous naloxone (Cross et al. 1987), but possibly it is undetectable when cows have moderately elevated physiological baseline concentration of this hormone.

Growth hormone

The pulsatile pattern of GH secretion on day 32 of lactation did not differ across treatments or breeds. The area under the concentration data and the average data value of GH were numerically greater in the *ad libitum* suckling treatment, but these differences did not reach statistical significance. GH is the main galactopoietic hormone in cows through stimulation of lipolysis and induction of glucose sparing in peripheral tissues (Burton et al. 1994). Although all cows were fed the same diet, the energy balance of cows maintained continuously with calves might have been more negative than in their restricted suckling counterparts according to live-weight variation within the first month post-partum. However, this fact did not trigger remarkably higher GH secretion in the *ad libitum* suckling treatment.

The concentration of GH in beef cows of the present experiment was lower than that in dairy cows at similar stage of lactation (Accorsi et al. 2005). As the steroidogenic and folliculogenic actions of GH are progonadal at physiological concentrations (Hull and Harvey 2000), the lower milk potential of beef compared to dairy genotypes suggest that pituitary GH secreted into the circulation had similar endocrine roles on the target reproductive organs of all animals.

It must be pointed out that differential ovarian response to gonadotropic signals might be regulated by intraovarian factors. While the number and affinity of pituitary GnRH binding sites do not change during the postpartum period (Moss et al. 1985), the ability of LH and/or GH binding to the ovary has been suggested to be affected by the absence of LH receptors (Inskeep et al. 1988) and/or GH receptors within the ovary (Kolle et al. 1998). For instance, the number of gonadotropin receptors on granulosa cells of developing follicles varies within the follicular growth wave (Bodensteiner et al. 1996).

Correlations and discriminant analysis

There was little association among the studied hormone measures, but the greater were peak width and peak area of LH, the greater was the peak number of GH. In fact, *ad libitum* and twice-daily suckled cows showed a tendency for greater peak width of LH than the once-daily suckled treatment, although peak number of GH was not remarkably increased.

The lack of correlation between the frequency of LH and GH pulses showed that even though both hormones are secreted from the anterior pituitary, their endocrine response during the early post-partum period is controlled by independent mechanisms. Nevertheless, both the AUC and the average data value of each hormone were crossed correlated with the interval to first post-partum ovulation, proving the negative relationship between the GH-induced supply of nutritional substrates and the LH-stimulated follicular maturation in suckled beef cows.

The multivariate analysis discriminated between breeds and between *ad libitum* and restricted nursing systems. Function 1 (Can1) clearly discriminated both breeds when nursing twice-daily. The centroid of Parda de Montaña cows managed under a twice-daily suckling system were allocated in the left quadrant (with low LH peak number), whereas the centroid of Pirenaica cows with the same management were depicted in the right quadrant (with great LH peak number). Concerning Function 2 (Can2), the centroid of Parda de Montaña cows nursing *ad libitum* was allocated in the upper quadrant (with long interval to first post-partum ovulation, low LH peak number and great GH mean concentration) whereas the centroids of restricted nursing treaments were depicted in the bottom quadrant (with short interval to first post-partum ovulation, great LH peak number and low GH mean concentration).

The difference between Parda de Montaña and Pirenaica genotypes nursing twice-daily was mainly attributed to LH peak number. The differences between nursing systems were mainly due to the interval from calving to first ovulation, LH peak number and the GH mean concentration within the 8-h sampling.

Conclusions

In conclusion, the pattern of secretion of LH on day 32 of lactation was more affected by breed than by suckling frequency, whereas the release of GH was not influenced by any of these parameters. The measure of the area under the concentration data and/or the average data value of LH and GH were the most related parameters with the resumption of post-partum luteal activity in suckled beef cows. The variables that best allowed discrimination between *ad libitum* and restricted nursing systems were the interval to post-partum first ovulation, LH peak number and the mean GH concentration.

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Conclusiones / CONCLUSIONS

6. Conclusiones / CONCLUSIONS

1. En condiciones moderadas de alimentación, las vacas de raza Parda de Montaña sometidas a uno o dos períodos de amamantamiento de 30 minutos al día mostraron ganancias de peso superiores que las de crianza libre. Esta recuperación pudo verse favorecida por la menor intensidad de amamantamiento observada en las vacas de ambos grupos de acceso restringido. La actividad luteal de las vacas no se vio significativamente afectada por el manejo del ternero.

