TESIS DOCTORAL

Influencia de las pautas de crecimiento en lactancia y recría sobre los rendimientos de novillas de dos razas de aptitud cárnica con parto a dos años

José Antonio Rodríguez Sánchez

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Memoria presentada por **José Antonio Rodríguez Sánchez**
para optar al grado de Doctor por la Universidad de Zaragoza

Mayo de 2016

CERTIFICACIÓN DE LAS DIRECTORAS DE TESIS

Isabel Casasús Pueyo y ***Albina Sanz Pascua***, Doctoras en Veterinaria e Investigadoras de la Unidad de Tecnología en Producción Animal del Centro de Investigación y Tecnología Agroalimentaria del Gobierno de Aragón (CITA),

HACEN CONSTAR

Que D. ***José Antonio Rodríguez Sánchez***, Ingeniero Agrónomo y Master Science en Nutrición Animal, ha realizado bajo nuestra dirección los trabajos correspondientes a su Tesis Doctoral titulada "***Influencia de las pautas de crecimiento en lactancia y recría sobre los rendimientos de novillas de dos razas de aptitud cárnica con parto a dos años***", que se corresponde con el proyecto de Tesis aprobado por la comisión de Doctorado, y que cumple con los requisitos exigidos para optar al grado de Doctor por la Universidad de Zaragoza, por lo que autorizan su presentación para que pueda ser juzgada por el Tribunal correspondiente.

Lo que suscribimos como directoras del trabajo en Zaragoza, a 10 de mayo de 2016

Isabel Casasús Pueyo   Albina Sanz Pascua

La presente Tesis Doctoral se ha realizado en el Centro de Investigación y Tecnología Agroalimentaria del Gobierno de Aragón (CITA-Aragón) en el marco del siguiente proyecto de investigación:

"*Repercusiones de las pautas de crecimiento en ganado vacuno de carne sobre la productividad en hembras de reposición y en animales para la producción cárnica*". Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria; INIA-RTA2010-00057. Investigador principal: Isabel Casasús Pueyo.

El doctorando ha disfrutado de una Beca PreDoctoral de formación de personal investigador del Subprograma FPI-INIA, en el tema "*Repercusiones de las pautas de crecimiento en ganado vacuno de carne sobre la productividad en hembras de reposición y en animales para la producción cárnica. RTA2010-2010-00057-C3-01*", según convocatoria de 1 de febrero de 2011 (BOE 5 de febrero de 2011), resolución del 28 de junio de 2011 (BOE 21 de julio de 2011), y de un contrato concedido por el CITA-Aragón el 11 de diciembre de 2015 en el marco de los siguientes proyectos de investigación:

"*Conservación "in situ" de razas autóctonas de vacuno y ovino del Pirineo aragonés*". *INIA RZP2012-00002-00.*

"Efectos de la alimentación materna sobre el desarrollo embrionario y la descendencia: implicaciones en la eficiencia productiva de la vaca nodriza". *INIA RTA2013-00059-C02-00.*

Todos sabemos que ésta es la última página que se escribe de una Tesis, pero la primera que se lee. Supongo que es así porque no quieres olvidar a nadie que deba salir en las siguientes líneas, pero como es imposible, por las muchas personas que han contribuido a que esta Tesis llegue a su fin, de antemano pido perdón a quien olvide citar. Me gustaría dar las gracias:

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"No vivas para que tu presencia se note,
sino para que tu ausencia se sienta"
Bob Marley

Esta Tesis Doctoral se planteó con el fin de analizar la posibilidad de adelantar el primer parto de las novillas de razas Parda de Montaña y Pirenaica a los dos años de edad. Para ello se aplicaron distintos manejos alimentarios durante la lactancia (0-6 meses) y la recría (6-15 meses), y se estudiaron en ambas razas las repercusiones de distintas pautas de crecimiento sobre parámetros productivos, reproductivos y fisiológicos de novillas paridas a dos años. Los ensayos se prolongaron hasta el final de la primera lactación de las novillas.

El primer ensayo se realizó con terneras de raza Parda de Montaña, a las que se aplicó un diseño factorial 2 × 2 con manejos de la alimentación orientados a obtener distintos crecimientos en las fases de lactancia (1,0 vs. 0,7 kg/d) y recría (1,0 vs. 0,7 kg/d). Las terneras llegaron a la pubertad con el mismo peso (55,9% del peso adulto), pero diferente edad dependiendo del ritmo de crecimiento impuesto tanto durante la lactancia como en la recría. Las novillas con mayor crecimiento durante la recría necesitaron un mayor número de inseminaciones para quedar gestantes, lo cual retrasó su primer parto. Las novillas con un crecimiento continuo de 0,7 kg/d en lactancia y recría tuvieron mayor dificultad al parto debido a su bajo peso, escaso desarrollo pélvico y alta desproporción materno-fetal. Los rendimientos al primer parto (producción lechera y peso del ternero al nacimiento y destete a los 4 meses de edad) no se vieron afectados por los manejos impuestos a cada lote. Las vacas primíparas con mayores crecimientos en recría tendieron a presentar una reactivación ovárica más temprana. Las concentraciones plasmáticas de los metabolitos analizados (glucosa, colesterol, ácidos grasos no esterificados, β-hidroxibutirato y urea) así como la de IGF-I presentaron una alta dependencia de la dieta ingerida. Las novillas con mayor nivel de glucosa e IGF-I en plasma al destete y mayor nivel de colesterol durante la recría alcanzaron antes la pubertad. Sin embargo, no se observó ninguna relación de los niveles de leptina y el inicio de la pubertad.

En el segundo ensayo se utilizaron terneras con un alto crecimiento durante la lactancia (1 kg/d). Siguiendo un diseño factorial 2 × 2, a partir del destete se suministraron dos dietas con el fin de conseguir dos crecimientos durante la recría hasta la primera cubrición (0,8 vs. 0,6 kg/d) en novillas de dos razas (Parda de Montaña vs. Pirenaica). Las terneras entraron en pubertad con el mismo peso (55,5 % del peso adulto), pero diferente edad, siendo más precoces las de raza Parda de Montaña pero sin observarse influencia del manejo alimentario aplicado en la recría. La fertilidad a la primera cubrición y el número de inseminaciones necesarias para quedar gestantes fue similar en todos los lotes, por lo que la edad al parto no fue diferente. Las novillas Pardas presentaron mayor dificultad al parto debido a la alta desproporción materno-fetal, consecuencia del mayor peso de sus terneros al nacimiento. La producción lechera en este ensayo fue moderada, presentando una interacción entre la raza y el manejo de la alimentación durante la recría. La diferente producción de leche no se tradujo en diferentes ganancias de los terneros durante la lactación. Tampoco se observaron diferencias entre lotes en el reinicio de la ciclicidad postparto. Los niveles plasmáticos de los metabolitos estudiados fueron fundamentalmente dependientes de la dieta ingerida, y similares en ambas razas. La concentración plasmática de leptina no presentó relación con la entrada en pubertad, sin embargo las novillas con mayor concentración de IGF-I en plasma durante la recría fueron más precoces.

A la vista de los resultados presentados en esta memoria, se concluye que es factible adelantar el primer parto de novillas de las razas Parda de Montaña y Pirenaica a los dos años si se asegura un crecimiento mínimo de 1 kg/d antes o después del destete. Si se consiguen estas ganancias en la lactancia, durante la recría será suficiente un crecimiento de 0,6 kg/d para no perjudicar los rendimientos de las novillas al primer parto.

The aim of this PhD thesis was to analyze the possibility of advancing the first calving of Parda de Montaña and Pirenaica heifers to two years. Different feeding managements were applied during the lactation (0-6 months) and the rearing periods (6-15 months) to study in both breeds the impact of different patterns of growth on productive, reproductive and physiological parameters in heifers calved at two years. The experiments lasted until the end of the first lactation of the heifers.

The first trial was conducted with Parda de Montaña heifers. A 2 × 2 factorial design was applied, with different feeding managements conducted to reach different growth rates during the lactation (1.0 vs. 0.7 kg/d) and the rearing periods (1.0 vs. 0.7 kg/d). The heifers reached the puberty with the same weight (55.9% of mature weight), but different age depending on the growth rate imposed both during the lactation and rearing periods. Faster growing heifers during the rearing phase needed more services to get pregnant, delaying the first calving. The heifers with continued growth of 0.7 kg/d during the lactation and rearing periods had a higher calving difficulty due to the low weight, low pelvic development and the high maternal-fetal disproportion. The performance at first lactation (milk yield and calf weight at birth and weaning at 4 months) was not affected by the feeding managements applied. The primiparous cows with greater growth rate during the rearing phase tended to show an earlier ovarian reactivation. The plasma concentrations of the analyzed metabolites (glucose, cholesterol, NEFA, β-hydroxybutyrate and urea) and IGF-I had a high dependence of the diet intake. The heifers with the greatest plasmatic concentration of glucose and IGF-I at weaning and cholesterol during the rearing period reached the puberty earlier. However, no relationship was found between the concentration of leptin and the onset of puberty.

In the second trial heifers with high growth rate during the lactation (1 kg/d) were used. Following a factorial design 2 × 2, from weaning to the first breeding, two diets were supplied in order to get two growth rates during the rearing period (0.8 vs. 0.6 kg/d) in heifers from two breeds (Parda de Montaña vs. Pirenaica). The calves reached the puberty with the same weight (55.5% of mature weight), but different age, the Parda de Montaña ones being more precocious, but without influence of the feeding management applied during the rearing phase. The fertility at the first service and the number of services necessary for gestation was similar in all lots, so the age at calving was not different. The Parda de Montaña heifers had greater difficulty at calving due to the high maternal-fetal disproportion, by the greater calf birth weight. The milk yield in this trial was moderate, showing an interaction between breed and feeding management during the rearing. The different milk production did not result in different calf gains during the lactation. No differences were observed between lots in the resumption of postpartum cyclicity. The metabolites studied were mostly dependent on the diet intake, and similar in both breeds. The plasma leptin concentration was not related to the onset of puberty, however the heifers with greater concentrations of IGF-I in plasma during the rearing phase were more precocious.

From the results displayed in this study, it is concluded that it is feasible to advance the first calving of the Parda de Montaña and Pirenaica heifers to two years if gains of at least 1 kg/d before or after weaning are guaranteed. If these gains are achieved in the lactation, during the rearing period a growth rate of 0.6 kg/d would be sufficient to provide adequate first calving performance.

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Anexos

LISTA DE ABREVIATURAS

a.s.l.	Above sea level
ADF	Acid Detergent Fiber
ADG	Average Daily Gain
ADL	Acid Detergent Lignin
AI/IA	Artificial Insemination
ANOVA	ANalysis Of VAriance
BCS	Body Condition Score
BW	Body Weight
CITA	Centro de Investigación y Tecnología Agroalimentaria
cm	Centimeter
CP	Crude Protein
CV	Coefficient of Variation
d	Día
DM	Dry Matter
dm^2	Square decimeter
ECM	Energy Corrected Milk
EDTA	Ácido Etilendiaminotetraacético
ELISA	Enzyme-Linked ImmunoSorbent Assay
g	Gramo
GH	Hormona de crecimiento
GLM	Modelos lineales generalizados
GnRH	Hormona liberadora de gonadotropinas
h	Hora
ha	Hectárea
IBR	Infectious Bovine Rhinotracheitis
IGF-I	Factor de Crecimiento similar a la Insulina-I
kg	Kilogramo
L	Liter
LSMEANS	Least-Square means
LH	Hormona luteinizante
MBW	Mature Body Weight
ME	Metabolic Energy
min	Minute
MJ	Megajulio
mL	Mililiter
mmol	Milimol
Mo	Month
NDF	Neutral Detergent Fiber
NEFA	Non-esterified fatty acids
ng	Nanogram
NRC	National Research Council
P	Probabilidad de error
PA	Parda de Montaña
$PGF_{2\alpha}$	Prostaglandina $F_{2\alpha}$
PI	Pirenaica
PMSG	Pregnant Mare Serum Gonadotropin
PPA	PostPartum Anestrus
PRID	Progesterone Releasing Intravaginal Device
PV	Peso Vivo
r	Coeficiente de correlación
RIA	Radioimmunoassay
SEM	Standard Error of the Mean
SFT	Subcutaneous Fat Thickness
UI/IU	Unidades Internacionales
vs	Versus
Yr	Year
µg	Microgramo

1. Introducción

El sector vacuno de carne en España representa el 5,7% de la Producción Final Agraria. Dentro de las producciones ganaderas es el cuarto en importancia económica, por detrás de los sectores porcino, lácteo y avícola, representando el 14,9% de la Producción Final Ganadera (Ministerio de Agricultura Alimentación y Medio Ambiente, 2015b). Dentro del sector vacuno de carne se distinguen dos subsectores, el de vacas nodrizas y el de cebo, con no muchas explotaciones de ciclo cerrado que integren los dos (García-Martínez *et al.*, 2006).

España cuenta con casi 2 millones de vacas nodrizas, ocupando el segundo lugar en la Unión Europea por detrás de Francia, con un 17% del total (Ministerio de Agricultura Alimentación y Medio Ambiente, 2015a). Geográficamente, casi el 70% de estas vacas madres se encuentran localizadas entre la zona de dehesa (Castilla y León, Extremadura y Andalucía) y Galicia. El 47% del censo total de vacas nodrizas españolas se explota en pureza de una gran variedad de razas, tanto autóctonas como foráneas (Ministerio de Agricultura Alimentación y Medio Ambiente, 2014).

Por su parte, Aragón registra el 2,8% de las vacas nodrizas españolas, siendo la Parda de Montaña y la Pirenaica las razas más representativas del Pirineo aragonés, por su adaptación al pastoreo de montaña.

La Parda de Montaña es una raza que proviene de la selección para producción cárnica de la Brown Swiss. Esta raza fue introducida en España hace más de dos siglos, como raza de doble aptitud (carne-leche). Por su parte, la Pirenaica es una raza rústica autóctona de los Pirineos y utilizada para la producción de carne. Las dos razas presentan un peso adulto similar, en torno a los 580 kg (Casasús *et al.*, 2002). A pesar de tener un formato adulto similar, a lo largo del ciclo productivo presentan diferencias en sus rendimientos. En general, el peso al nacimiento suele ser mayor en los terneros Pardos que en los Pirenaicos (Casasús *et al.*, 2002); las vacas Pardas suelen producir más leche que las Pirenaicas (Sanz *et al.*, 2003) y por tanto sus terneros presentan mayores ganancias de peso durante la lactancia, lo que da lugar a mayores pesos al destete (Villalba *et al.*, 2000). Tras el destete, las dos razas presentan similar capacidad de crecimiento, tanto en animales de cebo (Blanco *et al.*, 2009c) como en animales adultos y de reposición (Casasús *et al.*, 2004). Esto es debido a que las dos razas tienen similar capacidad de ingestión (Casasús *et al.*, 2004) e índice de conversión (Blanco *et al.*, 2009c). Donde sí se encuentran diferencias es en el ritmo de maduración, siendo más precoz en la deposición grasa la Parda que la Pirenaica (Piedrafita *et al.*, 2003). Esta diferencia, debida posiblemente al pasado lechero de la raza Parda de Montaña, puede dar lugar a una composición corporal diferente entre ambas razas, y por tanto, a unas necesidades alimentarias distintas durante la recría.

1.1 Objetivos técnicos en la producción de ganado vacuno de carne

En las explotaciones de vacas nodrizas la principal producción es el ternero, por tanto, la eficiencia reproductiva es determinante en su rentabilidad. Para maximizar la eficiencia reproductiva es necesario reducir los tiempos improductivos de las nodrizas a lo largo de su vida. Para ello, es importante que las vacas tengan un manejo que permita un intervalo entre partos no superior a 365 días, y así destetar un ternero al año. Por otro lado, el periodo más largo en el que una vaca se mantiene improductiva es desde su nacimiento hasta el primer parto. Adelantando el primer parto, además de reducir el tiempo en el que no se obtiene producción de la novilla, se reduce la cantidad de insumos necesarios para su mantenimiento hasta que comienza a producir. Teniendo en cuenta que las novillas suelen representar en torno al 20% del censo de una explotación de vacuno de carne (Blanco *et al.*, 2009a), esto supone un alto coste, el segundo mayor dentro de una explotación de vacas nodrizas, sólo por detrás de la alimentación (Freetly *et al.*, 2014).

Las novillas que paren a los 2 años de edad producen 0,7 terneros más a lo largo de su vida productiva que si paren por primera vez con 3 años (Day y Nogueira, 2013). Tradicionalmente las novillas no parían hasta que alcanzaban los 3 años, sin embargo, la edad al primer parto se ha ido reduciendo, y en países con un gran censo vacuno, como Estados Unidos, en este momento, lo normal es que las novillas tengan el primer parto con 2 años (Day y Nogueira, 2013). En España también se está produciendo este adelantamiento en la edad al primer parto, pero todavía un 50% de las novillas españolas paren por primera vez con más de 3 años (Ministerio de Agricultura Alimentación y Medio Ambiente, 2014).

El retraso en la edad al primer parto en las novillas españolas puede ser debido a la utilización de razas menos precoces que las razas británicas usadas en Estados Unidos (Diskin y Kenny, 2014). Además, el retraso se puede achacar a la creencia que tienen los ganaderos de que si las novillas paren demasiado jóvenes, tanto su crecimiento como sus rendimientos productivo y reproductivo se pueden ver afectados (Stygar *et al.*, 2014). Los ganaderos piensan del mismo modo que el autor del primer artículo científico encontrado sobre este tema (McCampbell, 1921), que si al primer parto las novillas son pequeñas, nunca llegarán a tener un tamaño adulto adecuado. Además, estas novillas producirán terneros más pequeños y tendrán más problemas reproductivos que si el parto se retrasa.

A estas creencias hay que añadirle el hecho de que al ser considerados animales "improductivos", suelen tener menos atención de la requerida. Según Derks *et al.* (2012), los ganaderos consideran la recría de las novillas como la fase menos importante dentro de su manejo. En unos casos, la falta de atención a las novillas es debida a que, por el pequeño tamaño de las explotaciones, son manejadas con el resto del rebaño, no atendiendo específicamente sus necesidades. En otros casos, aunque su manejo sea independiente del resto del rebaño, los requerimientos nutricionales de las novillas no son cubiertos, por no tener en cuenta que presentan unas necesidades especiales, ya que son animales en crecimiento.

A esta falta de atención sobre las novillas ha contribuido también la paulatina adaptación de las explotaciones a condiciones cada vez más extensivas impuestas por las Políticas Agrarias de la Unión Europea (García-Martínez *et al.*, 2009). Esta extensificación hace que la alimentación recibida a lo largo del año esté estrechamente ligada a la oferta de recursos forrajeros disponibles (Casasús *et al.*, 2002). La variabilidad a lo largo del año en la disponibilidad de alimento no siempre permite a los animales en crecimiento expresar su máximo potencial (Villalba *et al.*, 2000).

Asimismo, en este tipo de sistemas extensivos lo habitual es que la distribución de los partos no sea homogénea a lo largo de año, sino que haya una clara tendencia a la concentración de parideras (García-Martínez, 2008). Esto dificulta aún más el manejo de la recría, puesto que las novillas deben incorporarse al rebaño de vacas adultas tras el primer parto. Para ello es necesario tener en cuenta que las primíparas deberían parir antes que las vacas adultas para, por un lado, mejorar la atención de los partos, y por otro considerar la mayor duración de su anestro postparto (Sanz *et al.*, 2004; Álvarez-Rodríguez *et al.*, 2010b).

El manejo que reciben las novillas hasta su entrada en producción, durante la recría, se verá reflejado en las producciones futuras (Wathes *et al.*, 2014), por tanto, es necesario desarrollar programas específicos de manejo de las novillas en recría que permitan adelantar el primer parto a los 2 años. Estos programas deben describir unas pautas nutricionales específicas, para cubrir las necesidades de las novillas en cada fase de la recría. Para definir estos programas es necesario conocer cuáles son las necesidades de las novillas en cada momento, y en qué etapas de su desarrollo puede permitirse una reducción alimentaria, sin suponer perjuicio en su futuro productivo y reproductivo.

1.2 Desarrollo de las novillas de reposición

En el crecimiento de una ternera se distinguen dos etapas, separadas por la entrada en pubertad. La etapa prepuberal se caracteriza por un crecimiento básicamente lineal o isométrico. Tras la pubertad el crecimiento pasa a ser alométrico, modificándose principalmente la composición corporal y la relación entre los distintos tejidos (Hossner, 2005). Según Owens *et al.* (1995), el crecimiento y la composición tisular están controlados por la edad cronológica, la edad fisiológica (relacionada con el sexo, tamaño adulto y madurez), ingestión de energía, estado hormonal, deposición tisular relativa y número y actividad de células específicas de cada tejido. Por tanto, modificando la cantidad de energía ingerida por una ternera, se podría modificar su ritmo de crecimiento, así como la composición tisular y su estado tanto metabólico como endocrino.

Distintos ritmos de crecimiento en la fase pre y postpuberal pueden presentar distintas repercusiones a lo largo de la vida de la ternera, tanto a nivel productivo (producción de leche y peso del ternero) como reproductivo (entrada en pubertad, anestro postparto, fertilidad, etc.). El ritmo de crecimiento en estas dos fases es fácilmente graduable en explotaciones lecheras, donde la alimentación de las terneras es controlada desde que nacen. Sin embargo, en explotaciones de vacuno de carne la alimentación no suele estar tan controlada, por depender de la producción lechera de las madres, y ésta a su vez de los recursos naturales a su disposición en cada época.

En las explotaciones de vacuno de carne, se pueden distinguir dos periodos con grandes diferencias en cuanto al tipo de alimento y energía ingeridos por los terneros, antes y después del destete. Es habitual que el destete se produzca en torno a los 6 meses de edad, por lo que la fase postdestete comprenderá parte de ambas etapas, pre y postpuberal.

Durante la lactancia se pueden distinguir distintos manejos de las terneras. En algunas explotaciones las terneras únicamente tienen acceso a la leche producida por sus madres, mientras que en otras son suplementadas mediante pastoreo con las madres o con el suministro de concentrado. Desde el destete hasta la cubrición, las terneras se pueden mantener estabuladas, con una dieta controlada, o en el campo, a expensas de la disponibilidad de recursos naturales. En el primer caso, se puede imponer el ritmo de crecimiento deseado en cada etapa de la recría. En el segundo, el más comúnmente utilizado, el ritmo de crecimiento dependerá de la disponibilidad de recursos en cada época. También cabe la posibilidad de hacer una recría mixta, en la que las terneras aprovechan los recursos naturales y son suplementadas en épocas de escasez para conseguir los crecimientos deseados.

Los programas de recría se deben adecuar a las características de cada explotación, para aprovechar al máximo sus recursos propios y reducir al mínimo los costes que supone una novilla hasta su entrada en producción. El manejo de las novillas durante la recría debe asegurar que tanto el peso como el desarrollo corporal sean adecuados a la cubrición y al parto, para evitar que se vean perjudicados sus rendimientos futuros y su longevidad.

1.2.1 Peso a la primera cubrición y al parto

Tradicionalmente se ha considerado que las novillas debían llegar a la primera cubrición con un peso mínimo de entre el 60 y el 65% del peso vivo adulto (Patterson *et al.*, 1992). Sin embargo, Endecott *et al.* (2013) defienden que cubrir a las novillas por primera vez con un 65% del peso vivo adulto es excesivo, porque la genética ha evolucionado desde que se establecieron esas recomendaciones en los años 60-80. Varios trabajos proponen reducir el porcentaje tradicional hasta el 50-57% con el fin de disminuir los costes de la reposición (Funston y Deutscher, 2004; Roberts *et al.*, 2009b). Según dichos autores, esta medida hará que las novillas tengan menores necesidades de mantenimiento, por ser más ligeras, por lo que dispondrán de mayores oportunidades de obtener un balance energético positivo cuando la calidad del alimento sea baja en el momento de la cubrición.

Estas publicaciones señalan que la reducción en el peso con el que las novillas llegan a la primera cubrición no afectaría a su rendimiento reproductivo. Sin embargo, a pesar de que la fertilidad final no se vea afectada por la reducción de este peso (Funston y Deutscher, 2004), en cubriciones cortas (45 días), se reduce el número de novillas preñadas en los 30 primeros días por llegar menos novillas cíclicas al inicio de la cubrición (Perry y Cushman, 2013). Cuanto más temprana sea la cubrición de la novilla en la época de cubrición, más temprano será su primer parto en la paridera, lo que aumenta el tiempo que tiene para recuperarse del parto antes de la siguiente cubrición. Las novillas que paren al inicio de la paridera con 2 años suelen parir pronto durante toda su vida. Sin embargo, las que paren al final de la paridera suelen parir tarde el siguiente año, o no parir (Perry y Cushman, 2013) por quedarse vacías en esa cubrición y por tanto ser susceptibles de ser desechadas por baja productividad.

Por otro lado, el peso al parto es un importante punto de referencia en el manejo de la recría (Sejrsen y Purup, 1997), porque las novillas con pesos bajos tienen un mayor riesgo de distocias (Mee, 2008), producen menos leche, y tienen menor longevidad (Archbold *et al.*, 2012). Las recomendaciones sitúan este peso entre el 80 y el 90% del peso adulto, que indica el NRC (2000) para novillas de carne y Troccon (1993) para novillas de leche, respectivamente.

A la hora de definir el peso adulto de cada raza, existe una gran discrepancia, puesto que depende, entre otras cosas, del sistema de explotación en el que se encuentre. En vacuno lechero, se han descrito variaciones de hasta 100 kg en el peso al primer parto en una misma raza, según sea explotada bajo un sistema intensivo o extensivo basado en pastoreo (Roche *et al.*, 2015).

1.2.2 Desarrollo esquelético

El vacuno de carne alcanza la madurez, el formato adulto, entre los 4 (Cano *et al.*, *en prensa*) y los 5 años (Goldberg y Ravagnolo, 2015). Los huesos siguen creciendo hasta ese momento, aunque su crecimiento es máximo durante el primer año de vida. Un pobre desarrollo esquelético durante este primer año puede no ser recuperable más adelante (Wathes *et al.*, 2014). Por tanto, sería necesario que los programas de recría contemplaran el estudio del desarrollo corporal de las novillas a lo largo de la recría, para evitar problemas futuros.

Son muchas las mediciones que se pueden llevar a cabo con este fin, pero las más comunes, por su alta correlación con el peso durante la recría, son la altura a la cruz, el perímetro torácico y las dimensiones de la grupa (Brickell *et al.*, 2009b). La altura a la cruz es un buen indicador del desarrollo esquelético del animal, pudiendo ser una medida complementaria al peso, para determinar la madurez de la novilla. Por otro lado, el perímetro torácico presenta una alta correlación con el peso (Swali *et al.*, 2008), por lo que se podría utilizar para estimarlo en explotaciones que no disponen de medios para pesar a los animales (Wood *et al.*, 2015).

En el crecimiento del esqueleto de una novilla, es de vital importancia el desarrollo pélvico. Las dimensiones de la pelvis van a determinar el tamaño del ternero que puede parir una novilla. Por tanto, el estudio pélvico puede aportar una valiosa información a la hora de predecir distocias (Johanson y Berger, 2003), puesto que la mayor parte de las dificultades al parto en novillas son debidas a la desproporción entre el tamaño del ternero y el canal del parto (Hickson *et al.*, 2006). El examen de la zona pélvica debe hacerse antes de la primera cubrición, ya que esta medida está altamente correlacionada con el área pélvica al parto (r = 0,71; Johnson *et al.*, 1988). De este modo, se podrán descartar las novillas que por tener una pelvis demasiado pequeña puedan presentar problemas al parto. En general, el peso y la edad están positivamente relacionados con el tamaño de la pelvis al parto. Sin embargo, el peso por sí solo no es siempre un buen indicador del área pélvica. Debido a esto, sería necesario medir las dimensiones internas de la pelvis por medio de un pelvímetro. Esta medida debe ser hecha por personal especializado, lo que podría limitar su estudio a nivel de explotación. Sin embargo, según Murray *et al.* (2002), las medidas externas de la grupa, tanto longitud como anchura, están correlacionadas con la altura y anchura internas de la pelvis, siendo éstas medidas más sencillas de tomar.

1.2.3 Deposición del tejido graso

De los tres tejidos esenciales (muscular, óseo y adiposo) que componen el cuerpo animal, el último en desarrollarse es el tejido adiposo. Este tejido almacena el exceso de energía ingerida por el animal en forma de triglicéridos, para movilizarlos ante una subnutrición, con el fin de obtener la energía necesaria para su mantenimiento y producción. Este mecanismo, por el que durante los periodos con déficit energético se aumenta la lipólisis y se reduce la lipogénesis optimizando la movilización de ácidos grasos no esterificados para mantener el equilibrio fisiológico, se conoce como homeostasis (Roche *et al.*, 2009). Cuando la movilización de ácidos grasos es muy acelerada, el hígado los transforma en β-hidroxibutirato (Herdt, 2000), que puede ser una fuente de energía.

El tejido graso se relaciona con el equilibrio energético, el apetito y la reproducción (Hossner, 2005). Se distribuye en depósitos alrededor de vísceras (perineal, omental, cardíaco, mesentérico), entre el músculo y la piel (subcutáneo) y se infiltra entre los músculos (intermuscular) y dentro de los músculos (intramuscular). Los distintos depósitos adiposos no se almacenan al mismo tiempo (Eguinoa *et al.*, 2003), en primer lugar se deposita la grasa visceral, posteriormente la subcutánea y por último la intramuscular. Por otro lado, la deposición grasa se ve afectada por la entrada en pubertad, puesto que, antes de ésta suele darse un aumento en el número de depósitos adiposos (hiperplasia), mientras que tras la pubertad sólo se incrementa su tamaño (hipertrofia).

La grasa subcutánea es la más fácilmente medible y por ello se suele utilizar para determinar el estado corporal de las vacas. Ésta puede determinarse mediante la utilización de ultrasonidos, siendo la zona de las costillas 12-13 y la grupa (punto P8 australiano) las dos zonas más comúnmente utilizadas para dicho fin (Lazzaroni *et al.*, 2007). Sin embargo, a nivel de explotación, la condición corporal puede determinarse mediante apreciación visual y palpación de la zona de las apófisis transversas de las vértebras lumbares y de la base de la cola. Hay distintas escalas para determinar este parámetro como pueden ser las descritas por Lowman *et al.* (1976) (escala de 1-5) o Wagner *et al.* (1988) (escala de 1-9).

1.2.4 Pautas de crecimiento

Además de la importancia que tiene alcanzar un peso y desarrollo corporal adecuados en el momento de la cubrición y del primer parto, es importante el modo en que éstos se consiguen, es decir, las pautas de crecimiento que siguen las novillas durante la recría. Troccon (1993) proponía una evolución lineal del peso: 30, 60 y 90% del peso adulto a los 6, 15 y 24 meses, respectivamente. Sin embargo, cuando las novillas se mantienen en pastoreo, la cantidad y calidad de la alimentación oscila periódicamente, lo que puede influir en el potencial de crecimiento de la novilla, si ésta no recibe ningún suplemento. En estos casos, la estrategia alternativa es un manejo de la alimentación por fases (Ford y Park, 2001), en el que las novillas pasan periodos de restricción, seguidos de periodos de realimentación, para aprovechar el crecimiento compensatorio que esto provoca.

En este sentido, es necesario estudiar la repercusión de diferentes planos nutritivos en diversas fases, y determinar los momentos clave en que deben establecerse pautas de manejo específicas, o compensadoras de lo ocurrido en otros periodos (Grings *et al.*, 2007).

Es importante controlar los ritmos de crecimiento, tanto antes como después del destete, porque pueden afectar al rendimiento productivo y reproductivo de la novilla. A este respecto, puede ser interesante el estudio de los niveles plasmáticos de Factor de Crecimiento similar a la Insulina-I (IGF-I), puesto que esta hormona ha sido implicada tanto en el crecimiento (Blanco *et al.*, 2011), como en la reproducción (Samadi *et al.*, 2014). La concentración de IGF-I en vacuno puede variar con la edad, la nutrición, el estado fisiológico, la raza y el sexo. Esta hormona presenta unos niveles bajos al nacimiento, que van aumentando hasta alcanzar una meseta, en torno a los 9 meses, y vuelve a descender después de los 15–18 meses (Kerr *et al.*, 1991). La ingestión de energía y proteína están relacionadas positivamente con la concentración de IGF-I en plasma, así, cuando se restringe la alimentación de los terneros, se reduce el aumento de la IGF-I ligado con la edad (Blanco *et al.*, 2009c) retardando la llegada a la meseta descrita anteriormente. Al aumentar el aporte energético de las dietas, tras un periodo de restricción, la concentración de IGF-I puede incrementase pudiendo inducir un crecimiento compensador. En vacuno, los machos suelen presentar una concentración plasmática de IGF-I más elevada que las hembras. A su vez, las novillas suelen tener mayores niveles de IGF-I que las vacas adultas al parto, presentando ambas un precipitado descenso 15 días antes de este momento, que es recuperado tras el parto (Kerr *et al.*, 1991). También se han descrito diferencias raciales en la concentración de IGF-I, presentando mayores niveles las razas lecheras, como la Holstein, que las cárnicas, como la Charolesa, Blanca Azul Belga o Wagyu (Blanco, 2007).

1.3 Aptitud reproductiva de las novillas de reposición

En la vida reproductiva de una vaca nos encontramos con dos periodos fisiológicos críticos, el prepuberal y el postparto. Por tanto, entender los procesos que subyacen a los cambios fisiológicos en las novillas durante el desarrollo prepuberal, y en las vacas adultas durante y después del parto, puede ayudar a mejorar los protocolos de manejo en ambas fases. Los dos periodos se caracterizan por un estado de anestro, o ausencia de ciclos estrales. Curiosamente, muchos de los déficits hormonales que limitan la reproducción en la novilla prepúber, son similares a los observados en las vacas durante el anestro postparto. En las novillas los cambios hormonales y fisiológicos preceden a la primera ovulación, como consecuencia de la madurez del sistema nervioso central, lo que finalmente desencadena la función normal del ovario, el inicio de los ciclos estrales regulares, y la posibilidad de quedar gestante. Por su parte, durante el anestro postparto, los cambios hormonales reflejan el restablecimiento del sistema reproductivo tras la gestación y el parto (Williams y Amstalden, 2010).

1.3.1 Pubertad

El sistema reproductivo es el último de los sistemas y aparatos del cuerpo animal en madurar (Patterson *et al.*, 1992), siendo la entrada en pubertad un momento crítico en la recría de una ternera por ser el comienzo de su vida reproductiva. La pubertad es el proceso por el que una ternera adquiere competencias reproductivas, y se puede definir como la etapa de desarrollo de la novilla en la que sus ciclos ováricos comienzan a ser normales, y es capaz de quedarse preñada soportando una gestación (Diskin y Kenny, 2014).

Para poder adelantar el primer parto de una novilla, el primer paso es adelantar su pubertad, para que llegue púber a la época de cubrición. Para implementar óptimos programas de recría de novillas, que mejoren la eficiencia reproductiva, y por tanto la rentabilidad de la explotación, mediante el adelanto de la pubertad, es necesario conocer los factores implicados en ésta (Ahmadzadeh *et al.*, 2011).

A pesar de la tardía maduración del sistema reproductivo, su desarrollo comienza en la fase embrionaria, con fenómenos fisiológicos como la constitución de la población de folículos primordiales, y continúa tras el nacimiento hasta alcanzar la pubertad, con el crecimiento de folículos y ovarios (Revilla *et al.*, 1992). La aparición de la pubertad va a depender de la acción aislada o combinada de múltiples factores genéticos (raza) y ambientales (estado nutricional, estación de nacimiento, interacción social, etc.).

1.3.1.1 Factores genéticos

La pubertad suele alcanzarse a un determinado peso (*peso crítico*) para cada raza (Schillo *et al.*, 1992). Este peso ha sido descrito en torno al 55% del peso adulto para un amplio rango de razas (Freetly *et al.*, 2011).

La edad a la pubertad presenta una heredabilidad media de 0,40, aunque con una amplia variabilidad que va desde 0,07 a 0,67 (Diskin y Kenny, 2014). Según estos autores, la heterosis tiene una gran influencia en esta edad, siendo las terneras cruzadas más precoces que las explotadas en pureza. Se ha descrito una correlación negativa entre la producción de leche y la edad a la pubertad (r = –0,87; Diskin y Kenny, 2014), siendo, por tanto, las razas lecheras más precoces que las cárnicas.

En las razas de ganado vacuno de carne explotadas en el pirineo, se ha observado que la raza Parda de Montaña alcanza la pubertad a una edad más temprana que la Pirenaica (Revilla *et al.*, 1992), a pesar de tener un peso adulto similar (Casasús *et al.*, 2002), probablemente debido al pasado lechero de la Parda de Montaña.

A nivel individual, en la selección de terneras para futuras reproductoras, es importante tener en cuenta características como la circunferencia escrotal del padre, puesto que está positivamente correlacionada con precocidad de las hijas (Hopper, 2015).

1.3.1.2 Factores ambientales

El ***estado nutricional*** del animal es el factor más influyente de todos los factores ambientales y de manejo que pueden afectar a la pubertad. Teniendo en cuenta que el peso a la pubertad se puede considerar constante, el nivel de alimentación ejerce un papel importante sobre la edad al inicio de la pubertad, puesto que ésta se ve negativamente correlacionada con el ritmo de crecimiento que presenta la ternera desde el nacimiento hasta la entrada en pubertad (Patterson *et al.*, 1992).

