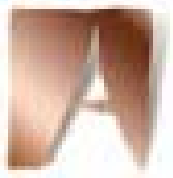

UNIVERSITAT AUTÒNOMA DE BARCELONA

Influence of maternal feed allowance during mid-gestation on progeny muscle fibre development and sow performance over three consecutive cycles

MEMÒRIA PRESENTADA PER ALBA CERISUELO GARCIA

PER ACCEDIR AL GRAU DE DOCTOR DINS DEL PROGRAMA DE DOCTORAT DE PRODUCCIÓ ANIMAL DEL
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certifiquen:

Que la memòria titulada **"*Influence of maternal feed allowance during mid-gestation on progeny muscle fibre development and sow performance over three consecutive cycles*"**, presentada per Alba Cerisuelo Garcia per optar al grau de Doctor en Veterinària, ha estat realitzada sota llur direcció i, considerant-la acabada, autoritzen la seva presentació per que sigui jutjada per la comissió corresponent.

I perquè consti als efectes oportuns, signen el present certificat a Bellaterra a 7 de Maig de 2007,

Dra. María D. Baucells Sánchez Dra. Roser Sala Pallarès

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A tu, mare

A ti Fran,

“*Bedtime reading for people who do not have time to sleep*”…

SUMMARY

The main objective of this PhD dissertation was to study the effects of increasing feeding allowance to pregnant sows from day 45 to day 85 of gestation on muscle fibres development of the progeny *in utero*, postnatal growth performance and carcass and meat quality traits at slaughter. The effects of the application of this feeding strategy over three consecutive cycles on sow body reserves and productive-reproductive performance, were additionally evaluated.

To achieve these objectives, a experiment involving an initial pool of 103 Landrace x Large White PIC sows from 0 to 4 parities was designed and conducted on a commercial sow farm. These sows were divided into two different treatment groups, control (C, n = 49) and supplemented (S, n = 54). Control sows were fed from 2.5 to 3.0 kg/day of a commercial feed (12.1 MJ ME/kg and 0.62 % lysine, on average), according to the feeding strategy routinely followed on the farm throughout gestation. Supplemented sows were provided with an extra feed allowance of + 50 % and + 75 % of the same feed for both gilts and multiparous sows, respectively, from day 45 to day 85 of gestation. The same feeding strategy was applied over three consecutive reproductive cycles. In cycles 2 and 3 a total of 30 and 20 gilts, respectively, were additionally incorporated into the study.

Male (castrated) progeny from cycles 1 and 3 was monitored during the nursery and growing-finishing periods (**Chapter 4**). Barrows were assigned at birth to one of the two treatments (Control, C and Supplemented, S) according to the dietary treatment received by their mothers during mid-pregnancy. At the beginning of the nursery period, the pigs were blocked into 5 weight groups (G) per treatment: G1 included the heaviest pigs and G5 the lightest and this classification was maintained until sacrifice. Growth performance throughout the nursery (n = 958; cycle 1 = 461 and cycle 3 = 497) and growing-finishing periods (n = 636; cycle 1 = 377 and cycle 3 = 259) was evaluated. Carcass and meat quality traits were studied in the second smallest group of weight (G4, n = 90) at slaughter. Muscle fibre characteristics (total number of muscle fibres, mean cross-sectional area and fibre types) were investigated in pigs from G4 using the histochemical myosinATP-ase demonstration (n = 70). Pigs born from supplemented mothers showed higher daily weight gains and feed consumption rates in cycle 1 ($P < 0.05$) and higher feed efficiencies in cycle 3 ($P < 0.05$) during the nursery period. However, no differences were found in growth performance during the growing-finishing period neither in cycle 1 nor in cycle 3. Carcass quality traits at slaughter were not affected either by the increase in maternal feeding level during mid-pregnancy. When studying the meat quality traits, the S pigs meats' consistently showed higher ultimate pHs (pH_{24h}) in the *semimembranosus* muscle and lower lightness (L*) in the cross-section of *longissimus thoracis* muscle compared to the C group of pigs, consistently in cycles 1 and 3 ($P < 0.05$). Differences in muscle fibre characteristics were also found between treatment groups. The pigs born from supplemented mothers showed a

lower number of muscle fibres with higher mean areas in both cycles ($P < 0.15$). This effect accounted for an invariable estimated developmental secondary to primary fibres ratio. In the adult animal, the lower number of muscle fibres in the S group of pigs was reflected in the lower number of type IIB fibres ($P < 0.05$), but with larger sizes ($P < 0.10$, cross-sectional area) compared to C pigs.

Regarding the sows, body weight (BW) and body reserves [backfat thickness (BF, mm); loin depth (LD, mm) and body condition score (BCS)] were measured at different times throughout the reproductive cycle (day 40 of gestation, day 85 of gestation, at farrowing and on day 18 ± 1 of lactation), over the three cycles studied. Pigs were counted and individually weighed at farrowing and on day 18 ± 1 of lactation. Sows rebreeding performance in the form of farrowing rate and weaning to oestrus interval, was also recorded (**Chapter 5**). Overall, both experimental groups of sows gained BW and LD after the three cycles studied. However, in contrast to the sows fed the level routinely used in the farm during gestation (C), S sows were able to accumulate backfat after three consecutive cycles of feed supplementation. Sows which started the study in their first parity (iPG) accumulated both, lean and fat tissue in response to the increased gestation feed allowance over the three consecutive cycles, while multiparous sows (> 2 parities) used this extra energy supply to mainly accrete fat tissue. In productive terms, the feeding strategy applied during mid-gestation did not show any significant effect on multiparous sows but it did enhance primiparous piglet weight at birth in cycle 3 (+300 g/pig in the S group of sows). This difference was maintained, although not statistically significant, at weaning. All the supplemented sows, but more specifically the multiparous sows showed an impaired lactation performance with a higher incidence of mamitis-metritis-agalactia syndrome. No differences were found between treatments regarding the rebreeding performance.

An exploratory analysis was conducted using the sows from cycle 1 (n = 103, **Chapter 6**) in order to determine the relationship between their body reserves at different times within the reproductive cycle and productive performance, under our conditions. This study revealed that sows BW and body reserves (BF and LD) levels at farrowing were positively associated with their respective losses during the lactation period, but also with their levels at weaning. Piglet weight at birth and at weaning was more influenced by sow parity than by sow body reserves at farrowing. In this way, first parity sows showed lower piglet weights at farrowing and at weaning compared to multiparous sows ($P < 0.05$). Sows' BW and BF losses were weak but positively associated with litter growth rates during lactation after the parity effect was removed from the statistical model (BW: r = 0.36, $P < 0.05$; BF: r = 0.27, $P < 0.10$). Additionally, BF losses also showed a low but positive association with the weaning to oestrus interval (WEI, r = 0.25, $P < 0.05$). Weaning to oestrus interval was also affected by sow parity since primiparous sows showed larger WEIs compared to the multiparous ones. Finally, in regard to the accuracy of BCS in predicting sow body reserves measured by ultrasounds, the results of the present experiment indicated that BCS and ultrasonic sow body reserves (BF and LD) were moderately

correlated (r_{BF} = 0.54 and r_{LD} = 0.49, $P < 0.01$). Additionally, the BCS appeared to be more reliable in predicting sow fat reserves in multiparous and extreme conditioned (thin and fat) sows.

To conclude, the increased maternal feeding level during mid-pregnancy in the present experiment did not affect in a consistent way progeny growth performance during the rearing period. However, this feeding regime did lead to histochemical differences in muscle fibre development *in utero* which, at the same time caused improved meat quality traits (less acid and darker meats) at 24 hours post-mortem. Moreover, this feeding strategy applied to well-managed sows led to an increase in body reserves after three cycles of feed supplementation. The greater body reserves in the S group had beneficial effects on the sows that started supplementation early in life (gilts in Cycle 1), while producing detrimental consequences in multiparous sows due to an impairment of mammary gland development. Finally, sow parity and body reserves management during gestation clearly affected body reserves management during lactation and also productive-reproductive performances.

RESUM

L'objectiu principal de la present tesi doctoral ha sigut l'estudi dels efectes d'un increment en el nivell d'alimentació en truges gestants entre els dies 45 i 85 de la gestació, en el desenvolupament de les fibres musculars de la progènie *in utero*, els seu creixement postnatal i diversos paràmetres de qualitat de la canal i de la carn al sacrifici. A més a més, els efectes d'aquesta estratègia d'alimentació aplicada durant tres cicles consecutius sobre el maneig de les reserves corporals i els rendiments productius i reproductius de les mares, varen ser avaluats.

Per tal de portar a terme aquests objectius es va realitzar un únic experiment en una granja comercial, amb una mostra inicial de 103 truges Landrace x Large White de genètica PIC, de entre 0 i 4 parts. Aquestes truges van ser distribuïdes en dos tractaments experimentals, control (C, n = 49) i suplementat (S, n = 54). Les truges control van ser alimentades amb una quantitat de 2.5-3.0 kg/dia d'un pinso comercial (12.1 MJ EM/kg i 0.62 % de lisina de mitja), seguint el patró normal d'alimentació de la granja durant el període de gestació. Les truges suplementades van rebre un suplement de + 50 % i de + 75 % del mateix pinso, en el cas de truges nulípares i multípares respectivament, en el període comprés entre els 45 i 85 dies de gestació. Aquest mateix tractament es va aplicar durant tres cicles consecutius. En els cicles 2 i 3, un total de 30 i 20 truges nulípares van ser introduides a l'estudi.

Els garrins mascles (castrats) provinents dels cicles 1 i 3 van ser controlats durant les fases de transició i engreix fins a l'escorxador (**Chapter 4**). Aquests garrins es van dividir al naixement en dos grups (Control, C i Suplementat, S) segons el tractament rebut per les mares durant la part mitja de la gestació. Al inici del període de transició, els garrins es van distribuir en 5 grups de pes (G) per tractament: G1 incloïa els garrins més pesats i G5 els de menor pes, i aquesta mateixa classificació va ser mantinguda fins al sacrifici. Es van avaluar els rendiments productius d'aquests animals en les fases de transició (n = 958; cicle 1 = 461 i cicle 3 = 497) i engreix (n = 636; cicle 1 = 377 i cicle 3 = 259). Diversos paràmetres de qualitat de la canal i de la carn varen ser estudiats en animals dins del grup de pes 4 (G4, n = 90) al sacrifici. L'estudi de les diferents característiques de les fibres musculars de la progènie (nombre total de fibres musculars, àrea mitja i tipus de fibres) es va realitzar també en garrins del G4, utilitzant la reacció histoquímica miosina-ATP-asa amb preincubacions a diferents pH (n = 70). Els garrins nascuts de mares suplementades presentaren majors guanys de pes diaris i consums mitjos en el cicle 1 ($P < 0.05$) i una superior eficiència de conversió en el cicle 3 ($P < 0.05$) durant el període de transició. Malgrat aquests resultats, no es varen trobar diferències en els rendiments productius entre tractaments en la fase d'engreix en cap dels dos cicles estudiats (Cicle 1 i Cicle 3). El increment del nivell d'alimentació durant la part mitja de la gestació no va tenir conseqüències en els paràmetres de qualitat de la canal al sacrifici, però sí va provocar diferències en alguns aspectes de la qualitat final de la canal. Al respecte, les carns dels porcs S van presentar un pH més elevat a les 24 hores després del sacrifici (pH_{24}) en el múscul

semimembranosus i un nivell més baix de lluminositat (L*) mesurada en una secció transversal del múscul *longissimus thoracis,* en relació amb els porcs del grup C i de forma consistent en els cicles 1 i 3 ($P < 0.05$). El tractament experimental també va provocar diferències en característiques histològiques de les fibres musculars. Els garrins nascuts de mares suplementades van desenvolupar un menor nombre de fibres musculars però de major àrea mitja en ambdós cicles estudiats ($P < 0.15$). La relació entre el nombre de fibres secundàries i el nombre de fibres primàries es va mantenir constant entre tractaments. En l'animal adult, el menor nombre de fibres musculars presentat pel grup de porcs S es va veure reflexat en un menor nombre de fibres tipus IIB ($P < 0.05$) de major àrea ($P < 0.10$, àrea transversal) en relació als porcs nascuts de mares C.

Pel que fa a les truges, el pes viu (BW) i els nivells de reserves corporals [Gruix de greix dorsal (BF, mm); profunditat de llom (LD, mm) i nota de condició corporal (BCS)] van ser mesurats en diferents moments del cicle reproductiu (40 dies de gestació, 85 dies de gestació, al part i als 18 ± 1 dies de lactació) en els tres cicles estudiats. Al moment del part i als 18 ± 1 dies de lactació s'enregistraren tant el nombre de garrins com el seu pes individual. El rendiment reproductiu de la truja després del deslletament es va determinar mitjançant paràmetres com l'índex de parts i el interval deslletament-cobrició (**Chapter 5**). En conjunt, tant les truges alimentades segons el nivell rutinari en granja (C) com les truges que van ser suplementades durant la meitat de la gestació guanyaren BW i LD al final dels tres cicles estudiats. D'altra banda, només les truges S van ser capaces d'acumular BF després de tres cicles consecutius de suplementació. Les truges que començaren l'experiment com a truges nulípares (iPG1) van ser capaces d'acumular teixit magre però també teixit lipídic, en resposta a l'augment del nivell d'alimentació en gestació durant tres cicles consecutius, mentre que les truges multípares (> 2 parts) dedicaren aquesta aportació extra d'energia, majoritàriament, al dipòsit de teixit gras. En termes productius, aquesta estratègia d'alimentació no va tenir un efecte significatiu en els rendiments de les truges multípares, encara que sí va resultar en un increment en el pes mig al naixement dels garrins nascuts de truges primípares (iPG1) en el cicle 3 (+300 g/garrí en el grup de truges S). Aquesta diferència es va mantenir, encara que no estadísticament significativa, al deslletament. Les truges suplementades, més específicament les truges multípares, demostraren uns menors rendiments durant el període de lactació conjuntament amb un increment de la incidència de la síndrome mamitis-metritis-agalaxia. No es van trobar diferències en el rendiment reproductiu post-deslletament entre tractaments experimentals.

Utilitzant el conjunt de dades de les truges del cicle 1 (n = 103, **Chapter 6**), es va realitzar un estudi exploratori amb la finalitat de determinar la relació entre els nivells i la gestió de les reserves corporals en diferents períodes del cicle reproductiu i els rendiments (re)productius, sota les nostres condicions. En aquest estudi es va demostrar que el pes viu de les truges i els nivells de reserves corporals (BF i LD) al part estaven positivament associats amb les seves respectives pèrdues durant el període de lactació, però també positivament associats amb els

seus nivells de reserves al deslletament. El pes mig dels garrins al naixement i al deslletament va resultar finalment més influenciat per la paritat de la truja que pel seu nivell de reserves corporals al part. Les truges primípares donaren lloc a garrins de menor pes al naixement i al deslletament en comparació amb aquells de les truges multípares ($P < 0.05$). Les pèrdues de BW i BF van estar relacionades dèbil però positivament amb el guany mig de la garrinada durant el període de lactació, un cop l'efecte part havia estat corregit dintre del model estadístic (BW: r = 0.36, $P < 0.05$; BF: r = 0.27, $P < 0.10$). A més a més, també es va trobar una associació dèbil però positiva entre la pèrdua de BF i el interval deslletament-cobrició (WEI, r = 0.25, $P < 0.05$). El interval deslletament-cobrició també es va veure afectat pel part de la truja, de manera que les truges primípares presentaren WEIs més llargs que les truges multípares. Finalment, en relació a l'exactitud de la nota de condició corporal com a variable predictora del nivell de reserves corporals de la truja mesurats mitjançant ultrasons (BF i LD), els resultats del present experiment indicaren l'existència d'una associació moderada entre aquestes variables ($r_{BF} = 0.54$ and $r_{LD} = 0.49$, $P < 0.01$). A més, la fiabilitat de la nota de condició corporal (BCS) en quant a la predicció de les reserves grasses sembla ser superior en truges multípares o en condicions corporals extremes (molt prima i molt grassa).

En conclusió, el increment del nivell d'alimentació aplicat durant la part central de la gestació en el aquest estudi no va demostrar un clar efecte en els rendiments productius de la progènie. D'altra banda, aquest règim alimentari sí va donar lloc a diferències histològiques en el desenvolupament de les fibres musculars *in utero* i a una millora en alguns dels paràmetres de qualitat de la carn estudiats (carns menys àcides i més fosques) a les 24 hores després del sacrifici. Addicionalment, aquesta estratègia nutricional portada a terme en truges correctament alimentades donà lloc a un augment del nivell de reserves corporals després de tres cicles d'aplicació del tractament experimental. Aquest augment de reserves corporals va tenir conseqüències positives en les truges en les que la suplementació es va iniciar aviat en la seva vida reproductiva (nulípares en el cicle 1), mentre que va donar lloc a conseqüències negatives en truges multípares degut a un efecte en el desenvolupament de la glàndula mamària. Finalment, tant el número de part com la gestió de les reserves corporals durant el període de gestació van exercir un clar efecte en el maneig de les reserves corporals durant el període de lactació, a més d'incidir també sobre els rendiments productius i reproductius de les truges.

RESUMEN

El objetivo principal del presente proyecto de tesis doctoral fue el estudio de los efectos del incremento en el nivel de alimentación en cerdas gestantes entre los días 45 y 85 de gestación, en el desarrollo de las fibras musculares de la progenie *in utero*, su crecimiento postnatal y diversos parámetros de calidad de la canal y de la carne al sacrificio. Adicionalmente, los efectos de esta estrategia de alimentación aplicada durante tres ciclos consecutivos en el manejo de las reservas corporales y los rendimientos productivos y reproductivos de las cerdas, fueron evaluados.

Con la finalidad de llevar a cabo estos objetivos se realizó un único experimento en una granja comercial, partiendo de una muestra inicial de 103 cerdas Landrace x Large White de genética PIC de entre 0 y 4 partos. Estas cerdas fueron distribuidas en dos tratamientos experimentales, control (C, n = 49) y suplementado (S, n = 54). Las cerdas control se alimentaron con niveles de 2.5-3.0 kg/día de un pienso comercial (12.1 MJ EM/kg y 0.62 % de lisina de media), siguiendo el patrón normal de alimentación de la granja durante el período de gestación. Les cerdas suplementadas recibieron un extra de + 50 % y + 75 % del mismo pienso, para cerdas nulíparas y multíparas, respectivamente, entre los días 45 y 85 de gestación. Este mismo tratamiento fue aplicado durante tres ciclos consecutivos. En los ciclos 2 y 3, un total de 30 y 20 cerdas nulíparas fueron incorporadas al estudio.

Los lechones macho (castrados) procedentes de los ciclos 1 y 3 fueron controlados durante las fases de transición y engorde hasta el sacrificio (**Chapter 4**). Estos lechones se dividieron al nacimiento en dos grupos (Control, C y Suplementado, S) en relación con el tratamiento recibido por sus madres durante la parte media de la gestación. Al inicio del periodo de transición, los lechones fueron distribuidos en 5 grupos de peso (G) por tratamiento: G1 incluye los lechones más pesados y G5 los de menor peso. Esta misma distribución fue mantenida hasta el sacrificio. Los rendimientos productivos de estos animales fueron evaluados en las fases de transición (n = 958; ciclo 1 = 461 y ciclo 3 = 497) y engorde (n = 636; ciclo 1 = 377 y ciclo 3 = 259). Diversos parámetros de calidad de la canal y de la carne fueron evaluados al sacrificio en animales del grupo de peso 4 (G4, n = 90). El estudio de las diferentes características de las fibras musculares de la progenie (número total de fibras musculares, área media y tipo de fibras) se llevó a cabo utilizando la reacción histoquímica miosinaATP-asa después de preincubar las muestras a diferentes pH (n = 70), también en lechones del G4. Los lechones nacidos de madres suplementadas presentaron mayores crecimientos y consumos medios en el ciclo 1 ($P < 0.05$) y una superior eficiencia de conversión en el ciclo 3 ($P < 0.05$) durante el periodo de transición. Sin embargo, no se encontraron diferencias entre tratamientos en los rendimientos productivos en la fase de cebo en ninguno de los dos ciclos estudiados (ciclo 1 y ciclo 3). El incremento del nivel de alimentación durante la parte media de la gestación no tuvo consecuencias en parámetros de calidad de la canal al sacrificio. Sin embargo, si provocó diferencias en algunos aspectos de calidad final de la carne, de manera

que las carnes de cerdos S presentaron un mayor pH a las 24 horas después del sacrificio (pH_{24}) en el músculo *semimembranosus* y una menor luminosidad (L*) determinada en una sección transversal del músculo *longissimus thoracis*, en comparación con las carnes de cerdos C y de manera consistente en los ciclos 1 y 3 ($P < 0.05$). El tratamiento experimental también provocó diferencias en las características histológicas de las fibras musculares. Los lechones nacidos de madres suplementadas desarrollaron un menor número de fibras musculares pero con un mayor tamaño en los dos ciclos estudiados ($P < 0.15$). La relación entre el número de fibras musculares secundarias y el número de fibras musculares primarias fue constante entre tratamientos. En el animal adulto, este menor número de fibras musculares presentado por el grupo de cerdos S se vio reflejado en un menor número de fibras tipo IIB ($P < 0.05$) de mayor área ($P < 0.10$, área transversal) con respecto a los animales nacidos de madres C.

En relación a las madres, el peso vivo (BW) y los niveles de reservas corporales [Espesor de grasa dorsal (BF, mm); profundidad de lomo (LD, mm) y nota de condición corporal (BCS)] fueron medidos en diferentes momentos del ciclo reproductivo (40 días de gestación, 85 días de gestación, al parto y a los 18 ± 1 días de lactación) en los tres ciclos estudiados. En el momento del parto y también a los 18 ± 1 días de lactación se registraron el número de lechones y su peso individual. Los rendimientos productivos de la cerda después del destete se determinaron utilizando índices como la tasa de partos y el intervalo destete-cubrición (**Chapter 5**). En conjunto, tanto el grupo de cerdas alimentadas con el nivel de alimentación rutinario en granja (C) como el grupo de cerdas suplementadas durante la mitad de la gestación ganaron BW y LD al final de los tres ciclos estudiados. Por otro lado, únicamente las cerdas del grupo S fueron capaces de acumular BF al final de los tres ciclos consecutivos de extra alimentación. Las cerdas que empezaron el experimento como cerdas nulíparas (iPG1) fueron capaces de acumular tejido magro aunque también tejido lipídico, en respuesta al incremento del nivel de alimentación en gestación durante tres ciclos consecutivos, mientras que las cerdas multíparas (> 2 partos) dedicaron este extra aporte de energía principalmente a depósito de tejido graso. En términos productivos, la aplicación de esta estrategia de alimentación no dio lugar a ventajas en los rendimientos productivos de las cerdas multíparas aunque si provocó un incremento del peso medio al nacimiento de la descendencia de las cerdas primíparas (iPG1) en el ciclo 3 (+300 g/lechón en el grupo de cerdas S). Esta diferencia en el peso del lechón se mantuvo, aunque no estadísticamente, al destete. Las cerdas suplementadas, más específicamente las cerdas multíparas, demostraron menores rendimientos durante el periodo de lactación y un incremento de la incidencia de mamitis-metritis-agalaxia en granja. No se encontraron diferencias en los rendimientos reproductivos post-destete entre tratamientos experimentales.

Utilizando los datos de las cerdas que participaron en el ciclo 1 (n = 103, **Chapter 6**) se realizó un estudio exploratorio con el fin de determinar la asociación entre los niveles y la gestión de

las reservas corporales en diferentes periodos del ciclo reproductivo y los rendimientos (re)productivos bajo nuestras condiciones. En este estudio se demostró que el peso vivo de las cerdas y los niveles de reservas corporales (BF y LD) al parto se encontraban positivamente relacionados con sus respectivas pérdidas en el periodo de lactación, aunque también positivamente asociados con sus niveles de reservas al destete. El peso medio de los lechones al nacimiento y al destete resultó más influenciado por la paridad de la cerda que por el nivel de reservas al parto. Así pues, las cerdas en su primer parto presentaron lechones con un menor peso al nacimiento y también al destete en comparación con la descendencia procedente de cerdas multíparas ($P < 0.05$). Las pérdidas de BW y BF se relacionaron débil pero positivamente con la ganancia media de la camada durante la lactación, aún cuando el efecto parto fue eliminado del modelo estadístico (BW: $r = 0.36$, $P < 0.05$; BF: $r = 0.27$, $P < 0.10$). También se encontró una relación débil, aunque positiva, entre la pérdida de BF y el intervalo destete-cubrición (WEI, $r = 0.25$, $P < 0.05$). El intervalo destete-cubrición también se vio influenciado por el parto de la cerda, de manera que las cerdas primíparas presentaron WEIs más largos que las multíparas. Finalmente, con respecto a la exactitud de la nota de condición corporal como variable predictora de los niveles de reservas corporales de la cerda determinados mediante ultrasonidos (BF y LD), los resultados del presente experimento indicaron una asociación moderada entre variables ($r_{BF} = 0.54$ y $r_{LD} = 0.49$, $P < 0.01$). La fiabilidad de la nota de condición corporal (BCS) como predictora de las reservas grasas de los animales pareció ser superior en cerdas multíparas o con condiciones corporales extremas (muy delgadas o muy grasas).

En conclusión, un incremento del nivel de alimentación aplicado durante la parte central de la gestación en el presente estudio no mostró un efecto claro en los rendimientos productivos de la progenie. Sin embargo, la aplicación de este régimen alimentario dio lugar a diferencias histológicas en el desarrollo de las fibras musculares *in utero* y a una mejora en algunos de los aspectos de la calidad de la carne estudiados (carnes menos ácidas y más oscuras) 24 horas después del sacrificio. Adicionalmente, esta estrategia nutricional llevada a cabo en cerdas correctamente alimentadas dio lugar a un aumento del nivel de reservas corporales después de tres ciclos de suplementación. Este aumento de reservas en las cerdas S repercutió positivamentedio en las cerdas en las que la suplementación se inició en el primer ciclo reproductivo (nulíparas en el ciclo 1), mientras que dio lugar a efectos negativos en cerdas multíparas debido a un efecto en el desarrollo de la glándula mamaria. Finalmente, tanto el número de parto como la gestión de las reservas corporales durante la gestación ejercieron un claro efecto en el manejo de las reservas corporales durante el periodo de lactación, así como sobre los rendimientos productivos y reproductivos de las cerdas.

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Chapter 1

Literature review

1.1 Nutrition and management of the pregnant sow

1.2 Why, How and When might sow body reserves be evaluated on the farm?

1.3 Main interactions between sow feeding strategy, body reserves and productive performance

1.4 The impact of maternal nutrition during gestation on the offspring

1.1 Nutrition and management of the pregnant sow

Sows gestation has a length of 114 days. Assuming that, on average, sows are capable of having 2.4 litters per sow per year, there will be about 280-290 days during the year (80% of the time) when the animal is pregnant. In spite of this, gestation is a frequently forgotten stage within the reproductive cycle by most farmers and nutritionists since, apparently, only "little things" occur in this period. Energy and nutrient requirements throughout gestation are low (1.3 times maintenance in average), and the feeding regime is restricted in order to maintain an optimum body condition at farrowing. Consequently, except in case of pathology, feed refusals are anecdotal on the gestation barns.

But, actually, over the last 30 years several studies from the literature have indicated the importance of proper nutritional and feeding management during gestation on the overall success and efficiency of pig production systems (Libal and Wahlstrom, 1977; Aherne and Kirkwood, 1985; Close and Cole, 1986). The feeding strategy applied during gestation appears to have a main role in assuring the adequate management of sow body reserves throughout the reproductive cycle, and also in optimizing sow productivity and reproductivity.

New considerations are needed to be taken into account nowadays, when a feeding strategy for pregnant sows has to be designed. Genetic selection for leaner and more prolific genotypes (MLC, 1999) and the new EU directive (Council Directive 2001/88/EC of 23 October 2001 amending Directive 91/630/EC), which lays down that sows shall be kept in groups from 4 weeks after the service to 1 week before the expected time of farrowing, replacing the individual stalls system widely used, add a concern to the practise of feeding pregnant sows.

Nonetheless, when feeding breeding sows it must be assumed that all phases in the reproductive cycle are related and, therefore, the feeding program in one phase will have significant effects on performance in another phase. Also, the effects of under or over-feeding in any one phase of the cycle may not be seen for several parities (Whittemore, 1996; Aherne et al., 1999). Although there is a lack of consensus as to the best strategy for feeding gestating and lactating sows, there is general agreement that targeting a moderate weight gain during gestation and a minimum weight loss during lactation is advisable.

The development of an adequate feeding strategy needs a clear identification of the main objectives aimed for this period. Thus, from field data and also following several studies' recommendations in the literature, the feeding program for pregnant sows should have the following aims:

- Prepare sows to be in proper body condition at farrowing. This implies recovering body reserves lost during the preceding lactation (fat, lean and minerals), and also

promoting maternal growth in young sows (until parity 3 or 4). These must all be carried out preventing adverse effects in voluntary feed intake during the next lactation. For this reason, to establish objectives in body reserves at farrowing, but also at mating, is a very important issue on the farm.

- Maximize productive performance at farrowing, by minimizing embryo mortality and maximizing piglet weight at birth. The aim is to obtain about 11.5-12 pigs born alive per litter on average, of at least 1.3-1.4 kg of weight per pig.

- Guarantee the bone integrity in young sows at farrowing, and to optimise mineral balance in adult sows throughout the consecutive reproductive cycles.

- Improve pregnant sow welfare in terms of gut health and stereotypic behaviour, through the use of high-fibre diets during gestation.

- Assure colostrum and milk production in quantity and quality, through achieving an optimum mammary gland development during the final third of pregnancy.

Moreover, a relatively new stream of consciousness arises as the so-called “Prenatal programming of postnatal performance” (Foxcroft and Town, 2004). It involves the knowledge of how prenatal events such as nutrition or non-nutritional causes (application of hormonal treatments, uterine crowding, placental efficiency and others), are able to impact on the developmental foetus physiology, and lead to permanent consequences in its postnatal life. This effect has been studied and reported in multiple species such as humans (Osmond et al., 1993; Hornstra et al. 2005; Kunz and King, 2007), pigs (Rehfeldt et al., 1993; Gatford et al., 2003) or sheep (Greenwood et al., 1998). For instance, existing literature in pigs indicates that the variation in growth performance after birth may be largely determined, and essentially pre-programmed, during foetal development in the uterus (Dwyer et al., 1994; Gatford et al., 2003). Also in humans, the theory of the “Foetal origins of adult disease” or “Thrifty phenotype” has been described from extensive epidemiological studies, such as marked famine or maternal obesity. It is based on the fact that maternal nutrition during pre-conception, gestation and lactation is linked to fertility, foetal growth and also to life-long health of the offspring after birth.

Consequently, nutritional management of gestating farm livestock, that generally aims to meet maternal requirements for maintenance, maternal and foetal growth, should be further evaluated as a programmer of postnatal traits. Because of its reported potential effects, this might become an important issue in the commercial swine production systems in the future.

Within the general framework of the preceding introduction, the following literature review attempts to briefly define the energy and nutrient requirements during gestation, to capture the

impact of the feeding pattern and strategy followed during gestation on foetal growth and development, and to establish the basis for muscle growth pre and postnatally and also its consequences on ultimate meat quality.

1.1.1 Energy and nutrient requirements and recommendations during pregnancy

Determining nutritional requirements is the first step in adapting the feeding program to any of the different productive phases defined. The knowledge of the nutritional requirements is especially important for pregnant sows, since their feeding is generally restrictedly fed, and the nutrient supply for each pregnancy stage must be fully covered by the daily feed allowance. Subsequently, pregnant sow requirements/recommendations in energy, protein and other nutrients (minerals, vitamins and fibre) will be shortly surveyed.

1.1.1.1 Energy and protein requirements

Energy and protein requirements of pregnant sows have been excellently studied and reviewed by several authors such as Close et al. (1985), Noblet et al., (1985), Noblet and Etienne (1987), Noblet et al. (1990), Speer (1990), Everts and Dekker (1994), Pettigrew (1995); Noblet et al. (1997), Close and Cole (2003) and others. Also, the official standards such as ITP (1991), NRC (1998), BSAS (2003) and FEDNA (2006) provide extended information on the requirements for feeding pregnant sows. Energy, protein and amino acid requirements are generally derived using the factorial approach, as the sum of requirements for maintenance, maternal weight gain and products of conception, following the subsequent considerations:

Energy

It is estimated that a conventional sow (150 to 300 kg body weight) in thermoneutral conditions needs from 28.2 to 34.9 MJ ME/day (NRC, 1998; BSAS, 2003, see Table 1.2) throughout pregnancy. Factorially, these requirements are divided in:

Requirements for maintenance

During pregnancy, maintenance represents as much as the 75 to 85 % of the total energy requirements (Noblet et al., 1990). In terms of energy cost, maintenance during pregnancy amounts to about 400 to 440 kJ ME/kg $BW^{0.75}$ per day, under thermoneutral conditions, and with moderate physical activity (Close et al., 1985; Whittemore and Yang, 1989; Noblet et al., 1990; ITP, 1991). Sows body weight (BW) plays then a very important role on the determination of energy requirements during pregnancy. In this respect, it is calculated that energy requirements for maintenance under thermoneutral conditions increase from 15-20 MJ ME to 25-30 MJ ME/day as body weight at mating increases from 120 to 250 kg (see Figure

1.1). Moreover, as the ultimate mature size is highly dependent on the genotype and the feeding level, then maintenance requirements during gestation will also ultimately depend on all these aspects.

Requirements for maintenance are additionally greatly affected by the ambient temperatures and the opportunity of exercise, and these are indirectly related to the rearing and housing systems used. Little has been published recently to amend the views of variation in requirements according to these factors, in spite of the importance that group-housed and free range production systems will have in the future.

The lower critical temperature (LCT) represents the lowest temperature at which heat loss is minimal, under any given set of environmental circumstances. Literature data suggest that the LCT of individually housed sows varies between 20 and 23ºC (Noblet et al., 1990) and that this increases in extremely thin sows (poor insulation of the animal) and in adverse housing conditions (concrete slats, drafts), while decreases with straw bedding, increasing feed allowances and in group-housing systems. Indeed, Geuyen et al. (1984) reported a 6ºC decrease in the LCT in group-housing systems compared to individually-housed sows. Compilation of data in the literature states that for each 1ºC below the sow's lower critical temperature, maintenance energy requirements increase about 7.5 kJ ME/kgBW$^{0.75}$ per day for animals living in groups, and 23.3 kJ ME/kgBW$^{0.75}$ per day for thin animals living individually in cold conditions (Geuyen et al., 1984; Kemp et al., 1987). Since the net efficiency of energy utilisation for maintenance has been established in 77 % (K_m, Noblet et al., 1997), in animals of 120 kg of BW this represents an increased energy requirement of 0.35 MJ ME/day and 1.10 MJ ME/day for group or individually housed systems, respectively. Additionally, for an animal weighing 250 kg of BW, it represents 0.61 MJ ME/day and 1.90 MJ ME/day for group or individually housing systems, respectively. In general, considering a standard commercial feed for pregnant sows (12.1 MJ ME/kg) it is calculated that a decrease in 1ºC below LCT requires from 30 to 150 g more of feed per day in order to satisfy the sows' energy requirements.

On the other hand, physical activity is also a major factor causing differences in energy balance between sows. Noblet et al. (1997) calculated from a literature survey that the energy cost of standing in growing pigs and sows ranged between 0.23 and 0.30 kJ ME/kg BW$^{0.75}$ and minute. Under practical conditions, the standing length and other physical activity varies greatly depending on the housing conditions (individual/group; indoor/outdoor), and also on the animal itself. It has been reported that stereotypy behaviour can represent an additional maintenance cost (Brouns et al., 1994). In this way, a mean of about 100 min daily difference on the standing's length will represent from about 80 to 140 g more of a conventional commercial feed (12.1 MJ ME/kg) in sows averaging 120 and 250 kg of BW/respectively. This observation means that attention should be paid to pregnant sow welfare, since its repercussions on feed requirements might be considerable.

Other factors such as sow parity, pregnancy and the stage of pregnancy do not significantly affect maintenance requirements, when expressed per kilogram of $BW^{0.75}$ (Close et al., 1985; Noblet et al., 1990).

Requirements for pregnancy gains of conception products

Energy requirements for conceptus growth and mammary development account for about 5% of the energy requirements of the sow during gestation (Noblet et al., 1990). Some 4.8 MJ of energy and 2.7 kg of protein are deposited in the total gravid uterus between conception and term (Noblet et al., 1985). Unlike rates of energy accretion within the uterus that can be estimated rather accurately, efficiency of feed energy utilization is difficult to determine. Values between 50 and 30 % (K_g) have been reported depending on the calculation approach used (Close et al., 1985; Noblet and Etienne, 1987; Noblet et al., 1990). Little information is available on development and energy deposition in the mammary gland. From measurements conducted by Noblet et al. (1985), it is clear that the mammary gland starts to grow significantly at the beginning of the final third of pregnancy.

Total energy or protein requirements are not constant over the gestation period. The developing conceptus makes little nutritional demand during the first two months of pregnancy and, during this period, most of the dietary nutrients retained are deposited in the maternal tissue. During the last month of pregnancy, when conceptus growth accelerates, a change in both the priority and the demand for nutrients appear (see Figure 1.1). Then, at a fixed nutrient intake, the increase in requirements for conceptus gain is achieved at the expense of tissue deposition within the maternal body (Close et al., 1984; Close et al., 1985). Considering that the net efficiency of energy utilisation for reproductive tissue altogether (including mammary tissue) is 0.6, the energy requirement for growth of the reproductive tissue during pregnancy was calculated to increase from 0 to 0.7, 1.2, 2.5 and 4.8 MJ ME/day on days 0, 28, 56, 84 and 112 of gestation (Noblet et al., 1985; Whittemore and Morgan, 1990, see Figure 1.1).

Requirements for maternal growth and recovery

The net weight gain of the sow during gestation is the difference between the total body weight gained during gestation and the reproductive weight gained (conceptus and mammary gland tissues), and it is generally called “maternal anabolism”. Maternal net weight gain represents approximately 15-20% of the sows’ energy requirements during gestation. It constitutes the most variable part of the energetic requirements, since it includes the inherent maternal growth but also the recovery of the maternal body reserves lost during the preceding lactation.

Maternal growth

Sows grow from first conception (120-150 kg of BW) over 3-4 parities to double their size and achieve approximately 280-300 kg of BW as adults. Energy requirements for maternal

growth can be calculated by making assumptions about the amount of gain expected and its composition which, in turn, vary among genetic lines, needs for maternal recovery (reported below) and the sow age/parity. Maternal anabolism is greater in young compared to elder sows, since they have a higher drive for maternal growth than adults (see Figure 1.1). Moreover, to this long-standing convention, must now be added the substantial requirements for growth necessitated by the modern large-maturing genotypes, which conceive at only 50 % (or less) of their mature size. In the recent literature, Young et al. (2005) reported average net maternal body weight gains during gestation of 39.0, 29.4, 28.2, 16.8 and 14.6 kg for gilts and parity 1, 2, 3 and 4 sows, respectively.

The composition of the maternal gain will also vary with parity and with the amount of gain accrued. Little information is available on the composition and energy content of the maternal pregnancy weight gain in sows, and most of the results concern gilts. For primiparous sows gaining 30 kg of BW throughout gestation, the composition of the maternal weight gain is approximately 75% lean and 25% lipid (Whittemore and Yang, 1989; Pettigrew and Yang, 1997). The proportion of lean gain is expected to decrease while the proportion of lipid gain is expected to increase as the sow attains maturity (increasing parity) and as the level of body protein nears the adult target (Whittemore and Yang, 1989; Whittemore and Morgan, 1990; Aherne et al., 1999). Whittemore and Morgan (1990) reported modelled conception to conception absolute protein gains of about 11.1, 7.8, 4.5 and 2.4 kg and lipid gains of 16.2, 11.7, 7.0 and 3.7 kg, approximately, in sows from parities 1 to 4, respectively.

In general, maternal tissue deposition is assumed to have the same protein to fat ratio throughout gestation. But, at a fixed age, this ratio decreases as the amount of weight gain during gestation increases. Therefore, higher feeding levels of feed intake during pregnancy may increase fat accretion, and protein deposition may be less dependent on the level of feeding, being more determined by a biological target (Close et al., 1985; Whittemore and Yang, 1989; Clowes et al., 1994).

Reconstitution of body reserves

Both energy and protein supply during gestation needs to be modulated according to the mobilization of body reserves during the previous lactation in multiparous sows. Very few direct measurements of chemical and energy contents of pregnancy net weight gain have been carried out in multiparous sows. Dourmad et al. (1996) measured changes in tissue weight from mating to farrowing in sows from 3 to 5 parities with increasing levels of energy allowance during gestation (29.8, 35.3 and 41.8 MJ ME/d). They reported that both, muscle weight (3.3 kg, 13.8 kg and 17.9 kg, respectively) and dissectable fat (1.0 kg, 8.8 kg and 17.5 kg, respectively) increased with energy supply, but that the ratio muscle to fat deposited also decreased with increasing energy intake. Additionally, from this study, it was concluded that when losses during the preceding lactation reached as much as 35 kg of body weight (high-producing sows),

adequate restoration of body energy reserves during gestation was achieved by providing about 34.1 MJ ME/day. Therefore, an allowance for further energy but also further protein gains considering the amount of body weight or body reserves loss during lactation, is necessary to obtain an adequate body reserves balance at the end of the whole reproductive cycle.

To calculate ME requirements for maternal gain, an energy content of lean and fat of 9.5 and 32 MJ ME/kg, respectively, can be assumed (Noblet et al., 1997), and it must be considered that ME is used for deposition of maternal tissues with a mean efficiency of 75 % (Close et al., 1985; Noblet and Etienne, 1987; Noblet et al., 1997; BSAS, 2003).

The mentioned partition of nutrients between maternal and foetal tissues during pregnancy for first parity and adult (parity 4) sows kept under thermoneutral conditions, is illustrated in figure 1.1.

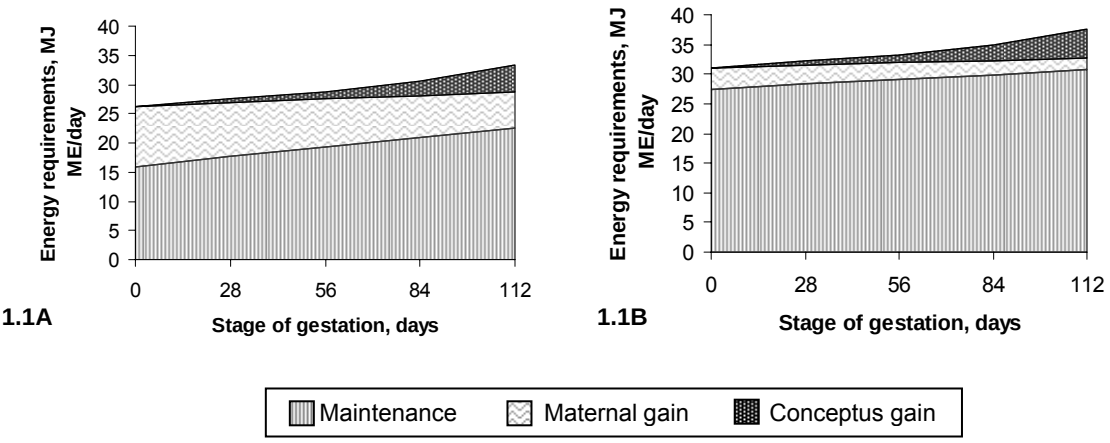


Figure 1.1 Contribution of maintenance, maternal gain and conceptus gain to the total energy requirements (MJ ME/day) throughout gestation. Figure 1.1A represents energy partition in first parity sows (Body weight at mating = 120 kg and 70 kg of total body weight gain during pregnancy) and figure 1.1B represents parity 4 sows (Body weight at mating = 250 kg and 40 kg of total body weight gain during pregnancy). Animals kept under thermoneutral conditions (Adapted from Noblet et al., 1985; Whittemore and Morgan, 1990; Close and Cole, 2003).

Protein and amino acid requirements

The protein needs of pregnancy can also be divided into requirements for maintenance, deposition of reproductive tissue, especially conceptus tissue, and for maternal gain (Speer, 1990). Compared to energy, there is less information on the requirements for protein and amino acids in pregnant sows. As a whole, it is accepted that the amino acid and protein requirements for pregnancy are low and that once the requirements of foetal development have been satisfied the remaining dietary protein can be used for maternal growth and repair.

The pregnant sow is extremely able to withstand protein deprivation by protecting the developing litter at the expense of maternal tissue. Trials have shown that only severe nitrogen (N) restriction (2-6 g N per sow per day) has been shown to reduce the nutrient accretion in products of conception (Pond, 1973; Hammell et al., 1976). Moreover, pregnancy can apparently be maintained in gilts fed protein-free diets during gestation, by dependence of maternal tissue stores of amino acid for growth of the foetus (Pond, 1968). However, lower litter sizes and individual pig weights has been reported for gilts deprived of dietary protein (Pond, 1968).

Speer (1990) stated that, overall, differences in protein supply in the diet during pregnancy affect maternal body composition, but not the composition of the products of conception. The maximum rate of maternal nitrogen retention depends on the level of dietary protein, but also on the energy supply in the diet (Hovell et al., 1977; Dourmad et al., 1996) and probably also on parity or age of the sow (Everts and Dekker, 1994).

According to foetal growth, protein and amino acid requirements also increase with increasing gestation, being maximal during the last month of pregnancy. Dourmad et al. (1996) reported that N retention increased steadily after 53 days of mating in multiparous sows and that was maximal on day 104 of gestation. Noblet and Etienne (1987) and Everts and Dekker (1994) also reported an increase in N retention over gestation after 50 to 60 days of pregnancy in first parity sows. A more recent study in gilts (McPherson et al., 2004) reported that protein accretion in the foetus increased from day 70 of gestation, being especially important the last month of gestation. In this study, a protein phase feeding was suggested during gestation, since protein deposition changed as much as from 3 g/d before day 70 of gestation to about 56 g/d after this day. Everts and Dekker (1994) reported that a protein supply of about 263 g per day in mid-pregnancy and 313 g per day in late pregnancy guaranteed a normal development of the products of conception and the udder, but decreased maternal N retention. In general, according to the higher necessity of young sows for increasing protein accretion, maternal nitrogen retention also will be higher in first parities (Everts and Dekker, 1994).

To calculate protein requirements during gestation, values for maintenance requirements of 2.8 g/ kg $BW^{0.75}$ and day and an efficiency for ingested nitrogen to nitrogen retention of 60 % (Everts and Dekker, 1994) must be considered. In general, it is estimated that a conventional sow will need from 275 to 350 g of protein per day (Mateos y Piquer, 1994) throughout pregnancy.

Also important in protein nutrition during pregnancy, is to cover amino-acid requirements according to the “ideal protein” concept. This concept was initially designed for growing pigs but, although less suitable, it has been also extrapolated to breeding sows nutrition. This concept consists in covering lysine requirements and all the other essential amino acids proportionally to

lysine (Table 1.1). Lysine is usually the first limiting essential amino acid in commercially available diets. The major component that influences lysine requirements during gestation is the maternal gain and its composition (NRC 1998). It is estimated that a mature sow of 300 kg of BW gaining 15 kg of BW and 20 kg of conceptus weight gain has a total lysine requirement of 10.6 g/day (Table 1.2). However, gilts (about 150 kg of BW at mating) which have an element of true growth with increased muscle deposition, total lysine requirements during pregnancy are estimated as 16 g/day (Table 1.2). Kusina et al. (1999a) reported higher piglet growth rates during lactation in first-parity sows when 8 to 16 g of lysine/day was fed during pregnancy, compared to those fed 4 g of lysine/day. The same authors in a latter study (Kusina et al., 1999b) suggested that the increased milk production resulted from the achievement of greater protein stores at farrowing, which could be called upon to support a higher level of milk production during lactation. This evidently shows the importance of lean tissue in the new improved genotypes for productivity.

The ideal balance of essential amino acids is dynamic since the amount of amino acids retained in the different compartments is different, although difficult to measure (Everts, 1998; Table 1.1). The maintenance function requires more threonine than other essential amino acids. However, in other functions such as conceptus growth and maternal recovery, lysine remains as the major amino acid retained. Therefore, in mid-pregnancy, when maintenance requirements are dominating, requirements in threonine must be higher than in other pregnancy stages. However, in late pregnancy, when the maximum foetal growth occurs, but also in gilts in which maternal growth has a higher importance compared to adult sows, requirements on lysine are higher.

Table 1.1 Balance of essential amino acids in ideal protein of diets for gestating sows (lysine=1.00).

Amino acid	Maintenance[1]	Conceptus[2]	Maternal body[3]	Total requirements[4]
Lysine	**100**	**100**	**100**	**100**
Methionine	**25**	**24**	**29**	**37**
Cystine	**111**	**22**	**15**	**-**
Threonine	**147**	**56**	**53**	**71**
Tryptophan	**30**	**12**	**12**	**20**
Isoleucine	**44**	**49**	**55**	**70**

Source: Adapted from Everts (1998)

[1]Growing pigs (Fuller et al., 1989)

[2] Conceptus at day 109 of pregnancy (Everts and Dekker, 1995a)

[3] First and third parity sows (Everts and Dekker, 1995a,b)

[4] BSAS (2003)

In practical conditions, the balance of essential amino acids recommended for pregnant sows is that of the combination of maintenance, maternal gains and reproduction functions.

Table 1.2 summarizes pregnant sows' energy and total lysine requirements according to sow body weight at mating and their anticipated body weight gain during gestation and following the official BSAS (2003) standards. From this table, it is calculated that primiparous sows (150 kg of BW at mating) require 6.7 MJ ME/day less and 5.4 g of lysine/day more than multiparous (300 kg BW at mating).

Table 1.2 Daily energy and lysine requirements for pregnant sows[1].

Body weight at mating, kg		**150**	**225**	**300**
Body net weight gain[2]		**40**	**27.5**	**15**
Total	Energy, MJ ME/d	28.2	34.1	34.9
	Total Lysine, g/d	16.0	11.4	10.6
Feeding level[3] kg/d		2.3	2.8	2.9

Source: Adapted from BSAS (2003)

[1] In individual feeding and thermoneutral conditions

[2] Do not considers the weight of the products of conception

[3] As-feed basis, it is considered energetic concentration of 12.1 MJ ME/kg of feed

1.1.1.2 Requirements and practical feeding recommendations in minerals and vitamins

For foetal development to proceed normally, high concentrations of specific nutrients are often required during specific periods of pregnancy. An example would be the case of the minerals and vitamins, since they are required in small amounts but they are essential for the normal metabolism and health of animals. The main minerals and vitamins required for pregnancy and its biological function are briefly described below.

Mineral requirements and recommendations

Calcium and phosphorus and some trace minerals such as sodium, chloride, zinc, iron and selenium are involved in diverse biological functions as the correct development of the skeleton, the osmotic regulation of body fluids and other metabolic processes. Table 1.3 shows calcium, digestible phosphorus and other microminerals requirements established for pregnant sows by different sources (NRC 1998, BSAS 2003 and FEDNA 2006). In the past, diets have been formulated with mineral levels on the basis of preventing acute deficiency symptoms. But, the mineral requirements for sows of modern genotypes have attracted more attention in recent years due to their potential influence also on reproduction (anoestrus, conception rate and litter size; Close and Cole, 2003). Mineral requirements are highly dependent on the level of production. Mahan and Newton (1995) reported a de-mineralization of sow bones and tissues (less Ca, P, Mg, Fe, Zn, Cu and Se content) of sows after completing three reproductive cycles, compared to sows from similar ages but that remained non-pregnant. Thus, recent advances in

animal breeding and improvements in productivity as well as sows health status may have increased the mineral requirements of modern hyperprolific genotypes.

Recent works demonstrate that mineral retention in the foetal tissue, especially calcium and phosphorus retention, is doubled during the last two weeks of gestation (Mahan, 2006). Thus, feeding strategy for pregnant sows must account for the fact that late gestation is a critical stage that could lead to mineral imbalances (more important for Ca and P) during the subsequent lactation. Fully supply of calcium requirements is important, especially in young animals since they must develop a solid skeleton, but also in adult sows in order to avoid limp and lameness problems. These leg problems appear as a consequence of the excessive calcium and other mineral loss during lactation and the physical restriction to which sows are subjected up to now (stall-housed).

Other minerals (microminerals) such as zinc, iron and selenium are reported to play an active role on foetal development, and also reproduction and piglet performance. But, in general, microminerals are supplied in very low amounts in the diet on order to avoid a potential toxic effect when they are given in excess.

Table 1.3 Macro and micromineral requirements (% or amount/kg of diet) for pregnant sows according to three different sources (NRC, 1998; BSAS, 2003 and FEDNA, 2006).

	NRC 1998[1]	**BSAS 2003[2]**	**FEDNA 2006[1]**
Calcium, %	0.75	0.72	0.85-1.10
Digestible phosphorus (min), %	0.35	0.23	0.28
Sodium (min), %	0.15	0.17	0.18
Chloride (min), %	0.12	0.14	0.16
Zinc, ppm	50	80	100
Iron, ppm	80	80	75
Selenium, ppm	0.15	0.2	0.3

Sources: Nutient Requirement Council, 1998 (NRC); British Society for Animal Sciences, 2003 (BSAS); Fundación Española para el Desarrollo de la Nutrición Animal, 2006 (FEDNA)

[1] As-feed basis

[2] In dry matter basis

In addition to meeting the animal's requirements, minerals need to be considered for their contribution to electrolyte balance (EB, balance of dietary cations and anions expressed in milliequivalents). The acid-base balance of pigs may be altered through the productive phase tending towards an acid metabolism while growing, and the diet may be a contributing factor to acid-base balance after consumed nutrients are absorbed and metabolized (Patience and Chaplin, 1997). Evidences of improved growth rates have been reported in growing and weaned pigs when dietary EB was about 150-250 mEq/kg (NRC, 1998). However, little information

exists for the breeding sow. Research in dairy cattle (West et al., 1991) and laying hens (Balnave and Muheereza, 1997) indicated positive effects on metabolism such as improved milk yield and feed intake in lactation or greater eggshell quality, when the dietary cation-anion difference (Na + K – Cl - S) was increased. Recent investigations in pregnant and lactating sows reported pronounced effects on the physiological status of sows through a decreased urine pH and decreased bacterial counts in urine, when the electrolyte balance was reduced in the diet (DeRouchey et al., 2003; Roux et al., 2006), with a possible positive effect in piglet survavility at birth (DeRouchey et al., 2003). But, in general, little effects have been demonstrated until now in variables such as sow feed intake during lactation and on litter performance.

Vitamins

Vitamin requirements are difficult to ascertain but its importance in reproduction has long been recognized. Standards provided for vitamins are not requirements but allowances in order to insure pig health and welfare. Levels presented in table 1.4 according to different standards (NRC 1998, BSAS 2003 and FEDNA 2006) are indicative of the mid point of a range of allowances.

Table 1.4 Vitamin requirements and recommended allowances for pregnancy (% or amount/kg of diet) according to three different sources (NRC, 1998; BSAS, 2003 and FEDNA, 2006).

	NRC (1998)[1]	BSAS (2003)[2]	FEDNA (2006)[2]
Vitamin A, I.U.	4000	8500	10500
Vitamin D_3, I.U.	200	800	1600
Vitamin E, I.U.	44	50	45
Vitamin K (menadione, ppm)	0.50	1.5	1.6
Thiamin (B1, ppm)	1.00	2	1.6
Riboflavin (B2, ppm)	3.75	5	5
Nicotinic acid, ppm	-	20	-
Pantothenic acid, ppm	12	15	13
Pyridoxyne (B6, ppm)	1.00	3	2.5
Cyanocobalamin (B12, ppb)	0.015	0.03	0.025
Biotin, ppb	200	200	130
Folic acid, ppm	1.30	3	2.1
Choline, ppm	125	300	260

Sources: Nutient Requirement Council, 1998 (NRC); British Society for Animal Sciences, 2003 (BSAS); Fundación Española para el Desarrollo de la Nutrición Animal, 2006 (FEDNA)

[1] Requirements

[2] Recommended levels, amount of vitamins to be added per kg final compounded air-dry feed

In general, fat soluble vitamins (such as Vitamin A, D, and E), not only may have a role in the attainment and maintenance of pregnancy, but also in foetal development. For instance, retinoids (Vitamin A) may regulate early embryonic elongation and placentation and maintenance of pregnancy (Chew, 1993), by influencing ovarian progesterone production. Deficiencies may lead to weak piglets born, lower growth rates and pigs less resistant to disease.

An insufficient supply of vitamin D may lead to poor absorption and utilisation of dietary calcium, resulting in reduced bone mineral content (Thompson and Robinson, 1989). This is clinically defined as rickets in young pigs, while pregnant and lactating sows may develop osteoporosis.

Tocopherols (vitamin E) act mainly as an intracellular antioxidant, protecting cells from the action of free radicals on unprotected unsaturated lipids. Beneficial effects of supplemental vitamin E in improving litter size in pigs have been reported. Mahan (1994) suggested that 44 to 66 I.U. vitamin E/kg of diet approached the optimum level for maximum litter size in sows. Tocopherols are also involved in various immune response pathways and then, deficiencies may also result in reduced survival and increased incidence of diarrhoea (Mahan et al., 1986).

Additionally, from the group of water soluble vitamins, Thiamin, Riboflavin and Folacin can also play an important role on reproduction. For instance, folic acid compounds are involved in protein and amino acid metabolism. A deficiency has been reported to decrease embryo growth. Additional folacin given by injection or in feed appeared to prevent some embryo mortality and thus, increase litter sizes (Lindemann and Kornegay, 1989).

1.1.1.3 Fibre in diets for pregnant sows

Economic and animal welfare considerations faced by modern pork production systems have increased the interest of producers in diets with elevated concentrations of fibre. Restricting voluntary feed intake in pregnant sows is necessary to prevent excessive weight gain and fat deposition, which could adversely affect the progress of farrowing and lead to problems in voluntary feed intake during lactation or with locomotion. However, limit feeding only to achieve nutritional requirements during gestation does not allow the sow the chance to feel satisfied after eating, and can lead to frustration and, ultimately, the exhibition of stereotypic behaviours (Terlouw et al., 1991); stereotypic behaviours have been interpreted as an indication of impaired welfare. The inclusion of high levels of fibrous ingredients in the diet allows increasing feed supply without increasing energy and nutrient allowances, and may reduce hunger and feeding motivation thus having positive effects on their wellbeing (Robert et al., 1993; Brouns et al., 1994; Ramonet et al., 1999; van der Peet-Schwering et al., 2003).

Providing sows with more bulky or high fibre diets during gestation has also been related with an improvement of gestating sows welfare in terms of gut health, in view of increasing gut motility before farrowing (Tabeling et al., 2003) and the maintenance of an equilibrated mycrobiota (Roediger, 1982).

From a compilation of diverse studies made by Reese (1997) it was suggested that feeding fibre during gestation has also potential benefits on the number of pigs farrowed and weaned per litter, and also on lactation feed intake. However, results on this area described large variations between studies related to various parameters of the experimental diets such as the nature and the incorporation rate of the fibre sources, together with the amino acid, vitamin and trace mineral content of the diets.

Fibrous ingredients such as sugar-beet pulp (Brouns et al., 1994), oat hulls (Robert et al., 1993) or soybean hulls (van der Peet-Schwering et al., 2003) are being used in sow high-fibre diets (see Table 1.5). Satiety provided by the different feedstuffs depends on their non-starch polysaccharides (NSP) content (dietary fibre fraction that is not digested by endogenous secretions of the digestive tract), and is highly related to their physicochemical properties, specially the water retention capacity (Kyriazakis and Emmans, 1995; Reese, 1997). Dietary NSPs include cellulose, hemi-cellulose, pectin, arabinoxylans and other carbohidrates. Some of them, but especially the latest, are fermented in the hindgut by resident microbes, contributing in this way to the maintenance of an equilibrated mycrobiota and a healthy epithelium via fermentation products as short chain fatty acids (Roediger, 1982). Gestating sows utilize fibre better than growing pigs, and so, are able to derive more energy from fibrous feedstuffs than growing pigs (LeGolff and Noblet, 2001). Moreover, the pregnant sow has the capacity to consume four to five times the amount of feed that is required to meet gestation energy requirements. All these characteristics make gestating sows well suited to utilize high-fibre, low-density diets (Reese, 1997).

Table 1.5 Composition of the main fibrous ingredients included in pregnant sows diets (DM: Dry matter; CF: crude fibre; CP: crude protein; NDF: neutral detergent fibre; ME: Metabolic energy).