2. En vacas de raza Parda de Montaña, la restricción del acceso del ternero a uno o dos períodos de amamantamiento al día y la edad de la madre no provocaron una respuesta adrenal que afectara a la concentración de metabolitos de glucocorticoides en heces en los días siguientes al parto.

3. Las vacas de raza Parda de Montaña sometidas a dos períodos de amamantamiento al día mostraron un perfil de metabolitos sanguíneos indicadores del balance energético más parecido a las vacas con acceso libre al ternero que a las sometidas a un período de amamantamiento al día. Sin embargo, este hecho no se tradujo en diferencias remarcables en los parámetros reproductivos de las vacas.

4. En condiciones de crianza libre, las vacas adultas mostraron distintas características metabólicas a las vacas de primer parto, especialmente en el perfil de ácidos grasos no esterificados, colesterol y factor de crecimiento similar a la insulina-I (IGF-I). Además, las hembras multíparas con acceso libre al ternero mostraron una reactivación ovárica más temprana que las primíparas con el mismo manejo.

5. El acceso libre del ternero mejoró la producción de leche de las madres y las ganancias de las crías en comparación con el amamantamiento restringido a un período al día. Estos resultados se obtuvieron en detrimento de un retraso en la primera ovulación post-parto en las vacas sometidas a crianza libre.

6. En las razas Parda de Montaña y Pirenaica, la respuesta al sistema de manejo del ternero en los metabolitos sanguíneos utilizados para evaluar el estado nutricional de la vaca difirió en función de la raza, pero ninguno de ellos

alteró la concentración endocrina de factor de crecimiento similar a la insulina-I (IGF-I) durante el post-parto.

7. Las vacas de raza Parda de Montaña y Pirenaica mostraron una duración del amamantamiento similar cuando se sometieron a un sistema de crianza restringida a un período al día o a crianza libre.

8. En condiciones de dos períodos de amamantamiento al día, el aislamiento visual, táctil y olfativo entre vacas y terneros no desencadenó cambios en su comportamiento durante el amamantamiento o en los parámetros reproductivos de estas razas.

9. El patrón de secreción de la hormona luteinizante al final del primer mes de lactación se vio menos afectado por el sistema de amamantamiento que por la raza, mientras que la secreción de hormona de crecimiento no se vio afectada por ninguno de estos factores. Las variables que mejor permitieron discriminar entre los sistemas de amamantamiento y las razas fueron la duración del anestro post-parto y la frecuencia de pulsos de la hormona luteinizante.

IMPLICACIONES

Los efectos del sistema de amamantamiento del ternero sobre la recuperación de peso vivo, los metabolitos sanguíneos indicadores del balance energético, el patrón de secreción de la hormona luteinizante y la duración del anestro post-parto indican la necesidad de intensificar su manejo durante la crianza para no comprometer los resultados productivos y reproductivos de la vaca nodriza, principalmente en hembras jóvenes. Esta intensificación no afectaría al establecimiento del vínculo materno-filial. En los sistemas extensivos, esta premisa apuntaría la necesidad de planificar las épocas de partos y las instalaciones para permitir la separación diaria del ternero durante los primeros meses de lactación.

La ausencia de diferencias en la mayoría de los parámetros analizados en las razas Parda de Montaña y Pirenaica reflejaría una trayectoria paralela de selección hacia la producción de carne en ambos genotipos a lo largo de las últimas décadas.

CONCLUSIONS

1. In moderate feeding conditions, cows from Parda de Montaña breed managed under once or twice a day nursing regimes for 30 minutes showed greater live-weight gains than those nursing *ad libitum*. This recovery may be favoured by the lower suckling intensity in cows from both restricted nursing treatments. The luteal activity of dams was not significantly affected by the type of calf management.

2. In Parda de Montaña breed, the suckling restriction to once or twice a day short periods and age of dams were factors that did not trigger an adrenal response which affects the concentration of faecal glucocorticoid metabolites after calving.

3. Cows from Parda de Montaña breed managed under twice a day nursing periods showed a profile of blood metabolites indicators of energy balance more similar to *ad libitum* nursing cows than to once a day nursing cows. Nevertheless, these differences did not trigger remarkable differences in the reproductive parameters of cows.