Day y Anderson (1998) propusieron que el tiempo desde el nacimiento hasta la pubertad se puede dividir en cuatro periodos: infantil (desde el nacimiento hasta los 2 meses), desarrollo (2-6 meses), estático (6-10 meses) y peripuberal. La edad a la pubertad se ve influida por la alimentación que tiene la ternera en cada uno de estos periodos, excepto en el infantil (Day y Nogueira, 2013). A partir de aquí, existen discrepancias a la hora de determinar en qué medida influyen los distintos ritmos de crecimiento, antes y después del destete, sobre la pubertad. Según Roberts *et al.* (2009b), la pubertad se ve afectada en gran medida por las ganancias de peso registradas, tanto durante la lactancia como durante el comienzo de la recría. Sin embargo, otros autores no encontraron influencia del ritmo de crecimiento después del destete (Wiltbank *et al.*, 1966; Gasser *et al.*, 2006; Cardoso *et al.*, 2014). Gasser *et al.* (2006) concluyeron que aumentando la energía ingerida, mediante el suplemento de concentrado entre los 4 y 6,5 meses de edad, la pubertad se adelanta independientemente de la dieta que tengan las terneras tras esa fecha. Este hecho puede ser debido a que un estado nutricional y metabólico favorable durante ese periodo puede provocar cambios funcionales en el sistema neuroendocrino reproductivo, que persistirán a pesar de que se restrinja la alimentación tras dicho periodo (Cardoso *et al.*, 2014).

El ritmo de crecimiento en cada fase vendrá determinado por el nivel y tipo de alimentación, y este ritmo va a determinar la composición corporal de la ternera. No está claro el mecanismo por el que se relacionan, pero ya Brody (1945) sugirió que la pubertad ocurre en el punto de inflexión de la curva de crecimiento, correspondiente al cambio de deposición magra a deposición grasa. Debido a esto, Wiltbank *et al.* (1966) proponían que hay un porcentaje de grasa crítico que se debe superar para asegurar suficientes reservas para soportar la transición a la pubertad. Por esto, el retraso en la pubertad en novillas más magras se ha asociado a una menor deposición grasa (Randel y Welsh, 2013).

El tejido adiposo desempeña un papel dinámico en los mecanismos fisiológicos y en la homeostasis del cuerpo, siendo considerado el mayor órgano endocrino y paracrino, por regular gran cantidad de factores de crecimiento y hormonas (Hausman *et al.*, 2012). Una de estas hormonas es la leptina puesto que, a pesar de ser producida por varios tejidos, mayoritariamente se origina en el tejido adiposo. La leptina ejerce acciones endocrinas sobre rutas metabólicas que regulan el apetito y el gasto de energía, así como sobre la secreción de hormonas reproductivas y metabólicas, especialmente de hormona luteinizante (LH) y de hormona de crecimiento (GH) (Zieba *et al.*, 2005).

Según Hausman *et al.* (2012), a medida que se acerca la pubertad, la concentración en leptina va aumentando, y hay un umbral a partir del cual se permite la activación del sistema reproductivo. Por otro lado, Block *et al.* (2003) defienden que el aumento peripuberal registrado en la concentración plasmática de leptina es debido al aumento del tejido adiposo, y no porque esta hormona provoque el inicio de la pubertad. En este sentido, la leptina se podría considerar como una señal del estado nutricional del animal.

Hall *et al.* (1995) proponían que dentro de una misma raza la pubertad se alcanza a un peso similar, pero no ocurre con una composición corporal o estado metabólico y endocrino similar en todas las novillas. Según estos autores, no se produce un cambio brusco en la composición corporal y en el estado metabólico de la novilla por los que se puede desencadenar la entrada en pubertad.

Otro de los factores ambientales estudiados, en relación con la entrada en pubertad, es la ***estación de nacimiento***. A pesar de que el vacuno no se considera una especie estacional, existen evidencias de variaciones en la actividad reproductiva en función de la época de nacimiento, independientes del estado nutricional. Los efectos de la estación sobre la edad a la pubertad se han atribuido a la duración de las horas de luz y la temperatura ambiental (Hopper, 2015). El principal intérprete del fotoperiodo es la glándula pineal, que produce melatonina en respuesta a la oscuridad. La glándula pineal está implicada en la transducción de la luz, en una señal neuroendocrina, que podría provocar un incremento de la frecuencia de pulsos de LH (Schillo *et al.*, 1992). La pubertad se puede adelantar mediante la aplicación de melatonina exógena, o incluso, sometiendo a las novillas a una extensión artificial del fotoperiodo (Hopper, 2015). También se ha descrito en ovino, especie sexualmente estacional, que la salida del anestro postparto puede ser modificada por medio del manejo de la alimentación (Forcada y Abecia, 2006). Se ha sugerido, además, que las diferencias entre genotipos en edad a la pubertad podrían interaccionar con el fotoperiodo, existiendo una "*edad crítica*" para la raza Pirenaica, que podría estar relacionada con la duración creciente de las horas de luz en primavera (Revilla *et al.*, 1993).

Otro factor que puede influir en el inicio de la pubertad es la ***interacción social***. Aunque el efecto macho no sea tan marcado como en ovejas (Abecia *et al.*, 2015) o cabras (Delgadillo *et al.*, 2015), las novillas que son expuestas a la presencia de un toro pueden adelantar su pubertad (Roberson *et al.*, 1991). Este efecto es mayor en las novillas con un ritmo de crecimiento alto que en las que presentan un crecimiento moderado (Ahmadzadeh *et al.*, 2011). Tal vez, esto significa que la presencia del toro no puede acelerar la entrada en pubertad si las novillas no han recibido una dieta adecuada y/o no han conseguido el peso óptimo. El mecanismo fisiológico por el cual la presencia del toro puede acelerar la entrada en pubertad no está claro. Sin embargo, se cree que las feromonas afectarían al descenso en el feedback negativo creado por el estradiol sobre la sensibilidad del hipotálamo, por lo que aumentaría la frecuencia de LH antes, adelantando la edad a la pubertad (Whittier *et al.*, 2008). También se ha descrito la estimulación que supone la presencia de otras hembras activas sexualmente para el inicio de la ciclicidad (Berardinelli y Joshi, 2005), conociendo este fenómeno como efecto hembra o celo por simpatía.

Por último, el ***tipo de alojamiento*** y el ***tamaño del lote*** donde se encuentra la ternera también pueden influir en la pubertad. Según Cappellozza *et al.* (2014), para que las novillas tengan una adecuada función reproductiva también podría ser necesario el ejercicio, mediante el cual se generan opiodes endógenos que modulan la secreción de gonadotropinas y la consiguiente entrada en pubertad.

1.3.2 Anestro postparto

Un parámetro clave en la rentabilidad de una explotación de vacuno de carne es el tiempo que tarda una vaca en volver a estar cíclica tras el parto (duración del anestro postparto). Según Sanz *et al.* (2004), la duración del anestro postparto depende de múltiples factores, entre los que destacan: la alimentación pre y postparto, la frecuencia de amamantamiento y la dificultad del parto. Los tres primeros son factores de manejo independientes de fases previas en la vida de la novilla. Sin embargo, la incidencia de distocias, especialmente al primer parto, depende en gran medida del manejo que las novillas han tenido durante la recría, como se ha explicado anteriormente.

Hay que tener en cuenta que la duración del anestro en las vacas primíparas suele ser más larga que en las multíparas. Este alargamiento se debe a que al estrés causado por el parto y la producción de leche se le suma que las primíparas todavía están en crecimiento (Boldt *et al.*, 2015).

La fertilidad conseguida a lo largo de toda la época de cubrición de las novillas no se ve afectada por el ritmo de crecimiento durante la recría (Roberts *et al.*, 2009b), sin embargo, las novillas sometidas a ritmos bajos de crecimiento pueden no llegar púberes a la cubrición, por lo que, como se ha apuntado anteriormente, se reducirá el número de novillas gestantes en los primeros días de la cubrición. Este hecho retrasará el primer parto de la vaca, por lo que puede no estar cíclica durante la época de cubrición siguiente, quedando vacía.

1.4 Productividad de las vacas nodrizas

Una vez garantizado que las novillas llegan de forma satisfactoria al primer parto, lo importante es asegurar su productividad a largo plazo. Teniendo en cuenta que la principal producción en una explotación de vacas nodrizas es la venta de terneros, su productividad dependerá del número de crías que pueda producir una vaca y del rendimiento de éstas.

1.4.1 Rendimiento de los terneros

En España, las explotaciones de vacas nodrizas suelen vender los terneros al destete, para ser cebados o recriados en otra explotación, por tanto, es importante que tanto el peso al nacimiento como la ganancia de peso durante la lactancia sean altos, para alcanzar así el mayor peso de venta posible. Estos dos parámetros dependen, en gran medida de factores genéticos, pero también están influidos por el manejo de la alimentación que reciben las vacas durante la gestación y la lactación. De igual modo, las pautas de alimentación que siguen las novillas durante la recría, antes de la primera cubrición, pueden afectar al rendimiento de sus terneros.

1.4.1.1 Peso del ternero al nacimiento

Uno de los factores que más influye en la incidencia de distocias al parto es la desproporción entre el tamaño de la vaca y del ternero, especialmente en primíparas. Para evitar distocias, es necesario adecuar el peso del ternero al de la vaca al parto mediante una óptima selección del semental.

El peso del ternero al nacimiento se ve influido por la alimentación de la vaca en el último tercio de la gestación, puesto que, en este periodo es cuando el ternero gana el 70-75% del peso (Hopper, 2015).

En el caso de primíparas, e incluso multíparas hasta que alcanzan el tamaño adulto, el peso del ternero al nacimiento también podría estar influido por la alimentación que reciben hasta la primera cubrición, puesto que ésta influye en el peso con el que llegan al parto. Distintos ritmos de crecimiento durante la recría, pueden influir en el tamaño de la novilla al primer parto. Aunque algunos autores han indicado que restringir la alimentación de las novillas durante la recría no afectaría al peso del ternero al nacimiento (Roberts *et al.*, 2009a).

1.4.1.2 Crecimiento del ternero

Durante la lactancia, el factor que más influye en la ganancia de peso del ternero, y por tanto en el peso al destete, es la producción de leche de la madre (Park *et al.*, 1998; Villalba *et al.*, 2000).

Durante la primera lactación, la producción lechera está positivamente correlacionada con el peso al parto (Dobos *et al.*, 2001), por lo cual, las ganancias de peso hasta ese momento afectarán a dicha producción. Por otro lado, no afectarán de igual modo los crecimientos pre y postpuberales. Tras la pubertad, el ritmo de crecimiento sólo afectaría al peso con el que la novilla llega al primer parto. Sin embargo, los crecimientos prepuberales pueden influir sobre el desarrollo mamario, lo que podría afectar al potencial lechero de la vaca a lo largo de su vida productiva (Lohakare *et al.*, 2012). En este punto hay discrepancias entre autores, dado que algunos describen efectos negativos de ritmos de crecimiento prepuberales altos (Buskirk *et al.*, 1996), mientras que otros los encuentran positivos (Buskirk *et al.*, 1995) o no encuentran efecto (Freetly *et al.*, 2001) sobre la producción lechera.

En el desarrollo mamario pueden distinguirse cuatro etapas, en dos de ellas la glándula mamaria crece al mismo ritmo que el resto del cuerpo (etapas isométricas; desde el nacimiento hasta los 3 meses y desde los 10-12 meses hasta los 3 meses de gestación) y dos en las que crece a mayor velocidad que el resto del cuerpo (etapas alométricas; desde los 3 hasta los 10-12 meses y a partir del tercer mes de gestación) (Lohakare *et al.*, 2012). El final de la primera fase alométrica coincidiría con el inicio de la pubertad (Sejrsen *et al.*, 2000), por tanto, ritmos altos de crecimiento que adelanten la entrada en pubertad reducirán la duración de esta fase con la consiguiente reducción en el desarrollo mamario. Además, un ritmo de crecimiento prepuberal alto, debido a dietas basadas en concentrado o con altos niveles de energía, puede provocar un engrasamiento excesivo de la ubre en detrimento del parénquima secretor (Sejrsen y Purup, 1997). En vacuno de leche se ha estudiado ampliamente el crecimiento prepuberal adecuado para maximizar la producción lechera (Zanton y Heinrichs, 2005). Sin embargo, en el caso de vacas nodrizas, son escasas las referencias en este sentido, por lo que sería de gran interés determinar el ritmo de crecimiento óptimo en distintas razas y/o sistemas de producción de vacuno de carne.

1.4.2 Longevidad

Otra aproximación para evaluar los factores que afectan a la eficiencia de una explotación de vacuno de carne es el estudio de la longevidad. Hay numerosas razones por las que una vaca se desecha (infertilidad, pérdida del ternero, mala aptitud maternal, aplomos, etc.) pero la principal es la baja fertilidad (Roberts *et al.*, 2015). Esta causa de desvieje cobra mayor importancia si cabe en el caso de las primíparas, puesto que, como se ha explicado anteriormente, la duración de su anestro posparto suele ser mayor que el de las multíparas.

Una vaca necesita destetar de 3 a 5 terneros para amortizar los costes de recría (Perry y Cushman, 2013). Si es desechada antes de producir estos terneros, los costes de su recría se repartirán entre las demás novillas, reduciendo así el beneficio de la explotación.

Por otro lado, aumentando la longevidad de las vacas se reduce la tasa de reposición, es decir, el número de terneras a recriar para mantener el tamaño de la explotación, lo que aumentaría el número de terneras disponibles para la venta. Además, la reducción en el número de primíparas que entran en el rebaño cada año hará que el peso global de los terneros al destete sea mayor, puesto que este peso va en aumento hasta que la vaca tiene 5-6 años (Roberts *et al.*, 2015). Lo mismo sucedería con el peso de las vacas desviejadas, que aumentará a medida que la vaca tiene mayor edad.

En resumen, son varios los factores a tener en cuenta a la hora de adelantar el primer parto de las novillas a los dos años. Además, la influencia de cada uno de estos factores no es independiente, por lo que es necesario su estudio en conjunto para desarrollar adecuados programas de manejo de la reposición que mantengan el equilibrio entre la reducción de costes y el rendimiento de las vacas a lo largo de su vida productiva.

2. Objetivos

Esta Tesis Doctoral se planteó para analizar la posibilidad de adelantar el primer parto de las novillas de razas autóctonas españolas de carne (Parda de Montaña y Pirenaica) a los dos años de edad.

Para abordar dicho objetivo general, se propusieron los siguientes objetivos parciales:

1.- Evaluar el efecto de distintas estrategias de alimentación durante la lactancia (0–6 meses) y la recría (6–15 meses) sobre:

- La evolución del ***peso*** y ***desarrollo corporal*** de las novillas, desde el nacimiento hasta el destete de su primer ternero.
- Aspectos ***reproductivos***, como la entrada en pubertad, fertilidad y la duración del anestro postparto de las novillas.
- Aspectos ***productivos***, como el peso del ternero al nacimiento y al destete, así como la producción lechera.
- La evolución de los ***perfiles metabólicos*** (glucosa, colesterol, ácidos grasos no esterificados, β-hidroxibutirato y urea) y ***endocrinos*** (IGF-I y leptina) de las novillas, desde el nacimiento hasta el destete de su primer ternero.

2.- Evaluar el efecto de la interacción de la raza con el manejo de la alimentación durante la recría de las novillas (6–15 meses), sobre los parámetros expuestos en el anterior objetivo.

3. Ensayos de la Tesis

Se plantearon dos ensayos con el objeto de evaluar la posibilidad de adelantar el primer parto de novillas de razas cárnicas autóctonas españolas a los dos años de edad:

1.- Con el primer ensayo se pretendió analizar, en novillas de raza Parda de Montaña, el efecto de diferentes pautas de manejo alimentario, desde el nacimiento de las terneras hasta su primera cubrición (15 meses), sobre distintos parámetros productivos, reproductivos y fisiológicos, hasta el destete de su primer ternero.

2.- Por su parte, el segundo ensayo pretendía estudiar la repercusión de la raza (Parda de Montaña y Pirenaica) y la aplicación de distintas dietas, desde el destete de las terneras (6 meses) hasta su primera cubrición (15 meses), sobre los mismos parámetros analizados en el primer ensayo.

Los ensayos se llevaron a cabo con terneras nacidas en dos parideras de otoño consecutivas, en el rebaño de la Finca Experimental La Garcipollera del Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA). Esta finca se localiza en Bescós de la Garcipollera (Jaca; 42°37' N, 0°30' O), a 945 m de altitud, con una temperatura media anual de 10,2 ± 0,2 °C y una precipitación anual de 1059 ± 68 mm.

En el primer ensayo, partiendo de 62 vacas Parda de Montaña multíparas (7,6 ± 3,5 años), se utilizaron las 29 hembras nacidas. Tras el nacimiento, las terneras se distribuyeron, de manera aleatoria, en cuatro lotes para aplicar un diseño factorial 2 × 2 de dos objetivos de crecimiento en lactancia (0–6 meses: 1 vs. 0,7 kg/d) y dos en recría (6–15 meses: 1 vs. 0,7 kg/d). Estos lotes se equilibraron teniendo en cuenta la fecha (12 de octubre ± 13 días) y peso de nacimiento de las terneras (41 ± 3 kg), así como el peso (580 ± 65 kg) y condición corporal de las madres (2,6 ± 0,1).

En el segundo ensayo se partió de 25 vacas multíparas (7,5 ± 3,6 años) de cada raza, y se utilizaron las 13 hembras Parda de Montaña y las 12 Pirenaicas nacidas en la paridera. En este caso, durante los 6 meses de lactancia todas las terneras tuvieron el mismo manejo, encaminado a favorecer un crecimiento alto en esta fase, y los tratamientos se aplicaron al destete. En este momento, las terneras de cada raza se dividieron en dos lotes, de manera aleatoria, para aplicar dos objetivos de crecimiento durante la recría (6–15 meses: 0,8 vs. 0,6 kg/d). Estos cuatro lotes se equilibraron teniendo en cuenta la edad (6,4 ± 0,3 meses) y peso de las terneras (238 ± 41 kg) al destete.

3.1 Manejo de las novillas

A continuación se detallan las 5 fases en que consistió el manejo de las novillas, desde su nacimiento hasta el destete de su primera cría (Figuras 1 y 2).

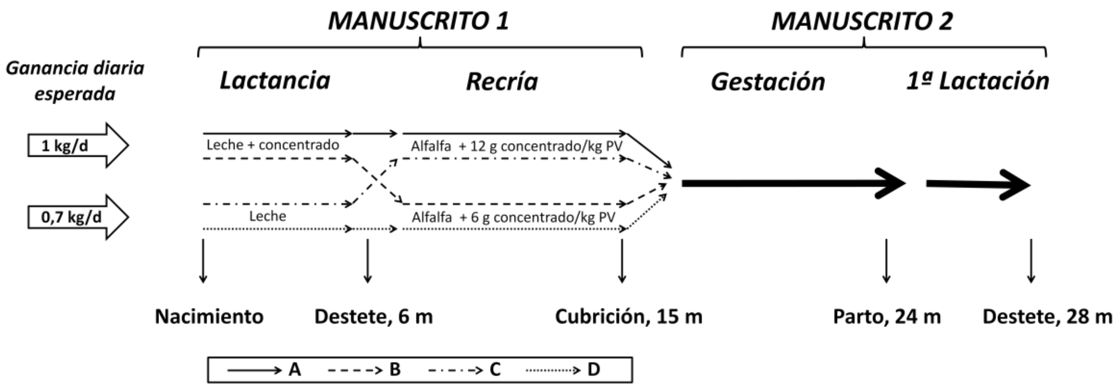

Figura 1. Diseño experimental del ensayo 1. A: Novillas con una ganancia diaria esperada de 1 kg/d desde el nacimiento a la cubrición; ***B***: Novillas con una ganancia esperada de 1 y 0,7 kg/d durante la lactancia y recría, respectivamente; ***C***: Novillas con una ganancia esperada de 0,7 y 1 kg/d durante la lactancia y recría, respectivamente; ***D***: Novillas con una ganancia diaria esperada de 0,7 kg/d desde el nacimiento a la cubrición.

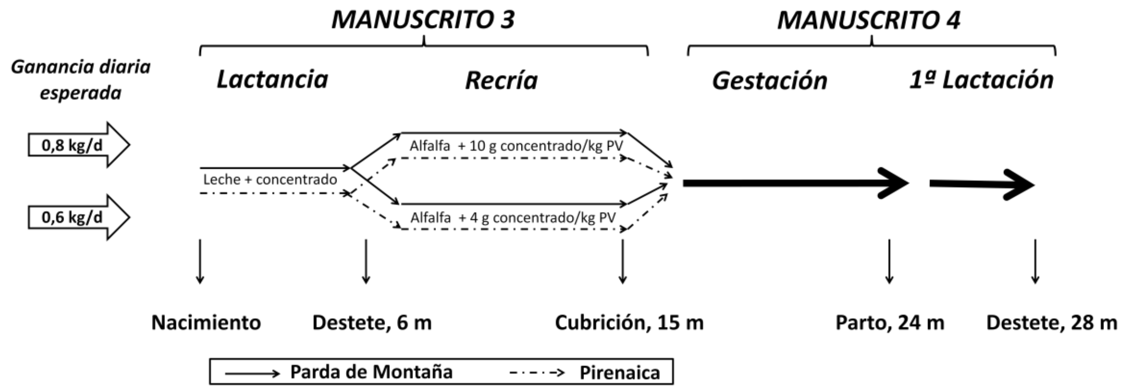

Figura 2. Diseño experimental del ensayo 2.

3.1.1 Lactancia

Desde el nacimiento hasta el destete de las terneras, en torno a los 6 meses de edad. Durante esta fase vacas y terneras permanecieron estabuladas en parques separados según el lote. Las vacas recibieron una dieta completa comercial calculada para cubrir sus necesidades de mantenimiento y producción de leche. Las terneras permanecieron en cubículos adyacentes a los parques de las madres, permitiéndoles mamar durante 30 minutos dos veces al día (08:00 y 16:00 h). Las terneras con un objetivo de crecimiento mayor (1 kg/d) tuvieron acceso libre a un pienso de arranque suministrado diariamente para controlar la cantidad ingerida por el lote. En el segundo ensayo, todas las novillas tuvieron este último manejo, presentando una ganancia media diaria de 1,039 ± 0,176 kg.

3.1.2 Recría

Tras el destete, las terneras fueron trasladadas a las instalaciones del Centro de Investigación y Tecnología Agroalimentaria de Aragón en Zaragoza (41°43' N, 0°48' O), a 225 m de altitud, con una temperatura media anual de 15,2 ± 0,2 °C y una precipitación anual de 318 ± 63 mm. Esta fase duró 9 meses (6-15 meses), terminando con el inicio de la época de cubrición. Durante este periodo, las terneras se alimentaron a base de heno de alfalfa y concentrado de crecimiento. La alfalfa fue suministrada a libre disposición para todos los animales de ambos ensayos. Por su parte, el concentrado se suministró en función del objetivo de crecimiento de cada lote. En el primer ensayo, se ofrecieron 12 y 6 g/kg de PV, para los objetivos de crecimiento de 1 ó 0,7 kg/d, respectivamente. En el segundo ensayo, la cantidad de concentrado administrada fue de 10 y 4 g/kg de PV para conseguir unos crecimientos de 0,8 y 0,6 kg/d, respectivamente.

3.1.3 Cubrición

En torno a los 15 meses de edad se inició una época de cubrición de 90 días, con la sincronización de celos de las novillas. Dicha sincronización se hizo siguiendo un protocolo Ovsynch, más la aplicación de un progestágeno (progesterone releasing intravaginal device, PRID): el día 0 se puso el PRID y 10 µg de GnRH; el día 10 se inyectaron 25 mg de prostaglandina $F_{2\alpha}$; el día 12 se pusieron 500 unidades internacionales de PMSG al retirar el PRID; a las 48 horas de la retirada del PRID se administraron otros 10 µg de GnRH y 8 horas más tarde se procedió a la inseminación artificial (Figura 3).

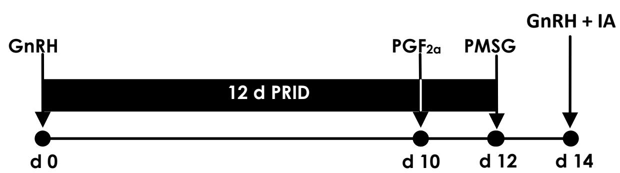

Figura 3. Protocolo utilizado para la sincronización de celos e inseminación artificial a tiempo fijo de las novillas a los 15 meses.

Después de la inseminación, y hasta el final de la época de cubrición, se hicieron controles visuales diariamente (07:00 y 19:00 h), para detectar los celos de las novillas no cubiertas en la primera y sucesivas inseminaciones, y proceder a su reinseminación.

3.1.4 Gestación

Tras la confirmación de la gestación, un mes después de finalizar la época de cubrición, las novillas fueron trasladadas de nuevo a la Finca Experimental La Garcipollera. Durante este periodo, todas las novillas fueron manejadas como un único lote, pastando praderas de montaña. En este tiempo se mantuvo una carga ganadera adecuada para cubrir las necesidades de mantenimiento, crecimiento y gestación de las novillas (4 novillas/ha). Un mes antes de la fecha prevista para el parto, las novillas fueron estabuladas y alimentadas a base de heno de hierba.

3.1.5 Primera lactación

Todas las vacas primíparas tuvieron el mismo manejo durante esta fase. Tras el parto recibieron una dieta completa comercial estimada para cubrir sus necesidades, tanto de mantenimiento y producción lechera como de crecimiento. Los terneros tuvieron acceso libre a las madres durante los 4 meses que duró la lactación, sin contar con ningún otro suplemento.

La composición y calidad nutritiva de las dietas se describe en los correspondientes apartados de los manuscritos.

3.2 Medidas y toma de muestras

3.2.1 Peso

A lo largo de los 30 meses de duración de cada ensayo, las novillas se pesaron semanalmente, para registrar la evolución del peso y determinar la ganancia media diaria en cada fase. Sus terneros también se pesaron semanalmente, desde el nacimiento hasta el destete, con 4 meses, con el mismo fin.

3.2.2 Zoometría

Se tomaron medidas zoométricas al destete, cubrición, parto y destete del primer ternero, para estudiar el desarrollo esquelético de las novillas. Se midió la altura a la cruz, el perímetro torácico, y la anchura y longitud de la grupa siguiendo las indicaciones de Aparicio Sánchez (1960). A partir del producto de las medidas de la grupa se calculó el área pélvica como describieron Murray *et al.* (1999).

3.2.3 Condición corporal

Se determinó la condición corporal de las novillas en el momento de la cubrición, al parto y al destete de los terneros. Esta determinación se hizo siguiendo el método descrito por Lowman *et al.* (1976), cuya escala va del 1 al 5 y se determina mediante la palpación de la zona de las apófisis transversas de las vértebras lumbares y de la base de la cola. Al mismo tiempo, se llevó a cabo la medición del espesor de la grasa subcutánea mediante ultrasonidos en los dos lugares más comúnmente utilizados para dicho fin, la zona de las costillas 12-13 y la grupa o punto P8 australiano.

3.2.4 Producción lechera

Para determinar la cantidad de la leche ingerida por las terneras se llevó a cabo un ordeño mensual, durante los 6 meses que se prolongó la lactancia. De igual modo, para conocer la cantidad de leche producida por las novillas tras su primer parto, se ordeñaron mensualmente durante los 4 meses de lactación.

Esta determinación se llevo a cabo utilizando la técnica descrita por Le Du *et al.* (1979), que consiste en realizar dos ordeños mecánicos, con un intervalo de 6 horas en las que los terneros no tienen acceso a la madre, tras la aplicación de 40 unidades internacionales de oxitocina (Gineamin, Laboratorios Maymó, Barcelona) para favorecer la bajada de la leche.

Para determinar la calidad de la leche, se analizó su contenido en grasa, proteína y caseína mediante infrarrojos (Milkoscan 4000™; Fosselectric Ltd., Hillerod, Dinamarca). Para comparar los resultados, las distintas producciones de leche se corrigieron al 3,5% de grasa y 3,2% de proteína, con la fórmula oficial para la leche corregida en energía según describieron Casasús *et al.* (2004).

3.2.5 Muestras de sangre

Desde el nacimiento hasta el parto de las novillas, se tomaron muestras de sangre trimestrales, para determinar la concentración en metabolitos (glucosa, colesterol, ácidos grasos no esterificados, β-hidroxibutirato y urea) y hormonas (IGF-I y leptina). Durante la primera lactación, las muestras se tomaron mensualmente.

Las concentraciones de glucosa (método glucosa oxidasa/peroxidasa), colesterol (método enzimático-colorimétrico), β-hidroxibutirato (método enzimático-colorimétrico) y urea (test cinético UV) se determinaron con un analizador automático GernonStar (RAL/TRANSASIA, Dabhel, India). Para la determinación del contenido en ácidos grasos no esterificados se utilizó un kit comercial (Randox Laboratories Ltd., Crumlin Co., Antrim, Reino Unido) basado en un método enzimático. La concentración en IGF-I se cuantificó mediante un ensayo inmunométrico quimioluminiscente en fase sólida con el analizador Immulite (Siemens Medical Solutions Diagnostics Limited, Llanberis, Gwynedd, Reino Unido). Por su parte, la concentración plasmática de leptina se determinó mediante un kit comercial RIA multiespecies (Multispecies Leptin Ria kit; LINCO Research, St. Charles, MO, EE.UU.).

Estas concentraciones se utilizaron para analizar la evolución de los perfiles metabólicos y endocrinos, generados por los distintos manejos alimentarios.

A lo largo de la fase de recría se tomaron muestras semanales de sangre para analizar el contenido en progesterona, y así determinar cuando las novillas alcanzaban la pubertad. Esta misma hormona se analizó durante la primera lactación de las novillas, tomando dos muestras semanales, para determinar la duración del anestro postparto. La concentración plasmática de progesterona se determinó mediante un kit comercial ELISA (Ridgeway Science, Lydney, Reino Unido).

3.3 Análisis estadístico de los datos

Los datos obtenidos en los ensayos se analizaron con el paquete estadístico SAS (SAS Institute Inc., Cary, NC, EE.UU.).

Los datos de pesos y concentraciones, tanto de metabolitos como de hormonas, se analizaron mediante modelos lineales generalizados, con medidas repetidas de las diferentes fechas (Procedimiento Mixed), con una matriz de covarizanza no-estructurada. Se consideró la novilla como efecto aleatorio en los dos ensayos. Como efectos fijos del primer ensayo se consideraron los manejos de la alimentación durante la lactancia y la recría, la fecha de la muestra y sus interacciones. En el segundo ensayo, los efectos fijos fueron la raza, el manejo de la alimentación durante la recría, la fecha de la muestra y sus interacciones.

Se utilizaron los modelos lineales generalizados (Procedimiento GLM) para el análisis de las ganancias de peso, las medidas zoométricas y espesores grasos, edad y peso en momentos clave (pubertad, primera inseminación, concepción, primer parto, peso del ternero al nacimiento y destete), número de inseminaciones necesarias para la cubrición, cantidad y calidad de la leche o la duración del anestro postparto. Se consideraron como efectos fijos los mismos que en el análisis de medidas repetidas, exceptuando la fecha de la muestra.

En ambos análisis estadísticos (Mixed y GLM) se obtuvieron las medias mínimo-cuadráticas y el error estándar de la diferencia, y posteriormente se realizó la prueba de separación de medias, con un nivel de confianza del 95% de los efectos fijos evaluados.

Los datos de fertilidad, asistencia al parto y duración de primer ciclo estral tras el parto fueron analizados mediante el test de frecuencias χ^2 (Procedimiento FREQ).

La relación entre variables se calculó mediante el coeficiente de correlación de Pearson.

En la Figura 4 se describe la nomenclatura utilizada en cada uno de los manuscritos.

Efectos Fijos	**Manejo alimentario en lactancia**		**Manejo alimentario en recría**		**Raza**	
Niveles	Crecimiento Alto	Crecimiento Bajo	Crecimiento Alto	Crecimiento Bajo	Parda de Montaña	Pirenaica

ENSAYO 1

Manuscrito 1. Metabolic, endocrine, and reproductive responses of beef heifers submitted to different growth strategies during the lactation and rearing periods

LACT		**REAR**	
LactHI	LactLO	RearHI	RearLO

Manuscrito 2. *First calving performance and physiological profiles of 2-year-old beef heifers according to their prebreeding growth rate*

PRE		**POST**	
PRE-HI	PRE-LO	POST-HI	POST-LO

Lotes resultantes: HI–HI, HI–LO, LO–HI y LO–LO

ENSAYO 2

Manuscrito 3. *Postweaning feeding management of beef heifers to be bred at 15 months: I. Growth, puberty and fertility in two genotypes*

Manuscrito 4. *Postweaning feeding management of beef heifers to be bred at 15 months: II. Gestation and first calving performance in two genotypes*

FEED		**BREED**	
HIGH	LOW	PA	PI

Lotes resultantes: PA–HIGH, PA–LOW, PI–HIGH y PI–LOW

Figura 4. Nomenclatura de los efectos fijos y sus niveles en los distintos manuscritos.

4. Manuscrito 1

Rodríguez-Sánchez JA, Sanz A, Tamanini C, Casasús I. 2015. **Metabolic, endocrine, and reproductive responses of beef heifers submitted to different growth strategies during the lactation and rearing periods.** *Journal of Animal Science* 93: 3871-3885.

Abstract

The effects of different feeding strategies (1.0 kg/d target ADG [HI] and 0.7 kg/d target ADG [LO] during the lactation period (LACT; 0–6 mo) and the rearing period (REAR; 6–15 mo; HI–HI, HI–LO, LO–HI and LO–LO treatments) on the growth and reproductive parameters of beef heifers bred by fixed-time AI at 15 mo were analyzed. Animal weights were recorded weekly (from birth to 18 mo), and size measures at 6 and 15 mo. Heifers were bled to determine the onset of puberty and the metabolic and endocrine (IGF-I and leptin) status. During lactation, calves in the high lactation treatment (LactHI) had greater weight ($P < 0.001$), weight gain ($P < 0.001$) and body size ($P < 0.001$) than calves in the low lactation treatment (LactLO). The greater energy balance of LactHI heifers at weaning was reflected in greater concentrations of plasma glucose ($P < 0.001$), urea ($P < 0.001$) and IGF-I ($P < 0.001$); plasma levels of NEFA were lower ($P < 0.001$). During REAR, LactLO heifers had a greater growth rate than did LactHI heifers ($P < 0.001$), partially overcoming the lower gains during lactation. The differences in size measurements registered at weaning were also compensated, with the exception of LO–LO heifers. The IGF-I profile was highly correlated with animal performance traits and metabolic profiles, providing a useful indicator of growth, nutritional, and metabolic status at key points in development. By contrast, the function of leptin as an indicator of growth and reproductive development of heifers was less clear. All treatments had similar weights at puberty onset (55.9% mature BW), although LactLO ($P < 0.01$) and the low rearing treatment (RearLO; $P < 0.001$) heifers were older than the others. The animals with greater glucose and IGF-I levels at weaning and greater cholesterol concentrations during the REAR reached puberty earlier. The fertility rate (86%) was similar among treatments. The heifers in the high rearing treatment (RearHI) required more AI services to become pregnant and were older at conception ($P < 0.05$). The age of conception was positively correlated with glucose (r = 0.57, $P < 0.01$) and cholesterol (r = 0.68, $P < 0.001$) at 9 mo. Our results show that a 0.7 kg/d gain from birth allowed the first breeding at 15 mo, 6 mo earlier than usual for these conditions, without any negative effect on heifer reproductive performance.

Key words: cattle, efficiency, management, puberty, replacement, reproduction

4.1 Introduction

The development of heifers is a key component of a beef production enterprise because it is crucial to future dam productivity. In systems based on seasonal feeding, it is not uncommon that first calving occurs at 30 to 36 mo of age (Le Cozler *et al.*, 2010). Particularly in Spain and according to official data from Ministerio de Agricultura Alimentación y Medio Ambiente (2014), approximately 50% of heifers are older than 3 yr at the first calving. This delay is due to the belief of farmers that earlier calving impairs future growth, production, and reproductive performance (Stygar *et al.*, 2014). Additionally, because of the extensification of beef cattle production systems (García-Martínez *et al.*, 2009) and/or the small herd size, replacement heifers are often managed with the rest of the herd and do not receive specific care to ensure optimal rearing.

The cost of rearing replacements could be reduced if heifers calved at 2 yr, if heifers reaching puberty before 12 to 13 mo, and if heifers had their first breeding at approximately 15 mo of age (Wathes *et al.*, 2014). Optimum nutritional management is required to ensure the success of this strategy, and specific replacement programs have yet to be developed. The lifetime productivity of a cow begins at the onset of puberty and depends on subsequent critical events such as age at first breeding and calving and fertility rate (Diskin and Kenny, 2014). These aspects could be influenced by growth rates both before and after weaning, which depend on nutritional management. Therefore, different patterns of feeding may influence the metabolic and endocrine profiles during lactation and rearing, which, consequently, may modify development and reproductive performance (Brickell *et al.*, 2009b).

The objective of this experiment was to evaluate the effects of different feeding strategies during the lactation period (**LACT**, 0–6 mo) and the rearing period (**REAR**, 6–15 mo) on the patterns of growth, onset of puberty, fertility rate, and metabolic (glucose, NEFA, cholesterol, ß-hydroxybutyrate and urea) and endocrine status (IGF-I and leptin) of beef heifers bred at 15 mo.

4.2 Materials and methods

The Animal Ethics Committee of the Centro de Investigación y Tecnología Agroalimentaria (**CITA**) approved the experimental procedures, which were in compliance with the guidelines of the European Union (Directive No. 86/609/CEE, 1986) on the protection of animals used for experimental and other scientific purposes.

4.2.1 Animals, management and diets

The study was conducted at the CITA-La Garcipollera Research Station in the mountain area of the central Pyrenees (northeastern Spain, 42°37' N, 0°30' W, 945 m above sea level, mean annual temperature 10.2 ± 0.2 °C, and mean annual rainfall 1059 ± 68 mm) and at the CITA-Montañana Research Station (41°43' N, 0°48' W, 225 m above sea level, mean annual temperature 15.2 ± 0.2 °C, and mean annual rainfall 318 ± 63 mm).