	DM, %	CF, %	CP, %	NDF, %	ME, MJ/kg	L, %[1]
Wheat bran	88.1	8.0	14.9	35.0	10.3	35
Oat hulls	90.9	28.3	3.8	69.0	4.1	5
Alfalfa hay	91.4	25.9	15.6	46.1	6.9	8
Sugar-beet pulp	89.7	17.8	10.1	42.8	10.8	10
Soybean hulls	89.0	32.7	13.0	57.5	7.5	8

Source: Tablas FEDNA, 2006

[1]L: Maximum inclusion levels

Nonetheless, it is difficult to establish recommendations in fibre content [crude fibre (CF) or neutral detergent fibre (NDF)] in diets for pregnant sows under gut health and animal welfare

basis. Inclusion levels will depend on their relative economic cost and on their potential negative effects on feed palatability. In fact, there is a maximum level allowed for almost each fibrous ingredient according to its composition and effects on consumption (Table 1.5). Pregnant sows diets are currently formulated to contain a level of CF of about 5-7 % but, levels of 12 % of CF (Mateos and Piquer, 1994) or higher than 18 % of NDF (Normas FEDNA, 2006) are generally tolerated by pregnant sows. Some European countries such as Holland, have already applied a normative that regulates fibre inclusion levels in the diet. In this respect, the Dutch law (National Reference Centre, 1998) states that gestating sows should receive 14% CF in their diet.

Once the main nutritional requirements and recommendations for pregnant sows have been reviewed, table 1.6 summarizes the main nutrient requirements of a typical of herd average sow according to BSAS (2003) [215 kg of BW at breeding and 27.5 maternal gain (20 kg lean tissue and 7.5-8.55 fat tissue, approximately)] during pregnancy provided by different official sources (NRC, 1998; BSAS, 2003), and the practical feeding recommendations also provided by different sources (FEDNA tables, PIC and Vall Companys Group).

Table 1.6 Nutrient requirements[1] (NRC, 1998; BSAS, 2003) and practical recommendations (VC, PIC, FEDNA) for pregnant sows.

Nutrients	Units	NRC 1998	BSAS 2003	VC	PIC	FEDNA
Intake	Kg/d	2.9	2.9	2.9-3.0[2]	2.8-2.9[2]	2.9[2]
Energy	MJ ME/kg feed	11.8	11.8	11.7	12.1	12.0
Crude fibre	%	-	3.1-4.4[3]	7.5 - 9.0	6-12	5.6-10
NDF, Mín.	%	-	11.4-24.6[3]	-	22	18
Crude protein	%	10.8	9.3-10.0	14.0 - 16.0	13.0-15.0	14.0-15.5
Total lysine	%	0.36	0.39	0.60	0.60	0.60
Methionine	%	0.10	0.14	0.21	0.17	0.21
Meth+Cys		0.25	0.26	0.42	0.39	0.39
Threonine	%	0.29	0.28	0.42	0.44	0.42
Tryptophan	%	0.07	0.08	0.12	0.12	0.12
Isoleucine	%	0.21	0.28	-	-	0.42
Linoleic acid	%	0.10	0.19	-	0-1.6	> 0.10

Sources: 1) Nutrient requirements standards: Nutient Requirement Council, 1998 (NRC); British Society for Animal Sciences, 2003 (BSAS); 2) Recommendations: Vall Companys Group (VC); Pig Improvement Company (PIC); Fundación Española para el Desarrollo de la Nutrición Animal, 2006 (FEDNA)

[1] For a typical of herd average sow: 215 kg at breeding, 27.5 maternal gain (20 kg lean tissue and 7.5-8.55 fat tissue, approximately). Average daily feed intake in order to calculate minimum feed specifications was set at 2.9 kg feed/day

[2] Optimum daily feed intake estimated in order to cover daily energy requirements established by NRC (1998) and BSAS (2003), according to the feed energy concentration recommended

[3] Values represent guidelines but not requirements

From the data reported in this table, it is deduced that a 14-16% of crude protein diet containing 11-13 MJ ME/kg and 0.60% lysine is adequate for feeding pregnant sows. Increases in 3-5% of lysine levels are recommended for nulliparous sows when formulating commercial feeds (Normas FEDNA, 2006).

1.1.2 Practical feeding pattern during gestation

The pattern of feed allowance during gestation could be of greater importance than the total amount of feed intake *per se*. Sows' requirements increase over gestation, being especially important in the last month, and the implementation of a phase feeding strategy throughout pregnancy has been advised. It involves feeding the required amount of diet at each stage and three stages have been defined (Coma, 1997; Boyd et al., 2002; McPherson et al., 2004). The first stage (early pregnancy) involves embryo implantation in the uterus and lasts about three-four weeks. The second stage (mid-pregnancy) comprises from the third week of pregnancy until the last month of gestation. In this phase foetus make little demands and it is one of the most important periods in which body reserves should be rebuilt. The third stage (late pregnancy) includes the last 30-35 days of gestation, and involves the exponential growth of the reproductive tissue. Detailed implications of sow feeding in each of these stages are given below.

1.1.2.1 Early pregnancy (*conception to day 30 of gestation*)

Overall, achieving an optimal number of implanted embryos *in utero*, but also to start modulating sow body reserves in order to obtain the desired body condition at farrowing, are the main aims of this pregnancy stage. Litter size is a major component of the reproductive output and, in turn, the ability of embryos to survive the first three weeks of pregnancy and to make a successful implantation onto the endometrium is crucial. In pigs, ovulation rate is usually high (a multiparous sows can release up to 20-25 ova at every ovulation), but the mean incidence of embryo mortality pre-implantation (prior to day 30 of pregnancy) in commercial breeds is also generally elevated (20-30%, reviewed by Ashworth, 1998). The causes for this high embryo loss have diverse origins such as stress, genetic factors, husbandry and management factors, hormonal status and maternal nutrition (Ashworth, 1998).

The results of many studies have shown that undernutrition during early gestation has to be very severe to reduce embryo survival (Pond et al., 1968), because when an ovum is fertilized, the embryo is given a high priority in nutrient supply. In contrast, the results of many experiments have shown that high levels of feed intake during this period of gestation may increase embryo mortality (den Hartog and van Kempen, 1980; Dyck and Strain, 1983; Baidoo et al., 1992). Risks periods up to 15-20 days post-mating have been suggested (Dyck and Strain, 1983; Baidoo et al., 1992), but the first 72 hours after mating appear to be the most

critical (Jindal et al., 1996). It has been proposed that a major mechanism involved is from the way in which feed intake increases hepatic blood flow and, therefore, the metabolic clearance rate of progesterone and other steroids processed by hepatic enzymes (Einarsson and Rojkittikhun, 1993; Jindal et al., 1996). However, this effect shows different responses depending on sow parity. While in primiparous sows the practise of maintaining energy intake at low levels has been well established for some years, in order to avoid detrimental effects on embryo survival (Dyck and Strain, 1983; Jindal et al., 1996), in multiparous sows the case is not so clear cut (Toplis et al., 1983; Kirkwood et al., 1990). Kirkwood et al. (1990) reported even positive effects of increasing feeding levels in early gestation (from 1.8 kg to 3.6 kg/day) on the number of embryos and percentage of embryo survival, when multiparous sows had been underfed during the preceding lactation.

In more recent studies it has been also argued that, despite the lower progesterone levels found in plasma, increasing feeding level during early gestation might have beneficial effects on pregnancy performance in group-housed animals (Virolainen et al., 2004). This is because an increase in feeding level beyond the recommended restricted feeding schedule, appears to reduce behavioural problems of group housed sows at feeding (Brouns and Edwards, 1994), and to improve reproductive performance during seasonal infertility (Virolainen et al., 2004).

Thus, there is little contemporary evidence to support the view that low level feeding in early pregnancy may somehow be beneficial to subsequent litter size. Even so, the practice of feeding gilts and sows with low levels (1.5-2.0 kg/day) for the first 15-20 days post-mating is followed by many producers (see Figure 1.2). But, in the new leaner genotypes which account for smaller fat reserves levels and higher body reserves losses during lactation, it is important to start body reserves replenishment as earlier as possible during gestation. Thus, feeding sows with low levels for the first 3 days post-mating and feeding multiparous sows according to body condition from mating might be advised in modern genotypes.

1.1.2.2 Mid-pregnancy (*day 30 to day 80 of gestation*)

Current understanding of this period during gestation is poor. The general recommendation is to feed a constant level [2.0-2.9 kg/day of feed (12.1-12.6 MJ ME/kg)] sufficient to meet the energy requirements and assure a good body condition of the sow at farrowing. But recent research indicates that maternal nutrition may be critical in this period for muscle differentiation of the developing foetuses.

Physiologically, foetal muscle fibre development comprises the period between 25-30 and 90 days of gestation (Wigmore and Stickland, 1983) and, although the number of muscle fibres is mainly determined by genetic factors, also environmental factors seem capable to influence prenatal myogenesis. In this respect, either severe undernutrition or extra feed allowance during

this period has been experimentally related to a decrease and, although less agreed by consensus in the literature, an increase in the number of prenatally formed muscle fibres, respectively (Dwyer and Stickland, 1992; Dwyer et al., 1994; Gatford et al., 2003). Also, the application of porcine somatotropin (pST) treatment to the mother during the first quarter of pregnancy, has been capable to increase muscle fibre hyperplasia *in utero,* placental weight and weight of the lightest foetuses (Rehfeldt et al., 1993; Sterle et al. 1995; Gatford et al., 2003).

The mechanisms responsible for the influence of maternal nutrition on foetal myogenesis may involve alteration in maternal and foetal milieu, concretely glucose, insulin-like growth factor levels and other myogenic factors, but also alteration in placental morphology and efficiency. Recently, other studies have suggested that supplementation with β-agonist drugs (such as Ractopamine; Hoshi et al., 2005) or L-carnitine (a vitamin-like compound involved on the energy metabolism; Musser, 1999; Waylan et al., 2004) during this period of gestation, could also affect muscle fibre development of the progeny and increase subsequent litter size. The effect of the uterine environment (maternal nutrition, hormone release and others) on foetal muscle fibre development is reviewed with more detail in the section 1.4 of this chapter.

The number of muscle fibres, which is fixed at birth in mammals, correlates positively to postnatal growth (Pedersen et al., 2001). Thus, any factor capable of affecting muscle fibre development is, in turn, able to interact with the postnatal growth performance of the progeny (Dwyer et al., 1993; Rehfeldt et al., 2004b; Gondret et al., 2005) and probably, final meat quality traits at slaughter (Larzul et al., 1997). All these effects take part of the so-called “Foetal programming of postnatal performance”. Concretely, the effect of maternal nutrition during gestation on muscle fibres development of the offspring, postnatal growth performance and meat quality at slaughter constitutes the central objective of the present PhD dissertation.

If future investigations are able to confirm this effect, it would represent a new opportunity for the pig industry for improving growth performance and meat quality traits and also, a new objective in farms when developing the feeding strategy for pregnant sows.

Another important developmental event that takes place during mid-gestation is the mammary tissue development. Mammary gland development mainly occurs in the period between day 75 and 90 of gestation (Kensinger et al., 1982). This period has been identified as critical to assure an optimal milk production performance in the subsequent lactation. Weldon et al. (1991) reported that a high dietary energy intake by primiparous sows during this period (43.9 MJ ME/day) had deleterious effects on total parenchyma deoxyribonucleic acid (PCM DNA). This reduction in DNA reflected reduced mammary cell number. The same authors suggested that the increase in energy allowance during the period of mammary gland development may have derived in a replacement of mammary secretory tissue by fat cells, thus reducing capacity for

milk production. However, this negative effect on milk yield was not detected by other authors when the increased feeding plane involved late pregnancy (from day 100 of gestation until farrowing, Miller et al. 2000). On the other hand, Kusina et al. (1999a) reported that increasing protein levels from 5.5 to 15.6 % of CP (0.25-0.80 % of lysine) during gestation positively affected milk production and litter growth during lactation. However, no effects in the mammary gland structure were detected in order to explain this effect (Kusina et al., 1999b).

1.1.2.3 Late pregnancy (*last month of gestation*)

Energy and nutrient requirements for pregnant sows increase with advancing pregnancy, following the pattern of foetal development (see Section 1.1.1.1). Foetal weight is doubled over the last month of pregnancy with growth acceleration occurring, especially, in the last 10 days. Current feeding programs in the farm recommend increasing feeding plane in about 0.5-1 kg per day from day 90-100 of gestation until farrowing in order to optimize piglet weight at birth (see Figure 1.2). However, efforts to increase birth weight of piglets by increasing the rate of pregnancy feeding in the last trimester have lead to variable and unspectacular results in the literature. From the results of several studies, it is suggested that dietary treatments that increase birth weight or glycogen and fat stores of the newborn pig, may have effects on pigs with less than 1.0 kg at birth (Hillyer and Phillips, 1980; Cromwell et al., 1989; Aherne and Kirkwood, 1985). Aherne and Kirkwood (1985) reported that increasing sow feed intake by 1.0 to 1.5 kg/day for the last week of gestation increased piglet birth weight by 50 to 100 g, and prevented or reduced backfat thickness loss in the sow. Hovell et al. (1977) and Etienne (1991) supported the contentions of Aherne and Kirkwood (1985), but demonstrating that increases in birth weight of small pigs can be just as readily achieved by increasing the amount of feeding throughout pregnancy, as increasing it in the last quarter. Cromwell et al. (1989) reported that piglet weight at birth was enhanced by sow feed intake but only after 2 reproductive cycles, suggesting the possibility that this result were due to a positive effect on maternal body reserves, more than to a direct effect on foetal growth.

Overall, Whittemore (1998) concluded that piglet birth weight should not be a problem in sows which are not suffering from reproductive disease, and which are adequately fed throughout pregnancy. It is then suggested that the major advantage of increasing feed allowance during late gestation accrues to the sow herself, assuring an adequate sow condition prior to lactation (Aherne et al., 1999; Miller et al. 2000). When feeding programs during gestation do not fit the exponential growth being made by the foetal load during the last trimester of gestation, sows may become catabolic and lose BW and body lipid stores before lactation with the consequent negative impact on lactation and even lifetime performance. Close et al. (1985) showed that primiparous sows fed 20.1 MJ ME/day mobilized fat from day 87 of pregnancy, resulting in loss of up to 20% of the sow's fat reserves by the time of farrowing. Noblet et al. (1990) suggested

that the daily energy intake required to prevent mobilization of body fat in late gestation is 29.3 MJ ME/day in first parity sows.

But high levels of feed intake during late gestation are reported to predispose to agalactia, mastitis, dystocia and metritis (Göransson, 1989; Head et al., 1991). Whittemore (1998) suggested that the high levels of fatness presented by the sows from the above mentioned studies [Göransson, 1989; Head et al., 1991, Backfat (P2): 25-36 mm], more than the increased feeding level during this period, may be the real cause of this effect. In fact, other studies in which this feeding strategy has been applied showed no deleterious effects in farrowing and lactation performance (Cromwell et al., 1989; Miller, 1996; Miller et al., 2000). Detrimental effects on milk production seem then, restricted to increased feeding allowances during the period of mammary gland development (day 75 to day 90 of gestation; Kensinger, 1982) and to overconditioned sows at farrowing. Additional benefits of increasing feeding level during the last part of gestation have been reported, such as an increased feed intake during the subsequent early lactation (Neil, 1996).

Figure 1.2 shows the typical suggested feeding pattern for breeding sows throughout gestation and lactation (Coma, 1997; Boyd et al., 2002; Close and Cole, 2003; PIC, 2007).

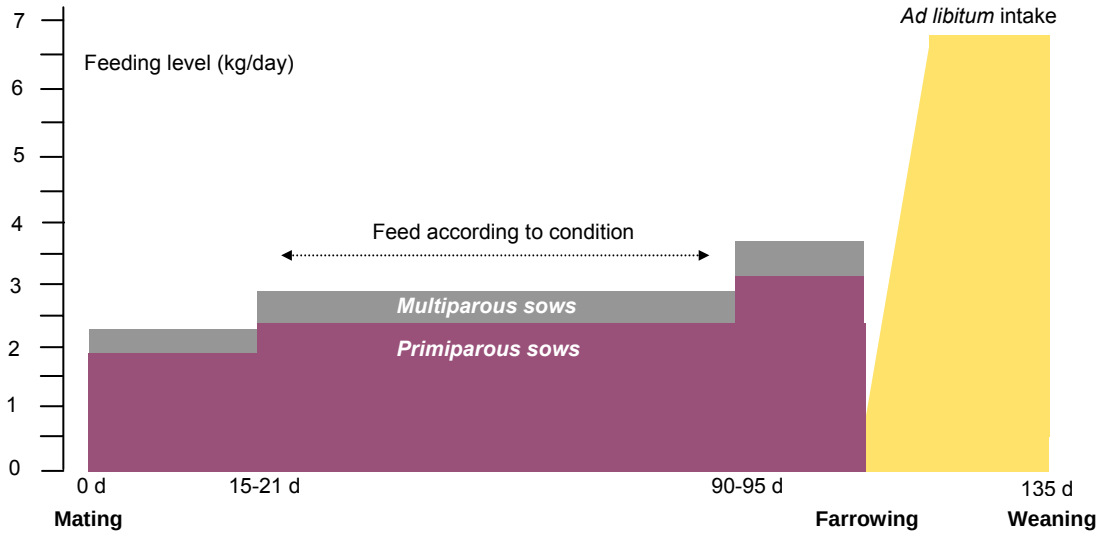


Figure 1.2 Feed intake pattern suggested for sows throughout gestation and lactation. Feeding level considers a commercial feed with an energetic concentration of 12.1 MJ ME/kg of feed.

The correct application of this scheme, as well as the optimal provision of the calculated energy and nutrient requirements for each animal, needs for an individualized control of feed intake to be effective. Only when it is possible, an optimum maintenance of body reserves from cycle to cycle and a low feed wastage will be achieved.

1.1.3 Future research in gestation feeding

The potential effects described of maternal nutrition on the offspring development have lead to the appearance of more specialized research associated with foetal development that will probably grow in popularity in the future. Specifically, functional research including the supplementation with omega-3 fatty acids as eicosapentaenoic (EPA, C20:5) and docosahexaenoic (DHA, C22:6) acids is being conducted in recent times (Brazle et al., 2005). Drawing comparison with its effects in human neonates and infants (Hornstra, 2005), these fatty acids are believed to assist in the development of brain function, which may lead to a more viable offspring at birth.

Also, trials involving the use of dietary conjugated linoleic acid have been conducted with positively results on immunologic variables in lactating sows and piglets (Bontempo et al., 2004).

Similarly, organic selenium sources are now available for use in sow diets. It has been reported that sows fed organic selenium derived higher levels of selenium in sow colostrum and milk, and in piglet tissue (Mahan and Kim, 1996), with potential consequences on piglet immunology. However, real impacts on foetal development are currently unknown.

These are examples of future research areas influencing foetal development and sow productivity, which will continue to improve gestating sow performance.

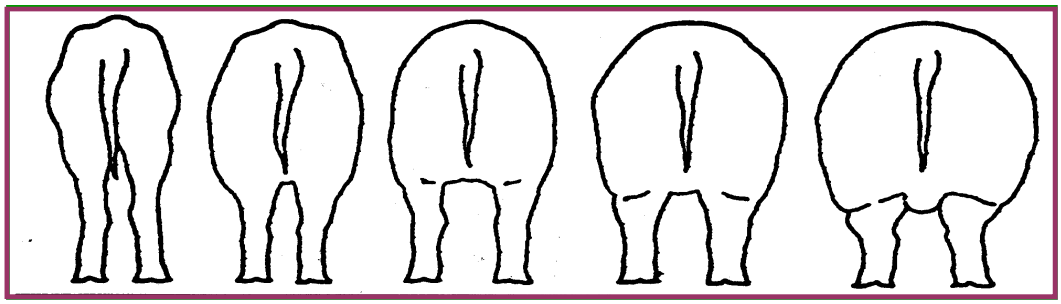
1.2 Why, How and When might sow body reserves be evaluated on the farm?

Feeding gestating sows should be focused on providing nutritionally adequate diets and to maintain optimal body condition. It has been well documented that both, overfeeding and not feeding sufficiently during gestation can create serious problems within the breeding herd (Dourmad et al., 1994; Young et al., 2004). In one hand, too little backfat reserves result in sows which are more susceptible to early culling due to reproductive failure, and also can be an animal welfare concern as thin sows have a greater chance of developing shoulder sores. On the other hand, overconditioned sows present farrowing difficulties, increases in the feed waste and lower performances in their subsequent lactation. Thus, developing a feeding strategy that lead to the maintenance of sow body condition over several parities is necessary in order to maximize sow productivity and longevity. As a part of the feeding strategy, once the requirements and the recommended feeding pattern during gestation have been addressed (Sections 1.1.1 and 1.1.2), the different means of monitoring sow body reserves and, indirectly, the feeding strategy applied are contemplated. In this section, also a brief description of the

normal pattern of body weight and body reserves changes, and a suggested schedule for body reserves evaluation, as well as targeted levels based on BF and BCS are provided.

1.2.1. Body reserves evaluation systems

In many commercial swine production systems, the body condition scoring method (BCS) is being used as a visual appraisal of sow body reserves from a long time, in order to adequate and regulate sow feed intake according to their condition in the farm. Usually, sows are assigned to a BCS ranging from 1 being very thin (emaciated) to 5 being very fat (grossly fat), as judged by a visual assessment and palpation of the hip and rib bones (Figure 1.3). Often, also intermediate 0.5 levels scale is used.



BCS	1	2	3	4	5
BF, mm	10- 12	16-18	22-24	28-30	+34

Figure 1.3 Body condition score (BCS) pattern according to a scale from 1 to 5 and its suggested equivalence in mm of backfat (BF, Close and Cole, 2003).

Due to its high subjectivity, it is advised that the determination of BCS must be done always by the same operator. The mean BCS desired depends on the physiological stage within the reproductive cycle but, in general, a BCS of 3 has been considered optimum in a sow herd. A BCS above or below this target should be adjusted by the daily feed allowance.

Despite its feasibility for being conducted in field conditions and, although having been used for a long time in most sow farms, its reliability in predicting body fat reserves has been recently questioned. Because of its inherent variability from one assessor to the next and also, due to the conformational changes suffered by the genetically leaner strains that have modified body shape making more difficult the differentiation of fat from lean tissue, BCS accuracy in predicting body reserves might not be the optimal in the new leaner strains. When comparing BCS with direct backfat levels measured ultrasonically at the level of the last rib (BF, mm, P2), recent studies have suggested that BCS and BF are poorly associated (Young et al., 2001;

Hughes and Smits, 2002; Maes et al., 2004). Correlation coefficients of 0.43 (Young et al., 2001) and 0.48 (Maes et al., 2004) have been reported. Young et al. (2001) observed a high variability in BF for each level of BCS measured (Figure 1.4). In agreement, Young et al. (2004) found, when comparing different methods of feeding gestating sows (based in BCS against based in BF+BW), that the feeding system based in sow BCS accounted for a considerable variation in sow condition at farrowing. However, when more objective methods such as BF or BW were introduced in body composition evaluation, feed wastage was minimized.

Thus, it seems that breeding sows production systems require from a more objective and accurate method for monitoring sow body reserves and in order to develop an appropriate control of feeding allowance on the farm.

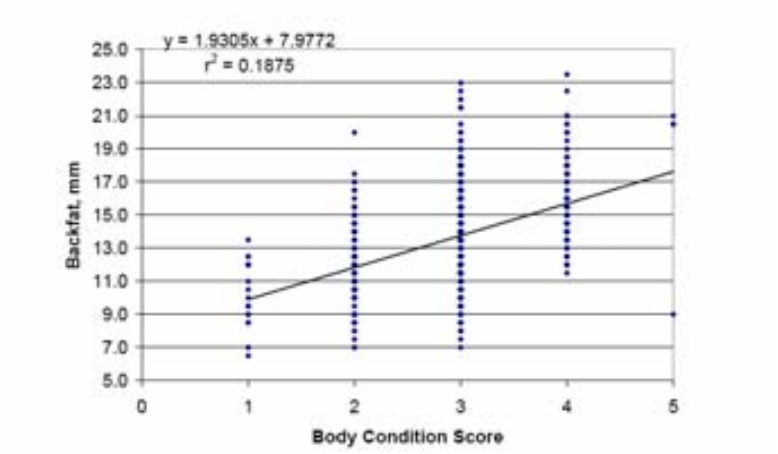


Figure 1.4 Relationship between body condition score and backfat thickness (mm) for gestating sows. A total of 731 sows were ultrasonically scanned at the last rib and correlated with a body condition score (1 = thin; 5 = fat) that was assigned by the farm manage (Young et al., 2001).

The ability to measure body condition changes in the live animal has been improved by the use of ultrasonic techniques. Ultrasounds, which are widely introduced in commercial farms to detect pregnancy status, are becoming a common tool on-farm also to determine body composition due to its objectivity, reliability and also easy and fast applicability in field conditions. Actually, the application of ultrasound technology in the swine industry dates to the late 1950s when Claus (1957) reported research results evaluating the feasibility of using ultrasound on the live pig to evaluate carcass composition, as an alternative of the invasive metal backfat probe method used to measure backfat depth (BF) since then. The initial findings in this area corresponded closely with the increased interest in producing leaner pigs according to the market demands and the establishment of swine testing centres throughout Europe and North America. Enhancing reproductive efficiency within the swine industry using ultrasound instrumentation to detect pregnancy status of breeding females (Fraser and Robertson, 1968) was, in fact, a second focal point for ultrasonic research and application.

There is a wide range of ultrasound equipments available in the market (Aloka, Renco Lean-meater, Pig-log,...). Although BF thickness is the parameter more frequently measured, some of them are also capable of measuring loin depth (LD).

Earlier ultrasound devices utilized amplitude-depth (A-mode) ultrasound technology (Renco Lean-meater, Krautkramer, Scanmatic, Scanoprodbe 731A), involving a sound emitted from a single quartz crystal located within the ultrasound transducer. Sound waves emitted travel through the biological tissues (skin, fat, fascia and muscle) and differences in acoustic density resulted in sound being reflected back through the transducer. Using the time-distance relationships, the reflected waves are converted into signals that are interpreted.

The introduction of B-mode ultrasound (Aloka, Technicare, Danscanner), consisting in multiple sound-emitting quartz crystals that produced a two dimensional image of the tissue being investigated on a video screen at a real time, represented a significant advance on the ultrasound technology. This allowed the entire loin and individual fat layers covering the loin to be viewed, and thus to increase the accuracy in measure composition of the live animal and carcass. However, experimentally, little differences in prediction ability have been reported between A-mode and real time ultrasound measures (Busk, 1986 cited by Moeller et al., 2002), probably due to the high variability still existing among B-mode ultrasound devices and the interpretation by the operator. Consequently, and also due to the relatively low investment cost and ease of operation make A-mode ultrasound devices widely used by seedstock selection, central testing programs, university and industry research and on-farm applications). In fact, recent studies encourage the use of BF levels in order to develop adequate feeding programs for breeding sows and give references in BF levels in order to optimize sow productive and reproductive performance (see the following section 1.2.3, Young et al., 1990; Tummaruk et al., 2001; Luborda, 2002; Close and Cole, 2003; Marco, 2004; Young et al. 2004; Kongsted, 2006).

Ultrasonic BF and LD can be measured at different locations within the animal such as in mid-line at the shoulder level (P1), at 6-6.5 mm of the dorsal line (P2) at the level of the last rib, and on the tail base (P3). However, the most commonly used and verified location is the P2 site. Results from our research in the Universitat Autònoma de Barcelona also showed higher correlation coefficients between two consecutive measurements from the same sows in P2 site compared to P1 and P3.

In swine, BF determined by ultrasounds at the P2 location is closely related to dissectable fat both, in growing pigs (Whittemore, 1993) and breeding sows (Whittemore at al., 1980). A work at the University of Nottingham (Harker and Cole, unpublished, cited in Cole, 1990) showed good relationships between ultrasonic BF measurement at P2 site on the life animal and BF measurement at P2 site on the carcass ($r = 0.93$), dissected fat in the body ($r = 0.82$) and chemically determined lipid in the whole body ($r = 0.96$) in primiparous sows. In a review paper

from Moeller et al. (2002), relatively high correlations of 0.89, 0.91 and 0.89 between live ultrasound, live ruler and carcass ultrasound backfat in relation to carcass backfat, respectively, where also reported when fat was measured at the centre of the pigs' backs.

But, as in the case of BCS, differences due to animals, species, technician and instrumentation has also been noted by many trials using ultrasonic devices as predictors of body composition. Summarized across trials, Moeller et al. (2002) reported that A-mode ultrasound may be less accurate at locations and on pigs where fat depth is large, because of its inability to define the third layer of fat that covers the loin muscle of the pig (Sather et al., 1986). Also, it has been reported that BF might not be as representative of total fat content in multiparous sows compared to primiparous, because of higher variations in body weight (King et al., 1986). Whittemore and Yang (1989) reported a good body lipid prediction by the combination of BF and BW in both, gilts and fourth litter sows.

Early attempts to measure loin muscle area with A-mode ultrasonic devices were reviewed by Moeller et al. (2002) and revealed moderate correlations with carcass loin muscle area measurements (Price et al., 1960, r = 0.74; Stouffer et al., 1961, r = 0.70). Differences in muscle shape, the lack of highly trained technicians animal movement and restraint and the inability to consistently differentiate the third layer of fat over the loin from the muscle has also been reported as possible factors of variation.

1.2.2 General pattern of live weight and body reserves changes in breeding sows

Sows normally gain body weight in pregnancy and loss it during lactation (Figure 1.5). Sows also accumulate fatty tissue in pregnancy and lose it in lactation, but for fat there is not the same overall positive balance as the sow increases in age and body size (Figure 1.6; Whittemore et al., 1980; Whittemore 1993). The expected ideal patterns of sow body weight changes and body fatness are represented in figures 1.5 and 1.6, respectively.

Whittemore (1993) reported in a review that over the first parities, pregnancy gains may be assumed to be both, lean and fat. Later, however, pregnancy gains are likely to be almost entirely of fatty tissues. In all parities (regardless of sow age), lactation losses are most likely to be fat alone, as this is the prime substrate for which there is likely to be a shortfall in relation to the requirements for milk synthesis. There may also be some protein mobilisation during lactation, but large amounts of body protein losses are generally restricted to situations of undernutrition during lactation (Clowes et al., 2003a; Quesnel et al., 2005a). At this point, it has been suggested that when they occur, protein losses are more detrimental on post-weaning performance than fat losses (King, 1987; Clowes et al., 2003a). This is probably due to the fact that they are more difficult to mobilise but also more difficult to built up once depleted. Also,

protein reserves are essential in order to achieve the mature drive for protein accretion and, consequently, an optimum reproductive efficiency in the breeding sow (Clowes et al., 1994; Everts and Dekker, 1994).

When the feeding strategy followed fail to allow the recovery of depot stores by the provision of energy supply in excess, a progressive decline of fat levels from cycle to cycle will occur. Unfortunate consequences for sows health and productivity will be then unavoidable (Yang et al., 1989; Whittemore, 1993; Eissen et al., 2003).

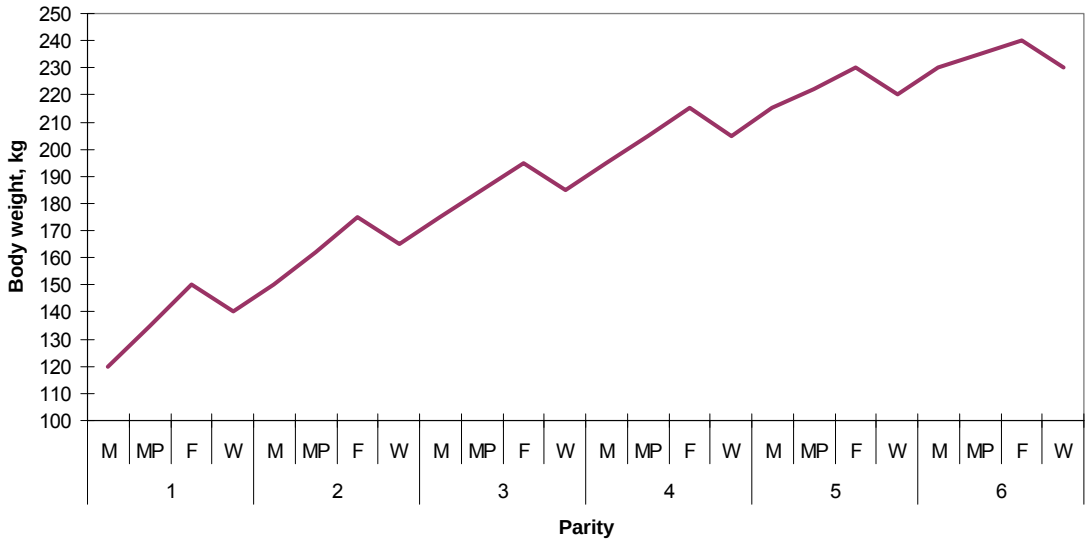


Figure 1.5 Expected pattern of sow body weight changes (M: mating, MP: mid-pregnancy; F: farrowing; W: weaning) from parity 1 to parity 6 sows (adapted from Whittemore, 1993 and Close and Cole, 2003).

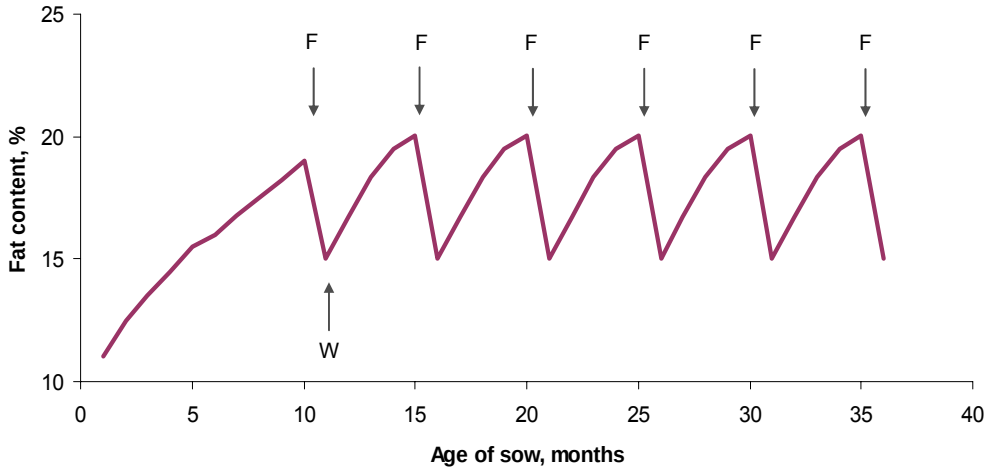


Figure 1.6 Expected pattern of sow changes in body fat content (F: farrowing; W: weaning) from parity 1 to parity 6 sows (adapted from Whittemore, 1993 and Close and Cole, 2003).

The optimum feed supply in order to maintain sows body reserves throughout their lifetime is sow-specific and requires from individual feeding strategies. In fact, this is one of the most difficult issues to be achieved when feeding group-housed sows.

It has been demonstrated that the variability among sow body reserves in sows allocated in different group-housing systems, may have adverse consequences on productive-reproductive traits (Kongsted, 2006). Therefore, special efforts are being conducted in the pig industry nowadays, in order to find the best feeding systems that really fit individual sow needs, having then no negative consequences on sow body reserves and on productivity (Chapinal, 2006).

1.2.3 Timing for body reserves evaluation and target levels on farm

In order to maintain sows in optimal body condition during all the reproductive cycle and avoid having extreme (very thin and very fat) sows in the herd, it is necessary to establish target levels at different times within the reproductive cycle, and to systematize the timing for body reserves evaluation in the breeding herd.

The recommended key times for body reserves evaluation on-farm are generally set at mating (gilts), at the time of the pregnancy test (30-35 days of gestation), before farrowing (at approximately 10 days before) and at weaning (Luborda, 2002; Marco, 2004; Marco and Barceló, 2006).

Suggesting optimum BF levels at different times is not an easy neither a safe task, since target BF levels may also depend on the sow age, genetics and other environmental factors. Whittemore et al. (1980) described substantial genotypic differences in sows fatness between breeding companies; BF (P2) at first mating ranging from 13.8 to 18.7 mm. Also, as BF represents an indirect measure of body fatness its variability will also depend on the selected methodology (A-mode *vs* B-mode ultrasound technology). So, previously to the establishment of target BF levels on-farm, an evaluation of the specific genetic line used and the methodology selected in order to carry out sow body condition measurements is recommended in order to adjust the desired levels.

Figure 1.7 represents an approximation to the ideal pattern of BF changes within one reproductive cycle.

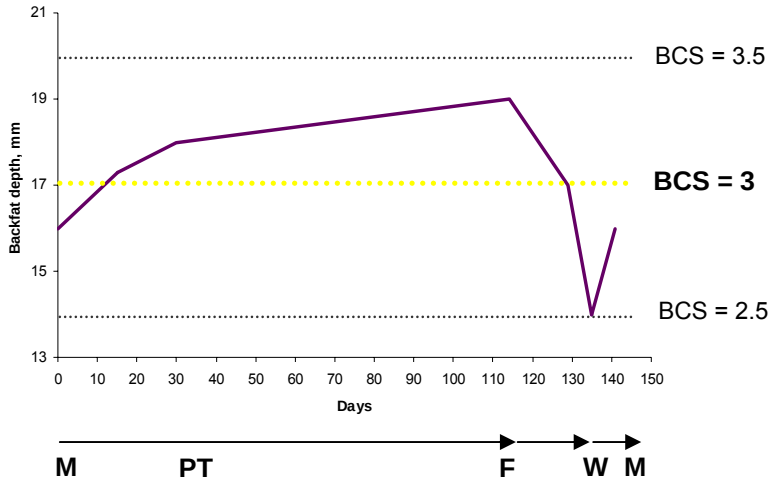


Figure 1.7 Suggested levels of backfat thickness (mm) along the reproductive cycle (M:mating; PT:pregnancy test; F:farrowing; W:weaning). Adapted from Young et al. (1990), Tummaruk et al. (2001), Luborda (2002), Marco (2004), Young et al. (2004), Kongsted (2006) and Marco and Barceló (2006).

Following the recommendations and suggestions of different studies and reviews in the literature (Young et al., 1990; Whittemore, 1993; Tummaruk et al., 2001; Luborda, 2002; Marco, 2004; Young et al., 2004; Kongsted, 2006; Marco and Barceló, 2006), the general suggested target levels at different times within the reproductive cycle are described as:

Gilts. Some minimum of body reserves (fat and lean tissue) and BW is generally required at first mating. If they are not adequately prepared in terms of body reserves at this time, the pursuit of a non fat-depleting regime throughout their subsequent life will be intolerable. But the appropriate weight, age and body reserves at first mating are much dependent upon the genotype of the pig. For improved genotypes, Close and Cole (2003) recommended that gilts must be first mated at, approximately 120 days of age, 120-150 kg of body weight and 18-20 mm of BF and these should coincide with the appearance of the third reproductive oestrus. But, these levels of fatness are unlikely to be achieved in our conditions in which a lower minimum, around 15 mm have been recommended. In gilts, evaluating body condition near the end of the growing-finishing phase (100 kg) also might serve as criteria for selecting them as replacement animals.

Sows: Assessing body condition of multiparous sows at the time of the pregnancy test might be useful in order to correct any deficit in the plane of feeding during the first part of gestation, where body reserves recovery must have started. It is generally set that sows might present at this time, the desired condition for the farrowing time. At approximately 100 days of gestation, sow body reserves control is again advisable in order to detect catabolic sows at the end of gestation and also overconditioned sows and to modify their feeding supply as convenient.

Some minimum of body reserves is necessary at farrowing in order to not to fall under 14 mm of BF at weaning. Also overconditioned sows (> 22 mm) must be avoided since they are susceptible to have a difficult parturition and also to present lower feed intakes during lactation. The desired body condition at farrowing is set between 17 to 22 mm of BF in our conditions. Body condition should be evaluated again at weaning, in order to know to which extent sows have mobilised body reserves during lactation, and in order to plan the most adequate feeding strategy for the weaning to oestrus interval period, and during next gestation. Backfat levels lower than 10-12 mm of BF at weaning are related to extended weaning to oestrus intervals, higher percentages of anoestrus or sows returning to oestrus after mating, and also a lower productive performance in the subsequent cycle.

1.3 Main interactions between sow feeding strategy, body reserves and productive performance

The implementation of a feeding strategy that leads to maintain condition over several parities is necessary to maximize sow productivity and longevity (Whittemore, 1993; Luborda, 2002). In this regard, it is important to remember that all the phases within the reproductive cycle are related, and that a deviation on body condition in one phase will have carry-over effects on subsequent phases and also in subsequent reproductive cycles. It is not possible, then, to design an appropriate feeding strategy for pregnant sows without considering its effects on lactation performance, post-weaning performance and, therefore, on lifetime performance.

The most important interactions described in the literature include the relationship between body reserves at first mating and lifetime productive performance and sow “stayability” in the herd, the potential negative effects of feeding plane during pregnancy on voluntary feed intake (VFI) during lactation and, in the last place but not for this less important, the relationship between the amount of body reserves lost during lactation and body reserves at weaning on subsequent productivity and post-weaning performance.

1.3.1 The influence of sow body reserves at first mating on productivity and longevity

In recent times, a concern exists about the increased sow early culling rates reported in the current commercial farms (more than 50% annually, PigCHAMP®, 1998-2002). The main reported causes for culling are failure to cycle or to conceive, failure to farrow, poor productive performance or death. Although underlying biological reasons for this to happen are complex, it is speculated that sows durability has suffered with the genetic selection for lower fat content that started at the decade of 1980’s (Whittemore et al., 1980).

In this regard, body condition (body reserves and body weight) of gilts when they begin their reproductive life at first mating, are key points for the success in productivity and lifetime performance thereafter in their life. To correct or compensate a deficiency or an excess of body reserves when gilts are inside the productive chain is extremely difficult. Brisbane and Chesnais (1996) reported that BF levels of gilts at 100 kg of body weight were positively related to their survival percentage in the herd over 6 consecutive parities (Figure 1.8). In their study, the difference in survival of sows until after the fourth parity was 10% higher in gilts from the highest BF category (> 18 mm) when compared to gilts from the leanest BF category (< 10 mm).

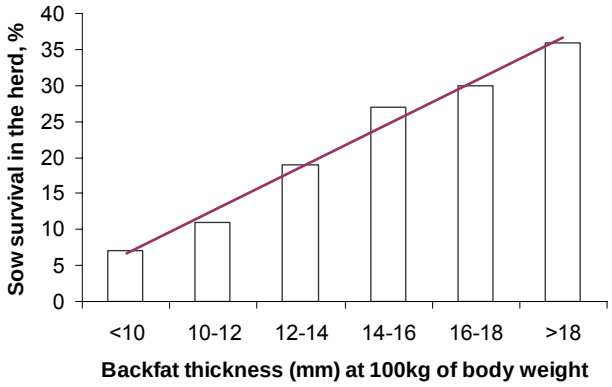


Figure 1.8 Sows survival percentage in the herd after 6 parities according to their backfat thickness (mm) at 100 kg of body weight (Brisbane and Chesnais, 1996).

Some years ago, Gaughan et al. (1995), Kerr and Cameron (1995) and Challinor et al. (1996) demonstrated the influence of backfat depth at first mating also on subsequent productive performance. In the study of Challinor et al. (1996), sows showing intermediate body weight and backfat levels (BW = 125-145 kg and BF = 18-20 mm) obtained a total of 9 additional live born piglets over five consecutive parities, compared to the thin (<15 mm BF) or fat (>20 mm BF) sows (Table 1.7).

Table 1.7 Sows productive performance according to body weight (BW, kg) and backfat thickness (BF, mm) at first mating (Challinoir et al., 1996).

BW	BF	Litter size at birth in the first parity	Average number of pigs over 5 parities[1]
117	14.6	7.1	51.0
126	15.8	9.8	57.3
136	17.7	10.3	56.9
146	20.0	10.5	59.8
157	22.4	10.5	51.7
166	25.3	9.9	51.3

[1] Only sows that completed the 5 parities

A more recent study (Tummaruk et al., 2001) reported that gilts with high BF levels at 100 kg of body weight (> 14-18 mm), had shorter weaning-to-first-service interval (< 6 days) in their first parity. Moreover, these authors obtained a positive relation between BF at 100 kg of body weight and the number of total born pigs in the second parity.

The number of studies about breeding herd longevity and productivity has increased in the recent past (Serenius and Stalder, 2004; Stalder et al., 2005; Tarrés et al., 2006). Stalder et al. (2005) conducted a very interesting study about the effects of the compositional traits in gilts on their lifetime (total) reproductive performance. In general lines, they demonstrated by sorting gilts according to BF and loin muscle area (LMA), that gilts in the lowest BF group (< 9.0 mm) had fewer lifetime number of piglets born alive when compared to females with higher BF. Additionally, females from the highest BF group (> 25 mm) also had more lifetime number of piglets born alive when compared to females in the medium group of BF (17 to 25 mm). In this study lifetime number of piglets born alive also increased with increasing sow LMA (from ≤ 31.5 cm^2 to ≥ 54 cm^2).

Overall, it seems evident that some minimum of BF and muscle is need before entering in the breeding herd in order to maximize productivity in terms of number of pigs born and thus, profitability of a commercial pork operation.

1.3.2 The influence of pregnancy feeding upon lactation performance

It is said that the challenge of lactation starts at conception and not at parturition. Sows fed a high level during gestation generally show lower voluntary feed intakes (VFI) during lactation (Mullan and Williams, 1989; Dourmad, 1991; Weldon et al., 1994; Revell et al., 1998a) and, consequently, higher body reserves losses during lactation. This effect seems more apparent throughout the two first weeks of lactation (Revell et al., 1998a).

This negative effect appears to be mediated through the level of fatness of the sow at parturition (Dourmad, 1991; Weldon et al., 1994; Revell et al., 1998a). There is a great variation among studies, however, in the quantitative importance of this phenomenon. In a review by Eissen et al. (2000) the effect of body fatness at farrowing on daily feed intake during lactation reported by different studies in the literature was illustrated by regression analyses [y = a + bx, where y = daily feed intake during lactation and x = BF thickness at farrowing]. Decreases of 18 and 129 g of daily feed intake during lactation for each 1 mm of increase in backfat thickness at farrowing were obtained for primiparous and multiparous sows, respectively, by Yang et al. (1989). Also for primiparous sows, Dourmad (1991) estimated a slope of -63 g of daily feed intake during lactation and mm of BF. Koketsu et al. (1996a) obtained a diminution of 19 g of feed intake per day and mm of BF at farrowing across all parities.

Overall, from these results it is deduced that although this effect seems evident, its final repercussion on feed intake appears to be small. Additionally, it is possible that the effect of fatness on VFI during lactation is not linear with increasing BF thickness at farrowing. In this respect, Mullan and Williams (1989) reported no significant reductions in voluntary feed intake during lactation up to levels of 25 mm of BF (P2) at farrowing. Similarly, Miller (1996) suggested that the effect of fatness might not be significant up to levels of 20 mm of BF (P2).

Mechanisms suggested to explain the effect of body composition at farrowing on lactation feed intake of sows are diverse. Increases of free fatty acids and glycerol, increased levels of insulin and leptin in blood, metabolic insulin resistance and glucose intolerance during lactation are some of the proposed mechanism (Weldon et al., 1994; Xue et al., 1996; Eissen et al., 2000). Perhaps, the most supported are those related with an increase in serum concentration of glucose by either increased insulin resistance (Weldon et al., 1994) or a reduced insulin secretion (glucose intolerance, Xue et al., 1996) after farrowing. As a result, the use of peripheral glucose is likely decreased and voluntary feed intake may be reduced to maintain blood glucose concentrations. Recent studies also suggest that leptin concentrations in blood could also play a role on modulating lactation feed intake. Leptin is a hormone synthesized by the fat cells and that exerts an effect on reproduction, immunology and control of voluntary feed intake (Barb, 1999). A positive relation has been found between leptin concentration in serum and BF levels at farrowing in primiparous (Estienne et al., 2003), and second parity sows (Estienne et al., 2000).

Contrary to fat reserves, body protein reserves at farrowing seem not to impair VFI during lactation. In this respect, Sinclair et al. (2001) demonstrated that VFI during lactation decreased when fat reserves increased at farrowing but not when this increase in fat reserves was accompanied also by an increase in protein reserves. Also Clowes et al. (2003b) reported no differences in feed intake during gestation when lean content was increased at farrowing.

Therefore, while fatness may negatively influence VFI during lactation, leanness may not. However, Whittemore (1998) reported that in modern genotypes with little tendency to excessive body fatness (25 mm of BF are hardly achieved), the positive benefits of attaining parturition in good body condition, and carrying lipid reserves available to support lactation, may far outweight the small negative effects of the undoubted, but slight, inverse relationship between pregnancy and lactation feed intakes.

1.3.3 Relationship between body reserves mobilization during lactation and post-weaning performance

Sows energy and nutrient requirements are high in lactation and, generally, lactating sows are notable to meet them through their VFI during lactation (Noblet et al., 1990; Eissen et

al., 2000). Consequently, mobilisation of body reserves during lactation seems unavoidable (Noblet et al., 1990). Feeding pattern during gestation may, then, assume that the animal will be unable to supply lactation demands and there will be an absolute need to draw on endogenous body reserves to resource the deficit. However, when the amount of BW and body reserves losses during lactation is excessive, this generally has a negative impact on subsequent reproduction performance and fertility (Aherne and Kirkwood, 1985; Einarsson and Rojkittikhun, 1993; Whittemore, 1996; Prunier and Quesnel, 2000; Jones et al., 2006). The main described consequences of this mobilisation can be summarized as:

- Extended weaning to oestrus interval (WEI)
- Dip in fertility
- Decreased subsequent litter size:
    - reduced ovulation rate
    - reduced embryo survival

What remains undefined in this area is the level of body weight or body condition losses and hence weight and body condition at weaning below which, this reduction in productive-reproductive efficiency occurs, but also the level of dietary energy intake necessary to prevent it. In this regard, Noblet et al. (1990) considered that about 10 kg of body weight loss during lactation was acceptable for mobilization, in order to not to impair subsequent performance.

Aherne and Kirkwood (1985) postulated that a reduction in subsequent reproductive performance occurred in sows after a loss of 10% to 15% of their body weight (a loss of 18 to 26 kg of BW in a 180 kg sow at farrowing) during lactation. Recently, efforts have been devoted in order to clarify or give additional information about the relation between body weight and body reserves lost and subsequent productive performance in the new leaner genotypes (Clowes et al., 2003a; Clowes et al., 2003b; Maes et al., 2004; De Rensis et al., 2005; Thaker and Bilkey, 2005; Weldon et al., 2006).

Thaker and Bilkey (2005) examined the effect lactation weight loss on subsequent performance in sows from different parities and concluded that, overall, lactation weight losses up to 15% (in respect to their weigh at farrowing) permitted sows returning to oestrus within 7 days post-weaning and to have farrowing rates at first service higher than 70%. However, higher losses (>15%) increased WEI and decreased farrowing rates. They also reported, in accordance with other studies (Vesseur et al., 1994; Weldon et al., 2006), that tolerance to BW loss during lactation varied across parities. In this regard, WEI increased when lactation weight losses increased above 5 % for parity 1 sows, but not until lactation weight losses exceeded 15 % for animals of parity 2 and more.

Additionally, it has been suggested that more than BW loss *per se*, this effect could be directly related to the amount of fat or protein tissue depleted. Large losses of fat tissue during lactation are clearly associated with a decline in lactation and reproductive performance (Yang et al., 1989; Whittemore and Yang, 1989). In fact, levels lower than 10 mm of BF at weaning have been related to higher culling rates due to reproductive failure (Young et al., 1990; Kongsted, 2006). However, King (1987) suggested that the loss of body protein during lactation may be of greater relevance to subsequent reproductive performance than the loss of fat tissue. A review of several data sets (in Aherne et al., 1999) shows that the fractional loss (%) of body protein during lactation accounts for almost half the variation in WEI (R^2 = 0.47). In contrast, less than a quarter of the variation in the same measure was accounted by the loss of body fat (R^2 = 0.24). Clowes et al. (2003a) reported that first parity sows could sustain losses of 9 to 12 % of their previously existing body protein mass at parturition, without any detriment on piglet growth and ovarian function. However, beyond this amount of protein loss (>12 %), piglet growth rate and many indices of ovarian function started to decline.

But it is important not to forget that the optimal body condition is represented by the equilibrium among fat and protein reserves. In this regard, Whittemore and Yang (1990) reported ideal protein-to-lipid ratios of 1:1.5 and Whittemore (1996) suggested that if protein-to-lipid ratio in primiparous sows falls below 1:1, reproductive function may be impaired.

Finally, Pettigrew (1998) proclaimed the existence of two schools of thought. The first one, supported by den Hartog and van Kempen (1980), holds that reproduction is impaired when body fat (or protein) content falls below some threshold level similarly to what has been described for women (Frisch and McArthur, 1974). The second, supported by Pettigrew and Tokach (1993), suggests that the reproductive system respond to metabolic cues which reflect the current metabolic state of the animal. However, in experimental conditions these two pathways are normally confounded because, inherently, lower final levels of body reserves may be related to a more catabolic state during lactation.

The physiological mechanisms proposed to explain the above outlined relationship between nutrition during lactation, body reserves loss and reproduction, have not been clearly determined. Briefly, the relationship between nutrition during lactation and WEI may involve LH (induced by GnRH) release pattern. Several studies have shown that LH levels and pulse frequencies at weaning are inversely related to WEI (Tokach et al., 1992; Kemp et al., 1995). In addition, lower feed intakes during lactation have been related to lower LH pulsatility. Tokach et al. (1992) reported that sows with prolonged WEI had fewer LH peaks per 6-hours and lower LH concentrations on day 14, 21 and 28 of lactation. But, sows with prolonged intervals not only had lower LH concentrations but also lower serum insulin concentrations (Tokach et al., 1992; Koketsu et al., 1996a). Therefore, insulin may also play a role in the influence of diet in reproductive function.

The causes of reduced subsequent litter size are focused on low ovulation rates and embryo survival post-mating. During the course of lactation, a gradual increase in follicle development is seen. Low feeding levels during lactation impair follicle development during and after lactation resulting in a lower number of follicles recruitable for ovulation (which result in a lower ovulation rate), and an impaired quality of eggs and follicular fluid (which may explain increased embryo mortality) (Koketsu et al., 1996a; Zak et al., 1997). The possible link of this effect with LH or plasma progesterone concentrations remains unknown.

1.3.4 Global strategy

To define the best global feeding strategy during the reproductive cycle, sows' requirements, feeding pattern and their interactions must be taken into account.

Generally, restricted feeding levels during gestation are advised during gestation. The reason is that high levels of fatness at farrowing lead to a lower voluntary feed intake during lactation. Moreover, excessive weight loss during lactation has been related to several common reproductive problems post-weaning. However, this feeding strategy has been questioned by some studies. Mullan and Williams (1989) and Dourmad (1991) reported that voluntary feed intake during lactation does not compensate for the restricted intake during gestation. Feed intake and body weight gains over the total reproductive cycle (pregnancy + lactation) was significantly higher when pregnancy feed intake was increased. Low gestation feeding level decreased backfat thickness and body weight at weaning, and tended to delay the return to oestrus after weaning especially in high producing sows (Dourmad, 1991).

Additionally, the efficiency of direct utilization of dietary energy for milk production ($K \approx 65\%$) is similar to the efficiency with which energy is stored as body fat during gestation and mobilized during lactation (Noblet et al., 1990). Therefore, it seems that the commonly described strategy of providing low energy intake during gestation and high energy intake during lactation is based on the demonstrated negative effects of gestation feed intake on feed intake during lactation and not on the efficiency of energy utilization.

Overall, it seems that the best feeding strategy for breeding sows is not universal, and must be adapted to each herd and situation. The common thing that one must born in mind is that long-term performance of sows is best served by minimising fluctuations in body weight and fat reserves, so avoiding the extremes of body condition and subsequent poor performance (Aherne and Kirkwood, 1985; Whittemore, 1993; Dourmad et al., 1994).

1.4 The impact of maternal nutrition during gestation on the offspring

The theory of the “Foetal origins of adult disease” or “Barker hypothesis” was originally published in humans more than 15 years ago (Osmond et al., 1993). It was based on that maternal nutrition and metabolism during gestation are major mechanisms by which the intrauterine environment programs the health of the offspring. In humans, from extensive epidemiological studies such as marked famine (i.e. “Dutch winter famine 1944-1945” or urban indian women situation) or maternal obesity (i.e. 30% of pregnancies in USA), it has been described that inadequate nutrition, as well as overnutrition or uncontrolled diabetes during pregnancy were linked to an increase in the neonate’s risk of metabolic disease (hypertension, diabetes and coronary heart disease) in their adult life (Hornstra et al. 2005; Kunz and King, 2007). This is believed to be a consequence of intrauterine metabolic adaptations that are thought to persist during the adult life. Foetal programming occurs not by changing the genes themselves but by altering the manner in which they are expressed during all life (Kunz and King, 2007).

This relatively new stream of consciousness has been also reported for livestock animals (pigs and sheep) as the so-called “Prenatal programming of postnatal performance” (Foxcroft and Town, 2004). In livestock species, growth performance characteristics and meat production are of high economic importance. Therefore, the knowledge of how prenatal events are able to influence foetal muscle fibre formation and its final consequences on postnatal growth performance, and on meat quality are of great interest for the pig industry. The above cited prenatal events include maternal nutrition, uterine crowding conditions, and the application of hormonal treatments *in utero*. In order to derive the effects of maternal nutrition during pregnancy on the offspring, the role of muscle fibres characteristics (number, size and type) on postnatal growth performance, as well as on the ultimate meat quality will be reviewed in the following lines. Also, the different sources of variation of muscle fibre traits will be documented.

1.4.1 Principles of postnatal muscle growth

Muscle growth rate is a major determinant of performance in livestock animals, since it is positively related to the daily weight gain, gain to feed ratio and meat percentage of the carcass (Dwyer et al., 1993; Rehfeldt et al., 2004b). Consequently, the understanding of growth and development of skeletal muscle is one of the most important goals in livestock animals and meat science. Skeletal muscle contains about 75% water, 19% protein, about 10 % lipids and 1% glycogen. It is mainly composed of muscle fibres, which are long, cylindrical and multinucleated cells that are embedded in connective tissue, a capillary network, nerve fibres and intramuscular adypocites. From the principles of skeletal muscle growth, it becomes clear

that muscle mass is largely determined by the number of muscle fibres, their size and also by their length.

In general, muscle fibres number is set at birth in mammals (Staun, 1963; Wigmore and Stickland, 1983; Ashton et al. 2005) and at hatching in birds (Ashton et al. 2005), but continues during all life in fish muscle (Stickland, 1983; Ashton et al. 2005). Hyperplasia does not occur to any significant extent after birth. Only the undifferentiated satellite cells may show a proliferative activity as a source of new nuclei incorporated into the muscle fibres, although with a very limited repercussion in muscle growth postnatally. Thereafter, any postnatal muscle growth in mammals relies largely on protein accretion and increased muscle fibre size leading to muscle fibre hypertrophy (Rehfeldt et al., 2004b).

The number of muscle fibres at birth has been markedly related to increased growth potential of skeletal muscle post-weaning, and also has been reported to increase muscle adaptability to environmental stress in farm animals (Dwyer et al., 1993; Rehfeldt et al., 1999; Pedersen et al., 2001). But, total muscle mass or lean meat percentage has been positively correlated with both, the number of prenatally formed fibres and also the degree of their postnatal hypertrophy (Miller et al., 1975; Larzul et al., 1997; Henckel et al., 1997; Fiedler et al., 1997).

On the other hand, postnatal muscle fibre hypertrophy is inversely correlated with the total number of muscle fibres within a muscle. As summarized by Rehfeldt et al. (2000), this negative correlation in the literature ranges from r_p = -0.3 to -0.8. Thus, the postnatal growth rate of the individual muscle fibre is lower when there are high numbers of fibres and higher when there are low numbers of fibres. Consequently, as the total number of muscle fibres is determined at birth, postnatal muscle fibre hypertrophy will strongly depend on the total number of muscle fibres formed within a given muscle.

But, it has been described that the postnatal increase in muscle fibre size is limited by genetic and physiological reasons, and that it increases towards a plateau (Rehfeldt et al., 1999). A clear example of this fact is found in pig genotypes with large muscle fibre types as it is the case of improved genetics and high conformed breeds as is Piétrain, which represent the pig breeds with the largest muscle fibres (Lefaucheur et al., 2004). These pigs are reported to be less responsible to the increased fibre size due to treatments with somatotropin than other genotypes with smaller fibres (Rehfeldt and Ender, 1995). Therefore, the ultimate potential for lean growth will largely depend on the number of prenatally formed muscle fibres. Nevertheless, current pig production systems may not allow achieving the maximum potential for growth of the animals (Rehfeldt et al., 1999) so that, in most cases, muscle fibre hypertrophy may be of greater interest than fibre number on achieving a maximum meat percentage (Larzul et al., 1997).

The negative correlation reported between the number of muscle fibres and size may also be present at birth. In this regard, Handel and Stickland (1984) early pointed out that the smallest pigs in the litter (runt pigs) exhibited larger muscle fibres at birth, as a result of the lower number of fibres developed *in utero*, when compared to their heavier littermates. More recent studies confirmed this effect in small but not necessarily runt pigs (Nissen et al., 2004; Gondret et al., 2006; Rehfeldt and Khun, 2006). Small pigs at birth also showed larger fibres than their littermates at a given body weight, in association with an advanced age and they also present fatter carcasses, denoting a higher physiological maturity. In this regard, Rehfeldt et al. (2000) hypothesised that the plateau of muscle fibres growth was achieved earlier at lower fibre numbers, and afterwards, available nutrients are preferentially used for fat deposition. Not surprisingly then, pigs small at birth will hardly achieve growth rates or live weights similar to those of their heavier littermates, even when a catch-up growth phenomena exists.