4. In *ad libitum* nursing conditions, multiparous cows nursing *ad libitum* showed different metabolic traits compared to primiparous cows with the same type of calf management, especially the profile of non-esterified fatty acids (NEFA), cholesterol and insuline-like growth factor-I (IGF-I). Furthermore, these multiparous cows resumed cyclicity earlier than primiparous cows.

5. The free access of calves to cows improved the milk yield of dams and calf gains compared to once a day restricted nursing. These results were obtained to the detriment of delaying the first post-partum ovulation in *ad libitum* nursing cows.

6. In Parda de Montaña and Pirenaica cows, the response to calf management in blood metabolites indicators of nutritional status differed according to breed, but any of them altered the endocrine insuline-like growth factor-I (IGF-I) concentration during the post-partum period.

7. Parda de Montaña and Pirenaica cows showed a similar nursing duration when they had once a day or *ad libitum* calf access.

8. In the twice a day suckling regimen, the visual, tactile and olfactory cow-calf isolation between suckling periods did not trigger behavioural changes during nursing nor affected the reproductive parameters of these breeds.

9. The secretion pattern of luteinising hormone at the end of the first month post-partum was less affected by the nursing system than by breed, whereas the growth hormone measures of secretion were not affected by any of these factors. The variables that best allowed discrimination among nursing systems and between breeds were the duration of post-partum anoestrus and the LH pulse frequency.

IMPLICATIONS

The effects of the type of calf access on live-weight recovery, blood metabolites indicators of energy balance, the pattern of luteinising hormone secretion and the duration of post-partum anoestrus highlight the need of intensifying the calf management to avoid an impaired productive and reproductive performance in post-partum cows, especially in first-calf cows. Such intensification practice would not affect the establishment of the maternal bond. In extensive systems, this may suggest the need of planning the calving periods and the facilities to allow daily calf separation during the first months of lactation.

The lack of differences in most of the parameters analysed in Parda de Montaña and Pirenaica cattle breeds might reflect a parallel trajectory of selection towards beef production in both genotypes over the last decades.

Resumen / SUMMARY

7. Resumen / SUMMARY

Esta tesis doctoral se planteó para profundizar en los mecanismos fisiológicos a través de los que la crianza del ternero puede actuar sobre la reproducción en la vaca nodriza, cuando es sometida a distintos sistemas de amamantamiento desde el día siguiente al parto, en condiciones moderadas de alimentación. Los manejos evaluados fueron: acceso restringido del ternero a un amamantamiento al día (RESTR1), acceso restringido a dos amamantamientos al día (RESTR2) y acceso libre (ADLIB).

En la publicación 1, se evaluó el efecto de la frecuencia de amamantamiento sobre los resultados productivos-reproductivos y el comportamiento epimelético de vacas multíparas de raza Parda de Montaña (PA) (n=36). Se observó que las pérdidas de peso de las vacas durante los 3 primeros meses de lactación fueron mayores en ADLIB que en RESTR1 y RESTR2. La concentración sanguínea media de ácidos grasos no esterificados (AGNE) fue inferior en RESTR1 que en el resto de lotes. La mayor duración del amamantamiento se observó cuando las vacas tenían libre acceso a su ternero. La proporción de vacas que reiniciaron su actividad ovárica cíclica en los 3 meses siguientes al parto fue similar en los tres tratamientos.

En la publicación 2, se evaluaron los efectos del sistema de amamantamiento (en vacas multíparas) y de la edad sobre la concentración de metabolitos de glucocorticoides fecales después del parto (como indicadores de respuesta adrenal), así como sobre la respuesta productiva, metabólica y reproductiva de vacas de raza PA (n=64). La concentración de glucocorticoides fecales durante los 3 primeros días post-parto no se vio afectada por el manejo del ternero o la edad de la vaca, pero fue superior a las 12 horas que a 48 y 72 horas post-parto. Las ganancias medias de las vacas fueron superiores en el RESTR1 que en el resto de lotes. Este carácter no difirió entre multíparas y primíparas. La concentración sanguínea de triglicéridos y urea no se vio afectada por el manejo del ternero o la edad de la vaca. La concentración de colesterol y factor de crecimiento similar a la insulina-I (IGF-I) no difirió entre sistemas de amamantamiento, pero su concentración fue inferior en vacas multíparas que en primíparas. La concentración sanguínea de AGNE fue inferior en RESTR1 que en RESTR2 y ADLIB durante el segundo y tercer mes de lactación. Los anteriores metabolitos fueron superiores en las vacas multíparas que en las primíparas en la semana 1 y después de la semana 7 de lactación. La concentración media de β-hidroxibutirato fue inferior en

las vacas de RESTR1 y RESTR2 que en ADLIB. El sistema de amamantamiento del ternero no afectó significativamente al intervalo desde el parto hasta la primera ovulación o hasta el primer estro. Sin embargo, las vacas multíparas mostraron un intervalo hasta la primera ovulación más corto que las primíparas.