Sixty-two Parda de Montaña (selected from old Brown Swiss for beef purposes) multiparous cows (7.6 ± 3.5 yr) calved in autumn at CITA-La Garcipollera, but only female calves and their dams (29 pairs) were used for this study. At calving, cow–calf pairs were randomly assigned to 1 of the 4 feed management strategies in a 2 × 2 factorial experiment. Two growth rates were targeted in the LACT (0–6 mo: 1.0 kg/d target ADG [**HI**] and 0.7 kg/d target ADG [**LO**] treatments, respectively) and 2 in REAR (6–15 mo; HI and LO treatments). The experimental design of the study, the diets supplied, and the resulting 4 treatments (HI–HI, HI–LO, LO–HI and LO–LO) are presented in Fig. 1. The treatments were randomly balanced according to dam calving BW (580 ± 65 kg) and BCS (2.6 ± 0.1; Lowman *et al.*, 1976) and to calf birth date (October 12 ± 13 d) and birth weight (41 ± 3 kg).

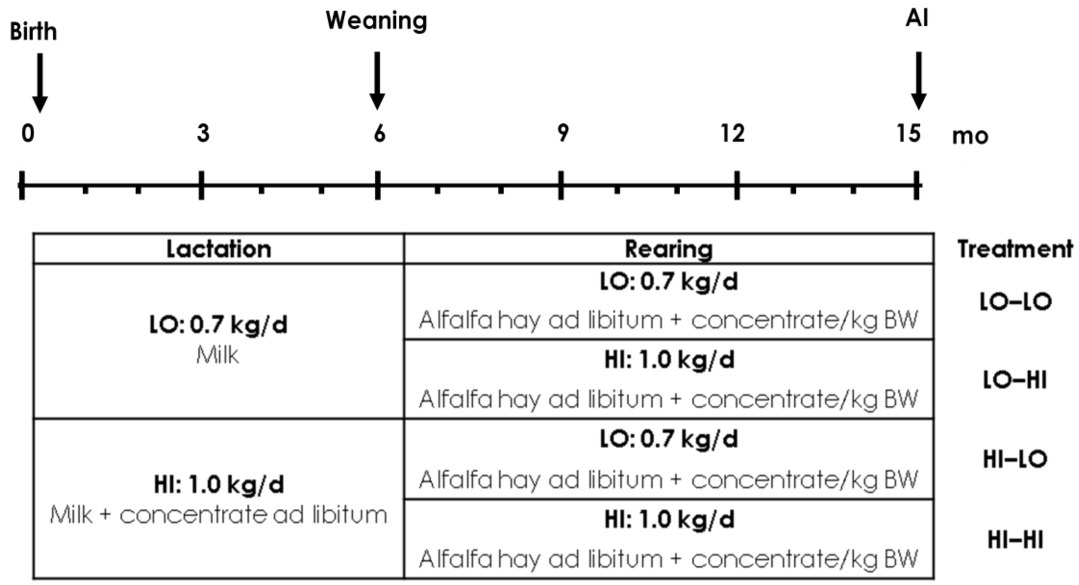

Figure 1. Experimental design with the treatment diets and the target ADG for each period and strategy.

The cow–calf pairs remained indoors throughout lactation in a loose housing system with straw-bedded pens. The dams were group-fed daily with 12 kg per animal of a total mixed ration (56% forage and 44% grains, with byproducts and vitamin and mineral supplements; 861 g/kg DM, 9.6 MJ ME/kg DM, 85 g CP/kg DM, and 499 g NDF/kg DM) to meet maintenance requirements for energy and protein in a 580-kg beef cow producing 9 kg of energy-corrected milk (**ECM**; NRC, 2000). The calves were kept in straw-bedded cubicles adjacent to their dams and were allowed to suckle twice daily for 30 min at 0800 and 1600 h. All heifers had access to fresh bed straw to ensure an adequate rumen development. To achieve the desired growth rates, heifers in the high lactation treatment (**LactHI**) had free access to starter concentrate (Table 1).

Before reaching 3 mo of age, the calves were vaccinated against infectious bovine rhinotracheitis (Bovilis IBR Marker; MSD Animal Health, Salamanca, Spain) and *Clostridium perfringens* (Polibascol; Schering-Plough, Kenilworth, NJ). At 6 mo (175 ± 13 d), the calves were weaned and transported to the CITA-Montañana facilities, where the REAR was conducted. The heifers were housed indoors in a loose housing system with straw-bedded pens with fresh and clean water supplied ad libitum. To achieve the targeted weight gains, heifers were group-fed alfalfa hay ad libitum and 12 (the high rearing treatment [**RearHI**]) or 6 g concentrate/kg BW (the low rearing treatment [**RearLO**] throughout this period (Table 1).

Table1. Ingredients and composition of concentrate and alfalfa hay provided to heifers during the lactation (0–6 mo) and rearing (6–15 mo) periods[1].

	Concentrate		***Alfalfa hay***
Item	***Lactation***	***Rearing***	
Ingredient (as-fed basis), %			
Corn	30.00	44.00	
Soybean flour	16.50	4.60	
Barley	15.50	21.60	
Corn gluten		15.00	
Extruded cereal	15.00		
Wheat bran	15.00		
Rapeseed flour		5.00	
Milk byproducts	3.00		
Beet pulp	2.00	3.00	
Palm oil	1.30	2.90	
Calcium carbonate	1.00	1.20	
Vitamin-mineral premix[2]	0.20	2.00	
Sodium chloride	0.50	0.20	
Urea		0.50	
Nutrient composition			
DM, g/kg	894	900	851
ME, MJ/kg DM	15.1	15.2	9.2
CP, g/kg DM	166	147	98
NDF, g/kg DM	214	252	462

[1]Lactation concentrate was provided ad libitum to heifers in the high lactation treatment. Rearing concentrate was provided in amounts of 12 g/kg BW to the high rearing treatment and 6 g/kg BW to the low rearing treatment heifers. Alfalfa hay was provided ad libitum in the rearing period to all heifers.

[2]Vitamin A, 7,000 IU/kg; Vitamin D3, 1,500 IU/kg; Copper (cupric sulfate pentahydrate), 2 mg/kg; Iodine (potassium iodide), 0.5 mg/kg; Cobalt (cobaltous carbonate monohydrate), 0.5 mg/kg; Zinc (zinc oxide), 40 mg/kg; Manganese (manganese oxide), 30 mg/kg; Selenium (sodium selenite), 0.2 mg/kg; Iron (ferrous carbonate), 5 mg/kg; Butylhydroxytoluene, 2 mg/kg.

At 15 mo, a 90-d breeding season began (Fig. 2). One month before breeding, the heifers were vaccinated against bovine viral diarrhea (Bovilis BVD; MSD Animal Health). All heifers were synchronized at 15 mo with an Ovsynch + progesterone releasing intravaginal device (**PRID**) program (Fig. 2) in which they simultaneously received 1.55 mg of progesterone in a PRID (CEVA, Barcelona, Spain) and a 10 µg injection of GnRH (Busol; INVESA, Barcelona, Spain) followed 10 d later by 25 mg of prostaglandin $F_{2\alpha}$ (Enzaprost; CEVA). After 12 d, the PRID was removed, and 500 IU of pregnant mare serum gonadotrophin (Foligon; Intervet, Salamanca, Spain) was administered followed 48 h later by a second injection of GnRH (10 µg). Eight hours after the final GnRH injection, the heifers were randomly inseminated from 1 of 4 bulls by an expert technician.

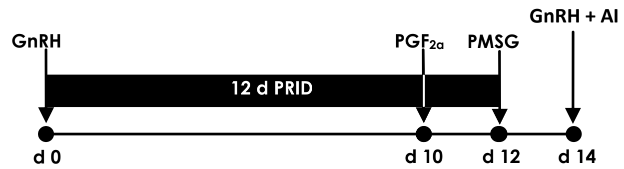

Figure 2. Synchronization protocol used in beef heifers at 15 mo of age managed with different feeding treatments. PRID = progesterone releasing intravaginal device.

After the first AI, estrus detection was recorded twice daily (0700 and 1900 h) until the end of the breeding season. The heifers were inseminated approximately 12 h after estrus was detected. Return to estrus after each AI was considered a diagnostic indicator of nonpregnancy status. Pregnancy was confirmed by ultrasonography (Aloka SSD-500V; equipped with a linear-array 7.5 MHz transducer; Aloka, Madrid, Spain) 31 d after the end of the breeding season.

The day of the first timed AI was used to determine the age and BW at first breeding, and the day of the effective AI was used to determine the age and BW at conception. The first-service fertility rate was determined as the number of pregnant heifers at the first AI divided by the total number of heifers. The number of AI necessary to become pregnant was calculated considering only heifers that were pregnant at the end of the breeding season. The fertility rate was determined as the number of pregnant heifers in the breeding season divided by the total number of heifers.

4.2.2 Measurements and blood sampling

The dams were milked monthly during lactation to determine the quantity and composition of daily milk intake by calves using the oxytocin and machine milking technique (Le Du *et al.*, 1979). The milk fat and protein were analyzed with an infrared scan (Milkoscan 4000™; Foss Electric Ltd., Hillerod, Denmark), and these data were used to calculate ECM yield (adjusted to 3.5% fat and 3.2% protein content), as described by Casasús *et al.* (2004).

The starter concentrate intake of LactHI heifers was recorded by group daily. The feed refusal was removed and weighed weekly. Throughout REAR, concentrate intake was recorded by group daily and adjusted monthly by average group weight. The intake of alfalfa hay was recorded by pen at weekly intervals. The actual daily intake along the experiment was calculated as feed provided minus feed refused. Feed samples were collected at weekly intervals and were pooled on a monthly basis for chemical analyses. The samples were dried at 60 °C until a constant weight and mill-ground (1 mm screen) and DM, ash, ether extract and CP (N × 6.25) contents were determined according to the Association of Official Analytical Chemists (1990; Method 942.05, 920.39, 968.06). Analyses of NDF, ADF and ADL were conducted according to the sequential procedure of van Soest *et al.* (1991). All values were corrected for ash-free content.

The heifers were weighed once a week throughout the 18 mo of the experiment before morning feeding, without prior deprivation of feed and water. The weight at key points (3, 6, 9, 12 and 15 mo, puberty onset, first breeding and conception) was calculated as the average of 3 consecutive weights. The ADG during LACT, REAR, and birth-to-puberty period were calculated with linear regression of weight against time. The ADG at 3-mo intervals from birth to 15 mo were used for further analysis.

Body development was studied using size measurements at the end of LACT and REAR. The height at withers (from the highest point of the shoulder blade to the ground), rump length (from the ischial tuberosity to the iliac tuberosity), and rump width (the maximum distance between iliac tuberosities) were recorded with a height stick. The heart girth (the body circumference immediately posterior to the front legs) was measured with a flexible tape.

The heifers were bled monthly throughout the experiment to determine both metabolites and hormones. The blood samples were collected before morning feeding from the jugular vein during the LACT and from the coccygeal vein during REAR. Additionally, heifers were bled weekly during REAR to determine the onset of puberty based on plasma progesterone concentration. The samples to determine progesterone, ß-hydroxybutyrate, IGF-I and leptin concentrations were collected into 9 mL heparinized tubes (Vacuette España S.A., Madrid, Spain). The samples to determine plasma glucose, NEFA, cholesterol, and urea concentrations were collected into 9 mL tubes containing EDTA (Vacuette España S.A., Madrid, Spain). Blood samples were centrifuged at 1,500 g for 20 min at 4 °C immediately after collection, and the plasma was harvested and frozen at −20 °C until analysis.

4.2.3 Assays

Plasma progesterone concentrations were measured using an ELISA kit (Ridgeway Science, Lydney, UK), according to the manufacturer's instructions. The mean intra- and interassay CV were 8.0 and 10.4%, respectively. The sensitivity was 0.27 ng/mL. The onset of puberty occurred when progesterone levels were ≥1.0 ng/mL in at least 2 consecutive samples (normal estrus cycle, ≥14 d; Álvarez-Rodríguez *et al.*, 2010b). The age at puberty was defined as the date of collection of the first blood sample that contained ≥1.0 ng/mL of plasma progesterone. To ensure the continuation of estrous cycles, blood samples analyzed after the attainment of puberty were confirmed by the observation of at least 1 subsequent estrous cycle of normal duration, based on progesterone concentration. The first day of the synchronization protocol was used as the date for onset of puberty for prepubertal heifers.

Plasma concentrations of glucose (glucose oxidase/peroxidase method), cholesterol (enzymatic colorimetric method), ß-hydroxybutyrate (enzymatic colorimetric method) and urea (kinetic UV test) were determined with an automatic analyzer (GernonStar, RAL/TRANSASIA, Dabhel, India). The reagents for glucose, cholesterol and urea analyses were provided by the analyzer manufacturer (RAL, Barcelona, Spain), and the reagents for ß-hydroxybutyrate were supplied by Randox Laboratories Ltd. (Crumlin Co., Antrim, UK). The mean intra- and interassay CV for these metabolites were <5.4% and <5.8%, respectively. The sensitivity was 0.056, 0.026, 0.030, 0.170 mmol/L for glucose, cholesterol, ß-hydroxybutyrate and urea, respectively. The plasma NEFA were analyzed with an enzymatic method using a commercial kit (Randox Laboratories Ltd.). Commercial reference plasma samples (bovine precision serum; Randox Laboratories Ltd.) were used to evaluate the accuracy of the analyses. The mean intra- and interassay CV were 5.1% and 7.4%, respectively. The sensitivity was 0.060 mmol/L.

Circulating IGF-I concentrations were quantified with a solid-phase enzyme-labeled chemiluminescent immunometric assay (Immulite; Siemens Medical Solutions Diagnostics Limited, Llanberis, Gwynedd, UK). The mean intra- and interassay CV were 3.1 and 12.0%, respectively. The sensitivity was 20 ng/mL.

Plasma leptin concentrations were determined by RIA with a multispecies commercial kit (Multispecies Leptin Ria kit; LINCO Research, St. Charles, MO). The mean intra- and interassay CV were 3.54 and 6.87%, respectively. The sensitivity averaged 1.30 ng/mL.

4.2.4 Statistical analyses

All data were analyzed as a completely randomized design with the SAS statistical software package (SAS Ins. Inc., Cary, NC). The heifer was the experimental unit. Data for BW and metabolic (glucose, NEFA, cholesterol, ß-hydroxybutyrate and urea) and endocrine (IGF-I and leptin) profiles collected at 3-mo intervals (3, 6, 9, 12 and 15 mo) were analyzed using the SAS MIXED procedure for repeated measures. The covariance structure was selected on the basis of the lowest Akaike information criterion. Therefore, an unstructured covariance matrix was used for the analysis of repeated measures, which included feeding treatment at lactation and rearing phases, time, and their interaction as fixed effects and with heifer as the random effect in a univariate linear mixed model.

The ADG (during LACT, REAR, and birth-to-puberty period, and at 3-mo intervals from birth to 15 mo) and size measurements (height at withers, heart girth, and rump width and length at 6 and 15 mo of age) were tested with ANOVA using the GLM procedure. The feeding treatment during LACT and REAR and their interaction were fixed effects. Similar analyses were performed to analyze the age and weight at puberty, at the first AI, and at conception and number of AI necessary to become pregnant. The fertility rate was analyzed using the FREQ procedure of SAS (χ^2 test). Pearson's correlation coefficients between variables were calculated using the CORR procedure of SAS. Means were separated using the LSMEANS procedure of SAS. For all tests, the level of significance was $P = 0.05$.

4.3 Results and discussion

4.3.1 Growth performance

The development of BW with time is shown in Fig. 3, and the ADG in each period is displayed in Table 2. During the lactation phase, LactHI heifers had greater ADG than the heifers in the low lactation treatment (**LactLO**; 1.063 vs. 0.672 kg/d, respectively, $P < 0.001$) and, consequently, were heavier at weaning (228 vs. 164 kg, respectively, $P < 0.001$). Being that the dam milk yield throughout lactation was similar in LactHI and LactLO feeding treatments (mean ECM yield: 7.66 vs. 7.81 kg/d, respectively, $P > 0.10$), the provision of concentrate ad libitum (mean intake: 1.26 DM kg/d) caused the differences in LactHI heifers. This result occurred both in the first half of lactation (0-3 mo; 0.892 vs. 0.711 kg/d in LactHI and LactLO heifers, respectively; $P < 0.001$) and, more intensively, in the second half (3-6 mo; 1.234 vs. 0.651 kg/d in LactHI and LactLO heifers, respectively; $P < 0.001$). The gains observed during the first 3 mo of age in the LactLO heifers were similar to those described in calves without supplements of the same breed under similar conditions by Álvarez-Rodríguez *et al.* (2009b, 2010c). The larger difference in the later stage of lactation was most likely caused by the increased intake of concentrate from 0.20 kg/d in the first month to 3.45 kg/d in the sixth month, which provided greater intake of both energy and protein for LactHI heifers (Fig. 4). The increase in rate of concentrate intake was similar to that described by Blanco *et al.* (2008a) for suckling calves from birth to the fifth month of lactation under similar conditions.

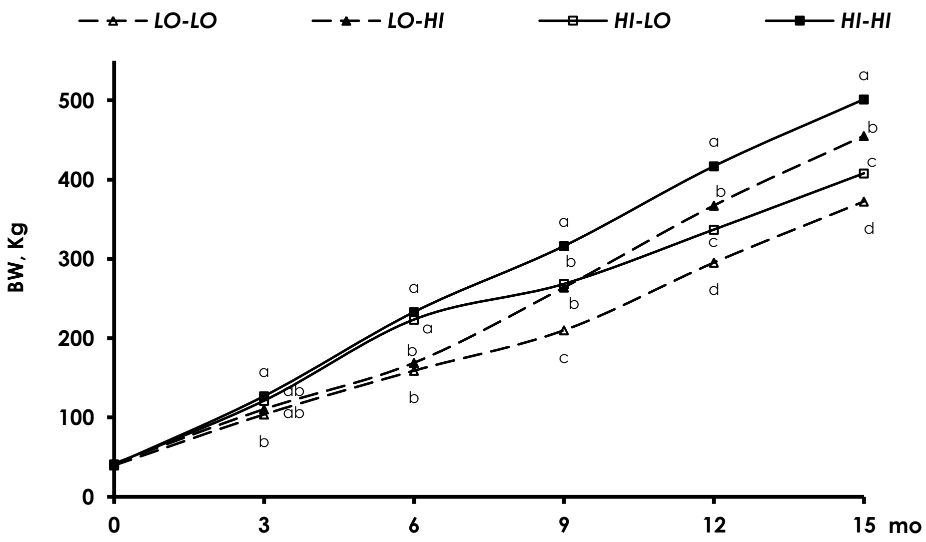

Figure 3. Development of heifer weight throughout the experiment according to the feed management applied in the lactation (0–6 mo) and rearing (6–15 mo) periods. LO = 0.7 kg/d target ADG; HI = 1.0 kg/d target ADG. [a–d]LSMeans within a row with different superscripts differ significantly (P < 0.05).

Table 2. Rate of weight gain (kg/d) of heifers from birth to first breeding according to the feed management applied in the lactation period (LACT) and the rearing period (REAR).

	LACT (0–6 months)				SEM	*P-value*		
	LO[1]		HI[1]					
	REAR (6–15 months)							
Item	**LO**	**HI**	**LO**	**HI**		**LACT**	**REAR**	**LACT×REAR**
LACT	0.643 [b]	0.699 [b]	1.046 [a]	1.080 [a]	0.03	<0.001	0.18	0.72
REAR	0.744 [c]	0.998 [a]	0.593 [d]	0.925 [b]	0.05	<0.001	<0.001	0.10
Birth to puberty	0.680 [c]	0.863 [b]	0.833 [b]	1.085 [a]	0.02	<0.001	<0.001	0.14

[a–d]LSMeans within a row with different superscripts differ significantly (*P* < 0.05).
[1]LO = 0.7 kg/d target ADG; HI = 1.0 kg/d target ADG.

As shown in Table 2, the gains during REAR were significantly influenced by feeding treatment during LACT (P < 0.001) and REAR (P < 0.001), with no significant interaction between them (P > 0.10). In the rearing phase, gains were influenced by previous performance during lactation, and LactLO heifers had a compensatory response that resulted in greater rate of gain than that of LactHI heifers (0.870 vs. 0.759 kg/d, respectively; P < 0.001), which partially corrected the lower gains in the previous phase. The compensatory growth was more intense in the first 2 trimesters of REAR (ADG at 6–9 mo: 0.649 vs. 0.767 kg/d in LactHI and LactLO heifers, respectively; P < 0.01; ADG at 9–12 mo: 0.899 vs. 1.002 kg/d in LactHI and LactLO heifers, respectively; P < 0.01), whereas in the last trimester, gains in both groups were similar (ADG at 12–15 mo: 0.849 vs. 0.891 kg/d in LactHI and LactLO heifers, respectively; P > 0.10). Because the average intake of concentrate (2.90 kg/d) and alfalfa hay (6.10 kg/d) was similar for the 2 treatments, the feed conversion efficiency might have been greater in the LactLO heifers that displayed compensatory growth (Hoch *et al.*, 2005). Despite this greater rate of growth, compensation was not complete in the LactLO treatment, and the large differences in weight at weaning remained to some extent at 15 mo (455 vs. 414 kg in LactHI and LactLO heifers, respectively; P < 0.01; Fig. 3).

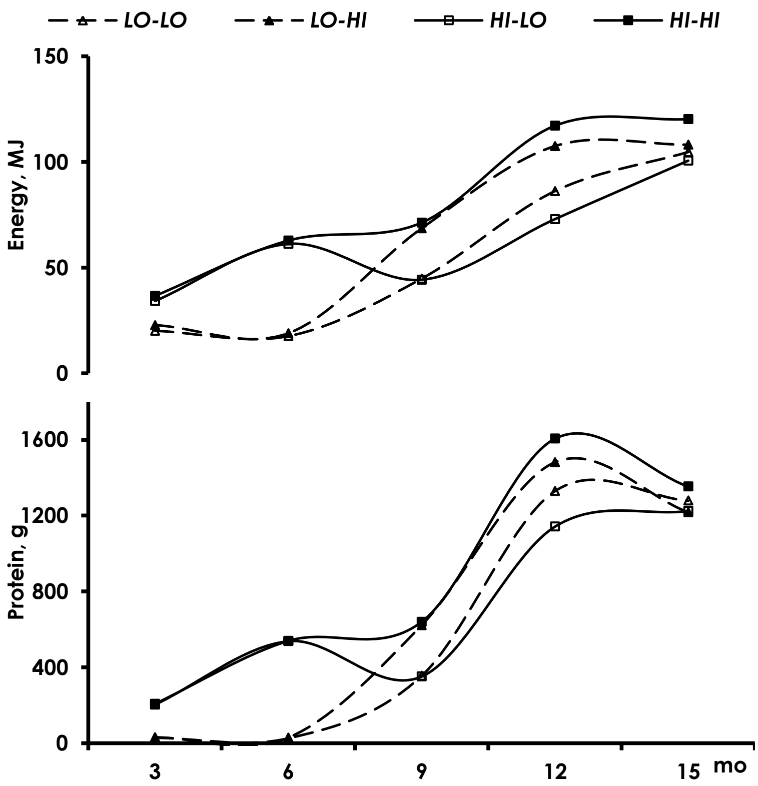

Figure 4. Estimated energy and protein intake by heifers with different nutrition treatments during the lactation (0–6 mo) and rearing (6–15 mo) periods. LO = 0.7 kg/d target ADG; HI = 1.0 kg/d target ADG.

The gains in REAR were also influenced by feeding treatment in this phase, with greater gains in RearHI than RearLO heifers (0.960 vs. 0.668 kg/d, respectively; $P < 0.001$). This difference is expected to result from greater intake of energy and protein by RearHI heifers, except for the last third of REAR (Fig. 4).

The size measurements at 6 and 15 mo are shown in Table 3. The greater BW and growth rate of LactHI heifers at weaning resulted in larger size, and LactLO heifers showed compensatory growth in some of the parameters during the rearing.

The height at withers in cattle is primarily a composite of the long bone measurement of the forelimb and is a good indicator of skeletal development. The height at withers at 6 mo was significantly affected by the lactation feeding treatment, and LactHI heifers were taller than LactLO heifers (104.2 vs. 98.5 cm, respectively; $P < 0.01$). This difference was compensated for during REAR (121.1 vs. 120.7 cm in LactHI and LactLO heifers at 15 mo, respectively; $P > 0.10$), although compensation was not complete for the LO–LO heifers, which remained smaller than their counterparts. The compensation in size was larger than that for BW, which confirmed the results of Swali *et al.* (2008) who reported that animals are less able to compensate for weight than for skeletal growth. Concomitantly, the relationship between height at withers and BW in our work was stronger at 6 mo ($r = 0.81$, $P < 0.001$) than at 15 mo ($r = 0.46$, $P < 0.05$). Therefore, our results show that height at withers is a better indicator of animal development and size than BW, consistently with previous works (Heinrichs *et al.*, 1992; Le Cozler *et al.*, 2010).

The heart girth provides an indirect measure of the development of the gastrointestinal tract and liver. In the current study, heart girth was influenced by feeding treatment in both LACT and REAR ($P < 0.001$). At 6 mo, LactHI heifers had greater heart girth than LactLO heifers (142.8 vs. 125.8 cm, respectively; $P < 0.001$), which was not fully compensated for after the rearing phase. Similarly, at the end of REAR, RearHI heifers showed greater heart girth than RearLO heifers (185.5 vs. 170.6 cm at 15 mo, respectively; $P < 0.001$). These results confirmed those reported by Abeni *et al.* (2012), who found differences in heart girth but not in height at withers after REAR. In the current work, strong and positive relationships between heart girth and weight both at 6 ($r = 0.98$, $P < 0.001$) and 15 mo were found ($r = 0.98$, $P < 0.001$), which confirmed that this measure provides an accurate indirect measure of BW. A similar relationship was found at weaning between heart girth and the immediately previous ADG (3–6 mo; $r = 0.90$, $P < 0.001$). However, at 15 mo of age, the relationship of this measure with the previous ADG (12–15 mo) was weaker ($r = 0.58$, $P < 0.01$) than at 6 mo.

Table 3. Size measures of heifers (cm) at 6 and 15 mo of age according to feed management applied in the lactation period (LACT) and the rearing period (REAR).

	LACT (0–6 months)					***P-value***		
	LO[1]		**HI[1]**					
	REAR (6–15 months)				**SEM**			
Item	**LO**	**HI**	**LO**	**HI**		**LACT**	**REAR**	**LACT×REAR**
Height at withers								
6 mo	98.0 ^{c}	99.0 ^{b}	103.9 ^{bc}	104.6 ^{a}	1.8	0.004	0.64	0.94
15 mo	118.6 ^{b}	122.8 ^{a}	120.1 ^{ab}	122.0 ^{a}	1.4	0.78	0.04	0.42
Heart girth								
6 mo	124.3 ^{b}	127.9 ^{b}	141.9 ^{a}	143.8 ^{a}	2.1	<0.001	0.24	0.78
15 mo	168.0 ^{d}	182.9 ^{b}	173.3 ^{c}	188.6 ^{a}	1.7	0.002	<0.001	0.79
Rump width								
6 mo	29.7 ^{b}	29.9 ^{b}	32.9 ^{a}	34.8 ^{a}	0.8	<0.001	0.20	0.28
15 mo	41.3 ^{b}	46.5 ^{a}	45.6 ^{a}	45.4 ^{a}	0.8	0.05	0.004	0.002
Rump length								
6 mo	32.5 ^{b}	32.9 ^{b}	36.1 ^{a}	37.5 ^{a}	1.0	<0.001	0.41	0.64
15 mo	43.4 ^{b}	47.4 ^{a}	46.3 ^{a}	48.0 ^{a}	0.7	0.02	<0.001	0.14
Height at withers								
6 mo	98.0 ^{c}	99.0 ^{b}	103.9 ^{bc}	104.6 ^{a}	1.8	0.004	0.64	0.94
15 mo	118.6 ^{b}	122.8 ^{a}	120.1 ^{ab}	122.0 ^{a}	1.4	0.78	0.04	0.42

$^{a–c}$LSMeans within a row with different superscripts differ significantly (*P* < 0.05).
[1]LO = 0.7 kg/d target ADG; HI = 1.0 kg/d target ADG.

The rump width and length provide an estimate of the internal pelvic area, which can influence the incidence and degree of calving difficulty in primiparous heifers. The rump width at weaning was affected by lactation treatment (33.8 vs. 29.8 cm in LactHI and LactLO heifers, respectively; *P* < 0.01). However, an interaction (P < 0.001) was found between feed treatments applied in LACT and REAR; at 15 mo of age, the rump width in HI–HI and HI–LO heifers did not differ, but those of LO–LO and LO–HI did (Table 3). Similarly, LactHI heifers had longer rump at weaning (36.8 vs. 32.7 cm, respectively; *P* < 0.001), and LO–LO heifers did not reach a similar rump length as their counterparts at 15 mo (Table 3).

All size measurements were positively correlated with each other at key points in time (6 and 15 mo). This relationship was stronger at weaning (r ≥ 0.69 among the different traits, *P* < 0.001) than at 15 mo (r ≥ 0.32, *P* < 0.05), perhaps because bone growth is maximal in the first year of life and ceases once the growth plates in the long bones and pelvic region have fused; therefore, compensation at a later date is not possible for poor early skeletal development (Wathes *et al.*, 2014).

In summary, heifer BW at 15 mo was different among treatments because of the different ADG in both lactation and rearing feeding treatments. The LactLO heifers compensated for the lower weight gains in lactation during REAR, and for body size as well, except for heifers that remained in a low feed treatment.

4.3.2 Reproductive performance

The results for reproductive performance are presented in Table 4. All heifers were pubertal before the breeding season, except for 3 heifers from the LO–LO treatment. The heifers reached puberty at a similar BW (324 ± 35 kg), which was 55.9% of the expected mature BW, considering the mature BW described for this breed (580 kg; Casasús *et al.,* 2002). The weight at puberty was similar to that described by Revilla *et al.* (1992) for Parda de Montaña heifers with similar ADG from birth to puberty to the LO–LO heifers herein. Moreover, these results confirm that puberty is reached at a critical BW (Schillo *et al.*, 1992). This critical BW depends on the breed and is approximately 55% of mature BW for a wide range of breeds (Freetly *et al.*, 2011), particularly for dual-purpose beef–dairy breeds (Larson, 2007; Diskin and Kenny, 2014), such as Parda de Montaña.

Table 4. Reproductive performance according to feed management applied in the lactation period (LACT) and the rearing period (REAR).

	LACT (0–6 months)					***P-value***		
	LO[1]		**HI[1]**					
	REAR (6–15 months)				**SEM**			
Item	**LO**	**HI**	**LO**	**HI**		**LACT**	**REAR**	**LACT×REAR**
BW at puberty, kg	330.6	313.7	326.2	328.8	14.18	0.71	0.62	0.50
Age at puberty, mo	13.5 [a]	10.2 [bc]	11.3 [b]	9.2 [c]	0.52	0.005	<0.001	0.26
%MBW[2] at puberty	57.0	54.1	56.2	56.3	0.02	0.75	0.56	0.53
Pubertal heifers[3]	4/7	8/8	7/7	7/7		0.08	0.06	
BW at first AI, kg	388.1 [d]	464.5 [b]	424.8 [c]	513.1 [a]	12.03	0.001	<0.001	0.62
Age at first AI, mo	15.8	15.6	15.7	15.9	0.15	0.67	0.93	0.26
Conception BW, kg	382.3 [d]	486.2 [b]	431.7 [c]	530.2 [a]	11.36	<0.001	<0.001	0.71
Conception age,mo	15.9 [b]	16.7 [a]	15.9 [b]	16.4 [a]	0.25	0.55	0.02	0.64
Number of AI	1.20 [b]	2.25 [a]	1.33 [b]	1.67 [a]	0.30	0.49	0.04	0.27
Fertility at first AI	4/7	2/8	4/7	3/7		0.58	0.19	
Fertility	5/7	8/8	6/7	6/7		0.94	0.25	

[a–d]LSMeans within a row with different superscripts differ significantly (*P* < 0.05).
[1]LO = 0.7 kg/d target ADG; HI = 1.0 kg/d target ADG. [2]%MBW = percentage of mature BW. [3]Pubertal heifers = number of heifers pubertal at first day of the synchronization protocol.

The age at the onset of puberty was significantly affected by the feeding treatments applied in LACT (10.3 vs. 11.9 mo in LactHI and LactLO heifers, respectively; *P* < 0.01) and REAR (9.7 vs. 12.4 mo in RearHI and RearLO heifers, respectively; *P* < 0.001); therefore, faster growing heifers reached puberty earlier. The age at puberty showed a strong negative correlation with ADG of heifers from birth to the onset of puberty (r = −0.75, *P* < 0.001), as was also described by Patterson *et al.* (1992). Considering the different phases, the age of puberty was correlated with gains in LACT (r = −0.50, *P* < 0.01) but not after weaning (weaning to puberty, r = −0.30, *P* = 0.12). Other studies also reported that the age of puberty was affected by energy intake during lactation, whereas feeding treatments during REAR did not exert a major effect (Wiltbank *et al.*, 1966; Gasser *et al.*, 2006; Cardoso *et al.*, 2014). The current study shows that feed management in the earlier juvenile period is critical, and supports the idea that the timing at onset of puberty can be nutritionally programmed by promoting high weight gains during targeted periods of heifer development (Amstalden *et al.*, 2014).

Two months before the breeding season, 90% of heifers were pubertal, thus achieving one of the main objectives of the replacement programs. In these programs, heifers should reach puberty 30 to 45 d before the breeding season (Gasser, 2013), because the fertility rate can be increased by up to 21% from the first to the third estrus (Byerley *et al.*, 1987). In the current experiment, only 3 LO–LO heifers were not pubertal at the start of the breeding season, despite having optimal BW to reach puberty (>55% of mature BW).

As shown in Table 4, the weight at the first AI and at conception in all groups was affected by feeding treatment in LACT ($P < 0.001$) and REAR ($P < 0.001$). However, all treatments had weights at the first AI that were greater than 65% of the expected mature BW (381 kg), which complied with previous recommendations (Perry, 2012; Gasser, 2013). Because all heifers were inseminated at the same time, no differences were found in age at the first AI. However, the age at conception was influenced by the feeding treatment during rearing ($P < 0.05$), with RearHI heifers older than RearLO heifers (16.5 vs. 15.9 mo, respectively; $P < 0.05$), because they needed more AI services to become pregnant (1.96 vs. 1.27 AI in RearHI and RearLO heifers, respectively; $P < 0.05$). This result supports the findings by Brickell *et al.* (2009a) and Summers *et al.* (2014), who reported that the fastest growing heifers required more services per conception. Funston *et al.* (2012) also found that developing obese heifers increased the number of services required for conception by 7% compared with leaner animals. In our work, the inclusion of the PRID in the synchronization protocol most likely induced ovulation in the 3 heifers that were not pubertal, which became pregnant at the first AI.

In the current study, no differences were found in fertility rate at the first AI and over the entire breeding season, as has been described in many other works (Funston and Deutscher, 2004; Roberts *et al.*, 2009b; Eborn *et al.*, 2013; Summers *et al.*, 2014).

In summary, the onset of puberty was attained at similar BW but different ages depending on the growth pattern during the LACT and REAR, and the onset of puberty occurred in 90% of animals at least 2 mo before targeted breeding at 15 mo. The faster-growing heifers came into puberty earlier, but they required more AI services per conception. At the end of the breeding season, the pregnancy rate did not differ among treatments.

4.3.3 Metabolic profiles

Circulating glucose, NEFA, cholesterol, ß-hydroxybutyrate and urea are indicators generally associated with ruminant energy metabolism, which were useful to characterize nutritional status of growing heifers in the current study. The profiles throughout the experiment are presented in Fig. 5.

The plasma glucose profile was affected by the lactation treatment ($P < 0.001$), with differences at weaning (6.42 vs. 4.75 mmol/L in LactHI and LactLO heifers, respectively; $P < 0.001$). These differences disappeared during REAER, and all treatments had similar glucose levels at 15 mo. The glucose concentration during LACT was greater than during the rearing phase (5.59 vs. 4.75 mmol/L in the LACT and REAR, respectively; $P < 0.001$), and the levels at the end of each period were greater than those described in other studies on dairy (Swali *et al.*, 2008; Brickell *et al.*, 2009b) and beef heifers (Cappellozza *et al.*, 2014). The greater glucose levels of LactHI heifers at weaning were most likely caused by the intake of concentrate, which increased the propionate available from ruminal fermentation to be metabolized into glucose (Agle *et al.*, 2010; Samadi *et al.*, 2014). By contrast, the lower nutrient intake of LactLO heifers led to lower plasma glucose concentrations, as observed in other studies (Chelikani *et al.*, 2004; Brickell *et al.*, 2009b; Le Cozler *et al.*, 2010).

In the current work, plasma glucose concentration at weaning was highly correlated to the previous ADG (3–6 mo; $r = 0.77$, $P < 0.001$), as described by other authors (Vizcarra *et al.*, 1998; Cappellozza *et al.*, 2014). The plasma glucose concentration was also significantly ($P < 0.05$) correlated with all skeletal measurements, as Swali *et al.* (2008) also observed, with correlation values (r) ranging from 0.46 (rump height) to 0.71 (heart girth). The strong relationship with heart girth confirmed the findings of Brickell *et al.* (2009b), who found that this parameter defines intake and digestive capacity and is thus related to metabolites reflecting intake, such as glucose and urea. This relationship for both ADG and size measurements was not evident at the end of REAR in the current work. The plasma glucose at weaning was negatively correlated with age at puberty ($r = -0.50$, $P < 0.01$), as Brickell *et al.* (2009a) also described, most likely because glucose is the primary source of energy for ovarian function and a major modulator of LH secretion (Bucholtz *et al.*, 1996). In our study, the number of services per conception was positively correlated with the plasma glucose concentration at 9 mo of age ($r = 0.57$, $P < 0.01$). As glucose is an indicator of energy balance, this result is consistent with other studies reporting that more services are necessary in faster growing (Brickell *et al.*, 2009a; Summers *et al.*, 2014) and obese heifers (Funston *et al.*, 2012).