Finally, in determining lean growth postnatally, the specific relationships between fibre type composition of a given muscle and growth performance is still highly controversial (Lefaucheur, 2006).

1.4.2 Muscle fibre type composition and classification in the adult pig

One of the unique features of skeletal muscle is its fibre architecture. In adult livestock animals, given muscles generally contain a mixture of different types of myofibres. Only few muscles such as the *soleus* muscle or the superficial part of the *semitendinosus* muscle, comprises only one fibre type (oxidative and glycolytic, respectively). Variation in myofiber types postnatally may be an important factor for adaptations to the functional demand, fatigability and fuel homeostasis. For instance, muscles used for postural maintenance tend to have more fibres with higher oxidative capacity (*Type I or slow-twitch oxidative fibres*), than those muscles solely used for rapid bursts of activity and forceful contractions such as running that normally show higher proportions of glycolytic fibres (*Type II or fast-twitch glycolytic fibres*). Examples of the different fibre type distribution observed between two different muscles with two different functions (*longissimus* muscle and dark portion of the *semitendinosus* muscle) are shown in figure 1.9.

Typically, in the adult pig different fibre types are organized in a clustered pattern with islets of slow fibres (*Type I or slow-twitch oxidative fibres*) surrounded by fast oxidative fibres (*Type IIA or fast-twitch oxidative glycolytic fibres*), and at the periphery by fast non-oxidative fybres (*Type IIB or fast-twitch glycolytic fibres*). This clustering pattern might have functional implications in the pig in view of the gradual recruitment of fibres in relation to the intensity of exercise (Lefaucheur et al., 2002). Other species such as cattle, goat, sheep and horse do not show this organized muscle fibre arrangement (see review by Reggiani and Mascarello, 2004).

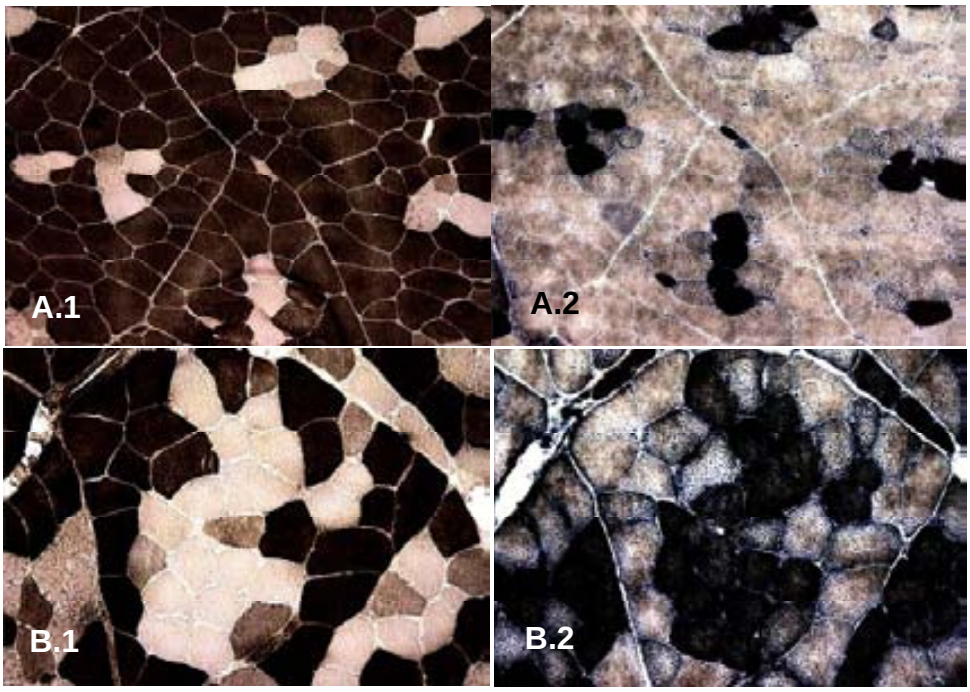


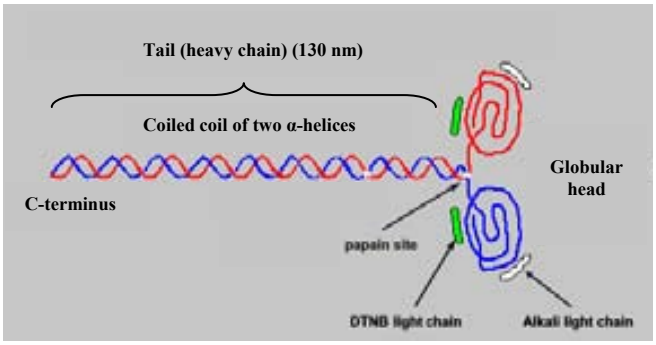
Figure 1.9 A. *Longissimus* muscle histochemical sections from an adult pig stained with alkaline myosin ATP-ase at pH 10.3 (A.1), and stained with the combined technique myosin ATP-ase + NADH (A.2); B. *Semitendinosus* muscle sections from an adult pig stained with alkaline myosin ATP-ase (B.1), and stained with the combined technique myosin ATP-ase + NADH (B.2). Images provided by the Research Institute for the Biology of farm animals (FBN, Dummerstorf).

Skeletal muscle fibres vary considerably and basically in their energy metabolism (oxidative and glycolytic), contractile properties (slow-twitch and fast-twitch) and colour (red and white), and these properties have been generally used to classify them. However, fibre typing is an extremely complex problem, because of the different perspectives scientists have brought to the matter. Anatomists early described muscle fibres as red or white (Yellin and Guth, 1970; Ashmore and Doerr, 1971), physiologists talk about fast and slow contraction speeds fibres (Brooke and Kaiser, 1970), and biochemists think in terms of metabolism types such as primarily oxidative or glycolytic (Solomon and Dunn, 1988). It has been even suggested that there are as many ways of naming fibre types as laboratories involved in their investigation. Whatever the system used for classification, it should be based, exclusively, on the parameter being studied and thus, will depend upon the technique used for identification (see Section 1.4.2.2).

1.4.2.1 Fibre type classification and myosin isoforms

Myosin is the predominant protein in skeletal muscle (40-50% of the total muscle proteins) and the determinant of both mechanical properties (contraction, slow or fast) and energy consumption of the muscle fibres (metabolism, oxydative or glycolytic). Some of the

biologically important properties of myosin are its ability to combine with actin forming the complex called actomyosin to produce muscle contraction and the hydrolysis of ATP to produce energy for muscle contraction and relaxation. Myosin is a hexameric protein that consists in four light chains (MyLC) and two heavy chains (MyHC) (Figure 1.10), each of which exist in numerous isoforms and can combine differently within a muscle fibre.



Adapted from King and Marchesini (2002).

Figure 1.10 Diagram of a myosin molecule. A long coiled-coil rod that carries the heavy chains connects to the globular domain by a flexible neck region that carries the light chains (alkali and DTNB light chains, so named for the methods used for their extraction). The globular domain has an ATP-ase function that is the basis for the power stroke during muscle contraction.

Myosin heavy chains isoforms are similar enough to replace each other, but also diverse enough to give the fibre distinct functional properties (such as contractile properties) since, as Schiaffino and Reggiani (1996) also reported, each myosin isoform is associated with specific kinetics of actomyosin (combination of myosin and actin) interaction and ATP hydrolysis. As such, myosin ATP-ase hystochemistry has proven to be useful for the delineation of muscle fibre types (see Section 1.4.2.2). Thus, MyHC isoforms are generally considered as the molecular marker of the fibre type, so that different fibre types exhibit different MyHC isoforms and are often indicated using the name of the myosin isoform that is expressed.

According to the major MyHC isoforms found in mixed adult pig muscles, four pure fibre types exist, as shown on the basis of RT-PCR amplification and sequencing of all four transcripts and subsequently confirmed by histochemistry (myosin ATP-ase reaction or combined use of myosin ATP-ase and NADH staining), immunohistochemistry using monoclonal antimyosin antibodies, and *in situ* hybridization (Chang and Fernandes, 1997; Gil et al., 2001; Lefaucheur et al., 2002; Toniolo et al., 2004). These four distinct pure fibre types identified in the pig are the classified as slow type I fibres (with MyHCI as the major MyHC isoform), and three fast types namely IIA (MyHCIIA isoform), IIX (MHCIIX isoform) and IIB (MHCIIB isoform). The coexpression of specific pairs of these major MyHC isoforms results in the formation of hybrid MyHC combinations such as IIA-IIX and IIX-IIB fibres (for recent review see Pette and Staron, 2000; Lefaucheur et al., 2002; Toniolo et al., 2004). These hybrid fibres bridge the gap between the pure fibre types. The fibre population of skeletal muscles, thus, encompasses a continuum of pure and hybrid fibre types.

Muscle fibres are dynamic structures capable of altering their phenotype in the adult animal by changing their contractile and metabolic properties, under various conditions as increased or decreased neuromuscular activity, mechanical loading or unloading, altered hormonal profiles, and aging. Thus, changes can be induced in MyHC isoform expression throughout life heading in the direction of either fast to slow or slow to fast (for recent review see Pette and Staron, 2000).

1.4.2.2 Methods for fibre type classification

To date, the most informative methods to delineate muscle fibre types are based on specific myosin profiles, especially the myosin heavy chain (MyHC) isoform expressed. Fibre typing methods consist either on the determination of muscle fibre contractile and metabolic properties (histochemistry), or on the assessment of the MyHC isoform expressed (immunohistochemistry, electrophoresis and molecular biology).

Histochemistry

Histochemical techniques are based on determining enzyme activities in histological muscle cross sections obtained, generally, from blocks of frozen muscles that are then cut in a cryostat. These techniques provide some morphological information of muscle fibres such as muscle fibre size (cross-sectional area or diameter) and total number, but also allow the identification of the different muscle fibre types. Fibre counting and typing from histochemical muscle cross sections need from modern image analysis systems that are currently available.

Histochemical methods constitute a qualitative assessment of the myosin complement and, therefore, the ability to delineate the multitude of potential hybrid fibres from these methods is limited. Normally, they lead to the identification of two or three fibre types. Nevertheless, histological methods are still the most widely used for counting and typing muscle fibres, at least, in developmental studies in which the simultaneous determination of muscle fibre number, morphology and fibre type is necessary.

In the literature, various methods of fibre type classification based on particular enzymes' histochemical reactions have been described (Table 1.8). Myosin ATP-ase reaction (mATP-ase) is based on the different contractile activity of the different fibre types and also on their sensibility to either alkaline or acid pre-incubation (Brooke and Kaiser, 1970). The interaction between the MyHC and actin molecules in the muscle fibre causes the hydrolysis of ATP, the rate of which is the major determinant of the speed with which a muscle fibre contracts. Myosin heavy chains act as isoenzymes in the hydrolysis of ATP, and the different MyHC isoforms found in adult muscle correlate with different speed of ATP hydrolysis and, consequently, with the speed of contraction of the muscle fibre (Wigmore and Evans, 2002). The mATP-ase activity

of type I fibres (slow twitch) is inhibited after the alkaline preincubation, while that of type II fibres (fast-twitch) is inhibited after the acid pre-incubation. The use of mATP-ase staining is normally restricted to the separation between type I (slow) and type II (fast). However, by pre-incubating muscle sections at various pHs, other muscle fibres subtypes (IIA and IIB) have been demonstrated in pigs (Peter et al., 1972; Suzuki and Cassens, 1980). Different pH lability has been reported for different species such as human, rat and rabbit (Brooke and Kaiser, 1970). Therefore, pre-incubation conditions (pH, time and temperature) when using mATP-ase demonstration must be pre-defined for different species, but also for different ages and laboratories.

Table 1.8 Main features of muscle fibre types classified using different nomenclature systems (Brooke and Kaiser, 1970; Asmore and Doerr, 1971; Solomon and Dunn, 1988).

Properties	***Reference***	***Fibre types***		
	Brooke and Kaiser, 1970	*I*	*IIA*	*IIB*
	Asmore and Doerr, 1971	*βR*	*αR*	*αW*
	Peter et al., 1972 *Solomon and Dunn, 1988*	*SO*	*FOG*	*FG*
Physiology	Speed of contraction	Slow	Fast	Fast
	Fatigue resistance	+++	++	+
Morphology	Colour	Red	Red	White
	Sectional area	+	+++	+++
Metabolites	Glycogen	+	+++	+++
	Lypides	+++	+++	+
Enzymatic properties	Myosin ATP-ase activity	+	+++	+++
	Glycolitic enzymes	+	++	+++
	Oxidative enzymes	+++	++	+

Adapted from Picard et al., (2002)

Myosin ATP-ase has been used alone or in combination with other histochemical techniques that determine the activity of some oxidative enzymes such as NADH tetrazolium reductasa (NADH-TR) or succinate dehydrogenase (SDH) (Moody and Cassens, 1968; Solomon and Dunn, 1988). These techniques are also important in so far as they reflect the utilisation of various metabolic intermediaries of the Krebs cycle and related pathways. They give an indication of the possible sources of energy in muscle metabolism. In combination (mATP-ase reaction + oxidative enzymes) lead to the identification of three types of fibres: slow oxidative (SO), fast oxidative-glycolytic (FGO) and fast glycolytic (FG) (Peter et al., 1972; Solomon and Dunn, 1988), or also: ATP-ase acido resistant and oxidative metabolism (βR), ATP-ase acido labile and oxido-glycolytic metabolism (αR) and ATP-ase acido labile and glycolytic metabolism (αW) (Ashmore and Doerr, 1971). All these classifications are the most commonly used (Table 1.8, Picard et al., 2002).

Studies comparing various conventional histochemical methods report nonidentical results, which is caused mainly by a lack of correspondence between the subgroups of type II fibres (type IIX and type IIB mainly), that are normally not separated through hystochemical demonstrations. However, further subtypes of fibres can be outlined by means of other methods such as immunohystochemistry, electrophoretic analysis and molecular biology, suggesting differences in the molecular composition of myosin within a class.

Immunohistochemistry

Immunohistochemical methods are based on the use of antibodies directed against MyHC isoforms. They constitute a qualitative and powerful approach to typing fibres. Some antibodies are very effective in identifying corresponding MyHC isoforms in different species. In this way, reactivity with specific antibodies shows that muscle fibres histochemically identified as type I or IIA in livestock animals, express a type I MyHC or, respectively, a type IIA MyHC similar to those expressed in the type I or IIA fibres of rat or rabbit muscles, suggesting that type I MHC and type IIA MHC are highly conserved among species (Lefaucheur et al., 2002).

However, antibody reactivity does not clarify completely the identification of the other two types of fast fibres, IIX and IIB (Lefaucheur et al., 2002; Toniolo et al., 2004). A specific IIX monoclonal antibody has been recently described in the mouse, guinea pig, rabbit, cat, and baboon (Lucas et al., 2000), but it is not yet commercially available and has not been tested in pig muscle fibres. Thus, the main limited factor in the pig remains the lack of a monoespecific type IIX antibody (Lefaucheur et al., 2002). Without any specific antibody for MHC-IIX, the precise identification of which fibres express IIX and which express IIB MyHC remain uncertain. However, immunohistochemistry appears to offer an advantage over mATP-ase histochemistry because this method allows the detection, at least in most cases, of hybrid fibres (Pette and Staron, 2000).

Gel electrophoresis and immunoblotting

As MyHC isoforms are assumed to be the molecular markers of fibre types, the electrophoretic separation of MyHC isoforms represents a direct approach to attribute a single muscle fibre to a given type or to determine the composition of a given muscle. Separation of MyHC isoforms by electrophoresis has been achieved in laboratory rodents and in human muscles. In livestock animals, results are still uncertain. Only type I MyHC seems to migrate consistently in all species until now. The type II MyHC changes their migration pattern from specie to specie. In pigs, electrophoresis has not achieved the separation of the corresponding four MHC isoforms (Bee et al., 1999). Also, it seems that the sequential order of myosin isoform migration is species-specific and it is possible that different isoforms migrate with the same speed and overlap each other (Picard et al., 1999; Toniolo et al., 2004). Consequently, although

electrophoretic analyses yield important information about the composition of porcine skeletal muscle, it needs from the combination with other methods (histochemical or immunohistochemical) or single fibre studies to accurately characterize porcine skeletal muscles.

Molecular biology

Molecular biology techniques are considered the most precise and accurate methods in order to identify muscle fibre types since they are based on the direct determination of their nucleotide and amino acid sequence. Using the variable parts of the MyHC molecule it is possible to design specific primers for RT-PCR, or probes for *in situ* hybridization. Expression studies based on RT-PCR clearly demonstrated that four distinct MyHC isoforms, identified as type I, type IIA, type IIX and type IIB for homology with other species are expressed in adult pig skeletal muscles. Thus, it seems that RT-PCR is a reliable tool used to determine which MyHC genes are transcribed in a given tissue sample (Pette et al., 1999). Sequences for all sarcomeric MyHC genes of all four adult skeletal muscle MyHC (type I, type IIA, type IIX and type IIB) are available in pigs (Chikuni et al., 2001).

1.4.3 Prenatal muscle development

Muscle fibre formation occurs prenatally and it is largely completed around the time of birth in mammals (Rehefldt et al., 1999; Picard et al., 2002). The early myogenesis is a multistep process. Briefly, during embryonic development, myoblasts form from myogenic precursor cells, which are cells of mesodermal origin determined to enter in the myogenic lineage (Figure 1.11).

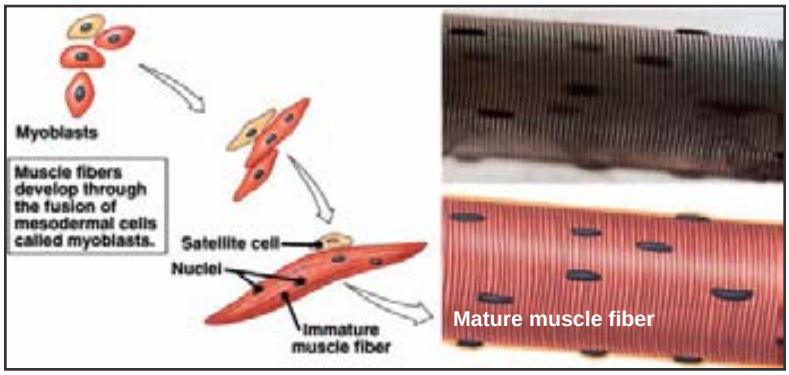


Figure 1.11 Myofibre hyperplasia and differentiation scheme during the prenatal period. *Source: Adapted from Randall et al. (1998).*

The determined myoblasts are then able to proliferate and to divide to establish a pool of myoblasts (hyperplasia). At some point of the development, special signals make the myoblast withdraw from the cell cycle, to stop dividing and to differentiate (differentiation). In this moment, myoblasts start to express skeletal muscle specific-genes, proteins (as developmental MyHC isoforms) and finally fuse with adjacent myoblasts to form multinucleated myotubes (myofibres) (for recent reviews see Rehfeldt et al., 2000; Maltin et al., 2001; Picard et al., 2002; Oksbjerg et al., 2004).

The final stage in the formation of muscle fibres is biphasic as it implies two waves of differentiation (Wigmore and Stickland, 1983). The first wave of myotubes gives rise to the formation of large primary muscle fibres. Primary muscle fibres provide a framework for the formation of the secondary fibres, in a second wave of differentiation of foetal myoblasts. Because the cross-sectional area of primary myofibers are up to six-fold larger than that of the secondary fibres (Wigmore and Stickland, 1983; Christensen et al., 2000), many more and smaller secondary myotubes than primary ones are formed in all mammals (Figure 1.12).

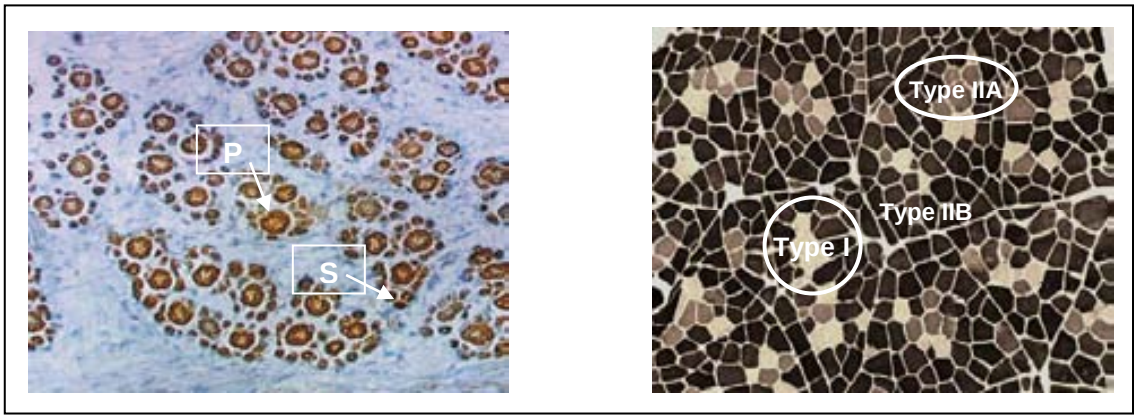


Figure 1.12 Pig muscle cross sections on day 62 of gestation (1.12A) and on day 178 of age (1.12B). A) *Semitendinosus muscle* with primary (P) and secondary (S) fibres (aniline-blue/orange G staining). B) *Longissimus muscle* with type I, type IIA and type IIB fibres (myosin ATP-ase reaction, pH = 10.3). Images provided by the Research Institute for the Biology of farm animals (FBN, Dummerstorf; 1.12A) and by the author (1.12B).

The number of secondary fibres around each primary fibre varies from 5 and 9 in the mouse and rat, respectively (Ross et al., 1987; Ontell et al., 1988) to over 20 in larger species such as the pig (Wigmore and Stickland, 1983; Stickland and Handel, 1986). Wigmore and Stickland (1983) reported that larger primary fibres were correlated with higher secondary fibre numbers. Thus, maternal treatments capable to increase primary fibres size *in utero* will support the formation of more secondary fibres (Rehfeldt et al., 2001).

The timing of formation of these two populations varies with the species according to their maturity. In pigs, primary fibres form from 35 until 60 days of gestation and secondary fibres population develop from 54 to 90-95 days of gestation (Wigmore and Stickland, 1983; Lefaucheur et al., 1995, see Figure 1.13). Also, during secondary muscle fibre formation the primary fibres continue to increase in size and to recruit nuclei (Zhang and Mclennan, 1995). Besides, the existence of a third wave for myotube formation around birth has been occasionally suggested in large mammals such as the pig (Lefaucheur et al., 1995). Finally, there is another population of myoblast that do not differentiate and remain mitotically quiescent during all life. These are satellite cells and they contribute to post-differentiated muscle fibres growth and they participate in regeneration processes (see Figure 1.11).

The total number of muscle fibres is generally considered to be established by 90-95 days of gestation in pigs, although this time can vary among different muscles, since not all muscles in the foetus are formed at the same time in gestation (Dwyer and Stickland, 1992). From late pregnancy through the first postnatal weeks, these fibres undergo a process of maturation from which the highly organized clustered pattern of slow central fibres surrounded by fast fibres encountered in the adult pig is established (Suzuki and Cassens, 1980; Fonseca et al., 2003, Figure 1.12). Generally, it is considered that in pigs, one of the fibres in each slow cluster would have been the primary myofibre during development. In most other species such as cattle, goat, sheep and horse the myofibres become mixed so that this pattern is not seen (Reggiani and Mascarello, 2004).

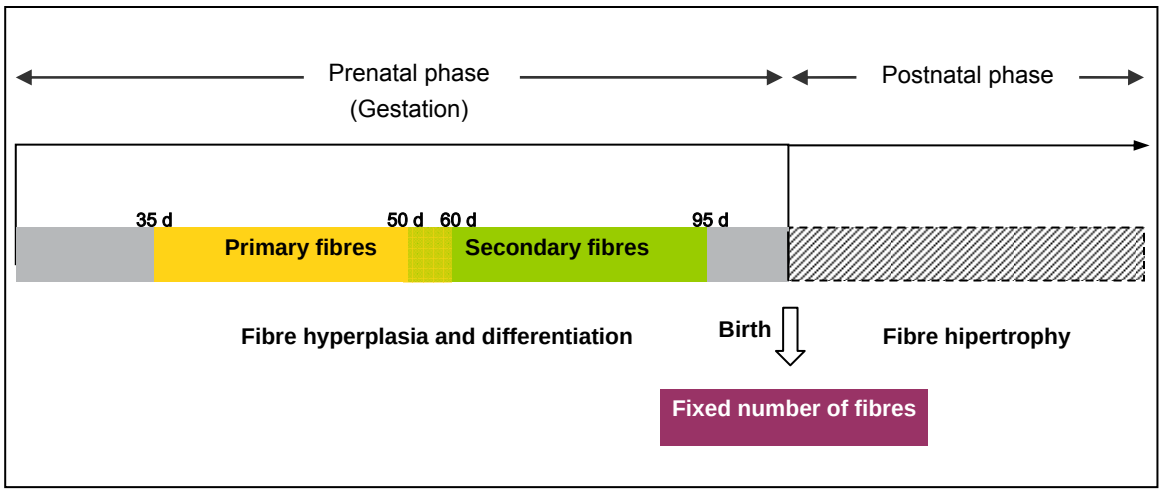


Figure 1.13 Scheme of the main characteristics of pig muscle fibres development *in utero*.

Numerous studies in the literature suggest that primary fibres are resistant to manipulation *in utero*, whereas secondary fibres are preferentially affected when variations in the uterus environment occur (Wigmore and Stickland, 1983; Ward and Stickland, 1991; Dwyer and Stickland, 1992; Tilley et al., 2007). But, although widely expected, this was not confirmed in

other studies in which increases in total number of fibres implied both, an increase in the number of primaries and the number of secondaries (Dwyer and Stickland, 1991; Rehfeldt et al., 2001; Fahey et al., 2005; Rehfeldt and Khun, 2006). Thus, it seems that although secondary fibres may be more influenced by factors in the uterus environment (nutrients; hormones; uterine restriction), under certain circumstances also type I fibres could be affected by environmental factors.

In both primary and secondary myofibers, developmental myosin heavy chains (MyHC) are progressively replaced by adult MyHC during the prenatal and perinatal periods. Primary fibres initially express developmental type I MyHC isoforms (slow myosin ATP-ase activity). They subsequently mature to type I (slow) fibres in most muscles, but can also give rise to fast type II fibres in pure fast-twitch muscles, such as the superficial white proportion of the *semitendinosus* muscle. Secondary fibres express developmental type II MyHC during the foetal period but they do express type I MyHC isoforms also at late gestation. As a result, a subpopulation of secondary fibres situated near primary fibres mature to type I fibres, perinatally, forming the so-called “type I clusters”. Adult fast type IIA MyHC is present in some secondary fibres during the foetal period, whereas IIX and IIB appear shortly after birth (Chang and Fernandes, 1997).

During the first postnatal weeks, secondary fibres which do not express type I MyCH mature to either type IIA, IIX or IIB fibres (Handel and Stickland, 1987; Picard et al., 2002). Thus, the secondary fibres mostly mature to fast fibres in fast muscles and to either fast or slow fibres in the mixed muscles (Lefaucheur et al., 1995; Picard et al., 2002).

During foetal life the oxidative metabolism represents the main source of energy in pigs. In fact, at birth, the majority of muscles are oxidative. But the oxidative potential decreases and the glycolytic potential increases with the age.

1.4.4 Sources of variation in skeletal muscle fibres number, size and type

The difference in muscle fibre number and size found between species, individuals and even among different muscles within the same individual, account for a number of genetic and environmental factors that are able to influence muscle fibre development prenatally and muscle growth and fibre type postnatally. The main factors affecting muscle fibres variation can be grouped into intrinsic factors (inherent to the animal), and extrinsic factors (environmental factors). As the number of muscle fibres is fixed at birth, this will be mainly determined by genetic factors and those environmental factors which are capable of influencing prenatal myogenesis. However, muscle fibre size and type will be also influenced by those environmental factors affecting postnatally.

1.4.4.1 Intrinsic factors of variation (natural variation)

Species, gender and breed

Specie-specific differences in the total number of muscle fibres and muscle fibre size exist among mammals. It has been reported that the number of muscle fibres increase with the size of the animal (Rehfeldt et al., 1999). However, this is not the case for muscle fibre size since the largest fibres are not from the largest mammals, but from the pig (Rehfeldt et al., 1999).

Also, sex-related differences in the number of muscle fibres have been reported in some studies in bovine (Seideman and Crouse, 1986) and pigs (Brocks et al., 1998; Bee, 2004). Apparently, intact males exhibit higher number of muscle fibres than females (Seideman and Crouse, 1986). Whether or not this effect is under the control of testosterone levels prenatally or other random ambient factors need to be clarified. In pigs, Bee (2004) obtained larger mean fibre areas in the *semitendinosus* muscle from gilts compared to boars. Also in pigs, Brocks et al. (1998) reported lower type I and higher type IIB fibres proportions in gilts compared to boars, and lower L* (lightness in meat colour) values in boars compared to gilts, demonstrating a certain influence of gender also on fibre type composition. Underlying reasons for these differences in fibre type composition also need further investigation.

Regarding differences in muscle fibres number and size among the different pig breeds, Serra et al. (1998) obtained differences in muscle fibre proportions when comparing Iberian (unimproved line) and Landrace pigs. They reported more oxidative muscles in the case of Iberian pigs with a higher proportion of type I and a smaller percentage of type II fibres. Similar results were obtained by Lefaucheur et al. (2004) when comparing muscle fibre characteristics of a lower growth efficiency-lower lean meat content breed (Meishan) and Large White pigs. Gil et al. (2003) also reported differences in the oxidative characteristics when five divergent porcine genetic lines of PIC were studied.

Mean fibre size between might also be affected by pig breeds. Early studies showed that obese selected strains of pigs had lower fibre hypertrophy than lean selected strains (Seideman et al., 1989). Lefaucheur et al. (2004) reported higher mean cross-sectional areas of type I fibres, although lower cross-sectional areas of type IIB fibres in Meishan females compared to Large White female pigs.

But the highest differences among breeds are generally found when comparing modern with ancient breeds, or breeds selected for leanness, as Landrace or Large White pigs are, with unimproved lines (Iberian and Meishan pigs). Among different modern meat-type breeds and crosses, reported differences in fibre number or size are generally weak (Ruusunen and

Puolanne, 1997; Kuhn et al., 1998). More information about the effects of intensive selection for lean muscle growth in modern pigs is provided in the next section (*Genetic selection* Section).

Genetic selection

During the last 30 years, an intense genetic selection in the swine industry has been carried out in order to obtain fast growing and leaner animals with lower fat content in the carcass due to market demands (MLC, 1999).

Research works focused on the impact of this selection on muscle architecture have suggested that selection for rapid growth and selection for lean meat content might have different consequences on histochemical characteristics of muscles fibres (Rehfeldt et al., 1999, Oksbjerg et al., 2000).

In one hand, selection for growth rate implemented in long-term studies (improved old breeds) seemed to increase both muscle fibre number and size. In agreement, Oksbjerg et al. (2000) demonstrated in a study comparing two Danish Landrace pigs representing the growth potentials of 1995 (fast-growing) and 1976 (slow-growing) that fast growing pigs had proportionately 0.20 higher number of muscle fibres in the *longissimus* muscle compared to slow growing. Weiler et al. (1995) also reported that the European domestic pig showed both larger and higher number of muscle fibres compared to the European wild pig, from which is descendant. There is some evidence that this response to selection for muscle growth rates is due to an increased satellite cell proliferation (more muscle fibres formed and no changes in DNA/protein ratio; Rehfeldt 2000; Oksbjerg et al. 2000).

In contrast, selection for protein deposition and muscle mass (low backfat thickness), as carried out in modern breeding programs, is reported to mainly increase muscle fibre size as indicated by a decreased muscle DNA/protein ratio in the pig (Nøstvold et al., 1979; Weiler et al., 1995; Larzul et al., 1997).

Some earlier and also recent studies indicate that selection for improved performance in pigs and other species may result in deterioration of some meat quality traits through correlated responses, since the desired traits are influenced by several interacting genes (Cameron, 1990; Larzul et al., 1997; Oksbjerg et al., 2000; Ramírez et al., 2004). Suggestions are made about the fact that meat quality characteristics that play an integral role in consumer acceptance, such as tenderness, colour, pH and intramuscular fat, have decreased as breeders have intensely selected for increased leanness (Schwab et al., 2006). Problems associated with these pigs include pork that is lighter in colour, less firm, lower in water holding capacity, and less marbled than normal.

This deterioration in meat quality has been mainly related to modern selection programs, which are preferentially directed to increase muscle mass and muscle fibre hypertrophy (Rehfeldt et al., 1999). Comparisons between improved and unimproved lines suggest that intensive selection for leanness in modern pigs may also have caused large genetic changes in fibre type composition, inducing a shift in muscle metabolism toward a more glycolytic and less oxidative fibre type (Karlsson et al., 1993; Weiler et al., 1995; Brocks et al., 2000; Lefaucheur et al., 2004). Brocks et al. (1998 and 2000) reported that pigs selected for leanness had higher percentages of type IIB (glycolytic) fibres but lower percentages of type I (oxidative) fibres compared to pigs selected for fast growing. They also observed that the effects of selection were different depending on the type of muscle involved. Karlsson et al. (1993) also reported that pigs with the highest lean percentage had the highest type IIB and the lowest type I and type IIA fibre volume percentage.

Therefore, because of the different properties of the diverse fibre types and its profound influence on postmortem changes in the conversion of muscle to meat, it is expected that changes in fibre type ratios influence meat quality characteristics. Actually, meat quality was negatively affected by the increase in glycolytic fibre type proportions in the above mentioned studies and in others (Brocks et al., 1998; Klont et al., 1998; Karlsson et al., 1999), leading to lighter and more acid meats.

Lefaucheur (2006) reported from a review of different studies, that myofibre traits are slightly to highly heritable with heritability (h^2) values ranging from 0.2 to 0.5 for total number of fibres, 0.2 to 0.35 for myofibre mean cross-sectional area, and around 0.4 for fibre type frequencies. Also, it has been reported that myofibre traits are highly variable between individuals with coefficients of variation of 20% for total number of fibres and mean fibre areas and 28% for the proportion of type I fibres (see also Larzul et al., 1997). Thus, both, the high heritability and variability of muscle fibre traits suggest that it is possible to select animals directly on myofibre characteristics (Rehfeldt et al., 2000; Lefaucheur, 2006). In this regard, Fiedler et al. (2004) concluded that adverse effects of lean selection on meat quality were minimized when a selection index that included total fibre number (+) and frequency of white fibres (-) was implemented.

Uterine capacity

Uterine capacity defined as the maximum number of foetuses a female can carry to term, may be a factor limiting litter size in many swine populations. It has been suggested that high ovulation rates in the new hyperprolyphic genetic lines (>30 ovulations), are associated with increased numbers of conceptuses at the implantation (day 30) that generally exceed uterine capacity of this lines (Foxcroft et al., 2006). This fact results in a uterine crowding phenomena in the early post-implantation period, that leads to detrimental effects on foetal

survival at this time causing a peak of prenatal piglet loss in the immediate post-implantation period (between day 30 and 50 of gestation, Town et al., 2004).

Additionally, as uterine crowding in the early postimplantation period (about day 30 of gestation) coincides with the period of muscle fibre formation prenatally, this phenomenon may also have an impact on foetal muscle development of the surviving conceptus (Foxcroft et al., 2006). Uterine crowding is though to affect developing pigs *in utero* in a manner analogous to the spontaneous intrauterine growth retardation (IUGR), that is generally restricted to a discrete population of foetuses namely runt pigs during gestation (Foxcroft et al., 2006).

In fact, several studies in the literature have demonstrated that, in polytocous species such as the pig, intralitter variation in muscle fibre number exist naturally (Wigmore and Stickland 1983; Nissen et al., 2004; Gondret et al. 2006; Tilley et al., 2007). In general, larger developing foetuses within a litter exhibit higher numbers of muscle fibres than their smaller littermates, although these differences in muscle development have been reported even without changes in piglet weights at birth (Town et al., 2004). The reduced foetal development of the smaller foetuses within a litter seems to be associated with an increased expression of several genes of the insulin-like growth factor system (IGF) in mid to late gestation (Tilley et al., 2007).

Therefore, as Foxcroft and Town (2004) have suggested uterine capacity and also placental efficiency might be able to pre-programme via its effects on muscle development, the variation in growth performance showed by large and small littermates postnatally.

1.4.4.2 Environmental factors of variation

Both the number of muscle fibres and their size are established following a complex sequence of pre and postnatal events. Thus, in each phase of this multistep process, a high number of factors not related to the genes are able to impact on muscle development and growth. Maternal nutrition, the application of GH and other growth promoters prenatally, postnatal nutrition, exercise and the administration of growth promoters, may exert a final effect on growth performance and carcass and meat quality traits at slaughter.

a) Prenatal factors

A great deal has been revealed about the mechanistic genetic regulation of myogenesis in the prenatal period (for review see Houba and te Pas, 2004). But, environmental influences that are able to have an impact on muscle tissue development *in utero* seem much more complicate to characterize. However, because of their recognized potential effects on myogenic

programming *in utero*, attention has recently focused on the influence of prenatal events such as maternal nutrition (Dwyer et al., 1994; Nissen et al., 2003) or the application of substances such as L-carnitine (Musser et al., 2001), growth hormone (Rehfeldt et al., 1993 and 2001; Kelly et al., 1995) or β-agonists (Hoshi et al., 2005) prenatally, on foetal muscle fibres development.

Maternal nutrition

During pregnancy, the optimal foetal growth and development is mostly depending on the maternal nutrients supply and her ability to utilize them, and also on the placental efficiency in transferring nutrients from the mother. Sows are generally fed according to requirements during pregnancy (between 28.2 and 34.9 MJ ME/day and between 10.6 and 16.0 g lysine/day throughout pregnancy, BSAS, 2003; see Section 1.1.1). The influence of the inadequate maternal nutrition on foetal growth or birth weight is well documented (for an excellent review see Robinson et al., 1999), although its effects on muscle fibre development are not as well established (Rehfeldt et al., 2004a).

Foetal growth and myogenesis

Many years ago, it was demonstrated that providing a low feed intake or a low protein diet to the sows during gestation had permanent negative effects on the postnatal growth of the offspring (Pond et al., 1985; Pond and Mersmann, 1988; Pond et al., 1991; Schoknecht et al., 1993). Also, it is known that severe maternal undernutrition (a 60% reduction) during pregnancy cause a reduction of approximately 20% in muscle fibre number in fast muscles (Ward and Stickland, 1991; Dwyer and Stickland, 1992) which appear to be irreversible. As in the case of uterine crowding, maternal undernutrition may simulate the undernutrition suffered *in utero* by some foetus (IUGR), especially in large litters in which the competition for nutrients is more evident. In this regard, it has been reported that undernourished pigs during gestation have lower birth weights and postnatal growth rates, showing signs of growth retardation (Wigmore and Stickland, 1983; Handel and Stickland, 1987), but also lower number of muscle fibres at birth (Dwyer and Stickland, 1991; Gondret et al., 2005; Rehfeldt and Khun, 2006).

Based on these findings, it was hypothesized that increasing feed allowance during gestation might increase nutrient availability in the uterus, increase proliferation rate of myoblasts and, consequently, increase the number of muscle fibres at birth. Also, a concomitant effect of higher litter homogeneity has been suggested as a consequence of this enhanced muscle development in the nutritionally disadvantaged pigs in the litter (Dwyer et al., 1994). If this is so, this higher number of prenatally formed muscle fibres might lead to increased growth rates postnatally (Dwyer et al., 1993; Rehfeldt et al., 1999), and also might have an impact on meat quality traits at slaughter. However, these effects are not that clear in the literature, since contradictory results have been reported.

In pigs, one of the first studies carried out with this hypothesis concluded that doubling feed intake from 25 to 50 days of gestation (100% above control) lead to an increased secondary to primary muscle fibres ratio, and a tendency to a higher total number of muscle fibres (Dwyer et al., 1994). In the same study, greater growth rates of pigs born from sows that were supplemented from day 25 to day 80 of gestation were reported, with differences in growth rates appearing from day 70 of age to market weight (day 130 of age in this case). Subsequently, Gatford et al. (2003) reported that supplementing sows from day 25 to 50 of pregnancy (+36% feed allowance than control) lead to an increased muscle cross-sectional area with an unchanged fibre number /mm^2, suggesting an overall increase on the total number of muscle fibres at birth. In this study, a positive effect of feed supplementation on postnatal growth performance of the offspring was also reported but, in this case, differences on body weight appeared on day 27 postnatally and disappeared thereafter (on days 34 and 61 of age).

Later studies have reported no clear effects of maternal nutrition on muscle fibres development, and no substantially better performance results in pigs born from supplemented sows during early to mid-gestation (Nissen et al., 2003; Bee et al., 2004; Heyer et al., 2004; Musser et al., 2006). Nissen et al. (2003) obtained no effects of *ad libitum* feeding of pregnant sows (approx. 150% above control) from day 25 to 50 or day 25 to 70 of gestation on muscle fibre number and mean areas of the offspring. Neither Bee (2004) found any effect on muscle fibre number when lowering (40% under control) or increasing (40% above control) the feed allowance for the first 50 days of gestation. Both, Nissen et al. (2003) and Bee (2004) reported negative effects of feed supplementation during early to mid-gestation (first 50 days of gestation) on postnatal growth. Heyer et al. (2004) and Musser et al. (2006) obtained no consistent effects of maternal feed allowance during early to mid-gestation (up to 100% above control) on postnatal growth performance, neither in meat quality traits at slaughter.

Thus, although it is obvious that restricted foetal access to nutrients has detrimental effects on foetal growth and myogenesis, and also on postnatal performance, increased maternal feed allowance has, apparently, low or inconsistent effects on foetal development. If an effect exists, it is very heterogeneous. In fact, the studies above mentioned differed in several ways, namely, sow parity, the magnitude of feed allowance during pregnancy, time and duration of the treatment, composition of the gestation diet, restricted feeding regime of the progeny during the growing-finishing period or even methodological characteristics such as muscle sampling location. All this factors could have lead to a variation and an inconsistency in the results obtained by different studies.

Regarding the time window when the treatment is applied, it has been suggested that early to mid-gestation (first 50 days of gestation, approximately) is the focal period during which foetal myogenesis is more susceptible to changes in the uterus environment (Rehfeldt et al., 1993 and 2001). In fact, most of the studies above mentioned include this time frame during gestation in

their experiments. However, Nissen et al. (2003) suggested in their study that supplementation during the period from day 50 to day 70 of pregnancy (including the formation of the secondary fibres) might have positive effects on growth performance of the offspring. They based their suggestion on that the negative effects they obtained on growth performance when supplementing during early to mid-gestation (25 to 50 days of gestation), disappeared when feed supplementation was extended from day 25 to day 70 of gestation.

It also has been reported that not all muscles are equally affected by a variation in the prenatal and postnatal nutrition in a fixed time window during gestation (Dwyer and Stickland, 1992; Greenwood et al., 2000; Bee, 2004). This may be a consequence of their developmental time gradient during gestation, which runs cephalocaudally and proximodistally, and also due to their different composition in fibre types (Ward and Stickland, 1991; Dwyer and Stickland, 1992). In this regard, Dwyer and Stickland (1992) showed that skeletal muscles with a high proportion of slow type fibres such as the *soleus* muscle were less affected by maternal undernutrition than other muscles with higher proportions of fast fibres as the *longissimus* or *semimembranosus* muscle.

In an attempt to determine whether muscle fibres development *in utero* responds to a selected component of the maternal diet, Dwyer and Stickland (1994) evaluated the effects of supplementing a restricted maternal diet (60% restricted diet) during gestation, with either protein or carbohydrates *ad libitum*. They concluded that both, carbohydrates and protein supplementation were equally effective on preventing myofibre reduction in the *biceps brachii* muscle, when maternal feed intake was restricted during gestation. Further information of the impact of either supplementation or restriction of specific components of the diet is scarce in the literature.

Additional investigations are being conducted nowadays in order to determine the impact of other specific substances such as L-carnitine applied prenatally, on muscle fibres development *in utero*. Carnitine is a water soluble, vitamin-like compound which primarily functions to transport free fatty acids (FFA) across the mitochondria membrane where they are processed to produce energy. The supplementation of gestating sows with L-carnitine have shown to increase the number of pigs born alive (Musser et al., 1999) and also to produce heavier piglets at birth (Ramanau et al., 2002) and at weaning due to a higher milk yield of the treated sows (Ramanau et al., 2004). Additionally, Musser et al. (2001) reported that the offspring of sows treated with L-carnitine had a higher number of muscle fibres with larger cross-sectional areas in the s*emitendinosus* muscle than piglets born from control sows. The underlying mechanisms for these effects seem to be related to increasing concentrations of the circulating insulin-like growth factor I (IGF-I) and other myogenic factors, due to changes in blood glucose (Musser et al., 1999; Waylan et al., 2005). Thus, the addition of L-carnitine in diets for pregnant sows could potentiate IGF-I actions on muscle fibre proliferation.

As subsequent research identifies the specific nutrient(s) and time period to elicit the optimal response, stage feeding during gestation for muscle development of the foetuses may become an important part of the commercial swine production.

Metabolic basis of the relation between maternal nutrition and prenatal muscle development

The metabolic regulation of prenatal growth is very complex and not sufficiently understood. It has been known for several decades that growth hormone (GH) exerts a major impact on muscle fibre development. Moreover, it has been recently recognized that the growth promoting effect of GH is mediated by the insulin-like growth factor (IGF) system (for recent review Oksjberg et al., 2004). However, the role of maternal nutrition on all these mechanisms and, therefore, on foetal muscle development is not sufficiently understood. It is reasonable to assume that the response of pig conceptuses to maternal feed intake is mediated by the milieu of hormones, nutrients, growth factors, and other constituents within the gravid uterus.

In pigs, as in other species, glucose is considered to be an important foetal growth-regulating factor (Père, 1995), and maternal glucose crossing the placenta is the primary nutrient for foetal production. But, maternal blood glucose seems not affected or either decreased in response to increased feed intake (Gatford et al., 2003; Musser et al., 2004; Nissen et al., 2005). However, increased levels of circulating IGF-I in maternal plasma have certainly been reported in response to increasing feeding levels in pregnant sows. Musser et al. (2004) demonstrated that *ad libitum* maternal feed intake during early to mid-gestation (30 to 50 days of gestation) altered maternal physiology by increasing concentrations of IGF-I and also urea N in plasma. They also reported that the conceptus of the *ad libitum* fed sows during early gestation showed greater concentrations of IGF-I and urea nitrogen (N) in plasma. Nissen et al. (2005) also obtained an increase in maternal IGF-I serum concentration when feeding pregnant sows *ad libitum* from day 25 to day 70 of gestation, although with no significant effects on serum growth factor concentrations in the foetuses. Similarly, maternal protein restriction during gestation has been shown to cause a decrease in the endocrine concentration of IGF-I in newborn pigs (Davis et al., 1997). Decreases on foetal IGF-I in response to maternal feed restriction has also been observed in guinea pigs (Dwyer and Stickland, 1992).

Thus, although IGF-I seems to certainly have a role on the relation between maternal nutrition and metabolism, further investigations are needed in order to elucidate whether mechanisms responsible for the influence of maternal nutrition on foetal myogenesis may also involve alteration in IGF levels.

Growth promoters: Growth hormone and β-agonists

The injection of porcine somatotropin (pST) to sows during early gestation (day 10 to day 24-27) has been reported to increase the total number of muscle fibres (primary and

secondary fibres) in the foetal *semitendinosus* muscle (Rehfeldt et al., 1993 and 2001). This increment in the number of muscle fibres was more intensely observed in the middle and light weight pigs within the litter, and was associated with higher birth weights of these pigs (Rehfeldt et al., 2001). Later on during gestation, the administration of pST not only alters embryonic development but also affects early postnatal growth (Kelley et al., 1995). Yet, although the mechanisms of action of growth hormone are largely unknown, increased nutrient availability and/or circulating hormones such as somatotropin, IGF-I or insulin in maternal and foetal blood may be involved in promoting foetal growth. Rehfeldt et al. (2001) suggested that the direct effects of increased GH and IGF-I in maternal plasma, as suggested for variations in maternal nutrition, can be largely excluded, because there is no physiologically important transfer of peptide hormones across the placenta (Fholenhag et al., 1994).

Some other works have reported beneficial effects of applying β-adrenergic agonists prenatally such as ractopamine or salbutamol on muscle fibre development *in utero* (Kim et al., 1994; Hoshi et al., 2005). These studies follow the hypothesis on that during the stage when muscle fibres undergo hyperplasia the number of membrane β-adrenergic receptors is increased (Parent et al., 1980). However the use of these substances in animal production is not allowed in the European countries.

b) Postnatal factors

The phenotype of a given muscle fibre is specified during muscle development (see Section 1.4.3), but it is generally open to changes during the adult life, i.e. muscle fibres can change their type in response to changes on functional demands induced by training or disuse. Factors such as physical activity or nutrition may affect muscle growth and fibre type distribution and then, probably, meat quality at slaughter. Generally, they affect muscle growth through increases in muscle fibre size hypertrophy although a certain muscle fibre hyperplasia can occur, occasionally, due to satellite cell proliferation postnatally (Rehfeldt et al., 1999).

The effects of physical activity on muscle traits acquire importance in free-range production systems (alternative production systems), where the growing pig is subjected to a variety of changing environmental influences and is allowed to perform more extensive physical activities (Lebret et al., 2002; Bee et al., 2004). Several researchers have determined that meat from pigs reared in outdoor pens had reduced tenderness (Enfält et al., 1997). Others studies reported that rearing outdoors could impact on muscle fibre type proportions with no significant effects on postnatal performance. Also postnatal nutrition has been reported to affect muscle fibre type proportions. Lefaucheur et al. (2003) reported more oxidative activities when feeding fewer carbohydrates and more lipids in restrictedly fed pigs. These facts suggest that the establishment of the adult muscle phenotype continues by myofiber type conversion postnatally.

1.4.5 Muscle fibres as factors for meat quality

Once the main factors affecting muscle fibres number, size and type variability have been considered, their relationship with meat quality will be studied. Firstly, a brief insight of what meat quality represents in given.

1.4.5.1 Meat quality

Optimal meat quality characteristics are to be considered in pork industry in order to stay competitive with the other major muscle foods and meet consumer demands but, objectively, it is difficult to determine. It has been suggested that ideally, pork must have a bright reddish pink colour, be free of surface exudate and firm in appearance and have adequate marbling (visible intramuscular fat; Kauffman et al., 1994). Obviously, pork must also be free of contaminants that could pose health risks for the consumer. And, more important, pork quality should be defined as consumer satisfaction. Most quality characteristics of pork are measured postmortem (immediately or after 24 hours, normally). Major variables important to assessing pork quality postmortem include intramuscular fat content, water holding capacity, colour stability, protein denaturation, pH, palatability and tenderness. Four pork quality categories have been defined in the literature, according to ultimate pH (pH at 24 h post mortem), drip loss percentage and colour of meats (Joo et al., 1995 and 1999; Table 1.9):

Table 1.9 Quality categories of pork and their characteristics.

Category	Characteristics	Ultimate pH[1]	Drip loss[2], %	Lightness[3], L*
PSE	Pale, soft and exudative	<5.5	> 6	>50
RSE	Reddish pink, soft and exudative	<5.6	> 6	≤50
RFN	Reddish pink, firm and nonexudative	5.6-5.9	≤ 6	≤50
DFD	Dark, firm and dry	>6.0	<2	≤43

Adapted from Joo et al. (1995;1999)

[1] At 24 hours postmortem

[2] At 48 hours postmortem

[3] At 24 hours postmortem

Nevertheless, these are suggested values since in field conditions they would depend on the genetic lines, the electronic devices used, the sampling methodology selected for the determination of the different meat quality traits, and other unspecified factors.

Among the quality categories above listed, pale, soft and exudative pork (PSE) is one of the most frequent quality defects in pigs (Oliver et al., 2001; Guàrdia et al., 2004). A recent study in which the incidence of PSE and DFD meats was assessed in different Spanish slaughterhouses, it was determined that PSE meats occur in a range of 19.3 to 2.7 % depending on the slaughterhouse, and that its incidence was higher in summer compared to colder seasons (Oliver et al., 2001). Typical PSE pork is pale grey to white in colour, has soft texture and exhibits excessive fluid loss from the muscle. It is undesirable to packers because of increased drip/purge loss, which represents value lost as "shrink", and the light colour makes it less appealing to the consumers. The PSE condition results from rapid postmortem metabolism of glycogen (carbohydrate reserves in the muscle) to lactic acid before muscle temperatures have cooled (Asghar and Pearson, 1980). The combination of a very rapid pH decline while carcass temperature is still elevated, results in partial denaturation of muscle proteins, which increases light reflectance and decreases water holding capacity (Freise et al., 2005).

Special effort has been undertaken in the literature in order to elucidate which are the most important risks factors in producing PSE condition. A major genetic contributor to the incidence of PSE is the so-called halothane gene. Pigs carrying the halothane-sensitivity allele (nn and Nn), are highly stress susceptible (PSS) and when they are exposed to stress, malignant hyperthermia causes a high percentage of PSE pork or even death. However, halothane positive pigs (nn and Nn) show, although with high controversy in the literature (Fábrega, 2002), advantages in carcass lean meat content compared to NN pigs (Guéblez et al., 1995; Oliver et al., 1993). It also has been suggested that the increased carcass yield and lean percentage observed in halothane carrier pigs is associated with increased muscle fibre cross-sections (Pedersen et al., 2001). Nevertheless, the halothane genotype cannot be considered as the unique determining factor of pork PSE condition. Other factors such as higher proportion of fibres with a glycolytic metabolism (Larzul et al., 1997) or lower number of capillary networks (Franck et al., 2007) have been described as partly responsible of the PSE expression within some muscles.

1.4.5.2 Role of myofibres in meat quality

There is a large individual variation in meat quality both within and between animals of the same breed, sex and environment and even within distinct muscles. Probably these are caused by differences in genetic and environmental factors that interact with the peri and postmortem biochemical processes. Based on their individual characteristics (Table 1.8), and through its influence on postmortem metabolism changes in the conversion of muscle to meat, myofibres are though to be important factors influencing meat quality. Hereby, both, muscle

fibres morphology (number and size) and muscle fibre type composition have been related with meat quality traits at slaughter (Karlsson et al., 1999; Klont et al., 1998; Rehfeldt et al., 1999).

Higher muscle fibre areas have been related to detrimental effects on meat quality, in particular for water holding capacity and tenderness characteristics (Rehfeldt and Khun, 1996; Rehfeldt et al., 1999; Gondret et al., 2006, see Section 1.4.4). Physiologically, strong fibre hypertrophy seems to reduce the capacity of the fibres to adapt to activity-induced demands, which in turn may be associated with stress susceptibility and poor meat quality in modern meat-type pig (Henckel et al., 1997; Rehfeldt et al., 1999). In fact, Lengerken et al. (1997) observed that muscles with a low fibre number but large fibres were prone to rapid postmortem pH decline and high drip losses, two factors known to alter meat tenderness.

Also, an association between fibre size and meat quality traits has been reported in small pigs at birth (Rehfeldt and Khun, 1996; Gondret et al., 2006). Because of interfering factors such as nutrient competition among littermates, low birth weight pigs have been reported to exhibit a lower total number of muscle fibres than their heavier littermates (Wigmore and Stickland, 1983; Rehfeldt and Khun, 1996; Gondret et al., 2006, see Section 1.4.4). In an attempt to compensate their lower growth rates postnatally, lower birth weight pigs generally show larger fibres and also, greater carcass fat content at market weight (Rehfeldt and Khun, 1996; Gondret et al., 2006). This higher fibre hypertrophy in small birth weight pigs has been negatively related with meat tenderness (Ryu and Kim, 2005; Gondret et al., 2006) and positively related with drip loss percentages (Rehfeldt and Khun, 1996). In this line, Maltin et al. (1997) also stated that as the mean diameter of FOG fibres increased, the force required in the instrumental texture analyses also increased, indicating tougher and less tender muscle.

But the specific relationship between myofibre area and meat quality still remains rather controversial (Lefaucheur, 2006). Nevertheless, Rehfeldt et al. (2000) suggested that from genetic correlation coefficients and results of selection experiments, selection for high fibre numbers at a moderate fibre size are most advantageous in achieving both meat content and good meat quality.

Among fibre types, in modern meat-type pigs, paradoxically, larger fibres tend to have lower numbers of mitochondria, and they belong to the white, fast-contracting type (Henckel et al., 1998; Cerisuelo et al., 2007). In pigs, type I fibres have a higher aerobic capacity, a lower glycolytic capacity, and contain a higher level of intracellular lipid and myoglobin than type II fibres (see Table 1.8). Because of the different properties of the diverse fibre types, it is expected that changes in fibre type proportions influence meat quality, and actually they do (Brocks et al., 1998; Klont et al., 1998; Karlsson et al., 1999; Ryu and Kim, 2006). As mentioned above (Section 1.4.4), modern genetic selection is reported to have detrimental effects on meat quality and, at the same time, to induce a shift in muscle metabolism increasing the proportion

of type IIB fibres. Additionally, higher proportions of type IIB fibres seem to predispose to PSE meat due to their glycolytic metabolism and their lower number of capillary networks (Lefaucheur et al., 2002).

Because of all these suggestions, it seems that at least in pigs, oxidative or oxidative-glycolytic fibres (type IIA and IIX) are desirable in meat quality, in conferring more intense colour characteristics (Brocks et al., 1998; Serra et al., 1998), higher ultimate pH (Sosnicki, 1987), better water holding capacity, and higher tenderness (Chang et al. 2003).

Despite the above mentioned evidence regarding the influence of muscle fibre types on meat quality, Lefaucheur (2006) supported that there are no studies enough or that there are too much limiting factors in these studies (such as muscle tissular heterogeneity, accuracy of fibre typing, choice of predictor muscle, sampling location, pertinence of biological markers, accuracy of meat quality evaluation and animal experimental designs), in order to be able to identify a superior fibre type for meat quality determination. Lefaucheur (2006) also suggested that because of their high genetic variability and heritability, selection experiments based directly on myofibre traits and the study of the correlated responses of growth and meat quality may be more adapted to study these relationships between fibre types and meat quality.

Chapter 2

Objectives

Objectives

The study presented in this PhD dissertation is involved within a major project entitled "Pautas de alimentación de cerdas prolíficas durante la gestación: optimización de rendimientos y adaptación a la normativa de bienestar animal" (PETRI 95.0639.OP), carried out in collaboration with Vall Companys Group, PIC España and SCA Ibérica.

To be specific, the general objectives of this study were to investigate:

1. The impact of increasing feeding level during mid-pregnancy (from day 45 to day 85 of gestation) on foetal muscle fibre development, postnatal growth performance and carcass and meat quality traits of the offspring at market weight in the pig.

2. The effects of increasing feeding level during mid-pregnancy (from day 45 to day 85 of gestation) over three consecutive cycles, on sows' body reserves management and their productive-reproductive performance.

A common trial, which lasted from January 2003 to August 2004, was carried out in order to asses these objectives. It involved an initial pool of 103 Landrace x Large White PIC sows that were followed through 3 consecutive reproductive cycles. Additionally, a total of 30 and 20 gilts were newly incorporated in cycles 2 and 3, respectively. From the reproductive cycles 1 and 3, the male progeny was followed through the nursery and growing-finishing periods until sacrifice.

Different partial objectives were pursuit in each of the results' chapters (Chapter 4 to Chapter 6):

The partial objective of **Chapter 4** ("*Effect of maternal feed allowance during mid-gestation on pig postnatal performance, carcass and meat quality traits at slaughter and muscle fibre characteristics*") was to asses the effects of the additional feed allowance during mid-pregnancy (day 45 to day 85 of gestation) on piglet growth performance throughout the lactation, nursery and growing-finishing periods, and its effects on some carcass and technological meat quality traits and on muscle fibre characteristics at slaughter, in the male progeny from cycles 1 and 3.

Chapter 5 ("*Effects of extra feeding during mid-gestation over three consecutive parities, on multiparous sows body reserves and productive and reproductive performance*") aimed to study the effects of this feeding strategy on sows' body reserves levels and changes over the three consecutive reproductive cycles followed (Section 5.2), and also the impact of this feeding strategy on sows' productive-reproductive performance on the three cycles studied (Section 5.3).

Finally, **Chapter 6** ("*Relationship between sow body condition and productive-reproductive efficiency. Accuracy of visual body condition score in predicting sows body reserves*") aimed to examine the relationship between sows' body weight and body reserves levels and changes at different times within the reproductive cycle, and diverse productive-reproductive traits in cycle 1.

Results regarding growth performance of the offspring and carcass and meat quality traits in cycles 1 and 3 were presented in the II Workgroup meeting of the European Cost Action 925 "The importance of prenatal events for postnatal muscle growth in relation to the quality of muscle based foods", and published as an special issue in Archiv für Tierzucht under the title "Effect of maternal feed intake during mid-gestation on pig performance and meat quality at slaughter" (*Archiv für Tierzucht 2006, 49:57-61*). Results regarding muscle fibres will be also presented in the next IV Workgroup meeting of the European Cost Action 925 (September, 2007).