En la publicación 3, se evaluó el efecto del sistema de amamantamiento y de la raza (PA vs. Pirenaica (PI)) sobre la función metabólica y reproductiva de vacas multíparas (n=50). Las vacas sometidas a RESTR1 mantuvieron peso durante los 3 primeros meses post-parto, mientras que las de ADLIB perdieron entorno a un 4% de su peso vivo inicial. Ambos genotipos mostraron pérdidas similares de peso durante ese período. La producción de leche y lactosa fue inferior en las vacas de RESTR1 que en las de ADLIB. La producción de leche y de proteína fue superior en la raza PA que en la PI. La concentración sérica de AGNE, proteínas totales y urea fue mayor en las vacas de raza PI en ADLIB que en el resto. Las vacas de raza PI mostraron mayores valores de AGNE que las de raza PA en la primera semana post-parto. La concentración de IGF-I no se vio afectada por la frecuencia de amamantamiento, la raza o su interacción. El tipo de acceso del ternero a la madre, pero no la raza, afectó al intervalo desde el parto a la primera ovulación, que fue inferior en un amamantamiento al día que en libre.

En la publicación 4, se evaluó el efecto de la raza sobre el vínculo maternal y se valoró su posible influencia sobre la duración del anestro post-parto, en vacas multíparas (n=24), sometidas a un período de amamantamiento al día y a crianza libre. Las razas PA y PI amamantaron a su cría durante un tiempo similar, tanto en un amamantamiento al día, como en crianza libre. Ambas razas reiniciaron su actividad cíclica ovárica de forma similar.

En la publicación 5, se evaluó el efecto del aislamiento visual, táctil y olfativo en un sistema de doble amamantamiento y de la raza sobre los resultados productivos, metabólicos, etológicos y reproductivos de vacas multíparas (n=52). Las vacas de ambos tratamientos (con y sin aislamiento) y razas mostraron similares ganancias de peso durante los 3 primeros meses post-parto. La producción de leche no se vio afectada por el sistema de manejo del ternero pero fue superior en la raza PA que en la PI. Los triglicéridos plasmáticos y la urea no se vieron afectados por el aislamiento del ternero o la raza. El colesterol plasmático se incrementó a partir de la semana 6 de lactación en las vacas de raza PA, mientras que dicho ascenso se retrasó hasta la semana 7 en las de raza PI. Los niveles plasmáticos de AGNE fueron mayores en las vacas de raza PA sin aislamiento que en el resto. La concentración de β-hidroxibutirato no se vio afectada por el manejo del ternero o la raza. Durante los períodos de amamantamiento, las vacas sin aislamiento entre amamantamientos tardaron menos tiempo en aproximarse hasta sus terneros que las que no tenían contacto entre estos períodos. Las vacas de ambos tratamientos amamantaron a sus terneros y se mantuvieron cercanos a ellos durante un tiempo similar en los períodos de amamantamiento. Las vacas de raza PA dedicaron mayor tiempo a lamer a su ternero que las de raza PI. El aislamiento del ternero y la raza no afectaron al intervalo hasta la primera ovulación o el primer estro en estas vacas.