The plasma NEFA concentrations were significantly affected by the treatment during lactation but opposite to the effect observed for plasma glucose (0.200 vs. 0.442 mmol/L in LactHI and LactLO heifers at weaning, respectively; $P < 0.001$). This opposite could be caused by the lower estimated energy intake by LactLO heifers during lactation (Fig. 4), because NEFA levels are inversely related to the level of nutrition (Chelikani *et al.*, 2009). Moreover, NEFA are released into circulation as a result of fat mobilization and lipid catabolism, and therefore, the increased NEFA concentrations indicate a lower energy balance in LactLO heifers. This relation explains the negative relationship between ADG and NEFA at weaning ($r = -0.59$; $P < 0.01$), which was also described in other works (Bell, 1995; Yelich *et al.*, 1995; Blanco *et al.*, 2011). Concomitantly, a negative relationship between NEFA and glucose concentrations was found at weaning ($r = -0.78$, $P < 0.001$). The NEFA concentrations were also negatively correlated with height at withers ($r = -0.53$, $P < 0.01$) and heart girth ($r = -0.56$, $P < 0.01$) at 6 mo, which indicate that animals with greater energy balance were larger, but this relationship disappeared at 15 mo. No relationship between NEFA concentrations at weaning and the rump measurements was found, but at 15 mo of age, rump width ($r = -0.52$, $P < 0.01$) and rump length ($r = -0.47$, $P < 0.05$) were inversely correlated with NEFA levels, thus indicating that heifers with a lower energy balance were less developed at the end of REAR.

The plasma cholesterol concentration was affected significantly by time ($P < 0.001$), with the highest values at weaning (3.966 ± 0.192 mmol/L) and the lowest values at 12 mo of age (1.310 ± 0.103 mmol/L), and overall, greater concentrations were found during LACT than during REAR (3.487 vs. 1.993 mmol/L, respectively; $P < 0.001$). The observed range of this metabolite was slightly wider than that previously described in Parda de Montaña adult cows (Álvarez-Rodríguez and Sanz, 2009).

In contrast with the previous metabolites, feed management did not influence plasma cholesterol levels during lactation ($P > 0.05$). During REAR, significantly greater concentrations were found in RearHI than in RearLO heifers both at 9 mo (2.896 vs. 2.054 mmol/L, respectively; $P < 0.001$) and at 15 mo of age (2.440 vs. 1.952 mmol/L, respectively; $P < 0.05$). Throughout the experiment, plasma cholesterol and glucose concentrations were positively correlated ($r = 0.61$, $P < 0.001$) because cholesterol level depends on the glucose concentration (Ndlovu *et al.*, 2007); both metabolites indicate a positive energy balance.

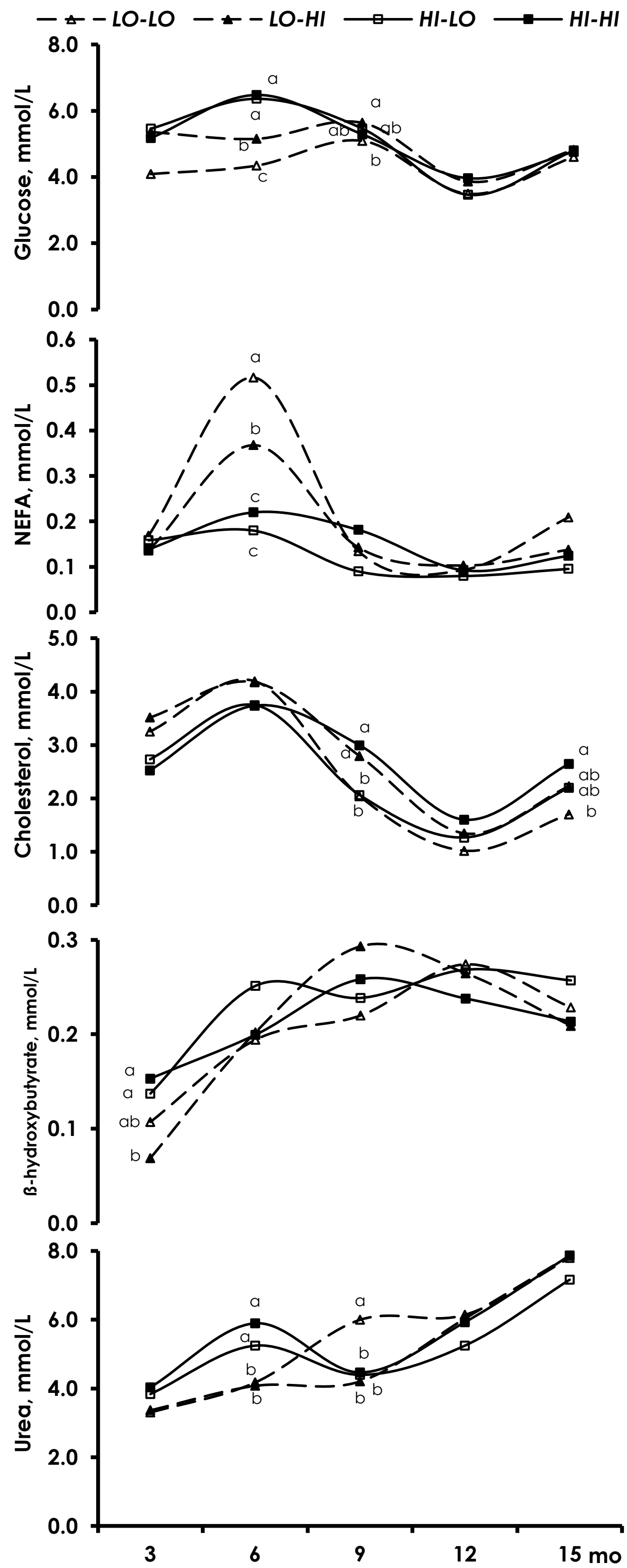

Figure 5. Plasma concentrations of glucose, NEFA, cholesterol, β-hydroxybutyrate and urea in beef heifers with different nutrition treatments in the lactation (0–6 mo) and rearing (6–15 mo) periods. *LO = 0.7 kg/d target ADG; HI = 1.0 kg/d target ADG. [a–c]LSMeans at a given age with different superscripts differ significantly (P < 0. 05).*

As the heifers reach sexual maturity, the ovaries become functional and increase progesterone production, with cholesterol uptake as the main source for the synthesis of steroid hormones (Yart *et al.*, 2014). Therefore, the lower level of plasma cholesterol during REAR could be explained by the negative relationship with plasma progesterone, a relationship also described by Talavera *et al.* (1985). Moreover, a negative relationship between plasma cholesterol concentration and age at onset of puberty was found (r = −0.56, −0.50, and −0.49 at 9, 12 and 15 mo, respectively; $P < 0.01$). The cholesterol concentration decreased with the onset of puberty, but a minimum level might be necessary to reach puberty. Similar to plasma glucose, at 9 mo of age, cholesterol was positively correlated with number of services needed per conception (r = 0.68, $P < 0.001$).

Plasma ß-hydroxybutyrate is a ketone body synthesized in the liver after adipose tissue catabolism and is commonly used to indicate a short-term negative energy balance (Ndlovu *et al.*, 2007). In the current study, ß-hydroxybutyrate was not influenced by management treatments, but it was affected significantly by time ($P < 0.001$) and increased from a low level at 3 mo of age until a plateau was reached at 12 mo (0.117 ± 0.012 and 0.261 ± 0.013 mmol/L, respectively). The high levels observed at 12 mo could be a response to the decrease in plasma glucose concentration at 12 mo (Lean *et al.*, 1992).

The treatment during lactation had a major effect on urea concentrations (5.570 vs. 4.129 mmol/L in LactHI and LactLO calves at weaning, respectively; $P < 0.001$). This was explained by the greater protein intake of LactHI calves receiving the concentrate (Fig. 4), because circulating urea is positively correlated with dietary protein intake (Walsh *et al.*, 2008; Kelly *et al.*, 2010). In REAR, the only differences were found at 9 mo of age, when urea levels were greater in LO–LO heifers (5.999 ± 0.500 mmol/L; $P < 0.05$) than in the other heifers (4.356 ± 0.500 mmol/L), which might indicate a deficit of energy intake and an increase in breakdown of endogenous proteins for energy production (Chimonyo *et al.*, 2002). The plasma urea concentration was also significantly influenced by time ($P < 0.001$) and increased throughout the experiment from 3.635 ± 0.150 mmol/L at 3 mo to 7.673 ± 0.403 mmol/L at 15 mo of age.

The plasma urea concentration at weaning was positively correlated with BW (r = 0.71, $P < 0.001$), previous ADG (3–6 mo; r = 0.76, $P < 0.001$) and glucose (r = 0.66, $P < 0.001$) and was negatively correlated with NEFA (r = −0.53, $P < 0.01$). These relationships suggest that LactHI heifers had a greater energy balance and more protein available to increase BW faster, as Hall *et al.* (1995) suggested, and would confirm the diagnostic value of urea as an indicator of energy and protein intake (Brickell *et al.*, 2009b; Abeni *et al.*, 2012). Similar to glucose, plasma urea concentrations at weaning had positive significant (*P* < 0.05) relationships with all size measurements, and the estimated correlation coefficients (r) ranged from 0.43 (height at withers) to 0.72 (heart girth); the relationships were not observed at 15 mo.

In summary, plasma glucose, NEFA and urea concentrations showed the greatest differences between treatments at weaning, when the estimated energy and protein intake were most markedly different according to the feed management. By contrast, plasma cholesterol levels differed more during REAR. Our results provide evidence that these metabolites are responsible for some of the underlying mechanisms regulating growth and reproductive development and are accurate indicators of the nutritional status of heifers.

4.3.4 Endocrine profiles

The profiles of plasma IGF-I and leptin during the experiment are presented in Fig. 6. The concentrations of IGF-I were significantly different between feed treatments during lactation. The concentrations increased 2.7-fold from 3 to 6 mo of age in LactHI heifers (106.0 vs. 288.9 ng/mL, respectively; *P* < 0.001), concomitantly with the intake of concentrate, as Blanco *et al.* (2008b) described for similar conditions. In the LactLO heifers, the IGF-I concentration remained stable throughout lactation, most likely because milk intake at weaning was not sufficient to support potential growth rates for Parda de Montaña calves (Blanco *et al.*, 2008b). Moreover, other studies showed that with feed restrictions the age-related increase in IGF-I concentrations of calves was attenuated (Elsasser *et al.*, 1989; Hayden *et al.*, 1993; Blanco *et al.*, 2009c).

As shown in Fig. 6, IGF-I concentrations declined immediately after weaning in LactHI heifers, whereas they increased in LactLO heifers but then were similar among treatments thereafter. These profiles reflected the compensatory growth shown by heifers in the 6 to 9 mo period, which confirmed that IGF-I concentration is a good indicator of growth at particular points in time (Cabaraux *et al.*, 2003; Blanco *et al.*, 2009c). In REAR, IGF-I concentrations were lower than those of feedlot cattle of similar age but fed high-concentrate diets (Blanco *et al.*, 2010), which was reflected in lower ADG.

The concentration of IGF-I at weaning was positively correlated with BW (r = 0.78, *P* < 0.001), as Kerr *et al.* (1991) also described, and with previous ADG (3–6 mo; r = 0.80, *P* < 0.001), as reported also by Blanco *et al.* (2009c). Simultaneously, the concentration of IGF-I was positively correlated with glucose (r = 0.78, *P* < 0.001) and urea (r = 0.63, *P* < 0.001) concentrations and was negatively correlated with NEFA (r = −0.57, *P* < 0.01) concentration, which reflected the response of IGF-I concentration to nutritional status (Cabaraux *et al.*, 2003). The greater energy and protein intake by LactHI heifers at the end of LACT led them to synthesize more glucose and urea and to reach greater ADG, which was reflected in greater IGF-I levels. By contrast, LactLO heifers had a lower energy balance at weaning, greater NEFA concentrations, lower weight gains and lower IGF-I levels. Concurrently, all size parameters were positively correlated with IGF-I at weaning (r ranged from 0.59 to 0.78, $P < 0.001$), as described in earlier works (Brickell *et al.*, 2009b), which reinforced the key role of IGF-I in the control of body growth as a regulator of skeletal and muscle development in growing cattle (Yelich *et al.*, 1995).

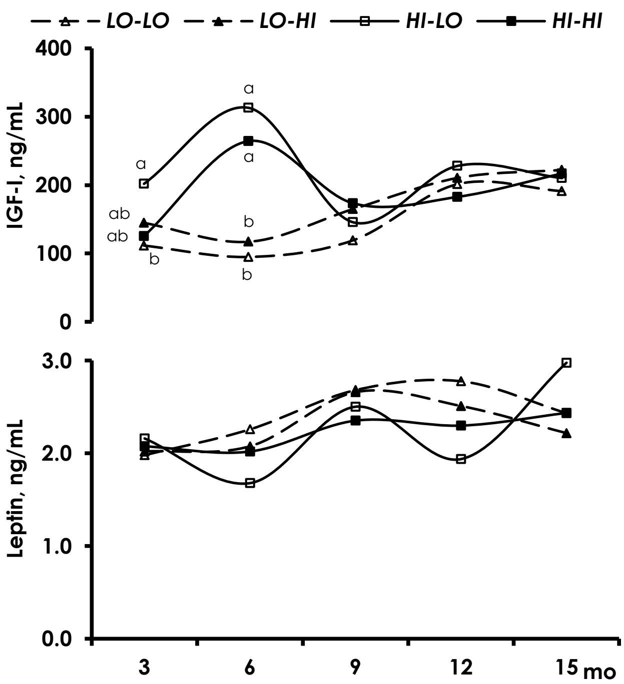

Figure 6. Plasma concentrations of IGF-I and leptin in beef heifers with different nutrition treatments in the lactation (0–6 mo) and rearing (6–15 mo) periods. LO: Low ADG (0.7 kg/d) and HI: High ADG (1.0 kg/d). [a,b]LSMeans at a given age with different superscripts differ significantly (P < 0. 05).

At 9 mo of age, IGF-I concentration was 36.7 ng/mL greater in RearHI heifers than in RearLO heifers (169.2 vs. 132.5 ng/mL, respectively), although the difference was not significant ($P > 0.10$). A negative relationship between IGF-I level at 9 mo and age at onset of puberty was found (r = −0.54, $P < 0.01$). Plasma IGF-I was described as a major metabolic mediator involved in the onset of puberty in heifers that could be delayed by low levels of IGF-I (Velazquez *et al.*, 2008), whereas greater concentrations could trigger an earlier start of ovarian function (Yelich *et al.*, 1995; 1996). Furthermore, IGF-I in the juvenile period was associated with subsequent cow longevity (Swali *et al.*, 2008), and therefore, IGF-I has predictive value.

In the current work, the mean plasma concentration of IGF-I was affected by time throughout the experiment and increased as heifers became older (from 146.0 ± 12.3 ng/mL to 210.1 ± 11.9 ng/mL at 3 and 15 mo of age, respectively; $P < 0.001$), most likely because of an increment in nutrient intake, as suggested by Blanco *et al.* (2008b). Furthermore, IGF-I concentrations in the current study were greater than those described for the same breed in the postpartum period of primiparous and multiparous cows (71.8 and 54.7 ng/mL, respectively; Álvarez-Rodríguez *et al.,* 2010b).

The circulating leptin was only influenced by the sample date ($P < 0.01$) and increased from the lactation to the rearing phase (2.04 vs. 2.48 ng/mL, respectively; $P < 0.01$). This age-related increment was due to the increase in deposition of fat (Geary *et al.*, 2003), because leptin is a key metabolic signal synthesized by fat cells that communicates information about body energy reserves, nutritional state, and metabolic shifts to reproductive axis (Hausman *et al.*, 2012). Unexpectedly, however, the concentration of leptin did not differ among treatments at any of the key points in time (Fig. 6).

Plasma leptin concentrations did not differ among treatments as puberty approached, and there was no evidence of a prepubertal increase. These results are in contrast with other studies that reported a linear prepubertal increase in plasma leptin concentrations in both beef (Garcia *et al.*, 2002; 2003) and dairy (Díaz-Torga *et al.*, 2001) heifers. Although leptin was proposed as a crucial hormone in determining the timing of puberty (Garcia *et al.*, 2002; 2003), leptin might not be a critical trigger for puberty in rapidly growing heifers, as Lents *et al.* (2013) have suggested. However, a certain threshold of leptin levels appears important for puberty (Cooke *et al.*, 2013), particularly in heifers with normal or restricted growth rates (Chelikani *et al.*, 2009), to ensure sufficient nutritional reserves to support the transition to puberty.

In summary, plasma IGF-I concentrations differed between feeding strategies at weaning and were associated with a wider range of estimated energy and protein intakes. The IGF-I profile was highly correlated with animal performance traits and metabolic profiles and provided a good indicator of growth, nutritional, and metabolic status at given key points in heifer development. Although plasma leptin levels were age related, concentrations were similar in all treatments throughout the study.

In the current experiment, both lactation and rearing feeding treatments resulted in different growth patterns. As a result, the onset of puberty was attained at a similar BW (55.9% of mature weight) but at a different age, with faster-growing heifers reaching puberty earlier but requiring more AI services to conceive. However, the fertility rate did not differ among treatments at the end of the 90-d breeding season. The profiles of plasma glucose, NEFA, urea and IGF-I were particularly different at weaning, when the ranges of energy and protein intake were larger; whereas cholesterol differed more among treatments in REAR, most likely because of its role as steroid hormone precursor. Therefore, these parameters were excellent indicators of growth, nutritional and metabolic status at particular key points in time. By contrast, the function of leptin in growth and reproductive development of heifers was less clear. Our results demonstrated that even a 0.7 kg/d gain from birth until breeding allowed the first breeding to be 6 mo earlier than usual for these conditions, without any negative effect on heifer reproductive performance. However, additional research is necessary to determine the impacts of the different growth strategies on size at maturity, production, reproductive performance at first and subsequent calvings, and lifespan of early-bred heifers.

5. Manuscrito 2

Rodríguez-Sánchez JA, Sanz A, Ripoll G, Casasús I. **First calving performance and physiological profiles of 2-year-old beef heifers according to their prebreeding growth.**

Abstract

Different feeding strategies were applied to beef heifers during the preweaning (PRE, 0–6 months) and the postweaning period (POST, 6–15 months) for two target gains (1.0 and 0.7 kg/day in HI and LO, respectively), in a 2 × 2 experimental design (HI–HI, HI–LO, LO–HI, LO–LO). After fixed-time artificial insemination at 15 months, the effect of these treatments on heifer performance and physiological parameters during the gestation and first lactation was evaluated. The heifer body weight (BW) at conception depended on the feeding treatment applied both in PRE (P < 0.001) and POST (P < 0.001), and ranged from 66 to 91% of the breed estimated mature BW. At calving all cows had similar BW (487 ± 24 kg BW), except those from LO–LO treatment, which were lighter (436 ± 39 kg BW). All heifers maintained the BW during the lactation. The size of the pelvic area at conception was affected by the interaction between PRE and POST (P < 0.01) and was smaller in LO–LO heifers than in the other treatments. The pelvic area at calving depended on the POST management (21.0 vs. 17.2 dm^2 in POST–HI and POST–LO treatments, respectively; P < 0.01). The feed management applied before breeding season did not affect the calf traits (BW at birth and at weaning, and daily gains) or the milk production. Although the animals complied with the general recommendations for the BW and size at conception and calving, the need for calving assistance was particularly high in LO–LO cows (80%), most likely because they had the smallest pelvic area and a large calf/cow BW ratio. The POST–HI cows tended to be cyclic earlier than POST–LO ones (82 and 106 days postpartum, respectively; P = 0.06). The feeding management applied in the preweaning and postweaning periods did not influence the metabolic (glucose, cholesterol, NEFA, β-hydroxybutyrate and urea) and endocrine (IGF-I and leptin) profiles. Our results suggest that advancing the first calving to 2 years is feasible in beef cattle, but gains of 1 kg/day either in the preweaning or postweaning period should be ensured to avoid impaired performance at the first calving.

Key words: beef cattle, feeding strategy, first lactation, prebreeding management, replacement heifers.

5.1 Introduction

Calving for the first time at 2 years is a primary goal, both in the dairy (Abeni *et al.*, 2012) and the beef operations (Diskin and Kenny, 2014), in order to minimize the cost of raising replacement heifers. To achieve this target, the rate of growth before breeding must be planned to ensure that heifers become productive without undermining lifetime productivity. This growth rate should allow to achieve 65% of mature body weight (**BW**) at breeding (Gasser, 2013) and 80% of mature BW at first calving (NRC, 2000), and enough skeletal development to avoid calving difficulties. In the particular case of extensively developed beef heifers, the decision on the most adequate phase for supplementation must consider both the economics of the cow enterprise and the effects on productivity and longevity (Endecott *et al.*, 2013).

There has been some controversy about optimal management in beef heifers, since earlier studies reported that diets that promoted high prepubertal gains could have deleterious effects on milk production and cow lifetime efficiency (Buskirk *et al.*, 1996). Recent research in dairy heifers indicates that higher preweaning growth results in greater milk yield at first lactation, establishing that both nutrition and management of the preweaned calf are major environmental factors influencing the expression of the genetic capacity for milk production (Soberon y Van Amburgh, 2013). In the case of beef heifers, faster growing animals attain puberty earlier (Rodríguez-Sánchez *et al.*, 2015), and it has been suggested that the provision of creep-fed concentrates to nursing heifers could result in a metabolic imprinting with consequences in the adult life (Reis *et al.*, 2015). These authors found both temporal and permanent effects of creep-feeding in mRNA expression of genes associated to nutrient metabolism, but could not demonstrate a permanent effect on physiological or biochemical variables beyond weaning, and did not look into further effects in adult cows.

Prebreeding feeding strategies can aim at a linear or a stair-step growth trajectory, with a phased nutrition regime, wherein heifers are subjected to periods of restriction and re-alimentation. The compensatory growth registered in the later periods increases the feed efficiency and can alter the secretion of hormones and metabolites (Ford and Park, 2001). This feeding management can affect the onset of puberty and the milk yield by modifying mammary-gland development. The influence of compensatory growth around puberty can be positive, by maximizing mammary cell proliferation (Park *et al.*, 1998), or negative, as hastening puberty may reduces the duration of the first allometric phase of development of the mammary-gland, which concludes with the puberty (Lohakare *et al.*, 2012).

Therefore, the aim of this study was to determine the effects of different feed management strategies applied during the preweaning (0–6 months) and postweaning periods (6–15 months) on the patterns of growth, calving difficulty, milk yield and quality, first calf performance, postpartum anestrus, and metabolic and endocrine status of beef heifers that were bred at 15 months.

5.2 Material and methods

The Animal Ethics Committee of the Centro de Investigación y Tecnología Agroalimentaria (**CITA**) approved the experimental procedures, which were in compliance with the guidelines of the European Union (Directive No. 86/609/CEE, 1986) for the protection of animals used for experimental and other scientific purposes.

5.2.1 Animals, management and diets

The study was conducted at La Garcipollera Research Station, in the mountain area of the central Pyrenees (northeastern Spain, 945 m a.s.l.).

Twenty-five Parda de Montaña (beef breed derived from old Brown Swiss) heifers were born in the autumn season (October 12 ± 13 day) and were randomly assigned to one of four management strategies in a 2 × 2 factorial experiment. Two growth rates were targeted in the preweaning period (**PRE**, 0–6 months: 1.0 kg/day and 0.7 kg/day in **PRE–HI** and **PRE–LO** treatments, respectively) and two in the postweaning period (**POST**, 6–15 months: 1.0 kg/day and 0.7 kg/day in **POST–HI** and **POST–LO** treatments, respectively), resulting in four experimental groups: **HI–HI, HI–LO, LO–HI** and **LO–LO**. This document presents the performance of the heifers from their conception (at 15 months) to weaning of their first calves after 4 months of lactation. A more detailed description of the management practices and the results of the heifer performance from birth to breeding is available in Rodríguez-Sánchez *et al.* (2015), and therefore only a brief description is presented here. To achieve the desired growth rates during PRE, all heifers suckled for 30 minutes twice daily, but only those in the PRE–HI treatment had free access to starter concentrate. The heifers were weaned at 6 months (175 ± 13 days) and were kept indoors in a loose-housing system with straw-bedded pens during POST. In this phase, the heifers were pen fed a diet that consisted of *ad libitum* access to alfalfa hay and received either 12 or 6 g concentrate/kg BW in POST–HI or POST–LO heifer treatments, respectively. Heifers were bred by artificial insemination over a 90-day breeding season. Throughout breeding and thereafter, heifers were managed as a single group. During the breeding season the heifers were fed 9 kg per animal of a dry total mixed ration (56% forages, 44% grains, by-products and vitamin-mineral supplements; 897 g/kg DM, 9.6 MJ ME/kg DM, 103 g CP/kg DM, 595 g NDF/kg DM). From the confirmation of pregnancy until a month before the expected calving date for each heifer, the animals grazed on mountain meadows, composed primarily of grasses *(Festuca arundinacea, Festuca pratensis* and *Dactylis glomerata*), legumes (*Trifolium repens*) and other species (4 heifers per hectare). In the last month of gestation, the heifers were housed and fed 9 kg per head of meadow hay.

During the 4 months of the lactation, the primiparous cows received 10 kg per animal of the same dry total mixed ration provided during the breeding season. The diet was, calculated to meet the requirements, for energy and protein, of maintenance, growth and milk production of a cow of 490 kg BW and 6.5 kg daily milk yield. The calves had free access to suckle their dams and received no other feed during the 4 months of lactation.

5.2.2 Measurements and blood sampling

The heifers were weighed once a week before morning feeding, without prior deprivation of feed and water. The weight of the heifers at calving and the calves at birth were taken few hours after calving. The average daily gain (**ADG**) during gestation and lactation was calculated by linear regression of weight against time. Their calves were weighed weekly from birth to weaning at 4 months of age to determine the ADG during lactation.

The body development of the heifers was studied using size measurements at conception, calving and weaning. The height at withers (from the highest point of the shoulder blade to the ground), rump length (from the ischial tuberosity to the iliac tuberosity), and rump width (the maximum distance between iliac tuberosities) were recorded with a height stick. The external rump measurements, which are easier to measure than internal ones, are correlated with the internal pelvic height and width (Murray *et al.*, 2002), and therefore they were used herein for practical purposes. The external pelvic area was estimated as the product of the rump length and width (Murray *et al.*, 1999). The heart girth (the body circumference immediately posterior to the front legs) was measured with a flexible tape.

The calving ease was classified into two categories, i.e., assisted or unassisted. The assisted calving included all types of assistance, from manual pull to caesarean section (Johanson y Berger, 2003). To determine the fetal-maternal disproportion, the ratio of calf/cow BW was calculated as the calf birth weight divided by the cow weight at calving (Johanson y Berger, 2003).

The body condition score (**BCS**) was assessed at conception, calving and weaning by two expert technicians, based on the estimation of the fat covering the loin, ribs and tailhead (0 to 5 scale; Lowman *et al.* (1976)). Simultaneously, the subcutaneous fat thickness (**SFT**) was measured by ultrasound scanning, with a multifrequency probe (7.5 MHz, Aloka SSD-900; Aloka Madrid, Spain), at the P8 rump site (**SFT_P8**) and at the 13th thoracic vertebra (**SFT_T13**). Vaseline was used for the contact of the transducer with the skin. The SFT_P8 was determined over the *gluteus* muscle on the rump at the intersection of a line through the pin bone parallel to the chine and one perpendicular through the 3rd sacral crest. To determine the SFT_T13, the transducer was placed perpendicular to the backbone, and the measurements were conducted above the rib.

The heifers were bled at 3 months intervals during the gestation and monthly during the lactation for determination of both metabolite and hormone profiles. The blood samples were collected before morning feeding from the coccygeal vein.

Additionally, the animals were bled twice a week during the lactation to determine the length of the postpartum anestrus (**PPA**), based on the plasma progesterone concentration. The samples to determine progesterone, ß-hydroxybutyrate, IGF-I and leptin concentrations were collected into 9 mL heparinized tubes (Vacuette España S.A., Madrid, Spain). The samples to determine plasma glucose, cholesterol, NEFA and urea concentrations were collected into 9 mL tubes that contained EDTA (Vacuette España S.A.). The blood samples were centrifuged at 1500 g × 20 min at 4 °C immediately after collection, and the plasma was harvested and frozen at −20 °C until analysis.

The heifers were milked monthly during the 4 months of the lactation using the oxytocin and machine milking technique (Le Du *et al.*, 1979) to determine the quantity and the composition of the milk produced daily. The milk fat, protein and casein contents were analyzed with an infrared scan (Milkoscan 4000™; Fosselectric Ltd., Hillerod, Denmark). The data for the fat and protein contents were used to calculate the energy-corrected milk (**ECM**) yield (adjusted to 3.5% fat and 3.2% protein), as described in Casasús *et al.* (2004).

5.2.3 Assays

Plasma progesterone concentrations were measured using an ELISA kit (Ridgeway Science, Lydney, UK), following the manufacturer's instructions. After calving, the onset of normal luteal activity began when the progesterone levels were ≥ 0.5 ng/mL in three or more consecutive samples to avoid considering first short cycles. Álvarez-Rodríguez *et al.* (2010b) reported that a first short estrous cycle (8 to 14 days) occurred before the second ovulation when a progesterone increase of ≥ 0.5 ng/mL was detected in two or fewer consecutive samples. The length of the PPA was defined as the number of days from calving to the first estrus. The animals that failed these criteria before weaning were assigned a value of 120 days for the length of anestrus; therefore, the data concerning the length of anestrus were right-censored.

Metabolic status was assessed by determination of the concentrations of glucose (glucose oxidase/peroxidase method), cholesterol (enzymatic colorimetric method), ß-hydroxybutyrate (enzymatic colorimetric method) and urea (kinetic UV test) with an automatic analyzer (GernonStar, RAL/TRANSASIA, Dabhel, India). The reagents for glucose, cholesterol and urea analyses were provided by the analyzer manufacturer (RAL, Barcelona, Spain), and the reagents for ß-hydroxybutyrate were supplied by Randox Laboratories Ltd. (Crumlin Co., Antrim, UK). The mean intra- and interassay CV for these metabolites were <5.4% and <5.8%, respectively. The sensitivity was 0.056, 0.026, 0.030, 0.170 mmol/L for glucose, cholesterol, ß-hydroxybutyrate and urea, respectively. The plasma NEFA concentrations were analyzed with an enzymatic method using a commercial kit (Randox Laboratories Ltd.). Commercial reference plasma samples (bovine precision serum; Randox Laboratories Ltd.) were used to evaluate the accuracy of the analyses. The mean intra- and interassay CV were 5.1% and 7.4%, respectively. The sensitivity was 0.060 mmol/L.

The concentrations of circulating IGF-I were quantified with a solid-phase enzyme-labeled chemiluminescent immunometric assay (Immulite; Siemens Medical Solutions Diagnostics Limited, Llanberis, Gwynedd, UK). The mean intra- and interassay CV were 3.1 and 12.0%, respectively. The sensitivity was 20 ng/mL.

Plasma leptin concentrations were determined by RIA with a multispecies commercial kit (Multispecies Leptin Ria kit; LINCO Research, St. Charles, MO, USA). The mean intra- and interassay CV were 3.54 and 6.87%, respectively. The sensitivity averaged 1.30 ng/mL.

5.2.4 Statistical analyses

All data were analyzed as a completely randomized design with the SAS statistical software package (SAS Institute Inc., Cary, NC, USA). The heifer was the experimental unit. Data for BW and metabolic (glucose, cholesterol, NEFA, ß-hydroxybutyrate and urea) and endocrine parameters (IGF-I and leptin) during pregnancy and first lactation were analyzed using the SAS MIXED procedure. The covariance structure was selected on the basis of the lowest Akaike information criterion. An unstructured covariance matrix (UN) was used for the analysis of repeated measures, including feeding treatment at PRE and POST, time and their interaction as fixed effects and with heifer as the random effect in a univariate linear mixed model.

The ADG, size measurements (height at withers, heart girth, rump width and length and pelvic area at conception, calving and weaning), BCS, SFT_P8, SFT_T13, ECM yield and milk quality at each key date, and PPA length in the heifers were tested with ANOVA using the GLM procedure. The feeding treatment during PRE and POST and the interaction were fixed effects. Similar analyses were performed to analyze calf performance (BW at birth and weaning, ADG) and calf/cow BW ratio where calf sex was considered as a fixed effect. The calf sex, calving assistance and type of first cycle length (short or normal) were analyzed using the FREQ procedure of SAS (χ^2 test). The Pearson correlation coefficients between variables were calculated using the CORR procedure of SAS. Means were separated using the LSMEANS procedure of SAS. For all tests, significance was set at $P < 0.05$ and tendencies were determined if $P \geq 0.05$ and $P < 0.10$.

5.3 Results and discussion

5.3.1 Growth performance

The development of BW with time is shown in Figure 1, and the ADG in each period is displayed in Table 1.

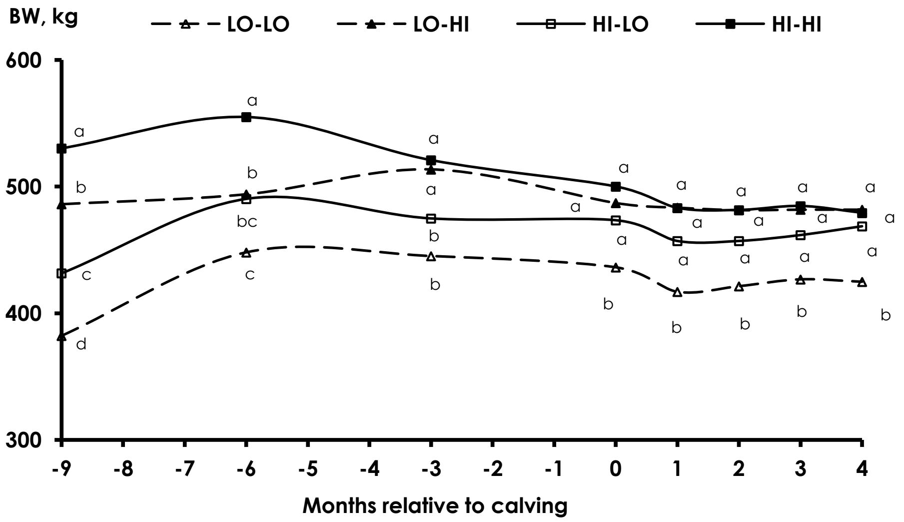

Figure 1. Weight of heifers throughout the gestation and lactation periods according to the feed management applied in the preweaning (0–6 months) and postweaning (6–15 months) periods. LO: 0.7 kg target average daily gain; HI: 1.0 kg target average daily gain. [a–d] LSMeans at a given age with different superscripts differ significantly (P < 0. 05).

The BW at conception at 15 months was influenced by the feeding treatments applied both during PRE (P < 0.001) and POST (P < 0.001). As expected, the highest and lowest percentage of mature BW (580 kg as Casasús *et al.* (2002) described for this breed) was reached by HI–HI (91%) and LO–LO (66%) heifers, respectively. All the treatments were above the threshold of 65% of mature BW recommended previously (Gasser, 2013), as the minimum to avoid future detriment to the performance of the heifer. Thereafter, gains during the gestation were also dependent on the feeding treatments applied in PRE (P < 0.05) and POST (P < 0.001). The POST–LO heifers gained weight (0.168 ± 0.077 kg/day) during gestation, whereas the POST–HI heifers either maintained (0.004 ± 0.085 kg/day, in the LO–HI treatment) or lost weight (−0.105 ± 0.031 kg/day, in the HI–HI treatment) in this phase. Because of this compensation, the differences in BW at conception were absent at calving, except for the LO–LO animals, which did not compensate for the large previous differences, being lighter than the rest of heifers (P < 0.05). At first calving the BW of LO–LO cows was 436 ± 39 kg, which was 75% of the expected mature BW for this breed. This proportion was below the 80% recommended by the NRC (2000), whereas it was at least 82% in the other treatments

(487 ± 24 kg BW at calving). However, Cano *et al. (in press)* questioned the use at an individual level of a maturity index calculated considering the average mature BW of this breed, because a large between-animal variability in mature BW was observed. The heifers in the current study were lighter at calving than those from the same herd described by Casasús *et al.* (2002) that calved for the first time at 2.5 years. After calving, all the animals maintained their weight (ADG = −0.053 ± 0.178 kg/day) during the lactation, regardless of the feed treatment applied in the first 15 months of life.

Table 1. Average daily gain (ADG) and size measures of heifers from conception (at 15 months) to weaning of their first calves (at 30 months) according to feed management applied in the preweaning (PRE) and the postweaning periods (POST).