The effects of the extra feed supplementation during mid-gestation on gilts' body reserves management, productive and reproductive performance over one reproductive cycle, including those gilts from cycle 1 and the newly introduced in cycles 2 and 3 (n = 90), were evaluated on the experimental study presented in order to obtain the master thesis degree the 5th of October, 2004. Results from this study have been submitted as an original research paper for publication in the Spanish Journal of Agricultural Research under the title "Effects of extra feeding during mid-gestation on gilts performance".

Chapter 3

General material and methods

General material and methods

3.1 Sows and experimental design

The experiment was conducted on a sow farm (Santa Ana, Soria, Spain) from January 2003 to February 2004, and received previous approval from the Animal Protocol Review Committee of the Universitat Autònoma de Barcelona (Spain). The study involved an initial pool of 103 Landrace x Large White PIC sows from 0 to 4 parities that were selected on day 40 of gestation (after positive pregnancy check) and allotted into two groups, Control (C, n=49) and Supplemented (S, n=54) sows. Sows were blocked by day of mating, body weight (BW, kg) and body condition [backfat thickness (BF, mm), loin depth (LD, mm) and body condition score (BCS)] on day 40 of gestation and assigned, randomly, to one of the two groups (treatments). Sows' distribution by parity and treatment and also their mean initial BW and body condition levels are recorded in table 3.1.

Table 3.1 Sows distribution by parity and treatment. Average body weight (BW), backfat thickness (BF), loin depth (LD) and body condition score (BCS) values[1] by treatment on day 40 of gestation in cycle 1.

Parity	***Treatment***	
	Control	*Supplemented*
0	18	22
1	9	13
2	9	10
3	10	6
4	3	3
Total	**49**	**54**
BW, kg	197.6 ± 5.79	192.1 ± 5.02
BF[2], mm	17.4 ± 0.56	16.7 ± 0.48
LD[2], mm	55.9 ± 0.63	53.9 ± 0.61
BCS[3]	3.1 ± 0.05	3.1 ± 0.05

[1] Values are expressed as means ± standard error

[2] Levels measured by ultrasounds at P2 location

[3] Measured according to a 1 to 5 scale with intermediate 0.5 levels

Treatments involved two different feeding levels allowed during mid-gestation (from day 45 to day 85 of gestation). Treatment group C was fed at the level routinely used in the farm (2.5-3.0 kg/d, depending on sows BCS at mating; 12.1 MJ ME/kg feed and 0.62 % lysine, in average) throughout gestation. Sows from the S group received an extra feed allowance of the same feed of + 50 % for gilts and + 75 % for multiparous sows above the C level, from day 45 to day 85 of gestation. Feed allowance of S sows was calculated individually as a 50 % or 75 % higher than the feeding level corresponding to each sow according to their BCS on day 40 of gestation, and

the desired feeding level was gradually reached in 4 days (from day 41 to day 45 of gestation). From day 85 of pregnancy until farrowing, the amount of feed provided to the S group was the same for all sows and was calculated as the average feeding level of the C sows at that time.

Animals were kept under study during 3 successive reproductive cycles (cycle 1, from January to May 2003; cycle 2, from April to September 2003; cycle 3, from October to February 2003-2004). The same treatment was applied over the three reproductive cycles. In order to maintain a total number of animals about 100 in each cycle, new gilts were incorporated into the experiment on day 40 of gestation in cycles 2 (n = 30; C = 15 and S = 15) and 3 (n = 20; C = 10 and S = 10). The introduction and distribution of these gilts within treatments followed the same criteria than those used in cycle 1 (day of mating and sows body weight and body condition).

Different gestation and lactation feeds were fed to the animals throughout the experiment. Their composition is given as ranges of minimum and maximum levels of the different ingredients included and the calculated nutrient analysis in table 3.2.

Table 3.2 Composition (minimum and maximum levels) of the gestation and lactation diets (as-fed basis)[1].

	Gestation diet		**Lactation diet**	
Ingredient, %	*Minimum*	*Maximum*	*Minimum*	*Maximum*
Barley	20.53	55.80	28.00	40.00
Wheat bran	11.50	43.80	8.00	24.26
Sugar beet molasses	2.00	6.50	3.00	6.00
Wheat	6.00	9.00	9.08	43.00
Cassava meal	-	-	15.00	17.50
Soybean meal, 44% CP	3.50	6.60	11.30	25.27
Sunflower meal	-	12.25	3.00	10.00
Animal fat	2.80	5.95	5.80	6.20
Calcium carbonate	1.18	1.48	1.36	1.75
NaCl	0.20	0.33	0.39	0.48
Monocalcium phosphate	-	0.55	0.50	0. 90
L-LysineHCl - 50%	-	0.15	-	0.38
Choline chloride - 75%	-	0.03	-	0.03
Vitamin and mineral premix[2]	0.50	0.50	0.50	0.50
Calculated composition, %				
ME (MJ/kg)	11.95	12.32	12.75	13.04
Crude Fat	6.79	8.12	7.21	8.20
Crude Fibre	7.31	7.73	6.28	6.60
Crude Protein	13.82	15.31	16.53	17.83
Lysine	0.59	0.65	0.88	0.90
Ca	0.68	0.71	0.90	0.92
Available P	0.23	0.28	0.30	0.35

[1] An acid-insoluble ash external marker was added to the gestation (1%) and lactation (1.5%) diets about 1 week prior to the digestibility balance

[2] Provided per kilogram of feed: 10000 IU of vitamin A; 2000 IU of vitamin D3; 40 mg of vitamin E; 6 mg of vitamin K; 1 mg of vitamin B1; 6 mg of vitamin B2; 0.02 mg of vitamin B12; 29 mg of nicotinic acid; 11.71 mg of pantothenic acid; 0.5 mg of folic acid, 0.06 mg of biotin; 80 mg of Fe; 25 mg of Cu; 0.40 mg of Co; 100 mg of Zn; 43.20 mg of Mn; 2.25 mg of I and 0.09 mg of Se

3.2 Sows management

The management, housing and husbandry conditions conformed the European Union Guidelines. During gestation, sows were housed in individual stalls and were fed twice a day (08:00 h and 14:30 h) with dry feed. They had free access to water. About one week before farrowing (110 days of gestation), sows were moved to the farrowing crates. The average lactation length was 22 ± 2 days. Feed during lactation was given as dry feed twice a day (08:00 h and 14:30 h), and the sows had free access to water via nipple drinkers that were placed in the feeder. Feeding level during lactation was increased gradually from 0 kg/d at the day of farrowing to a maximum of 7.7 kg/d (as-fed basis), reached on day 14 of lactation and maintained at this level until weaning (Figure 3.1). Room temperatures in the farrowing rooms were set from 23 to 18°C, depending on the stage of lactation.

Routine farm management procedures were followed in caring for the sow and litter during parturition and lactation. Litter size was adjusted to 10-11 pigs per litter at 24 h post-farrowing, and cross-fostering was allowed only for female piglets and between sows of the same treatment. No creep feeding was provided to the piglets. Male piglets were castrated on day 7 after birth. At weaning, sows were moved to the weaning barn and housed in individual gestation stalls. After 3-4 days post-weaning, sows were checked daily in order to detect oestrus. From the first insemination (day the oestrus was detected) until the pregnancy test (35-40 days of gestation), sows were controlled daily in order to detect return to oestrus, abortion, infections and other incidences.

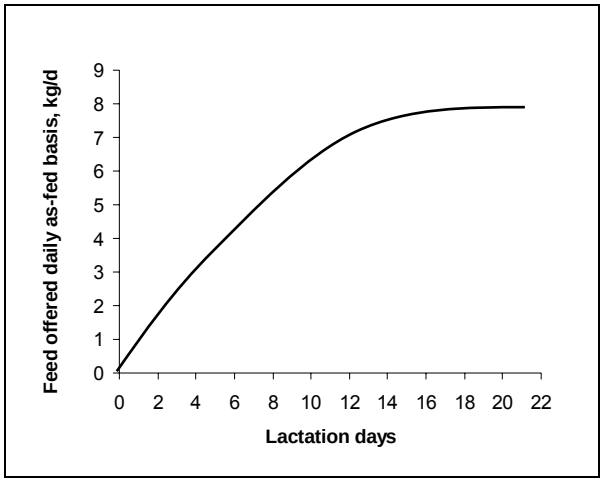


Figure 3.1 Feeding pattern followed throughout lactation on the farm.

The maximum and minimum temperatures in the gestation barn were recorded daily during the experimental period (days 45 to 85 of gestation) and throughout lactation from the three consecutive cycles studied.

3.3 Sows measurements

3.3.1 Body weight and body condition

Sows were weighed on day 40 of gestation, at 48 ± 24 h post-farrowing and at weaning. Backfat thickness and LD were measured at the P2 position (above the last rib at approximately 6.0-6.5 cm from the midline, Figure 3.2) using an A-mode ultrasound device (Renco sonograder 4.2, Renco Corporation, Minneapolis, MN) on days 40, 80 and 110 of gestation and on day 18 ± 1 of lactation[1]. The point of the initial scan was marked to ensure that subsequent scans were recorded at the same place. Subjective BCS was recorded visually, according to a 1 to 5 score subjective scale (Close and Cole, 2003, Figure 3.3 and Table 3.3) at the time of ultrasounding.

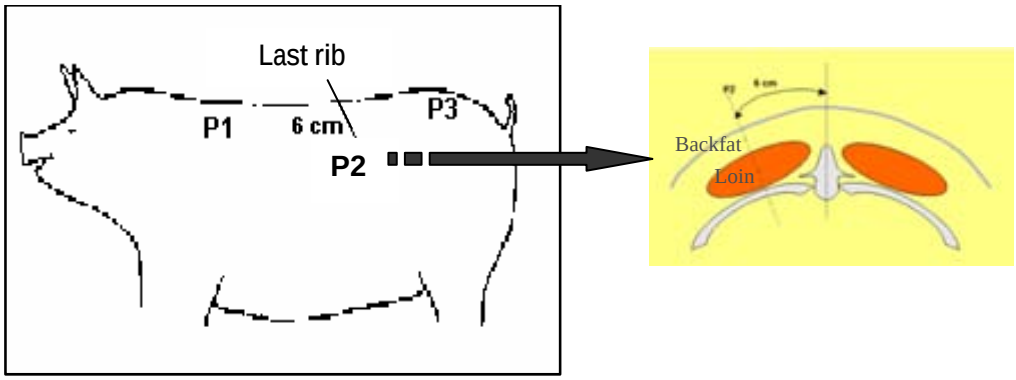


Figure 3.2 The P2 position is located at the level of the last rib, at 6.0-6.5 cm of the midline for measures of backfat thickness (BF) and loin depth (LD).

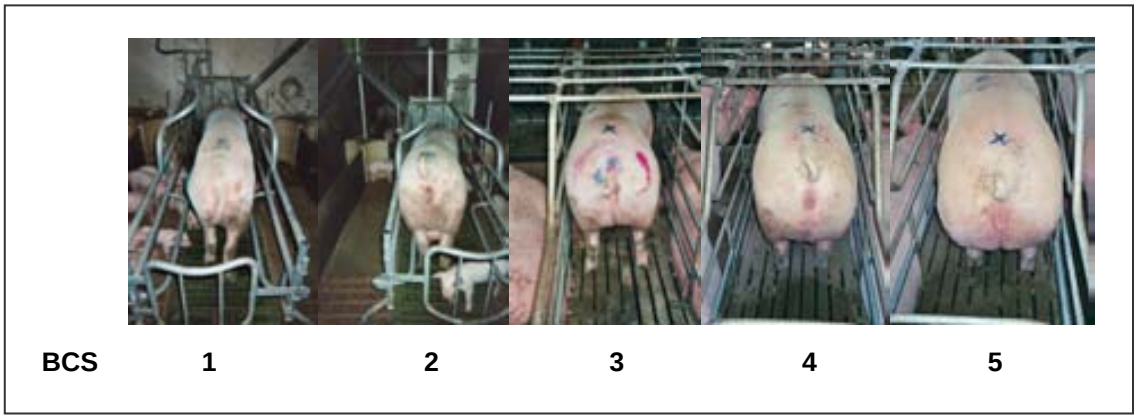


Figure 3.3 Visually body condition score (BCS) evaluation pattern adapted to the farm in which the present study was carried on (Santa Ana, Soria).

[1] Hereafter, measurements taken on day 18 ± 1 of lactation will be refereed to as measurements on day 18 of lactation

Table 3.3 Body condition score (BCS) evaluation pattern (Close and Cole, 2003).

Score condition	Pin bones and tail setting	Loin and backbone
1 Poor	Pin bones very prominent. Deep cavity around tail setting	Transverse spinal and vertebrae prominent
2 Moderate	Pin bones slight covered. Cavity around tail setting	Transverse spinal and vertebrae visible
3 Good	Pin bones only felt with firm pressure. No cavity around tail	Transverse spinal and vertebrae only felt with firm pressure
4 Fat	Pin bones hard to feel. Root of tail set in surrounding fat	Transverse spinal rounded, impossible to feel vertebrae
5 Grossly fat	Pin bones impossible to feel. Root of tail set deep in surrounding fat. Plenty of fatty folds	Mid-line appears as slight hollow between rolls of fat

In addition, sows' body lipid and protein mass was estimated using the equations of Dourmad et al. (1997) and the BW and BF measurements at different times within the reproductive cycle,

$$Lipid\ (kg) = -26.4 + 0.221 \times EBW + 1.331 \times BF, \quad CV=17.18\%$$
$$Protein\ (kg) = 2.28 + 0.178 \times EBW - 0.333 \times BF, \quad CV=7.79\%$$

where, EBW (Empty body weight) = 0,905 x $BW^{1,013}$ at weaning, and EBW = 0,912 x $BW^{1,013}$ at farrowing and on day 40 of pregnancy. These equations were originally established for primiparous sows at mating, farrowing and weaning.

3.3.2 Productive and post-weaning performance

At farrowing, piglets including total, alive, stillborn and mummies were counted and weighed individually. The number of pigs and their weight were recorded again, after the cross-fostering and on day 18 ± 1 of lactation [2]. Milk production was estimated using the average litter weight gain during lactation (after cross-fostering until day 18 of lactation), including the number and weight of the dead piglets throughout this period and applying the efficiency factor for milk of 4 L/kg (Pluske and Dong, 1998). Lactation incidences, piglet mortality and causes of death were also recorded. Piglet mortality causes were classified into diverse categories such as small at birth pigs (born with a less than 900 g of body weight), crushed by the sow, starvation (poor body condition after 3 or 4 days of birth) and others (splay-leg, illness, bitten by the mother).

[2] Hereafter, measurements taken on day 18 ± 1 of lactation will be refereed to as measurements on day 18 of lactation

Weaning to oestrus interval (WEI), defined as the interval from weaning to first mating, was also recorded. By program design, sows with prolonged interval (longer than 7 days), and sows that returned to oestrus after the first insemination were removed from the experiment.

3.3.3 Gestation and lactation feed intake and digestibility balances

During the experimental period (mid-gestation), refusals were controlled every day in order to confirm the different level of feed intake between the two groups of sows. In a sample of animals (cycle 1: n = 28, C = 14 and S = 14; cycle 2: n = 31, C = 14 and S = 17; cycle 3: n = 35, C = 18 and S = 17), apparent faecal digestibility (AFD_G) of organic matter during gestation was measured by day 60 of gestation using the acid-insoluble ash method (van Keulen and Young, 1977). An adaptation period of one week was given to the acid-insoluble ash external marker added to the gestation feed (1%). Calculations of apparent faecal digestibility were made as follows:

$$AFD = 1 - \left(\frac{AIM_{feed}}{AIM_{faeces}} \right)$$

where,
AFD = Apparent faecal digestibility (%)
AIM_{feed}= Acid-insoluble marker concentration in feed (%)
AIM_{faeces}= Acid-insoluble marker concentration in faeces (%)

During lactation in cycle 3, average daily feed intake (ADFI) was measured in a sample of sows (n = 51, C = 26 and S = 25). The study was done during 12 randomly selected and non-consecutive days of lactation, which corresponded to different lactation days for each sow. Feed refusals were weighed and one homogeneous sample of each refusal (200 g, approximately) was frozen to allow subsequent determination of dry matter content at 70°C, following AOAC (1995) procedures. Dry matter consumed each day was calculated as the difference between dry matters offered and refused. Data was pooled within treatments and ADFI for the two treatments was determined.

Apparent faecal digestibility of organic matter was also measured on day 15 ± 1 of lactation (AFD_L, n = 46, C = 21 and S = 25), after one week of adaptation period to the acid-insoluble ash external marker added to the lactation feed (1.5%).

3.3.4 Culling

During the experiment, culling and causes for culling of sows were recorded. Sows were culled for the following reasons: long weaning-to-oestrus interval (≥ 7 days), return to oestrus after the first insemination, non pregnant after pregnancy test at 35-40 days of gestation, abortion, sudden death, mamitis-metritis-agalaxia syndrome (MMA), severe lameness, prolapse, illness, age and others.

3.4 Progeny growth performance and carcass and meat quality measurements

The male progeny [(Landrace x Large white) x Duroc] coming from the sows studied in cycles 1 and 3 was followed post-farrowing until market weight in order to determine the effect of maternal nutrition during mid-gestation on growth performance and carcass and meat quality traits at slaughter. On the farm where the experiment was conducted (Santa Ana, Soria), female piglets were reared as the future replacement gilts for other commercial farms and thus, were not included in the study of post-weaning growth performance.

Barrows were assigned at birth to one of the two treatments (Control, C or Supplemented, S), according to the dietary treatment received by their mothers during mid-gestation. After weaning, they were reared conventionally in commercial nursery and growing-finishing facilities located in Lleida (Spain), and were fed diverse commercial diets until slaughter.

3.4.1 Progeny growth performance

For the study of growth performance, a total of 958 barrows (cycle 1 = 461 and cycle 3 = 497) were kept under evaluation during the nursery period and a total of 636 barrows (cycle 1 = 377 and cycle 3 = 259) were followed throughout the growing-finishing period. At the beginning of the nursery period, pigs were classified according to body weight into 5 weight groups (G, where G1 included the heaviest pigs and G5 the lightest). They were then organized according to groups of weight and maternal treatment in 20 pens (2 pens per weight group and maternal treatment, and 20-26 pigs per pen). The same distribution within weight groups was maintained until slaughter.

The nursery period lasted about 6 weeks in which pigs were fed 3 commercial diets. For the growing-finishing period, only one of the two replicates per group of weight and maternal treatment used in the nursery period was followed. Each of these replicates was then divided into two pens (12-13 animals each). The growing-finishing phase lasted 13 (cycle 1) and 16 (cycle 3) weeks and pigs were fed 3 different commercial feeds.

Pigs were weighed weekly in the nursery and every three weeks in the growing-finishing phases, and average daily weight gain (ADG) was calculated. Feed consumption (ADFI) was obtained from all pens in the nursery period and in weight groups G3 and G4 in the growing-finishing period. The reasons for selecting these two groups of weight were that G3 represented the mid-piglet weight group and G4 represented a lighter pig, in which the most important effects of maternal (prenatal) treatments are described in the literature (Dwyer et al., 1994; Rehfeldt et al., 2001).

3.4.2 Measurements of carcass quality and sample collection

Carcass, meat quality and muscle fibre measurements at market weight were studied in pigs from G4 (Carcass and meat quality: n=90, cycle 1 = 50 and cycle 3 = 40; muscle fibres study: n=70, cycle 1 = 40 and cycle 3 = 30). Pigs from G4 were all slaughtered the same day (cycle 1: 8th October, 2003 and cycle 3: 3rd August, 2004) in a commercial abattoir (Patel, SAU). The average live weights registered the day before slaughter were 104.1 ± 1.16 kg in cycle 1 and 120.9 ± 2.65 kg in cycle 3.

Different measures of carcass and meat quality were assessed immediately post-mortem and at 24 hours after slaughter. Measures were done on the left part of the carcass.

Carcass weight, percentage of lean meat content (Fat-O-Meat'er, SFK, Denmark) and mid-line fat thickness at the *gluteus medium* level (mm) were measured right after slaughter, before the chilling process. Forty five minutes after slaughter, pH (pH_{45}) was measured at the level of the last rib in the *longissimus thoracis* (LT) and *semimembranosus* (SM) muscles, using a hand held Crison micropH 2001 meter with a xerolite electrode.

About two hours after slaughter, the left ham and *longissimus* muscle were removed from the carcass and weighted. After weighing the ham, the SM muscle was also removed and weighed. Then, three consecutive cranial slices of the LT muscle were taken starting from the last rib level. The first one (from the last rib to the interface between 12th and 13th ribs) was used to perform some meat quality measurements (pH and meat colour) and to obtain samples for muscle fibres measurements (addressed below); the next slice (interface between 12-13th to interface between 11-12th ribs) was photographed in order to measure muscle cross-sectional area using an image analysis system (Digital Image System, S.L., Spain), and the last one (interface between 10-11th ribs) was taken in order to determine water holding capacity measured as drip loss percentage.

In addition, in cycle 3, one more 4 cm wide slice, caudal to the last rib, was obtained from the LT muscle of each animal and frozen immediately in order to carry out the texture profile analysis test (TPA) (Bourne, 1978).

The whole SM muscle, the first part of the LT muscle (from the last rib to the interface between the 12^{th} and 13^{th} rib) and the LT sample used for drip loss measurements (interface between 10-11^{th} ribs) were kept at 4ºC for 24 h.

3.4.3 Measurements of meat quality

Meat colour and pH

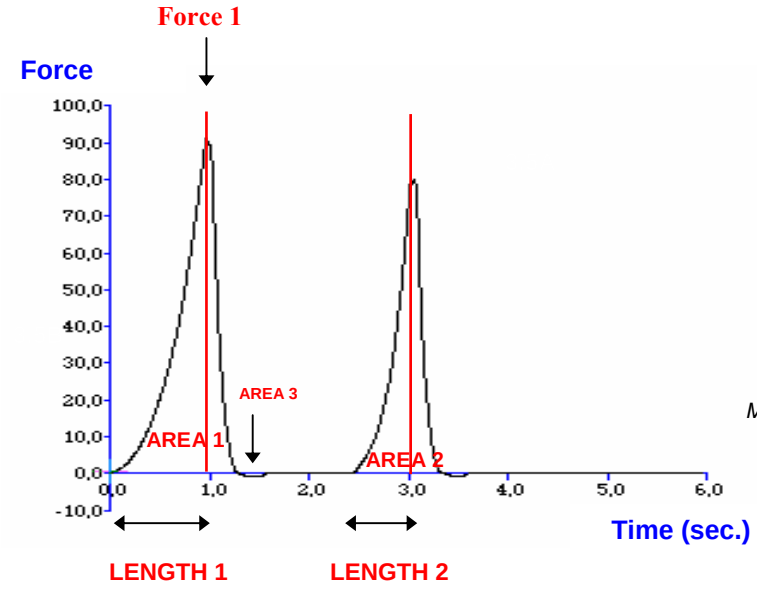
After 24 hours post-mortem, meat reflectance (Minolta Chroma Meter CR300) and pH_{24} measurements were obtained. The pH (pH_{24}) was measured, again, in the SM muscle and at the level of the last rib in the LT muscle. Meat colour determinations were made in the transverse cut of the LT muscle using the Comission Internationale de l'Eclairage (1976) (CIE) values (L*: lightness, a*: redness and b*: yellowness). The instrument was calibrated according to manufacturer instructions.

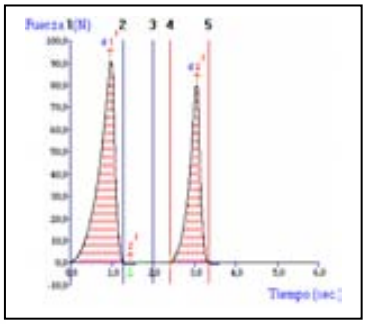
Drip loss

Water holding capacity was determined using the drip loss method (Rasmussen and Andersson, 1996). A cylindrical sample of 2.5 cm of diameter was obtained from the third slice of LT (interface between 10-11^{th} ribs) around 2 hours post-mortem. This was placed in a drip loss plastic tube (KABE Labortechnik), weighed and held horizontally at 4ºC for 24 h. Then, the plastic tube was reweighed without the meat sample to determine the percentage of moisture loss.

Texture profile analysis (TPA)

The TPA analysis was performed using a TA-TX2 Texture Analyzer (State Micro System, Survey, UK) with a 25 kg load cell and a crosshead speed of 5 mm/s. Before the TPA analysis, LT muscle slices were thawed for 24 hours at 4ºC. The slices (1 slice per animal) were then cooked in a gas oven until an internal temperature of 65ºC. This was controlled using a high temperature logger (Dickson HT 120). After cooking, meat was allowed to cool in a temperature controlled room (18ºC) for 60 minutes. After that, 6 to 8 sample cubs (1x1.5x1.5 cm^3) were obtained from each slice and used for the analysis. These sample cubes were compressed twice with a cylindrical probe to a 50% of their original height at a constant temperature of 18ºC. This analysis simulates the normal chewing when eating. Analyses were carried out under lubricated conditions in order to eliminate frictional effects. The study of the curve force-time resulting led to the extraction of six textural parameters: hardness, cohesiveness, adhesiveness, springiness, gumminess and chewiness (Figure 3.4). Mean values for each animal were obtained averaging all the samples per animal (n = 6-8).





Macro program developed using TA-TX2

Figure 3.4 Analysis of the curve force-time to extract six textural parameters: Cohesiveness: Area under second compression / Area under first compression (Area 2/Area 1); Adhesiveness: Area under the negative curve (Area 3); Springiness: Distance of the detected height of the product on the second compression/Original compression distance (Length 2/Length 1); Hardness: Peak force of the first compression (Force 1); Gumminess: Hardness x Cohesiveness; Chewiness: Hardness x Springiness x Cohesiveness.

3.4.4 Histochemistry

Sampling and histochemical reaction

Fifteen cube shaped muscle samples (1cm^3 aprox.) were taken evenly distributed over the cross-sectional area of the portion of LT used for muscle fibres study (from the last rib to the interface between 12th and 13th ribs). The cubes were cut parallel to the longitudinal myofibre axis, placed on a cork surface, embedded in optimum control temperature embedding medium (OCT, TissueTeck®) and talcum powder, snap frozen in liquid nitrogen and stored in a -80ºC ultracold freezer until histochemical analyses.

A statistical approach was made to know the minimum number of samples required in our experimental conditions in order to obtain reliable estimations of muscle fibre traits, using the calculated intraclass correlation coefficient (ICC; Cerisuelo et al., 2007). The results obtained from this investigation showed that 5 samples per animal in LT cross sectional area at the level of the 12th-13th rib, would be recommended in order to assure an acceptable repeatability and accuracy of the estimations in most of the muscle parameters measured (estimated total fibre number, fibre type composition and mean cross sectional area). Thus, for each animal, 5 of the fifteen cube shaped samples taken from the cross-sectional area of the LT were used for the study of muscle fibre traits.

Transverse serial sections (10 µm) were cut in a cryostat (Leica CM 1900, Nussloch, Germany) at -15ºC, placed on silane treated microscope slides and allowed to thaw and dry at room temperature for 1-2 hours. The sections were then stained for myosin adenosine triphosphatase activity (mATP-ase) after alkaline (pH 10.3) and acid (pH 4.40 and pH 4.45) incubation, using a modification of the method described by Latorre et al. (1993). As the pH sensitivity of the mATP-ase reaction differs slightly among species and sampling conditions, a preliminary investigation was conducted to optimize the alkaline and acid pH that better differentiated muscle fibre types in our samples, as well as, the optimal preincubation time in the acid reaction. Based on the results obtained, three pHs (10.3, 4.40 and 4.45) and a preincubation time of 4 minutes were carried out. Examples of the histochemical reaction are shown in figure 3.5. Fibres that stained negatively (light) for alkali-preincubated mATP-ase activity (alkali-labile mATP-ase) were classified as type I fibres (Figure 3.5A). Positively (dark) and less intensively stained (alkali-stable mATP-ase) fibres were classified as type IIB and type IIA fibres, respectively. Results of fibre typing were validated using the acid preincubated mATP-ase reaction from a consecutive transverse section, in which type I fibres were dark (acid-stable mATP-ase) and types IIA and IIB fibres were less intensely stained (acid-labile mATP-ase, Figures 3.5B and 3.5C). Alkaline incubated sections were used to calculate the number and the mean area of fibre type I, IIA and IIB, through a computer-assisted image analysis system (Digital Image System, S.L., Spain). All measurements were made by the same person in order to reduce any potential subjective variability to a minimum.

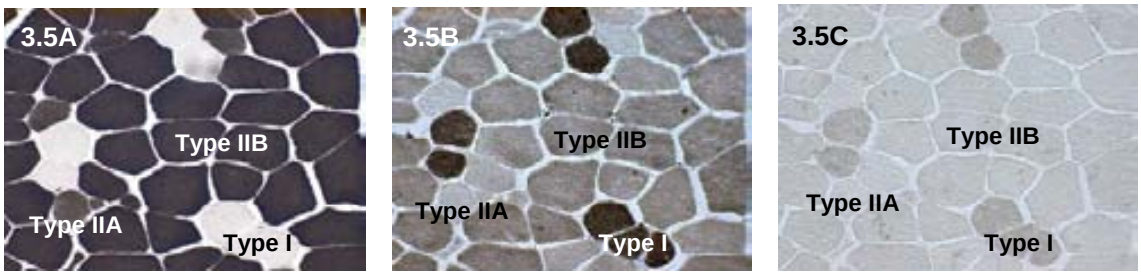


Figure 3.5 Demonstration of mATP-ase reaction in a section of *longissimus thoracis* muscle after alkaline (10.3, 3.5A) and acid (4.40 and 4.45, 3.5B and 3.5C, respectively) preincubation. Magnification 100x.

Image analysis procedure

The computer-assisted image analysis system used (Digital Image System, S.L., Spain) consisted of a standard microscope allowing for a magnification of x40, fitted with a camera (Leica DC300) and a computer for collection of data from all individual fibres of a sample. Within the image analysis system used, an interactive system based on the grey colour differentiation was developed to determine semi-automatically the number and the mean area of the different muscle fibre types. For each sample (5 samples per animal), measurements were made on at least 300 fibres (that means at least 1500 fibres per animal). Then, these data was used to

calculate the percentage of type I, IIA and IIB fibres and the mean area of each fibre type. Also, the total number of fibres was estimated as total number of fibres counted * muscle cross-sectional area divided by the sum of the areas of the total number of fibres counted.

The number of developmental primary and secondary fibres was estimated on the basis of the type I clusters. Mature pigs exhibit a unique arrangement of muscle fibres with central groups of slow type I fibres surrounded by large numbers of fast fibres (Type II). From previous studies (Wigmore and Stickland, 1983) it is known that one of the central slow fibres in each cluster was a primary myofibre, whereas all others developed as secondary fibres. Therefore, it means that it is possible to estimate the number of primary and secondary fibres formed prenatally through the number of type I clusters in the adult animal (= primary fibres) and their subtraction from the estimated total number of fibres (= secondary fibres).

3.5 Statistical Analyses

Data was analysed using SAS System® Software (Version 9.1, SAS Institute Inc., Cary, North Carolina, USA).

In all the analysis procedures, gestation feeding level (treatment, C and S) but also sow parity (Chapters 5 and 6) and piglet weight group (Chapter 4) served as main classification factors. The SAS procedures generally used in this study included GLM, MIXED, GENMOD, CORR and REG procedures.

Multiple comparisons between least square means were performed when a main classification effect was found statistically significant. Tukey correction for multiple contrasts was performed to control significance level.

Specific statistical procedures used for the different data are explained in detail within each of the following chapters of results.

Statistical significance level was set at 5% (0.05), and an alpha of $0.05 > P < 0.10$ was considered a trend.

Chapter 4

Effect of maternal feed allowance during mid-gestation on pig postnatal performance, carcass and meat quality traits at slaughter and muscle fibre characteristics

Related papers:

Effect of maternal feed intake during mid-gestation on pig performance and meat quality at slaughter
Published in:
Archiv für Tierzucht 2006 49:57-61

How many muscle samples are required to obtain reliable estimations of muscle fibre characteristics from pig longissimus muscle?
Published in:
Meat Science 2007 76:583-587
(Annex 1)

4.1 Introduction

Because of its potential effects, the knowledge of how environmental factors such as maternal nutrition during gestation affect foetal growth and muscle fibre development *in utero*, is a very interesting challenge for the pig industry nowadays. Prenatal feed restriction has been clearly related to a reduction in the number of muscle fibres and an impairment of piglet birth weight and postnatal growth (Pond et al., 1985; Pond and Mersmann, 1988; Dwyer and Stickland, 1992; Schoknecht et al., 1993; Dwyer and Stickland, 1994). However, the consequences of increased maternal feed intake during pregnancy are controversial in the literature. The hypothesis is that a higher nutrient supply during the period of muscle fibre formation may increase the amount of nutrients available for foetal growth and, in turn, stimulate muscle fibre development *in utero*. Thereby, as the number of muscle fibres largely determines postnatal muscle growth (Dwyer et al., 1993; Rehfeldt et al., 1999; Pedersen et al., 2001), it will consequently enhance postnatal growth performance of the offspring.

Myogenesis is a biphasic phenomenon with the sequential formation of a primary generation of fibres (from 35 to 60 days of gestation) followed by a secondary generation, which develop between 54 and 90-95 days of gestation, using primary fibres as templates (Swatlands and Cassens, 1973; Wigmore and Stickland, 1983). In the literature, an extra feed allowance during early to mid pregnancy (day 25-30 to day 50 of gestation) has been reported to increase foetal number of muscle fibres (Gatford et al., 2003), and also the secondary to primary muscle fibres ratio (Dwyer et al., 1994), improving growth performance during different periods of the postnatal growth. However, in other studies, feed supplementation during this part of gestation has lead to no clear or even opposite effects on muscle fibre development and growth performance (Nissen et al., 2003; Bee, 2004; Heyer et al., 2004; Musser et al., 2006). Early to mid gestation (prior to the period of secondary muscle fibre hyperplasia) is reported to be the most sensitive period in which foetal muscle tissue development *in utero* might be influenced by environmental factors (Rehfeldt et al., 1993; Dwyer et al., 1994; Hoshi et al., 2005). However, derived from the contradictory results obtained when supplementing at different window times during gestation, it has been speculated that the period of secondary fibre hyperplasia (50 to 70 days of gestation) could be of interest in order to enhance postnatal muscle growth in the pig (Nissen et al., 2003).

Muscle fibre type and morphology is thought to influence meat quality (Larzul et al., 1997; Oksbjerg et al., 2000). As reported by Karlsson et al. (1999), contractile and metabolic properties of skeletal muscle may strongly affect the pattern of energy metabolism in live animals, as well as during the postmortem conversion of muscle to meat and, finally, meat quality. Also, it has been indicated in the literature that muscle fibre number and area may have an impact on meat quality (Rehfeldt et al., 1999; Oksbjerg et al., 2000).

4.2 Partial objective

The purpose of the present chapter was to asses the implications of providing a higher feed allowance to sows during mid-gestation (from day 45 to day 85 of gestation), including the period of the secondary muscle fibres formation *in utero*, on piglet postnatal performance, carcass and technological meat quality characteristics and muscle fibre development.

4.3 Specific material and methods

Barrows coming from all the sows that joined the experiment in cycles 1 (n = 103; C = 49, S = 54) and 3 (n = 96; C = 46, S = 50) were used for this study. These barrows were assigned at birth to one of the two treatments (Control, C or Supplemented, S) according to the dietary treatment received by their mothers during mid-pregnancy (for treatment description see Chapter 3). After weaning, piglets were reared conventionally in commercial nursery and growing-finishing facilities and were fed commercial diets until slaughter. Growth performance[3] throughout the nursery (n = 958) and the growing-finishing (n = 636) periods was evaluated. At the beginning of the nursery period, pigs were blocked into 5 weight groups (G) per treatment; G1 included the heaviest pigs and G5 the lightest. The average initial piglet weight within each group of weight was similar between treatments (Table 4.1). In cycle 3, pigs started the nursery study about one week later than expected (30 days of age instead of 22); for this reason, their initial weight in the nursery period was higher than that of cycle 1 pigs.

Pigs were weighed weekly in the nursery and every three weeks in the growing-finishing phase. Feed intake was recorded in the nursery for all pens and in the growing-finishing period for the middle and the second lightest groups of weight (G3 and G4). At slaughter, carcass and technological meat quality traits were measured in the pigs from the second lightest group of weight (G4, n=90). The following traits were measured in the left carcass and the left *longissimus thoracis* (LT) and *semimembranosus* (SM) muscles: carcass weight and lean content, main cuts and muscle weight, pH at 45 minutes and at 24 hours post-mortem, meat colour, drip loss and textural properties. Texture profile analysis was performed in animals from cycle 3 (n = 40). Also from G4 pigs (n = 70), samples of the *longissimus thoracis* muscle were obtained in order to carry out the histological study of muscle fibres characteristics, as it is specified in detail in chapter 3. The total number and mean size of the different muscle fibre types (type I, type IIA and type IIB) were compiled. The number of developmental primary and secondary fibres was estimated from the number of type I clusters counted (Wigmore and Stickland, 1983).

[3] Average daily gain (ADG), average daily feed intake (ADFI) and feed efficiency (G:F)

Table 4.1 Initial piglet weight (wt) per groups of weight at the beginning of the nursery period and number of barrows controlled per treatment throughout the nursery and growing-finishing periods in cycles 1 and 3.

			Group of weight				
Cycle 1			**1**	**2**	**3**	**4**	**5**
Nursery	Initial wt, kg		8.2	7.4	6.7	5.8	4.6
	Pigs, No	C	50	50	50	40	40
		S	50	51	50	40	40
Growing-finishing	Pigs, No	C	37	38	49	25	39
		S	38	38	50	25	38
Cycle 3							
Nursery	Initial wt, kg		9.3	8.1	7.4	6.8	5.4
	Pigs, No	C	51	47	51	50	47
		S	50	50	51	51	49
Growing-finishing	Pigs, No	C	25	27	25	26	26
		S	26	26	26	26	26

C:control and S: supplemented

Statistics

Results were analysed using the SAS statistical package (Version 9.1, SAS Institute Inc., Cary, North Carolina, USA). In all cases, gestation feeding level (treatment) served as the main factor. In the post-weaning growth performance analysis, piglets group of weight (G) was also considered as a class factor.

For maternal data analyses sows were considered the experimental unit. The number of total born, born alive, stillborn and pigs at 18 days of lactation were analysed through a statistical model for count data (GENMOD procedure). The offset term was used for the born alive and for the number of pigs on day 18 of lactation, in order to refer these data to a total (total born and pigs after cross-fostering, respectively). Litter and average piglet weight at birth and on day 18 of lactation were evaluated through a two-way analysis of variance model using GLM procedure. Litter size was used as a covariate term for litter weight and for average piglet weight. Litter weight and average piglet weight on day 18 of lactation were also covariated by litter weight and average piglet weight after cross-fostering, respectively.

The general one-way ANOVA model used was the following:

$$y_i = \mu + T_i + \beta\, x + \varepsilon_i$$

where, y_i is the response variable studied (litter weight and average piglet weight), μ is the overall mean, T_i is the treatment effect, i = C and S; x indicates the covariate term used when necessary (litter size, litter weight and average piglet weight) and $\varepsilon_i \sim N(0, \sigma^2)$ represents the unexplained random error.

Intralitter variation was tested through the coefficient of variation (CV, %).

Regarding post-weaning growth performance (ADG, ADFI and G:F) pen was considered the experimental unit whereas for carcass, meat quality and muscle fibre traits analysis the experimental unit was the pig. Growth performance was analysed through a two-way ANOVA (SAS GLM procedure) following the model:

$$y_{ij} = \mu + T_i + G_j + (T * G)_{ij} + \varepsilon_{ij}$$

where, y_{ij} is the response variable studied; μ is the overall mean; T_i is the maternal treatment effect, i = C and S; G_j is the effect group of weight, j = 1 to 5; $(T * G)_{ij}$ is the interaction between treatment and group of weight and $\varepsilon_{ij} \sim N(0, \sigma^2)$ represents the unexplained random error. Meat pH at 24 hours post-mortem was additionally covariated by pH values at 45 minutes post-slaughter.

Differences in carcass, meat quality and muscle fibre measurements in pigs from the weight group 4 (G4) were tested through a one-way ANOVA (GLM procedure) in which treatment was the main and unique factor. For muscle fibre type analysis, when data were expressed as percentages, they were subjected to a square-root arcsine transformation to achieve a normalized distribution before ANOVA.

Pearson correlation coefficients (CORR procedure) were calculated in order to examine the relationships between diverse meat quality traits and muscle fibre characteristics.

4.4 Results

4.4.1 Sow and piglet performance

Sow performance in the form of number and weight of pigs total born, born alive, stillborn and pigs at 18 days of lactation in cycles 1 and 3 is recorded in table 4.2. No significant differences were found in the number and weight of pigs at birth (total, alive and stillborn) and on day 18 of lactation in cycle 1. However, in cycle 3, sows from the S group showed higher litter and average piglet weights at birth compared to those from the C group ($P < 0.10$). The intra-litter variation (CV) at birth did not differ between treatments.

Table 4.2 Litter performance at birth and on day 18 of lactation according to the experimental treatment, in cycles 1 and 3.

Cycles	***1***				***3***			
	Maternal treatment				**Maternal treatment**			
Variables	C	S	SEM	*P (T)*	C	S	SEM	*P (T)*
Sow, n	49	54		-	46	50		-
Total born								
Number	12.7	12.9	0.466	*0.782*	13.0	12.3	0.499	*0.376*
Litter wt, kg	19.0	19.5	0.388	*0.405*	17.3	18.2	0.351	*0.059*
APW_{TB}	1.55	1.59	0.031	*0.355*	1.35	1.42	0.030	*0.095*
Born alive								
Number	11.8	12.0	0.426	*0.679*	11.9	11.2	0.457	*0.918*
Litter wt, kg	18.0	18.2	0.361	*0.642*	15.8	16.8	0.334	*0.039*
APW_{BA}, kg	1.59	1.59	0.031	*0.874*	1.36	1.44	0.031	*0.078*
CV_{BA}, %	16.3	18.3	0.903	*0.089*	21.1	19.5	0.820	*0.121*
Stillborn, n	1.03	0.97	0.160	*0.809*	0.94	0.83	0.184	*0.599*
Day 18 of lactation								
Number	10.3	10.0	0.164	*0.109*	9.4	9.1	0.257	*0.823*
Litter wt, kg	60.4	59.2	1.304	*0.537*	46.7	48.4	0.954	*0.224*
APW_{LT}, kg	5.90	5.83	0.127	*0.724*	5.01	5.22	0.104	*0.147*
ADG, kg/d	2.49	2.37	0.083	*0.314*	2.05	2.08	0.088	*0.798*

Results are expressed as least square means and SEM; C: control; S: supplemented; in counting data (litter size) standard error was extracted from the ANOVA analysis; T: maternal treatment; APW_{TB}: Average piglet weight of total born; APW_{BA}: Average piglet weight of born alive; CV_{BA}: Coefficient of variation intralitter of born alive; APW_{LT}: Average piglet weight on day 18 of lactation; ADG: Average litter daily gain during lactation

4.4.2 Post-weaning growth performance

Growth performance throughout the nursery and growing-finishing periods in cycles 1 and 3 is recorded in tables 4.3 and 4.4, respectively. During the nursery period, the pigs born from the supplemented group of sows showed higher daily average feed intakes (ADFI) and weight gains (ADG) in cycle 1 and a higher feed efficiency (G:F) in cycle 3, compared to pigs born from control sows ($P < 0.05$). However, these differences between treatments did not follow a consistent trend, since they disappeared in the growing-finishing phase.

Table 4.3 Overall nursery and growing-finishing growth performance (average daily gain, ADG; average daily feed intake, ADFI; feed efficiency, G:F) in **cycle 1**.

Period	***Maternal treatment***			*P-value*		
	Control	Supplemmented	SEM	*T*	*G*	*T * G*
Nursery[1]						
Pigs, No	230	231		-	-	-
ADG, g/d	316	333	0.004	*0.011*	*<0.001*	*0.227*
ADFI, g/d	430	448	0.004	*0.008*	*<0.001*	*0.112*
G:F	0.73	0.74	0.005	*0.374*	*0.442*	*0.821*
Growing-Finishing[2]						
Pigs, No	188	189		-	-	-
ADG, g/d	789	774	0.012	*0.390*	*0.762*	*0.142*
ADFI[3], g/d	1670	1630	0.041	*0.550*	*0.320*	*0.940*
G:F[3]	0.47	0.48	0.005	*0.641*	*0.531*	*0.913*

Results are expressed as least square means and SEM; T: maternal treatment; G = piglet group of weight

[1] Nursery period: from 22 to 66 days of age, in average

[2] Growing-finishing period: from 67 to 157 days of age, in average

[3] Feed consumption controlled in groups of weight 3 and 4

Table 4.4 Overall nursery and growing-finishing growth performance (average daily gain, ADG; average daily feed intake, ADFI; feed efficiency, G:F) in **cycle 3**.

Period	***Maternal treatment***			*P-value*		
	Control	Supplemmented	SEM	*T*	*G*	*T * G*
Nursery [1]						
Pigs, No	246	251		-	-	-
ADG, g/d	327	333	0.005	*0.387*	*<0.001*	*0.964*
ADFI, g/d	455	455	0.006	*0.965*	*<0.001*	*0.804*
G:F	0.72	0.74	0.005	*0.038*	*0.113*	*0.128*
Growing-Finishing[2]						
Pigs, No	129	130		-	-	-
ADG, g/d	808	797	0.011	*0.501*	*0.056*	*0.912*
ADFI[3], g/d	1970	2010	0.038	*0.500*	*0.143*	*0.379*
G:F[3]	0.40	0.38	0.009	*0.185*	*0.486*	*0.416*

Results are expressed as least square means and SEM; T: maternal treatment; G = piglet group of weight

[1] Nursery period: from 30 to 58 days of age, in average

[2] Growing-finishing period: from 61 to 178 days of age, in average

[3] Feed consumption controlled in groups of weight 3 and 4

The interaction term between treatment and G (T * G) was not significant in any of the growth performance traits measured, neither in cycle 1 nor in cycle 3 (Tables 4.3 and 4.4). However, a numerical trend was observed in both cycles towards a higher benefit of the maternal feed supplementation in pigs from the lightest weight groups (G4 and G5, data not shown). In cycle 1, S pigs from groups of weight 4 and 5 showed numerically higher growth rates and higher feed consumption rates than C pigs from the same groups of weight (ADG: *G4*, C: 280 *vs* S: 306 g/d and *G5*, C: 272 *vs* S: 303 g/d; ADFI: G4, C: 381 *vs* S: 414 g/d and G5, C: 372 *vs* S: 400

g/d), whereas not even numerical differences were observed between treatments in mid and the heaviest groups of weight (Gs 1, 2 and 3). In cycle 3, the same trend was observed for gain:feed efficiency (G:F) although, in this case, differences were only evidenced in G5 (C: 0.71 *vs* S: 0.76 g/d, $P = 0.115$).

When growth performance within the nursery period was studied in shorter periods of time it was observed that in cycle 1, differences in piglet weight between treatments became statistically significant by day 53 of age ($P < 0.05$, Table 4.5). Similarly, in cycle 3, growth efficiency tended to be different among treatment groups by day 58 of age ($P = 0.074$, Table 4.6). Thus, differences in growth performance appeared at the end of the nursery period rather than in earlier stages in both cycles studied.

In cycle 1 (Table 4.5), the interaction between maternal treatment and G for piglet weight tended to be significant by day 53 of age ($P = 0.091$). Table 4.7 shows the results of this interaction on days 45, 53 and 66 of age (which correspond to the last three weight controls made during the nursery period). The main differences in piglet weight, although not significant, were, again, detected in the lightest groups of pigs in test (G4 and G5) and in favour of the S treatment. In cycle 3, no interactions between maternal treatment and group of weight were observed in growth efficiency when data were analysed through the time.

Table 4.5 Piglet weight (piglet wt, kg), average daily gain (ADG, g/d), average daily feed intake (ADFI, g/d) and feed efficiency (gain:feed ratio, G:F) throughout the **nursery period** (from day 22 to day 66 of age, in average) in **cycle 1**.

Age, d	***Trial, d***	***Item***	***Maternal treatment***			*P-value*	
			Control	Supplemented	SEM	*T*	*T * G*
22	**0**	**Piglet wt**	6.53	6.53	0.030	*0.945*	*0.462*
37	**14**	**Piglet wt**	7.77	7.99	0.093	*0.115*	*0.792*
		ADG	89	104	0.006	*0.084*	*0.838*
		ADFI	145	152	0.003	*0.143*	*0.079*
		G:F	0.61	0.69	0.035	*0.131*	*0.867*
45	**22**	**Piglet wt**	9.92	10.18	0.118	*0.151*	*0.212*
		ADG	263	273	0.007	*0.323*	*0.259*
		ADFI	315	333	0.007	*0.105*	*0.453*
		G:F	0.83	0.82	0.013	*0.617*	*0.848*
53	**30**	**Piglet wt**	13.54	14.13	0.127	*0.009*	*0.091*
		ADG	451	486	0.008	*0.010*	*0.243*
		ADFI	528	561	0.008	*0.010*	*0.124*
		G:F	0.86	0.87	0.010	*0.339*	*0.467*
66	**42**	**Piglet wt**	19.90	20.62	0.191	*0.018*	*0.124*
		ADG	530	542	0.009	*0.385*	*0.725*
		ADFI	788	812	0.008	*0.055*	*0.357*
		G:F	0.67	0.67	0.008	*0.541*	*0.922*

Results are expressed as least square means and SEM; T: maternal treatment; G = piglet group of weight

Table 4.6 Piglet weight (piglet wt, kg), average daily gain (ADG, g/d), average daily feed intake (ADFI, g/d) and feed efficiency (gain:feed ratio, G:F) throughout the **nursery period** (from day 30 to day 58 of age, in average) in **cycle 3**.

Age, d	*Trial, d*	*Item*	*Maternal treatment*			*P-value*	
			Control	Supplemented	SEM	*T*	*T * G*
30	**0**	**Piglet wt**	7.36	7.39	0.026	*0.500*	*0.328*
37	**7**	**Piglet wt**	9.14	9.14	0.058	*0.933*	*0.740*
		ADG	248	250	0.008	*0.817*	*0.853*
		ADFI	318	312	0.008	*0.621*	*0.552*
		G:F	0.78	0.81	0.023	*0.359*	*0.425*
44	**14**	**Piglet wt**	11.09	11.12	0.065	*0.746*	*0.479*
		ADG	280	283	0.009	*0.798*	*0.958*
		ADFI	389	410	0.007	*0.067*	*0.999*
		G:F	0.71	0.69	0.019	*0.462*	*0.849*
51	**20**	**Piglet wt**	13.52	13.48	0.101	*0.759*	*0.182*
		ADG	373	362	0.011	*0.466*	*0.213*
		ADFI	508	489	0.010	*0.204*	*0.071*
		G:F	0.74	0.74	0.013	*0.752*	*0.909*
58	**27**	**Piglet wt**	16.39	16.56	0.128	*0.356*	*0.918*
		ADG	410	441	0.014	*0.143*	*0.446*
		ADFI	610	613	0.010	*0.863*	*0.996*
		G:F	0.67	0.72	0.016	*0.074*	*0.238*

Results are expressed as least square means and SEM; T: maternal treatment; G = piglet group of weight

Table 4.7 Average piglet weight (kg) by maternal treatment and group of weight on days 45, 53 and 66 in the **nursery period** in **cycle 1**.

Age, d	*Maternal treatment*	*Groups of weight*					
		1	2	3	4	5	SEM
45	Control	11.9	11.5	10.5	8.7	7.1	0.239
	Supplemented	12.3	11.3	10.3	9.3	7.7	0.239
	P-value	*0.967*	*0.999*	*0.999*	*0.657*	*0.617*	-
53	Control	16.3	15.4	14.3	11.6	10.1	0.283
	Supplemented	16.8	15.5	14.2	12.6	11.6	0.283
	P-value	*0.954*	*1.000*	*1.000*	*0.326*	*0.080*	-
66	Control	23.3	21.8	20.8	17.5	16.1	0.362
	Supplemented	24.2	22.1	20.4	18.6	17.7	0.362
	P-value	*0.747*	*0.999*	*0.996*	*0.486*	*0.155*	-

Results are expressed as least square means and SEM

As stated before (Tables 4.3 and 4.4), no consistent differences were detected between treatment groups neither in ADG, ADFI nor in G:F throughout the growing-finishing period in cycles 1 and 3, even when this phase was more intensively studied by smaller time periods (data not shown).

Table 4.8 shows growth performance data of the different piglet weight groups (G) throughout the nursery and the growing-finishing periods, regardless of the treatment. As it was also deduced from tables 4.3 and 4.4, group of weight (G effect) exerted a strong effect on daily weight gain rates and feed consumption, at least, during the nursery period. Initially, the heavier the pigs were (group of weight 1), the higher growth and feed intake rates they had, consistently, in cycles 1 and 3 during the nursery phase ($P < 0.001$). In this period, pigs from G1 showed daily gains of 368 g/d and 391 g/d while pigs from G5 had daily gains of 289 g/d and 266 g/d in cycles 1 and 3, respectively. However, no consistent differences were found between groups of weight in gain:feed efficiency neither in cycle 1 nor in cycle 3. In the growing-finishing phase, differences in ADG between Gs were still evident but lower than in the nursery period ($P > 0.05$).

Table 4.8 Growth performance (average daily gain, ADG; average daily feed intake, ADFI and gain:feed efficiency, G:F) by groups of weight (G) during the nursery and growing finishing phases in cycles 1 and 3.

	Groups of weight						*P-value (G)*
	1	**2**	**3**	**4**	**5**	SEM	
Cycle 1							
Nursery							
ADG, g/d	368	344	330	293	289	0.006	*<0.001*
ADFI, g/d	489	473	449	398	386	0.006	*<0.001*
G:F	0.75	0.72	0.73	0.73	0.74	0.009	*0.442*
Growing-finishing							
ADG, g/d	796	784	791	769	766	0.019	*0.762*
ADFI[1], g/d	-	-	1680	1613	-	0.045	*0.320*
G:F	-	-	0.47	0.48	-	0.006	*0.531*
Cycle 3							
Nursery							
ADG, g/d	391	349	327	318	266	0.008	*<0.001*
ADFI, g/d	550	485	443	432	364	0.010	*<0.001*
G:F	0.71	0.72	0.74	0.74	0.73	0.008	*0.113*
Growing-finishing							
ADG, g/d	819	848	791	771	784	0.017	*0.056*
ADFI[1], g/d	-	-	2040	1942	-	0.038	*0.143*
G:F	-	-	0.39	0.40	-	0.009	*0.486*

Results are expressed as least square means and SEM; G: group of weight

[1] Feed consumption controlled in groups of weight 3 and 4

4.4.3 Carcass and meat quality

Pigs from G4 (n = 90) were slaughtered at 104.0 ± 1.16 and 120.9 ± 2.65 kg of body weight in cycle 1 and cycle 3, respectively. The most important carcass and technological meat quality traits measured from the selected pigs are summarized in table 4.9.

Increased maternal feed intake during mid-pregnancy did not lead to differences on carcass lean meat content and mid-line fat thickness at *gluteus medium* of the progeny, neither in cycle 1 nor in cycle 3. Regarding muscle and main cuts weight, S pigs showed significantly higher SM muscle and ham weights in cycle 1; this fact is in agreement with the tendency to a higher carcass weight also observed in this cycle ($P = 0.056$). No differences in carcass and main cuts weight were detected between treatments in cycle 3.

Regarding technological meat quality traits, values of pH measured at 24 h post-mortem (pH_{24}), and meat colour traits appeared to be influenced by the maternal treatment, consistently, in cycles 1 and 3. Pigs from the S group showed significantly higher pH_{24} levels ($P < 0.05$) in the SM muscle. In addition, S pigs showed lower lightness (L*) values in the LT muscle ($P < 0.66$) and, at the same time, tended to have higher redness values in cycle 3 ($P = 0.09$). No differences were found on water retention capacity (drip loss values) between treatments.

Table 4.9 Carcass and technological meat quality traits measured at slaughter in cycles 1 and 3 in pigs from the weight group 4.

	Cycle 1				***Cycle 3***			
Maternal treatment	**Control**	**Supplemented**	SEM	*P*-value	**Control**	**Supplemented**	SEM	*P*-value
Pigs, No	25	25		-	20	20		-
Carcass quality								
Carcass wt, kg	72.4	77.9	2.02	*0.056*	87.4	86.7	2.36	*0.835*
Lean meat, %	54.23	52.68	0.738	*0.146*	54.11	53.35	0.920	*0.563*
GM, mm	17.52	19.24	1.018	*0.238*	22.6	21.65	1.033	*0.520*
Muscle and main cuts weight, kg								
SM	0.972	1.059	0.028	*0.030*	1.177	1.165	0.033	*0.720*
LT	2.65	2.67	0.223	*0.695*	3.22	3.21	0.099	*0.920*
Ham	10.56	11.38	0.271	*0.036*	13.17	13.27	0.324	*0.840*
Meat quality								
pH_{45}								
SM	6.18	6.22	0.047	*0.546*	6.24	6.14	0.048	*0.145*
LT	6.17	6.30	0.051	*0.079*	6.19	6.12	0.035	*0.124*
pH_{24}								
SM	5.53	5.62	0.029	*0.047*	5.59	5.71	0.031	*0.013*
LT	5.52	5.53	0.019	*0.736*	5.58	5.62	0.016	*0.143*
Meat colour[1]								
*L**	53.89	52.38	0.567	*0.066*	51.88	49.39	0.755	*0.025*
*a**	5.36	5.43	0.242	*0.852*	5.61	6.13	0.213	*0.090*
*b**	3.86	4.32	0.271	*0.238*	4.47	3.98	0.227	*0.135*
Drip loss[2], %	1.84	1.87	0.294	*0.939*	0.87	1.22	0.178	*0.171*

Results are expressed as least square means and SEM; GM: Mid-line fat thickness at *gluteus medium*; SM: *semimembranosus* muscle; LT: *longissimus thoracis* muscle; pH_{45}: pH at 45 minutes post-mortem; pH_{24}: pH at 24 hours postmortem

[1] Meat colour (L*: lightness; a*: redness; b*: yellowness) was measured in LT muscle

[2] Measured at 24 hours post-mortem in LT muscle

Results from the texture profile analysis (TPA) performed in cycle 3 using a cooked portion of the *longissimus thoracis* muscle, are provided in table 4.10. These data show no differences between treatment groups in any of the instrumental texture variables studied [cohesiveness, adhesiveness (g/s), springiness, hardness (g), gumminess (g) and chewiness (g)].

Table 4.10 Instrumental texture analysed according to a texture profile analysis (TPA) test in the *longissimus thoracis* muscle in pigs from the weight group 4, in cycle 3.

Traits	***Maternal treatment***			P-*value*
	Control	Supplemented	SEM	
N	20	20	-	-
Cohesiveness	0.573	0.567	0.0040	*0.317*
Adhesiveness, g/s	6.22	5.72	0.578	*0.542*
Springiness	1.01	1.01	0.0003	*0.606*
Hardness, g	7976.0	8315.7	284. 0	*0.403*
Gumminess, g	4576. 7	4723.0	167. 2	*0.540*
Chewiness, g	4635. 3	4785. 3	169. 8	*0.536*

Results are expressed as least square means and SEM

4.4.4 Muscle fibre characteristics

The estimated total number of muscle fibres (total number and fibre density) and the number and percentage of primary and secondary fibres formed in the prenatal period (estimated through the number of type I clusters, see Chapter 3) are recorded in table 4.11. In general, results between cycles are coincident in outline. Increased maternal feeding level during mid-pregnancy led to lower estimated total number of muscle fibres in the LT muscle of the progeny, compared to the control treatment in cycle 1 ($P < 0.05$; Table 4.11). In cycle 3, these differences also existed numerically, although they were not significant ($P = 154$). Regarding the estimated number of developmental primary and secondary fibres formed, maternal supplemented pigs showed lower numbers of both primary and secondary fibres populations compared to pigs born from C sows in cycle 1. The results in cycle 3 followed the same trend as those in cycle 1 ($P < 0.158$). This finally led to a similar secondary to primary fibres ratio between treatments. When results were expressed in percentages, the same distribution among primary and secondary fibres was observed between treatments.

Table 4.11 Estimated total number of muscle fibres, total number and proportions of embryonic primary and secondary fibres, and secondary to primary fibres ratio (ratio 2:1) in the *longissimus thoracis muscle* of pigs from the weight group 4.

	Cycle 1				***Cycle 3***			
	C	**S**	**SEM**	*P*	**C**	**S**	**SEM**	*P*
N	20	20	-	-	15	15	-	-
Total number [1], ($x10^3$)	1964.0	1695. 5	70.5	*0.007*	1711.5	1560.3	73.0	*0.154*
Fiber density, nº/mm	285.4	254.4	8.71	*0.011*	236.6	219.6	9.32	*0.207*
Primary fibres, ($x10^3$)	75.3	66.1	2.9	*0.023*	66.6	59.4	3.0	*0.098*
Secondary fibres, ($x10^3$)	1888.7	1629.3	68.3	*0.007*	1644.9	1501.0	70.2	*0.158*
Ratio 2:1	25.2	25.0	0.737	*0.826*	24.7	25.4	0.463	*0.304*
Primary fibres, %	3.87	3.89	0.101	*0.890*	3.90	3.80	0.066	*0.308*
Secondary fibres, %	96.1	96.1	0.101	*0.890*	96.1	96.2	0.066	*0.308*

Results are expressed as least square means and SEM; C: control and S: supplemented

[1] Estimated total number of muscle fibres calculated as: [(total number of fibres counted x muscle cross-sectional area) / the area occupied by the fibres counted]

Figure 4.1 illustrates a clear example of two stained sections of LT muscle belonging to control (4.1A) and supplemented (4.1B) groups of pigs. These images denote that the difference in total number and size of muscle fibres between treatments was visually evidenced through a mATP-ase demonstration in most of cases.