En la publicación 6, se estudiaron los efectos de la frecuencia de amamantamiento y de la raza sobre la liberación episódica de hormona luteinizante (LH) y la hormona del crecimiento (GH) en vacas multíparas (n=28). Los efectos del sistema de amamantamiento se evaluaron en la raza PA, en la que se observó que la duración del anestro post-parto fue superior en ADLIB que en RESTR1, mientras que este período no difirió de los anteriores en el lote RESTR2. Las vacas de RESTR1 mostraron una tendencia a tener picos de LH más estrechos que las de ADLIB. El efecto de la raza se comparó en el régimen de dos amamantamientos al día (RESTR2). No se observaron diferencias en la duración del anestro entre las vacas de raza PA y PI de RESTR2. Las vacas de raza PA en RESTR2 tuvieron picos de LH ligeramente más anchos que sus homólogas de raza PI, así como una mayor área de dichos picos. No se observaron diferencias entre sistemas de amamantamientos o razas en ninguna de las medidas de secreción de GH. No se encontró correlación entre la frecuencia de pulsos de LH y GH. El área debajo de la curva y el valor medio de LH se correlacionaron negativamente con la duración del anestro post-parto, mientras que lo contrario ocurrió entre estas medidas de GH y la duración de dicho período. Las variables que mejor permitieron la discriminación entre los sistemas de amamantamiento y las razas fueron la duración del anestro post-parto y la frecuencia de pulsos de LH en las 8 horas de muestreo.

SUMMARY

This doctoral thesis aimed at studying several physiological mechanisms by which the suckling calf may module the reproductive function of beef cows, when they are managed under different nursing systems in moderate nutritional conditions. The managements were: once a day restricted access (RESTR1), twice a day restricted access (RESTR2) or *ad libitum* access (ADLIB), from the day after calving.

In manuscript 1, we evaluated the effects of nursing system on the performance and suckling behaviour of beef cows from Parda de Montaña breed (PA) (n=36). The live-weight losses throughout the first 3 months post-partum were greater in ADLIB than in RESTR1 and RESTR2 nursing cows. The mean blood concentration of non-esterified fatty acids (NEFA) was lower in RESTR1 than in the rest. The greater nursing duration was observed in ADLIB cows. The proportion of cows that resumed cyclic ovarian activity within 3 months post-partum was similar in the three treatments.

In manuscript 2, we studied the effects of nursing system (in multiparous cows) and dam age on the concentration of faecal glucocorticoid metabolites after calving (indicator of adrenal response), as well as on productive, metabolic and reproductive performance of PA cows (n=64). The concentration of faecal glucocorticoid metabolites during the first 3 days post-partum was not affected by calf management or cow parity, but it was greater at 12 hours than at 48 and 72 hours post-partum. The live-weight gains of dams were greater in RESTR1 than in RESTR2 or ADLIB treatments. This trait did not differ between multiparous and primiparous cows. The blood concentration of triglycerides and urea was not affected by calf management or dam age. The blood concentration of cholesterol and insulin-like growth factor-I (IGF-I) did not differ among nursing systems, but their mean concentration was lower in multiparous than in primiparous cows. Blood NEFA were lower in RESTR1 than in the rest during the second and third month of lactation. Blood NEFA were greater in multiparous than in primiparous cows on week 1 and after week 7 of lactation. The mean blood concentration of ß-hydroxybutyrate was lower in RESTR1 and RESTR2 than in ADLIB cows. The type of calf management did not affect significantly the interval from calving to first ovulation or to first oestrus. Nevertheless, multiparous cows showed a shorter interval to first ovulation than primiparous cows.

In manuscript 3, the effects of nursing system and breed (PA vs. Pirenaica (PI)) on the metabolic and reproductive function were studied in multiparous beef cows (n=50). RESTR1 cows maintained live-weight throughout the first 3 months post-partum, while ADLIB nursing cows lost around 4% of the initial live-weight. Both genotypes showed similar gains during this period. The milk and lactose yield was lower in RESTR1 cows than in their ADLIB counterparts. Milk and protein yield was greater in PA than in PI breed. Serum concentration of NEFA, total protein and urea was greater in PI cows nursing *ad libitum* than in the rest. Cows from the PI breed had greater serum NEFA than PA ones on the first week post-partum. Endocrine IGF-I was not affected by the nursing system, breed or their interaction. The type of calf management but not breed affected the interval from calving to first ovulation, which was shorter in once-daily than in *ad libitum* nursing cows.

In manuscript 4, the effect of breed on the maternal bond and on the duration of post-partum anoestrus were evaluated in multiparous cows (n=24) managed under once-daily or *ad libitum* nursing. PA and PI breeds nursed their offspring during similar time each day, regardless of being managed under a once-daily or *ad libitum* nursing system. Also, both breeds resumed cyclic ovarian activity in a similar way.