	PRE (0–6 months)				**SEM**	***P-value***		
	HI[1]		**LO**[1]					
	POST (6–15 months)							**PRE×**
Item	**HI**	**LO**	**HI**	**LO**		**PRE**	**POST**	**POST**
n	6	6	8	5				
Gestation ADG (kg)	−0.105 ^{c}	0.146 ^{a}	0.004 ^{b}	0.190 ^{a}	0.03	0.02	<0.001	0.28
Lactation ADG (kg)	−0.120	0.015	−0.04	−0.065	0.07	0.96	0.44	0.32
Height at withers (cm)								
Conception	122.8 ^{a}	120.3 ^{ab}	122.8 ^{a}	118.0 ^{b}	1.39	0.43	0.02	0.46
Calving	126.0 ^{ab}	127.2 ^{ab}	128.6 ^{a}	123.8 ^{b}	1.06	0.75	0.12	0.02
Weaning	127.5 ^{ab}	127.0 ^{ab}	129.5 ^{a}	125.4 ^{b}	1.25	0.88	0.10	0.19
Heart girth (cm)								
Conception	189.2 ^{a}	173.5 ^{c}	182.4 ^{b}	166.6 ^{d}	1.73	<0.01	<0.001	0.98
Calving	181.3 ^{ab}	177.2 ^{bc}	185.9 ^{a}	172.0 ^{c}	1.69	0.87	<0.001	0.014
Weaning	179.2 ^{ab}	175.8 ^{bc}	180.8 ^{a}	171.4 ^{c}	1.61	0.42	0.002	0.09
Rump width (cm)								
Conception	45.7 ^{a}	45.3 ^{a}	46.5 ^{a}	41.6 ^{b}	0.80	0.11	0.006	0.014
Calving	44.8 ^{ab}	41.3 ^{bc}	46.1 ^{a}	39.0 ^{c}	1.26	0.70	<0.001	0.20
Weaning	48.3 ^{a}	47.7 ^{a}	48.1 ^{a}	45.0 ^{b}	0.65	0.05	0.012	0.09
Rump length (cm)								
Conception	48.0 ^{a}	46.3 ^{a}	47.4 ^{a}	43.4 ^{b}	0.61	0.01	<0.001	0.58
Calving	45.7 ^{ab}	43.3 ^{ab}	46.1 ^{a}	42.2 ^{b}	1.20	0.80	0.03	0.54
Weaning	48.5	47.2	48.3	46.0	0.86	0.45	0.06	0.62
Pelvic area (dm^2)								
Conception	22.3 ^{a}	20.9 ^{a}	22.0 ^{a}	18.2 ^{b}	5.63	0.012	<0.001	0.005
Calving	20.6 ^{ab}	17.9 ^{bc}	21.4 ^{a}	16.5 ^{c}	10.3	0.76	0.002	0.33
Weaning	23.4 ^{a}	22.5 ^{ab}	23.2 ^{a}	20.7 ^{b}	6.25	0.15	0.012	0.25
Body condition score (0-5)								
Conception	4.38 ^{a}	3.75 ^{bc}	4.13 ^{ab}	3.40 ^{c}	0.14	0.07	<0.001	0.75
Calving	2.56	2.60	2.59	2.56	0.04	0.86	0.94	0.34
Weaning	2.56	2.60	2.50	2.55	0.07	0.40	0.52	0.94

$^{a–d}$LSMeans within a row with different superscripts differ significantly ($P < 0.05$). [1]LO: 0.7 kg target ADG; HI: 1.0 kg target ADG

The BCS at conception was affected by the POST treatments (4.25 vs. 3.58 in POST–HI and POST–LO heifers, respectively; $P < 0.001$), but during pregnancy the BCS loss depended inversely on BCS at conception (Table 1). Therefore, despite the differences found in BW, all the heifers had a similar BCS at calving (2.58 ± 0.10). During the lactation, as occurred with BW, they maintained the BCS until weaning (2.55 ± 0.15). Concerning subcutaneous fat thickness, at conception the SFT_P8 was affected by the POST treatments (5.8 vs. 3.6 mm, in POST–HI and POST–LO heifers, respectively; $P < 0.01$), and SFT_T13 was lower in LO–LO heifers than in the other treatments (7.3 vs. 10.0 mm, respectively; $P < 0.01$). The SFT_P8 was correlated with BCS (r = 0.66; $P < 0.001$), but not with SFT_T13, which makes the former a better indicator of fat reserves. The differences at conception were counterbalanced during pregnancy and all heifers had similar SFT_P8 (2.6 ± 0.9 mm) and SFT_T13 at calving (7.2 ± 0.8 mm). At weaning, the SFT_P8 was affected by PRE treatments (1.8 vs. 2.2 mm, in PRE–HI and PRE–LO heifers, respectively; $P < 0.05$), but no differences were found for the SFT_T13 (7.0 ± 0.7 mm).

The size measurements at conception, calving and weaning are shown in Table 1. At breeding, the height at withers differed between POST treatments (122.8 vs. 119.2 cm in POST–HI and POST–LO heifers, respectively; $P < 0.05$). At calving, an interaction between PRE and POST treatments was detected ($P < 0.05$), because LO–LO primiparous cows were shorter than LO–HI cows, but no differences remained between HI–HI and HI–LO animals. The measures observed in this study ranged from 94 to 97% of those described by Álvarez-Rodríguez *et al.* (2009a) in adult cows from the same breed, in which maturity is reached at around 4.5 years (Cano *et al., in press)*.

The heart girth at conception was influenced both by PRE ($P < 0.01$) and POST ($P < 0.001$) feeding treatments and was strongly correlated with BW (r = 0.97, $P < 0.001$). This relationship was slightly lower at calving (r = 0.74, $P < 0.001$), when the heart girth was influenced by the interaction between PRE and POST ($P < 0.05$).

The internal dimensions of the pelvis provide valuable information for the prediction of dystocia (Johanson y Berger, 2003), these measures being well correlated with the external ones (Murray *et al.*, 2002). Rump measures observed in the current work were mostly influenced by the POST treatments. The estimated size of the pelvic area was affected at conception by the interaction between PRE and POST ($P < 0.01$), with smaller values in the LO–LO heifers (18.2 ± 1.5 dm^2, $P < 0.001$) than in the rest (21.8 ± 1.4 dm^2). At calving, the pelvic area was influenced only by POST management (21.0 vs. 17.2 dm^2 in POST–HI and POST–LO heifers, respectively; $P < 0.01$). Although the physical changes that occur at calving can bias this measurement (Murray *et al.*, 1999), this effect persisted at weaning (23.3 vs. 21.6 dm^2 in POST–HI and POST–LO heifers, respectively; $P < 0.05$).

5.3.2 *Productive performance*

The ratio of calf sexes was similar among treatments ($P > 0.10$). The calf weight at calving and at weaning and ADG during the lactation was alike between sexes (data not shown). No other productive or reproductive trait analyzed herein was influenced by the calf sex. As shown in Table 2, the calf BW at birth (38.5 ± 5.0 kg) and at weaning (132.2 ± 21.1 kg) and the ADG during the lactation (0.781 ± 0.178 kg/day) were not affected by the PRE and POST treatments. Moreover, the BW at birth was similar to the weight reported by Casasús *et al.* (2002) for calves born to 2.5-years-old primiparous cows in the same herd (39.1 ± 0.9 kg BW). Calf gains were similar to those observed in their dams at 4 months from the unsupplemented treatment during lactation (PRE–LO; 0.775 ± 0.208 kg/day). The BW at weaning was similar to that described by Villalba *et al.* (2000) for 4-month-old calves from primiparous cows in the same breed.

Table 2. Productive and reproductive performance in the first calving of the heifers according to the feed management applied in the preweaning (PRE) and the postweaning periods (POST).

	PRE (0–6 months)				**SEM**	***P-value***		
	HI[1]		**LO[1]**					
	POST (6–15 months)							**PRE×**
Item	**HI**	**LO**	**HI**	**LO**		**PRE**	**POST**	**POST**
n	6	6	8	5				
Calf								
BW at birth (kg)	36.3	38.3	41.5	36.6	1.81	0.37	0.46	0.09
BW at weaning (kg)	145.5	126.7	127.7	130.1	7.95	0.24	0.40	0.23
ADG[2] (kg)	0.910	0.737	0.718	0.779	0.07	0.30	0.43	0.11
Cow								
Age 1st calving (month)	26.2 [a]	25.7 [b]	26.6 [a]	25.6 [b]	0.29	0.51	0.02	0.45
Calving assistance (%)	16.7	0.0	37.5	80.0		0.02	0.68	0.03
Calf/Cow BW ratio (%)	7.3 [b]	7.3 [b]	8.5 [a]	8.4 [a]	0.30	0.002	0.86	0.85
PPA[3] (day)	84	101	79	112	11.7	0.82	0.06	0.56
Non-cyclic cows[4] (%)	50	33	37	40		0.87	0.74	0.94

[a, b]LSMeans within a row with different superscripts differ significantly ($P < 0.05$). [1]LO: 0.7 kg target ADG; HI: 1.0 kg target ADG. [2]ADG: Average daily gain during the lactation. [3] PPA: *Postpartum* anestrus. [4]Non-cyclic cows at weaning, after 120 days of lactation.

The similar calf gains among the treatments was consistent with the alike milk yield (average ECM yield = 6.26 ± 1.46 kg/day) and milk composition (protein = 3.20 ± 0.28%; fat = 3.14 ± 0.51%; casein = 2.48 ± 0.23%) produced by their dams. The different prepubertal ADG reached by the treatments did not affect the milk production, composition or persistency, because the ECM yield decreased steadily from a peak in the first month (7.75 ± 1.69 kg/d) to the forth month (6.46 ± 1.48 kg/d) in all treatments. The lack of detrimental effects on milk yield or calf performance observed here agrees with the results of Abeni *et al.* (2012) in dairy cattle, but contradicts studies where high prepubertal gains increased the deposition of mammary adipose tissue or impaired the parenchymal development of the mammary gland (Sejrsen *et al.*, 2000), reducing the milk yield. In the case of beef heifers there is large variability in the literature concerning the direction and the extent of these effects (Buskirk *et al.*, 1996). Our results agree with those described by Freetly y Cundiff (1998) over a diverse group of beef breeds, who did not observe any difference in milk production traits between heifers raised on different planes of nutrition. Moreover, Freetly *et al.* (2014) could not demonstrate that modifying peripubertal nutrition could modify DNA methylation in the mammary gland and consequently alter milk production.

5.3.3 Reproductive performance

The data concerning reproductive performance are shown in Table 2. The heifers from all treatments had reached puberty at a similar BW but at a different age, depending on the growth patterns (Rodríguez-Sánchez *et al.*, 2015). After first breeding at 15 months, the age at first calving was 26.1 ± 0.8 months. This was 2 months above our target (24 months), but below the national average in Spain, where more than 50% of the beef cows are older than 3 years at first calving (Ministerio de Agricultura Alimentación y Medio Ambiente, 2014). The age at calving was affected by the POST treatments (26.1 vs. 25.3 months in POST–HI and POST–LO heifers, respectively; $P < 0.05$). This difference was due to the fact that POST–HI heifers became pregnant later than POST-LO ones (16.5 vs. 15.9 months, respectively), because of the former were fatter at conception and needed more AI services to become pregnant (Rodríguez-Sánchez *et al.*, 2015).

In the current experiment, 32% of the heifers were assisted at first calving, an incidence similar to that described by Johanson y Berger (2003) for dairy heifers (38%). These needed at most the use of a calving jack, with no incidence of caesarean sections. No differences related to calf sex or calving date were found among the treatments. The incidence of assisted calving was affected by the interaction between PRE and POST ($P < 0.05$). The greater incidence in the PRE–LO treatments might be because the calf/cow BW ratio was above the 7.5% described by Johanson y Berger (2003) as the threshold that could compromise the ease of calving. A particularly high proportion of LO–LO cows (80%) needed assistance, most likely due to their small pelvic area and large calf/cow BW disproportion (Hickson *et al.*, 2006).

Concerning the ovarian activity, 60% of the animals were cyclic at the end of lactation, and all of them had a short cycle before recovering normal ovarian activity as described by Álvarez-Rodríguez *et al.* (2009b) for mature cows. The cows that had not ovulated 120 days after calving were evenly distributed among the treatments ($P > 0.10$; Table 2). The animals from the POST–HI treatments tended to be cyclic 24 days before those from the POST–LO treatment (82 and 106 days, respectively; $P = 0.06$). The mean PPA was longer in all treatments than the mean values observed in 2.5- and 3-year-old primiparous cows of the same breed (54 days) by Sanz *et al.* (2004), who described that the length of PPA depended mainly on *pre* and postpartum feeding levels, suckling frequency and calving difficulty. In our case, the pre and postpartum diets were calculated to meet the requirements of the animals without impairing their performance, but BCS at calving was lower than that observed in their study (2.58 vs. 2.77). Despite this lower value, BCS at calving was not correlated with the length of PPA in the current experiment. The calving difficulty was not reflected in the PPA length (94.4 vs. 91.2 days in assisted or unassisted calving, respectively; $P > 0.10$), in contrast to findings by Boldt *et al.* (2015) in dairy cows. However, in our experiment calves had *ad libitum* access to their dams, which can delay the resumption of cyclicity; restricted suckling may have shortened the PPA interval, as suggested by Diskin and Kenny (2014) for beef cattle in moderate BCS at calving.

5.3.4 Metabolic profiles

The profiles of some metabolites commonly associated with ruminant energy metabolism are presented in Figure 2. All the metabolites had greater concentrations during gestation than during lactation ($P < 0.001$), except for the NEFA that had similar concentrations in both phases.

The plasma glucose concentrations were positively correlated with cholesterol levels during the whole experiment ($r = 0.81$, $P < 0.001$), given that both are indicators of energy balance. Both were affected by the PRE treatment ($P < 0.05$), with greater concentrations in PRE–HI than in PRE–LO heifers, probably due to the differences found in the first sample postpartum. The concentrations of both metabolites during lactation were lower than those reported by Vizcarra *et al.* (1998) and Álvarez-Rodríguez *et al.* (2010b) in primiparous beef cows. This might explain the long PPA observed in the present study, because hypoglycemia has been associated with lack of luteal activity and infertility in lactating cows (Vizcarra *et al.*, 1998) and cholesterol is a precursor of steroid hormones. Furthermore, the PPA was negatively correlated with plasma glucose ($r = -0.51$, $P < 0.01$) and cholesterol levels ($r = -0.47$, $P < 0.05$) at 3 months postpartum.

During the experiment, the NEFA concentrations were not affected by the previous feeding treatments. As expected, they increased in the first sample postpartum because of parturition stress and lipolytic activity for milk secretion (Vizcarra *et al.*, 1998). This was not appreciated in LO–LO heifers, despite the amount of BCS and the SFT at calving were similar among treatments. Maybe heifers from the LO–LO treatment had less fat in abdominal or visceral deposits, which are more readily mobilized in early lactation in primiparous cows (Weber *et al.*, 2013), and therefore concentrations of NEFA did not peak after calving.

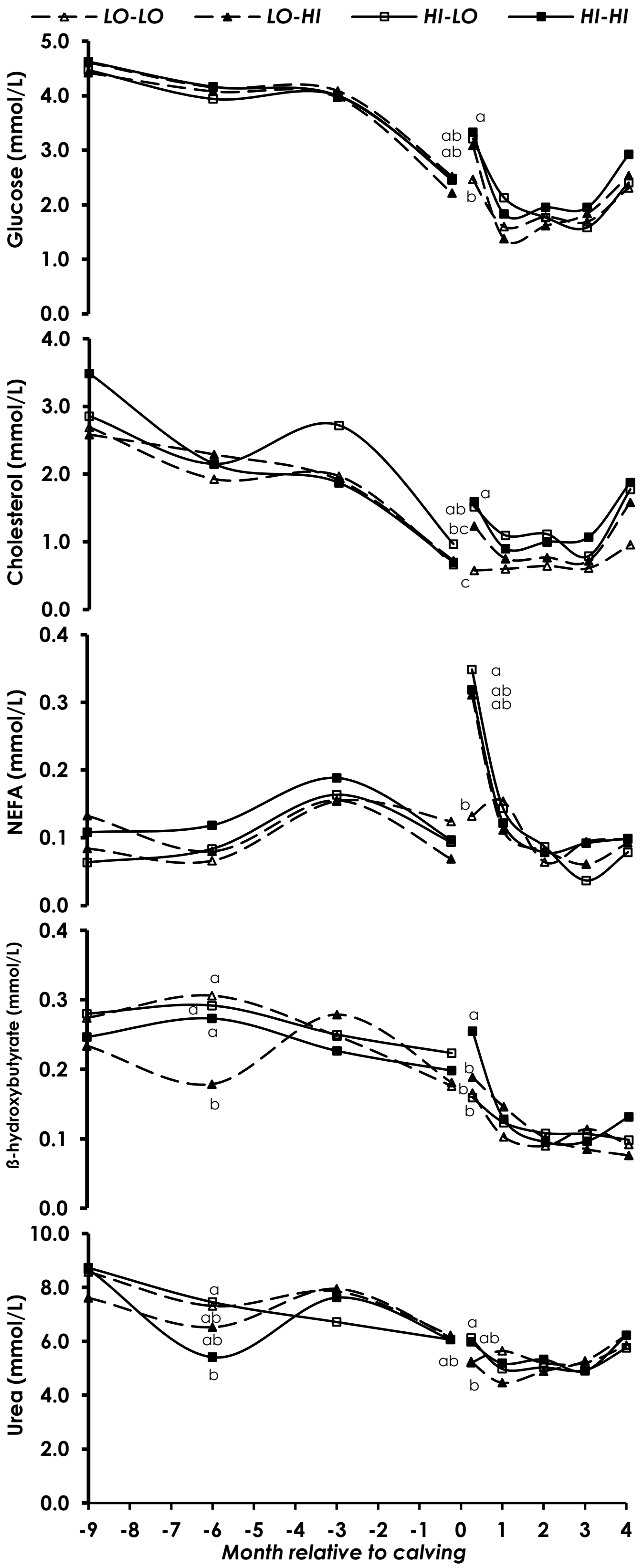

Figure 2. Plasma concentrations of glucose, cholesterol, NEFA, β-hydroxybutyrate and urea during the gestation and the lactation of heifers according to the feed management applied in the preweaning (0–6 months) and postweaning (6–15 months) periods. LO: 0.7 kg target average daily gain; HI: 1.0 kg target average daily gain. [a–c]LSMeans at a given age with different superscripts differ significantly (*P* < 0. 05).

The plasma concentrations of ß-hydroxybutyrate were affected by the interaction between POST and time. Unexpectedly, at 3 months postbreeding the LO–HI heifers had lower levels ($P < 0.05$) of this metabolite than their counterparts. Nevertheless, at calving levels were higher in the HI–HI cows ($P < 0.01$), maybe due to a poor adaptation to the lower energy balance at the onset of lactation or because they had more easy-to-mobilize fat reserves. No relationship was found among the concentrations of ß-hydroxybutyrate during lactation and the length of PPA, in contrast to the observations of Mulliniks *et al.* (2013), who indicated that this metabolite was a sensitive indicator of energy status that could be used to predict the rebreeding competence in young beef cows.

Throughout the experiment, the concentration of urea was positively correlated with that of glucose (r = 0.64, $P < 0.001$) and cholesterol (r = 0.50, $P < 0.001$), all of them dependent on nutrient intake. However, at 3 months postbreeding POST–HI heifers had lower concentration of plasma urea than POST–LO heifers (5.968 vs. 7.381 mmol/L, respectively; $P < 0.05$). This difference might be explained because urea is also a hepatic by-product of protein catabolism, and maybe the POST–HI heifers had less breakdown of endogenous protein for energy production because of their greater BCS at conception. This was confirmed by the negative relationship between urea concentration and BCS (r = –0.39, $P = 0.05$). During lactation, the levels of urea were always below 7 mmol/L, threshold described by Butler (1998) above which subsequent fertility might be impaired.

5.3.5 Endocrine profiles

The IGF-I and leptin concentrations were not affected by the experimental treatments (Figure 3). This would confirm the results of Reis *et al.* (2015), who found that the increased concentrations of IGF-I associated to improved nutritional status in the early life of heifers did not persist in subsequent phases with similar nutrient intake, and found no treatment effects on leptin concentrations at any time before puberty. They attributed the lack of effects to the short duration of their treatments, but in our case the heifers had different nutritional management until first breeding and no differences were found either on the first calving performance.

Both hormones were influenced by the time ($P < 0.001$), probably because they depend directly on the current energy intake (Ciccioli *et al.*, 2003). The concentration of IGF-I was correlated to the ECM at 3 months postpartum (r = 0.62, $P < 0.001$), confirming its role an indicator of energy status. The ECM also was related with the leptin concentration one month after calving (r = −0.56, $P < 0.01$), which might reflect the increased ability of the cows to mobilize body fat when energy is deficient and redirect this energy to milk production (Sullivan *et al.*, 2009).

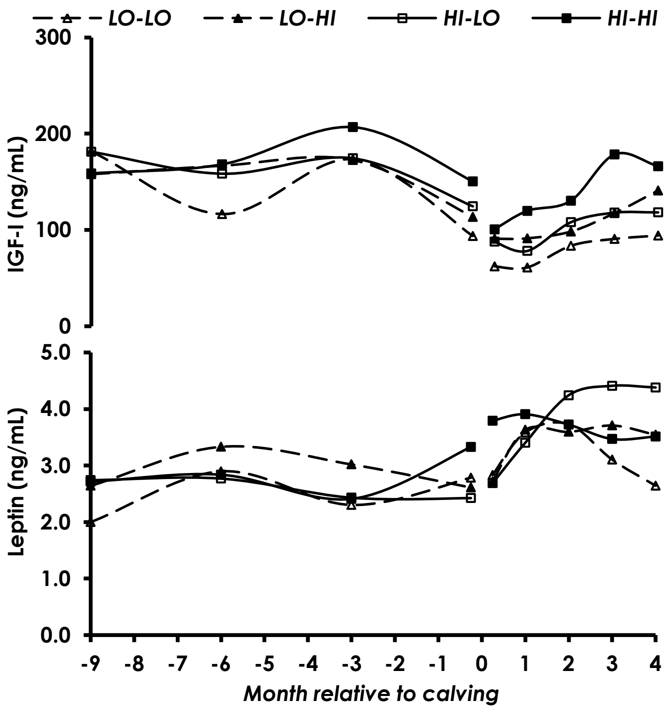

Figure 3. Plasma concentrations of IGF-I and leptin during the gestation and the lactation of heifers according to the feed management applied in the preweaning (0–6 months) and postweaning (6–15 months) periods. LO: 0.7 kg target average daily gain; HI: 1.0 kg target average daily gain.

Concerning the reproductive performance, the IGF-I concentrations in lactation were not related with the PPA length, in contrast to the findings of Ciccioli *et al.* (2003) in primiparous cows receiving different diets in the postpartum. The PPA length was not related with the concentrations of leptin, in accordance with Ciccioli *et al.* (2003) but not with Liefers *et al.* (2003), who described a relationship between leptin and PPA in dairy heifers. The differences in the role of leptin on the resumption of postpartum ovarian function between beef and dairy breeds might reflect different degrees of negative energy balance, associated with the great differences in milk yield (Sullivan *et al.*, 2009).

5.3.6 Conclusion

In this experiment, the commonly established recommendations for BW and size at first calving were met with the feeding levels applied during the PRE and POST. These feeding levels did not influence the milk yield, first calf performance or metabolic and endocrine profiles of the primiparous cows. Nevertheless, the reproductive performance of the LO-LO heifers might have been hindered because they were lighter and smaller than the rest, had a larger disproportion in calf/cow BW at calving and the smallest pelvic area, which could have induced the greater calving assistance and the longer PPA. Thus, the threshold recommendations for the BW at conception and calving should be reviewed for this breed. We can conclude that advancing the first calving to 2 years in beef cows is feasible, but it is recommended to optimize growth rates before breeding season. Gains of at least 1 kg/day either in the preweaning or the postweaning phase should be ensured to prevent impaired performance at calving. The decision on the period in which to provide this gain should be based on the relative feeding costs for each phase. Further investigations are needed to be sure that the prebreeding nutritional strategies have no effect on the productive lifetime of beef cows.

6. *Manuscrito 3*

Rodríguez-Sánchez JA, Casasús I, Ferrer J, Sanz A. **Postweaning feeding management of beef heifers to be bred at 15 months: I. Growth, puberty and fertility in two genotypes.**

Abstract

This experiment was developed during the postweaning phase, from weaning to breeding by fixed-time AI (6–15 mo). Two **feeding managements (FEED:** 0.8 [**HIGH**] vs. 0.6 [**LOW**] kg/d target ADG) were applied on beef heifers from 2 breeds (**BREED**: Parda de Montaña [**PA**] vs. Pirenaica [**PI**]) to evaluate the consequences on the growth, reproductive parameters and metabolic and endocrine status. The heifers had been creep fed with concentrates while nursing their dams. Animal weights were recorded weekly, and size measures were taken at 6 and 15 mo. The heifers were bled weekly to determine the onset of puberty, and every 3 mo for determining the metabolic (glucose, cholesterol, NEFA, β-hydroxybutyrate and urea) and endocrine status (IGF-I and leptin). During the experiment, the growth, size measurements and reproductive performance were not affected by the interaction between BREED and FEED. At 15 mo the BW ($P < 0.001$), ADG ($P < 0.001$), subcutaneous fat thickness ($P < 0.001$), concentrations of glucose ($P < 0.05$) and cholesterol ($P < 0.001$) were greater in heifers from the HIGH feeding treatment, but they were not affected by BREED ($P > 0.10$). The pelvic area was unaffected by feeding management but it differed between breeds ($P < 0.01$), because of the wider and longer rump of PA heifers. All the heifers reached puberty at similar BW (55% mature BW) but different age depending on the BREED, since PA heifers were 1.6 mo more precocious than PI ones ($P < 0.05$). Heifers from the HIGH feeding treatment tended ($P < 0.09$) to be pubertal earlier than those from the LOW treatment, all of them being pubertal at least 1 mo in advance of the breeding season. The heifers with greater plasma concentrations of urea at 6 mo reached puberty earlier. The age at puberty was negatively related with IGF-I (r = –0.43, $P < 0.001$), but not with leptin concentrations reached during the experiment. The fertility rate after a 3-mo breeding season (92.3%) and the number of services required to become pregnant (1.7) were similar among experimental groups, but the heifers with greater cholesterol concentrations at 9 and 12 mo needed less services for pregnancy. Regardless of the different precocity, our results confirmed the feasibility of advancing the first service at 15 mo in both breeds, even with moderate gains (0.6 kg/d) in postweaning, if preweaning gains are enhanced by creep feeding.

Key words: breed, first breeding, fixed-time AI, growth strategy, replacement cattle

6.1 Introduction

In order to decrease the expenses of rearing replacement heifers, it is recommended to minimize their non-productive period by advancing the first calving to 2 yr. To achieve this objective the heifers should be bred at 15 mo and be pubertal at least 6 wk before mating (Wathes *et al.*, 2014). The age at puberty depends on the energy intake from 4 to 6 mo (Gasser *et al.*, 2006), and also on the diet applied during the postweaning phase after weaning (Roberts *et al.*, 2009b). Different patterns of feeding during the postweaning period may influence the metabolic and endocrine profiles, which may consequently modify the development and reproductive performance (Brickell *et al.*, 2009b). Moreover, since breeds differ widely in age at puberty and productive performance, management during the postweaning phase should be tailored to each breed in order to avoid inefficient under or overfeeding at the different periods (Ferrell, 1982).

Parda de Montaña (**PA**) and Pirenaica (**PI**) are two beef cattle breeds widely spread in the mountain areas of the Spanish Pyrenees. Parda de Montaña breed comes from the old Brown Swiss, originally a dual purpose breed (milk-beef), but afterwards selected for beef production. Pirenaica is an autochthonous hardy breed from the Pyrenees area used for beef production. Although both breeds have similar mature BW, around 580 kg (Casasús *et al.*, 2002), their production traits differ throughout the production cycle. Calf BW at birth is greater in PA, and due to the greater milk yield of their dams (Sanz *et al.*, 2003), they grow faster during the lactation and they are heavier at weaning than PI ones (Villalba *et al.*, 2000). After weaning both breeds have similar gains but different maturing rate, PA being considered an intermediate-maturing breed and PI a late-maturing one (Piedrafita *et al.*, 2003). Due to these differences, it might be necessary to use breed-specific feeding programs during the postweaning phase, to ensure a timely match of their requirements.

The objective of this experiment was to evaluate the effects of two feeding managements designed to promote different growth rates (0.8 vs. 0.6 kg/d) during the postweaning period (6 to 15 mo) on two breeds (PA vs. PI), on the consequent patterns of growth, onset of puberty, fertility rate and metabolic (glucose, cholesterol, NEFA, β-hydroxybutyrate, and urea) and endocrine status (IGF-I and leptin) of beef heifers bred at 15 mo.

6.2 Materials and methods

The Animal Ethics Committee of the Centro de Investigación y Tecnología Agroalimentaria (**CITA**) approved the experimental procedures, which were in compliance with the guidelines of the European Union (Directive No. 86/609/CEE, 1986) on the protection of animals used for experimental and other scientific purposes.

6.2.1 Animals, management and diets

The study was conducted at the CITA-Montañana Research Station (41°43' N, 0°48' W, 225 m above sea level, mean annual temperature 15.2 ± 0.2 °C, and mean annual rainfall 318 ± 63 mm).

Twenty-five 6-mo-old female calves, from PA (13) and PI (12) breeds, were distributed in a 2 × 2 factorial arrangement: 2 breeds (**BREED:** PA vs. PI) × 2 feeding treatments to promote different growth rates (**FEED:** 0.8 kg/d [**HIGH**] and 0.6 kg/d [**LOW**] treatments, respectively) during the postweaning phase (from weaning to breeding at 15 mo), which resulted in four experimental groups: **PA–HIGH**, **PA–LOW**, **PI–HIGH** and **PI–LOW.** The experiment started when the calves were weaned at 6.4 ± 0.3 mo and 238 ± 41 kg BW. The treatments were randomly balanced according to calf BW and age. During the previous lactation phase, they were fed on their dams' milk (suckling twice daily for 30 min) and had free access to a starter concentrate, which resulted in pre-weaning gains of 1.039 ± 0.176 kg/d.

During the postweaning phase the heifers were kept indoors in a loose housing system in straw-bedded pens with fresh and clean water supplied ad libitum. To achieve the targeted weight gains, the heifers were group-fed alfalfa hay ad libitum and 10 (HIGH) or 4 g concentrate/kg BW (LOW) (composition in Table 1).

Table 1. Ingredients and composition of concentrate and alfalfa hay provided to heifers during the postweaning period (6–15 mo)[1]

Item	Concentrate	Alfalfa hay
Ingredient (as-fed basis), %		
Corn	44.00	
Barley	21.60	
Corn gluten	15.00	
Rapeseed flour	5.00	
Soybean flour	4.60	
Beet pulp	3.00	
Palm oil	2.90	
Vitamin-mineral	2.00	
Calcium carbonate	1.20	
Urea	0.50	
Sodium chloride	0.20	
Nutrient composition		
DM, g/kg	908	885
ME, MJ/kg DM	15.4	9.4
CP, g/kg DM	169	192
NDF, g/kg DM	240	459

[1]Concentrate was provided in amounts of 10 or 4 g/kg BW to the high or low targeted ADG feeding managements, respectively. Alfalfa hay was provided ad libitum to all heifers.

[2]Vitamin A, 7,000 IU/kg; Vitamin D3, 1,500 IU/kg; Copper (cupric sulfate pentahydrate), 2 mg/kg; Iodine (potassium iodide), 0.5 mg/kg; Cobalt (cobaltous carbonate monohydrate), 0.5 mg/kg; Zinc (zinc oxide), 40 mg/kg; Manganese (manganese oxide), 30 mg/kg; Selenium (sodium selenite), 0.2 mg/kg; Iron (ferrous carbonate), 5 mg/kg; Butylhydroxytoluene, 2 mg/kg.

At 14 mo the heifers were vaccinated against bovine viral diarrhea (Bovilis BVD; MSD Animal Health, Salamanca, Spain) and 1 mo later a 90-d breeding season began. All heifers were synchronized with an Ovsynch + progesterone releasing intravaginal device (**PRID**) program (Fig. 1) in which they simultaneously received 1.55 mg of progesterone in a PRID (CEVA, Barcelona, Spain) and a 10 µg injection of GnRH (Busol; INVESA, Barcelona, Spain) followed 10 d later by 25 mg of prostaglandin $F_{2\alpha}$ (Enzaprost; CEVA). The PRID was removed 12 d later, and 500 IU of pregnant mare serum gonadotrophin (Foligon; Intervet, Salamanca, Spain) was administered followed 48 h later by a second injection of GnRH (10 µg). Eight hours after the final GnRH injection, the heifers were randomly inseminated by an expert technician. Three different sires for each breed were selected for their calving ease.

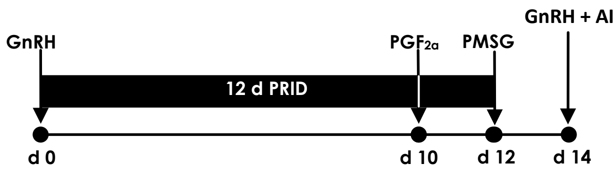

Figure 1. Synchronization protocol used in Parda de Montaña and Pirenaica heifers at 15 mo of age managed with different feeding management during the postweaning period (6–15 mo). PRID = progesterone releasing intravaginal device.

The heifers were checked twice daily (0700 and 1900 h) from the first AI to the end of the breeding season for detecting estrus of non-pregnant heifers. They were inseminated approximately 12 h after the estrus was detected. Return to estrus after each AI was considered as a diagnostic indicator of non-pregnancy status. Pregnancy was confirmed by ultrasonography (Aloka SSD-500V; equipped with a linear-array 7.5 MHz transducer; Aloka, Madrid, Spain) 31 d after the end of the breeding season.

The day of the first timed AI was used to determine the age and BW at first breeding, and the day of the effective AI was used to determine the age and BW at conception. The first-service fertility rate was determined as the number of pregnant heifers at the first AI divided by the total number of heifers. The number of AI needed to become pregnant was calculated considering only heifers that were pregnant at the end of the breeding season. The fertility rate was determined as the number of pregnant heifers in the breeding season divided by the total number of heifers.

6.2.2 Measurements and blood sampling

The concentrate intake was daily recorded by group and monthly adjusted by average group weight. The intake of alfalfa hay was recorded by pen at weekly intervals. The actual daily intake during the experiment was calculated as feed provided minus feed refused. Feed samples were collected at weekly intervals and were pooled on a monthly basis for chemical analyses. The samples were dried at 60 °C until a constant weight and mill-ground (1 mm screen) and DM, ash, ether extract and CP (N × 6.25) contents were determined according to the Association of Official Analytical Chemists (1990; Method 942.05, 920.39, 968.06). Analyses of NDF, ADF and ADL were conducted according to the sequential procedure of van Soest *et al.* (1991). All values were corrected for ash-free content.

The heifers were weighed once a week before morning feeding, without prior deprivation of feed and water. The weight at key points (6, 9, 12 and 15 mo and puberty onset, first breeding and conception) was calculated as the average of three consecutive weights. The ADG during the weaning-puberty period and the whole postweaning phase were calculated with linear regression of weight against time.

Body development was studied using size measurements at 6 and 15 mo. The height at withers (from the highest point of the shoulder blade to the ground), rump width (the maximum distance between iliac tuberosities) and rump length (from the ischial tuberosity to the iliac tuberosity) were recorded with a height stick. The external pelvic area was estimated as the product of the rump width and the rump length, as suggested by Murray *et al.* (2002). This trait was used as a proxy of the internal pelvic area, a trait of major importance in replacement heifers because of its influence on the incidence and degree of calving difficulty in primiparous cows. The heart girth (the body circumference immediately posterior to the front legs) was measured with a flexible tape. The increase of every size measure during the experiment was defined as the difference between the values registered at 6 and 15 mo.

The BCS was assessed at 15 mo by two expert technicians, according to Lowman *et al.* (1976) (0 to 5 scale). Simultaneously, the subcutaneous fat thickness was measured at the P8 rump site by ultrasound scanning with a multifrequency probe (7.5 MHz; Aloka SSD-900, Aloka, Madrid, Spain). This measure was determined over the *gluteus* muscle on the rump at the intersection of a line through the pin bone parallel to the chine and one perpendicular through the 3rd sacral crest (Robinson *et al.*, 1992), using vaseline for the contact of the transducer and the skin.

The heifers were bled weekly to determine the onset of puberty based on plasma progesterone concentration. Additionally, the heifers were bled every 3 mo throughout the experiment to determine the concentrations of both metabolites and hormones. The blood samples were collected before morning feeding from the coccygeal vein. The samples to determine progesterone, ß-hydroxybutyrate, IGF-I and leptin concentrations were collected into 9 mL heparinized tubes (Vacuette España S.A., Madrid, Spain). The samples to determine plasma glucose, cholesterol, NEFA and urea concentrations were collected into 9 mL tubes containing EDTA (Vacuette España S.A.). Blood samples were centrifuged at 1,500 × g for 20 min at 4 °C immediately after collection, and the plasma was harvested and frozen at −20 °C until analysis.

All measurements and samples taken at 6 mo were conducted before the postweaning diets were applied.

6.2.3 Assays

Plasma progesterone concentrations were measured using an ELISA kit (Ridgeway Science, Lydney, UK), following the manufacturer's instructions. The onset of puberty occurred when progesterone levels were ≥ 1.0 ng/mL in at least 2 consecutive samples (normal estrus cycle, ≥ 14 d; Álvarez-Rodríguez *et al.* (2010b). The age at puberty was defined as the date of collection of the first blood sample that contained ≥ 1.0 ng/mL of plasma progesterone. To ensure the continuation of estrous cycles, blood samples analyzed after the attainment of puberty were confirmed by the observation of at least 1 subsequent estrous cycle of normal duration, based on progesterone concentration.