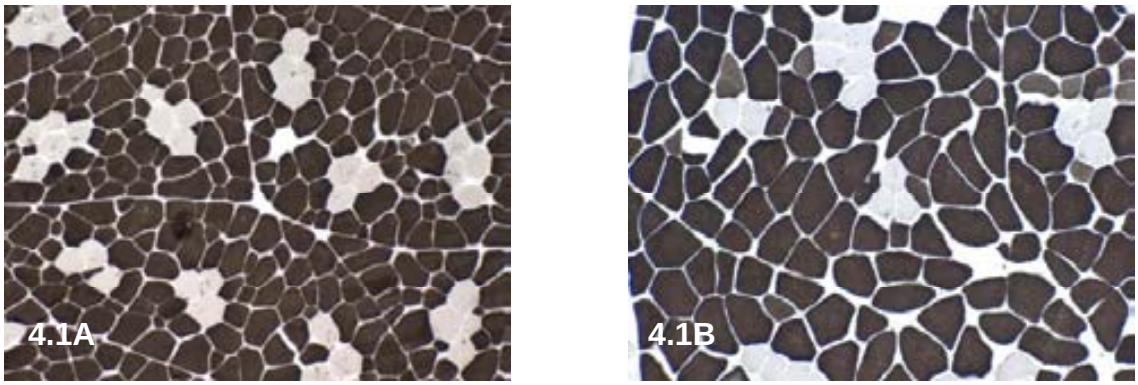


Figure 4.1 Examples of myosin ATP-ase staining demonstration in sections from *longissimus thoracis* muscle in pigs from the weight group 4 born from control (4.1A) or supplemented (4.1B) mothers. Magnification 40x.

Muscle fibres were classified according to the nomenclature provided by the mATP-ase technique in type I, type IIA and type IIB fibres. Results from the estimated total number of each fibre type, and also fibre type distribution (in percentages) within the LT muscle and mean cross sectional area of these fibres are recorded in table 4.12.

Overall, the results reflect the typical fast twitch glycolytic muscle fibre distribution of the *longissimus* muscle; type IIB fibres were quantitatively the most important fibre type compared to type I and IIA fibres.

Maternal treatment applied during mid-gestation (from day 45 to day 85 of gestation) led to lower estimated total number of type IIB fibres in both cycles ($P < 0.05$), apparently, not affecting the number of type I and type IIA fibres. From the calculated proportions of each fibre type, S group of pigs showed a lower percentage of type IIB fibres compared to those pigs from the C group, consistently in the two cycles studied ($P < 0.08$). Besides, in cycle 3, the pigs born from supplemented mothers showed a higher proportion of type I and type IIA fibres compared to the pigs born from control sows ($P < 0.05$). Thus, maternal nutrition during mid-pregnancy could have changed fibre type distribution in the adult offspring.

The lower number of muscle fibres in the S group of pigs (Table 4.11) was linked to a higher mean cross-sectional area of those fibres (Table 4.12). In cycle 1, mean cross-sectional area of muscle fibres was statistically higher in pigs from the S group compared to controls ($P < 0.05$). This difference between treatments derived, in fact, from the observed larger IIB fibres in the LT muscle of S pigs ($P < 0.01$). In cycle 3, mean overall fibre size was not statistically different between treatments, but IIB fibres did also show a tendency to be larger in S compared to C group of pigs ($P = 0.097$).

Table 4.12 Muscle fibre characteristics in the *longissimus thoracis* muscle from pigs in the weight group 4, in cycles 1 and 3 at market weight.

	Cycle 1				Cycle 3			
	Control	**Supplemented**	*SEM*	*P-value (T)*	**Control**	**Supplemented**	*SEM*	*P-value (T)*
Pigs, No	20	20			15	15		
Longissimus thoracis area, cm^2	69.4	66.7	2.23	*0.361*	72.5	71.2	2.11	*0.669*
Total number of muscle fibres[1], (x 10^3)								
Type I	162.9	154.0	10.5	*0.530*	149.3	161.6	9.8	*0.387*
Type IIA	123.0	117.8	12.3	*0.755*	106.7	132.0	11.9	*0.145*
Type IIB	1678.1	1423.7	58.4	*0.002*	1455.5	1266.4	62.8	*0.042*
Fibre type composition, %								
Type I	8.4	9.0	0.50	*0.379*	8.8	10.4	0.53	*0.046*
Type IIA	6.1	6.9	0.54	*0.378*	6.2	8.7	0.54	*0.011*
Type IIB	85.5	84.2	0.57	*0.076*	85.0	81.1	0.76	*0.058*
Mean fibre area, μm^2								
Total	3611.1	3983.8	109.7	*0.015*	4367.0	4692.5	190.2	*0.236*
Type I	3432.5	3551.9	133.3	*0.508*	4160.7	4085.0	206.9	*0.798*
Type IIA	1854.4	1930.5	84.6	*0.506*	2249.3	2288.3	114.3	*0.811*
Type IIB	3761.9	4202.0	117.8	*0.008*	4554.8	5056.0	206.7	*0.097*

Results are expressed as least square means and SEM

[1] Estimated total number of muscle fibres calculated as [(total number of fibres counted x muscle cross-sectional area) / the area occupied by the fibres counted]

4.4.5 Relationship between muscle fibre characteristics and meat quality traits

Due to the reported close relationship between skeletal muscle fibres and meat quality (Karlsson et al., 1999), the association between muscle fibres number, size and type and some of the main quality traits studied in this experiment were investigated in this section and recorded in table 4.13.

From our data (combined data from cycles 1 and 3), although only weak correlation coefficients were obtained, estimated total fibre number and the number of type IIB fibres in the LT muscle resulted positively related to meat lightness (L*). On the contrary, the amount of type IIA fibres was not related to L* but was positively associated with meat redness (a*). Besides, total number of fibres and total number of type IIB fibres resulted negatively correlated to ultimate pH (pH_{24}) in the LT muscle. Regarding fibre type distribution within LT muscle, the percentage of type IIB fibres was positively correlated to L* and, at the same time, negatively associated with a* so that, the higher the IIB proportion in the muscle, the paler is the meat.

The mean fibre cross-sectional area showed a negative association with meat L*, but a positive association with pH_{24} in the LT muscle. Finally, the water retention capacity expressed as a percentage of drip loss showed no significant relationship with any of the muscle fibre traits previously evaluated.

Table 4.13 Correlation coefficients between meat quality traits and diverse muscle fibres characteristics in the *longissimus thoracis* muscle in pigs from the weight group 4.

Muscle fibre traits	***Meat quality traits***				
	Minolta colour			**pH_{24}[1]**	**Drip loss[2]**
	L*	a*	B*		
n pigs	70	70	70	70	70
Estimated total number[3]					
Total	0.29	ns	ns	-0.30	ns
Type I	ns	ns	ns	ns	ns
Type IIA	ns	0.43	ns	ns	ns
Type IIB	0.32	ns	ns	-0.30	ns
Fibre type composition, %					
Type I	ns	ns	ns	ns	ns
Type IIA	ns	0.44	ns	ns	ns
Type IIB	0.27	-0.38	ns	ns	ns
Mean cross-sectional area	-0.36	ns	ns	0.27	ns

All the correlation coefficients showed are statistically significant ($P < 0.05$); ns: non significant

[1] pH24: pH measured at 24 hours postmortem

[2] Drip loss measured at 24 hours postmortem

[3] Estimated total number of muscle fibres calculated as: [(total number of fibres counted x muscle cross-sectional area) / the area occupied by the fibres counted]

4.5 Discussion

Maternal feeding level and litter performance

In the present study, sows performance at birth was not affected by the level of maternal feed intake during mid-pregnancy in cycle 1. However, a positive effect was observed in maternal feed supplementation during mid-gestation tending towards a higher litter and average piglet weight at birth in cycle 3. Several studies in the literature concluded that litter size and piglet weight at birth are unaffected by increased maternal feed intake throughout the different stages of pregnancy (Young et al., 1990; Dwyer et al., 1994; Sinclair et al., 2001; Heyer et al., 2004). However, positive effects of additional maternal feeding during the last month of gestation on birth weight may appear under very special conditions, such as litters with piglet weights lower than 1 kg at birth (Aherne and Kirkwood, 1985). It has been also suggested that more than an increased feed allowance *per se*, the accumulation of body reserves by the mother after several parities of feed supplementation might be the cause for increased piglet weights at birth in some studies (Cromwell et al., 1989). Of the 50 S sows that participated in cycle 3, a total of 28 started the global experiment in cycle 1 and then, received additional feed during mid-gestation for three consecutive reproductive cycles. Consequently, a carry over effect in sows from cycle 3 could have caused the higher litter and piglet weights observed in this case. This then lends support to the fact that maternal reserves might be more important than merely providing higher feed allowances during gestation on piglet weight at birth. This possibility has been also suggested and discussed with more detail in Chapter 5.

Dwyer et al. (1994) reported that a further consequence of increasing sow feed intake in gestation would be the decrease in the distribution of piglet weights at birth within a litter, by increasing foetal growth and fibre numbers of the nutritionally disadvantaged pigs at birth, without affecting larger pigs. This hypothesis could not actually be confirmed by the results on the coefficient of variance intralitter (CV_{BA}) obtained in the present study, since no differences between treatments were obtained.

Maternal feed allowance during mid-gestation did not affect litter performance during the suckling period neither in cycle 1 nor in cycle 3. The pigs born from supplemented mothers showed similar weights at 18 days post-farrowing and also similar growth rates during lactation, as those from the C group. This finding suggests that increasing feed allowance (complete diet, energy + protein) during gestation does not enhance pre-weaning piglet growth, nor sow milking ability. In agreement, neither Nissen et al. (2003) nor Bee (2004) found positive effects of increasing feed intake during different periods of gestation on piglet weaning weights.

Maternal feeding level, post-weaning performance and muscle fibre development of the progeny

Muscle fibre hyperplasia is completed around birth in mammals and postnatal muscle growth is merely hypertrophic (Staun, 1963; Wigmore and Stickland, 1983). Ultimate growth potential will be then largely determined by the number of muscle fibres at birth and their growth capacity thereafter (Miller et al., 1975; Dwyer et al., 1993; Rehfeldt et al., 1999). Because of the reported negative effects of maternal undernutrition during pregnancy on foetal muscle fibres formation (Dwyer and Stickland, 1992 and 1994), it has been hypothesised that increasing feed allowance during gestation might have a positive impact on muscle fibres development and, consequently, on postnatal growth. Pond et al. (1985) and Pond and Mersmann (1988) showed significantly lower progeny growth from week 10 of age onwards when maternal feed intake was restricted over gestation. Similarly, Dwyer et al. (1994) stated that an extra feed supply (100% more of the ration feed to controls) from day 25 to day 80 of gestation in third parity sows was able to increase progeny growth rate after day 70 of age. Also Gatford et al. (2003) reported higher live weights in pigs born from supplemented mothers from day 25 to day 50 of gestation compared to the progeny of control sows, but in this study differences disappeared after day 27 of age.

In the present research, slight differences in growth performance and growth efficiency between treatments were observed when supplementing at a +50 % and +75 % in respect to the C level to gilts and multiparous sows, respectively during mid-gestation (day 45 to day 85 of gestation). These differences appeared in ADG and ADFI (cycle 1) and G:F (cycle 3) throughout the nursery period in favour of the pigs born from supplemented mothers, but they were not consistent since they disappeared in the growing-finishing phase. In agreement, neither Nissen et al. (2003), Heyer et al. (2004), nor Musser et al. (2006) found firm and consistent differences in piglet growth rates when increasing maternal feed allowance during gestation. Contrary to the above mentioned studies (Dwyer et al., 1994; Gatford et al., 2003; Nissen et al., 2003; Heyer et al., 2004; Musser et al., 2006), in which sow feeding level was increased in the early to mid-gestation period (between day 25 to day 50) or between a longer time period during gestation (day 25 to day 70-80 of gestation), in the present experiment the feed supplementation covered the period of the secondary muscles formation (from day 45 to 85 of gestation). Therefore, evidence shows that increasing feed allowance during gestation has no clear or consistent effects on postnatal growth, whichever the stage of gestation involved.

Although no clear effects on growth performance were found, our results showed that the smallest groups of weight in the test were the most significantly affected by maternal nutrition. Several studies have previously reported that the most nutritional disadvantaged pigs *in utero* (smaller), were the most benefited from maternal nutritional and hormonal (growth hormone and somatotropin) treatments (Rehfeldt et al., 1993; Dwyer et at., 1994; Rehfeldt et al., 2001). As it

was suggested by Foxcroft et al. (2006), relative undernutrition of the smallest foetuses in the uterus may then be the driver of their low birth weight and poor postnatal performance. All this evidence led us to select pigs from weight group 4 in order to carry out the carcass, meat quality and muscle fibre measurements planned in the present study. The weight group 5 was discarded for this proposal because it may have included runt and less healthy pigs.

The effects of maternal nutrition during gestation on muscle fibres development *in utero* also remains controversial in the literature. Positive effects on the number of secondary muscle fibres have been reported when overfeeding during early to mid-gestation (Dwyer et al., 1994; Gatford et al., 2003). But, other studies have described no effects or even negative effects on muscle fibre number development when increasing feed allowance during this period (Nissen et al., 2003; Bee, 2004). It has been suggested that the most suitable stage to induce foetal muscle responses through nutritional and non nutritional (hormonal) prenatal strategies is early to mid-gestation, from day 25 to day 50 (prior to secondary muscle fibre formation; Dwyer et al., 1994; Rehfeldt et al., 1993 and 2001; Gatford et al., 2003). However, Nissen et al. (2003) speculated the possibility that increasing maternal feed intake from day 50 to day 70 had advantageous effects on muscle growth of the offspring. This suggestion derived from a study in which supplementing sows (140% above control) during early to mid-pregnancy (day 25 to 50 of pregnancy) led to negative effects on postnatal muscle growth; these negative effects were compensated when the period of feed supplementation was extended from day 25 to day 80 of gestation. At this point, it is important to bear in mind that secondary muscle fibres, which form from day 54 to day 90 of gestation, have been reported as being more susceptible to environmental factors such as maternal nutrition than primaries (Wigmore and Stickland, 1983; Gatford et al., 2003).

In the present work, in spite of finding no consistent effects in postnatal piglet growth performance by providing extra feed to sows within the period of secondary muscle fibres formation (day 45 to day 85 of pregnancy), some differences in muscle fibre traits in the LT muscle were observed between treatments. Contrary to what was expected, pigs born from supplemented mothers showed lower estimated numbers of muscle fibres compared to control pigs in both cycles studied [*cycle 1 (x10^3):* C = 1964.0 and S = 1695. 5, *P* = 0.007 and *cycle 3 (x10^3):* C = 1711.5 and S = 1560.3, *P* = 0.154]. Because of similar trends observed in both cycles, we believe that with a larger sample size than the used in cycle 3, variations in total number of muscle fibres would have reached signification.

Despite being quite variable, post-weaning growth performance was never in favour of C animals in the present study. Therefore, from our results it would be assumed that the number of muscle fibres was not positively related to postnatal growth. However, muscle mass and muscle growth does not only depend on the number of muscle fibres but also on the size of these fibres (Larzul et al., 1997; Rehfeldt et al, 1999). In the present study, the total number of

fibres and their mean cross-sectional area were negatively associated (Figure 4.2, r = - 0.72). Rehfeldt et al. (2000) also stated that the number of fibres and their mean size followed a negative correlation; the coefficients of correlation in their study ranged between -0.3 and -0.8. Therefore, the lower number of muscle fibres found in the maternal supplemented pigs could have been compensated by a higher hypertrophy of these fibres during the growing period. This fact could also be suggested from the results obtained in table 4.12, in which S pigs showed larger mean cross-sectional areas than C pigs. This might then give a suitable explanation to the inexistence of differences in growth performance despite the differences found in number of muscle fibres between treatments.

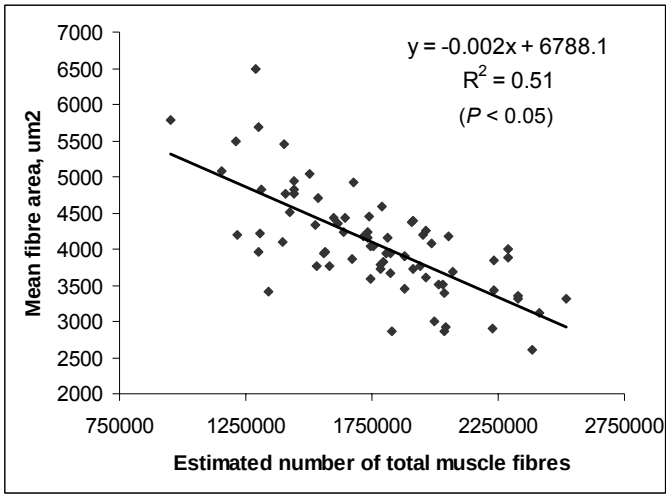


Figure 4.2 Relationship between the estimated number of muscle fibres in the *longissimus thoracis* muscle and their mean cross-sectional area.

Little evidence is provided in the literature regarding the impact of maternal feed allowance during the time window studied in the present experiment on foetal muscle tissue development. Dwyer et al. (1994) included this time frame in their investigation and, although not significant, they reported a lower number of fibres in the *semitendinosus* (ST) muscle when feed supplementation was provided from 50 to 80 days of gestation compared to control [C ($x10^3$) = 371.0 and S ($x10^3$) = 360.4]. Similarly, in a more recent study (Hoshi et al., 2005), the effects of the administration of ractopamine to pregnant sows from day 50 to day 80 of gestation resulted in a lower calculated total number of muscle fibres in the ST muscle compared to control [C ($x10^3$) = 396.6 and S ($x10^3$) = 388.8]. None of the above mentioned studies accounted for muscle fibre sizes. Thus, it seems that while maternal treatment during early to mid-pregnancy brought about no consistent effects on muscle fibre development and postnatal performance in the literature, when feed supplementation involves secondary muscle fibre generation in the foetus, the overall effect seems detrimental for muscle fibre hyperplasia.

Myogenesis is a biphasic phenomenon which includes the formation of two different populations of muscle fibres, namely primary and secondary fibres. Numerous studies have suggested that the primary fibre population is unaltered by nutritional manipulation (Wigmore and Stickland, 1983; Ward and Stickland, 1991; Dwyer and Stickland, 1992; Tilley et al., 2007). Ward and Stickland (1991) suggested that this was probably due to the fact that, at the timing of primary muscle fibres formation, foetuses are relatively small making few demands on maternal nutrition. However, in some other research on pigs (Dwyer and Stickland, 1991; Rehfeldt et al., 2001) and lambs (Fahey et al., 2005) indicated that primary fibres could also be modified *in utero* when applying growth hormone or nutritional treatments to the mother. In the current study both, the number of primary and the number of secondary fibres estimated from the number of type I clusters decreased in the pigs born from supplemented sows (cycle 1, $P < 0.05$; cycle 3, $P < 0.16$), leading to an invariable secondary to primary ratio (around 25) between treatments. This finding supports the suggestion that, under certain circumstances, the number of primary fibres may also vary among animals.

An explanation on the unexpected negative effect of increasing feed allowance from day 45 to day 85 of pregnancy on muscle fibre number is not clear. Various hypothesis based on the physiology of muscle fibre development *in utero* are ensuing formulated. The diminution in muscle fibre number in the present study involved both developmental fibre populations, primary and secondary fibres. As primaries constitute the framework for secondaries to be formed, it is speculated that the effect of maternal nutrition could have been directed to primary muscle fibres formation. Wigmore and Stickland (1983) studied the evolution of foetal muscle fibre development throughout pregnancy, and established that the number of primary fibres was fixed on day 60 of gestation. Then, increasing nutrient availability during this time could have both, prevented final formation or induced degeneration of the existing primary fibres. With less primary fibres, a lesser number of secondary fibres will be subsequently developed.

From a metabolic viewpoint, it is reasonable to think that the response of pig conceptuses to maternal feed intake is mediated by the milieu of hormones, nutrients, growth factors, and other constituents within the gravid uterus (Musser et al., 2004). Thus, it could be also speculated that some of these factors (insulin, GH, progesterone) may have been involved to this effect.

It has been reported that higher levels of nutrition during the first 72 h post-mating have negative consequences on embryo mortality (Jindal et al., 1996). A decrease in maternal serum concentrations of progesterone might be involved to this effect (Jindal et al., 1996). Progesterone and other hormones or metabolites might similarly respond to maternal feeding during mid-pregnancy, and interact with embryo or foetal development. In this line, Musser et al. (2004) reported that feeding sows *ad libitum* (complete diet, energy+protein) from day 30 to day 56 of gestation, lead to an increase in IGF-I (which is a significant myogenic factor) and urea nitrogen (N) both, in the maternal and foetal blood stream. However, a reduction in litter size at

birth was obtained when this treatment was applied in a related paper (Musser et al., 2006). The authors suggested that dietary protein might have originated excessive ammonia concentrations in the foetal compartment that, in turn, might have negatively affect foetal survival, metabolism and growth. It is well known for decades, that pregnant sows are able to buffer their foetuses during periods of feeding restriction, but also that protein retention does not generally increase with increasing feeding levels (Close et al., 1985). As a whole, it can be speculated that an excess in protein that is not retained in the foetal tissue, could have been involved in the negative effect observed on muscle fibre hyperplasia in the current study.

The real cause of this consistent effect needs further investigation. Muscle histological studies in foetal tissue around this period of gestation, or studies on maternal transfer of metabolites to the foetus, may help to elucidate the underlying mechanisms involved. Also it would be necessary to address whether sow parity and other related factors, that could contribute to differences on maternal metabolism may play a role on this effect.

In the present experiment, irrespective of the treatment, a “catch up” growth phenomenon was evidenced during the growing-finishing period (Table 4.8). As expected, in the nursery period the smallest pigs (G4 and G5) grew significantly slower than the largest pigs (G1 and G2) ($P < 0.05$). However, these differences in average daily gain between groups of weight decreased during the growing-finishing period ($P > 0.05$). The smallest pigs were then able to compensate growth and to achieve growth rates closer to those of the heaviest groups in the growing-finishing phase. Thus the competitive disadvantage for growth and feed consumption of the lightest pigs in the test was lowered in later phases of postnatal growth, in agreement with other studies (Hegarty and Allen, 1978; Dwyer et al., 1993). In the literature, the compensatory growth physiological capacity has been also related to the number of muscle fibres (Handel and Stickland, 1988), but this effect couldn’t be verified in the present experiment since muscle fibres were not measured in other weight groups other than G4.

Maternal feeding level, carcass and meat quality and muscle fibre development

There is evidence to suggest that a higher muscle growth may be explained either by a high level of muscle fibre hypertrophy of the individual fibres (Miller et al., 1975) and/or a higher total number of fibres (Henckel et al., 1997), both determining final lean meat content of the carcass. In the present study, as mentioned above, the lower number of muscle fibres in the S group might have been compensated by a higher cross-sectional area of such fibres. As a result, final carcass lean content at market weight was not affected by maternal treatment in the present study. In agreement, Nissen et al. (2003) and Heyer et al. (2004) found no effects of increasing maternal feed intake during gestation on carcass composition of the progeny at slaughter.

On the contrary, the different feed allowance provided to the sows in the present study, resulted in meat quality differences that were consistent in cycles 1 and 3. Pigs born from supplemented sows showed higher ultimate pH values in the SM muscle (pH_{24}) and darker LT meats (lower L* values) than pigs born from C sows. In the present study, ultimate pH in the SM muscle was positively correlated with pH values in LT at this time (r = 0.54, $P < 0.05$) thus, we consider that the differences between treatments found in SM could also have affected LT muscle, although it was not detected.

Much is known about pale, soft and exudative (PSE) meat development, which is related to accelerated metabolism of carbohydrate reserves in muscle during the early post-mortem period (Briskey et al., 1966; Oliver et al., 2001). *In vitro* studies that duplicate PSE meat condition, show that the resulting combination of low pH and high temperatures results in partial denaturation of muscle proteins, which increases lightness and decreases water holding capacity (Freise et al., 2005). In the present study, the lower ultimate pH together with the higher L* found in the C group, might be indicating a higher tendency of these pigs to present PSE meats, compared to those belonging to the S group. However, differences in pH_{24} and in L* between treatments were not followed by differences in drip loss percentages. But, when combining data from cycles 1 and 3, ultimate pH (pH_{24}) resulted negatively related to lightness (r = -0.41, $P < 0.001$) and to drip loss (r = -0.33, $P = 0.003$), and lightness resulted positively associated with drip loss (r = 0.24, $P = 0.030$).

As a whole, due to the concern about the incidence of PSE in pig meat, the meat quality features observed in the S group would be more desirable than those of the C group.

However, it is important to highlight that results in all the meat quality traits measured in the present experiment, either in the S or in the C group, were situated within the normal values suggested for pork (Joo et al. 1995 and 1999). To our knowledge, other studies where a supplemental feeding strategy has been applied during early gestation (25 to 50 days, approximately) or covering both, primary and secondary muscle fibres formation (25 to 70-80 days approximately), did not report differences on meat quality traits at slaughter (Heyer et al., 2004; Nissen et al., 2004).

Histochemical and biochemical properties of a given muscle, such as the number of muscle fibres, mean area and fibre type composition (oxidative and glycolytic capacities and glycogen and lipid contents), are factors susceptible to influence meat quality (Larzul et al., 1997; Karlsson et al., 1999; Rehfeldt et al., 1999; Oksbjerg et al., 2000). However, although evidences exist, the relationship of fibre type composition to meat quality is not fully established and validated for pigs (Lefaucheur et al., 2006).

In the present study, apart from the above mentioned differences in number and size of the muscle fibres, different maternal feed allowances during mid-gestation were able to impact on muscle type composition, consistently, in cycle 1 and cycle 3. Pigs born from supplemented sows showed a lower number of type IIB fibres ($P < 0.05$), compared to C pigs. The same pigs also showed a tendency to a lower percentage of type IIB fibres (cycle 1: $P = 0.085$; cycle 3: $P = 0.001$) and also in cycle 3, a significantly higher percentage of type I and IIA fibres ($P < 0.05$). These results agree with those found on meat quality traits, due to the glycolytic potential and the lighter colour of type IIB fibres (Larzul et al., 1997; Depreux et al., 2002). Therefore, the light colour and the lower ultimate pH found in animals from the C group might be originated by a higher total number and proportion of type IIB fibres. In fact, in the present experiment, estimated total number of type IIB fibres resulted positively related to lightness ($r = 0.32$, $P < 0.05$), and negatively associated with the ultimate pH ($r = -0.30$, $P < 0.05$) when combining data from cycles 1 and 3, irrespective of the treatment (see Table 4.17). In this manner, experiments focused on the effects of genetic selection for lean content on muscle fibre characteristics have reported that an increase in the proportion of type IIB fibres may be one of the causes of the deteriorated meat quality characteristics reported for the new leaner genotypes (Larzul et al., 1997; Oksbjerg et al., 2000).

Also, muscle fibre size has been negatively related to meat quality, in particular with decreased water holding capacity and tenderness (Karlsson et al., 1999; Rehfeldt et al., 1999; Gondret et al., 2006). Strong fibre hypertrophy seems to reduce the capacity of the fibres to adapt to activity-induced demands, which in turn may be associated with poor meat quality in modern meat-type pigs (Henckel et al., 1997; Rehfeldt et al., 1999). Gondret et al., (2006) reported impaired meat tenderness in the smaller pigs at birth, related to their lower number but larger muscle fibres. In the present study, S pigs tended to show muscle fibres with a higher mean cross-sectional area, derived from the higher cross-sectional area of type IIB fibres, compared to the control (cycle 1: $P = 0.008$; cycle 3: $P = 0.097$). But neither drip loss nor meat tenderness measured through the TPA test, were apparently affected by sow nutrition during mid-gestation. However, although not significant, S pigs showed numerically higher meat hardness from the TPA analysis compared to C pigs.

Overall, in the present experiment, supplementation during mid-gestation caused both adverse and favourable issues regarding meat quality in pigs belonging to weight group 4. First of all, S pigs showed lower numbers but larger (in area) fibres which, although not apparent in the current study, may exert negative effects on meat quality. On the other hand, S pigs showed a lower percentage of glycolytic fibres (type IIB) compared to C pigs, which have been associated with a reduced pork quality and higher incidence of PSE meats (Depreux et al., 2002; Frank et al., 2007). To our knowledge there is little evidence in the literature supporting the fact that maternal feeding allowance is able to affect meat quality of their offspring at slaughter.

4.6 Implications

From our results, we cannot conclude that increasing feed allowance during mid-gestation has a clear effect on growth performance. However, some changes in muscle fibre characteristics and meat quality traits have been observed, as a consequence of the different maternal feed allowances during this period. Morphologically, an extra feed supplementation during mid-pregnancy led to a lower estimated number, but larger (in area) muscle fibres. From a meat quality point of view, S pigs showed darker meats with a higher ultimate pH, making them less prone to show PSE problems, which could be of interest to the packing industry. This fact is related with the lower number and percentage of type IIB fibres found in pigs born from supplemented mothers. Therefore, feeding pregnant sows above requirements during the period of secondary muscle fibres formation, may impact muscle fibre development and differentiation *in utero*. To our knowledge, overfeeding in other different critical time windows during gestation did not result in meat quality differences at slaughter. However, the underlying mechanism for this effect and whether it is consistent in pigs from other less disadvantaged weight groups remains unknown, needing further investigations.

Chapter 5

Effects of extra feeding during mid-gestation over three consecutive parities, on multiparous sows body reserves, productive and reproductive performance

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5.1 Introduction

Lifetime, welfare and overall productive-reproductive efficiency of the breeding herd have been strongly related to nutrition and hence, the maintenance of an adequate level of body reserves throughout the reproductive cycle (Whittemore, 1993; Maes et al., 2004). A major concern for the modern lean genotypes is the sow body reserves *"wastage"* from cycle to cycle, which leads to early culling due to reproductive failure (Eissen et al., 2000). But feeding sows to maximize prolificacy and longevity is becoming increasingly difficult; the greater productivity of modern sows has increased the demand for nutrients. Moreover, genetic selection for leanness has developed animals with only small reserves of body fat (Challinoir et al., 1996) and, more important, small appetites mostly evident during lactation. Overall, this leads to a severe drain on body weight and body reserves in lactation and, consequently, an impairment on productive and reproductive performance (Whittemore, 1996; Clowes et al., 2003a). Therefore, it seems necessary to review sow feeding strategies and to adapt them to feeding requirements of the modern leaner sows.

Pregnant sows are generally fed restrictedly in order to avoid the well recognized detrimental impact of increasing feeding levels during gestation on feed intake during lactation (Dourmad, 1991; Revell et al., 1998a). But, the restricted feeding strategy used during gestation does not always allow the recovery of the body reserves lost in the previous lactation in modern sows (Young et al., 1990). Additionally, recent evidence demonstrates that larger amounts of body reserves at farrowing (fat and lean) may be able to buffer detrimental effects of lactation body reserves losses on reproductive performance (Dourmad et al., 1994; Clowes et al., 2003b; Quesnel et al., 2005b). Then, increasing feeding allowances during gestation could be beneficial in the new leaner genotypes in order to assure the maintenance of sow body reserves from cycle to cycle, and to improve (re)productive herd performance. In all likelihood, the most adequate time frame during gestation for recovering maternal body reserves through increasing feeding levels is the central part (25-30 to 90 days), thus avoiding possible embryo mortality in early gestation (Dick and Strain, 1983) and the high foetal demands in the final part of gestation. Moreover, increasing feeding level at this time would reflect the practices implemented in many farms. That is, as sows are usually found to be pregnant at approximately 30-35 days post mating then, measures are taken to correct shortcomings in body condition. Additionally, studies suggest that maternal feeding during this period might impact on foetal growth and development (Schoknecht et al., 1993; Dwyer et al., 1994).

The purpose of the present chapter was to determine the consequences of increasing feed intake during mid-pregnancy (day 45 to day 85 of gestation) over three consecutive cycles, on sow body reserves management and productive-reproductive performance. To the authors' knowledge this area of sow nutrition has not previously been addressed. The results in this chapter are further divided into two sections: section 5.2 includes those results related to body

weight and body reserves management, and section 5.3 exposes those results related to the effects of this feeding regime on productive and lactation and postweaning performance.

5.2 Effects of extra feeding during mid-gestation over three consecutive parities on body reserves performance in multiparous sows

5.2.1 Partial objective

The effects of an extra feed intake during mid-pregnancy (day 45 to day 85 of gestation) on body weight (BW), backfat thickness (BF), loin depth (LD), body condition score (BCS) and estimated body composition (lipid and protein) management over three consecutive parities (3 cycles) in multiparous sows, were studied in this section.

5.2.2 Specific material and methods

In order to achieve this objective, the data from the initial pool of 103 animals (Control, C = 49 and Supplemented, S = 54) from 0 to 4 parities which started the experiment in cycle 1, was analysed over three cycles (for treatment description see Chapter 3). In cycle 1, the sows were grouped in three parity groups within treatment named initial parity groups (iPG, iPG1: gilts, iPG2: sows from parity 1 and 2 and iPG3: sows from parities 3 and 4). This initial classification was maintained throughout the three cycles studied. Therefore, iPG defines the parity group to which the sows belonged when they started the experiment in cycle 1. The data from gilts that were subsequently incorporated in cycles 2 and 3 (see Chapter 3), is not included in the results of this present chapter. On a parallel, body reserves management was also studied from the total of 54 sows from the initial 103, that finally completed the three cycles (n = 54, C = 26 and S = 28). As an exception in this chapter, apparent faecal digestibility (AFD_G) was determined from a pool of about 30 sows per cycle, which also included the gilts that were newly incorporated in cycles 2 and 3. Overall, the experiment continued until day 40 of gestation of the cycle after the last experimental cycle (cycle 4). In this way, the culling rates of sows from cycle 1 to cycle 4 were calculated.

Sow BW, BF, LD and BCS were obtained on day 40 and day 80 of gestation, at farrowing and at weaning in each of the three cycles studied. Sow body protein and lipid content were estimated using the equations of Dourmad et al. (1997). Apparent faecal digestibility of organic matter was determined on day 60 of gestation in each of the three cycles.

Statistics

All the analyses were performed using the SAS statistical package (Version 9.1, SAS Institute Inc., Cary, North Carolina, USA). The two main classification effects considered were treatment (feed allowance during mid-gestation) and initial parity group (iPG, the parity group to which the sows belonged when they started the experiment in cycle 1). Also, the interaction between treatment and iPG (T * iPG) was considered in all the analysis carried out. Generally,

when a fixed effect or the interaction term was significant, means were separated and tested for significance using Tukey correction for multiple contrasts.

The evolution of sows' body condition (BW, BF, LD and BCS) levels throughout gestation and lactation over the 3 cycles was analysed according to a repeated measures model using the MIXED procedure and considering sows as the repeated factor. The covariance structure specified in the repeated statement that fitted this model best was type AR (1) (first-order autoregressive) covariance. The mixed model used was the following:

$$y_{ijk} = \mu + T_i + iPG_j + t_k + (T * t)_{ik} + S_l + \varepsilon_{ijkl}$$

where, y_{ijk} is the response variable studied (BW, BF, LD and BCS); μ is the overall mean; T_i is the treatment effect, i = C and S; iPG_j is the initial parity group effect, j = 1, 2 and 3; t is the effect of time when the measurements were performed, k = day 40 and day 80 of gestation, farrowing and weaning; $(T * t)_{ik}$ is the interaction between treatment and time; S_l is the variance component that accounts for the repeated measurements made on the same sow and $\varepsilon_{ijkl} \sim N(0, \sigma^2)$ represents the unexplained random error. Subsequently, also the evolution of the interaction between treatment and time by iPG $(T * t * iPG)_{ijk}$ was studied (not specified in the statistical model above mentioned).

Net changes of sows' body condition throughout gestation and lactation, and apparent faecal digestibility at day 60 of gestation (AFD_G), were analysed through a two-way analysis of variance model using SAS GLM procedure:

$$y_{ij} = \mu + T_i + iPG_j + (T * iPG)_{ij} + \varepsilon_{ij}$$

where, y_{ij} is the response variable studied (BW, BF and LD changes or AFD); μ is the overall mean; T_i is the treatment effect, i = C and S; iPG_j is the initial parity group effect, j = 1, 2 and 3; $(T * iPG)_{ij}$ is the interaction between treatment and initial parity group and $\varepsilon_{ij} \sim N(0, \sigma^2)$ represents the unexplained random error. In the case of AFD, the parity group considered was named PG and was different from the iPG, since the gilts introduced in each cycle were also considered. Thus, for the AFD_G analysis PG1 included gilts, PG2 included sows from parities 1 and 2 and PG3 included sows from 3 to 5 parities in each of the three cycles studied.

5.2.3 Results

5.2.3.1 General

Feed intake throughout the experimental period (day 45 to day 85 of gestation)

Feed intake was supervised daily, during the experimental period (from day 45 to day 85 of gestation), in order to control feed refusals. The maximum and minimum ambient

temperatures achieved in the gestation barn were recorded daily during the experimental period. In cycles 1 and 3, no feed refusals were observed in any of the two experimental groups. Maximum and minimum temperatures in the gestation barn ranged between 13-24ºC and 6-19ºC, respectively, in cycle 1 and 15-22ºC and 11-19ºC, respectively, in cycle 3. In cycle 2, however, the maximum temperatures in the barn exceeded the 30ºC (maximum of 23-35ºC and minimum of 15-26ºC), and this caused a decrease in feed intake in the S group during the experimental period. In this respect, S sows consumed, approximately, a 20% less of the amount of feed expected for this group during the experimental period (+1.50 and +1.75 above control level in primiparous and multiparous sows, respectively). On the contrary, no feed refusals were observed in the C group during the experimental period in cycle 2.

The extra feed allowance imposed during mid-gestation led to slight differences on AFD_G of organic matter between treatments in cycles 1 and 3, tending to be lower in the S group of sows, although only significant in cycle 1 ($P < 0.10$, Table 5.1). In cycle 3, a parity group effect appeared, indicating that sows from PG1 had a lower coefficient of digestibility of organic matter at day 60 of gestation compared to sows from PGs 2 and 3 (PG1 = 74.4 %, PG2 = 76.8 % and PG3 = 77.7 %, SEM = 0.47, $P < 0.001$).

Table 5.1 Apparent faecal digestibility of organic matter (%) at day 60 of gestation by cycle.

Treatment		***Control***	***Supplemented***	***SEM***	**P-*value***		
Cycle	n				*T*	*PG*[1]	*T * PG*
1	28	78.1	77.0	0.41	*0.077*	*0.981*	*0.836*
2	31	79.9	80.1	0.60	*0.845*	*0.868*	*0.245*
3	35	76.6	76.0	0.33	*0.204*	*<0.001*	*0.507*

Results are expressed as least square means and SEM; T: dietary treatment; PG: parity group

[1]Parity group: PG1 = gilts; PG2 = parity 1 and 2; PG3 = parities 3 to 5

Sows culling rates

From the total of 103 sows from 0 to 4 parities that were initially assigned to this study, 54 animals (C = 26 and S = 28) remained until weaning in cycle 3, and 37 animals (C = 19 and S = 18) remained until day 40 of gestation in cycle 4. The number of animals per treatment, initial parity group (iPG) and cycle that were culled, and the main causes for culling throughout the three cycles, are shown in tables 5.2 and 5.3, respectively.

Overall, a total of 30 sows (61.2%) from the C group and 36 sows (66.7%) from the S group were removed from the experiment in the period comprised between day 40 of gestation in cycle 1 and day 40 of gestation in cycle 4 (Table 5.2). Across parity categories, iPG1 sows from the S group showed greater culling rates compared to their counterparts from the C group

during this period of time. In general, most of the removals took place in the period post-mating until day 40 of gestation of the same cycle. In percentages, the number of sows removed in this period [4] increased with the cycle in the C group but not in the S group, in which the percentage of sows removed was more or less constant over the three cycles studied (cycle 2, C = 16.3 % and S = 17.0 %; cycle 3, C = 20.6 % and S = 16.2 % and cycle 4, C = 24.0 % and S = 18.2 %).

Table 5.2 Number of sows per treatment and initial parity group (iPG) remaining at different stages within the reproductive cycle over the 3 cycles studied.

Dietary treatment	***Control***				***Supplemented***			
iPG[1]	*1*	*2*	*3*	*Total*	*1*	*2*	*3*	*Total*
Initial number of sows	18	19	12	49	22	22	10	54
Sows on trial, n								
Cycle 1								
Day 40 of gestation	18	19	12	49	22	22	10	54
Farrowing	18	18	11	47	22	22	9	53
Weaning	17	18	9	44	20	22	9	51
Cycle 2								
Mating	16	18	9	43	17	22	8	47
Day 40 of gestation	13	15	8	36	15	17	7	39
Farrowing	13	15	8	36	14	17	7	38
Weaning	13	15	8	36	14	17	7	38
Cycle 3								
Mating	12	14	8	34	13	17	7	37
Day 40 of gestation	10	11	6	27	10	15	6	31
Farrowing	10	10	6	26	9	14	6	29
Weaning	10	10	6	26	9	13	6	28
Cycle 4								
Mating	10	10	5	25	9	11	2	22
Day 40 of gestation	8	8	3	19	7	9	2	18
Total culled (%)[2]	10 (55.6)	11 (57.9)	9 (75)	30 (61.2)	15 (68.2)	13 (59.1)	8 (80)	36 (66.7)

iPG: initial parity group

[1] iPG: parity group to which the sows belonged when they started the experiment in cycle 1 (iPG1 = parity 0, iPG2 = parity 1 and 2 and iPG3 = parity ≥ 3)

[2] Totally of culled sows expressed as a percentage (%) of the initial number of sows

It is important to highlight that in the present study, the overall culling rates over the three cycles (about 60%) did not represent the real culling rates on this farm. This is because due to pre-established criteria in the experimental protocol, sows returning to oestrus after the first insemination and sows that showed WEI higher than 7 days were eliminated from the experiment; in commercial conditions these sows would have remained, at least, for one more mating.

[4] The number of sows removed from mating until day 40 of gestation of the same cycle was calculated as follows: [(number of sows at day 40 of gestation / number of sows at mating of the same cycle) * 100)] for each cycle.

Table 5.3 shows the different causes for culling according to treatment and cycle. The main reason for sow removal in all the three cycles studied was returning to oestrus after mating (RTE). The percentage of sows that returned to oestrus after mating [5] was higher in the C compared to the S group of sows over the three cycles studied (cycle 2, C = 16.3 % and S = 14.9 %; cycle 3, C = 17.6 % and S = 13.5 % and cycle 4, C = 24.0 % and S = 18.2 %). By iPG (data not shown in the table), the C group of sows showed higher final percentages of sows returning to oestrus than the S group, systematically, in all parity groups (iPG1, C = 18.4 % and S = 15.4 %; iPG2, C = 19.0 % and S = 16.0 %; iPG3, C = 18.2 % and S = 11.8 %).

Table 5.3 Number of sows and causes for culling from cycle to cycle[1] by dietary treatment.

Cause	*Cycle 1 to Cycle 2*[1]		*Cycle 2 to Cycle 3*[1]		*Cycle 3 to Cycle 4*[1]	
	C	S	C	S	C	S
N	49	54	36	39	27	31
Long WEI	-	2	-	-	-	2
RTE	7	7	6	5	6	4
NP	-	1	-	-	1	-
Abortion	1	-	-	-	-	1
Sudden death	1	3	-	-	-	1
MMA syndrome	-	2	-	1	-	-
Severe lameness	-	-	1	1	-	1
Prolapse	2	-	-	-	-	-
Illness	1	-	-	-	-	-
Age	-	-	1	-	1	4
Others	1	-	1	1	-	-

C: control and S: supplemented; n: number of sows at day 40 of gestation of the first cycle; WEI: Weaning-to-oestrus interval; RTE: sows that return to oestrus after mating; NP: found non pregnant after pregnancy test (30-35 days of gestation); MMA syndrome: Mamitis-Metritis-Agalactia syndrome

[1] Considers the period between day 40 of gestation in one cycle and day 40 of gestation of the next cycle, including weaning to oestrus interval and non confirmed gestation period of the subsequent cycle

Further causes for culling were extended weaning to the next detected oestrus interval (WEI, > 7 days), and sows eliminated due to Mamitis-Metritis-Agalactia syndrome (MMA). A total of 4 sows from the S group (2 sows from cycle 1 and 2 sows from cycle 3) were eliminated from the experiment because of long WEI. However, in 3 of the 4 cases, WEI ranged from 19-21 days post-weaning, suggesting that oestrus at 5-6 days post-weaning might have existed but not detected. On the other hand, the 3 sows eliminated due to MMA syndrome belonged to the S group, 2 of the 3 cases were gilts and the other one was a sow from parity 2. Regarding the remaining causes for culling (i. e. abortion, sudden death, age, illness, Table 5.3), they seemed not related to dietary treatments. Some of them (i.e. age eliminations) were planned culling by the producer and others (i.e. abortion, sudden death, lameness,...) were evenly distributed across treatments.

[5] The percentage of sows that returned to oestrus (RTE) after mating was calculated as [(number of sows RTE / number of sows mated in the same period) * 100)]

5.2.3.2 Sow body weight and body reserves management over the three cycles

a) Sow body weight and body reserves levels

Body weight and body reserves levels of the initial 103 sows

The evolution of sow condition over the three cycles studied was analysed through a repeated measures model. According to this model, in which sows were the repeated item, the interaction between treatment and time (T * t) is represented in figure 5.1 (5.1A, 5.1B and 5.1C). At allocation (day 40 of gestation in cycle 1), BW (Figure 5.1A), BF (Figure 5.1B) and LD (Figure 5.1C) levels were not different across treatment groups ($P > 0.10$). Then, the general tendency was to increase BW and body reserves levels during gestation and to decrease them during the lactation period. In general, the increased feeding level during mid-gestation did not lead to significant differences on BW, BF and LD levels between treatments in cycle 1. However, dietary treatment was able to cause significant differences on body reserves management at determined stages within the reproductive cycle, in cycles 2 and 3.

Figure 5.1A shows that after three cycles (from day 40 of gestation in cycle 1 until weaning in cycle 3), sows from both treatments experienced an overall increase in BW. Statistical differences between treatments appeared at weaning in cycle 2 (C = 224.6 kg and S = 236.8 kg, $P = 0.023$) and after farrowing in cycle 3 (C = 251.9 kg and S = 265.6 kg, $P = 0.033$). Thereafter, although the S group maintained higher weight values, the differences between treatment groups were not statistically significant ($P > 0.10$).

Backfat was the most influenced body condition trait by the increased feeding level during mid-gestation. Figure 5.1B illustrates that in the supplemented group of sows (S) BF reserves increased during gestation and decreased during lactation, with an overall tendency to have a higher backfat at the same stage in subsequent cycles. However, in the C group there was a tendency towards maintaining or even decreasing BF levels at the end of each reproductive cycle, as time progressed. Indeed, S sows showed significantly higher BF levels at weaning than C sows in cycles 2 (C = 15.4 mm and S = 17.2 mm, $P = 0.017$) and 3 (C = 15.6 mm and S = 18.3 mm, $P = 0.004$). Thus, only the sows supplemented during mid-gestation were able to accumulate fat reserves after three cycles. Supplemented sows showed higher, while C sows showed lower levels at weaning in cycle 3 compared to those they had at day 40 of gestation in cycle 1 (C = 15.6 mm to 17.5 mm and S = 18.3 mm to 16.4 mm, respectively).

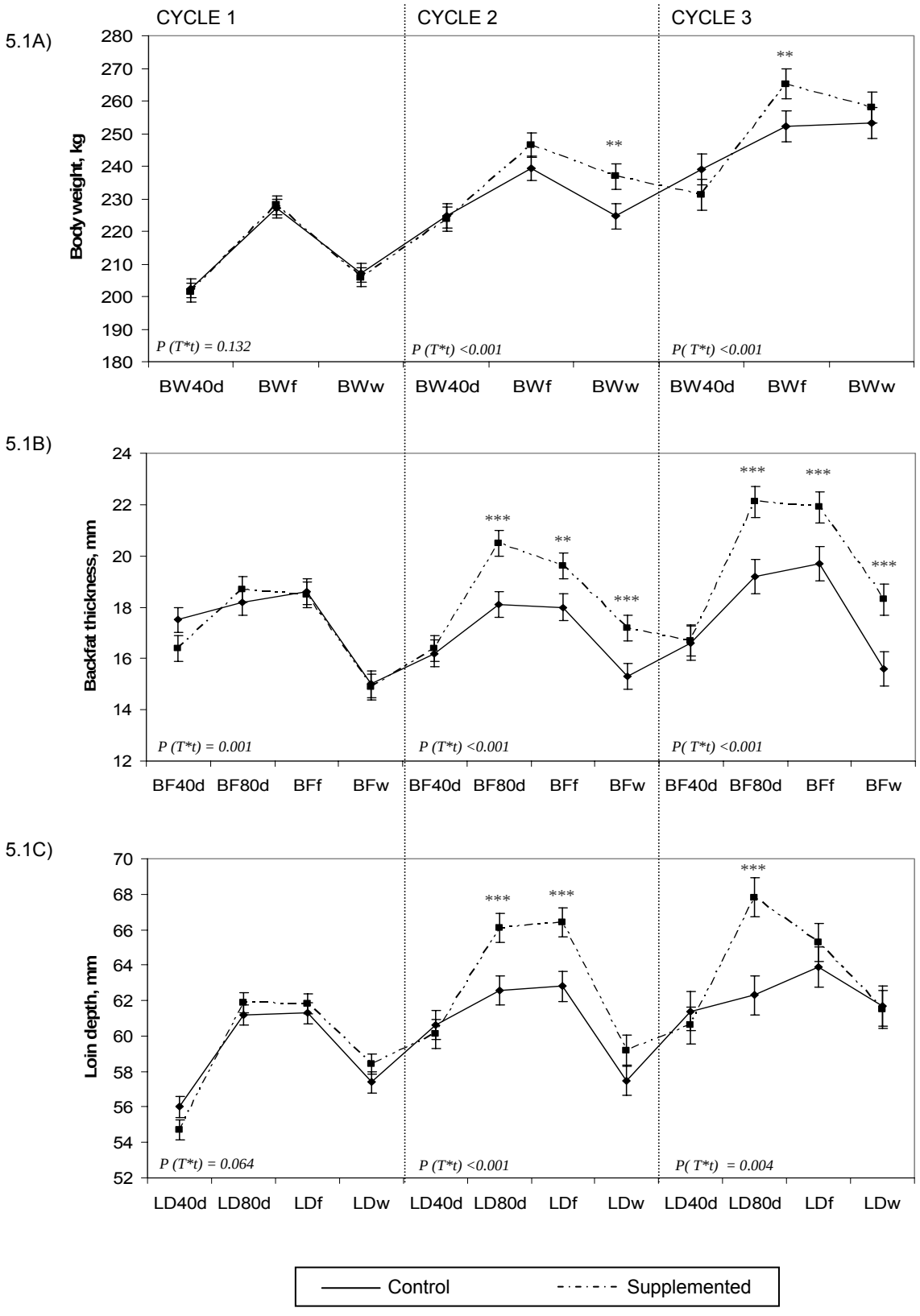


Figure 5.1 Body weight (5.1A), backfat thickness (5.1B) and loin depth (5.1C) levels at 40 days of gestation (BW40d, BF40d and LD40d), day 80 of gestation (BF80d and LD80d), 48 ± 24 h post-farrowing (BWf, BFf and LDf) and on day 18 of lactation (BFw and LDw) or at weaning (BWw) over three consecutive cycles in the control (n = 48) and supplemented (n = 53) groups of sows. Significance is marked as: * if $P < 0.1$, ** if $P \leq 0.05$ and *** if $P \leq 0.01$ and error bars represent the standard error of the least square means.

Regarding LD measures (Figure 5.1C), their evolution was similar to that of the BW showing a total increase in LD levels from cycle 1 to cycle 3 in both dietary treatments. The supplemented group of sows showed higher levels of LD at day 80 of gestation (C = 62.6 mm and S = 66.1 mm, *P* = 0.003) and at farrowing (C = 62.8 mm and S = 66.4 mm, *P* = 0.002) in cycle 2, but these differences finally disappeared at weaning due to higher LD losses throughout lactation in the S compared to the C group of sows.

Body condition score was maintained around 3.0 throughout gestation and lactation in cycles 1 and 2 (data not shown). In cycle 3, differences were found between treatment groups on day 80 of gestation (C = 3.1 and S = 3.7, *P* = 0.001) and at farrowing (C = 3.3 and S = 3.6, *P* = 0.060), but they disappeared statistically at weaning (C = 2.9 and S = 3.1, *P* = 0.246).

Body weight and body reserves levels of the initial 103 sows by initial parity group

Figure 5.2 (5.2A, 5.2B and 5.2C) represents the pattern of sow body reserves changes along the three consecutive cycles studied, according to the triple interaction between dietary treatment, time and initial parity group (T * t * iPG).

The graph representing BW values (Figure 5.2A) shows a data grouping by parity group. As it was expected, the youngest sows in both treatments (iPG1) showed a marked increase in BW over the three cycles (from 157.3 kg on day 40 of gestation in cycle 1 to 241.6 kg at weaning in cycle 1). Nevertheless, the sows from iPGs 2 and 3 showed slight increases in BW from the beginning of the experiment until the end (iPG2: from 210.4 kg to 249.4 kg and iPG3: from 237.8 kg to 275.6 kg). The dietary treatment significantly affected sows from iPG1 but not from the other parity groups. The supplemented sows (S) from iPG1 showed significantly higher BW values at weaning in cycle 2 (C = 191.8 kg and S = 213.4 kg, *P* = 0.015) and at farrowing in cycle 3 (C = 231.3 kg and S = 251.1 kg, *P* = 0.068) than C sows from the same iPG (Figure 5.2A). These differences were kept numerically but not statistically significant at weaning in cycle 3.

Contrary to BW, BF (Figure 5.2B) and LD (Figure 5.2C) measures were mainly grouped by dietary treatment, instead of by iPG. In general, S sows showed a higher amount of BF reserves than C sows, especially in cycles 2 and 3 (Figure 5.2B). By initial parity groups, sows from iPG1 and 2 were the most benefited in terms of body reserves by the experimental treatment. In this respect, S sows from iPG1 showed higher BF levels at weaning in cycle 2 and at weaning in cycle 3, than the C sows of the same iPG (C = 13.4 mm and S = 15.9 mm, *P* = 0.032 and C = 14.7 mm and S =17.7 mm, *P* = 0.037, respectively). Besides, the S sows from iPG2 showed higher BF levels at weaning in cycle 3 than C sows from the same iPG (C = 14.2 mm *vs* S =18.7 mm, *P* = 0.001).

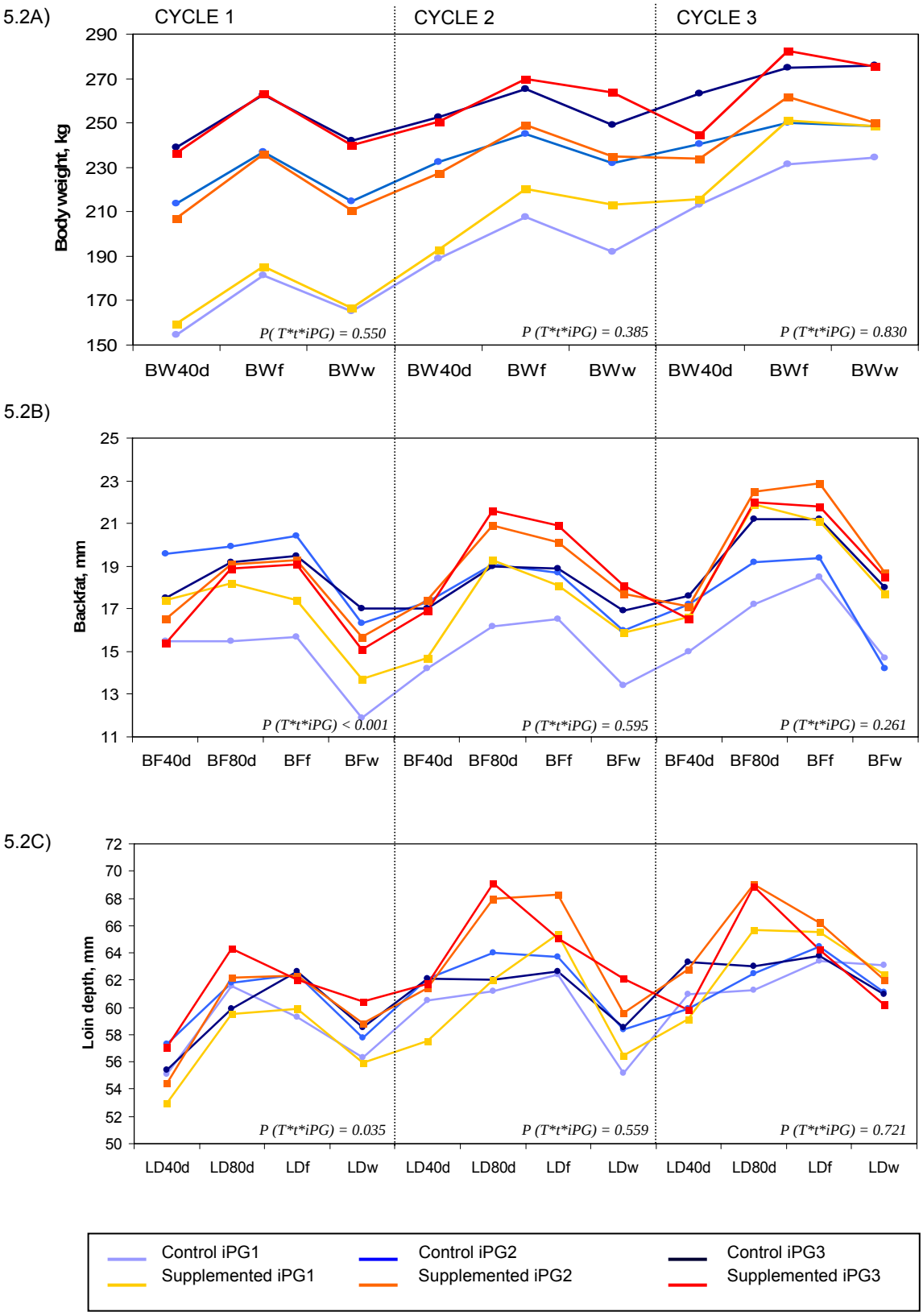


Figure 5.2 Body weight (4.2A), backfat thickness (4.2B) and loin depth (4.2C) values on day 40 of gestation (BW40d, BF40d and LD40d), day 80 of gestation (BF80d and LD80d), 48 ± 24 h post-farrowing (BWf, BFf and LDf) and at weaning (BWw, BFw and LDw) over three consecutive parity cycles by initial parity group (iPG) in control (iPG1, n=18; iPG2, n=18 and iPG3, n=12) and supplemented (iPG1, n=22; iPG2, n=22 and iPG3, n=9) groups of sows.

Loin depth levels did not show this cumulative pattern in the supplemented group *versus* the control group of sows at the end of each cycle (Figure 5.2C). The advantage in LD levels achieved by S sows at the end of the experimental period (day 80 of gestation) within each cycle, disappeared thereafter due to a higher amount of LD losses during lactation in this treatment group. By parity groups, all the sows increased their LD levels in the end of the 3 cycles, with no clear benefits from the experimental treatment (feed supplementation) and between parity groups.

Body weight and body reserves levels of the sows completing the three cycles

A retrospective analysis of body reserves evolution from the sows that had completed the three cycles consecutive cycles studied (n = 54) was performed (Figure 5.3: 5.3A, 5.3B and 5.3C). In general, the body reserves evolution of this group of sows throughout the three cycles was very similar to that of the initial 103 sows (see Figure 5.1). However, the retrospective analysis of the subgroup of 54 sows completing the three cycles showed significant differences between treatments in mean body reserves levels at the initial point of the experiment (day 40 of gestation in cycle 1). The S sows that remained after the three cycles showed lower average BF (C = 18.7 mm and S = 15.8 mm, P = 0.003) and LD (C = 56.7 mm and S = 54.1 mm, P = 0.019) initial levels compared to C sows. This indicates that the body reserves profile at the initial point of the experiment was different between the control and supplemented sows that completed the three cycles.