In manuscript 5, the effects of visual, tactile and olfactory contact in a twice-daily nursing system and of breed on productive, metabolic, ethologic and reproductive traits were evaluated in multiparous beef cows (n=52). Cows from both treatments (with and without contact) and breeds showed similar gains throughout the first 3 months post-partum. Milk yield was not affected by calf management but it was greater in PA than in PI breed. Plasma triglycerides and urea were not affected by calf contact or breed. Plasma cholesterol was increased from week 6 of lactation onwards in PA breed, whereas this rise was delayed until week 7 in PI breed. Plasma NEFA were greater in PA cows with contact with their calves than in the rest. Plasma ß-hydroxybutyrate was not affected by calf contact or breed. At the beginning of the suckling periods, cows with visual, tactile and olfactory contact with their offspring took less time to approach them than those dams without contact between nursing periods. Cows from both treatments nursed their calves and were in close proximity with them during a similar time within the suckling periods. Cows from PA breed devoted more time to lick their calves than those from PI breed. The limitation of visual, tactile and olfactory contact with calves and breed were factors that did not affect the interval to first ovulation and to first oestrus in these cows.

In manuscript 6, the effects of nursing system and breed on the episodic release of luteinising hormone (LH) and growth hormone (GH) were studied in multiparous beef cows (n=28). The effect of nursing system was evaluated in PA breed. The duration of post-partum anoestrus was longer in ADLIB than in RESTR1 cows, while this period did not differ from the rest in the RESTR2 treatment. RESTR1 cows showed a tendency to have shorter LH peaks than their ADLIB counterparts. Breed effect was compared in the RESTR2 regimen. There were no differences in the duration of post-partum anoestrus between twice-daily nursing PA and PI breeds. RESTR2 cows from the PA breed had slightly longer LH peaks and greater LH peak area than those from PI breed. There were no differences among nursing systems or between breeds in any measure of GH secretion. There was no correlation between LH and GH pulse frequency. The area under the concentration data and the average data value of LH were negatively correlated with the duration of post-partum anoestrus, whereas the contrary occurred between these measures of GH and the duration of this period. The variables that best allowed discrimination among nursing systems and between breeds were the duration of post-partum anoestrus and the LH pulse frequency within the 8-h sampling.

Apéndice

8. Apéndice

Acreditación de la aceptación de las publicaciones en las revistas científicas

Las revistas donde se han publicado los trabajos incluidos en esta tesis doctoral están incluidas en el área temática *Agriculture, Dairy and Animal Science* del *Journal Citation Reports* (ISI Web of Knowledge):

1) Álvarez-Rodríguez J., Palacio J., Casasús I., Revilla R., Sanz A. (2009). Performance and nursing behaviour of beef cows with different types of calf management. *Animal* 3 (6): 871-878. *Factor de impacto año 2008: 0,994.*

2) Álvarez-Rodríguez J., Palacio J., Sanz A. (*aceptado*). Effects of nursing frequency and parity on the productive, metabolic and reproductive parameters of beef cows. *Livestock Science* (ref. LIVSCI-D-09-1895). *Factor de impacto año 2008: 1,091.*

3) Álvarez-Rodríguez J., Palacio J., Sanz A. (*en prensa*). Metabolic and luteal function in winter-calving Spanish beef cows as affected by calf management and breed. *Journal of Animal Physiology and Animal Nutrition* (disponible en: DOI: 10.1111/j.1439-0396.2009.00919.x). *Factor de impacto año 2008: 1,171.*

4) Álvarez-Rodríguez J., Palacio J., Casasús I., Sanz A. (*aceptado*). Does breed affect nursing and reproductive behaviour in beef cattle? *Canadian Journal of Animal Science* (ref. CJAS09033). *Factor de impacto año 2008: 0,659.*

5) Álvarez-Rodríguez J., Sanz A. (2009). Physiological and behavioural responses of cows from two beef breeds submitted to different suckling *strategies. Applied Animal Behaviour Science* 120: 39-48. *Factor de impacto año 2008: 1,823.*

6) Álvarez-Rodríguez J., Palacio J., Tamanini C., Sanz A. (*en prensa*). Luteinizing hormone and growth hormone secretion in early lactating Spanish beef cows. *Journal of Animal Physiology and Animal Nutrition* (Disponible en: DOI: 10.1111/j.1439-0396.2009.00961.x). *Factor de impacto año 2008: 1,171.*