The plasma concentrations of glucose (glucose oxidase/peroxidase method), cholesterol (enzymatic-colorimetric method), ß-hydroxybutyrate (enzymatic-colorimetric method) and urea (kinetic UV test) were determined with an automatic analyzer (GernonStar, RAL/TRANSASIA, Dabhel, India). The reagents for glucose, cholesterol and urea analyses were provided by the analyzer manufacturer (RAL, Barcelona, Spain), and the reagents for ß-hydroxybutyrate were supplied by Randox Laboratories Ltd. (Crumlin Co., Antrim, UK). The mean intra- and interassay CV for these metabolites were <5.4% and <5.8%, respectively. The sensitivity was 0.056, 0.026, 0.030, 0.170 mmol/L for glucose, cholesterol, ß-hydroxybutyrate and urea, respectively. The plasma concentrations of NEFA were analyzed with an enzymatic method using a commercial kit (Randox Laboratories Ltd.). Commercial reference plasma samples (bovine precision serum; Randox Laboratories Ltd.) were used to evaluate the accuracy of the analyses. The mean intra- and interassay CV were 5.1% and 7.4%, respectively. The sensitivity was 0.060 mmol/L.

Circulating IGF-I concentrations were quantified with a solid-phase enzyme-labeled chemiluminescent immunometric assay (Immulite; Siemens Medical Solutions

Diagnostics Limited, Llanberis, Gwynedd, UK). The mean intra- and interassay CV were 3.1 and 12.0%, respectively. The sensitivity was 20 ng/mL.

Plasma leptin concentrations were determined by RIA with a multispecies commercial kit (Multispecies Leptin Ria kit; LINCO Research, St. Charles, MO). The mean intra- and interassay CV were 3.54 and 6.87%, respectively. The sensitivity averaged 1.30 ng/mL.

6.2.4 Statistical analyses

All data were analyzed as a completely randomized design with the SAS statistical software package (SAS Institute Inc., Cary, NC). The heifer was the experimental unit. Data for BW and metabolic (glucose, cholesterol, NEFA, ß-hydroxybutyrate and urea) and endocrine (IGF-I and leptin) samples collected at 3-mo intervals (6, 9, 12 and 15 mo) were analyzed using the SAS MIXED procedure for repeated measures. The covariance structure was selected on the basis of the lowest Akaike information criterion. Therefore, an unstructured covariance matrix was used for the analysis of repeated measures, which included BREED, FEED, sampling date and their interaction as fixed effects and with heifer as the random effect in a univariate linear mixed model.

The ADG (during the weaning-to-puberty period and in the whole postweaning phase), size measurements (height at withers, heart girth, rump width and length and pelvic area at 6 and 15 mo of age), BCS and subcutaneous fat thickness in the heifers were tested with ANOVA using the GLM procedure. BREED, FEED and their interaction were fixed effects. Similar analyses were performed to analyze the age and weight at puberty, at the first AI, and at conception, and number of AI necessary to become pregnant. The fertility rate was analyzed using the FREQ procedure of SAS (χ^2 test). The Pearson correlation coefficients between variables were calculated using the CORR procedure of SAS. Means were separated using the LSMEANS procedure of SAS. For all tests, the level of significance was $P < 0.05$ and tendencies were determined if $P \geq 0.05$ and $P < 0.10$.

6.3 Results and discussion

6.3.1 Growth performance

The interaction between BREED and FEED was not significant for any of the growth traits; therefore, the main effects were examined separately.

The BW at keypoints and the ADG during the postweaning phase are displayed in Table 2. At the start of the study PA heifers were 18 kg heavier than PI ones ($P > 0.10$). This numerical difference would confirm previous research comparing both breeds, where greater calf birth weight and cow milk yield resulted in greater weaning weight of Parda de Montaña calves (Villalba *et al.*, 2000). During the postweaning phase, heifers of both breeds had similar gains, as described in other works both with heifers and growing bulls (Piedrafita *et al.*, 2003), due to their similar intake capacity (Casasús *et al.*, 2004) and feed conversion efficiency (Blanco *et al.*, 2009c). Therefore, PA heifers remained heavier at 15 mo, although not significantly ($P > 0.10$). The BCS (4.1 vs. 4.2 in PA and PI, respectively, $P > 0.10$) and the subcutaneous fat thickness (4.7 vs. 4.5 mm in PA and PI, respectively, $P > 0.10$) at 15 mo were also similar between breeds.

Table 2. Weights and ADG of heifers according to the breed (BREED) and the feeding management (FEED) applied in the postweaning period (6–15 mo).

	BREED		FEED		SEM	*P-value*	
Item	**PA[1]**	**PI[1]**	**HIGH[1]**	**LOW[1]**		**BREED**	**FEED**
Weight, kg							
6 mo	247	229	235	241	17.57	0.30	0.76
9 mo	320	296	315	301	18.53	0.20	0.45
12 mo	383	357	383	357	20.33	0.21	0.20
15 mo	441	410	452^{x}	400^{y}	21.28	0.15	0.02
ADG, kg/d							
Postweaning	0.737	0.700	0.814^{x}	0.624^{y}	0.03	0.27	<0.001
6 mo-puberty	0.997	0.827	1.067^{x}	0.757^{y}	0.09	0.07	0.002

^{x,y}LSMeans within a row with different superscripts differ significantly among feeding managements ($P < 0.05$). [1]PA = Parda de Montaña; PI = Pirenaica; HIGH = 0.8 kg/d target ADG; LOW = 0.6 kg/d target ADG.

The target ADG during the postweaning phase was reached in both FEED treatments, which differed significantly ($P < 0.001$). Consequently heifers from the HIGH treatment were significantly heavier than those from the LOW one at 15 mo ($P < 0.05$), and BCS also tended to be greater (4.3 vs. 3.9 in HIGH and LOW, respectively; $P = 0.06$). This trend was confirmed by the subcutaneous fat thickness measured at the same time (5.5 vs. 3.4 mm in HIGH and LOW heifers, respectively, $P < 0.01$), since both measures were correlated ($r = 0.59$, $P < 0.01$).

The size measurements at 6 and 15 mo are shown in Table 3. At the beginning of the trial the size measures were similar between breeds, except for the wider rump of PA heifers ($P < 0.05$). When heifers reached 15 mo of age, the height at withers was alike between breeds, indicating a similar skeletal development. They also had similar heart girth, a trait which has been proposed as an accurate indirect measure of BW, which was confirmed by the strong and positive relationship between both traits ($r = 0.93$, $P < 0.001$). At 15 mo both rump width and length were larger in PA heifers than in PI ones ($P < 0.05$), and consequently so it was the external pelvic area ($P < 0.01$), which could have further consequences on the ease of calving in primiparous cows (Murray *et al.*, 2002).

No differences were observed at 6 mo for any of the size measures among heifers that received different FEED during the postweaning phase. At 15 mo, height at withers only tended to be greater in heifers from the HIGH feeding treatment (P = 0.09), but they had significantly greater heart girth (P < 0.01), which corresponded with their greater BW at this point. They also had greater rump width (P < 0.05) and tended to have longer rumps (P = 0.09) at the time of breeding, which resulted in a larger pelvic area (P < 0.05), confirming the results described by Rodríguez-Sánchez *et al.* (2015) in heifers under different postweaning programs. The lower pelvic area reached at breeding by the heifers from LOW treatments could increase the difficulties at calving because this measure is highly correlated with the pelvic area at calving (Johnson *et al.*, 1988).

Table 3. Size measures of heifers at 6 and 15 mo of age according to the breed (BREED) and the feeding management (FEED) applied in the postweaning period (6–15 mo).

	BREED		FEED		SEM	*P-value*	
Item	**PA[1]**	**PI[1]**	**HIGH[1]**	**LOW[1]**		**BREED**	**FEED**
Height at withers, cm							
6 mo	100.9	100.3	100.8	100.4	2.2	0.81	0.87
15 mo	121.0	120.1	122.4	118.8	2.1	0.65	0.09
Heart girth, cm							
6 mo	136.0	132.9	133.7	135.2	3.6	0.40	0.69
15 mo	178.5	173.5	181.2 ^{x}	170.8 ^{y}	3.0	0.11	0.002
Rump width, cm							
6 mo	31.6 ^{a}	28.8 ^{b}	29.8	30.6	1.1	0.01	0.47
15 mo	45.9 ^{a}	42.2 ^{b}	45.5 ^{x}	42.6 ^{y}	1.3	0.007	0.03
Rump length, cm							
6 mo	35.9	34.8	35.3	35.5	1.2	0.36	0.83
15 mo	47.1 ^{a}	44.9 ^{b}	46.9	45.2	1.0	0.03	0.09
Pelvic area, dm^2							
6 mo	11.4	10.1	10.6	10.9	0.7	0.05	0.62
15 mo	21.7 ^{a}	19.0 ^{b}	21.4 ^{x}	19.3 ^{y}	0.9	0.009	0.04

^{a,b} LSMeans at a given age with different superscripts differ significantly among breeds (P < 0.05); ^{x,y} Means at a given age with different superscripts differ significantly among feeding managements (P < 0. 05). [1] PA = Parda de Montaña; PI = Pirenaica; HIGH = 0.8 kg/d target ADG; LOW = 0.6 kg/d target ADG.

6.3.2 Reproductive performance

No interaction between BREED and FEED was found and therefore results of both effects are presented separately in Table 4. All the heifers were pubertal before the breeding season, reaching puberty at similar BW (322 ± 38 kg), which was 55.5% of the expected mature BW, 580 kg for both breeds as described Casasús *et al.* (2002). The weight at puberty was similar to that described by Revilla *et al.* (1992) for both breeds with different ADG during the postweaning phase. These data confirm that puberty is reached at a critical BW around 55% of mature BW (Freetly *et al.*, 2011).

The age at puberty was significantly different between breeds, PA heifers being more precocious ($P < 0.05$). This was also described by Grings *et al.* (1999) among beef heifers of three sire breeds differing in potential muscularity and raised on different dietary regimes. The fact that PA heifers were more precocious, also reported by Revilla *et al.* (1992), could be ascribed to its origin in the old Brown Swiss dual purpose breed, since dairy breeds reach puberty earlier than beef breeds (Patterson *et al.*, 1992).

Table 4. Reproductive performance of heifers according to the breed (BREED) and the feeding management (FEED) applied in the postweaning period (6–15 mo).

	BREED		FEED		SEM	*P-value*	
Item	**PA[1]**	**PI[1]**	**HIGH[1]**	**LOW[1]**		**BREE**	**FEED**
Weight at puberty, kg	321.2	324.8	320.5	325.5	15.6	0.81	0.75
Age at puberty, mo	9.1 [b]	10.7 [a]	9.4	10.5	0.62	0.01	0.09
% MBW[2] at puberty	55.4	56.0	56.1	55.3	0.03	0.81	0.75
Weight at first AI, kg	458.3	422.8	467.2 [x]	413.9 [y]	22.0	0.12	0.02
Age at first AI, mo	15.7	15.8	15.8	15.8	0.14	0.55	0.99
Conception BW, kg	465.8	426.1	471.5	420.3	24.4	0.13	0.06
Conception age, mo	16.6	16.3	16.4	16.5	0.38	0.56	0.65
Number of AI	1.92	1.45	1.62	1.75	0.32	0.17	0.69
Fertility at first AI, %	30.8	50.0	30.8	50.0		0.33	0.33
Fertility[3], %	92.3	91.7	84.6	100.0		0.95	0.16

[a,b]LSMeans at a given age with different superscripts differ significantly among breeds ($P < 0.05$); [x,y]Means at a given age with different superscripts differ significantly among feeding managements ($P < 0.05$). [1]PA = Parda de Montaña; PI = Pirenaica; HIGH = 0.8 kg/d target ADG; LOW = 0.6 kg/d target ADG. [2]MBW = mature BW. [3]Fertility rate in a 90-d breeding season.

The dietary treatments received during the postweaning period did not affect age at puberty, although there was a trend for heifers from the HIGH feeding treatment to be pubertal 1 mo earlier ($P = 0.09$) than those from the LOW one. This trend was confirmed by the strong negative correlation between the age at puberty and the weaning-to-puberty ADG of the heifers ($r = -0.70$, $P < 0.001$). The fact that the differences between treatments did not reach significance could be due to the small numbers of animals, and also to the fact that all the heifers had similar and high pre-weaning gains (1.039 ± 0.176 kg/d), which were greater than those observed in previous works with non creep-fed calves of both breeds (Casasús *et al.*, 2002, 2004). These fast gains associated to a high nutrient intake during lactation could have induced an earlier puberty, as suggested by Day and Nogueira (2013). Besides, Cardoso *et al.* (2014) reported that a favorable nutritional and metabolic status between 4 and 6.5

mo of age induces functional changes in the neuroendocrine reproductive system that persist after a period of feed intake restriction between 6.5 and 9 mo of age. Furthermore, Rodríguez-Sánchez *et al.* (2015) indicated a major role of nutrient intake during the early juvenile period in the control of puberty, whereas feeding management afterwards was less important. Consequently, the heifers were pubertal more than 2 mo before the first AI, except for one PI–LOW heifer who was pubertal only 1 mo before. Hence, one of the main objectives of heifer replacement programs was achieved, to reach puberty 30–45 d before the breeding season (Gasser, 2013), because fertility increases with the number of estrous cycles preceding breeding (Byerley *et al.*, 1987).

Taking into account that the heifers reach the puberty at fixed proportion of mature BW, and both breeds have similar mature BW, our purpose was to increase the ADG during the postweaning period to advance the puberty in later maturing heifers. However, the age at puberty was not influenced by the interaction between BREED and FEED, in accordance with Ferrell (1982) who reported that even though breed and feeding management influenced age at which puberty was reached, these factors did not interact.

As shown in Table 4, the BW at the first AI did not differ between breeds, despite PI heifers were 35 kg lighter, most likely because of the low BW reached by PI–LOW heifers (389 ± 51 kg). As expected, the BW at the first AI was affected by FEED ($P < 0.05$), heifers from the HIGH feeding treatment being 53 kg heavier. Despite this difference, the BW in all experimental groups was greater than 65% of the expected mature BW (381 kg), which complied with previous recommendations (Gasser, 2013).

No differences were found in the age at the first AI, because all heifers were inseminated at the same time. The fertility rate at the first AI was similar between experimental groups, in contrast with the findings by Roberts *et al.* (2009b), who described a tendency to be reduced in restricted heifers. The age at conception was similar between experimental groups because a similar number of AI services was needed for pregnancy (1.7 ± 0.7). This result contradicts the findings by Brickell *et al.* (2009a), who reported that the fastest growing heifers required more services per conception. The fertility rate at the end of the breeding season was similar among treatments, as described by Roberts *et al.* (2009b) between restricted or fed to appetite heifers during the postweaning phase.

6.3.3 Metabolic profiles

In order to characterize the nutritional status of the heifers, the glucose, cholesterol, NEFA, ß-hydroxybutyrate and urea concentrations were considered because they are indicators generally associated with ruminant nutrient intake and metabolism. The estimated energy and protein intake depending on the feeding management are presented in Fig. 2. The profiles of the different metabolites are shown in the Fig. 3. The effects of BREED and FEED were considered separately, due to the lack of interaction between both effects.

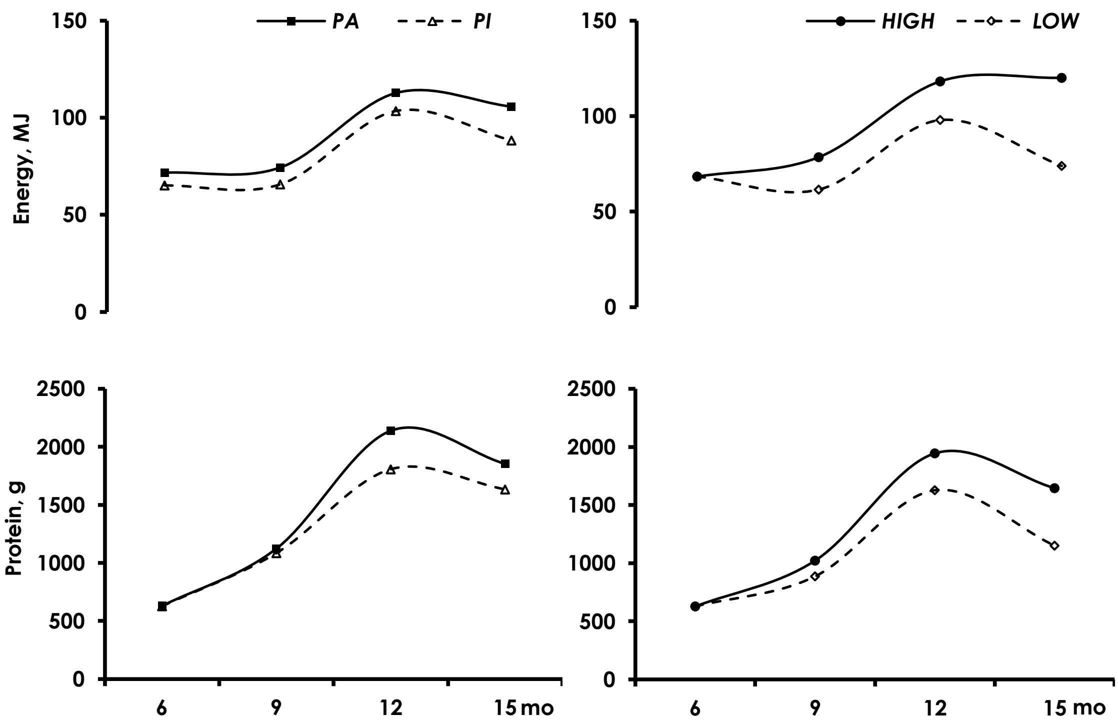

Figure 2. Estimated energy and protein intake by heifers during the experiment according to the breed and feeding management applied in the postweaning period (6–15 mo). PA = Parda de Montaña; PI = Pirenaica; HIGH = 0.8 kg/d target ADG; LOW = 0.6 kg/d target ADG.

The plasma glucose profile was affected by sampling date (*P* < 0.001). The highest levels of glucose (6.25 ± 0.53 mmol/L) occurred in the sample taken at 6 mo, before the postweaning diets were applied, when the estimated energy intake per kg BW was greater, and then values were lower in the rest of the samples (4.51 ± 0.33 mmol/L). The concentrations of glucose in this study (4.94 ± 0.80 mmol/L) were close to those described by Rodríguez-Sánchez *et al.* (2015) under similar dietary treatments, but greater than the observed in other studies on dairy (Brickell *et al.*, 2009b) and beef heifers (Cappellozza *et al.*, 2014), most likely due to their lower energy intake.

Glucose concentrations did not differ between breeds (*P* > 0.10), but mean values were greater in the heifers from the HIGH feeding management (5.04 vs. 4.85 mmol/L, in HIGH and LOW, respectively, *P* < 0.05). This difference was significant only at 15 mo, owing to the larger differences in energy intake at this point (Fig. 2). Besides, the greater concentrate intake by HIGH heifers resulted in a greater ruminal production of propionate (Casasús *et al.*, 2015), which once absorbed is the main source for glucose synthesis at the liver (Schoonmaker *et al.*, 2003).

As regards to the concentrations of cholesterol, no BREED effect was observed but there was an interaction between sampling date and FEED (*P* < 0.001). At the start of the study at 6 mo the cholesterol concentrations were similar, but thereafter they were greater in the HIGH feeding management. Moreover, cholesterol and glucose were strongly correlated (r = 0.68, *P* < 0.001), both being indicators of a positive energy balance. The nadir of cholesterol concentration was found at 12 mo (2.89 ± 0.44 mmol/L), when most of the heifers had started cycling, because cholesterol uptake from plasma is increased when ovaries use it for the synthesis of steroid hormones such as progesterone (Yart *et al.*, 2014).

The number of services per conception was negatively correlated with the cholesterol concentration at 9 (r = –0.41, *P* = 0.05) and 12 mo (r = –0.48, *P* < 0.05), confirming the findings by Westwood *et al.* (2002), who reported a positive association between cholesterol and reproductive performance in dairy cows.

The plasma NEFA concentrations were significantly affected by the sampling date (*P* < 0.001). The highest NEFA concentrations were observed at 6 mo, probably because weaning is stressful and increases the level of catecholamines, which stimulate the lipolysis (Blanco *et al.*, 2012). Thereafter, they were significantly affected by breed, with greater values in PI heifers throughout the postweaning phase (0.112 vs. 0.154 in PA and PI heifers, respectively, *P* < 0.05). This greater basal NEFA concentrations of PI heifers could reflect the greater reactivity of young animals of this breed to handling practices (Blanco *et al.*, 2009b).

No differences were observed in NEFA levels between FEED treatments (0.133 ± 0.057 mmol/L), maybe because the energy provided by the two diets was not different enough, provided that NEFA concentrations are inversely related to the energetic status (Chelikani *et al.* (2009). However, at the individual level, at 9 mo they were negatively related to the concentrations of glucose (r = –0.43, *P* < 0.05), as Blanco *et al.* (2011) described, and also to ADG weaning-to-puberty (r = –0.49, *P* < 0.05), as Rodríguez-Sánchez *et al.* (2015) reported.

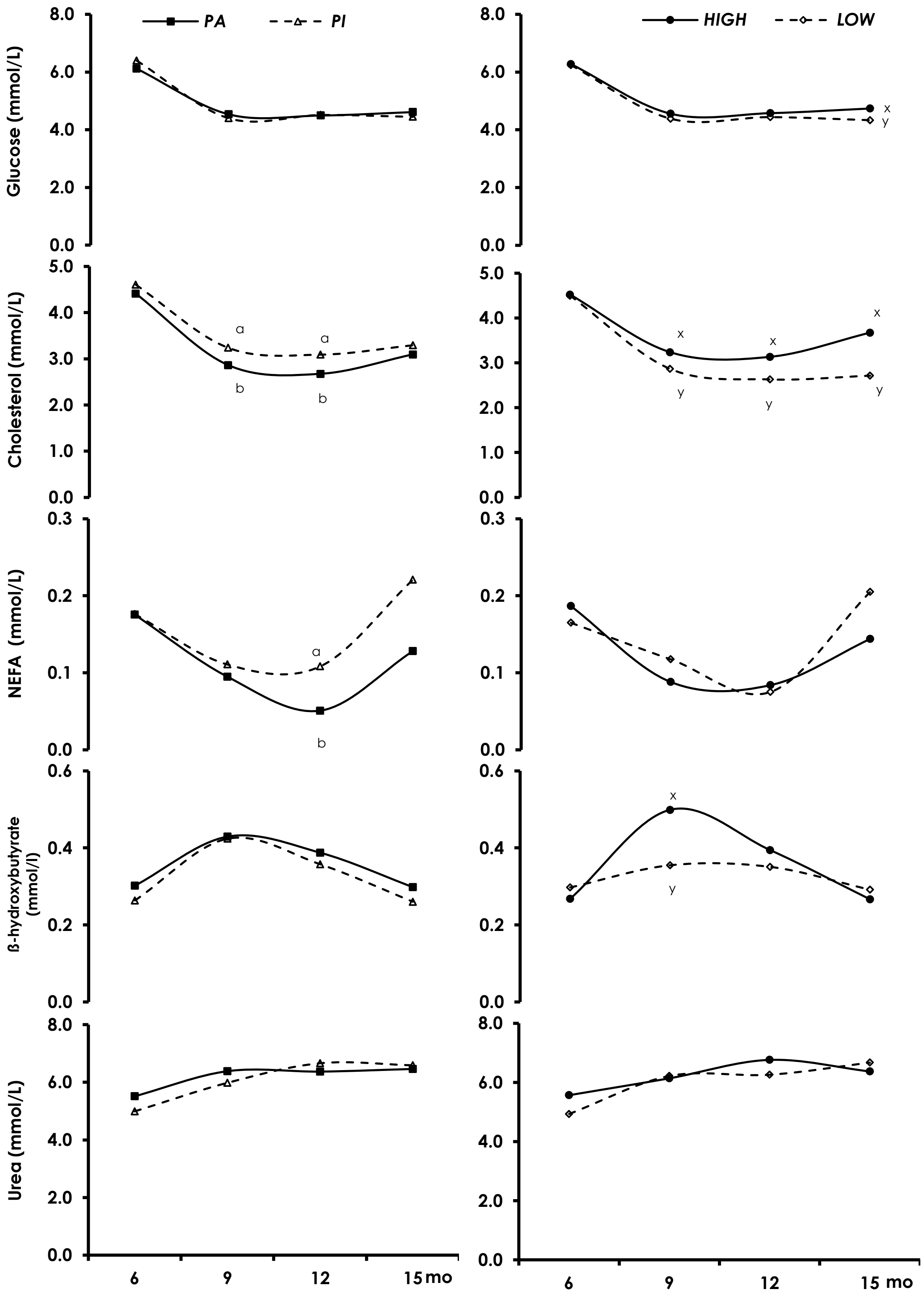

Figure 3. Plasma concentrations of glucose, cholesterol, NEFA, β-hydroxybutyrate and urea in beef heifers according to the breed and feeding management applied in the postweaning period (6–15 mo). PA = Parda de Montaña; PI = Pirenaica; HIGH = 0.8 kg/d target ADG; LOW = 0.6 kg/d target ADG; [a,b]LSMeans at a given age with different superscripts differ significantly among breeds ($P < 0.05$); [x,y]LSMeans at a given age with different superscripts differ significantly among feeding managements ($P < 0.05$).

Regarding the concentration of ß-hydroxybutyrate, the influence of FEED depended on sampling date (P < 0.001), with greater values in the HIGH feeding treatment only at 9 mo of age (0.499 vs. 0.355 mmol/L in HIGH and LOW treatments, respectively; *P* < 0.001). Plasmatic levels of ß-hydroxybutyrate depend on the energy balance and the ruminal fermentation pattern, since both internal lipolysis and absorption of butyric acid from the ruminal wall (Kristensen *et al.*, 2007) are its major sources. In our study, the greater concentrate intake induced greater ruminal production of butyric and propionic acid in the HIGH feeding treatment (Casasús *et al.*, 2015), which was reflected in greater ß-hydroxybutyrate and glucose concentrations, and in their positive relationship at 9 mo (r = 0.44, *P* < 0.05). At the same time, the concentrations of ß-hydroxybutyrate and NEFA were negatively correlated (r = –0.51, *P* < 0.01). This relationship could be explained because, although both metabolites indicate short-term negative energy balance and adipose tissue catabolism, ß-hydroxybutyrate can come from dietary sources (Agenäs *et al.*, 2006).

The plasma urea concentration during the trial was affected by sampling date (*P* < 0.001). The lowest level was found at weaning at 6 mo (*P* < 0.05), and thereafter it increased in the same way as the estimated protein intake, both being positively correlated (Kelly *et al.*, 2010). The urea concentration at 6 mo was positively related with cholesterol concentration (r = 0.47, *P* < 0.05) and negatively with age at puberty (r = –0.58, *P* < 0.01). Since puberty is hastened in heifers with better nutritional status at weaning (Roberts *et al.*, 2009b), the levels of cholesterol and urea registered at 6 mo could be helpful indicators to estimate heifer precocity.

6.3.4 Endocrine profiles

The profiles of plasma IGF-I and leptin during the experiment are presented in Fig. 4. During the trial, the concentration of both hormones was affected by the sampling date (*P* < 0.001), but not by BREED, FEED or their interaction (*P* > 0.10).

The greatest level of IGF-I was observed at the start of the study at 6 mo, due to the high pre-weaning weight gains. Thereafter, the level of IGF-I remained steady throughout the rest of the experiment and did not differ between breeds but for the 15 mo sample. This lack of differences has been reported in previous works for suckled cows (Álvarez-Rodríguez *et al.*, 2010c) and growing bulls (Blanco *et al.*, 2009c) of both breeds, which is associated to a similar growth potential. The concentrations of IGF-I were significantly greater in HIGH heifers only in the last sample (15 mo), which was associated to the relatively small difference between gains in both treatments. Despite the lack of significant differences found in the level of IGF-I during the experiment, the heifers with greater concentrations of IGF-I during the experiment were more precocious (r = 0.43, *P* < 0.001). This fact confirms that IGF-I is an important metabolic mediator involved in the onset of puberty in heifers (Velazquez *et al.*, 2008). The positive relationships found between IGF-I and glucose (r = 0.46; $P < 0.001$) and cholesterol (r = 0.47, $P < 0.001$) during the trial reflect that the heifers in a positive energy balance present greater IGF-I levels and larger, and with more progesterone, *corpora lutea* (Velazquez *et al.*, 2008). Although it is not the case of this trial, this fact could affect the fertility of the heifers in negative energy balance.

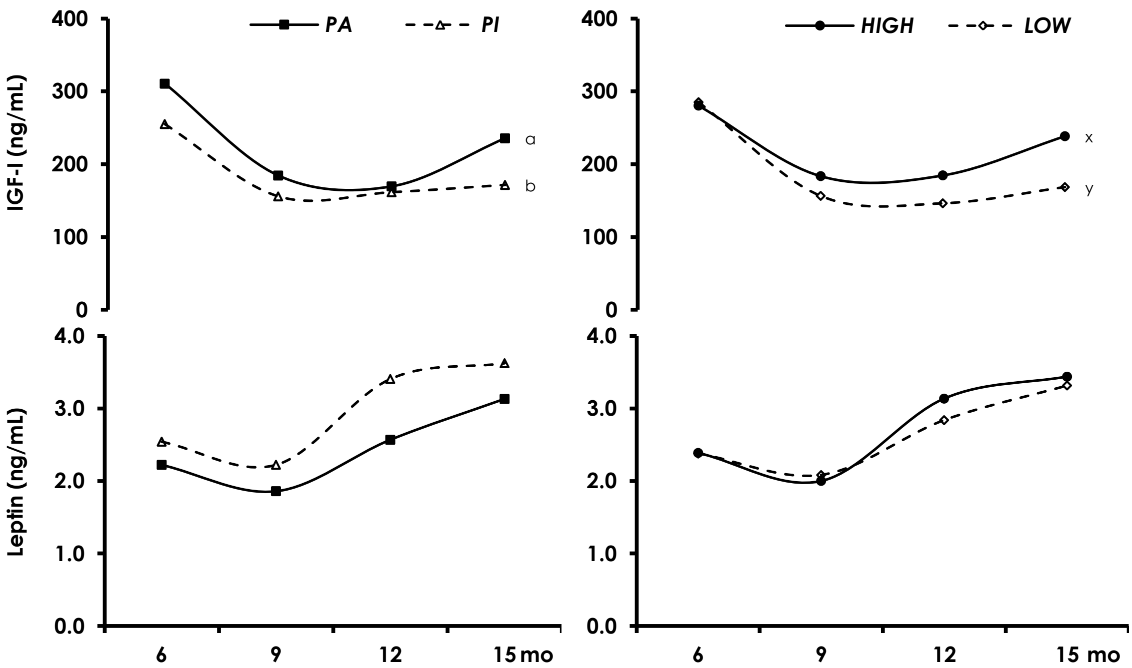

Figure 4. Plasma concentrations of IGF-I and leptin in beef heifers according to the breed and feeding management applied in the postweaning period (6–15 mo). PA = Parda de Montaña; PI = Pirenaica; HIGH = 0.8 kg/d target ADG; LOW = 0.6 kg/d target ADG; ^{a,b}LSMeans at a given age with different superscripts differ significantly among breeds ($P < 0.05$); ^{x,y}LSMeans at a given age with different superscripts differ significantly among feeding managements ($P <$ 0. 05).

The circulating leptin increased through the postweaning phase, which was associated to fat deposition, because leptin is a key metabolic signal synthesized by fat cells in white adipose tissue that communicates information about body energy reserves and nutritional status (Hausman *et al.*, 2012). There were no differences between breeds, which agrees with the fact that BCS and subcutaneous fat thickness were alike. Leptin was not influenced by FEED, probably because the dietary restriction applied in the LOW treatment was not sufficient to reduce leptin synthesis.

The plasma leptin concentration during the trial was not related with age at puberty, despite it has been described by Hausman *et al.* (2012) as a permissive signal for the onset of puberty. The peripubertal rise in plasma leptin reported by Garcia *et al.* (2002) most likely reflects fat deposition but is not a mandatory condition for attainment of puberty, which can occur over a wide range of plasma leptin concentrations (Block *et al.*, 2003).

In conclusion, regardless of the different precocity, our results confirmed the feasibility of advancing the first service to 15 mo in both breeds, even with moderate gains (0.6 kg/d) in the postweaning phase, if preweaning gains are enhanced by creep feeding.

7. Manuscrito 4

Rodríguez-Sánchez JA, Casasús I, Ferrer J, Sanz A. **Postweaning feeding management of beef heifers to be bred at 15 months: II. Gestation and first calving performance in two genotypes.**

Abstract

The objective of this experiment was to study the effects of the postweaning (6–15 mo) **feeding managements (FEED:** 0.8 [**HIGH**] vs. 0.6 [**LOW**] kg/d target ADG) on the gestation and first calving performance at 2 yr of 2 beef breeds (**BREED**: Parda de Montaña [**PA**] vs. Pirenaica [**PI**]). The heifers had been creep fed with concentrates while nursing their dams. Animal weights were recorded weekly, and size measures were taken at conception, calving and subsequent weaning. In order to determine the metabolic (glucose, cholesterol, NEFA, β-hydroxybutyrate and urea) and endocrine status (IGF-I and leptin), the heifers were bled every 3 mo during the gestation, and monthly throughout the lactation. Likewise, in the lactation period, they were bled twice weekly to determine the length of postpartum anestrus (**PPA**). During the experiment, the BREED had more influence than the FEED or their interaction on the parameters studied. The average BW at conception (452 ± 59 kg) and at calving (471 ± 51 kg) were above common recommendations for beef heifers (65% and 80%, respectively) for all the treatments ($P > 0.10$). The BCS at conception (4.1 ± 0.6) and at calving (2.8 ± 0.3) did not differ among treatments. During the lactation the PA primiparous cows lost more BCS than PI ones, BCS at weaning being affected by BREED (2.6 vs. 2.8, respectively, $P < 0.01$). At conception the pelvic area was larger in PA heifers than in PI ones (21.7 vs. 19.3 dm^2, respectively, $P < 0.05$), but this difference was offset during the pregnancy. The PA calves were heavier at birth than PI ones (38.0 vs. 33.0 kg, respectively, $P < 0.01$), resulting in a higher calf/cow BW ratio (8.1% vs. 7.1, respectively, $P < 0.05$), which could explain the greater incidence of calving assistance registered in the PA cows (58% vs. 10%, respectively, $P < 0.05$). No differences were found among treatments in calf ADG (0.669 ± 0.104 kg) during the lactation or BW of calves at weaning (116.1 ± 12.9 kg). The length of the PPA was similar for all treatments (64 ± 37 d), although the PI cows were cyclic 15 d earlier than the PA ones. The BREED and FEED had little effect on the physiological variables analyzed, which depended mostly on sampling date. In conclusion, the threshold weights at conception (15 mo) and at calving (2 yr) were reached, even with postweaning gains of 0.6 kg/d. Advancing the age at first calving to 2 yr was feasible in both breeds without impairing the physiological status and the performance of primiparous cows, if the preweaning gains are enhanced by creep feeding.

Key words: breed, first lactation, prebreeding growth strategy, replacement cattle

7.1 Introduction

Replacing cows is the second most important cost in cow-calf beef production, behind nutrition (Freetly *et al.*, 2014). In order to reduce this cost, the heifers should calve for the first time at 2 yr, minimizing the non-productive period. To achieve this target, the BW gains during the postweaning phase (weaning to breeding) must ensure to achieve 65% (Gasser, 2013) and 80% of mature BW (NRC, 2000) at breeding and at first calving, respectively; likewise, adequate skeletal development should be attained to avoid calving difficulties and not undermine the lifetime productivity of the heifer. Overnutrition during heifer development has been associated with decreased milk production (Buskirk *et al.*, 1996), because it hastens the puberty and reduces the duration of the first allometric phase of development of the mammary gland (Lohakare *et al.*, 2012). This effect has been widely studied in dairy cows (Le Cozler *et al.*, 2008), but it is also of major interest in beef cows, since milk yield is one of the main factors that determine the weaning weight of calves.

As described in the companion manuscript (Rodríguez-Sánchez *et al.*, manuscript 3), Parda de Montaña (**PA**) and Pirenaica (**PI**) are two beef cattle breeds widely spread in the mountain areas of the Spanish Pyrenees. Both breeds reach similar mature BW, but their growth pattern and productive performance differ throughout the production cycle (Casasús *et al.*, 2002). The main hypothesis of the experiment was that feeding management during the postweaning phase should be tailored to each breed to ensure a timely match of their requirements in order to avoid inefficient under or overfeeding, which may have a negative impact in the first calving performance.

The objective of this experiment was to study the effects of the postweaning (6–15 mo) **feeding managements (FEED),** designed to promote different growth rates (0.8 vs. 0.6 kg/d), on the gestation and first calving performance at 2 yr of 2 beef breeds (**BREED**: PA vs. PI). The development, first calving performance and metabolic (glucose, cholesterol, NEFA, β-hydroxybutyrate, and urea) and endocrine status (IGF-I and leptin) were analyzed.

7.2 Materials and methods

The Animal Ethics Committee of the Centro de Investigación y Tecnología Agroalimentaria (CITA) approved the experimental procedures, which were in compliance with the guidelines of the European Union (Directive No. 86/609/CEE, 1986) for the protection of animals used for experimental and other scientific purposes.

7.2.1 Animals, management and diets

The study was conducted at the CITA-La Garcipollera Research Station in the mountain area of the central Pyrenees (northeastern Spain, 42°37' N, 0°30' W, 945 m above sea level, mean annual temperature 10.2 ± 0.2 °C, and mean annual rainfall 1059 ± 68 mm).

Twenty-two replacement heifers, from PA (12) and PI (10) breeds, were born in the autumn season (October 11 ± 10 d). The heifers were distributed in a 2 × 2 factorial arrangement: 2 breeds (PA vs. PI) × 2 feeding managements to promote different growth rates (0.8 kg/d [**HIGH**] and 0.6 kg/d [**LOW**] treatments, respectively) during the postweaning period (from weaning to first breeding at 15 mo), which resulted in four experimental groups: **PA–HIGH, PA–LOW, PI–HIGH** and **PI–LOW**.