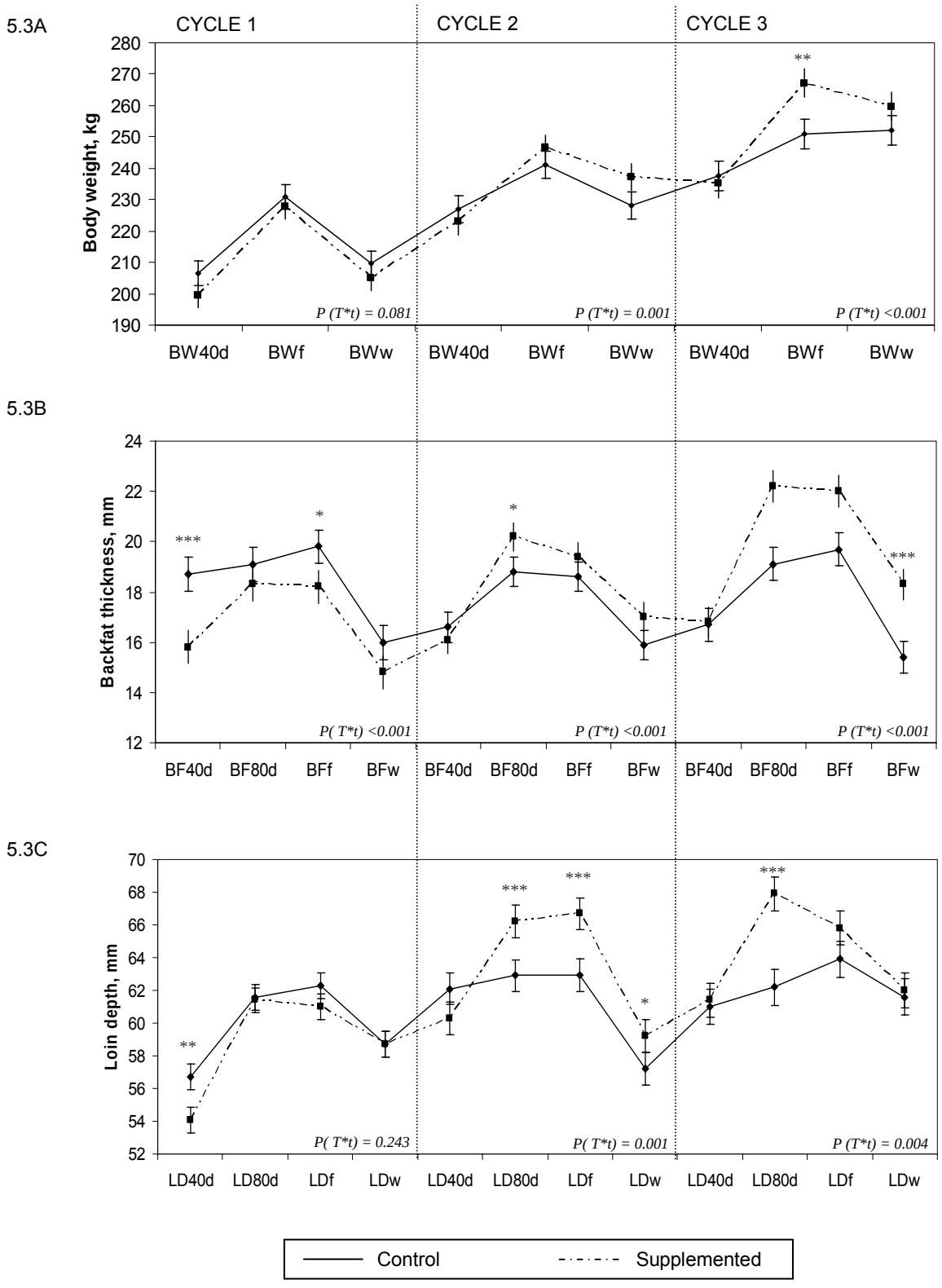


Figure 5.3 Body weight (5.3A), backfat thickness (5.3B) and loin depth (5.3C) levels on day 40 of gestation (BW40d, BF40d and LD40d), day 80 of gestation (BF80d and LD80d), 48 ± 24 h post-farrowing (BWf, BFf and LDf) and at weaning (BWw, BFw and LDw) over three consecutive cycles from the sows completing the three cycles studied (n=54, C = 26 and S = 28). Significance is marked as: * if $P < 0.1$, ** if $P \leq 0.05$ and *** if $P \leq 0.01$ and error bars represent the standard error of the least square means.

b) Changes of body weight and body reserves

The magnitude of sow BW and body reserves changes over the three cycles was investigated from the animals that completed the three cycles (n = 54). Overall sows BW, BF and LD changes over the three cycles studied (from day 40 of gestation in cycle 1 until weaning in cycle 3), total balances by cycle (from day 40 of gestation until weaning in each cycle), and changes throughout gestation (from day 40 of gestation until farrowing) and lactation [from farrowing until day 18 of lactation (BF and LD), or until weaning (BW)] also by cycle, are presented in tables 5.4, 5.6 and 5.7, respectively.

Overall changes through the three cycles studied

Body reserves balance from the beginning of the experiment (day 40 of gestation in cycle 1) to the weaning time in cycle 3, including lipid and protein estimations, were recorded in table 5.4. The supplemented group of sows during mid-pregnancy showed higher BW and estimated lipid content gains than C sows ($P < 0.02$). The increases of LD and estimated protein content were numerically higher in the S compared to the C group, although not statistically significant. Furthermore, S sows exhibited a positive final BF balance while C sows lost BF at the end of the three reproductive cycles (S = 1.96 mm and C = -3.17 mm, $P < 0.001$).

Table 5.4 Overall changes of body weight (BW), backfat thickness (BF), loin depth (LD) and estimated lipid and protein content from weaning in cycle 3 to day 40 of gestation in cycle 1.

Treatment	***Control***	***Supplemented***		P-*value*		
n	27	31	SEM	*T*	*iPG*[1]	*T * iPG*
BW, kg	45.7	60.7	4.26	*0.016*	*<0.001*	*0.834*
BF, mm	-3.17	1.96	0.677	*<0.001*	*0.521*	*0.001*
LD, mm	5.47	7.33	1.353	*0.332*	*0.124*	*0.208*
Lipid, kg[2]	5.61	15.40	1.783	*0.001*	*0.005*	*0.050*
Protein, kg[2]	8.73	9.46	0.690	*0.457*	*<0.001*	*0.752*

Results are expressed as least square means and SEM; T: dietary treatment; iPG: Initial parity group

[1] iPG: parity group to which the sows belonged when they started the experiment in cycle 1 (iPG1 = parity 0, iPG2 = parity 1 and 2 and iPG3 = parity ≥ 3)

[2] Body lipid and protein content estimated from the equations of Dourmad et al. (1997)

The initial parity group effect was found significant for BW and estimated lipid and protein gains. In this respect, the youngest sows in the experiment (iPG1) gained significantly more BW and also lipid and protein tissue compared to the other parity groups ($P < 0.005$, Figure 5.4).

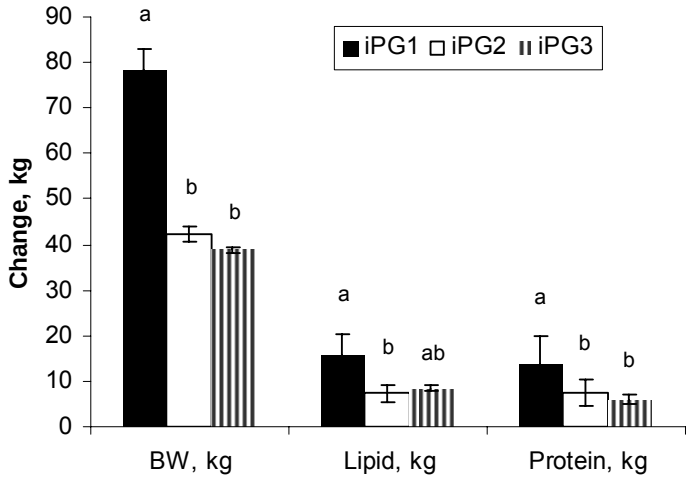


Figure 5.4 Overall changes of body weight (BW) and estimated lipid and protein content from weaning in cycle 3 to day 40 of gestation in cycle 1, by initial parity group (iPG1 = parity 0, iPG2 = parity 1 and 2 and iPG3 = parity ≥ 3). Different letters between iPGs mean $P < 0.06$.

A significant interaction effect between treatment and iPG was found for BF and estimated lipid overall balance (Table 5.4). This interaction is showed in table 5.5 and reveals that sows from iPG2 results benefited most in terms of fat reserves by the increase in feed allowance during mid-pregnancy after three cycles ($P < 0.001$).

Table 5.5 Body weight (BW), backfat thickness (BF) and loin depth (LD) overall changes (from weaning in cycle 3 to day 40 of gestation in cycle 1), by dietary treatment and initial parity group (iPG).

Item	*BW, kg*			*BF, mm*			*LD, mm*		
Treatment	C	S	*P*-value	C	S	*P*-value	C	S	*P*-value
iPG1	72.5	84.4	*0.781*	-1.5	-0.11	*0.933*	7.3	10.3	*0.922*
iPG2	32.6	52.0	*0.273*	-6.0	3.6	*<0.001*	4.0	9.5	*0.419*
iPG3	32.0	45.7	*0.884*	-2.0	2.4	*0.273*	5.2	2.2	*0.994*

Results are expressed as least square means; C: control and S: supplemented

[1] iPG: parity group to which the sows belonged when they started the experiment in cycle 1 (iPG1 = parity 0, iPG2 = parity 1 and 2 and iPG3 = parity ≥ 3)

Total body reserves balance per cycle

Changes of BW, BF and LD from day 40 of gestation until weaning were calculated within each cycle and shown in table 5.6. The general tendency indicated positive changes in BW, although variable results in BF and LD balances throughout each of the 3 cycles studied.

At the end of the first reproductive cycle (cycle 1), BF and lipid balances were negative while LD balance was positive in both dietary treatments. Across treatments, S sows tended to loose less BF and lipid content than group C (1.4 mm of BF and 2.68 kg of lipids of difference). In subsequent cycles, BW, BF, and also LD (cycle 2) changes resulted clearly improved ($P < 0.01$) by the extra feed supplementation strategy implemented during mid-gestation in the S group of sows.

The interaction between dietary treatment and iPG was significant for BF in cycles 1 and 3 ($P < 0.07$). The study of this interaction revealed that the positive effect found on BF balance in the S group in those two cycles was, again, mainly due to sows from iPG2, since they were able to maintain (cycle 1) or even gain BF (cycle 3) at the end of the cycle, while their counterparts from the C group presented negative balances at this time (cycle 1, C = -3.9 mm and S = -0.5 mm, P = 0.022 and cycle 3, C = -3.1 mm and S = 1.5 mm, P = 0.001). This was not observed in other parity groups.

Table 5.6 Total balance of body weight (BW), backfat thickness (BF) and loin depth (LD) from day 40 of gestation until weaning by cycles from sows completing the three cycles (n = 54).

Treatment	***Control***	***Supplemented***		P-*value*		
n	26	28	*SEM*	*T*	*iPG*[1]	*T * iPG*
Cycle 1						
BW, kg	1.37	5.32	2.425	*0.223*	*0.097*	*0.156*
BF, mm	-2.3	-0.86	0.524	*0.063*	*0.036*	*0.063*
LD, mm	2.3	3.7	1.019	*0.346*	*0.381*	*0.209*
Lipid, kg	-3.18	-0.50	1.155	*0.088*	*0.457*	*0.101*
Protein, kg	0.667	0.937	0.453	*0.656*	*0.006*	*0.380*
Cycle 2						
BW, kg	0.59	14.6	2.728	*0.001*	*0.386*	*0.413*
BF, mm	-0.62	0.94	0.379	*0.004*	*0.539*	*0.554*
LD, mm	-4.7	-0.558	1.089	*0.009*	*0.780*	*0.955*
Lipid, kg	-1.47	4.050	0.918	*<0.001*	*0.755*	*0.868*
Protein, kg	-0.28	1.95	0.472	*0.001*	*0.331*	*0.135*
Cycle 3						
BW, kg	14.2	26.1	2.522	*0.001*	*0.001*	*0.494*
BF, mm	-1.0	1.5	0.457	*0.001*	*0.040*	*0.047*
LD, mm	0.33	0.94	1.174	*0.712*	*0.245*	*0.557*
Lipid, kg	1.39	7.902	0.946	*<0..001*	*0.001*	*0.425*
Protein, kg	2.47	3.60	0.430	*0.065*	*0.002*	*0.198*

Results are expressed as least square means and SEM; T: dietary treatment; iPG: Initial parity group

[1] iPG: parity group to which the sows belonged when they started the experiment in cycle 1 ((iPG1 = parity 0, iPG2 = parity 1 and 2 and iPG3 = parity ≥ 3)

Changes throughout gestation and lactation per cycle

In general, as it was expected, sows gained BW and body reserves during gestation and they later lost, at least part of them, during the lactation period (Table 5.7). In cycle 1, pregnant sows from the S group gained significantly more BF than C sows (P = 0.052), but not significantly more BW or LD. Also in this cycle, the interaction between treatment and iPG tended to be significant for LD (P = 0.082) and numerical for BF (P < 0.20). In this manner, S sows from iPGs 1 and 2 accumulated more LD and S sows from iPGs 2 and 3 accumulated more BF than their control counterparts, suggesting that the extra feeding went on increasing LD reserves in the younger sows, whereas it went on increasing BF reserves in the older sows under treatment. In cycle 2, S sows evidenced higher BW, BF and LD gains during gestation than C sows (P < 0.05). In cycle 3, S sows continued gaining more BW, BF and LD during gestation than C sows, although LD gains were not statistically significant.

Throughout the lactation period, all BW, BF and LD losses were similar between treatments in all cycles, with the exception of cycle 3, where the sows from the S group showed higher BW losses than C sows.

Estimated lipid and protein balances during gestation and lactation showed similar tendencies as BF and LD balances. For that reason, in this case, they were not included in the table.

Table 5.7 Changes in body weight (BW), backfat thickness (BF) and loin depth (LD) during gestation and lactation from sows that completed the three consecutive cycles (n = 54). The percentage of change is shown in brackets.

Treatment	***Control***	***Supplemented***		P-*value*		
n	26	28	SEM	*T*	*iPG*[1]	*T * iPG*
Cycle 1						
Gestation gains[3]						
BW, kg	24.1 (12.1)	28.0 (14.6)	1.970	*0.158*	*0.985*	*0.439*
BF, mm	1.4 (7.9)	2.7 (19.6)	0.482	*0.052*	*0.020*	*0.156*
LD, mm	6.0 (11.0)	6.5 (12.6)	1.019	*0.730*	*0.382*	*0.082*
Lactation losses[4]						
BW, kg	-21.2 (-9.1)	-23.0 (-10.2)	1.156	*0.321*	*0.001*	*0.648*
BF, mm	-3.6 (-18.0)	-3.5 (-19.2)	0.355	*0.787*	*0.378*	*0.151*
LD, mm	-3.7 (-5.6)	-2.9 (-4.7)	0.918	*0.572*	*0.696*	*0.320*
Cycle 2						
Gestation gains[3]						
BW, kg	14.0 (6.5)	23.3 (10.8)	1.855	*0.001*	*0.131*	*0.864*
BF, mm	2.1 (13.5)	3.3 (21.4)	0.350	*0.013*	*0.694*	*0.768*
LD, mm	0.70 (1.2)	5.4 (9.5)	1.165	*0.006*	*0.657*	*0.990*
Lactation losses[4]						
BW, kg	-13.4 (-5.7)	-8.5 (-3.4)	2.370	*0.146*	*0.926*	*0.173*
BF, mm	-2.6 (-14.0)	-2.5 (-12.9)	0.291	*0.734*	*0.653*	*0.456*
LD, mm	-5.4 (-8.1)	-6.4 (-9.4)	1.198	*0.576*	*0.415*	*0.995*
Cycle 3						
Gestation gains[3]						
BW, kg	13.3 (5.8)	33.7 (14.4)	2.051	*<0.001*	*0.060*	*0.379*
BF, mm	3.1 (19.2)	5.2 (31.7)	0.504	*0.004*	*0.802*	*0.201*
LD, mm	2.7 (4.8)	4.3 (7.4)	1.428	*0.440*	*0.724*	*0.364*
Lactation losses[4]						
BW, kg	1.2 (0.51)	-6.2 (-2.2)	2.578	*0.042*	*0.361*	*0.605*
BF, mm	-4.1 (-20.6)	-3.6 (-16.9)	0.443	*0.460*	*0.120*	*0.749*
LD, mm	-2.4 (-3.3)	-3.9 (-5.6)	1.085	*0.313*	*0.460*	*0.946*

Results are expressed as least square means and SEM; T: dietary treatment; iPG: initial parity group

[1] iPG: parity group to which the sows belonged when they started the experiment in cycle 1 (iPG1 = parity 0, iPG2 = parity 1 and 2 and iPG3 = parity ≥ 3)

[2] Gestation gains accounted from day 40 of gestation until post-farrowing

[3] Lactation losses accounted from farrowing until day 18 of lactation (BF and LD), or until weaning (BW)

5.3 Effects of extra feeding during mid-gestation over three consecutive parities on productive and reproductive performance in multiparous sows

5.3.1 Partial objective

The main purpose of this chapter was to determine the extent to which an extra feed intake during mid-pregnancy (day 45 to day 85 of gestation) over three consecutive parities (3 cycles) affected sow productive and reproductive performance.

5.3.2 Specific material and methods

Productive and post-weaning performance was recorded from the same initial pool of 103 sows (C = 49 and S = 54) that were evaluated for body weight and body reserves changes in section 5.2. Data from the gilts that were incorporated in cycles 2 and 3 were not included in the present section. But as an exception, lactation feed intake and apparent faecal digestibility during lactation (AFD_L) determined in cycle 3 implied a pool of sows (n = 51 and n = 46, respectively) which did also indeed include the gilts introduced in cycles 2 and 3, in order to have representation of parity 1 and 2 sows in those two traits. The lactation feed intake was measured over 12 non-consecutive days of lactation and pooled by treatment, and AFD_L of organic matter was measured on day 15 ± 1 of lactation.

The number of pigs and litter and average piglet weights were recorded at farrowing and on day 18 of lactation. Furthermore, lifetime piglet production over the 3 cycles studied was calculated from the 54 sows that finally completed the three cycles (C = 26 and S = 28). After weaning, rebreeding performance (weaning to oestrus interval) and farrowing rates were recorded.

Statistics

All the analysis were performed using the SAS statistical package (Version 9.1, SAS Institute Inc., Cary, North Carolina, USA). The two main classification factors considered for all the analysis were treatment (feed allowance during mid-gestation) and initial parity group (iPG, the parity group to which the sows belonged when they started the experiment in cycle 1, see table 3.1 in Chapter 3). The interaction between treatment and iPG was also considered in all the analysis performed. Generally, when a fixed effect or the interaction term was significant, means were separated and tested for significance using Tukey correction for multiple contrasts.

The number of total born, born alive, stillborn and pigs on day 18 of lactation were analysed using a statistical model for counting data (GENMOD procedure). The offset term was used for born alive, stillborn and number of pigs at day 18 of lactation, in order to refer this data to a total (total born and pigs after cross-fostering, respectively).

Litter and average piglets weight of total born, born alive and pigs on day 18 of lactation were evaluated through a two-way analysis of variance model using GLM procedure. Litter size was used as a covariate term for litter weight and average piglet weight. Litter weight and piglet weight on day 18 of lactation were also covariated by litter weight and piglet weight after cross-fostering, respectively.

Differences on average litter daily gain (ADG) during lactation (from 24 h post-farrowing until 18 days of lactation), weaning to oestrus interval (WEI) and apparent fecal digestibility in lactation (AFD_L) were also tested through a two-way analysis of variance model (GLM procedure). For litter ADG, the litter weight at 24 hours post-farrowing was used as a covariate term.

The general two-way analysis of variance model used was the following:

$$y_{ij} = \mu + T_i + iPG_j + (T * iPG)_{ij} + \beta\, x + \varepsilon_{ij}$$

where, y_{ij} is the response variable studied (litter weight, average piglet weight, ADG of the litter and WEI), μ is the overall mean, T_i is the treatment effect, i = C and S; iPG_j is the initial parity group effect, j = 1, 2 and 3; $(T * iPG)_{ij}$ is the interaction between treatment and initial parity group; x indicates the covariate term used when necessary (litter size, litter weight and APW) and $\varepsilon_{ij} \sim N(0, \sigma^2)$ represents the unexplained random error.

In order to test the differences in the average daily feed intake during lactation (ADFI), a GLM procedure was performed by taking into consideration the day of lactation as an additional classification effect:

$$y_{ijk} = \mu + T_i + iPG_j + L_k + \varepsilon_{ijk}$$

where, y_{ij} is the response variable studied (ADFI), μ is the overall mean; T_i is the treatment effect, i = C and S; PG_j is the parity group effect, j = 1, 2 and 3; L_k is the lactation day effect and $\varepsilon_{ijk} \sim N(0, \sigma^2)$ represents the unexplained random error.

In the case of AFD_L and ADFI measured during lactation in cycle 3, the parity group was named PG and was different from the iPG, because the gilts introduced in each cycle were considered. However, the criteria for parity grouping were the same as those followed at the beginning of the experiment in cycle 1: PG1 included gilts, PG2 included sows from parities 1 and 2 and PG3 included sows from 3 or more parities (3 to 6 in this case).

5.3.3 Results

5.3.3.1 Birth and lactation performance over the three cycles studied

The results of productive performance at birth and throughout lactation over the three cycles studied are stated below. It is important to mention that in cycle 3, this farm suffered a porcine respiratory and reproductive syndrome (PRRS) outbreak. No evident clinical symptoms in reproductive performances (abortions, return to oestrus) nor in litter performance at birth (stillbirths, mummified foetuses) appeared but the outbreak might have affected productive performance during lactation (pig mortality rates and litter weights at weaning).

a) Litter performance at birth

The litter performance at farrowing from the three cycles studied is recorded in table 5.8. In cycles 1 and 2, the number of total born, born alive, stillborn and mummies and average litter and piglet weights were not affected by the feeding level during mid-pregnancy ($P > 0.10$). Nevertheless, in cycle 3 the supplemented sows presented higher litter and average piglet weights at birth (Total born, $P < 0.10$; Born alive, $P < 0.05$) compared to group C.

The parity at which sows started the experiment (iPG effect) had a strong influence on litter and average piglet weights at birth in cycle 1 ($P < 0.001$, Table 5.8). In this respect, sows from iPG1 showed lower piglet weights at birth ($P < 0.01$) compared to iPG 2 and 3 sows. Because this effect was only found in cycle 1 it seems that the age of the sow and not the iPG itself could be the main cause of this difference. More detailed information about this effect will be provided in chapter 6.

Additionally, in cycles 2 and 3, the number of pigs born alive and stillborn was also affected by iPG ($P < 0.01$, Table 5.8 and Figure 5.5). In this respect, sows from iPG3 showed the highest number of total born pigs per litter, but the lowest percentage of born alive and the highest percentage of stillbirths (in respect to total born) compared to their counterparts from iPGs 1 and 2 in cycle 2 (Figure 5.5A). In cycle 3, iPG3 sows continued having the lowest percentage of piglets born alive and the highest percentage of stillborn (in respect to total born) compared to the other parity groups (Figure 5.5B). This fact, revealed the existence of an aging effect also in the number stillbirths but, in this case, it was in detriment to the older sows in the experiment (iPG3).

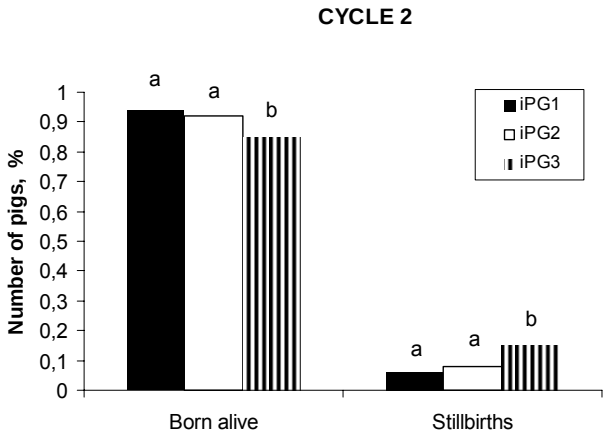
Table 5.8 Litter performance per sow at farrowing by dietary treatment and cycle.

Treatment	***Control***	***Supplemented***		P-*value*		
			SEM	*T*	*iPG*[1]	*T * iPG*
Cycle 1						
n sows	48	53				
Total born						
Litter size	12.7	12.9	0.47	*0.782*	*0.156*	*0.209*
Litter weight, kg	19.0	19.5	0.39	*0.405*	*<0.001*	*0.180*
Piglet weight, kg	1.55	1.59	0.031	*0.355*	*<0.001*	*0.229*
Born Alive						
Litter size	11.8	12.0	0.43	*0.679*	*0.624*	*0.417*
Litter weight, kg	18.0	18.2	0.36	*0.642*	*<0.001*	*0.259*
Piglet weight, kg	1.59	1.59	0.031	*0.919*	*<0.001*	*0.314*
Stillborn	0.89	0.77	0.160	*0.574*	*0.548*	*0.357*
Mummies	0.15	0.15	0.067	*0.923*	*0.509*	*0.692*
Cycle 2						
n sows	36	39				
Total born						
Litter size	13.4	13.3	0.45	*0.846*	*0.061*	*0.826*
Litter weight, kg	19.2	19.5	0.43	*0.703*	*0.112*	*0.859*
Piglet weight, kg	1.48	1.50	0.03	*0.642*	*0.120*	*0.894*
Born Alive						
Litter size	12.3	11.8	0.47	*0.261*	*0.008*	*0.890*
Litter weight, kg	17.4	17.7	0.41	*0.678*	*0.145*	*0.796*
Piglet weight, kg	1.49	1.50	0.035	*0.855*	*0.159*	*0.644*
Stillborn	1.07	1.33	0.229	*0.244*	*0.003*	*0.999*
Mummies	0.29	0.34	0.141	*0.707*	*0.225*	*0.116*
Cycle 3						
n sows	27	31				
Total born						
Litter size	13.7	13.5	0.73	*0.831*	*0.945*	*0.542*
Litter weight, kg	18.7	19.8	0.48	*0.091*	*0.152*	*0.016*
Piglet weight, kg	1.38	1.49	0.038	*0.056*	*0.075*	*0.014*
Born Alive						
Litter size	12.2	11.9	0.63	*0.741*	*0.014*	*0.859*
Litter weight, kg	16.8	18.1	0.44	*0.038*	*0.129*	*0.005*
Piglet weight, kg	1.39	1.52	0.037	*0.012*	*0.036*	*0.005*
Stillborn	1.41	1.51	0.257	*0.700*	*0.032*	*0.850*
Mummies	0.45	0.46	0.158	*0.965*	*0.466*	*0.558*

Results are expressed as least square means and SEM; In counting data (litter size) standard error was extracted from an ANOVA analysis; T: dietary treatment; iPG: initial parity group

[1] iPG: parity group to which the sows belonged when they started the experiment in cycle 1 (iPG1 = parity 0, iPG2 = parity 1 and 2 and iPG3 = parity ≥ 3)

5.5A



5.5B

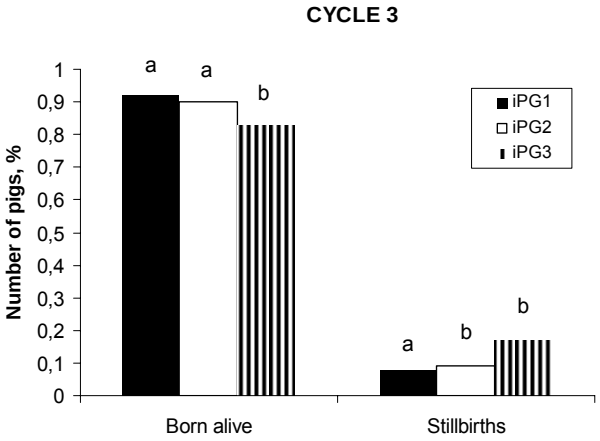


Figure 5.5 Percentage of pigs born alive and stillborn in respect to the total born per litter in cycles 2 (5.5A) and 3 (5.5B) by initial parity group. Initial parity group (iPG): iPG1 = parity 0, iPG2 = parity 1 and 2 and iPG3 = parity ≥ 3. Statistical comparisons were made between iPGs for each trait analysed (GENMOD procedure of SAS). Different letters between iPGs mean $P < 0.06$.

The interaction between dietary treatment and iPG (T * iPG) became significant in cycle 3 for litter and piglet weight at birth (total born and born alive, Table 5.8). This interaction is demonstrated in table 5.9 and reveals that the treatment effect observed and previously commented was caused by a specific effect in iPG1 sows. Supplemented sows from iPG1 presented greater litter and piglet weights at birth compared to their counterparts from the C group ($P < 0.05$), while no statistical differences were found between treatments in sows from iPGs 2 and 3.

Table 5.9 Litter and average piglet weight at birth (total born and born alive) by dietary treatment and initial parity group (iPG[1]) in cycle 3.

Traits	***Litter weight, kg***				***Piglet weight, kg***			
Treatment	*Control*	*Supplemented*	*SEM*	P-*value*	*Control*	*Supplemented*	*SEM*	P-*value*
Total born								
iPG1	17.8	21.1	0.78	0.032	1.28	1.58	0.061	0.014
iPG2	20.5	19.5	0.74	0.903	1.54	1.48	0.058	0.968
iPG3	17.8	18.9	1. 04	0.970	1.33	1.40	0.082	0.980
Born Alive								
iPG1	15.9	19.5	0.71	0.008	1.29	1.62	0.060	0.002
iPG2	18.6	17.6	0.68	0.877	1.57	1.51	0.057	0.974
iPG3	15.8	17.1	0.96	0.908	1.30	1.43	0.081	0.855

Results are expressed as least square means and SEM; iPG: initial parity group

[1] iPG: parity group to which the sows belonged when they started the experiment in cycle 1 (iPG1 = parity 0, iPG2 = parity 1 and 2 and iPG3 = parity ≥ 3)

b) Lactation performance

Lactation performance, expressed in the form of number of pigs, litter and average piglet weights at day 18 of lactation, piglet mortality rates and estimated litter average daily gain during lactation (ADG), is recorded by cycles in table 5.10. Litter-weight gain was used as a measure of milk production since it has been demonstrated that there is a strong relationship between milk production and litter gain (Noblet et al., 1990).

The maternal dietary treatment only caused slight differences in some of these parameters in cycles 1 and 2. In cycle 3, sows from the S group tended to breed 1 piglet less than C sows on day 18 of lactation (C = 9.3 and S = 8.3, P = 0.078). This result was supported by the fact that, in spite of the unusual higher piglet mortality found in this cycle, the S group of sows showed higher mortality rates compared to the C group (C = 16.0 % and S = 23.9 %, P = 0.064). Litter growth rates and thus, estimated milk production were not different between treatments in any of the three reproductive cycles studied (P > 0.10). However, the S group showed systematically, lower average litter weight gains and thus, lower estimated milk yields.

In addition to this evidence, a total or partial MMA syndrome was diagnosed in 9 sows from the S group (2 in cycle 1, 5 in cycle 2 and 2 in cycle 3). These were sows that showed lower piglet growth rates or sows that had less than 4 pigs remaining at day 18 of lactation or were eliminated from the experiment due to agalactia (see Table 5.3). Six of the 9 sows suffering from the MMA syndrome belonged to parity 3 or onwards. None of these lactation incidences were detected in the C group of sows. So the increased feed allowance from day 45 until day 85 of gestation could have been detrimental to mammary gland development, more evidently in adult sows. It is important to highlight that, when calculating the average litter weight gain during lactation per treatment and cycle (Table 5.10), the data of those sows with total or partial MMA (9 sows) was not included. Therefore, the real negative impact of feed supplementation during mid-pregnancy on the estimated milk yield should be, even higher than the one currently detected.

Litter weight at day 18 of lactation in cycle 1 was also affected by the iPG. Sows from iPG1 presented lower litter weights compared to the other iPGs (iPG1 = 55.7, iPG2 = 61.8 and iPG3 = 61.9, P = 0.020). This fact was supported by numerically lower litter growth rates during lactation (data not shown). This effect was not observed, however, in cycles 2 and 3 which suggests that an aging effect might have existed also in milk production, with a clear differential factor for gilts. These results will be also commented in depth in chapter 6.

Table 5.10 Litter performance per sow on day 18 of lactation by dietary treatment and cycle.

Treatment	***Control***	***Supplemented***		P-*value*		
Cycle 1			*SEM*	*T*	*iPG*[1]	*T * iPG*
n sows	48	53				
n pigs	10.2	10.0	0.16	*0.109*	*0.814*	*0.430*
Litter weight, kg	60.4	59.2	1.30	*0.537*	*0.020*	*0.759*
Piglet weight, kg	5.9	5.8	0.12	*0.715*	*0.133*	*0.736*
ADG[2], kg	2.50	2.36	0.081	*0.208*	*0.271*	*0.897*
Milk production[3], L/d	10.0	9.5	0.32	*0.208*	*0.271*	*0.897*
Piglet mortality, %	3.2	5.3	1.28	*0.212*	*0.630*	*0.880*
Cycle 2						
n sows	36	39				
n pigs	9.5	9.3	0.228	*0.378*	*0.509*	*0.213*
Litter weight, kg	52.3	52.0	1.00	*0.808*	*0.530*	*0.209*
Piglet weight, kg	5.6	5.4	0.10	*0.272*	*0.424*	*0.067*
ADG[2], kg/d	2.18	2.13	0.059	*0.484*	*0.915*	*0.387*
Milk production[3], L/d	8.7	8.5	0.24	*0.484*	*0.915*	*0.387*
Piglet mortality, %	11.2	6.5	1.64	*0.042*	*0.245*	*0.401*
Cycle 3						
n sows	27	31				
n pigs	9.3	8.3	0.326	*0.078*	*0.565*	*0.942*
Litter weight, kg	47.5	48.7	1.36	*0.550*	*0.638*	*0.640*
Piglet weight, kg	5.3	5.5	0.14	*0.333*	*0.826*	*0.408*
ADG[2], kg/d	2.22	2.03	0.095	*0.149*	*0.873*	*0.959*
Milk production[3], L/d	8.9	8.1	0.38	*0.149*	*0.873*	*0.959*
Piglet mortality, %	16.0	23.9	2.98	*0.064*	*0.568*	*0.936*

Results are expressed as least square means and SEM; In counting data (litter size) standard error was extracted from the ANOVA analysis; T: dietary treatment; iPG: initial parity group

[1] iPG: parity group to which the sows belonged when they started the experiment in cycle 1 (iPG1 = parity 0, iPG2 = parity 1 and 2 and iPG3 = parity ≥ 3)

[2] ADG: Litter average daily gain

[3] Estimated value calculated using ADG and a conversion factor for milk of 4 L/kg piglet body weight growth (Pluske and Dong, 1998)

Throughout lactation, the pig mortality rates before and after cross-fostering and the main causes of piglet death were recorded (Table 5.11). In no case did piglet mortality rates exceed 10 % during the first 24 hours post-farrowing (before cross-fostering). The main causes of piglet death during the first 24 hours post-farrowing were being crushed by the sow and being small at birth. During lactation (after cross-fostering), mortality rates reached the unusual high levels of 15 and 25% respectively, in the C and the S group of sows in cycle 3; this was probably due to the pathological process (PRRS) detected on the farm in this cycle. After cross-fostering, the main cause of piglet death registered in all cycles was starvation. Overall, causes for pig mortality during lactation were variable between treatments, indicating no clear tendency for a treatment effect on them.

Table 5.11 Piglet mortality rates and causes of death before cross-fostering (CF) and during lactation (after cross fostering), by dietary treatment.

Causes of pig mortality	***Cycle 1***		***Cycle 2***		***Cycle 3***	
	C	**S**	**C**	**S**	**C**	**S**
Number of pigs, n						
Born alive	372	369	429	381	307	337
After CF	345	338	385	356	277	304
Mortality before CF, %[1]	5.38	8.67	3.73	2.62	0.98	1.48
Small at birth, %[2,3]	40.0	68.8	43.8	10.0	0	40.0
Crushed by the sow, %[3]	50.0	28.1	56.3	70.0	66.7	40.0
Others, %[3]	10.0	3.1	0	20.0	33. 3	20.0
Mortality in lactation, %[4]	5.51	8.28	12.21	13.48	15.16	24.67
Small at birth, %[2,5]	0	10.7	0	10.4	9.5	8.0
Crushed by the sow, %[5]	15.8	32.1	21.3	16.7	7.1	5.3
Starved, %[6,5]	47.4	39.3	78.7	68.8	73.8	80. 0
Others, %[3]	36.8	17.9	0	4.2	9.5	6.7

C: control and S: supplemented

[1] Expressed as a percentage of the number of piglets born alive

[2] Small at birth: piglet born with a weight less or equal to 900 g

[3] Expressed as a percentage of the overall mortality before cross-fostering

[4] Expressed as a percentage of the number of piglets after cross-fostering

[5] Expressed as a percentage of the overall mortality after cross-fostering

[6] Starved: piglet small for its age, with poor body condition after 2 or 3 days of age

c) Total productive performance over the three cycles

From the sows that completed the three cycles (n = 54), lifetime (sum of the 3 cycles) productive performance at birth and at day 18 of lactation was studied and summarized in table 5.12. These data were given as the sum of the number of pigs and litter weights (total born, total born alive and at day 18 of lactation) and the average piglet weight per sow, over the three cycles studied.

The dietary treatment did not affect the total number of pigs farrowed and alive at day 18 post-farrowing, neither the total litter nor the average piglet weight. Regardless of the level of feed intake during mid-gestation, the parity at which the sows started the experiment had little influences on these traits. In this respect, sows from iPG3 showed a lower percentage of pigs born alive (iPG1 = 93.4 %, iPG2 = 92.4 % and iPG3 = 86.3 %, P = 0.007). Regarding litter and piglet weight, the sows from iPG2 showed higher total born litter weights (iPG1 = 56.3 kg, iPG2 = 60.7 kg and iPG3 = 56.3 kg, P = 0.046) and also higher average piglet weights at birth (*total born*: iPG1 = 1.46 kg, iPG2 = 1.58 kg and iPG3 = 1.47 kg, P = 0.021 and *born alive*: iPG1 = 1.49 kg, iPG2 = 1.60 kg and iPG3 = 1.48 kg, P = 0.044).

Table 5.12 Total productive performance at the end of the experiment (total from the 3 cycles) of sows completing the three cycles studied (n=54)[1]

Treatment	***Control***	***Supplemented***		P-*value*		
n sows	26	28	*SEM*	*T*	*iPG*[2]	*T * iPG*
Total born						
N	39.1	40.2	1.495	*0.566*	*0.617*	*0.876*
Litter weight, kg	57.3	58.2	1.249	*0.600*	*0.046*	*0.039*
Piglet weight, kg	1.49	1.52	0.031	*0.611*	*0.021*	*0.036*
Born alive						
N	35.5	36.4	1.328	*0.976*	*0.007*	*0.505*
Litter weight, kg	52.3	53.6	1.099	*0.393*	*0.113*	*0.059*
Piglet weight, kg	1.51	1.53	0.031	*0.572*	*0.044*	*0.032*
Day 18 of lactation						
N	28.9	28.2	0.581	*0.383*	*0.509*	*0.839*
Litter weight, kg	163.3	166.2	3.464	*0.539*	*0.725*	*0.343*
Piglet weight, kg	5.62	5.55	0.090	*0.617*	*0.348*	*0.147*

Results are expressed as least square means and SEM; T: dietary treatment; iPG: initial parity group

[1] Calculations were made as follows: number of pigs and litter weight as the sum of the number of pigs and litter weights over the three consecutive cycles per sow, and piglet weight as the average of the average piglet weight per sow over the three cycles

[2] iPG: parity group to which the sows belonged when they started the experiment in cycle 1 (iPG1 = parity 0, iPG2 = parity 1 and 2 and iPG3 = parity ≥ 3)

The interaction between treatment and iPG was significant for litter and piglet weights at birth. The general trend for this interaction followed that observed in cycle 3, in favour of the S sows from iPG1. Sows from iPG1 seemed to accumulate the beneficial effects of the supplemental feeding strategy applied during mid-pregnancy in terms of litter and average piglet weights at birth and at day 18 of lactation. On the contrary, in multiparous sows the extra feeding strategy seemed to have negative effects, although not significant ($P > 0.10$), in terms of average litter and piglet weight at birth and total number of pigs at day 18 of lactation over three consecutive cycles (Table 5.13).

Table 5.13 Overall productive performance at the end of the experiment (total from the 3 cycles) of the sows completing the three cycles studied (n=54)[1], by treatment and initial parity group.

	Initial parity group									P-*value* *(T * iPG[2])*
	1			2			3			
Dietary treatment	Control	Supplemented	SEM	Control	Supplemented	SEM	Control	Supplemented	SEM	
n sows	10	10		11	14		5	4		
Total born										
n	37.8	39.2	2.469	39.8	39.6	2.343	39.8	41.7	3.024	*0.876*
Litter weight, kg	52.8	59.8	1.923	61.2	60.2	1.916	58.0	54.7	2.572	*0.039*
Piglet weight, kg	1.38	1.54	0.051	1.63	1.54	0.048	1.48	1.46	0.062	*0.036*
Born alive										
n	35.0	36.9	2.132	37.3	36.1	2.132	34.1	36.3	2.860	*0.505*
Litter weight, kg	49.1	55.6	1.761	55.1	55.3	1.858	52.7	50.1	2.359	*0.059*
Piglet weight, kg	1.40	1.58	0.051	1.63	1.56	0.051	1.50	1.46	0.069	*0.032*
18 ± 1 days of lactation										
n	29.9	29.9	0.867	29.6	28.3	0.867	27.3	26.4	1.300	*0.839*
Litter weight, kg	159.6	164.4	6.530	161.2	172.0	4.913	169.1	162.2	7.904	*0.343*
Piglet weight, kg	5.33	5.62	0.151	5.68	5.44	0.141	5.84	5.60	0.182	*0.147*

Results are expressed as least square means and SEM; T: dietary treatment

[1] Calculations were made as follows: number of pigs and litter weight as the sum of the number of pigs and litter weights over the three consecutive cycles per sow, and piglet weight as the average of the average piglet weight per sow over the three cycles

[2] Initial parity group: parity group to which the sows belonged when they started the experiment in cycle 1 (Initial parity group 1 = parity 0, Initial parity group 2 = parity 1 and 2 and Initial parity group 3 = parity ≥ 3)

5.3.3.2 Rebreeding performance

The impact of the extra feed allowance during mid-pregnancy on weaning to estrus interval (WEI) and farrowing rate is summarized in table 5.14. For the study of WEI, the three cases with reported long WEI (between 19 and 21 days) were removed from the database since it was considered that the dietary treatment was not implied on this effect.

Table 5.14 Post-weaning performance by treatment in the three cycles studied[1].

Cycle	**1**			**2**			**3**		
Treatment	*C*	*S*	*SEM*	*C*	*S*	*SEM*	*C*	*S*	*SEM*
n	49	54	-	36	39	-	27	31	-
WEI[2], days	4.5	4.6	0.112	4.4	4.3	0.109	4.2	4.0	0.179
Farrowing rate[3], %	-	-	-	83.7	81.0	-	76.5	78.0	-

C: control and S: supplemented; WEI: weaning to oestrus interval

[1] Only WEI was treated statistically. Results are expressed as least square means and SEM. Overall, treatment effect was not significant, but a parity group effect was found in cycle 1 (P = 0.023)

[2] Considered sows showing oestrus within 7 days after weaning

[3] Farrowing rate was calculated as [(farrowed sows/mated sows)*100)]

Overall, WEI was not affected by the increase in feed allowance during mid-gestation in any of the three cycles studied. A statistical tendency was observed in cycle 1 for the iPG factor (not shown in the table) indicating that gilts (sows from iPG1 in cycle 1) had significantly longer weaning to oestrus intervals compared to the older sows (iPG1 = 4.8 ± 0.12 days, iPG2 = 4.4 ± 0.11 days, iPG3 = 4.4 ± 0.17 days, P = 0.023). Farrowing rates [6] calculated in cycles 2 and 3 were not affected either by dietary treatment.

5.3.3.3 Feed consumption and apparent faecal digestibility in lactation

Average daily feed intake (ADFI) and apparent faecal digestibility (AFD_L) of organic matter were measured in lactation from cycle 3, in a sample of 51 (C = 26 and S = 25) and 46 (C = 21 and S = 25) sows, respectively. In this case parity group 1 included gilts (ADFI, n = 19; AFD_L, n = 18), parity group 2 included sows from parity 1 and 2 (ADFI, n = 17; AFD_L, n = 13) and parity group 3 included sows from 3 to 6 parities (ADFI, n = 15; AFD_L, n = 15).

Regarding ADFI, due to the fact that the feed allowance during lactation was not *ad libitum*, it was not possible to indicate the maximum level of feed intake that the sows achieved by treatment. Nonetheless, having taken this fact into account, the average level of feed intake

[6] The farrowing rate was calculated as [(farrowed sows/mated sows)*100)]

during lactation based on the feeding curve applied was estimated (Table 5.15). The supplemented sows showed lower ADFIs than C sows throughout the lactation period and also, from day 14 of lactation until weaning, which corresponds to the period when the maximum feeding level during lactation was offered (see Chapter 3, S = 6.74 kg /d and C = 7.20 kg /d, SEM = 0.139 and *P* = 0.010).

Across the parity groups, results showed that although all the parity groups seemed to be negatively affected, S sows from PG1 (gilts) and PG3 (parities 3 to 6) were the groups that reduced their ADFI most significantly during lactation as a consequence of the dietary treatment applied during gestation.

Table 5.15 Average daily feed intake during lactation by treatment and parity group[1]

Treatment	***Control***	***Supplemented***	SEM	P-*value*
Feed intake, kg /d[2]	5.87	5.49	0.05	*<0.001*
Parity group[3]				
1	5.85	5.48	0.09	*0.027*
2	5.85	5.60	0.08	*0.194*
3	5.91	5.37	0.08	*<0.001*

Results are expressed as least square means and SEM

[1] Average daily feed intake was measured on 12 non consecutive days per sow during the lactation period. Overall data were then pooled by treatments

[2] As-fed basis

[3] Parity group (Parity group 1 = gilts, Parity group 2 = parity 1 to 2 and Parity group 3 = parity 3 to 6)

The differences detected in the average feed consumption during lactation did not lead to significant differences on AFD_L of organic matter between treatment groups on day 15 ± 1 of lactation (C = 78.1 % and S = 77.2 %, SEM = 1.21 and *P* > 0.10). However, a parity group effect was found in which sows from PG1 (gilts) showed lower AFD_L of organic matter compared to multiparous sows (PGs 2 and 3) (PG1 = 73.7 %, PG2 = 78.6 % and PG3 = 80.7 %, SEM = 1.52, *P* = 0.002).

5.4 Discussion

Effects of gestation feeding regime on sow body weight and body reserves

The extra feed provided during mid-gestation in the present experiment represented a mean feed intake of approximately 4.5 kg/d (54.6 MJ ME/d) and 5.25 kg/d (63.7 MJ ME/d) in gilts and multiparous sows, respectively, from day 45 to day 85 of gestation, compared to the 2.5-3 kg/d (33.4-36.4 MJ ME/d) that consumed control sows throughout gestation. The restricted feeding regime normally used in the farm (C) covered full well (1.5-1.7 times maintenance, approximately) the energy requirements established by BSAS (2003) standards for both gilts (28.2 MJ ME/d) and multiparous sows (34.1-34.9 MJ ME/d) in gestation.

During the experimental period, diet digestibility (AFD_G) of organic matter showed slight differences between treatments. These differences accounted for 1.1 and 0.6 units in percentage terms lower in the S group of sows at cycles 1 and 3, but they are considered of low relevancy in the present study. In cycle 2, when S sows suffered a dip in feed consumption due to environmental high temperatures in the gestation barn, not even these small differences appeared. Overall, coefficients of digestibility of the organic matter found in the present experiment are in the line of those reported by LeGolff and Noblet (2001) and van der Peet-Schwering et al. (2002) in adult sows fed commercial diets.

The experimental plane of feeding proposed in the present experiment did influence positively BF (+1.7, +2.6 and +3.1 mm in the S group compared to the C sows in cycles 1, 2 and 3, respectively, $P < 0.01$) and LD (+2.6, +5.5 and +5.2 mm in the S group compared to the C sows in cycles 1, 2 and 3, respectively, $P < 0.05$) gains during the experimental period (from day 45 to day 85 of gestation). This denotes that sows are able to grow in both, lipid and lean reserves when feed supplementation is provided during gestation.

At the end of the three cycles (for overall changes, see Table 4.5), both treatment groups gained BW and LD, making evident the inherent maternal growth from cycle to cycle also described in several works (Whitemore and Yang, 1989; Young et al., 1990; Whittemore, 1993). This maternal anabolism was more evident in gilts (iPG1) than in multiparous sows (iPGs 2 and 3). Indeed, gilts were able to gain an average of +38 kg more of BW and also +7.1 kg more of estimated protein content in both treatments compared to multiparous sows after 3 cycles (see Figure 5.4). Also Whittemore and Yang (1989) reported conception to conception protein gains increasing with parity (12, 8.5 and 2 kg in parity 1, 2, and 3 animals, respectively). In fact, this was a quite foreseeable result since it is known that gilts of lean genotypes have a biological need to attain a target protein body mass to reach mature body size (Clowes et al., 1994; Everts and Dekker, 1994; Whittemore, 1996). This drive for body weight and protein accretion

decreases with aging until the adult weight, which is generally attained at the end of the third or fourth parity (Whittemore, 1996; Aherne et al., 1999).

On the other hand, in the modern sow fat tissue is less likely to be accumulated from cycle to cycle. But, from an evolutionary viewpoint, it is said that fat tissue is more easily manipulated by nutrition than lean tissue, at least once sows have attained its adult weight (Whittemore and Yang, 1989; Whittemore, 1993). In the present experiment, weight and LD gains were accompanied by no BF gains in the control group. However, as planned, supplemented sows were really able to largely accumulate BF after three cycles. Backfat changes from the beginning of the experiment (day 40 of gestation in cycle 1) until weaning in cycle 3 were positive for the supplemented sows whilst negative for control sows (C = -3.17 mm and S = 1.96 mm, $P < 0.001$). The same trend was observed when these changes were calculated from day 40 of gestation in cycle 1 to day 40 of gestation in cycle 4 (n = 37), in an attempt to consider differences among similar stages of different cycles (C = -1.49 mm and S = 1.37, $P = 0.032$). Young et al. (1990) reported losses of about 1.6 mm of BF after three cycles (breeding to breeding) when feeding pregnant sows a regime with a similar energy content to that of the C group in the present experiment (35 MJ ME/d). Also, Whittemore and Yang (1989) reported reductions in 1.86 mm of BF in average after 4 cycles (breeding to weaning) in sows fed conventionally during gestation. Overall, these results evidence the sow wastage in terms of backfat reserves that occur after 3 or 4 reproductive cycles in normal conditions and that is not desirable in terms of productivity and stayability within a herd. Dietary treatment in the present study was able to overcome this body reserves depletion from cycle to cycle in modern sows.

The influence of first parities on the selective partitioning of energy and nutrients towards maternal growth (protein accretion), which is widely reported in the literature (Whittemore, 1996; Pluske et al., 1998; Aherne et al., 1999), has also been demonstrated in the present experiment. In cycle 1, when sows were younger, both, S and C groups gained LD but were not able to accumulate BF (see Table 5.6). A parity group effect in this case revealed that the youngest sows devoted dietary nutrients during gestation to accumulate lean tissue demonstrating the maternal growth anabolism, whilst the oldest sows derived dietary energy on fat reserves (Figure 5.6). At latter cycles (2 and 3), this maternal anabolism was not as important and the experimental treatment clearly improved BW and body reserves levels.

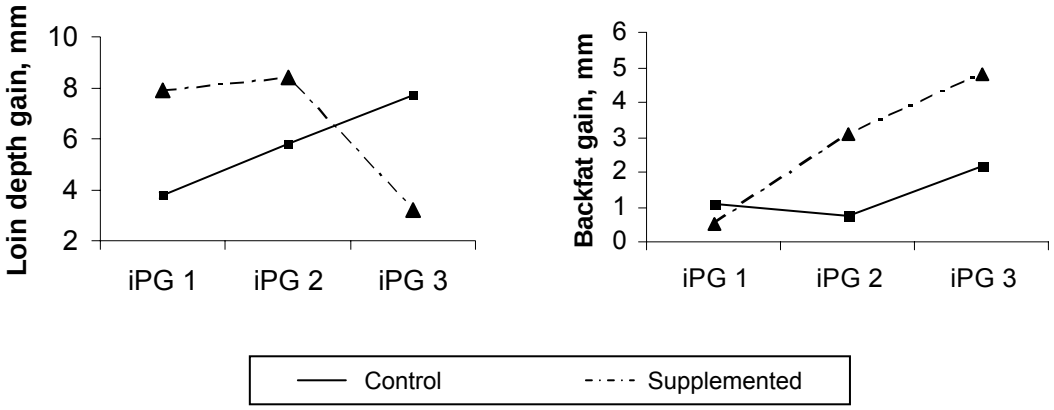


Figure 5.6 Backfat thickness and loin depth gains through gestation (from day 40 of gestation to farrowing) in cycle 1, by treatment and initial parity group (iPG: iPG1 = parity 0, iPG2 = parity 1 and 2 and iPG3 = parity ≥ 3) from sows completing the three consecutive cycles studied (n=54).

As a whole, as it was reported by many authors (Whittemore et al., 1980; Whittemore and Yang, 1989; Dourmad, 1991), an increase in BW or LD is not always accompanied by an increase in BF, at least once sows are already incorporated in the breeding herd. In second place, feeding plane or feeding strategy used throughout gestation in this farm (C), might not assure the maintenance of sow BF from cycle to cycle; sows belonging to iPG2 were the ones that lost the highest amount of BF after the three cycles. An important feature associated with the current lean genotypes is the so-called “Thin Sow Syndrome”. This syndrome arises as a gradual declining in body condition in the herd over the cycles, which finally affects sows reproductive performance and sows stayability in the farm. In the present study, feed supplementation during mid-gestation, more specifically in parity 2 sows, prevented sows from suffering this “Thin Sow Syndrome” in subsequent cycles.

Also expectable were the results of the compositional pattern (lipid and protein profile) of body weight gains over the three cycles studied, according to parity and treatment. Figure 5.6 illustrates the composition (in lean and lipid tissue) of the overall body weight gained through the three cycles, by parity group and treatment. It shows that the extra feed allowance provided during mid-gestation over three consecutive cycles led to different consequences depending on the parity. When the supplementation started early in life (iPG1), these sows were able to derive the extra feed intake to both lean and fat tissue accretion as it was also evidenced by Jones et al. (2006) in a choice-feeding trial with gilts. However, in later parities, the amount of lean tissue gained remained constant and the extra feed intake ended up with benefit of body fat content. This denotes, as reported by Noblet and Etienne (1987) and Whittemore and Yang (1989), that while the relation between body weight and body lipid is readily altered by nutrition, the relation between body weight and body protein gain is a particularly intransigent value.

From the graph (Figure 5.7), it is also noticed that sows from iPG2 gained more lean tissue than those in iPG3, suggesting that the drive for protein gain was not already achieved in sows from

parity 2 and 3 (Whittemore, 1996; Aherne et al., 1999). The previously defined "Thin Sow Syndrome" in iPG 2 sows is also graphically supported by figure 5.7. This indicates that sows that started the experiment in parities 2 or 3 were the worst hit in terms of BF reserves at the end of the three cycles and, at the same time, the most benefited in terms of body reserves by the increase plane of feed intake during gestation.

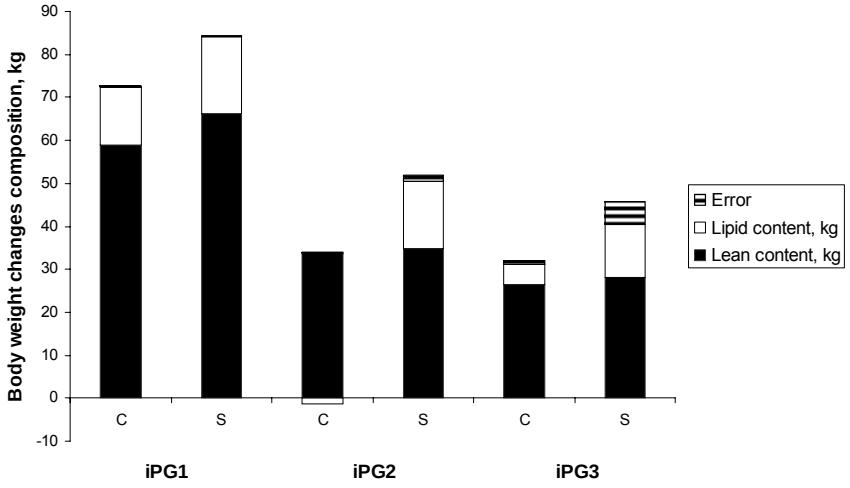


Figure 5.7 Composition of body weight changes from day 40 of gestation in cycle 1 until weaning in cycle 3 by dietary treatment (C: control and S: supplemented) and initial parity group (iPG1 = parity 0, iPG2 = parity 1 and 2 and iPG3 = parity ≥ 3). Protein and lipid content were estimated using the equations proposed by Dourmad et al. (1997), and lean content was estimated from protein content assuming that lean tissue contains 22% of protein. The error term indicates the difference between body weight gain and the sum of body fat and lean tissue estimated contents.

Mean body reserves levels may not always reflect the real status in the farm since the presence of thin sows might be compensated by the presence of fat sows; it is recognized that both, thin and fat sows in a herd, will have a negative impact on productive-reproductive parameters (Dourmad et al., 1996). Alternatively, body reserves status should be studied by using frequency distribution graphs (Barceló, 2005). Figure 5.8 represents sows BF distribution on day 40 of gestation in cycle 1 and at farrowing in each of the three cycles studied in the present experiment, according to three BF categories (on day 40 of gestation in cycle 1: THIN: < 15 mm, MEDIUM: 15-20 mm and FAT: > 20 mm; at farrowing: THIN: < 17 mm, MEDIUM: 17-21 mm and FAT: > 21 mm). These categories were defined according to recent recommended BF levels in the literature at these times (Tummaruk et al., 2001; Luborda, 2002; Marco, 2004, Young et al. 2004). In the modern leaner genotypes, it is normally set that sows may have more than 15 mm of BF at mating (16-18 mm optimum) and that all sows should have between 17 mm and 21 mm of BF at farrowing to allow them to lose 2 to 3 mm of BF during lactation. It is desirable not to fall below 14 mm at weaning in order to avoid reproductive problems, and also to avoid excessive BF at farrowing (> 21-22 mm) that might cause a diminution on lactation feed intake.

On day 40 of gestation, both experimental groups showed a similar BF distribution (THIN: 26 %, MEDIUM: 53 % and FAT: 21 %, Figure 5.8). However, a tendency was observed for the S group to gradually increase the percentage of sows with more than 21 mm at farrowing. In cycles 1 and 2, both groups showed the highest percentage of sows within the medium (ideal) BF interval at farrowing but, in cycle 3, S sows went towards a FAT profile with a 65.5 % of sows showing 22 or more mm of BF in the S group. Some authors raise a warning about the fact that too fat sows at parturition have a higher risk to suffer from dystocia associated with high stillbirth rate, and agalactia, mastitis and metritis syndrome (Göransson, 1989; Head et al., 1991); so this is an undesirable situation for producers in a commercial farm. In fact, also in our study, S sows showed a higher incidence of milking problems compared to C sows (9 sows in the S group and none in the C group). On the other hand, the disadvantages of S sows at farrowing become an advantage at weaning since a lower percentage in the S-group sows showed BF levels under 14 mm compared to those in the C group, at weaning in cycles 2 and 3 (C: 29 and 32% and S: 6 and 14% in cycles 2 and 3, respectively, data not shown).