The management practices and the results of the heifer performance from weaning to conception, are detailed in the companion manuscript (Rodríguez-Sánchez *et al.*, manuscript 3), therefore, only a brief description is presented in this report. During lactation all heifers suckled their dams twice daily and had free access to starter concentrate, which resulted in preweaning gains above 1 kg/d. The heifers were weaned at 6.4 ± 0.3 mo and were kept indoors in a loose-housing system in straw-bedded pens. In this period, the heifers were pen-fed a diet consisting of ad libitum access to alfalfa hay and received either 10 or 4 g concentrate/kg BW in the HIGH or LOW feeding treatments, resulting in growth rates of 0.814 and 0.624 kg/d, respectively. The heifers were synchronized at 15 mo with an Ovsynch + progesterone releasing intravaginal device (**PRID**) program. They were inseminated randomly from 1 of 3 sires for each breed selected for their calving ease, and repeatedly when they were observed in heat, during a 90-d breeding season. Throughout breeding and thereafter, heifers were managed as a single group. During the breeding season the heifers were fed 9 kg per animal of a dry total mixed ration (56% forages, 44% grains, by-products and vitamin-mineral supplements; 897 g/kg DM, 9.6 MJ ME/kg DM, 103 g CP/kg DM, 595 g NDF/kg DM).

From the confirmation of pregnancy until a month before the expected calving date for each heifer, the animals grazed on mountain meadows, which were composed primarily of grasses *(Festuca arundinacea, Festuca pratensis* and *Dactylis glomerata*), legumes (*Trifolium repens*) and other species at a stocking rate of 4 heifers per hectare. In the last month of gestation, the heifers were housed and fed 9 kg per animal of meadow hay. One month before calving, the heifers were dewormed (Ivomec; Merial, Barcelona, Spain) and were vaccinated against bovine viral diarrhea (Bovilis BVD), infectious bovine rhinotracheitis (Bovilis IBR Marker), *Rotavirus*, *Coronavirus* and *E. coli (Rotavec Corona) (all from MSD Animal Health; Madrid, Spain)*.

During the 4 mo of the lactation, the primiparous cows received 10 kg per animal of the same dry total mixed ration provided along the breeding season. The diet was calculated to meet the requirements for energy and protein of maintenance, growth and milk production of a cow of 490 kg BW and 6 kg daily milk yield. The calves had free access to suckle their dams and received no other feed during the lactation period.

7.2.2 Measurements and blood sampling

The heifers were weighed once a week throughout the 13 mo of the experiment, before morning feeding and without prior deprivation of feed and water. The weight of the heifers at calving and the calves at birth were taken a few hours after calving. The ADG during the gestation and lactation was calculated by linear regression of weight against time. The calves were weighed weekly from birth to weaning at 4 mo of age to determine the ADG during lactation.

The BCS was assessed at conception, calving and weaning by two expert technicians, according to Lowman *et al.* (1976) (0 to 5 scale).

The body development of the heifers was studied using size measurements at conception, calving and weaning. The height at withers (from the highest point of the shoulder blade to the ground), rump length (from the ischial tuberosity to the iliac tuberosity) and rump width (the maximum distance between iliac tuberosities) were recorded with a height stick. The external pelvic area was estimated as the product of the rump length and width, these measures being correlated with the internal ones but easier to take (Murray *et al.*, 2002). The heart girth (the body circumference immediately posterior to the front legs) was measured with a flexible tape.

The calving ease was classified into two categories, i.e., unassisted or assisted. The assisted calving included all types of assistance, from manual pull to a caesarean section as described by Johanson and Berger (2003). The ratio of calf/cow BW was estimated to determine the fetal-maternal disproportion, as the calf birth weight divided by the cow weight at calving expressed as a percentage (Johanson and Berger, 2003).

The heifers were bled every 3 mo during the gestation and monthly during the lactation for determination of both metabolite and hormone profiles. The blood samples were collected before morning feeding from the coccygeal vein. Additionally, the animals were bled twice a week during the lactation to determine the length of the postpartum anestrus (**PPA**), which was based on the plasma progesterone concentration. The samples to determine progesterone, ß-hydroxybutyrate, IGF-I and leptin concentrations were collected into 9 mL heparinized tubes (Vacuette España S.A., Madrid, Spain). The samples to determine plasma glucose, cholesterol, NEFA and urea concentrations were collected into 9 mL tubes that contained EDTA (Vacuette España S.A.). The blood samples were centrifuged at 1,500 g × 20 min at 4 °C immediately after collection, and the plasma was harvested and frozen at −20 °C until analysis.

The heifers were milked monthly during the lactation, using the oxytocin and machine milking technique (Le Du *et al.*, 1979), to determine the quantity and the composition of the milk produced daily. The milk fat and protein contents were analyzed with an infrared scan (Milkoscan 4000™; Fosselectric Ltd., Hillerod, Denmark). The energy-corrected milk (**ECM**) yied (adjusted to 3.5% fat and 3.2% protein) was calculated as Casasús *et al.* (2004) described.

7.2.3 Assays

Plasma progesterone concentrations were measured using an ELISA kit (Ridgeway Science, Lydney, UK), following the manufacturer's instructions. After calving, the onset of normal luteal activity began when the progesterone levels were ≥ 0.5 ng/mL in at least 3 or more consecutive samples. Short estrous cycle (8 to 14 d) were defined by a progesterone increase of ≥ 0.5 ng/mL was detected in 2 or fewer consecutive samples before the second ovulation (Álvarez-Rodríguez *et al.*, 2010b). The length of the PPA was defined as the number of days from calving to the first estrus. The cows that were acyclic at weaning were assigned the length of lactation as the length of PPA; therefore, these data were right-censored.

The plasma concentrations of glucose (glucose oxidase/peroxidase method), cholesterol (enzymatic-colorimetric method), ß-hydroxybutyrate (enzymatic-colorimetric method) and urea (kinetic UV test) were determined with an automatic analyzer (GernonStar, RAL/TRANSASIA, Dabhel, India). The plasma concentrations of NEFA were analyzed with an enzymatic method using a commercial kit (Randox Laboratories Ltd., Crumlin Co., Antrim, UK). Circulating IGF-I concentrations were quantified with a solid-phase enzyme-labeled chemiluminescent immunometric assay (Immulite®; Siemens Medical Solutions Diagnostics Limited, Llanberis, Gwynedd, UK). Plasma leptin concentrations were determined by RIA with a multispecies commercial kit (Multispecies Leptin Ria kit; LINCO Research, St. Charles, MO). Detailed descriptions of protocols, reagents and sensitivity of the analyses are presented in the companion manuscript (Rodríguez-Sánchez *et al.*, manuscript 3).

7.2.4 Statistical analyses

All data were analyzed as a completely randomized design with the SAS statistical software package (SAS Institute Inc., Cary, NC). The heifer was the experimental unit. Data for BW and metabolic (glucose, cholesterol, NEFA, ß-hydroxybutyrate and urea) and endocrine profiles (IGF-I and leptin) were analyzed using the SAS MIXED procedure. The covariance structure was selected on the basis of the lowest Akaike information criterion. Thus, an unstructured covariance matrix (UN) was used for the analysis of repeated measures, which included BREED, FEED during the postweaning phase, sampling date and their interaction as fixed effects and with heifer as the random effect in a univariate linear mixed model.

The ADG, size measurements (height at withers, heart girth, rump width and length and pelvic area at conception, calving and weaning), BCS, ECM yield and milk quality at each key date, and PPA length in the heifers were tested with ANOVA using the GLM procedure. The BREED and the FEED during the postweaning period and the interaction were fixed effects. Similar analyses were performed to analyze calf performance (BW at birth and weaning, ADG) and calf/cow BW ratio where calf sex was considered as a fixed effect. The calf sex, calving assistance and type of first cycle length (short or normal) were analyzed using the FREQ procedure of SAS (χ^2 test). The Pearson correlation coefficients between variables were calculated using the CORR procedure of SAS. Means were separated using the LSMEANS procedure of SAS. For all tests, the level of significance was set at $P < 0.05$ and tendencies were determined if $P \geq 0.05$ and $P < 0.10$.

7.3 Results and discussion

7.3.1 Growth performance

The main effects, BREED and FEED, were analyzed separately because the interaction between them was not significant at any date and for any growth trait assessed.

The BW at keypoints, ADG and BCS during the gestation and the lactation are displayed in the Table 1. The BW at conception was not significantly affected by BREED or FEED, although the PA heifers were 31 kg heavier than the PI ones and the heifers of the HIGH feeding strategy were 43 kg heavier than those from the LOW treatments. Consequently, the PI-LOW heifers were the lightest at conception (412 kg, 71% of their expected mature BW), but even these animals exceeded the minimum maturity recommended in previous works (65% mature BW) to avoid future detriment to the performance of the cow (Gasser, 2013).

During the gestation, the LOW heifers showed a compensatory growth resulting in significantly greater gains than HIGH ones ($P < 0.05$). Because of the compensatory growth, particularly in the PI-LOW heifers, no significant differences in BW were found at calving (471 ± 51 kg). In the current study, the BW at calving was similar to that described by Rodríguez-Sánchez *et al.* (manuscript 2) in PA heifers with the first calving at the same age than herein, but they were lighter than those described by Casasús *et al.* (2002), which calved for the first time at 2.5 yr. Taking into account that both breeds have similar mature BW, 580 kg according to Casasús *et al.* (2002), the weight at calving was 81% of the expected mature BW, which matched the 80% recommended by the NRC (2000) for beef primiparous cows. After that, the primiparous cows maintained the weight during the lactation (0.047 ± 0.169 kg/d), irrespectively of BREED or FEED, because they were fed a diet formulated to meet their requirements.

Table 1. Weights, ADG and BCS of heifers according to the breed (BREED) and the feeding management (FEED) applied in the postweaning period (6–15 mo).

	BREED		FEED		SEM	*P-value*	
Item	**PA[1]**	**PI[1]**	**HIGH[1]**	**LOW[1]**		**BREED**	**FEED**
Weight, kg							
Conception	466	435	472	429	23.3	0.23	0.10
Calving	477	464	480	461	21.9	0.58	0.43
Weaning	479	475	489	465	20.2	0.84	0.28
ADG, kg/d							
Gestation	0.040	0.102	0.030 [x]	0.111 [y]	0.041	0.07	0.02
Lactation	0.017	0.083	0.071	0.030	0.073	0.39	0.59
BCS							
Conception	4.1	4.2	4.3	4.0	0.22	0.76	0.11
Calving	2.7	2.8	2.8	2.7	0.11	0.60	0.56
Weaning	2.6 [b]	2.8 [a]	2.8	2.7	0.06	0.008	0.34

[a,b]LSMeans at a given keypoint with different superscripts differ significantly among breeds (P < 0.05); [x,y]LSMeans at a given keypoint with different superscripts differ significantly among feeding managements (P < 0. 05).

The BCS at conception was not significantly affected by BREED or FEED (4.1 ± 0.6), although the values were greater for heifers of the HIGH feeding strategy than in the LOW ones (P > 0.10). During the pregnancy, a similar loss of BCS was observed in all the treatments, and BCS at calving did not differ (2.8 ± 0.3) among them. Throughout the lactation, PA primiparous cows lost BCS whereas PI ones maintained it, and therefore BCS at weaning was affected by BREED (P < 0.01). This difference might be explained by the lower ability of PA cattle to maintain BCS compared to suckler PI cows (Álvarez-Rodríguez *et al.*, 2010c), both under grazing (Casasús *et al.*, 2002) or confinement conditions (Sanz *et al.*, 2003). This disadvantage presented by PA cows could come from its past as dairy breed, because the beef breeds have lower maintenance energy requirements due to the greater body fat composition (Reynolds and Tyrrell, 2000). This fact also confirms the different pattern of energy distribution in both breeds, whilst the PA cows direct the energy mainly for milk production, the PI ones destine the energy to the accumulation or maintenance of body reserves (Sanz *et al.*, 2003).

The size measurements at conception, calving and weaning are shown in Table 2. The height at withers was not affected by the BREED or the FEED treatments at any keypoint assessed, confirming results of Álvarez-Rodríguez *et al.* (2009a) for mature cows of both breeds, and Rodríguez-Sánchez *et al.* (manuscript 2) for PA heifers under similar feeding treatments in the postweaning phase. In the current study, the height at withers was 92% and 97% at conception and calving, respectively, of those described by Álvarez-Rodríguez *et al.* (2009a) (131 cm), and therefore skeletal development at these keypoints could be considered as adequate for all the experimental treatments.

Table 2. Size measures of heifers (cm) at conception, calving and weaning according to the breed (BREED) and the feeding management (FEED) applied in the postweaning period (6–15 mo).

	BREED		**FEED**		**SEM**	***P-value***	
Item	**PA[1]**	**PI[1]**	**HIGH[1]**	**LOW[1]**		**BREED**	**FEED**
Height at withers							
Conception	121.0	120.7	122.1	119.7	1.96	0.87	0.24
Calving	128.1	127.4	128.6	126.9	1.84	0.73	0.37
Weaning	128.1	128.0	128.1	128.0	1.85	0.97	0.99
Heart girth							
Conception	178.5	173.7	180.4 [x]	171.9 [y]	2.83	0.12	0.009
Calving	177.1	176.1	178.4	174.8	2.72	0.73	0.23
Weaning	177.1	175.8	178.1	174.8	2.56	0.64	0.23
Rump width							
Conception	45.9 [a]	42.4 [b]	45.3	43.0	1.25	0.01	0.08
Calving	49.4	48.1	49.1	48.5	1.44	0.39	0.69
Weaning	50.3	49.5	49.7	50.1	1.22	0.57	0.79
Rump length							
Conception	47.1	45.3	46.9	45.5	0.97	0.08	0.18
Calving	49.6	49.8	49.7	49.7	0.83	0.81	0.92
Weaning	50.3	49.5	50.4	49.4	0.99	0.43	0.35
Pelvic area (dm^2)							
Conception	21.7 [a]	19.3 [b]	21.3	21.3	0.92	0.02	0.09
Calving	24.5	24.0	24.5	24.5	1.05	0.63	0.76
Weaning	25.3	24.6	25.1	25.1	1.03	0.49	0.77

[a,b]LSMeans at a given keypoint with different superscripts differ significantly among breeds (*P* < 0.05); [x,y]LSMeans at a given keypoint with different superscripts differ significantly among feeding managements (*P* < 0. 05). [1]PA = Parda de Montaña; PI = Pirenaica; HIGH = 0.8 kg/d target ADG; LOW = 0.6 kg/d target ADG.

The heart girth was only affected by the FEED at conception (*P* < 0.01), when it was strongly correlated with BW (r = 0.92, *P* < 0.001). Thereafter, under similar diets it was similar among treatments at calving (176.6 ± 6.6 cm) and at weaning (176.5 ± 6.2 cm), and also was correlated with BW (r = 0.84 and r = 0.89, respectively, P < 0.001).

Johanson and Berger (2003) described that the internal dimensions of the pelvis could help to predict the future calving difficulties, these measures being well correlated with the external ones (Murray *et al.*, 2002). The PA heifers had greater pelvic area at conception (*P* < 0.05), because their rump was wider (*P* < 0.05) and tended to be longer (*P* = 0.08) than in PI ones. Throughout the gestation the differences were offset and at calving (24.3 ± 2.4 dm^2) and at weaning (25.0 ± 2.4 dm^2) no influences of the BREED neither the FEED treatments were found, which may reflect a later pelvic development in the PI heifers.

7.3.2 Productive performance

The ratio of calf sexes was similar among treatments (*P* > 0.10). The calf weight at calving and at weaning and ADG during the lactation was alike between sexes (data not shown). No other dam productive or reproductive trait analyzed herein was influenced by the calf sex. As shown in Table 3, the PA calves were 5 kg heavier at birth than PI ones (*P* < 0.01). In both breeds, the calves were 4 kg lighter at birth than those born from multiparous cows from the same herd, but similar to those of 2.5-yr-old primiparous cows (Casasús *et al.*, 2002). The BW of the calves at birth was not affected by FEED (*P* > 0.10) as was described by Rodríguez-Sánchez *et al.* (manuscript 2). Thereafter, calf performance was unaffected by BREED or FEED treatments, with similar gains during the 120 d of lactation (0.669 ± 0.104 kg/d), which resulted in similar weight at weaning (116.1 ± 12.9 kg). These parameters were lower than those reported by Villalba *et al.* (2000) for both breeds, probably due to the low milk yield of primiparous dams. In the current study, the milk yield was unexpectedly low, especially in the PA primiparous cows, which had similar production than the PI ones (5.05 vs. 5.11 kg/d respectively, *P* > 0.10), although it is usually lower in the latter breed (Casasús *et al.*, 2004).

Table 3. Productive and reproductive performance in the first calving of the heifers according to the breed (BREED) and the feeding management (FEED) applied in the postweaning period (6–15 mo).

	BREED		FEED		SEM	*P-value*	
Item	**PA[1]**	**PI[1]**	**HIGH[1]**	**LOW[1]**		**BREED**	**FEED**
Calf							
Birth BW, kg	38.0 [a]	33.0 [b]	35.4	35.6	1.68	0.01	0.90
Weaning BW, kg	119.0	114.5	118.7	113.8	5.28	0.36	0.41
ADG, kg	0.675	0.675	0.694	0.655	0.04	0.99	0.39
Cow							
Age 1st calving, mo	26.0	25.8	25.8	26.1	0.39	0.61	0.39
Calf/Cow BW ratio, %	8.1 [a]	7.1 [b]	7.4	7.1	0.42	0.04	0.42
PPA[2], d	71	56	65	62	15.8	0.39	0.85

[a, b]LSMeans within a row with different superscripts differ significantly (*P* < 0.05). [1]PA = Parda de Montaña; PI = Pirenaica; HIGH = 0.8 kg/d target ADG; LOW = 0.6 kg/d target ADG. [2]PPA: Postpartum anestrus.

As shown in Table 4, the milk yield was affected by the interaction between BREED and FEED (P < 0.001). All the groups, except PI-LOW, had lower milk production than expected. Apart from the fact that primiparous cows produce less milk than mature ones, the reduced milk production could respond to the high rate of gains reached by these lots before puberty, over 1 kg/d, whilst the PI-LOW reached 0.89 kg/d. This fact would confirm that prepubertal gains around 0.8 kg/d can maximize the milk yield, as Zanton and Heinrichs (2005) described in dairy heifers, and that higher prepubertal gains could increase the deposition of mammary adipose tissue or impair the parenchymal development of the mammary gland (Sejrsen *et al.*, 2000).

Table 4. Milking performance in the first calving of the heifers according to the breed (BREED) and feeding management (FEED) applied in the postweaning period (6–15 mo).

	BREED				SEM	***P-value***		
	PA[1]		**PI[1]**					
	FEED					**BREED**	**FEED**	**BREED × FEED**
Item	**HIGH[1]**	**LOW[1]**	**HIGH**	**LOW**				
Yield, kg/d	5.51 [a]	4.60 [b]	4.36 [b]	5.86 [a]	0.58	0.84	0.33	<0.001
ECM[2], kg/d	5.22 [ab]	4.20 [c]	4.72 [bc]	5.98 [a]	0.61	0.048	0.71	<0.001
Protein content, %	3.44 [ab]	3.34 [b]	3.62 [a]	3.42 [b]	0.12	0.049	0.02	0.45
Fat content, %	3.56 [b]	3.42 [b]	4.51 [a]	4.11 [a]	0.35	<0.001	0.15	0.50

[a–c]LSMeans within a row with different superscripts differ significantly (*P* < 0.05). [1]PA = Parda de Montaña; PI = Pirenaica; HIGH = 0.8 kg/d target ADG; LOW = 0.6 kg/d target ADG. [2]ECM = Energy corrected milk.

The milk fat content was significantly lower in PA in comparison to PI cows (3.49 vs. 4.31%, respectively, *P* < 0.001) in accordance with previous works using these breeds (Sanz *et al.*, 2003; Casasús *et al.*, 2004). On the opposite, these authors did not find the differences in milk protein content between breeds registered herein (3.39 vs. 3.52% in PA and PI, respectively, *P* < 0.05). This trait was also affected by the FEED (3.53 vs. 3.38% in HIGH and LOW, respectively, *P* < 0.05) in contrast with Rodríguez-Sánchez *et al.* (manuscript 2) who did not find influence of postweaning feeding management on the milk composition.

Usually, the greater milk yield of PA cows is offset when the milk production is standardized, because of the greater milk fat content produced by PI ones, but in this case the standardization increased the difference between breeds until significant difference was reached (4.71 vs. 5.35 kg/d in PA and PI cows respectively, *P* < 0.05).

7.3.3 Reproductive performance

The reproductive traits analyzed were not affected by the interaction between BREED and FEED effects, thus, they were examined separately (Table 3). Despite the earlier puberty in PA heifers and in those of the HIGH feeding treatments shown in the companion study (Rodríguez-Sánchez *et al.*, manuscript 3), as all heifers were bred at the same age and they needed a similar number of services to become pregnant, age at first calving was similar regardless of BREED or FEED (25.9 ± 0.9 mo), 10 mo earlier than usually in Spain (Ministerio de Agricultura Alimentación y Medio Ambiente, 2014).

In the present study, 36% of the primiparous cows were assisted at calving, which was a similar incidence to that described for beef (32%; Rodríguez-Sánchez *et al*., manuscript 2) and dairy heifers (38%; Johanson and Berger, 2003). The assistance consisted at most in the use of a calving jack, no caesarean section was needed. The incidence of calving difficulty was affected by the interaction between BREED and FEED ($P < 0.05$). The PA primiparous cows needed more help at calving than the PI ones, particularly the PA-LOW heifers (83%). This fact might be due to the disproportion between cow and calf BW. The calf/cow BW ratio was higher in PA cows than in PI ones (8.1% vs. 7.1% respectively, $P < 0.05$) because of the greater BW of the PA calves at birth. The disproportion between the calf and cow BW at calving in PA couples was above 7.5%, value reported by Johanson and Berger (2003) as the threshold which could compromise the ease of calving in dairy heifers. Therefore, even though sires had been selected for their ease of calving, special emphasis should be made on this trait in the breeding program of this breed (Villalba and De la Fuente, 2012) to avoid the fetal-maternal disproportion, particularly in primiparous dams.

Regarding the postpartum ovarian activity, 91% of the animals were cyclic at the end of lactation (120 d). All the primiparous cows had a short cycle before recovering the normal ovarian activity, as described by Rodríguez-Sánchez *et al.* (manuscript 2) and Álvarez-Rodríguez *et al.* (2009b) for primiparous and mature cows, respectively. The transient increase in progesterone in these short cycles may occur to prepare the reproductive tract for rebreeding (Werth *et al.*, 1996).

The PPA length was not statistically affected by the FEED ($P > 0.10$) or BREED ($P > 0.10$), but PA heifers were cyclic 15 d later than PI ones. This delay could be caused by the greater incidence of calving difficulty and the greater loss of BCS during lactation registered in the PA cows. Although no significant differences in PPA length were found between heifers assisted or unassisted at calving, the latter group needed 22 d less to resume the ovarian activity (78 vs. 56 d, respectively, $P > 0.10$), in accordance with previous works (Hickson *et al.*, 2012; Boldt *et al.*, 2015). On the other hand, ad libitum suckling, which can delay the resumption of postpartum activity

(Crowe *et al.*, 2014), has been described as more stressful for PA than for PI cattle due to a different nursing behavior and maternal-offspring bond (Álvarez-Rodríguez *et al.* (2010a). The PPA length registered herein was similar to that reported by Sanz *et al.* (2004) and Álvarez-Rodríguez *et al.* (2010b) in primiparous cows 10 mo older at calving, confirming the feasibility of advancing the first calving in these genotypes, without impairing the resumption of postpartum ovarian activity.

7.3.4 Metabolic profiles

In the current study, some metabolites, commonly associated with ruminant energy metabolism, were analyzed in order to characterize the nutritional status of the heifers (Fig. 1). The concentrations were influenced by the sampling date ($P < 0.001$) because of their dependence on the current energy intake.

At conception ($P < 0.01$) and at calving ($P < 0.05$), HIGH lots showed greater level of glucose than LOW ones. Glucose concentrations decreased through gestation to a nadir observed one week before calving (3.49 ± 0.26 mmol/L), which could be caused by the reduced intake capacity in late gestation as foetus size increases (Casasús *et al.*, 2004).

The cholesterol profiles were related with those of glucose during the gestation (r = 0.59, $P < 0.001$), as both are indicators of energy balance. This relationship disappeared during the lactation, where cholesterol was positively associated to the milk yield (r = 0.38, $P < 0.001$), as shown by Ruegg *et al.* (1992). The concentration of cholesterol during the lactation was affected by the FEED (P < 0.01). The highest level of cholesterol was registered in the PI-LOW primiparous cows, reflecting of their higher milk yield. Likewise they had high level of NEFA concentrations at calving, which were correlated with milk yield on the first month of lactation (r = 0.55, $P < 0.01$). This fact could reflect the mobilization of fat body reserves for milk secretion.

The NEFA concentrations were positively correlated with ß-hydroxybutyrate (r = 0.54, P < 0.01) 3 mo before calving. At this sampling date the HIGH heifers had greater concentrations of NEFA (0.45 vs. 0.29 mmol/L, respectively, $P < 0.05$) and ß-hydroxybutyrate, although without significant differences (0.36 vs. 0.30 mmol/L, respectively, $P > 0.10$), than LOW ones. This fact could reflect the compensatory growth reached at this time by the LOW pregnant heifers and the greater mobilization of fat body reserves of the HIGH ones.

The concentrations of ß-hydroxybutyrate were also negatively related with PPA at 2 and 3 mo postcalving (r = −0.47, $P < 0.05$), contradicting the findings by Mulliniks *et al.* (2013), who reported that young beef cows with greater level of ß-hydroxybutyrate resume the estrus later.

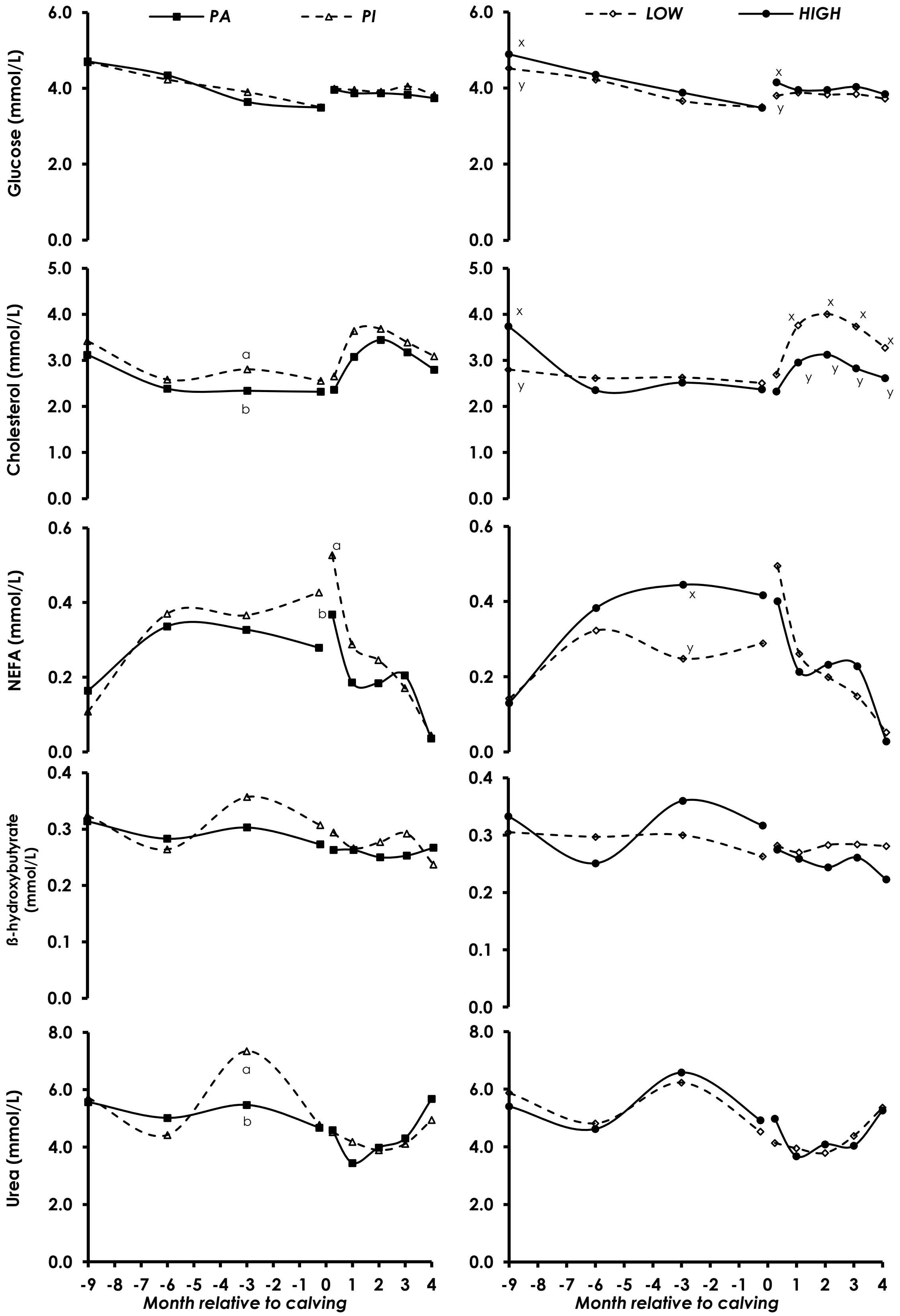

Figure 1. Plasma concentrations of glucose, cholesterol, NEFA, β-hydroxybutyrate and urea during the gestation and the first lactation of beef heifers according to the breed and feeding management applied in the postweaning period (6–15 mo). PA = Parda de Montaña; PI = Pirenaica; HIGH = 0.8 kg/d target ADG; LOW = 0.6 kg/d target ADG; ^{a,b}LSMeans at a given age with different superscripts differ significantly among breeds (*P* < 0.05); ^{x,y}LSMeans at a given age with different superscripts differ significantly among feeding managements (*P* < 0.05).

The plasma urea concentration was affected by the interaction between BREED and sampling date ($P < 0.05$), due to values observed 3 mo prepartum (5.47 vs. 7.34 mmol/L in PA and PI heifers, respectively, $P < 0.001$). No differences were found among treatments at any other date, because this metabolite is correlated with dietary protein intake (Kelly *et al.*, 2010), and the heifers received the same diet throughout this study. During the lactation, plasmatic urea was below 7 mmol/L, level indicated by Butler (1998) as maximum to avoid impair subsequent fertility.

7.3.5 Endocrine profiles

The IGF-I and the leptin profiles during the gestation and the lactation periods are displayed in Fig. 2. No effects of BREED or FEED were found in the concentration of IGF-I because, as Lents *et al.* (2005) related, the current nutrient intake has a greater influence than body energy reserves, and thus it was only affected by sampling date ($P < 0.001$). The concentrations of IGF-I showed a decreasing tendency throughout the gestation (from 190 to 75 ng/mL, at conception and at the last month of gestation). The nadir of IGF-I concentration was reached at calving because of the negative energy balance caused by the stress of the calving (Kessel *et al.*, 2008), and increased thereafter, as described in other works (Roberts, 2008; Maillo *et al.*, 2012). During the first lactation, IGF-I showed a range of values similar to those described by Álvarez-Rodríguez *et al.* (2010b) in older primiparous cows. In the current experiment the IGF-I concentrations were negatively correlated at 1 mo postpartum ($r = -0.44$, $P < 0.05$) with the PPA length, as was previously described by Ciccioli *et al.* (2003) in primiparous and Samadi *et al.* (2013) in multiparous beef cows, confirming IGF-I as a good indicator of reproductive precocity.

In the current experiment, the concentration of circulating leptin was fairly stable (3.61 ± 1.59 ng/mL), without influence of any treatment or the sampling date. This is probably due to the similar nutrient intake during gestation and lactation among treatments, in line with the relationship of leptin with the current intake described by Samadi *et al.* (2013). These authors also described a relationship between leptin and BCS that was not found herein, maybe because higher differences in BCS are needed to affect plasma leptin concentration. No relationship was found between leptin concentrations and the PPA length, as Ciccioli *et al.* (2003) described for beef primiparous cows. Nonetheless, this fact contrasts with the relationship described by Liefers *et al.* (2003) between both traits for dairy cows, most likely because of their greater negative energy balance due to their higher milk yield (Sullivan *et al.*, 2009). Hence, the lack of treatment effects on leptin levels during gestation and lactation in primiparous cows suggest that leptin is not an adequate indicator of nutritional past of heifers, in line with the results of Reis *et al.* (2015) in a metabolic imprinting study.

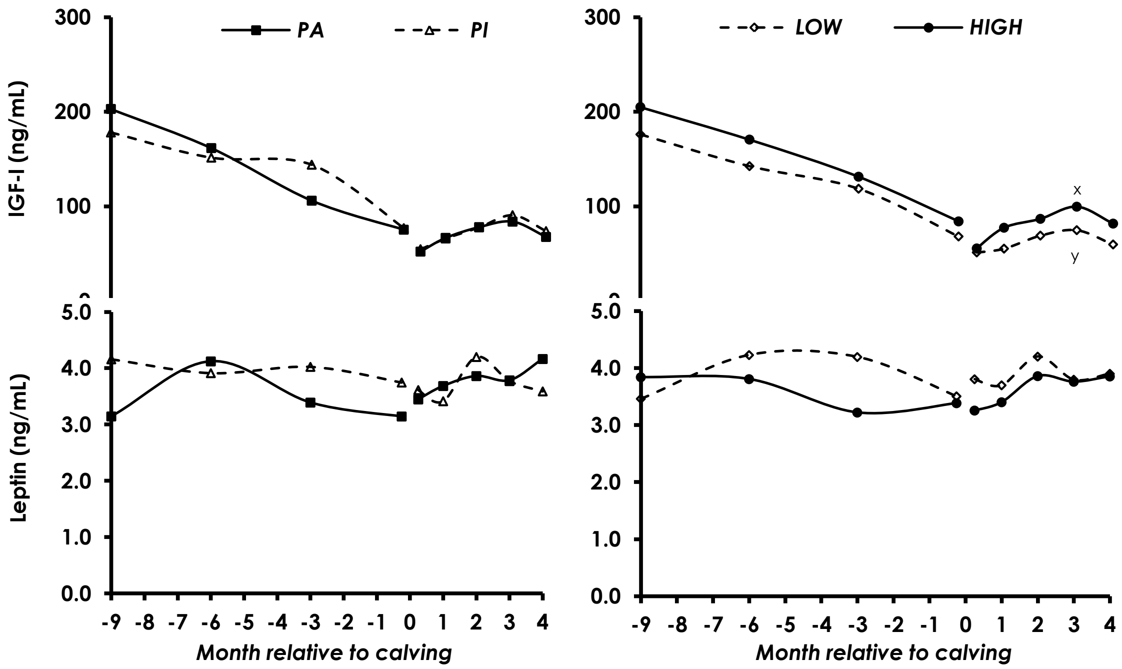

Figure 2. Plasma concentrations of IGF-I and leptin during the gestation and the first lactation of beef heifers according to the breed and feeding management applied in the postweaning period (6–15 mo). PA = Parda de Montaña; PI = Pirenaica; HIGH = 0.8 kg/d target ADG; LOW = 0.6 kg/d target ADG; [x,y]LSMeans at a given age with different superscripts differ significantly among feeding managements ($P < 0.05$).

In conclusion, the threshold weights at conception (15 mo) and at calving (2 yr) were reached, even with postweaning gains of 0.6 kg/d. Advancing the age at first calving to 2 yr was feasible in both breeds without impairing the physiological status and the performance of primiparous cows, if the preweaning gains are enhanced by creep feeding. However, the use of proven calving ease sires should be ensured in replacement heifers. Further investigations are recommended to assure that discontinuous prebreeding nutritional strategies have no effect on the productive life time of beef cows.

8. Consideraciones Finales

Esta Tesis Doctoral se planteó para analizar la posibilidad de adelantar el primer parto de novillas de razas cárnicas españolas a los dos años de edad, con el objeto de reducir el tiempo improductivo en su vida útil. Para ello se sometió a diferentes manejos de la alimentación, desde el nacimiento hasta la primera cubrición con 15 meses, a terneras de las dos razas más representativas del Pirineo aragonés, la Parda de Montaña y la Pirenaica. A su vez, se analizaron las repercusiones de las distintas pautas de crecimiento sobre aspectos productivos, reproductivos y fisiológicos de las novillas hasta el final de su primera lactación.

Los ***ritmos de crecimiento*** impuestos a las terneras durante la lactancia en ambos ensayos estuvieron basados en experiencias previas llevadas a cabo en el mismo rebaño. En el primer ensayo se evaluaron las repercusiones de aumentar el ritmo de crecimiento durante la lactancia por medio de la ingestión de concentrado *ad libitum*. Este manejo hizo que las terneras suplementadas pesaran 64 kg más al destete con 6 meses que las que dispusieron sólo de la leche materna (Anexo 1). En el segundo ensayo, a pesar de que todas las terneras dispusieron de concentrado *ad libitum*, las Pardas fueron 18 kg más pesadas al destete que las Pirenaicas debido a su mayor peso al nacimiento y una mayor, aunque no estadísticamente significativa, ganancia de peso durante la lactancia.

Durante el periodo de recría las novillas permanecieron alojadas en nave y alimentadas a base de heno de alfalfa *ad libitum* y concentrado en distintas proporciones según el objetivo de crecimiento de cada lote. Este manejo no es el comúnmente utilizado por los ganaderos, pero se eligió esta opción para controlar las cantidades ingeridas y así conseguir las ganancias de peso deseadas. En el segundo ensayo se aplicaron distintos objetivos de crecimiento (0,8 vs. 0,6 kg/d) que en el primero (1,0 vs. 0,7 kg/d) para disponer de un mayor abanico de ganancias durante esta fase.