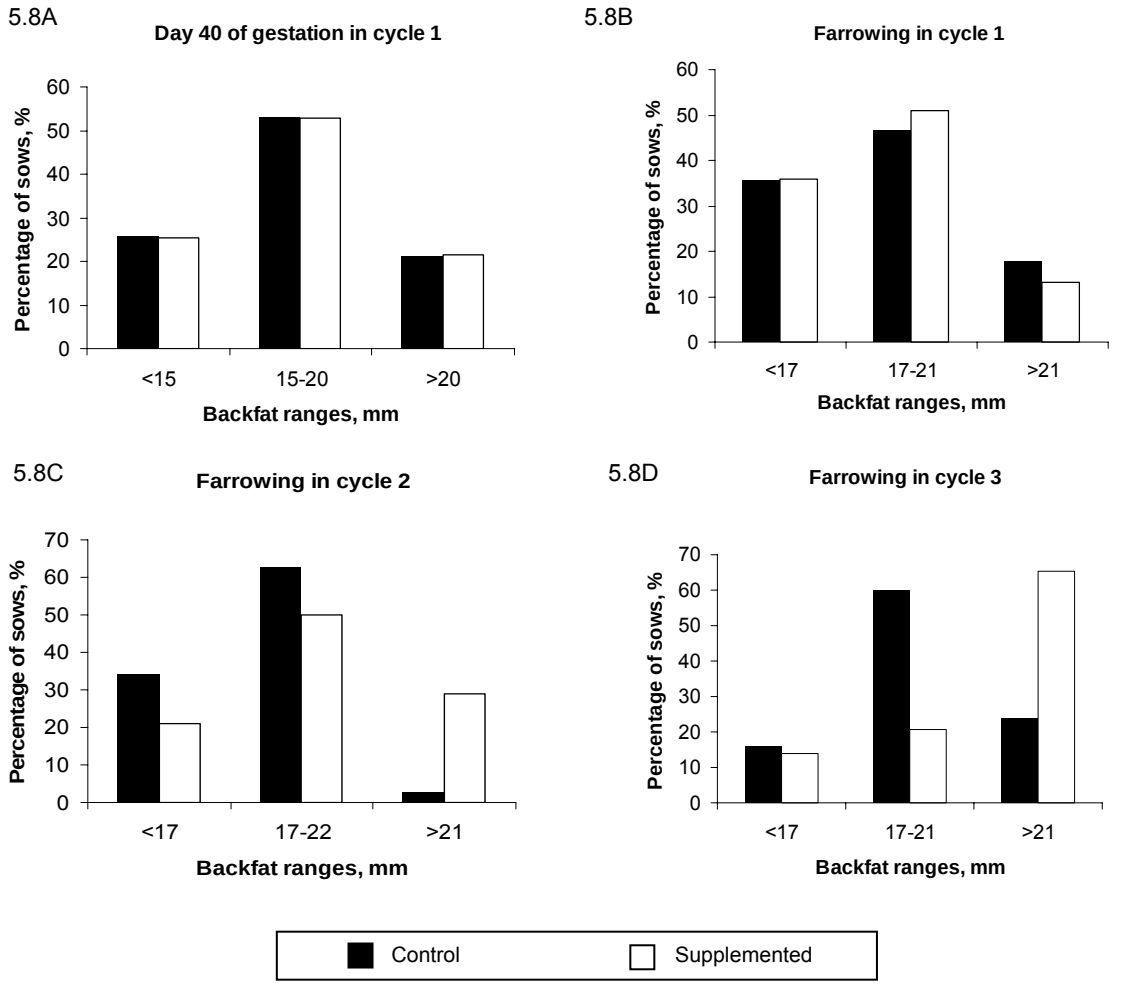


Figure 5.8 Percentage of sows (%) plotted by backfat thickness range and experimental treatment on day 40 of gestation (5.8A) and at farrowing in cycles 1 (5.8B), 2 (5.8C) and 3 (5.8D).

So far, it is important to notice that BF levels reached by sows in this study and in others described above using lean genotypes (Marco, 2004, Young et al. 2004) are far from the 24 to 26 mm of BF suggested by Close and Cole (2003) at farrowing, in sows from first (140 kg BW at mating) to sixth parity (245 kg BW at mating). From this, it is deduced that universal target levels in BF reserves are difficult to set and that they must be adapted to different genotypes and husbandry conditions in each herd.

Compositional traits of sows completing the three cycles

Taken into account the retrospective analysis made in terms of body reserves from sows that finally completed the three cycles in this study (n = 54), it was detected that sows supplemented during mid-gestation had significantly lower BF and LD levels at the initial point of the experiment (day 40 of gestation in cycle 1), compared to C sows ($P < 0.05$). This fact was also evident when these levels were compared to those from the initial pool of 103 sows under treatment (Table 5.16). Thus, feeding level during mid-gestation might have selected different sow body composition profiles to stay in the herd after three cycles.

From these results, it is deduced that, when applying the restrictedly dietary regime normally imposed in the farm (C sows), a minimum of 18.7 mm of BF in average were required on day 40 of gestation in order to guarantee sows survival in the herd at least for three cycles. However, when an extra feed supplementation was provided during mid-gestation, sows that were thinner on day 40 of gestation (15.8 mm of BF) and thus, at mating, were able to stay in the herd at least during three consecutive cycles.

Table 5.16 Comparison between mean body weight, backfat and loin depth levels on day 40 of gestation in cycle 1 from the initial pool of sows that started the experiment (all sows, n = 103) and from the sows that completed the three cycles studied (three cycles, n = 54).

Treatment	***Control***			***Supplemented***		
	All sows	*Three cycles*	*SEM*	*All sows*	*Three cycles*	SEM
	49	26		54	28	
Body weight, kg	202.6	206.5	4.030	201.1	199.4	3.953
Backfat, mm	17.5	18.7	0.669	16.4	15.8	0.655
Loin depth, mm	55.9	56.7	0.788	54.8	54.1	0.786

Results expressed as least square means and SEM (n = 26 and 28)

Interestingly, the same analysis made by parity group revealed that this interpretation differed depending on sow parity (Table 5.17). In this respect, BF levels required in order to survive three consecutive parities showed a lower margin of error in first parity sows, compared to higher parities. First parity sows with less than 16-17 mm on day 40 of gestation did not stay in the herd more than three cycles, even though an extra feed allowance was provided during

gestation (supplemented group). This result is in agreement with the fact that sows need a minimum of fat reserves in order assure an optimum sow longevity and lifetime productive performance (Brisbane and Chesnais, 1996; Stadler et al., 2005). In this regard, Brisbane and Chesnais (1996) concluded that the survival of sows within a herd until after the fourth parity was 10% higher in gilts from the highest BF category (> 18 mm) when compared to gilts from the leanest BF category (< 10 mm).

Table 5.17 Body weight, backfat thickness and loin depth levels at the beginning of the experiment (day 40 of gestation in cycle 1) by initial parity groups, from the sows completing the three cycles (n=54).

Treatment	***Control***	***Supplemented***	SEM	P-*value*
Trait				
Body weight, kg				
iPG1	162.0	168.4	6.665	0.486
iPG2	214.5	199.9	6.323	0.093
iPG3	245.8	229.8	8.382	0.176
Backfat, mm				
iPG1	16.2	17.9	1.045	0.245
iPG2	20.5	14.8	0.992	<0.001
iPG3	19.3	14.3	1.316	0.008
Loin depth, mm				
iPG1	56.3	52.2	1.318	0.026
iPG2	57.1	53.1	1.250	0.023
iPG3	55.8	58.0	1.614	0.344

Results expressed as least square means and SEM; iPG: initial parity group

[1] iPG: parity group to which the sows belonged when they started the experiment in cycle 1 (iPG 1 = parity 0, iPG 2 = parity 1 and 2 and iPG 3 = parity ≥ 3)

On the other hand, higher parity sows in this experiment (parity 2 onwards) required a higher minimum amount of BF at day 40 of gestation when feeding the regime normally imposed in the farm (C sows, 19-20 mm) than when receiving an extra feed supplementation during mid-gestation (S sows, < 15 mm), in order to survive over three consecutive cycles in the herd. However, these 15 mm of BF could also be suggested as a maximum level for supplemented sows, since sows higher than 15 mm on day 40 of gestation didn't achieve the three cycles either. Overall, this means that, under the conditions of the present experiment, the advantages conferred in longevity within the herd by feed supplementation when sows are thin (< 15 mm), turn into disadvantages when sows were in good body reserves condition.

At the same time, C group showed a higher percentage of sows removed due to returning to oestrus compared to S sows, and S sows presented 9 cases of totally or partially diagnosed MMA while C group of sows didn't. When analyzing body condition of the removed sows, it was observed that 54.3% of the sows that returned to oestrus in the subsequent cycle showed BF levels lower than 14 mm at weaning. Additionally, a 55.6% of the sows eliminated due to MMA problems showed BF levels of 22 mm or higher at farrowing. Thus, it seems that sows with less

than 16-17 mm (first parity) or 19-20 mm (multiparous) of BF at day 40 of gestation in the C group had higher risks of returning to oestrus after mating, while sows (especially multiparous sows) from the supplemented group that were in optimal body condition at the beginning of the experiment (> 15 mm), were more prone to suffer from MMA syndrome. In fact, it has been well reported in the literature that low BF levels (< 10-14 mm) at weaning have been related with higher sow culling rates (Young et al.,1990; Marco, 2004; Kongsted, 2006), and also that too fat sows at parturition have higher risks to suffer from agalactia, mastitis and metritis (Göransson, 1989; Head et al., 1991; Weldon et al., 1994).

Lean reserves (loin depth measurement) an day 40 of gestation also determined the ability of sows to stay in the herd in the present study. A minimum of 56-57 mm of LD was required in the C group of sows in order to remain in the herd for, at least, three consecutive cycles (Table 5.16). Stadler et al. (2005) also reported some minimum level of muscle needed in gilts to maximize the number of live born pigs over their lifetime (lifetime live born pigs). In this respect, Stadler et al. (2005) showed that sows with more than 37.1 cm^2 of loin muscle area averaged from 3.2 to 3.5 of increase on live born pigs compared to sows with lower values. By parities, unlike BF levels (commented above), first parity (iPG1) and also second and third parity (iPG2) sows in this case required lower levels of LD at the beginning of the experiment when they received a feed supplementation during gestation (52-53 mm), but this was not the case for higher parity sows (iPG3). Thus, this may be indicating a higher response of lean accretion through nutrition in first parities compared to higher parity sows, demonstrating again that young sows are able to derive more energy to lean than to fat growth (Whittemore and Yang, 1989; Aherne et al., 1999).

In short, these results imply that a supplemental feeding strategy during mid-gestation allows to be less restrictive in setting minimum BF levels for multiparous sows and minimum LD levels in young sows at the beginning of the reproductive cycle (40 days of gestation in this case), in order to put up with at least three consecutive cycles. However, feed supplementation when sows are in good condition could, additionally, be detrimental in terms of sow stayability within the herd.

Productive performance

Many studies in the literature have demonstrated that the amount of feed or energy intake and feeding pattern during gestation has no effects on piglet weight at birth and performance (Elsey et al., 1971; Young et al., 1990; Sinclair et al., 2001). However, some others suggest that there may be crucial periods within gestation when food intake may have profound effects on litter performance (Aherne and Kirkwood, 1985; Schoknecht et al., 1993; Dwyer et al., 1994; Gatford et al., 2003). Also, preservation or increased sow body reserves in

the long term has been recognized to have an effect on litter and sow lifetime performance (Cromwell et al., 1989; Noblet et al., 1990; Whittemore, 1993). Increasing feed allowance during mid-pregnancy in the present experiment, did not confer an immediate effect (cycle 1 and cycle 2) in litter and pig performance at birth or at weaning (day 18 of lactation), but did result in a positive effect on productive performance at birth in cycle 3.

In cycle 1, a parity group effect appeared indicating that first parity sows had lower litter and average piglet weight at farrowing and also at weaning (18 days of lactation in this case), compared to multiparous sows. A lower productive and reproductive performance for primiparous sows has been previously reported by several authors (Whittemore, 1996; Miller et al., 2000; Guedes et al., 2001; Tummaruk et al., 2001). This effect will be treated with more detail in chapter 6. Likewise, in cycles 2 and 3, a parity group effect was also evidenced but, in this case, with negative consequences for iPG3 sows. In these two cycles, the oldest group of sows in the experiment (iPG3) came up with a higher number of stillborn piglets, regardless of the treatment. In agreement with other studies in the literature, the probability of presenting high stillbirth rates is greater in later parities (Leenhouwers et al., 1999; Canario et al., 2006). These authors suggest that such an increase in stillborn piglets might result from excessive fatness of old sows, as well as from aging of the uterus which, having reduced its muscular tone, becomes less efficient for the farrowing process; or from both factors. In the present study, it is plausible that both causes were implicated.

Despite not showing any effect on litter performance in cycles 1 and 2, in cycle 3, dietary treatment influenced positively litter performance at birth. Sows which started the experiment as first-parity sows in cycle 1 (iPG1), showed an increase of about 300 g in piglet birth weight in response to high feed allowances during mid-gestation in cycle 3. Efforts devoted to increase piglet birth weight in the literature by increasing sow feed allowance have been focused on the last month of gestation since it is the period of the maximum foetal growth, but they have generally led to little success (Hillyer and Phillips, 1980; Cromwell et al., 1989; Cole, 1990; Miller et al., 2000). When existing, the effect has been usually found at birth weights lower than 1.0 kg (Aherne and Kirkwood, 1985; Whittemore, 1996). Cromwell et al. (1989) reported larger litters of heavier piglets at birth (+ 39 g) with subsequently higher weaning weights from primiparous sows receiving increased food intake in late gestation, but these effects became significant after two consecutive cycles of feed supplementation. They, consequently, suggested the possibility of a long-term effect mediated through an improved body condition of sows, rather than an immediate effect of maternal nutrition in late gestation on the developing foetus.

In the present study, since the positive effect was only seen after being on the treatment for 3 parities, it also suggests a relationship between the improved sow condition and a higher productive capacity. The fact that only gilts were able to respond to the nutritional treatment

could have a physiological basis. It has been reported that the increasing (re)productive performance that sows experience with age is attributed, at least partly, to the increase in lean tissue content through parities and the achievement of a target protein level at parities 3-4, when sows show their higher performances (Clowes et al., 1994; Everts, 1994). In the present experiment, gilts were able to accumulate extra lean tissue in response to the feed supplementation applied during mid-gestation (S group), while no other parity group did (see Figure 5.7). Thus, the increase in lean tissue content in the S group might have contributed to the success of gilts at farrowing after three cycles. Also, since first parity sows performance never exceeded that of the iPGs 2 and 3, it is plausible that increasing feed intake had simply accelerated body muscle accretion in achievement of the marked lean target but not conferred an additional benefit to the other groups of sows.

The increased birth weight of pigs from supplemented gilts after three cycles did not fully disappear by weaning, since it resulted in an increased piglet weight also at day 18 of lactation in gilts although not statistically significant (C: 5.11 kg and S: 5.60 kg, SEM: 0.226, $P > 0.10$).

Regarding lactation performance, supplemented sows bred about one piglet less than C sows and showed noticeable high piglet mortality rates through lactation in cycle 3, just when mean BF reserves were greater (see Figure 5.7). This suggests that piglet survival and milking ability of the sow were impaired by higher feeding levels during gestation after 3 cycles. As stated before, there were clinical evidences of MMA syndrome in the supplemented group of sows (9 sows from the S treatment). Thus, the failure on lactation performance found in this study could be partly caused by a detrimental effect of the dietary treatment applied on mammary gland development during gestation. Physiologically, mammary gland differentiation and development take place from day 75 to day 90 of gestation (Kensinger et al., 1982) that match, in a large extent, the period of feed supplementation in the current experiment. Weldon et al. (1991) reported that higher energy or feed allowance during this period may contribute to the replacement of mammary tissue by fat, which would also affect negatively milk production and explain both higher incidences of MMA syndrome and high pig mortalities found in the S group. However, other studies have reported no effects of feed or energy supplementation during late gestation (> 100 days) on mammary development (Miller et al., 2000). This suggests that this complication appears when supplementation concerns the critical period of mammary gland development.

Although there was no statistical evidence of that, clinical casuistry indicated that multiparous sows were the ones who suffered mostly from MMA syndrome, compared to first parity sows. The different nutrient partitioning reported in the literature (Whittemore, 1993) and also evidenced in the present study (discussed above) between primiparous and multiparous sows, might have played a very important role on this effect. Therefore, as multiparous sows based their gains more on lipids and less on protein, they would be more prone to derive dietary

energy into fat; specifically, fatty tissue in the mammary parenchyma, and suffer from MMA syndrome when energy is provided above requirements.

Under the conditions of the present experiment, sows farrowed a total of 39-40 pigs, 35-38 of which were born alive, and weaned about 26-30 pigs per sow at the end of the three cycles. Results of total performance reflected the same abovementioned advantage of the supplemented feeding regime in iPG1 sows and also the apparent disadvantage in terms of number of pigs weaned per sow in iPG2 and iPG3 sows, although differences were not significant. Thus, benefits that the experimental treatment exerted on sows from iPG2 in terms of BF balance from cycle to cycle were not reflected in an increment on productive performance neither at farrowing nor at weaning in a three-consecutive cycle period.

As stated before, body reserves of gilts (protein and fat) when entering to the breeding herd are likely influencing subsequent productivity. In the current experiment, BF at the initial point of the experiment (day 40 of gestation in cycle 1) resulted positively and significantly correlated with the total number of pigs born alive after 3 cycles in gilts, but not in multiparous sows (r_{gilts} = 0.49, P = 0.030; r_{sows} = 0.29, P = 0.100). In addition, initial BF (day 40 of gestation in cycle 1) was positively correlated with the total number of born alive litter weight after 3 cycles in gilts, but not in multiparous sows (r_{gilts} = 0.66, P = 0.002; r_{sows} = 0.19, P = 0.309).

Lactation feed intake and rebreeding performance

The practise of increasing feeding level during gestation has been largely criticized due to the well known detrimental impact of fat levels at farrowing on voluntary feed intake during lactation (Mullan and Williams, 1989; Dourmad, 1991; Revell et al., 1998a). In this respect, Mullan and Williams (1989) documented a negative correlation between BF levels on day 1 of lactation and feed intake during lactation in primiparous sows (r = -0.52, P < 0.001). It is clear that inadequate intake of energy and/ or amino acids during lactation leads to a drain on body weight and body reserves that may impair subsequent reproduction (Einarsson and Rojkittikhun, 1993; Whittemore, 1996; Pettigrew, 1998; Prunier and Quesnel, 2000; Jones et al., 2006).

Lactation ADFI in the current study was calculated in cycle 3. As feed intake during lactation was not *ad libitum*, average intake values in the present experiment cannot be taken as representative but may well serve as a comparative between the two treatments, when feed intake is adjusted to the same lactation feeding curve. Even so, the average feed intake obtained in the conditions of the present experiment is in line with other studies in the literature (Dourmad, 1991; Weldon et al., 1994; Sinclair et al., 2001). As a result of the pooled analysis of 12 non-consecutive days of lactation, in the present experiment it was shown that

supplemented sows during mid-gestation consumed in average, 380 g less of feed per day than the restrictedly feed sows (C). Considering only the sample of sows used for ADFI estimations (n = 51), S sows showed higher BW, BF and LD gains during gestation ($P < 0.05$) and, consequently higher BW (C = 211.8 kg and S = 226.6 kg, $P < 0.001$), BF (C = 19.3 mm and S = 20.6 mm, $P = 0.015$) and LD (C = 61.5 mm and S = 64.4 mm, $P = 0.073$) levels at farrowing comparing to C sows. Further, in the present study, an increase of BF from 19.3 to 20.6 mm resulted in a decrease of 0.380 kg /d. Therefore, it is calculated that each +1 mm of BF at farrowing is equivalent to -0.292 kg of feed intake/day during lactation. Revell et al. (1998a) found that an increase in backfat thickness at parturition from 17.9 to 24.3 mm resulted in a decrease in lactation feed intake of 1.6 kg/d. Thus, 1 mm thicker at farrowing resulted in a 0.25 kg/day lower feed intake during lactation. Overall, there is lack of consensus in the literature about the quantification of this impact that, in turn, may also depend on the genetics and the fattening capacity of each animal.

However, this effect on feed consumption didn't result in a higher BW and body reserves loss during lactation in the supplemented group of sows, be it in the subgroup of 51 sows or in general (all sows). Body weight and body reserves levels at weaning were, consequently, higher in the S compared to the C group of sows controlled for feed consumption during lactation (n = 51, BW: 210.3 kg *vs* 225.0 kg; BF: 14.8 mm *vs* 16.6 mm and LD: 58.1 mm *vs* 61.0 mm). Sows nursing large litters lose generally more BW and body reserves than sows nursing small litters (Eissen et al., 2003). As S sows showed smaller litters during lactation in cycle 3, and also some milking problems, the lower feed intake during lactation may have been compensated by lower milking demands preventing S sows from showing higher BW and body reserves losses during lactation.

According to the lack of differences in body tissue mobilization, rebreeding performance in terms of weaning to oestrus interval and farrowing rate were not affected by the gestation feeding treatment applied. It is important to point out that the mean values of WEI obtained were lower (not exceeding 5 days) than what is normally described in the literature (Maes et al., 2004; Thaker and Bilkei, 2005; De Rensis et al., 2005; Weldon et al., 2006). This could be, in part, due to the fact that sows with prolonged WEI (19-21 days) were removed and not considered for the analysis.

5.5 Implications

In conclusion, gestating sows that received an extra feed supplementation during mid gestation (day 45 to day 85 of gestation) during three successive reproductive cycles were able to accumulate BF at the end of the three cycles. However, restricted feeding sows were not able to accumulate fat reserves although, similarly to S sows, they gained BW and LD throughout the

three cycles. This feasibility for fat accretion made possible to keep multiparous sows (> 2 parities) with less than 15 mm of BF at day 40 of gestation within the herd for, at least, three reproductive cycles. In young sows, the minimum BF levels in order to stay in the herd for three reproductive cycles were invariably set on 16-17 mm, not depending on the feed allowance during gestation. Backfat accumulation in multiparous sows did not finally confer advantages on productive-reproductive performance after three cycles, but appeared to be detrimental for milk production or piglet survival during lactation in a long term. On the other hand, lean and probably also fat tissue accumulation in primiparous sows after three cycles of feed supplementation improved productive performance in terms of litter weight at farrowing (+0.300 kg/piglet). No effects were demonstrated of this feeding strategy on body weight and body reserves losses during lactation, nor in weaning to oestrus interval, although lactation feed intake was reduced. Further investigations are needed in order to assess whether the manipulation of body composition of young sows is adequate as a strategy for producers to increase piglet weight at birth. At the same time, an economical evaluation of this effect should be carried out to reach the most efficient definition of this strategy.

Chapter 6

Relationship between sows body condition and productive-reproductive efficiency. Accuracy of visual body condition score in predicting sows body reserves

6.1 Introduction

Sows that are able to maintain an adequate level of body reserves throughout the reproductive cycle and over different cycles show a greater lifetime and overall productive-reproductive efficiency in the herd (Noblet et al., 1990; Whittemore, 1993). But, the maintenance of an optimal body condition for all sows in a herd is not always easy, especially in group-housing systems where, eventually, individual control of feed intake is not always allowed (Kongsted, 2006). Because all the phases in a reproductive cycle are related, deviations of the optimal body condition in one phase can have significant effects on performance in another phase (Whittemore, 1998; Aherne et al., 1999). For instance, it is well established that sows with an excessive amount of backfat (BF) reserves at farrowing have a lower voluntary feed intake during lactation (Mullan and Williams, 1989; Sinclair et al., 2001). Many years ago it was suggested that this effect was significant only when BF was 25 mm or greater (Mullan and Williams, 1989). But, in the current leaner genotypes which have smaller BF levels, this threshold can have changed or might have different consequences.

Furthermore, it is generally recognized that excessive body weight and body reserves (fat and lean) losses during lactation tend to compromise subsequent fertility and prolificacy (Aherne and Kirkwood, 1985; Pettigrew, 1998; Weldon et al., 2006). But there is little agreement as to what really constitutes excessive weight or body reserves loss. Also, it might not be the weight loss *per se* which is important, but the role and pattern of lipid and protein loss (Cole, 1990; Clowes et al., 2003a,b). It has also been suggested that it is the actual weight or body reserves at weaning which influence subsequent reproductive performance (Mullan and Williams, 1989; Young et al., 1991; Tantasuparuk et al., 2001). Considerable effort has been recently devoted in order to elucidate which is the real impact of sow body condition on productive-reproductive efficiency (Miller et al., 2000; Maes et al., 2004; De Rensis et al., 2005; Weldon et al., 2006; among others).

So that, the evaluation of sows' body reserves status in modern pig herds has become an issue of considerable importance, in order to optimise sows feeding programs and productivity targets. In many commercial swine production systems, the subjective method of body condition score (BCS) is being used as a means of predicting sow body reserves visually, and also as a guide to adapt feeding levels to the animal reserves. However, because of the inherent variability from one assessor to the next and the conformational changes suffered by the new leaner strains that make it difficult to distinguish fat from lean tissues, the accuracy of BCS in predicting body reserves may not be the optimal. In fact, recent studies have reported that BCS and backfat are only moderately related (Young et al., 2001; Maes et al., 2004).

6.2 Partial objectives

The first objective of this chapter was to explore whether sow body weight (BW), backfat thickness (BF) and loin depth (LD) at different times within the reproductive cycle, were associated with sow body reserves management during lactation, and with sow productive-reproductive efficiency. Also the role of sow parity on those associations was examined. The second objective aimed to investigate the reliability of visual BCS used as a subjective prediction method of sow body reserves compared to body reserves measured by ultrasounds, in sows of different parities and during different stages within the reproductive cycle.

6.3 Specific material and methods

The high variability of the data generated from the study presented in this PhD document, allowed us to establish multiple relations among sow body reserves and productive performance traits, irrespective of the treatment. Also, the experimental design carried out (two feed allowance during mid-gestation) provided us with a wide range of sow body reserves status among individuals that may make the associations obtained, valid for different physiological phases and situations.

In order to achieve the first objective of this chapter, data from the initial pool of 103 sows (cycle 1) was included in the study [n, parity 0 (gilts): 40; parity 1: 21; parity 2: 19; parity 3: 15 and parity 4: 6]. Only the data taken throughout the course of one reproductive cycle (cycle 1) was used, in order to avoid the misleading effect of having repeated measures within the same sow from cycle to cycle. In order to simplify the results' outcome, sows were grouped according to parity into three parity groups; parity group 1 (PG1: gilts, n =40), parity group 2 (PG2: sows from parity 1 and 2, n =40) and parity group 3 (PG3: sows from parity 3 and 4, n =21). Body weight and body condition of the sows [determined through ultrasonic BF and LD measured at the level of the last rib, at 6.5 cm of the midline (P2)] were recorded on day 40 of gestation, at farrowing and at weaning. The following productive traits were investigated: the number and weight of total born and pigs on day 18 of lactation; litter gain and pig mortality during lactation, and weaning to oestrus interval (WEI, d). As the main purpose of this first section was to carry out an exploratory analysis of the data, all the possible associations between the variables of interest were investigated but only the most important and relevant were highlighted and then discussed to draw conclusions.

Additionally, in order to asses the second objective within this chapter, the relationship between all the measures obtained simultaneously of BW, BF, LD, and BCS throughout the whole experiment (on day 40 and day 80 of gestation, at farrowing and at weaning of the three cycles), regardless of the individual, the treatment and the cycle were determined (n measures = 1216). Body condition score was measured visually, according to a 1 (extremely thin sows) to 5 (overly

fat sows) score scale, using intermediate (0.5) levels (Close and Cole, 2003). These data included sows from 0 to 6 parities.

Statistics

Sows body condition data (BW, BF and LD) were subjected to summary statistics for obtaining means, standard deviation and minimum-maximum range using the MEAN procedure of SAS (Version 9.1, SAS Institute Inc., Cary, North Carolina, USA). The differences in BW, BF and LD levels among parity groups were tested using an analysis of variance (GLM procedure of SAS), in which the parity group was included in the model as a fixed factor and means were separated and tested for significance using Tukey correction for multiple contrasts.

The association between sow body condition and the different productive-reproductive performance traits studied was explored using Pearson's correlation coefficients (gross correlations; CORR procedure of SAS). Additionally, partial correlations (CORR procedure of SAS) were calculated for all these parameters in order to elucidate the potential effect of sow parity in each association, being sow parity the partial term. If appropriate, the results of the gross correlations were analysed separately for each parity group.

The differences in productive-reproductive variables among parity groups were tested using an analysis of variance (GLM procedure) and the GENMOD procedure, in which the parity group was included in the model as a fixed factor and covariables (litter size and litter weight) were used, when necessary, according to the methodology proposed in chapter 5. Means were separated and tested for significance using Tukey correction for multiple contrasts.

Pearson's correlation coefficients (CORR procedure of SAS) and linear regression (REG procedure of SAS) were used to determine the precision and accuracy of the established relationship between BW, BF and LD measurements and the subjective scoring of body condition (BCS). Criteria for determining best predictive equations included the greatest magnitude of coefficient of determination (R2) and the smallest coefficient of variation (CV).

6.4 Results

6.4.1 Description of body weight and body reserves data

Table 6.1 summarizes the descriptive statistics (mean, standard deviation, maximum and minimum) for body weight (BW, kg), backfat (BF, mm) and loin depth (LD, mm) measurements. Overall, sow BW ranged from 127.5 kg to 288.0 kg; BF from 7.0 mm to 30.0 mm and LD from 44.0 mm to 79.0 mm.

Table 6.1 Descriptive statistics for body weight (kg), backfat thickness (mm) and loin depth (mm) from the 103 sows included in this study on day 40 of gestation, at farrowing and at weaning.

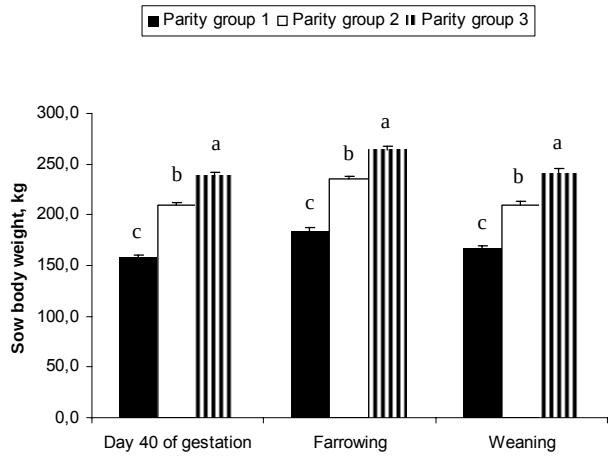
Descriptive parameters	***Mean***	***SD***	***Minimum***	***Maximum***
Body weight on day 40 GT	194.6	37.51	129.5	268.0
Body weight at farrowing[1]	219.9	37.10	157.5	288.0
Body weight at weaning	198.8	35.26	127.5	269.0
Backfat on day 40 GT	17.0	3.63	8.0	26.0
Backfat at farrowing	18.2	3.89	9.0	30.0
Backfat at weaning[2]	15.0	3.55	7.0	25.0
Loin depth on day 40 GT	54.8	4.50	44.0	69.0
Loin depth at farrowing	61.5	4.62	49.0	79.0
Loin depth at weaning[2]	57.6	4.08	48.0	68.0

GT: gestation; SD: Standard deviation

[1] 48 ± 24 h post-farrowing

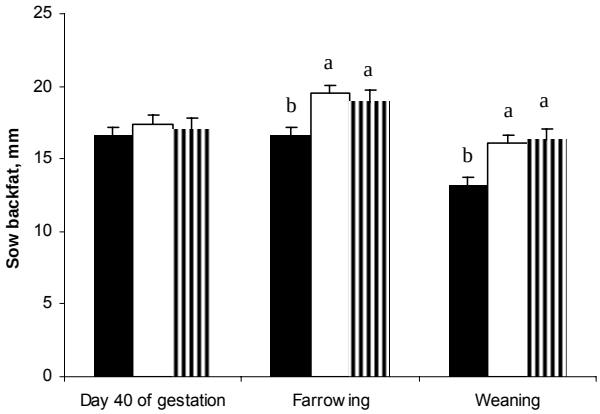
[2] Day 18 ± 1 of lactation

Mean body weight and body reserves levels according to parity group and production stage are presented in figure 6.1, and the maximum and minimum ranges are provided in the joined tables. As a whole, the lowest levels of BW and LD were found on day 40 of gestation and the lowest levels of BF were found at weaning. There was a significant influence of parity group on sow BW ($P < 0.001$) and body reserves (BF and LD) levels at farrowing and at weaning ($P < 0.01$), with gilts showing the lowest levels all over these periods.



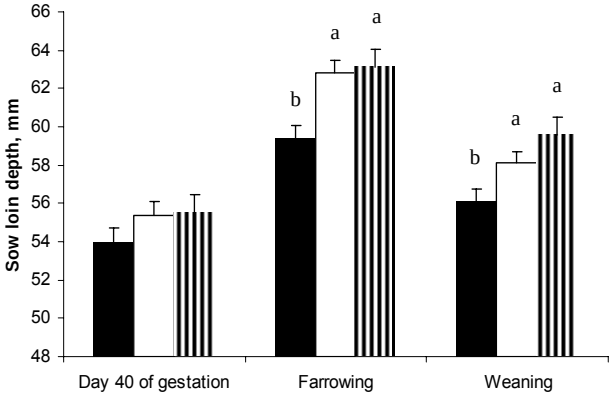
BW, kg **6.1A**

Stage	PG	Max	Min
Day 40 GT	1	189.0	129.5
	2	259.0	159.5
	3	268.0	193.5
Farrowing	1	221.0	157.0
	2	282.0	182.0
	3	288.0	237.0
Weaning	1	209.0	127.0
	2	265.0	153.0
	3	269.0	209.0



BF, mm **6.1B**

Stage	PG	Max	Min
Day 40 GT	1	22	8
	2	26	12
	3	24	10
Farrowing	1	24	9
	2	28	12
	3	30	13
Weaning	1	21	7
	2	22	9
	3	25	10



LD, mm **6.1C**

Stage	PG	Max	Min
Day 40 GT	1	66	46
	2	69	44
	3	68	47
Farrowing	1	66	49
	2	79	55
	3	77	57
Weaning	1	66	48
	2	68	51
	3	68	52

Figure 6.1 Sow body weight (BW, 6.1A), backfat thickness (BF, 6.1B) and loin depth (LD, 6.1C) at different stages within the reproductive cycle by parity group (PG). Parity group 1 includes parity 1 sows (n = 40), PG2 includes parities 2 and 3 (n = 40) and PG3 includes sows from parities 4 and 5 (n = 21). Different letters within physiological stage (day 40 of gestation, farrowing and weaning) mean statistical differences ($P < 0.05$). Error bars are expressed in standard error terms.

6.4.2 Sow body reserves management throughout gestation and lactation, and their association with productive-reproductive efficiency

6.4.2.1 Relationship between sow body reserves in gestation and body reserves in lactation

In general, sows tend to gain body weight and body reserves during gestation and they lost, at least part of them, in the subsequent lactation. The way in which body reserves during gestation were able to influence their later management during lactation was explored in this section. First of all, the association between sow BW and body reserves (BF and LD) levels and their net gains during gestation (from day 40 of gestation until weaning) and BW and body reserves losses during lactation was investigated. The correlation coefficients obtained are shown in table 6.2. Secondly, the association of all these traits with BW and body reserves levels recorded at weaning were calculated and presented in table 6.3.

Table 6.2 Correlation coefficients (r) between sow body weight (BW, kg), backfat thickness (BF, mm) and loin depth (LD, mm) mean levels on day 40 of gestation, at farrowing and their net gains during gestation, and their losses during lactation (n sows= 103).

Time of measurement	***Losses during lactation***		
	BW[1]	**BF[2]**	**LD[2]**
Values at day 40 of gestation			
BW	0.27**	0.01	0.13
BF	0.11	0.22**	0.13
LD	0.08	0.15	0.03
Values at farrowing			
BW[3]	0.29**	0.01	0.08
BF	0.20*	0.35**	0.17
LD	0.20*	0.06	0.62**
Net gains during gestation[4]			
BW	0.08	-0.07	-0.05
BF	0.09	0.15	0.05
LD	0.11	-0.07	0.52**

Significance is indicated in the table as follows: ** statistically significant ($P < 0.05$) and * tendency ($P < 0.10$).

[1] From 48 ± 24 h post-farrowing until weaning

[2] From the day of farrowing to 18 ± 1 of lactation

[3] 48 ± 24 h post-farrowing

[4] From day 40 of gestation until farrowing

Body weight loss during lactation was positively and significantly associated ($P < 0.05$) with sow BW on day 40 of gestation and at farrowing (Table 6.2). There was also a positive correlation between BF and LD losses during lactation and their levels on day 40 of gestation (BF, $P <$

0.05) and at farrowing (BF and LD, $P < 0.05$), which indicates that the higher the levels of body reserves at farrowing, the higher will their losses be during lactation.

Overall, the amount of BW and body reserves (BF and LD) lost during lactation was more highly associated to their absolute levels at farrowing (+0.29 > r < +0.62, $P < 0.05$) than to the respective net gains during gestation (+0.08 > r < +0.52) (Table 6.2).

As can be seen in table 6.3, sows BW, BF and LD levels at weaning were also positively and significantly correlated with their levels on day 40 of gestation and at farrowing ($P < 0.05$), which then suggests that sows with higher amounts of body reserves during gestation will also reach higher levels at weaning. Furthermore, BW, BF and LD levels at weaning were again, more highly correlated to their absolute levels at farrowing (+0.45 > r < +0.97, $P < 0.05$), than to the respective net gains from day 40 of gestation until farrowing (+0.13 > r < +0.31, Table 6.3).

Table 6.3 Correlation coefficients (r) between sow body weight (BW, kg), backfat thickness (BF, mm) and loin depth (LD, mm) mean levels on day 40 of gestation, at farrowing and their net gains during gestation, and their levels at weaning (n = 103).

Time of measurement	***Levels at weaning***		
	BW	**BF[1]**	**LD[1]**
Values at day 40 of gestation			
BW	0.94**	0.59**	0.45**
BF	0.27**	0.55**	0.21**
LD	0.29**	0.30**	0.32**
Values at farrowing			
BW[2]	0.97**	0.53**	0.53**
BF	0.43**	0.80**	0.28**
LD	0.53**	0.41**	0.45**
Net gains during gestation[3]			
BW	0.12	-0.16	0.22**
BF	0.18*	0.31**	0.07
LD	0.19*	0.12	0.13

Significance is indicated in the table as follows: ** statistically significant ($P < 0.05$) and * tendency ($P < 0.10$).

[1] Day 18 ± 1 of lactation

[2] 48 ± 24 h post-farrowing

[3] From day 40 of gestation until farrowing

Therefore, it seems that the magnitude of sows BW, BF and LD future losses during lactation and their levels reached at weaning were better predicted by their levels at farrowing than by the calculated net gains during gestation. In this way, higher body reserves gains throughout gestation do not necessarily lead to greater losses during lactation, and neither guarantee higher body reserves levels at weaning. However, LD is an exception to this since the amount

of LD gained during gestation is also highly associated with their subsequent losses during lactation (r = +0.52, $P < 0.05$, Table 6.2).

All these relations between sows BW and body reserves throughout gestation and lactation were also subjected to a partial correlation analysis (Tables 6.4). This was in order to investigate the possible interaction of parity/age in all these associations. It was considered that the parity/age was partially involved in the studied effect when the previously established correlations in the gross data (Tables 6.2 and 6.3) were lowered or became insignificant in the partial analysis.

From the associations presented in table 6.4 it is worth noticing that the correlation between sows BW at farrowing and BW losses during lactation disappeared when the parity group was considered. This indicates that sows age/parity may be interfering on the BW effect observed in table 6.2. Body fat and lean reserves (BF and LD) at farrowing continued to be moderately correlated with their losses during lactation when corrected by parity. Also, BF and LD gains during gestation maintained a positive relationship with their losses during lactation when corrected by parity. Thus, it seems that, sow body reserves levels at farrowing and their increases during gestation partially explain the subsequent losses during the lactation period, regardless of the parity.

On the other hand, when looking at the body reserves levels at weaning, BW, BF and LD levels on day 40 of gestation and at farrowing continued to be highly correlated with their levels at weaning, when the parity group was taken into account thus showing no parity effect in this case.

Additionally, from table 6.4 it is also deduced that body reserves levels at farrowing were more highly correlated with both, the amount of body reserves lost during lactation and their mean levels at weaning, than sows body reserves net gains during gestation. When these correlations were analysed across parity categories (data not shown), it was detected that this trend was followed by sows from PGs 1 and 2 but not in sows from PG3. In higher parity sows (PG3) the importance of body reserves changes during gestation increased, achieving even similar correlation coefficients with losses during lactation than their absolute levels at farrowing.

Table 6.4 Partial correlation coefficients (r_p) between sow body weight (BW, kg), backfat thickness (BF, mm) and loin depth (LD, mm) mean levels on day 40 of gestation, at farrowing and their mean net gains during gestation, and their losses throughout lactation and final levels at weaning (n = 103). Sow parity is the partial factor.

Time of measurements	***Losses during lactation***			***Levels at weaning***		
	BW[1]	**BF**[2]	**LD**[2]	**BW**	**BF**[3]	**LD**[3]
Values at day 40 of gestation						
BW	0.06	0.17	0.08	0.83**	0.54**	0.37**
BF	0.10	0.22*	0.11	0.37**	0.63**	0.22**
LD	0.01	0.20*	0.02	0.32**	0.27**	0.28**
Values at farrowing						
BW[4]	0.13	0.13	0.05	0.93**	0.47**	0.50**
BF	0.12	0.41**	0.18	0.38**	0.78**	0.24**
LD	0.11	0.10	0.63**	0.47**	0.40**	0.42**
Net gains during gestation[5]						
BW	0.14	-0.07	-0.05	0.19*	-0.17	0.24**
BF	0.02	0.21*	0.08	-0.01	0.15	0.01
LD	0.08	-0.09	0.49**	0.11	0.09	0.10

Significance is indicated in the table as follows: ** statistically significant ($P < 0.05$) and * tendency ($P < 0.10$).

[1] From 48 $\pm$ 24 h post-farrowing until weaning

[2] From the day of farrowing to 18 ± 1 of lactation

[3] Day 18 ± 1 of lactation

[4] 48 $\pm$ 24 h post-farrowing

[5] From day 40 of gestation until farrowing

6.4.2.2 Association between sow body reserves at different times within the reproductive cycle and productive-reproductive performance

The correlation coefficients between sows BW and body reserves (BF and LD) on day 40 of gestation, at farrowing and at weaning, and litter performance at farrowing and on day 18 of lactation are recorded in table 6.5.

In general and, under our experimental conditions, the correlation coefficients obtained were moderate-low, not going beyond 0.50 in any case. Sows BW on day 40 of gestation and at farrowing showed a positive effect on piglet weight at farrowing (r = +0.37 and +0.38, respectively, $P < 0.05$), and BW at farrowing also exerted a positive effect on piglets weight on day 18 of lactation (r = +0.46, $P < 0.05$). A negative effect was found, although not always significant, between BW on day 40 of gestation and at farrowing and litter size at birth and on day 18 of lactation. Sows BW at weaning resulted also positively correlated with piglet weight on day 18 of lactation (r = +45, $P < 0.05$), and negatively correlated with the number of pigs at this time (r = -0.32, $P < 0.05$).

Similarly, sows BF and LD levels on day 40 of gestation (LD) and at farrowing (BF and LD) seemed to have, simultaneously, a weak but negative role on the number of pigs born and the number of pigs on day 18 of lactation ($-0.20 > r < -0.28$, $P < 0.05$), but a positive effect on the average piglet weight at birth and on day 18 of lactation ($+0.22 > r < +0.30$, $P < 0.05$). This might suggest that the fatter the sow is at farrowing, the higher the probability of having fewer pigs but the heavier they would be at farrowing and at weaning. Interestingly enough, sow BF reserves at weaning also resulted negatively correlated with litter size at this time (r = -0.39, $P < 0.05$).

In an attempt to investigate whether sows' BW or body reserves losses during lactation were related with litter performance on day 18 of lactation, the corresponding correlation coefficients were also determined (Table 6.5). In general, BW and BF losses throughout lactation were positively associated with the number of pigs and litter and piglet weights at weaning (on day 18 of lactation). However, these associations differed slightly when either absolute or in percentage terms (relative to their levels at farrowing) losses were considered.

The partial correlations of all these traits were also calculated (Table 6.6) in order to explore the degree of implication of sow parity/age in all these associations. Overall, the negative relationship observed in table 6.5 between sow BW and body reserves (BF and LD) at farrowing and litter size at birth and on the lactation's 18^{th} day was preserved, although not always statistically significant, when corrected by parity. This may indicate that body reserves variation within each parity group may be partially explained by these associations.

Table 6.5 Correlation coefficients (r) between body weight (BW, kg), backfat thickness (BF, mm) and loin depth (LD, mm) levels at different times in the reproductive cycle and change values during lactation, and productive performance at birth and on day 18 of lactation (n sows = 103).

Time of measurement		***Total born[1]***			***Day 18 of lactation***		
		Number	***Litter weight***	***Piglet weight***	***Number***	***Litter weight***	***Piglet weight***
40 days of GT	*BW*	-0.09	0.20*	0.37**	-	-	-
	BF	-0.09	-0.01	0.11	-	-	-
	LD	-0.24**	-0.15	0.22**	-	-	-
Farrowing	*BW*[2]	-0.17*	0.10	0.38**	-0.30**	0.18	0.46**
	BF	-0.24**	-0.05	0.30**	-0.28**	-0.13	0.22*
	LD	-0.20**	-0.06	0.22**	-0.13	-0.12	0.19
Weaning	*BW*	-	-	-	-0.32**	0.16	0.45**
	BF[3]	-	-	-	-0.39**	-0.21	0.17
	LD[3]	-	-	-	-0.15	0.09	0.21
Losses during LT[4]	*BW*	-	-	-	0.09 (0.23**)	0.48** (0.35**)	0.39** (0.10)
	BF	-	-	-	0.04 (0.23*)	0.19 (0.30*)	0.25* (0.14)
	LD	-	-	-	-0.01 (0.03)	-0.22* (-0.21)	-0.02 (-0.04)

Significance is indicated as follows: ** statistically significant ($P < 0.05$) and * tendency ($P < 0.10$).

[1] Total born: born alive + stillborn piglets

[2] 48 ± 24 h post-farrowing

[3] Day 18 ± 1 of lactation

[4] In brackets, coefficient of correlation calculated using the percentage of body weight, backfat and loin depth lost during lactation, in respect to their levels at farrowing

However, the positive association found between sow body reserves at farrowing and piglet weight at farrowing and at weaning (on day 18 of lactation) when analysing gross data (Table 6.5), lost its consistency when parity was taken into account. This might suggest that sow parity was, at least in part, contributing to the average piglet weight both, at farrowing and at weaning. Even so, piglet weight on day 18 of lactation remained positively related to sow BW at farrowing, when data was corrected by sow parity.

Regarding BW and body reserves at weaning, the inverse relation between BW and BF levels and litter size at this time was maintained when analysed across parities, suggesting that the level of sow body reserves is implied on this effect.

Finally, the relation between BW, BF and LD losses during lactation followed a similar trend as the found in the gross data. Thus, body reserves mobilisation during lactation was, actually, associated with weaning performances, regardless of the parity. Body weight losses of the sows during lactation were positively related to the number of pigs, and also their mean weights at weaning (day 18 of lactation). Additionally, BF losses also correlated positively with piglet weight at weaning. However, LD losses during lactation correlated negatively with the number of pigs at this time.

Table 6.6 Partial correlation coefficients (r_p) between body weight (BW, kg), backfat thickness (BF, mm) and loin depth (LD, mm) levels at different times in the reproductive cycle and change values during lactation, and productive performance (n sows = 103). Sow parity is the partial factor.

Time of measurement		***Total born[1]***		***Day 18 of lactation***	
		Number	***Piglet weight***	***Number***	***Piglet weight***
40 days of GT	*BW*	-0.19	0.18	-	-
	BF	0.05	-0.09	-	-
	LD	-0.37**	0.27*	-	-
Farrowing	*BW*[2]	-0.34**	0.21	-0.18	0.31**
	BF	-0.13	0.12	-0.40**	0.10
	LD	-0.23*	0.05	-0.38**	0.01
Weaning	*BW*	-	-	-0.26*	0.23
	BF[3]	-	-	-0.47**	0.01
	LD[3]	-	-	-0.15	0.01
Losses during LT[4]	*BW*	-	-	0.30** (0.35**)	0.24* (0.12)
	BF	-	-	-0.02 (0.20)	0.24* (0.24*)
	LD	-	-	-0.24* (-0.19)	-0.07 (-0.07)

Significance is indicated as follows: ** statistically significant ($P < 0.05$) and * tendency ($P < 0.10$).

[1] Total born: born alive + stillbirth piglets

[1] 48 ± 24 h post-farrowing

[2] Day 18 ± 1 of lactation

[3] In brackets, coefficient of correlation calculated using the percentage of body weight, backfat and loin depth lost during lactation, in respect to their levels at farrowing

Overall, while body reserves seemed to exert an important effect on litter size and lactation performance, sows parity/age might partially explain the positive relation between variation of sow body reserves at farrowing and piglet weight.

More focused on lactation and post-weaning performance, the influence of sows body weight and body reserves (BF and LD) levels at farrowing, at weaning and their losses during lactation (either in absolute or in percentage terms relative to their levels at farrowing) on litter weight gain and piglet mortality throughout lactation, and on weaning to oestrus interval (WEI) was studied and reported in table 6.7.

Average litter growth rates during lactation were positively related to sows BW at farrowing and at weaning ($P < 0.10$), and also to the amount of BW ($P < 0.05$) and BF ($P < 0.10$) lost during lactation, both in absolute and in percentage terms.

Table 6.7 Correlation coefficients (r) between body weight (BW, kg), backfat thickness (BF, mm) and loin depth (LD, mm) levels at farrowing and at weaning and their losses during lactation, and litter weight gain (g/d), piglet mortality during lactation (%) and weaning to oestrus interval (WEI, d) (n sows = 103).

Time of measurement		***Litter gain***	***Pig mortality***	***WEI***
Farrowing	*BW*[1]	0.26*	-0.09	-0.22**
	BF	-0.01	0.03	-0.06
	LD	0.02	0.02	-0.07
Weaning	*BW*	0.25*	-0.05	-0.26**
	BF[2]	-0.10	-0.01	-0.22**
	LD[2]	0.17	0.04	-0.04
BW loss during lactation[3,5]		0.46** (0.28**)	-0.08 (-0.07)	0.08 (0.25*)
BF loss during lactation[4,5]		0.22* (0.25*)	-0.05 (-0.06)	0.35** (0.37**)
LD loss during lactation[4,5]		-0.15 (-0.15)	-0.01 (-0.01)	-0.02 (-0.02)

Significance is indicated as follows: ** statistically significant ($P < 0.05$) and * tendency ($P < 0.10$).

[1] 48 ± 24 h post-farrowing

[2] Day 18 ± 1 of lactation

[3] From 48 ± 24 h post-farrowing until weaning

[4] From day of farrowing to day 18 ± 1 of lactation

[5] In brackets, the correlation coefficient calculated using the percentage of body weight, backfat and loin depth lost during lactation, in respect to their levels at farrowing

Piglet mortality throughout lactation was not significantly affected neither by sows BW nor body reserves at farrowing and their management throughout lactation. Nevertheless, when the different causes of piglet death during this period were studied (data not shown), the percentage of piglet mortality due to starvation tended to be positively associated with sow BF levels at

weaning (r = 0.21, $P < 0.10$), probably indicating that sows mobilizing a lower amount of fat reserves during lactation may be less able to maintain litter size at weaning.

After weaning, the interaction between sows body reserves and WEI length was evaluated (Table 6.7). Weaning to oestrus interval was low but negatively correlated with sows BW at farrowing and at weaning, and with BF levels at weaning ($P < 0.05$). So that, lower weaning levels of BW and BF lead to longer WEIs. The percentage of BW ($P < 0.10$) and BF ($P < 0.05$) lost during lactation were positively associated with WEI. In this way, larger BW and BF losses caused delayed WEIs. However, under the conditions of the present experiment, no relationship was obtained between LD losses and WEI.

Once more, all these associations were studied considering parity categories, and the partial correlation coefficients obtained were recorded in table 6.8.

Table 6.8 Partial correlation coefficients (r_p) between body weight (BW, kg), backfat thickness (BF, mm) and loin depth (LD, mm) levels at farrowing, at weaning and their losses during lactation, and litter weight gain (g/d), piglet mortality during lactation (%) and weaning to oestrus interval (WEI, d). Sow parity is the partial factor.

Time of measurement		***Litter gain***	***Pig mortality***	***WEI***
Farrowing	*BW*[1]	0.30**	-0.02	-0.10
	BF	-0.01	-0.03	-0.06
	LD	0.01	0.09	0.09
Weaning	*BW*	0.19	0.01	-0.11
	BF[2]	-0.15	-0.01	-0.21
	LD[2]	0.07	0.01	0.06
BW loss during lactation[3,5]		0.36** (0.27*)	-0.08 (-0.09)	0.02 (0.09)
BF loss during lactation[4,5]		0.27* (0.34**)	-0.04 (-0.03)	0.25* (0.34**)
LD loss during lactation[4,5]		-0.07 (-0.07)	0.08 (0.08)	0.03 (0.02)

Significance is indicated as follows: ** statistically significant ($P < 0.05$) and * tendency ($P < 0.10$).

[1] 48 ± 24 h post-farrowing

[2] Day 18 ± 1 of lactation

[3] From 48 ± 24 h post-farrowing until weaning

[4] From day of farrowing to day 18 ± 1 of lactation

[5] In brackets, the correlation coefficient calculated using the percentage of body weight, backfat and loin depth lost during lactation, in respect to their levels at farrowing

In general, the association between BW and BF levels at farrowing and at weaning with litter daily gain during lactation and WEI were lowered and turned out not to be significant when parity effect was corrected. Therefore, these relations might be more influenced by sow parity than by the levels of body reserves themselves. Only a positive relation between sows BW at

farrowing and litter weight gain during lactation remained significant and even higher when sow parity was taken into account in the correlation analysis.

Regarding the changes in sows' condition during lactation, the positive association between the sows BW and BF losses during lactation and the average daily litter weight gain was maintained when the parity effect was removed from the correlation. Moreover, WEI continued to be positively related to BF losses during lactation regardless of the parity, indicating that the higher the BF losses throughout lactation, the longer it will take for the WEI to be expected.

As a whole, sow parity was partially able to explain the relation between body reserves levels and WEI, but body reserves losses during lactation also exerted a noticeable effect on litter growth rate during lactation and WEI after weaning.

6.4.3 Study of the relationship between body condition score (BCS), and sow body weight and ultrasonic body reserves (BF and LD)

The second objective within this chapter was to investigate the reliability of the body condition scoring method in predicting sows body reserves (BF and LD) and also its relation with sow weight, under our experimental conditions. Table 6.9 summarizes BW, BF, LD and BCS descriptive statistics (mean, standard deviation and minimum-maximum range) from all the data included in the present analysis (n = 1216).

Table 6.9 Descriptive statistics for body weight (kg), backfat thickness (mm), loin depth (mm) and body condition score (BCS) of the total data studied in this section (n = 1216 values).

Descriptive parameters	***Mean***	***SD***	***Minimum***	***Maximum***
Body weight	214.1	40.4	122.5	319.0
Backfat	17.5	3.69	7.0	31.0
Loin depth	60.6	5.73	44.0	82.0
BCS	3.1	0.61	1	5

SD: Standard deviation

The correlation coefficients obtained between BCS, BW and body reserves (BF and LD) measurements are recorded in table 6.10. This table also shows the correlation coefficients when data was split up by parity groups (PG). In general (all parities), a positive and significant relationship between BW, BF, LD and BCS existed ($P < 0.05$). Among all the variables correlated with BCS, sow BF showed the highest correlation coefficients (r = 0.54) and BW the lowest (r = 0.44).

Across parity groups (PG), sows from PG1 (first parity sows) showed lower correlation coefficients compared to those of PGs 2 and 3 sows, in all the three traits studied (BW, BF and LD). But, specifically, BW was the trait that increased its association more with the BCS note assigned to each sow with the increasing parity.

Table 6.10 Correlation coefficient values (r) between sow body condition score (BCS) and sow body weight (BW, kg), backfat thickness (BF, mm) and loin depth (LD, mm), using all data and by parity group[1]

	n	r	*P*-value
All data			
BW, kg	900	0.44	*<0.001*
BF, mm	1216	0.54	*<0.001*
LD, mm	1216	0.49	*<0.001*
Parity group 1			
BW, kg	259	0.25	*<0.001*
BF, mm	354	0.44	*<0.001*
LD, mm	354	0.36	*<0.001*
Parity group 2			
BW, kg	348	0.58	*<0.001*
BF, mm	476	0.52	*<0.001*
LD, mm	478	0.51	*<0.001*
Parity group 3			
BW, kg	293	0. 51	*<0.001*
BF, mm	386	0.56	*<0.001*
LD, mm	384	0.46	*<0.001*

n = number of data included in each analysis

[1] Parity group 1: gilts; parity group 2: sows from parities 1 and 2; parity group 3: sows from 3 to 6 parities

In order to quantify the equivalence of each unit of variation in BCS in terms of sow BW, BF and LD in the present experiment, all they were regressed on BCS ($P < 0.001$, Eq. 1, 2 and 3). From the equations obtained it could be estimated that each unit of BCS (evaluated using a 5 score scale with intermediate 0.5 levels) was equivalent to 29 kg of BW, 3.3 mm of BF and 4.7 mm of LD. However, in spite of being statistically significant, the low coefficient of determination (R^2) and the high coefficient of variation (CV, %) indicated that the equations would be unsatisfactory to use for predicting BW, BF and LD from BCS, at least, under our experimental conditions.

Body weight (kg) = 124.9 (±6.13) + [29.0 (±1.95) x BCS],
$P < 0.001$; $R^2 = 0.20$; CV = 16.9 % *(Eq.1)*

Backfat (mm) = 7.2 (±0.47) + [3.3 (±0.15) x BCS],
$P < 0.001$; $R^2 = 0.29$; CV = 17.8 % *(Eq.2)*

Loin depth (mm) = 46.0 (±0.75) + [4.7 (±0.24) x BCS],
$P < 0.001$; $R^2 = 0.24$; CV = 8.23 % *(Eq.3)*

In order to find out whether the sensitivity of BCS when measuring BF reserves was different depending on the range of sow BF levels considered, total BF data were distributed in three groups: THIN (7-15 mm), MEDIUM (16-21 mm) and FAT (22-31 mm), and the correlation between BF and BCS was again calculated within each of these groups (Table 6.11). The results show that correlation coefficients were higher in the extreme BF groups (THIN and FAT) compared to the MEDIUM levels which should represent, in normal conditions, the greatest part of the sows on a commercial sow farm.

Table 6.11 Correlation coefficient (r) between sow body condition score (BCS) and backfat thickness (BF) within three BF groups (Thin, medium and fat).

BF groups	***THIN***	***MEDIUM***	***FAT***
BF interval, mm	7-15	16-21	22-31
n	382	671	164
r	0.39	0.23	0.31
P-*value*	*<0.001*	*<0.001*	*<0.001*

n = number of data included within each BF range

6.5 Discussion

The body weight is inherently related to the parity group since it increases with age and, generally, age increases with parity. This relationship is demonstrated in figure 6.1A. In the present study, an interaction between the parity group and body reserves was also demonstrated (see Figures 6.1B and 6.1C). As long as the multiparous sows in this herd were able to accumulate BF reserves throughout gestation, first parity sows (PG1) were not. Thus, in spite of having similar levels on day 40 of gestation, BF reserves at farrowing were significantly lower in first parity sows compared to the multiparous sows. Consequently, BF reserves at weaning were also lower in first parity sows compared to those of the multiparous sows, and even lower than those the primiparous had on day 40 of gestation. These results evidently demonstrated the lower ability of the first parity sows to accumulate body reserves in the form of fat, when compared to multiparous sows.

Regarding lean body mass (LD), in the present experiment first parity sows also showed lower LD levels than the multiparous sows at farrowing and also at weaning. However, all the sows (primiparous as well as multiparous) were able to generate LD reserves during gestation showing, consequently, higher levels at the end of the reproductive cycle when compared to the initial levels (day 40 of gestation). It is known that sows have a biological need for growing which is at its maximum in the first reproductive cycle and that need lasts until parity 3 or 4 (Whittemore, 1996; Aherne et al, 1999). In this respect, it has been described that the drive for maternal growth takes the young sow to selectively partition nutrients into lean tissue growth at the expense of fat (Aherne et al, 1999). Thus, as it was also demonstrated and concluded within the preceding chapter of this document (see Chapter 5), the maternal gains in primiparous sows will be mainly composed of lean tissue while well-managed multiparous sows (>3 parities) will gain fat and not lean tissue from one cycle to the next.

The above mentioned differences in BW and body reserves levels between parity groups at different times within the reproductive cycle might be interacting, to a large extent, to the associations evaluated in this chapter. Thus, the possibility of a parity effect will be considered, when necessary, within the following discussion of the results.

Sow body reserves interaction between different stages within the reproductive cycle

Because all the phases within the reproductive cycle are related therefore, the feeding program or sows reserves management in one phase will have significant consequences on performance in others (Dourmad et al., 1994; Coffey et al., 1994; Whittemore, 1998; Revell et al., 1998a,b; Sinclair et al., 2001; Prunier et al., 2001). As an example of this interrelation between different physiological stages, there is the well documented negative effect of feed

intake during gestation on voluntary feed intake throughout lactation and, subsequently, on body weight and body reserves losses during lactation (Mullan and Williams, 1989; Cole, 1990; Dourmad, 1991; Weldon et al., 1994; Revell et al., 1998a; Sinclair et al., 2001). Several studies in the literature have stated that this negative effect may be mediated through the level of fatness at parturition (Mullan and Williams, 1989; Weldon et al., 1994; Sinclair et al., 2001; Young et al., 2004).

The results of the present experiment confirm that the well established relationship between body reserves management during gestation and body reserves during lactation was still evident in the new leaner genotypes. Sows BF and LD levels at farrowing resulted positively related to their mobilisation during lactation in the present study, thus supporting the theory that BF levels at farrowing but also LD levels in this case promote BF (r = 0.35, $P < 0.05$) and LD (r = 0.62, $P < 0.05$) losses during lactation, respectively. The amount of BW lost during lactation also tended to be positively but weakly related to both, BF and LD at farrowing (r = 0.20, $P < 0.10$).