La ***entrada en pubertad*** de una novilla representa su activación sexual, siendo el primer paso necesario para que comience su vida productiva. Según Freetly *et al.* (2011) este estado se alcanza en torno al 55% del peso adulto, independientemente del manejo previo que reciban las terneras y de la raza. En nuestro caso, las terneras llegaron a la pubertad con una media del 55,7% (323 kg) del peso adulto. La pubertad se alcanzó con un peso similar en ambas razas debido a que las dos presentan un peso adulto cercano a los 580 kg (Casasús *et al.*, 2002).

Puesto que el peso a la pubertad se puede considerar fijo, la edad con la que ésta se alcanza dependerá del tiempo que cueste obtener ese peso, es decir, del ritmo de crecimiento desde el nacimiento. Según varios autores (Wiltbank *et al.*, 1966; Gasser *et al.*, 2006; Cardoso *et al.*, 2014), el inicio de la pubertad depende de las ganancias registradas durante la lactancia y no tanto de las obtenidas tras el destete. Este hecho fue corroborado en el segundo ensayo, en el que la edad a la pubertad fue independiente del ritmo de crecimiento impuesto en la recría, pero no en el primer ensayo, en el que la edad a la pubertad dependió del manejo alimentario aplicado tanto durante la lactancia como tras el destete. Esto podría deberse por un lado a que durante la lactancia del primer ensayo no todas las novillas tuvieron altos ritmos de crecimiento, y por otro a una menor diferencia de crecimiento entre lotes con distinto manejo en el segundo ensayo.

La raza Parda de Montaña suele alcanzar el peso crítico que determina la entrada en pubertad a los 12 meses de edad (Cano *et al.*, *en prensa*), por tanto con los manejos aplicados en la presente Tesis la pubertad se ha adelantado en algunos lotes hasta 3-4 meses.

En el segundo ensayo también se comprobó cómo afecta la raza a la entrada en pubertad; a pesar de que las novillas de ambas razas tuvieron el mismo manejo y alcanzaron la pubertad a un peso similar, las Pardas fueron 1,6 meses más precoces que las Pirenaicas. Esto podría deberse al pasado lechero de la raza Parda de Montaña, a su mayor peso al nacimiento y/o a las mayores ganancias conseguidas durante la lactancia, proporcionadas por la mayor producción de leche de sus madres (Sanz *et al.*, 2003).

En la presente Tesis se analizaron las concentraciones plasmáticas de varios ***metabolitos y hormonas***, como indicadores para el control del óptimo desarrollo de la novilla. También se cuantificaron estos parámetros para estudiar las repercusiones de los distintos manejos aplicados sobre su vida productiva. A lo largo de los dos ensayos se observó que la mayoría de los metabolitos y hormonas estudiados tienen una alta dependencia de la ingesta en el momento del muestreo.

En el primer ensayo se encontró una correlación negativa entre la concentración de colesterol en plasma y la edad a la pubertad en las muestras cercanas a la entrada en pubertad de las novillas. El descenso del colesterol en torno a la pubertad pudo deberse a que éste es el principal precursor de la progesterona, cuya producción aumenta cuando los ovarios empiezan a ser funcionales (Yart *et al.*, 2014). Confirmando esta teoría, en el segundo ensayo la mínima concentración de colesterol en sangre se registró cuando las novillas estaban comenzando a ciclar.

En estos ensayos se ha corroborado que las concentraciones de colesterol y glucosa están altamente correlacionadas, por ser los dos indicadores de un balance energético positivo. De los resultados obtenidos se desprende la existencia de un posible umbral en los niveles de colesterol y glucosa, y por tanto de energía ingerida, necesarios para que se inicie la pubertad.

Por otra parte, la entrada en pubertad se ha relacionado con la deposición grasa. Autores como Wiltbank *et al.* (1966) proponían que es necesario superar un mínimo de grasa que asegure suficientes reservas para soportar la transición a la pubertad. Esto explicaría el retraso en la pubertad en novillas de razas más magras o con una deposición de grasa más tardía (Randel y Welsh, 2013), es decir, en nuestro caso explicaría el retraso observado en las novillas Pirenaicas (Piedrafita *et al.*, 2003).

En la presente Tesis se ha analizado la concentración plasmática de leptina, puesto que esta hormona se produce mayoritariamente en el tejido adiposo. Hausman *et al.* (2012) describieron un aumento de la concentración de leptina a medida que se acerca la pubertad, siendo necesario un mínimo para que se produzca la activación del sistema reproductivo. En ninguno de los dos experimentos se observó dicho aumento en torno a la pubertad. Esto podría deberse a que en novillas con crecimientos rápidos la concentración de leptina no sea un desencadenante crítico de la pubertad (Lents *et al.*, 2013) por tener cubierto el mínimo necesario; o puede deberse a que, como defienden Block *et al.* (2003), el aumento peripuberal de la leptina se deba simplemente a un aumento en el tejido adiposo y no a que esta hormona provoque el inicio de la pubertad. Tras la pubertad tampoco se encontraron diferencias en la concentración de leptina entre los distintos manejos, debido probablemente a las escasas diferencias de engrasamiento entre lotes. Por todo lo anterior, y teniendo en cuenta la complejidad de su determinación, la leptina no parece un indicador de interés en estudios de novillas con niveles de engrasamiento similares, especialmente si estos son altos.

El inicio de la pubertad puede estar influido por ***otros factores*** no analizados en la presente Tesis, como es el tipo de alojamiento. El ejercicio genera opiodes endógenos que modulan la secreción de gonadotropinas y que podrían adelantar la pubertad (Cappellozza *et al.*, 2014). En nuestro caso, la recría de las novillas se llevó a cabo en condiciones de confinamiento con espacio reducido, lo que no les permitió hacer mucho ejercicio, por lo que sería interesante estudiar en futuros experimentos la recría en condiciones más extensivas.

También sería interesante estudiar la utilización de progestágenos, especialmente en novillas que, a pesar de superar el peso crítico de entrada en pubertad, no se encuentren cíclicas como sucedió en el primer ensayo. Según describieron Anderson *et al.* (1996), la madurez sexual de una novilla se puede adelantar mediante la administración de progestágenos que suprimen los receptores de estradiol en el hipotálamo, y tras su retirada hacen que se acelere la secreción de LH hasta niveles de la fase folicular. Sin embargo, para que esto suceda es necesario que la aplicación del progestágeno se haga cuando el feedback negativo creado por el estradiol haya comenzado su descenso, es decir, que la novilla esté próxima a la pubertad puesto que dicho descenso comienza aproximadamente 50 días antes de la entrada en pubertad.

Otro factor a estudiar podría ser cómo influye el fotoperiodo en la entrada en pubertad. En la zona donde se desarrollaron los ensayos de la Tesis los partos suelen agruparse en primavera y otoño, concentrando un 28,5% en marzo y abril (Cortés-Lacruz *et al.*, *enviado*). Sin embargo, Schillo *et al.* (1992) describieron que las terneras nacidas en otoño son sexualmente más precoces que las de primavera, debido a la influencia del fotoperiodo, a pesar de que el vacuno no sea una especie estrictamente estacional. En este sentido, Revilla *et al.* (1993) definieron que la raza Pirenaica presenta una "edad crítica" para entrar en pubertad dependiente del fotoperiodo, además de un peso crítico. Esto podría determinar de qué paridera (otoño o primavera) sería más aconsejable dejar la reposición para adelantar la pubertad. Incluso podrían plantearse diferentes programas de recría para las terneras nacidas en una u otra época, puesto que en ovino, especie sexualmente estacional, se ha demostrado que la salida del anestro puede ser modificada por la alimentación recibida (Forcada y Abecia, 2006).

Por último sería interesante analizar cómo influye la interacción social en el inicio de la pubertad, especialmente estudiar el efecto macho y el efecto hembra. En ovino y caprino se ha probado que la presencia de machos sexualmente activos acortan el periodo de anestro estacional (Abecia *et al.*, 2015; Delgadillo *et al.*, 2015). La frecuencia de LH en las hembras aumenta con los machos presentes hasta niveles óptimos para la ovulación. Este efecto también puede ser obtenido mediante la presencia de vacas cíclicas (Berardinelli y Joshi, 2005).

Con respecto a la ***fertilidad a la primera cubrición,*** la edad media a la concepción fue de 16,3 meses, un mes más tarde de lo deseado para adelantar el parto a los dos años. Este retraso se debió a la utilización de un protocolo de sincronización de celos, con inseminación artificial a tiempo fijo, para agrupar las cubriciones, y a que la fertilidad de la inseminación artificial suele ser menor que en la monta natural. Teniendo en cuenta la edad a la pubertad y los pesos a la primera cubrición de las novillas habría sido factible adelantar el protocolo de sincronización para que la concepción fuera más cercana a los 15 meses.

Antes de comenzar la época de cubrición, ya sea ésta mediante inseminación artificial o monta natural, sería necesario controlar algunos parámetros para conseguir una cubrición y posterior vida productiva de la novilla adecuadas.

En primer lugar sería conveniente que la mayoría de las novillas estuvieran púberes de 30 a 45 días antes del inicio de la época de cubrición. Esto aumenta la fertilidad en la primera cubrición (Gasser, 2013) y el número de cubriciones al principio de la época de cubrición. En los ensayos presentados en esta Tesis la mayoría de las novillas habían alcanzado la pubertad al menos dos meses antes de la primera cubrición. Atendiendo a este dato, la pubertad no sería un problema para adelantar el parto a los dos años en estas razas y con los manejos descritos.

Las novillas que llegaron impúberes a la sincronización de celos pertenecían al lote LO–LO del primer ensayo y no estaban púberes aunque superaban el peso crítico de entrada en pubertad. A pesar de esto, dichas novillas quedaron gestantes en la primera inseminación, debido probablemente a que el progestágeno que incluía el protocolo de sincronización hizo que los niveles de LH aumentaran tras su retirada hasta niveles óptimos para la ovulación.

Las novillas deben llegar a la cubrición con un peso mínimo del 60-65% del peso adulto para no perjudicar su vida productiva (Patterson *et al.*, 1992). Todas las novillas utilizadas en este estudio superaron ese límite, independientemente de la raza y del manejo recibido hasta ese momento. Es decir, cualquiera de las combinaciones de manejo alimentario durante la lactancia y la recría, incluso la más restrictiva aplicada al lote LO–LO del primer ensayo, permitiría llegar con un peso adecuado a la primera cubrición (>377 kg). Tampoco es conveniente llegar a este momento con un peso excesivo puesto que las novillas con un mayor ritmo de crecimiento suelen necesitar un mayor número de cubriciones para quedar gestantes (Summers *et al.*, 2014), reduciendo la fertilidad a la primera cubrición. Esto sucedió en el primer ensayo, en el que las novillas con un ritmo de crecimiento mayor durante la recría necesitaron una media de 0,7 inseminaciones más que las novillas con menores ganancias en ese periodo.

Antes del comienzo de la época de cubrición es necesario analizar el ***desarrollo esquelético*** de las novillas puesto que las dificultades al parto dependen en gran medida del desarrollo corporal del animal. Tiene especial interés el estudio del tamaño de la pelvis debido a que la mayor parte de las distocias en novillas son debidas a la desproporción entre el tamaño del ternero y el canal del parto (Hickson *et al.*, 2006). La medida de la pelvis a la cubrición está altamente correlacionada con la del parto (r = 0,71; Johnson *et al.,* 1988), y por tanto, es un buen momento para identificar las novillas que presenten un área pélvica extremadamente reducida que pueda derivar en dificultades al parto. Dependiendo de la estrategia y las circunstancias de cada explotación, estas novillas pueden reservarse para cubrir con mayor edad en posteriores épocas de cubrición o descartarse definitivamente.

En el primer ensayo las novillas de lote LO–LO presentaron el menor desarrollo pélvico a la cubrición y al parto, lo que pudo contribuir a que presentaran la mayor necesidad de asistencia en el parto. En el segundo ensayo se encontraron diferencias en el momento de la cubrición entre los tamaños pélvicos de las novillas Pardas y Pirenaicas, que desaparecieron durante la gestación. Esto puede indicar que las novillas Pirenaicas tienen un desarrollo esquelético más tardío que habría que tener en cuenta a la hora de analizar el desarrollo pélvico.

El ***primer parto de las novillas*** se produjo con una edad media de 25,8 meses, 10 meses antes que la media nacional (Ministerio de Agricultura Alimentación y Medio Ambiente, 2014). Según Hickson *et al.* (2010), la incidencia de dificultades al parto en primíparas con 2 años es mayor que cuando éste se produce a los 3 años, normalmente debido a un insuficiente desarrollo corporal. Para reducir las necesidades de ayuda en el parto las primíparas deben alcanzar un peso cercano al 80% del peso adulto (464 kg en ambas razas). Todos los manejos alimentarios proporcionados a las novillas en la presente Tesis hicieron que se superara esta recomendación, a excepción del manejo LO–LO. En dicho lote, a pesar del crecimiento compensador que registraron las novillas durante la gestación, no fueron capaces de alcanzar el mismo peso que el resto de novillas y parieron con un 75% del peso adulto.

Las dificultades al parto también dependerán del peso del ternero al nacimiento. Para adelantar el parto de las novillas a los dos años es imprescindible hacer una adecuada selección del semental, siendo necesario que éste tenga una facilidad de parto probada o incluso utilizar toros de razas con bajo peso al nacimiento. En este sentido también es importante tener en cuenta la facilidad de parto de la novilla. A la hora de seleccionar la reposición habría que elegir las terneras que provengan de vacas sin problemas al parto, lo que aumentará su capacidad de parir sin ayuda (Teichert, 2016).

El peso del ternero que pueda parir una novilla va a depender de las características descritas anteriormente, es decir, de que ésta tenga un adecuado desarrollo esquelético y un peso óptimo al parto. En el primer ensayo las novillas con más dificultades al parto fueron las del tratamiento LO–LO debido a una alta desproporción materno-fetal. Estas novillas presentaban un peso bajo y un pobre desarrollo pélvico, mientras que el peso del ternero fue similar al del resto de lotes. Sin embargo, en el segundo ensayo las novillas con mayores dificultades al parto fueron las PA–LOW, siendo estas debidas a un mayor peso del ternero al nacimiento.

En cuanto a los ***rendimientos de las novillas en su primera lactación***, el peso de los terneros al nacimiento no se vio afectado por el manejo alimentario que habían tenido sus madres antes de la cubrición en ninguno de los dos ensayos como apuntaban Roberts *et al.* (2009a). En el segundo ensayo, como era de esperar, los terneros de las primíparas Pirenaicas fueron más ligeros que los de las Pardas, reduciéndose así la desproporción materno-fetal y las dificultades al parto en estas vacas.

El peso de los terneros al destete no estuvo influido por los distintos manejos que tuvieron sus madres antes de la cubrición, puesto que sus ganancias de peso fueron similares durante la lactación. Estos ritmos de crecimiento similares estuvieron ligados a unas producciones lecheras también parecidas, dentro de cada ensayo.

La producción de leche en las primíparas del primer ensayo y en las del lote PI–LOW del segundo fue similar. Sin embargo, las primíparas de los otros tres lotes del segundo ensayo presentaron una menor producción lechera. La reducción en la producción de leche de estas novillas podría deberse a que su mayor ritmo de crecimiento previo a la pubertad impidiera un óptimo desarrollo de la ubre, como describieron Sejrsen *et al.* (2000). Este efecto no se detectó en las novillas del lote HI–HI, con crecimientos prepuberales similares, posiblemente porque éstas fueron más pesadas al parto y según Roche *et al.* (2015) las vacas más pesadas producen más leche. Este hecho podría indicar que, al igual que describieron Zanton y Heinrichs (2005) en novillas Holstein, el crecimiento prepuberal adecuado para maximizar la producción lechera en novillas de razas cárnicas podría estar en torno a los 0,8 kg/d.

El último aspecto clave tras el parto de las vacas primíparas es la ***reactivación ovárica*** para volver a quedar gestantes. El parto de las novillas se debería programar para que sucediera antes que el de las vacas adultas. Por un lado para tener un control más exhaustivo sobre estos partos, y por otro porque las primíparas suelen presentar un anestro postparto más largo que las multíparas (Sanz *et al.*, 2004). De este modo las primíparas contarán con más tiempo para recuperarse del parto antes del comienzo de la siguiente época de cubrición.

En el primer ensayo de esta Tesis se encontró un alargamiento del anestro postparto en las novillas que habían tenido unas menores ganancias de peso durante la recría. Sin embargo, esta influencia del manejo durante la recría no se reflejó en el segundo ensayo, debido quizás a que en el segundo ensayo las novillas presentaron una condición corporal al parto algo superior que las novillas del primer ensayo y además tuvieron una menor producción de leche.

Para intentar reducir la duración del anestro postparto en las primíparas se pueden aplicar prácticas como limitar el acceso de los terneros a las madres para reducir la frecuencia de amamantamiento, especialmente en las Pardas (Sanz *et al.*, 2003). Siendo éste uno de los factores que tras el parto más influye en la recuperación de la ciclicidad (Sanz *et al.*, 2004), sería aconsejable que los terneros de las primíparas mamaran sólo una o dos veces al día.

Otra forma de favorecer la aparición del estro en las vacas primíparas podría ser mediante un destete precoz de los terneros. El cese de la producción lechera permitiría utilizar más recursos energéticos para recuperar la ciclicidad (Waterman *et al.*, 2012). Además, debido a la reducida producción lechera de las primíparas sus terneros tal vez no lleguen a expresar su máximo potencial de crecimiento. Por tanto, un destete precoz podría mejorar el rendimiento de estos terneros (Blanco *et al.*, 2009c).

Por último, para que el crecimiento de los terneros no sea tan dependiente de la producción lechera de las primíparas, y favorecer el destete precoz si fuera necesario, éstos pueden ser suplementados con pienso de arranque. Esto aumentaría las ganancias de peso de los terneros destetados a la edad habitual y mejoraría su rendimiento en cebo (Blanco *et al.*, 2008b).

Todas estas propuestas dirigidas a la reducción de la duración del anestro en las vacas primíparas también se deberían aplicar en las secundíparas, puesto que el segundo parto puede ser incluso más crítico que el primero. Tras el primer parto las vacas se incorporan al rebaño de adultas y reciben el mismo manejo que éstas. Aunque se les considere animales adultos, siguen siendo animales en crecimiento (Cano *et al.*, *en prensa*). En el momento del parto tienen que dividir los nutrientes tanto para la producción de leche como para su propio crecimiento. Esto hará que se retrase la reactivación ovárica, aumentando así las posibilidades de quedar vacía y con ello las de ser desechada precozmente por falta de productividad.

Por último, sería de gran interés analizar cómo los distintos manejos aplicados a las novillas en esta Tesis pueden influir en su ***longevidad***. La presente Tesis finaliza con el destete del primer ternero de las novillas, pero para amortizar su coste deben destetar al menos 3 terneros (Perry y Cushman, 2013). La vida productiva de las vacas se debería alargar hasta los 10-12 años, cuando comienza a reducirse su productividad (Genho, 1984). Como indican Roberts *et al.* (2015), mejorando la longevidad en una explotación indirectamente se aumenta su rentabilidad, puesto que la amortización anual de la recría será menor. Además se reducirá la tasa de reposición necesaria para mantener el tamaño del rebaño, aumentando el número de terneras disponibles para la venta. El peso al destete aumentará por tener una mayor proporción de vacas adultas, al igual que el peso de las vacas desviejadas.

El fin último de adelantar el parto de las novillas a los dos años es reducir su tiempo improductivo para aumentar la rentabilidad de la explotación. Sin embargo, es necesario tener en cuenta el ***coste económico*** que dicho adelanto supone. En el Anexo 2 se muestran los costes variables de cada uno de los tratamientos analizados en esta Tesis.

Generalmente, las novillas mantenidas en un plano alto de alimentación antes de la cubrición tienen mayores ganancias de peso, lo que permite llegar antes a los pesos críticos de pubertad, cubrición o parto, pero este manejo incrementa los costes en alimentación. Por tanto, es necesario determinar cuando los costes en alimentación exceden las ganancias derivadas del adelanto del parto.

En la presente Tesis las novillas pertenecientes a los lotes con crecimientos continuamente altos antes de la cubrición (HI–HI, PA–HIGH y PI–HIGH) presentaron, además de unos altos costes en alimentación, algunas repercusiones negativas a nivel productivo y reproductivo. Por el contrario, las novillas del lote LO–LO, con crecimientos previos a la cubrición constantes de en torno a los 0,7 kg/d, presentaron los menores costes en alimentación, pero también varias desventajas respecto al resto de lotes, mayoritariamente debidas a un escaso desarrollo corporal. Por tanto, en las condiciones en que se han desarrollado estos trabajos, para adelantar el parto de las novillas a los dos años parece más aconsejable la recría de las terneras con crecimientos por fases.

Parece necesario asegurar crecimientos cercanos a 1 kg/d al menos durante la lactancia o la recría; la elección del momento dependerá de las circunstancias de cada explotación y de los precios de los insumos necesarios.

Atendiendo a los resultados obtenidos en esta Tesis, la mejor elección sería suplementar a las terneras durante la lactancia, pudiendo tener un crecimiento más limitado durante la recría. Esta opción sería la más adecuada puesto que, a pesar de que el precio del concentrado suministrado durante la lactancia suele ser alto, el tiempo y cantidad de suministro son reducidos. Así durante la recría, que supone un periodo más largo, se podrían conseguir los crecimientos necesarios para alcanzar los pesos críticos y el desarrollo adecuado de la novilla con menos recursos. En este sentido, varios autores describen la posibilidad de reducir los costes de las hembras de reposición durante esta fase restringiendo la alimentación tras el destete (Roberts *et al.*, 2009b; Funston y Larson, 2011). En el primer trabajo la restricción se aplicaba en la cantidad de alimento suministrada a las novillas en cebadero, mientras que en el segundo la restricción venía dada porque las novillas eran manejadas de una manera más extensiva, pastando rastrojo de maíz o pasto de invierno. Al igual que en la presente Tesis, la restricción tras el destete no afectó a los rendimientos de las novillas.

Por otro lado, las novillas recriadas mediante pastoreo aprenden a aprovechar estos recursos (Summers *et al.*, 2014), adaptándose mejor en el futuro al medio en el que van a desarrollar su vida productiva que las recriadas en confinamiento.

Este tipo de manejo, con suplementación durante la lactancia y menores crecimientos durante la recría, podría ser incluso más aconsejable en el caso de las novillas Pirenaicas para compensar su menor peso al nacimiento y menores ganancias en lactancia por su menor producción lechera.

Los resultados obtenidos en esta Tesis confirman la viabilidad de adelantar el primer parto de novillas de raza Parda de Montaña y Pirenaica a los dos años de edad, aunque algunas estrategias de alimentación aplicadas durante la lactancia y la recría influyeron en factores que podrían limitar este adelanto. Será necesario proseguir con el estudio de las repercusiones que puedan tener estas estrategias de alimentación sobre la productividad de las vacas nodrizas a lo largo de su vida útil.

9. Conclusiones

En las condiciones en las que se han realizado los ensayos de la presente Tesis Doctoral, se pueden extraer las siguientes conclusiones generales sobre los efectos del manejo de la alimentación recibida por novillas de razas cárnicas desde el nacimiento hasta la primera cubrición, así como de la raza:

1. Las terneras de ambas razas iniciaron la ***pubertad*** con un peso similar, en torno al 55,7% (323 kg) del peso adulto, independientemente del manejo alimentario previo. La edad a la que se alcanzó este peso dependió del ritmo de crecimiento prepuberal, siendo más precoces las novillas con mayores ganancias de peso, especialmente si éstas fueron durante la lactancia. Esta edad también se vio afectada por la raza, siendo las novillas Pirenaicas 1,6 meses más tardías que las Pardas. Cualquiera de los manejos aplicados en la presente Tesis permitió alcanzar la pubertad con una edad adecuada para adelantar la primera cubrición de las novillas a los 15 meses de edad.

2. La ***fertilidad*** en la primera cubrición de las novillas con crecimientos de en torno a 1 kg/d durante la recría se redujo, necesitando mayor número de inseminaciones para quedar gestantes. Al final de la época de cubrición la proporción de novillas gestantes fue similar en todos los tratamientos de ambos ensayos.

3. La ***dificultad al parto*** aumentó en las novillas con crecimientos bajos desde el nacimiento a la cubrición, debido a un peso al parto menor al recomendado para novillas de razas cárnicas (80% del peso adulto), unido a un escaso desarrollo pélvico que derivó en una alta desproporción materno-fetal. Las vacas primíparas Pirenaicas presentaron menor dificultad al parto que las Pardas, debido al menor peso al nacimiento de sus terneros.

4. El ***peso del ternero*** al nacimiento no estuvo afectado por el manejo alimentario que recibieron sus madres desde el nacimiento hasta la primera cubrición. Sin embargo, se observó un efecto racial en este peso, siendo los terneros de las vacas primíparas Pirenaicas 5 kg más ligeros.

5. La ***producción lechera*** de las vacas primíparas no dependió de la pauta de crecimiento registrada durante la lactancia o la recría, aunque en el segundo ensayo se observó una mayor producción en las vacas de raza Pirenaica con un crecimiento más moderado durante la recría. Esta mayor producción de leche no se reflejó en el crecimiento de los terneros a lo largo de la lactación, posiblemente debido a su corta duración.

6. La duración del ***anestro postparto*** no estuvo afectada en gran medida por el manejo aplicado a las novillas antes de la cubrición, ni tampoco por la raza. En el primer ensayo la reactivación ovárica de las vacas primíparas fue más tardía que en el segundo ensayo, debido probablemente a que presentaron una condición corporal inferior al parto y una mayor producción láctea.

7. Las concentraciones plasmáticas de ***metabolitos*** relacionados con el metabolismo energético dependieron principalmente de la dieta ingerida en el momento del muestreo. Fueron necesarios unos niveles mínimos de glucosa y colesterol, y por tanto una cantidad mínima de energía ingerida, para que se iniciara la pubertad.

8. La concentración plasmática de ***IGF-I*** presentó una alta dependencia de la dieta recibida y fue un buen reflejo del ritmo de crecimiento, así como del estado nutricional y metabólico de las novillas. Esta hormona estuvo positivamente correlacionada con la precocidad reproductiva de las novillas.

9. La concentración de ***leptina*** en plasma no fue diferente entre las novillas de los distintos manejos alimentarios ni entre las razas, por el similar estado de engrasamiento entre éstas. Esta hormona no parece ser un indicador de interés en estudios de novillas de razas cárnicas con niveles de engrasamiento altos, por no ser un desencadenante crítico del inicio de la pubertad.

10. Esta Tesis muestra que es factible el ***adelanto del primer parto*** de novillas de raza Parda de Montaña y Pirenaica a los dos años de edad si se asegura un crecimiento mínimo de 1 kg/d antes o después del destete. Si se consiguen estas ganancias en la lactancia, durante la recría sería suficiente un crecimiento de 0,6 kg/d para no perjudicar los rendimientos de las novillas al primer parto.

10. Anexos

Anexo 1. Síntesis de resultados zootécnicos obtenidos en la presente Tesis Doctoral

	ENSAYO 1				ENSAYO 2			
	LO-LO	**LO-HI**	**HI-LO**	**HI-HI**	**PA-HIGH**	**PA-LOW**	**PI-HIGH**	**PI-LOW**
Edad, meses								
Destete	5,8	5,6	5,7	5,9	6,4	6,4	6,5	6,5
Pubertad	13,5	10,2	11,3	9,2	8,4	9,7	10,3	11,2
1ª cubrición	15,8	15,6	15,7	15,9	15,7	15,7	15,8	15,8
Concepción	15,9	16,7	15,9	16,4	16,5	16,6	16,2	16,5
Parto	25,3	26,3	25,3	25,9	26,0	26,1	25,5	26,2
Peso vivo, kg								
Destete	159	169	224	233	247	248	224	234
Pubertad	331	314	326	329	307	335	334	316
1ª cubrición	388	465	425	513	478	439	457	389
Concepción	382	486	432	530	487	447	458	412
Parto	436	487	474	500	486	468	474	455
Proporción del peso vivo adulto, %								
Pubertad	57,0	54,1	56,2	56,3	52,9	57,8	57,6	54,4
1ª cubrición	66,9	80,1	73,2	88,5	82,4	75,6	78,7	67,1
Concepción	65,9	83,8	74,4	91,4	84,0	77,0	79,0	71,0
Parto	75,2	84,0	81,6	86,2	84,0	81,0	82,0	78,0
Ganancia media diaria, kg/d								
Lactancia (0-6 m)	0,643	0,699	1,046	1,080	1,109	1,071	0,941	1,022
Recría (6-15 m)	0,744	0,998	0,593	0,925	0,798	0,677	0,829	0,571
Prepuberal	0,680	0,863	0,833	1,085	1,127	1,087	1,034	0,886
6 m-pubertad	0,714	0,995	0,518	0,850	1,101	0,893	1,032	0,622
Gestación	0,190	0,004	0,146	-0,105	0,005	0,074	0,055	0,148
Lactación	-0,065	-0,048	0,015	-0,120	0,030	0,003	0,111	0,056
Condición corporal (1-5)								
Cubrición	3,40	4,13	3,75	4,38	4,42	3,79	4,25	4,10
Parto	2,56	2,59	2,60	2,56	2,83	2,65	2,77	2,82
Destete	2,55	2,50	2,60	2,56	2,63	2,63	2,87	2,76
Área pélvica, dm²								
Cubrición	18,2	22,0	20,9	22,3	22,5	20,8	20,1	18,4
Parto	16,5	21,4	17,9	20,6	24,5	24,6	24,4	23,7
Destete	20,7	23,2	22,5	23,4	25,5	25,2	24,8	24,4
Rendimiento reproductivo								
Nº inseminaciones	1,2	2,3	1,3	1,7	1,8	2,0	1,4	1,5
APP, días	112	79	101	84	71	70	59	53
PVT/PVV, %	8,4	8,5	7,3	7,3	8,1	8,2	6,8	7,4
Partos asistidos, %	80,0	37,5	0,0	16,7	33,0	83,0	0,0	20,0
Rendimientos primer parto, kg								
PVT nacimiento	37	41	34	36	39	38	32	34
PVT destete	131	122	125	149	122	113	115	113
GMD ternero	0,779	0,718	0,737	0,910	0,692	0,628	0,691	0,667
ECM	5,7	6,6	6,0	6,6	5,2	4,2	4,7	6,0

LO-LO: GMD de 0,7 kg/d de nacimiento a cubrición. LO-HI: GMD de 0,7 y 1 kg/d en lactancia y recría; HI-LO: GMD de 1 y 0,7 kg/d en lactancia y recría; HI-HI: GMD de 1 kg/d de nacimiento a cubrición; PA: Parda de Montaña; PI: Pirenaica; HIGH: GMD de 0,8 kg/d de destete a cubrición; LOW: GMD de 0,6 kg/d de destete a cubrición; APP: Anestro postparto; PVT: Peso Vivo del Ternero; PVV: Peso Vivo de la Vaca; GMD: Ganancia Media Diaria; ECM: Energy Corrected Milk.

Anexo 2. Estimación del coste económico de los manejos aplicados en la presente Tesis Doctoral

El estudio de la conveniencia económica de los diferentes tratamientos analizados en la presente Tesis se realizó evaluando los costes variables de alimentación y manejo que diferían entre tratamientos, considerando que no había diferencias en los ingresos obtenidos de los terneros producidos en el primer parto.

Los costes considerados para este estudio se determinaron a partir de los precios reales de los insumos utilizados a lo largo de cada uno de los ensayos. A continuación se describen dichos costes:

- El precio del ***pienso*** de arranque suministrado durante la ***lactancia*** de las novillas fue de 0,45 €/kg en ambos ensayos.
- El ***pienso*** de crecimiento suministrado durante la fase de ***recría*** de las novillas tuvo un precio medio de 0,32 €/kg.
- El ***heno de alfalfa*** consumido durante la recría por las novillas del primer ensayo tuvo un precio medio de 0,12 €/kg. El precio de este heno se vio incrementado hasta los 0,15 €/kg a lo largo del segundo ensayo debido a la coyuntura económica del momento, con una reducción de producción por problemas meteorológicos y un aumento de las cotizaciones en los cereales.
- El coste unitario por ***inseminación artificial*** se fijó en 10 €.
- El ***consumo preparto*** fue definido como el sobrecoste en alimentación debido al retraso en el parto de cada novilla, y se calculó con respecto a la fecha del primer parto de cada ensayo (LO-LO y PI-LOW para el primer y segundo ensayo, respectivamente). Se determinó a razón de 9 kg/d de heno por vaca. El precio de este heno fue de 0,14 €/kg en el primer ensayo y 0,15 €/kg en el segundo.

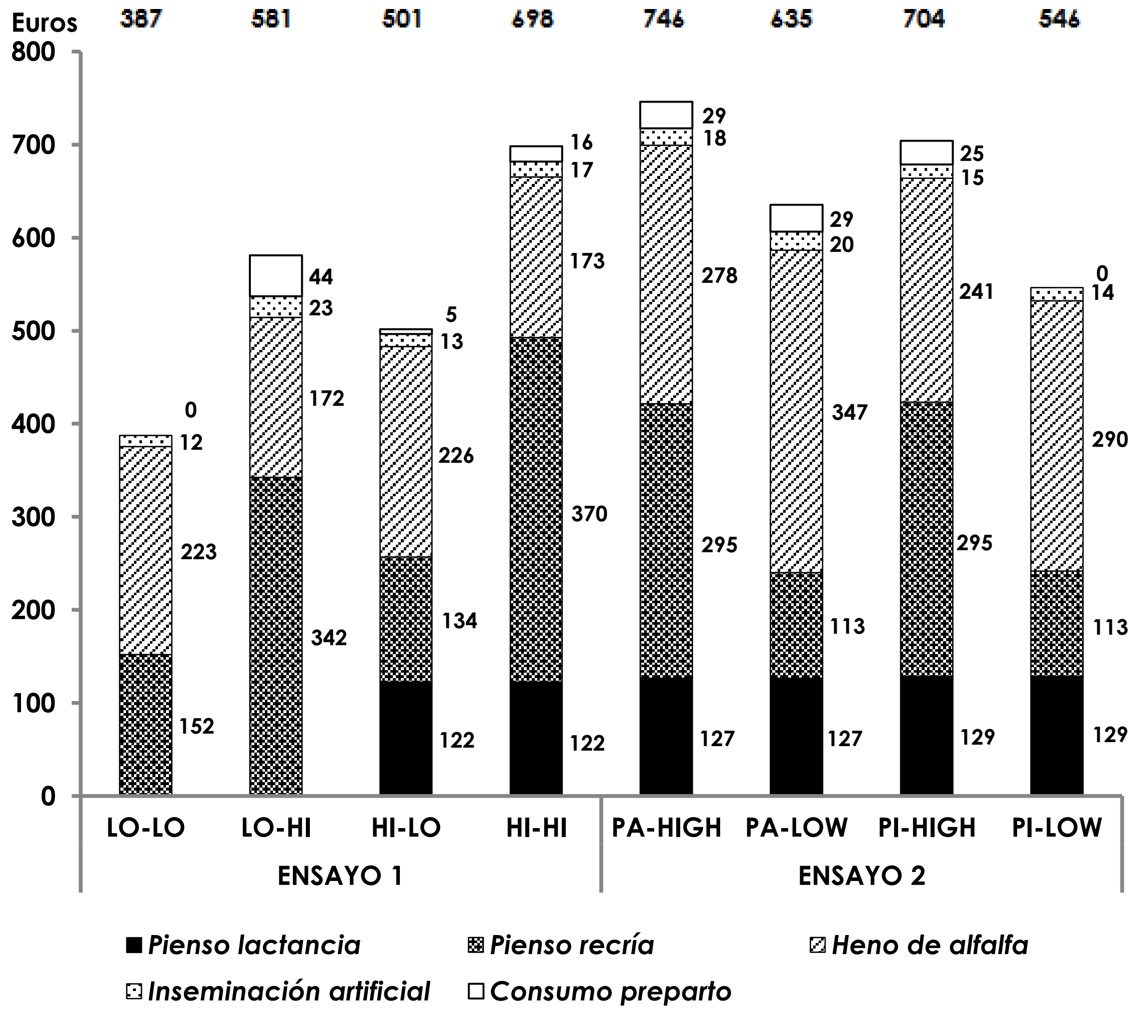

Figura 1. Estimación del coste económico de los manejos aplicados en la presente Tesis Doctoral. LO-LO: Ganancias diarias esperadas de 0,7 kg/d de nacimiento a cubrición. LO-HI: Ganancias diarias esperadas de 0,7 y 1 kg/d en lactancia y recría; HI-LO: Ganancias diarias esperadas de 1 y 0,7 kg/d en lactancia y recría; HI-HI: Ganancias diarias esperadas de 1 kg/d de nacimiento a cubrición; PA: Parda de Montaña; PI: Pirenaica; HIGH: Ganancias diarias esperadas de 0,8 kg/d de destete a cubrición; LOW: Ganancias diarias esperadas de 0,6 kg/d de destete a cubrición. Números en negrita de la parte superior: Coste total estimado del lote.

Anexo 3. Publicaciones a las que ha dado lugar la Tesis

Publicaciones incluidas en el Journal Citation Reports (ISI)

Rodríguez-Sánchez JA, Sanz A, Tamanini C, Casasús I. 2015. Metabolic, endocrine, and reproductive responses of beef heifers submitted to different growth strategies during the lactation and rearing periods. *Journal of Animal Science* 93: 3871-3885.

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Ea, Ea…ya pasó, ya pasó…