The positive association between BF levels at parturition and the amount of BF lost during lactation could be explained by means of a quadratic relationship (Figure 6.2A, $P < 0.180$), whilst LD levels at farrowing clearly exerted a linear effect on the amount of LD lost during lactation (Figure 6.2B, $P < 0.05$). De Rensis et al. (2005) suggested that BF losses higher than 4 mm during lactation markedly decreased the percentage of sows returning to oestrus before 6 days post-weaning and decreased pregnancy rates in the subsequent cycle. From figure 6.2A it may be estimated, although with a considerable high variability among data, that this 4 mm of BF losses were reached at about levels of 20-21 mm of BF at farrowing. These results are in agreement with those of Miller (1996) and Young et al. (2004) who reported no negative effects of BF levels at parturition on lactation appetite up to 20-21 mm of BF levels at farrowing. However, earlier studies suggested that this effect was only significant when BF was about 25 mm or greater (31.6 mm) (Mullan and Williams, 1989). This might be indicating that in the actual leaner genotypes, this BF threshold, when existing, might have been lowered compared to that of fattier animals. It was also noticeable from the relation found between BF levels at parturition and the amount of BF lost during lactation under our experimental conditions is that a certain amount of BF loss during lactation of about 3 mm seems unavoidable.

Further, from figure 6.2B it is deduced that, within the range of data obtained in the present study, LD losses throughout lactation increased linearly with increasing levels of LD at farrowing. As BF is closely related to dissectable fat in sows (Whittemore et al., 1980; Whittemore and Yang, 1989), LD is used as a measure of loin muscle area in the carcass (reviewed by Moeller et al., 2002) and thus, as an approximation to lean body mass.

High levels of body protein mass at parturition have been suggested to increase milk production (Kusina et al., 1999b) and to ensure a higher litter growth rates during lactation (Clowes et al., 2003b). If this is so, higher demands for milk synthesis in this situation could have explained the increase of lipid and protein mass losses during lactation. However, in the present study, higher LD losses during lactation were not apparently accompanied by increases in milk production or litter growth rates (see Table 6.7) and, therefore, the decline on LD reserves with increased LD levels at farrowing was not expected.

6.2A

6.2B

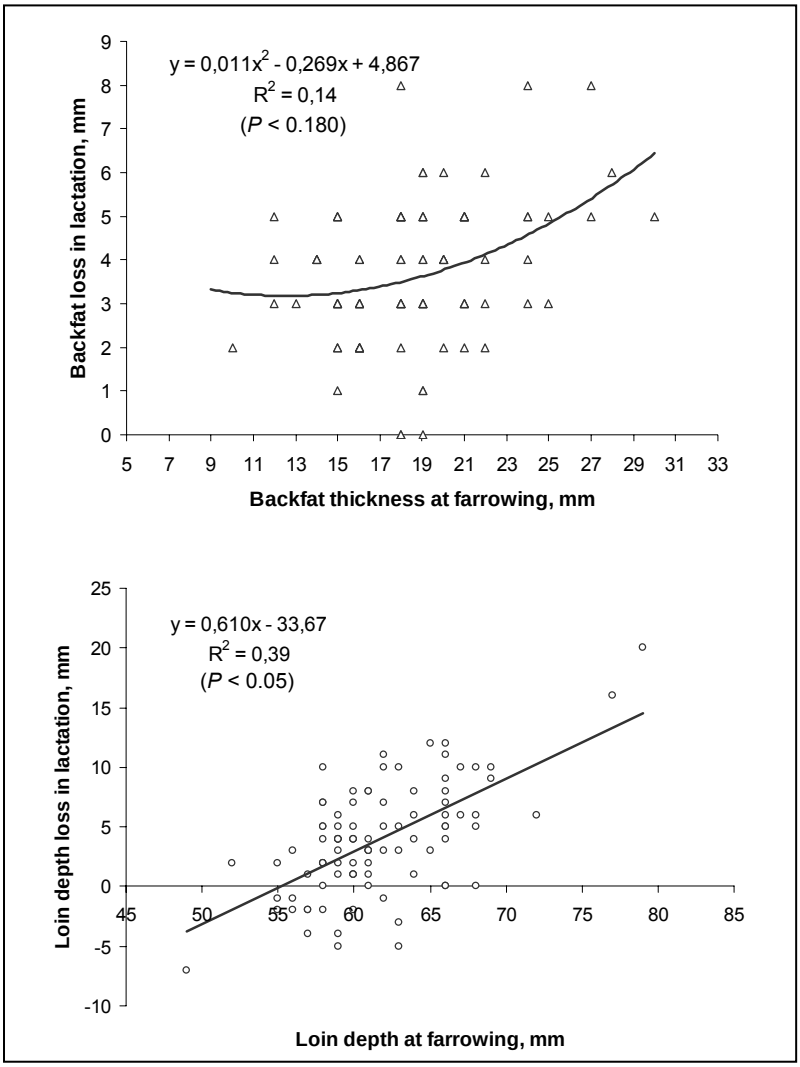


Figure 6.2 Relation between backfat and loin depth levels at farrowing and the amount of backfat and loin depth lost during lactation (6.2A and 6.2B, respectively).

Also, from figure 6.2B it is observed that the amount of LD mobilised during lactation may reach levels up to 20 mm of LD losses and it is estimated that at an average level of 55 mm of LD at farrowing, LD mobilization during lactation will be approximately zero. Thus, contrary to BF, LD losses may reach zero mobilisation during lactation. Progressive mobilization of body protein during lactation may have negative consequences on milk production and subsequent

reproduction (King, 1987; Aherne et al., 1999; Clowes et al. 2003a). Therefore, it is speculated that, under the conditions of the present study, and whatever the reason for lean tissue mobilisation may have been, 55 mm is the threshold level of LD at farrowing below which sows would have to mobilize LD in order to support lactation and not to impair post-weaning performance.

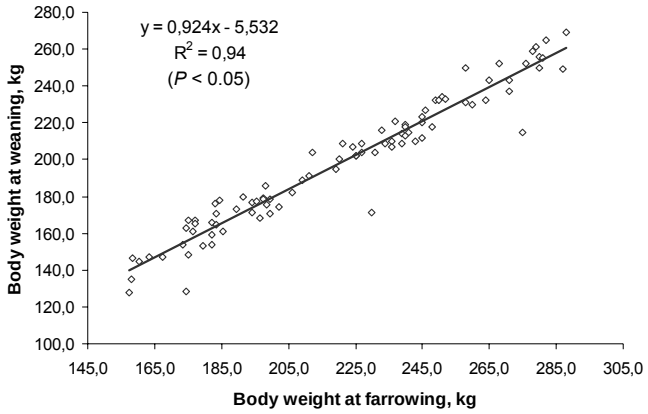
In order to guarantee a high subsequent productivity and a long lifetime performance, it is also essential to assure an optimum level of body reserves (lean and fat) at weaning, specially in lean genotypes (Mullan and Williams, 1989; Young et al., 1991; Whittemore, 1993; Kongsted, 2006). In the present study, BF and LD levels at farrowing together with BW values at this time, exerted a positive and strong lineal effect on their levels at weaning (Figure 6.3). This positive correlation was maintained when partial correlations accounting for sow parity were applied (see Table 6.4), suggesting that parity was not acting as a misleading effect in this case.

Overall, BW showed the highest correlation coefficients compared to BF and LD (Figure 6.3A). Indeed, sows BW at farrowing was able to explain a 94% of its variability at weaning, demonstrating the extremely high reliability of BW as a predictor of BW values in other stages within the reproductive cycle in sows. In this respect, it is important to take note that in the growing pig context, the BW correlation among the different growing stages is much lower than those found in sows. From our data, the correlation coefficients between pigs body weight measurements in similar time distances to those considered for sows (about 3 weeks) ranged from 0.44 to 0.49 (data not shown). This makes evident the high influence of the environmental factors throughout the fattening period, which disappear afterwards, when the animal has already reached its sexual and chemical maturity.

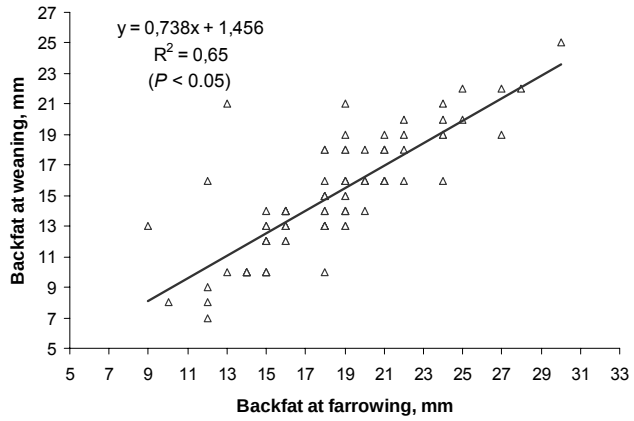
In terms of BF reserves, it has been suggested that levels lower than 14 mm at weaning may compromise subsequent reproductive performance (Young et al., 1991; Tantasuparuk et al., 2001; Marco, 2004). From figure 6.3B it is estimated that, within our particular body reserves range, a minimum of 17 mm of BF at farrowing is required in order allow sows to lose 3 to 4 mm of BF and not to fall below 14 mm of BF at weaning. In accordance, this BF target (17 mm) was the minimum permitted at farrowing in the experimental study of Young et al. (2004) and from Spanish field data (Luborda, 2002; Marco, 2004; Marco and Barceló, 2006).

In figure 6.3C it is shown, consistently with figure 6.2B, that under the conditions of the present study an average of 55 mm of LD at farrowing will lead to zero mobilization of LD during lactation and, consequently, to LD levels at weaning similar to those reported at farrowing. Little research has been conducted using LD levels measured by ultrasounds in pigs, thus it is difficult to compare our results with others. Even so, the LD levels reported in the present experiment seem to be within the normal levels for sows commercial genotypes (Stalder et al., 2005; PIC, 2007).

6.3A



6.3B



6.3C

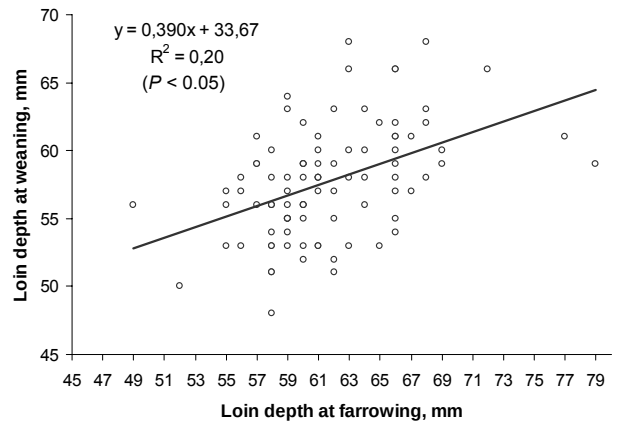


Figure 6.3 Relationship between body weight, backfat thickness and loin depth levels at farrowing and their levels at weaning (6.3A, 6.3B and 6.3C, respectively).

Overall, although the sows having greater body reserves at farrowing were sentenced to lose more body reserves during lactation, higher levels at farrowing also guarantied, even with greater accuracy (see Table 6.5), higher body reserves levels at weaning. In agreement, Whittemore et al. (1980) also stated that there was clearly a tendency for fat sows to remain fat, with correlation values among BF measurements at different times ranging from 0.42 to 0.92.

The results of the present study also suggested that, in general, BF and LD losses throughout lactation and their levels at weaning were more accurately predicted (higher correlation coefficients) by their final levels at farrowing, rather than by their net gains during gestation. In agreement, Young et al. (2004) reported that a suggested BF gain does not always cope with the desired (targeted) levels at weaning. However, when relations were measured across parity groups (data not shown), this trend was observed in sows from PG1 and PG2 but not in sows from PG3 (data not shown).

Consequently, these results may derive the thought that feeding programs for gestating sows should be based on setting a minimum of BW and body reserves at farrowing for each sow (approx. 17 mm of BF and more than 55 mm of LD), and not on a suggested BW, BF and LD change during gestation, in order to reach the optimum levels at weaning at least in young sows. When sows have already attained their final adult weight (parities 3 and 4, Whittemore, 1996), setting absolute or change values will similarly affect their levels at weaning (see Table 6.3), probably because the changes may then represent their reserves recovery and not maternal growth.

Additionally, as BW, BF and LD levels on day 40 of gestation were also positively correlated to their levels at weaning, defining the optimum BW, BF and LD levels by the time of the pregnancy test (days 30-35 of gestation) could also contribute to achieve the body condition desired at weaning. The latter, when extrapolated to nuliparous sows at first mating suggests, in agreement with Challinoir et al. (1996) and Brisbane and Chesnais (1996), that the level of body reserves at first mating or at earlier stages (selection) may be crucial in determining body reserves maintenance and subsequent reproductive and lifetime performance success.

Influence of sow body reserves during gestation and lactation on productive efficiency

Recently, numerous studies in the literature have been devoted to determine the effects of weight and body reserves (both fat and/or protein) levels and changes within all phases of the reproductive cycle, on sow productive-reproductive efficiency (Maes et al., 2004; De Rensis et al., 2005; Stalder et al., 2005; Thaker and Bilkei, 2005; Kongsted, 2006; Weldon et al., 2006). This is not surprising since the negative consequences of a wrong body reserves management on the subsequent performance, may be of higher magnitude in the genetically improved leaner sows used nowadays.

According to the results of the present experiment both, sow body reserves (BF and LD) levels and sow parity exerted an effect on litter performance at farrowing and at weaning although, in general, weak correlations were obtained. From the calculated partial correlations, it was determined that more than body reserves *per se*, sow parity was partially involved on productive

performance at birth and at weaning. That is, the positive relationship found between sow BW and body reserves at farrowing on the average piglet weight at farrowing and on day 18 of lactation ($0.19 > r < 0.46$, $P < 0.05$) lost its consistency when analysed corrected by parity. Indeed, first parity sows in the present study (PG1, lower BW) showed lower litter and average piglet weight both, at farrowing and on day 18 of lactation compared to multiparous sows (Figure 6.4, PGs 2 and 3, $P < 0.05$).

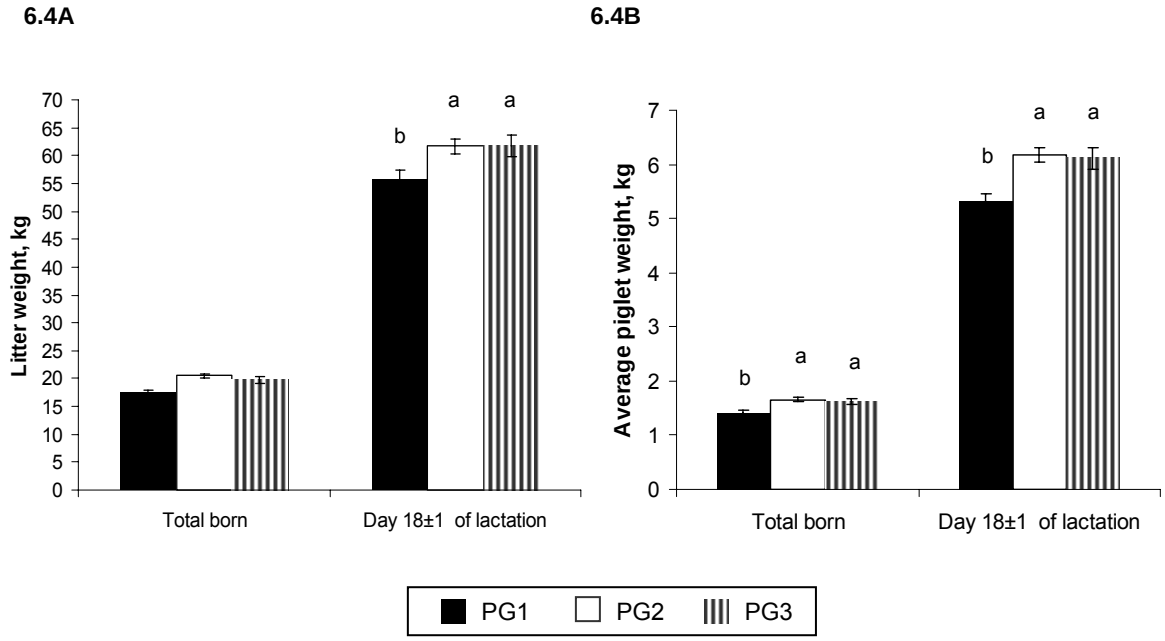


Figure 6.4 Litter weight (6.4A) and average piglet weight (6.4B) at birth (total born and born alive) and on day 18 of lactation by parity group (PG). Parity group 1 includes parity 1 sows (n = 40), PG2 includes parity 2 and 3 sows (n = 40) and PG3 includes sows from parities 4 and 5 (n = 21). Differing letters within the same variable mean statistical differences between parity groups.

The first reproductive cycle has been considered a challenge for the sow productivity and lifetime performance. It is generally recognized that first parity sows show lower productive and reproductive performances when compared to multiparous sows (Whittemore, 1996; Miller et al., 2000; Guedes et al., 2001). Many theories have been proposed in terms of body reserves, in order to give an explanation to this fact. As previously mentioned, it is suggested that breeding sows have a biological need to attain a target of at least 35 kg of body protein mass to reach maturity (Everts, 1994), and that the reproductive efficiency is expected to increase as the animal approaches this target of protein mass (Everts and Dekker, 1994; Clowes et al., 1994). Therefore, because of its physiological necessity for maternal growth and because of its generally lower fattening levels (see Figure 6.1), the primiparous sow is less capable of devoting energy into foetal growth, and also less able to mobilize body reserves in order to sustain lactation compared to the more mature multiparous sow.

Aditionally, primiparous sows have approximately 20% lower feed intake in lactation than multiparous sows (Whittemore, 1996; Young et al. 2004). So this will prevent these sows from consuming the amount of energy and nutrients required to support milk yield. In the current experiment, apart from the reported lower piglet weights at birth and at weaning, a lower ability for milk production in first parity sows but also in higher parities (4 and 5), was also detected (Quadratic, $P < 0.01$, Figure 6.5). As a result, primiparous sows will come up with lighter piglets at farrowing and also, lighter and immunologically challenged piglets at weaning that generally tend to grow less after weaning, at least in early stages (Dwyer et al., 1993).

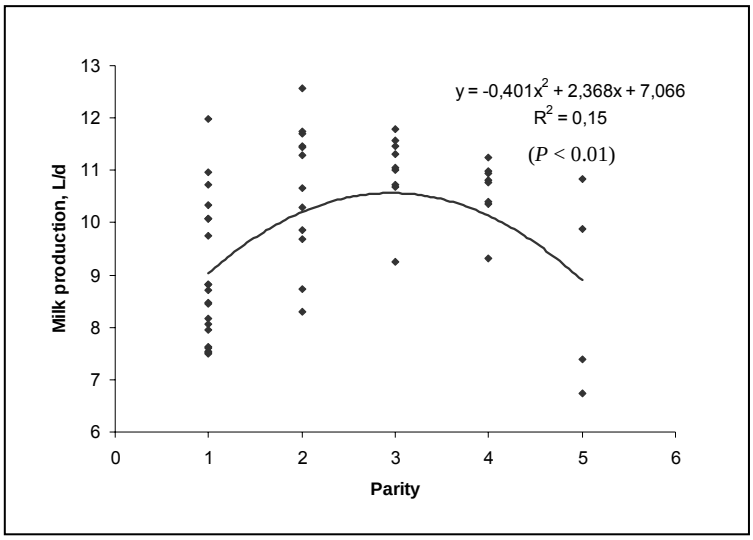


Figure 6.5 Milk yield (L/d) according to sow parity. Milk yield is estimated from the litter weight gain during lactation and applying an efficiency factor of 4 L/ kg of piglet body weight (Pluske and Dong, 1998).

Thus, based on these suggestions and on our own results, the lower productivity performance in first parity sows seems, in part, inherent to their biology and therefore unavoidable. For this reason, some companies have proposed the use of parity segregated management systems for breeding sows, in which primiparous sows and their offspring are managed separately from that of multiparous sows. This production system has already been introduced in some herds with satisfactory results (Boyd et al., 2002).

On the other hand, litter size at birth and on day 18 of lactation were not clearly associated with sow parity in the present experiment (Figure 6.6). Sows BW and body reserves at farrowing exerted a negative effect on these variables which, although not always significant, appeared systematically even after the parity effect was removed from the model. Consequently, the sows with higher body condition at parturition in the present experiment would probably farrow and wean fewer, but heavier piglets.

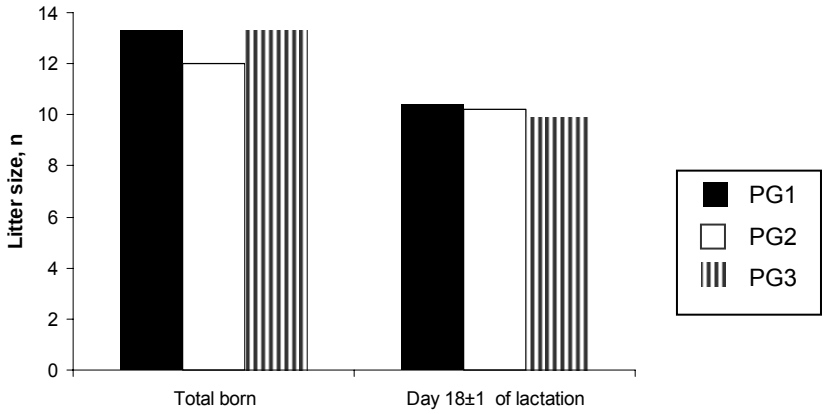


Figure 6.6 Litter size at birth (total born and born alive) and on day 18 of lactation by parity group (PG). PG1 includes first parity sows (n = 40), PG2 includes parity 2 and 3 sows (n = 40) and PG3 includes sows from parity 4 and 5 (n = 21). *P*-value (PG) was > 0.10 in all cases.

The precise cause for this relation between sow BW and body reserves at farrowing and litter size at birth (BW, r = - 0.34; BF, r = - 0.13; LD, r = - 0.23) is not known. Higher embryo mortality before implantation (implantation at 30 days of gestation, approximately) associated to higher body condition could be suggested. On the other hand, an excessive body condition (fatness) at farrowing is known to increase the risks of suffering from dystocia and associated high stillbirth rate, agalactia, mastitis and metritis (Göransson, 1989; Weldon et al., 1991). This is not likely in the present study, since the total born also included the number of stillborn pigs. In the present study, a negative association was found between the number of pigs born per litter and their mean weight (r = 0.4, $P < 0.001$). Thus, the higher birth weights found with increasing sows body condition might be explained, in part, by this lower number of pigs at birth.

At weaning, this negative relation between sow BW and body reserves and the number of pigs (BW, r = - 0.18; BF, r = - 0.40; LD, r = - 0.38) might be related to a lower milking ability of sows when increasing weight and LD but, mainly, fat reserves at farrowing. An increase in feed allowance during the period of mammary gland development (Weldon et al., 1991) or, simply high levels of BF at farrowing (> 25 mm, Head et al., 1991) have been observed to cause an impairment of mammary gland function and a lower milk yield. In the present study, fat reserves at weaning reduced litter size at weaning but did not affect litter growth rate during lactation (Table 6.8) indicating that high sow body reserves, specially BF levels at farrowing might lead to lower number but heavier pigs at weaning. A detrimental effect of feeding level during gestation on mammary development has been previously described in the present experiment (see Chapter 5).

Milk production in the lactating sow represents, at least, a 70% of their total energy requirement in this phase (Noblet et al., 1990). If sows are not able to eat sufficiently to fulfil its considerable high requirements during lactation, these will be supplied from maternal body reserves. The

most marked effect is therefore on the weight change of the sow since substantial reserves of body fat may have to be mobilized to maintain milk production (Close and Cole, 1986). However, fat is not the only tissue available for mobilisation, and there is recent evidence indicating that muscle tissue may be used under certain circumstances to meet the metabolic demands during lactation (Prunier et al., 2001; Clowes et al., 2003a,b). Also, there is some evidence that protein losses during lactation are more highly related to post-weaning reproductive failure than fat mobilisation (Aherne et al., 1999). In the current study sows lost, in average, 22.3 kg of BW (10% relative to values at farrowing) and 3.4 mm of BF (18% relative to levels at farrowing) and 3.9 mm of LD (6% relative to levels at farrowing) during the lactation period.

The percentage of BW and BF losses in the current experiment was weakly but positively correlated with the number of pigs, litter and piglet weights at weaning, even when data was corrected by parity group (see Table 6.6). Maes et al. (2004) also reported a positive relation between lactation performance and sow BW and BF losses, thus indicating that the greater the litter demands during lactation, the higher the sow BW and fat reserves losses. In the present experiment, these results also matched the fact that litter growth rates during lactation were positively correlated with BW and BF losses after correcting by parity effect (see Table 6.8). However, no correlation was found between litter growth rate and LD losses during lactation. Thus, it seems, in accordance with Whittemore (1993) and Prunier et al., (2001) that although both, lean (LD) and adipose (BF) tissue were mobilised during lactation, BF reserves were more likely to respond to increasing milk production than LD reserves. Another possibility to this lack of association between LD and litter growth rate is that maybe, LD measures were not able to appreciate whole body protein changes.

Influence of sow body reserves during lactation on reproductive performance

The negative effects of excessive amounts of live weight or body reserves (protein or fat) losses during lactation, especially in primiparous sows, on remating intervals, pregnancy rates and embryo survival in the subsequent cycle have been widely reported (Aherne and Kirkwood, 1985; Einarsson and Rojkittikhun, 1993; Whittemore, 1996; Weldon et al., 2006; Clowes et al., 2003b). However, there is no consensus in the literature about what constitutes an excess of body weight or body reserves loss. Neither is there agreement about whether it is the threshold level of body reserves at weaning (Young et al., 1991; Tantasuparuk et al., 2001) or the metabolic state of the animal (amount of body reserves loss) the last, indeed, modulated by the type and the amount of body reserves mobilised during lactation (Pettigrew, 1998) which most affect reproductive performance.

In the present experiment, the association between BW and BF levels and WEI was mediated, at least in part, by a parity effect since it turned out to be non significant when partial correlations were applied (see Table 6.8). Indeed, PG1 sows in the present experiment showed a greater WEI compared to multiparous sows (PGs 2 and 3), (PG1 = 4.8 days, PG2 = 4.4 days and PG3 = 4.4 days, $P < 0.10$). This is in agreement with other studies (Einarsson and Rojkittikhun, 1993; Miller et al., 2001; Guedes and Nogueira, 2001; Thaker and Bilkei, 2005), in which first parity sows presented extended remating intervals after weaning. This condition seemed to be associated to primiparous sows, as well as the lower productivity at birth and at weaning previously discussed.

Regardless of the parity, sows' BF changes throughout lactation were low but positively related to WEI (r = 0.25-0.34, $P < 0.01$, Table 6.8). Similar correlation coefficients between BF losses during lactation and WEI were reported by Mullan and Williams (1989) and Maes et al. (2004). Under our conditions, this relation is noteworthy considering the very low variation among existing in WEI data (from 3 to 7 days). Also under our experimental conditions, LD losses showed no relation with WEI indicating that in well managed sows, as it is our case, lipid content contribute to a larger proportion of the mobilised tissue.

Overall, these results suggest that, at least under the conditions of this experiment, the reproductive success measured in terms of WEI depends less on the achievement of a recommended backfat depth at weaning than on the maintenance of body condition during lactation, supporting the theory based on the implication of the metabolic status on sows reproductive performance proclaimed by Pettigrew (1998). Moreover, LD losses were not implied on WEI.

Other post-weaning parameters likely to be affected by excessive body weight and body reserves losses during lactation are farrowing rates and the subsequent litter performance (Einarsson and Rojkittikhun, 1993; Weldon et al., 2006) probably, due to an impairment of the quality of the growing follicles during lactation (Zak et al., 1997). In the present study, the analysis of this data (not shown) demonstrated no relation of number of total born in the subsequent parity with sow BW and body reserves losses during lactation, indicating that BW and body reserves lost during lactation were not "excessive" enough to affect subsequent litter performance. In consequence, the results of the present experiment illustrated that WEI might be earlier impaired than subsequent litter by the BF reserves loss during lactation. However, because of experimental design, half of the sows used (S sows, n = 47) repeated the experimental treatment (higher feed allowance during mid-gestation) also in the subsequent cycle (cycle 2). Thus, the possibility of having interactive or additive effects caused by the double experimental treatment must be born in mind when interpreting these data.

Accuracy of body condition score (BCS) in the prediction of sow body reserves

Breeding sow herds require a reliable method of monitoring sows body reserves on the farm in order to assure low body condition variability among individuals, and to optimise feeding strategy. Visual scoring of the body condition on the farms might not be adequate enough due to its subjectivity and to the conformational changes suffered from the new leaner genotypes. In fact, recent studies showed that BCS and backfat appeared to be only moderately correlated (r = 0.43, Young et al., 2001; r = 0.48, Mes et al., 2004). In the present experiment, a moderate, but slightly higher relationship was found between BCS and BF (r = 0.54, $P < 0.001$). Similarly to the study of Young et al. (2001), this low association between BCS and BF came from the wide range in backfat at each assigned body condition score (Figure 6.7). However, in spite of the spreader of the BF levels within each BCS value, but according to the positive correlation found between them, as the average backfat thickness increased the body condition score also increased.

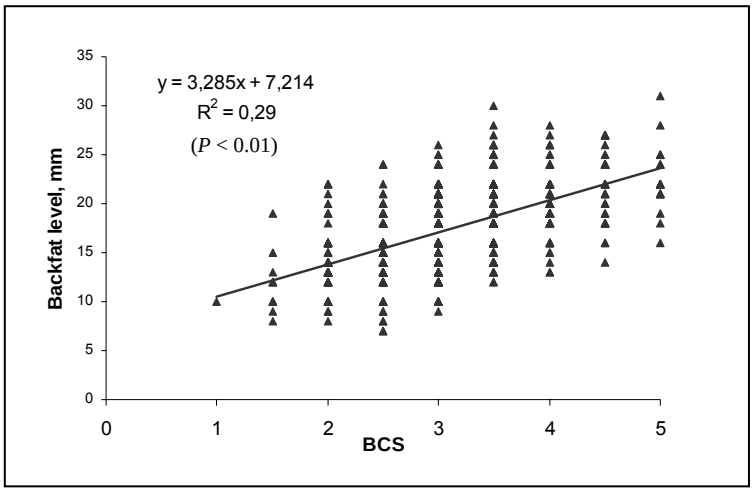


Figure 6.7 Relationship between body condition score (BCS) and backfat thickness (n = 1216 values). Body condition score was obtained according to a 1 to 5 scale (1 = very poor condition and 5 = very fat), using intermediate (0.5) levels and backfat thickness was measured by ultrasounds (Renco sonograder) at the level of the last rib, at 6.0-6.5 cm of the midline (P2).

Within the present work, apart from BF, also the correlation between BW, LD and BCS was obtained. To our knowledge, although BCS may also be influenced by sow BW and LD, their relationship has not been evaluated recently. In the present study, BSC was mostly influenced by sow fatness (BF), but positive correlations were also observed between BCS and BW and LD indicating that, as expected, BCS do not predict purely fat reserves, but may be also associated with lean reserves (ultrasonic LD) and sow size. In fact, all this variables together (BW, BF and LD) were able to explain a 38% in front of the 29% of the BCS variability that explained BF alone (Equations 4 and 2, respectively).

BCS = - 0.139 + 0.06 BF (mm) + 0.03 LD (mm) + 0.002 BW (kg),
$P < 0.001$; $R^2 = 0.38$; CV = 15.7 % *(Eq. 4)*

Across parity categories, in general, first parity sows showed the lowest association between BCS and BW and body reserves. It is possible that the morphological homogeneity within the primiparous group of sows prevented BCS from noticing small body reserves differences between individuals. On the contrary, the heterogeneity in body reserves levels presented by multiparous sows may lead to a more accurate perception of body condition status among individuals. Thus, special attention should be paid to the youngest collective of sows in order to optimise body reserves prediction, when using BCS.

Whittemore et al. (1980) established the following equation relating BCS and BF,

BF (mm) = -0.7 + 5.8 BCS

This equation states that each change in BCS equates to a 6 mm change in BF. As they used a 10 score BCS scale in their study, it is perceived that in a 5 BCS score scale the equivalence would be, approximately, of 3 mm by each BCS unit which is similar to that obtained in the present study (3.3 mm). However, from the equation calculated by Young et al. (2001) (see Chapter 1, Figure 1.6), it was determined that each BCS corresponded to 1.9 mm of BF in a similar data range to ours. This suggests that the equivalence between BCS and BF may vary among studies probably depending on the operator, sow condition and genetics, among others.

Results from the present experiment show that BCS association with BF reserves changes depend on the BF category being higher in extreme BF groups (thin and fat, table 6.11). In an attempt to investigate if the reliability of BCS on predicting BF reserves also varies along the different stages within the reproductive cycle, BCS was regressed on BF at day 40 of gestation, at farrowing and at weaning (Table 6.12). A general tendency was observed in the relationship (slopes, b) between BCS and BF to be fairly constant over the different stages in the reproductive cycle (b = 2.6-3.3, P = 0.261). Thus, the use of a unique equation for all the reproductive cycle (Eq. 1) would be recommended.

The determination coefficients (R^2) indicated that the estimates of BF through BCS were more accurate at farrowing and at weaning, in agreement with the fact that correlation coefficients between BF and BCS were higher when BF levels were carried to the highest and lowest extremes (Table 6.11). However, again, the low coefficient of determination (R^2) indicates that the equations would be unsatisfactory to use as predictive, at least, under our experimental conditions.

Table 6.12 Linear regression relationships between backfat thickness (BF) and body condition score (BCS) at different times within the reproductive cycle [BF = a + (b x BCS)].

Time of measurement	***Regression equation***			
	a	b	R^2	*P*-value
Day 40 of gestation	8.3	2.6	0.18	< 0.001
Farrowing	8.2	3.3	0.29	< 0.001
Weaning	7.4	3.0	0.31	< 0.001

As a whole, it is clear that body condition score alone is not an accurate method on which to base a feeding program, at least in gilts and sows with medium BF values. Thus, these results emphasize the need to find a more accurate or a combined method for feeding sows in order to reduce variation in sow condition. In agreement, recent studies in which different feeding methods during gestation based on either BCS or BF, and BW were compared showed that feeding based on BF and BW provided more homogeneous body reserves status and a higher proportion of sows in the optimal backfat category than the BCS feeding system alone (Young et al., 2004).

6.6 Implications

From the results obtained in this study, it can be highlighted that a close interaction between pregnancy and lactation body reserves management is still evident in the new genotypes. Higher levels of body reserves at farrowing led to higher losses throughout lactation but not impairing final levels at weaning, since higher levels at farrowing also assured higher levels of body reserves at weaning. This finding adds a concern to the controversy about which is the best feeding strategy during gestation that reduce body weight and body condition losses during lactation and that, at the same time, guarantees higher levels of body reserves at weaning. Another important outcome of the current study is that primiparous sows showed lower productive (piglet weight at farrowing and at weaning) and reproductive performances (extended WEI) compared to multiparous sows, denoting that their reproduction function is certainly compromised in the first parities. The lower levels of fat and lean reserves at farrowing and the lower capacity for body reserves mobilisation during lactation of the primiparous sows, might have played a role on these effects. From the results of this study, we also recommend limiting backfat losses during lactation in order not to detriment the WEI length. Finally, regarding the use of BCS as a method of predicting sow body reserves, the correlation obtained among BF and BCS was not high enough to consider BCS a good predictor, at least, when used alone. Furthermore, when applied, especially attention must be paid to gilts since their homogeneity may be playing a negative role on the relationship between BCS and body reserves.

Chapter 7

General discussion

7.1 Introduction

The main point of the study presented in this PhD dissertation was to investigate the consequences of an extra feed allowance during mid-pregnancy (day 45 to day 85), on foetal muscle tissue development. In 1962, McCance and Widdson proposed the concept that during pregnancy, the foetus moves through windows of vulnerability, meaning that the stage of pregnancy during which a nutritional imbalance is imposed determines the ability of the animal to respond. Until recently, studies of the impact of nutrition on foetal growth tended to concentrate on late pregnancy when most of the increase in foetal size takes place (Aherne and Kirkwood, 1985; Cromwell et al., 1989). However, through its effects on placental development, early foetal organogenesis and tissue hyperplasia (including muscle tissue) nutrition in mid- and indeed early-pregnancy can have profound effects on foetal growth and development (Rehfeldt et al., 1993; Dwyer et al., 1994; Gatford et al., 2003). There is increasing evidence that alterations in nutrient supply during critical periods of embryonic and foetal life may cause permanent changes in foetal programming and development *in utero,* imparting a legacy of growth and developmental changes that affect neonatal survival and adult performance. This has been reported in humans (reviewed by Hornstra et al., 2005), but also in livestock animals (reviewed by Robinson et al., 1999).

The hypothesis of the present study was that, on one hand, providing a higher nutrient supply during the period of secondary muscle fibre formation (day 45 to day 85) may enhance the number of foetal muscle fibres developed *in utero*. Consequently, postnatal growth performance from birth to slaughter and meat quality traits at market weight might also be influenced. Additionally, as the present study involved three consecutive sow reproductive cycles, the consequences of this feeding regime during gestation on sow body reserves management were also determined.

This chapter is focused on the capture of the most important practical consequences and implications of the feeding strategy carried out in the present study on the progeny, as well as, on their mothers and therefore, its impact on animal production. Also, a recommended feeding pattern based on a compilation of different data in the literature and our own results is provided.

7.2 Implications of "Prenatal programming of postnatal performance" concept in muscle fibre development

In pig production, the number of live-born piglets per litter, piglet survival, daily weight gain, feed conversion ratio and lean meat percentage are the most important traits for economic efficiency (Leman, 1992). Growth rate and muscle mass are predominantly a function of muscle fibre number and size. Additionally, due to their different contractile and metabolic properties,

muscle fibre types may also be involved in determining final pork quality. Thus, the understanding of growth and development of skeletal muscle is one of the most important goals in animal production and meat science. Studies on muscle development of livestock animals are relevant not only to the optimization of meat quantity and quality (Wray-Cahen et al., 1998), but also to the health and disease of a wide range of mammals, including companion animals and humans (Dauncey, 1997). The young pig makes a particularly good developmental, nutritional, hormonal and metabolic model for the human infant (Tumbleson and Schook, 1996).

A series of studies in the pig have demonstrated that somatotropin administration (Rehfeldt et al., 1993 and 2001; Sterle et al., 1995), β-agonist application (Hoshi et al., 2005) and nutrition (Dwyer and Stickland, 1992; Dwyer et al., 1994; Gatford et al., 2003) prenatally, are potential factors affecting muscle development *in utero*. The results from the present experiment (Chapter 4) confirm the existence of a foetal programming of postnatal performance in terms of muscle tissue development, at least in the pigs classified as the second smallest group of weight. However, the results obtained were not the expected from the initial hypothesis. Prenatal nutrition during the period of the secondary muscle fibres formation in the present study was able to impact on muscle fibre characteristics (number, size and type proportion). Postnatally, these developmental modifications did not clearly affect growth performance, although led to consistent effects on meat quality traits at market weight.

Pigs born from supplemented sows in the current study showed a lower number of muscle fibres in the *longissimus thoracis* muscle that involved both developmental fibre types (primaries and secondaries) but, at the same time, these fibres were larger compared to the control. Although, apparently, no clear effects were found on growth performance the combination of a low number of fibres with higher sizes has been reported to be less efficient in growth potential in the literature (Rehfeldt et al., 1999). A muscle with a lower number of fibres that grow mainly due to increasing size of these fibres has a lower potential for growth than a muscle with a higher number of fibres. This is because a genetic and physiological limit for muscle fibre growth in size has been actually described (Rehfeldt et al., 1999). Nevertheless, it has also been reported that pig slaughter ages in our current commercial conditions (100-110 kg BW) might not allow to reach this limit for muscle fibre enlargement, thus leading to a compensatory growth in size with decreasing in fibre number. We hypothesized that this was the reason for the lack of growth performance differences in our study.

Moreover, a lower number of fibres but higher in size have been considered less desirable for meat quality, specially showing impaired meat tenderness (Gondret et al., 2006; Rehfeldt and Khun, 2006). But, limits in fibre number and size are not further established. In the present study, the larger fibres found in the maternal supplemented group of pigs did not seem to negatively affect meat quality. On the contrary, maternal supplemented animals show positive

effects on meat quality traits (pH and colour), indicating that the limit in size might not have been achieved in this case.

Generally, leaner meat-type pig breeds such as Piétrain or Large White, show higher fibre sizes than other fatter and less selected breeds such as Iberian or Meishan pigs (Seideman et al., 1989; Serra et al., 1998; Lefaucheur et al., 2004). Thus, it can be speculated that effects of the feeding strategy proposed in the present study, if reproduced, might be different depending on pig breed. In leaner breeds, it might lead to negative effects on meat quality if muscle fibre continues growing in size or, it might have even no effects if they are at the limits of leanness selection and fibre size as it is suggested by Rehfeldt et al. (2000). Less improved lines would presumably show higher responses in fibre sizes but probably no reaching the maximum allowed in order to deteriorate meat quality.

One of the most important and most interesting outcomes of the present study for the pig producer and also for the packing industry was that maternal treatment resulted in distinctive histological characteristics that lead to differences in meat quality traits. *Longissimus thoracis* muscle of pigs born from supplemented mothers, showed a serial of meat quality features that made them less vulnerable to produce pale, soft and exudative (PSE) meats, due to their darker colour and higher ultimate pH at 24 hours post-mortem, compared to control pigs. In accordance to these findings, a lower number of type IIB fibres in the *longissimus thoracis* muscle were found when supplementing sows during mid-pregnancy. As it was reviewed in Chapter 1, selection for improved lean meat performance during the last decades in pigs and other livestock species may have resulted in deterioration of some meat quality traits (Cameron, 1990; Oksbjerg et al., 2000; Ramírez et al., 2003). This effect has been linked, amongst others, to a correlated response between selection for growth and lean performance and the increase of type IIB fibres proportion (Larzul et al., 1997). Due to their glycolytic metabolism and their lower number of capillary networks, type IIB fibres seem to be detrimental in pork quality, and particularly susceptible to PSE development (Lefaucheur et al., 2002 and 2003). As a whole, through changes in fibre type proportions, the feeding strategy carried out in the current study might help to improve meat quality and prevent PSE condition.

The PSE condition is one of the main concerns for the packing industry, not only in pig industry (Oliver et al., 2001; Guàrdia et al., 2004) but also in poultry (Sams, 1999). Many investigations have been carried out in an attempt to identify and characterize the main risk factors associated with the appearance of PSE meat, such as halothane sensitivity, stressful handling processes pre-slaughter (transport, lairage, and slaughter conditions) or even muscle fibre types. It is well known that pigs homozygous for the halothane sensitivity allele (nn) present a higher frequency of this effect than normal animals (Monin et al., 1998; Oliver et al., 2001). Breeding programs have eliminated “nn” genotypes from commercial farms in order to avoid malignant hyperthermia in response to a stress factor that will cause a high percentage of PSE pork, or

even death. However, although with high scientific controversy, halothane positive pigs (Nn) are of interest for the pig industry because they show advantages in carcass lean meat content compared to NN pigs (Oliver et al., 1993; Larzul et al., 1997). So, although in the negative form, halothane allele is still mantained on many commercial farms nowadays.

In addition, it has been described that PSE meat susceptibility vary among the different muscles according to their muscle fibre composition, and being the more glycolytic (higher number of type IIB fibres, i.e. *Longissimus; Semimembranosus muscles*) the most prone to PSE condition (Franck et al., 2007).

Throughout a more exhausted verification of these findings, the feeding practise applied in the present study might be used as an alternatively way of preventing PSE appearance in high incidence cases of these types of meats in the packing plants, at least in glycolytic muscles as is *longissimus thoracis*. Furthermore, in the halothane gene carrier animals (Nn), that represent the extreme conditions for developing PSE meats in the current pig industry, this strategy might also be of interest. Overall, the observed positive relationship between maternal feed allowance and fibre type composition could form a basis of fibre type manipulation to improve meat quality.

Mammalian muscles do not all develop at the same time, but in a cephalocaudal and proximodistal direction (Dwyer and Stickland, 1992). So, the effects of the increased feeding allowance proposed in the current study may not be the same depending on the composition in muscle fibres or the anatomical location. Thus, it is encouraged to further investigate whether this effect in reducing PSE susceptibility in pigs born from supplemented mothers during mid-pregnancy duplicates when other muscles with a more oxidative profile (i.e. *Triceps brachii muscle; Soleus muscle*) and in muscles formed in earlier and later developmental stages during pregnancy.

There is a high controversy in the literature regarding the effects of maternal supplementation during pregnancy on foetal muscle fibre development. As suggested by some authors (Rehfeldt et al., 2004b; Bee 2004), apart from the real effects *per se* that do not seem to be fully clear, some other factors implying methodology and sow factors might be largely implied on this variability among studies.

An important point to be considered in studies based on muscle fibre characteristics (counting and typing) is that analytical determination of muscle fibre number includes a series of methodological problems and, therefore, differences in the results obtained between works in the literature may depend, at least in part, on the fibre counting technique used (Rehfeldt et al., 2004b). Fibre counting from histological sections is a very tedious and time-consuming methodology but still it is the technique most widely used. Inaccuracies arise from the fact that in livestock species, such as sheep, cattle and pig, the impracticability of preparing and counting

entire cross sections of large muscles necessitates a sampling procedure based on subsampling techniques (Bee, 2004). The number of muscle fibres and their mean area are obtained from small samples and then extrapolated to the whole muscle cross section. In addition, there is no consensus in the literature about the number of muscle samples necessary or the minimum number of muscle fibres counted per sample that minimizes the level error when estimating these traits.

From the methodological approach performed in the present study (see Annex 1), it was derived that, under our experimental conditions, 5 samples per muscle (300 fibres per sample) in the *longissimus thoracis* muscle were necessary in order to obtain reliable estimations of muscle number, fibre and type. This study also revealed that total number of fibres could be estimated with high confidence using only 3 samples per muscle. More studies in this area are needed in other different muscles and also varying the number of muscle fibres counted per muscle. More sophisticated techniques involving immunohistochemistry, immunoblotting or molecular biology are currently being developed in some laboratories. It is clear that they will enhance the knowledge of fibre type molecular composition and classification. However, developmental studies into which muscle fibres need to be quantitatively and morphologically determined will probably still require histochemical methods.

Additionally to the counting methodology, studies on the effects of maternal nutrition on muscle fibre development *in utero* differ also in several other conditions namely sow parity, the magnitude of feed allowance during pregnancy, time and duration of the supplementation, composition of the gestation diets, genetics, and sow condition (Bee, 2004). The current experiment demonstrated that maternal factors such as sow age/parity may have important effects on productivity and pattern of energy utilisation during pregnancy (Chapter 5). Thus, we also suggest that “maternal factors” might be taken into account when determining the consequences of maternal nutrition on foetal development. The so-called “maternal factors” found in our study and its implications on foetal growth will be briefly discussed in the following section.

7.3 Implication of “maternal factors” on the prenatal programming effects

Pregnancy is a time when nutrient supply interacts with maternal factors such as size (maintenance), body condition (ability for recovery-mobilisation) and degree of maturity (age). These features may influence on the partition of nutrients between the uterus and maternal body tissue but also they may affect the growth and function of the placenta and, consequently, alter the growth response of the foetus to fluctuations in maternal nutrition (Robinson et al., 1999).

In the present study (Chapters 5 and 6), interactions between age/parity group and level of feed allowance during gestation have been reported. Primiparous sows are challenged during their first reproductive cycle since they need to cope with high prolificities and also keep growing accounting, at the same time, for smaller backfat reserves and then, opportunity to mobilise (Whittemore, 1996). This is especially evident in the modern large-maturing genotypes since they conceive at only 50 % (or less) of their mature size and, therefore, under a higher impulsion simultaneously to grow and reproduce (Whittemore, 1998). Additionally, primiparous sows present about a 20% lower capacity of feed intake during lactation (Young et al., 2004). Altogether, these conditions make them less able to achieve the desired (re)productive performances and make them more susceptible to early culling, compared to the more physiologically prepared and reproductively efficient multiparous sows.

In the current study, primiparous sows' body weight on day 40 of gestation represented a 66 % of their body weight as adults (more than 3 parities), in average. Also from our results a lower productive and reproductive performance for primiparous sows was demonstrated (Chapters 5 and 6). First parity sows showed lower litter and piglet weights at birth, and also on day 18 of lactation, compared to multiparous sows. Additionally, primiparous sows presented longer weaning to oestrus intervals compared to multiparous. So, also from our results it seems that because of their higher maternal requirements for growth, and their lower ability to cover them through body reserves mobilization and feed intake, primiparous sows are less efficient when using dietary energy for foetal growth and milk production.

Because sows are nutritionally compromised in their first parities, increasing feed allowance (dietary energy and nutrients) during pregnancy may then lead to different consequences depending on parity (Schoknecht et al., 1993). In the current work, increasing feed allowances during mid-pregnancy led to positive consequences on productivity (higher average piglet weight at birth) in primiparous sows after 3 cycles. However, no evident effects were found in productivity output of sows that started the experiment in cycle 1 at higher parities.

We hypothesized that this effect was probably due to an increase in body reserves levels, specially lean content, in this sows after three cycles of feed supplementation (see Chapter 5). Gilts need to achieve a target of around 35 kg of body protein in order to attain their maximum productive and reproductive efficiency (Clowes et al., 1994; Everts, 1994). Under this hypothesis, it was speculated that supplemented primiparous sows in the present study might have reached their desired target earlier than the control sows fed the routinely level provided in the farm during gestation.

Table 7.1 records the estimated body protein content from sows of different parity groups by cycles, using the equations proposed by Dourmad et al. (1997). From field data and also from experimental data, there is a general agreement that sows attain chemical maturity (target in

body protein) in their 3rd and 4th parity and that, at this time, they exhibit the highest productivity rates in their lifetime (Whittemore, 1993; Aherne et al., 1999). In the current experiment, mature sows (iPG2 in cycle 3 and iPG3 in cycle 1) showed 39 to 42 kg of body protein estimated through the equations of Dourmad et al. (1997). Interestingly, from table 7.1 it is deduced that supplemented sows from iPG1 were able to fully reach this target in body protein in cycle 3 (after 3 cycles of feed supplementation, 39.8 kg of body protein). However, control sows from iPG1 remained at a lower level of protein (36.2 kg) in this cycle. This finfing partly confirms our initial hypothesis on that increases in feed allowance during gestation may have accelerated the achievement of chemical maturity in young sows.

Table 7.1 Estimated body protein content (kg) at farrowing in cycles 1, 2 and 3 by initial parity group[1]

	Maternal treatment					
	Control			Supplemented		
	iPG1	**iPG2**	**iPG3**	**iPG1**	**iPG2**	**iPG3**
Cycle 1	28.6	36.9	41.9	28.7	36.6	41.9
Cycle 2	32.8	38.8	41.8	35.0	38.6	42.5
Cycle 3	36.2	39.4	44.0	39.8	40.3	45.2

iPG: initial parity group

[1] Initial parity group: parity group to which the sows belonged when they started the experiment in cycle 1 (Initial parity group 1 = parity 0, Initial parity group 2 = parity 1 and 2 and Initial parity group 3 = parity ≥ 3)

[2] Body protein estimated from the equations of Dourmad et al. (1997)

In adult multiparous sows growth is nearly completed. Consequently, the extra energy provided to these sows in the present study, was mainly devoted to body fat accretion, minimizing lean deposition from cycle to cycle. Table 7.2 shows the estimated lipid composition of sows from different parities and cycles in the present study. Under our experimental conditions, this particularity derived in detrimental effects on mammary gland development (higher incidence of mamitis-metritis-agalactia syndrome) in the supplemented group of multiparous sows.

Table 7.2 Estimated body lipid content (kg) at farrowing in cycles 1, 2 and 3 by initial parity group[1]

	Maternal treatment					
	Control			Supplemented		
	iPG1	**iPG2**	**iPG3**	**iPG1**	**iPG2**	**iPG3**
Cycle 1	34.3	52.2	56.5	36.7	50.0	56.1
Cycle 2	40.6	51.5	55.5	46.2	54.0	59.9
Cycle 3	47.7	53.8	62.3	57.0	60.7	67.7

iPG: initial parity group

[1] Initial parity group: parity group to which the sows belonged when they started the experiment in cycle 1 (Initial parity group 1 = parity 0, Initial parity group 2 = parity 1 and 2 and Initial parity group 3 = parity ≥ 3)

[2] Body lipid estimated from the equations of Dourmad et al. (1997)

Overall, as primiparous sows are higher nutritionally compromised, their progeny might be expected to be more vulnerable than progeny of multiparous dams to maternal feeding restriction, given the consequences of foetal-maternal competition for nutrients. In human adolescent pregnancy, growth is continuing in the mother (Scholl and Hediger, 1993) and maternal growth is also associated with decreased infant birth weight. Thus, when performing nutritional restriction but also supplementation studies, parity is an important factor that may add variability to the results obtained and then must be taken into account.

7.4 Practical sows feeding management and global strategy

There are a number of priority areas that must be given attention if we are to optimise output from the breeding sow. These include making certain the gilt is properly prepared prior to first mating, maximising milk production, optimising health status of the sow and litter and reducing the number of days in which the sow is non-productive. All of these are influenced by the feeding program and types of diets that sows are fed, and ultimately by attention to detail by management.

Feeding programs in order to maintain sow body condition from cycle to cycle and maximize sow (re)productive performance should be carefully designed based on sow requirements, but also in the reported interactions between different phases within the reproductive cycle (see Chapter 6).

The information obtained in terms of sow body reserves levels at different times within the reproductive cycle and about their interactions in chapters 5 and 6, as well as additional information from recent data in the literature, will be used in the present section in order to make suggestions about possible adaptations of the feeding program typically recommended for gestating sows on most farms. Our recommendations will be focused on gestation since it is the period studied in the present investigation, and suggested body condition levels from our results will be given on day 40 of gestation and at farrowing.

Figure 7.1A shows the standard feeding program during gestation and lactation for primiparous and multiparous and figure 7.1B illustrates the suggested adaptations to the standard feeding program, according to our results. Also, the mean body condition score notes, backfat thickness and loin depth levels recommended at different times within the reproductive cycle are provided in the graphs. In figure 7.1A, optimum body reserves levels represent a compilation of recent studies in the literature (Close and Cole, 2003, Marco, 2004, Young et al., 2004; Marco and Barceló, 2006; PIC, 2007, among others). The actual feeding levels will depend on the energy content of the diet (that is set on 12.1-12.6 MJ ME/kg in this case).

The important aspects of the typically suggested feeding pattern on most farms (Figure 7.1A) are that feeding level is reduced immediately after mating for a period of 15 to 20 days (about 1.9 to 2.4 kg feed/day) to enhance embryo survival, maintained according to condition during mid-gestation (2.3-2.8 kg feed/day) and increased about 0.5-1.0 kg feed/day in the last trimester of gestation when most foetal growth occurs. Then, it is increased gradually but to as high a level as possible during lactation.

In figure 7.1B we suggest reducing feeding level after mating but only in primiparous sows and in overconditioned multiparous sows, and only during the first 72 hours post mating. This suggestion is based on that clear evidence to this effect in the literature are generally restricted to primiparous sows, and that the most critical period of time seems to be the first three days post-mating (Jindal et al., 1996). Also, it seems that lower feeding levels during the preceding lactation may have higher consequences on embryo mortality than feeding plane during the first period of gestation (Kirkwood et al., 1990). Thus, thin sows at mating will need to recover body reserves lost during lactation as soon as possible, making use of the pregnancy anabolism.

The practise of increasing feeding level from day 90-95 of gestation to farrowing is generally directed to increase or maintain piglet birth weight on most farms. However, literature does not report clear effects on this. It seems that this practise is efficient in cases of high incidences of low birth weights (Aherne and Kirkwood, 1985). Recent research suggest that, in fact, this practise is more beneficial to maintain sow body reserves than to increase piglet weights at birth, at least in short term and in well-managed sows (Millller et al., 2000). For this reason, we recommend to consider this strategy exclusively for sows with low body condition in late gestation (<17 mm BF), in order to avoid becoming catabolic before lactation (see Figure 7.1B). However, increases of about 200 g/day of feed are still advised in figure 7.1B in order to cope with the calculated higher requirements of primiparous (120 kg BW at mating) and multiparous sows (250 kg BW at mating) during this period of gestation.

In the current investigation an increase in mean piglet birth weight was observed in primiparous sows after receiving additional feed allowance during mid-gestation over three consecutive cycles. A suggestion has been made regarding the implication of maternal body lean reserves and the earlier achievement of the chemical maturity of young sows on this effect (see Chapter 5 and Section 7.3 of the present Chapter). So, the possibility of moving the widely recommended increase of feeding levels during late pregnancy (about 0.5-1 kg of extra feed) to mid-pregnancy, in order to achieve higher piglet birth weights in primiparous sows in a long term period, is then given as "*optional"* in the suggested feeding graph (Figure 7.1B). This change in the feeding strategy may always assume that feeding level in late pregnancy fully covers the established requirements at this time.

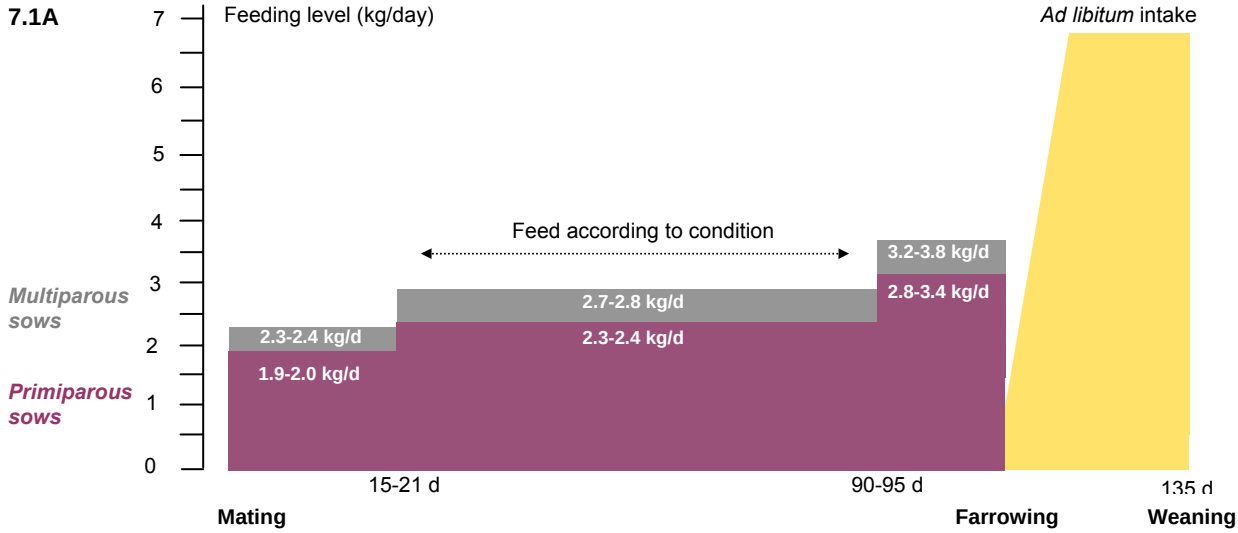
In chapter 5, the retrospective analysis performed in terms of body condition from the collective of sows that completed the three cycles showed that, under our experimental conditions, primiparous sows rigorously required a minimum of 16-17 mm of backfat thickness on day 40 of gestation in order to complete three consecutive cycles within the herd (Figure 7.1B). This minimum did not vary when an extra feed supplementation was given during mid-pregnancy. When extrapolated to the time of mating these levels are very similar to those recommended in the literature (≥ 15 mm, Figure 7.1A).

In multiparous, the retrospective study suggested a minimum of 19 mm of backfat required on day 40 of gestation in order to stay three consecutive cycles within the herd, when following the routine feeding management for gestation in this farm. Moreover, our study deals with the possibility of recovering multiparous sows with less than 15 mm of backfat on day 40 of gestation, by giving extra feed supplementation during mid-pregnancy. This demonstrates the capacity of multiparous sows to recover body reserves during gestation, which seems less easy in primiparous sows. It was suggested that feed supplementation prevented these sows from being culled due to return to oestrus after mating (see Chapter 5). But, attention must be paid to those multiparous sows in good-high condition at mating (>20-21 mm of BF, approximately), since they also failed to complete the three consecutive cycles under the feed supplementation regime applied in gestation. From our results, it is deduced that they have increased risks of suffering MMA syndrome during lactation when increasing feeding level during mid-pregnancy. In the present work, it has been speculated that, as feeding supplementation covered the period of mammary gland development (day 75 to 90; Kensinger et al., 1982), this may have lead to a substitution of the mammary cells by fat as supported by Weldon et al. (1991). In short, we recommend giving extra feed supplementation at around 0.5-1 kg above requirements to those multiparous sows with ≤ 15 mm of backfat thickness on day 40 of gestation but, moving the period of supplementation to earlier stages (i.e. 35-40 to 70 days instead of 45 to 85 days of gestation), in order to avoid the period of mammary gland development. The rest of the sows (16 to 19 mm or > 20 mm of backfat on day 40 of gestation) might be fed according to their condition and requirements, in order to reach 17 to 20 mm of backfat levels at farrowing.

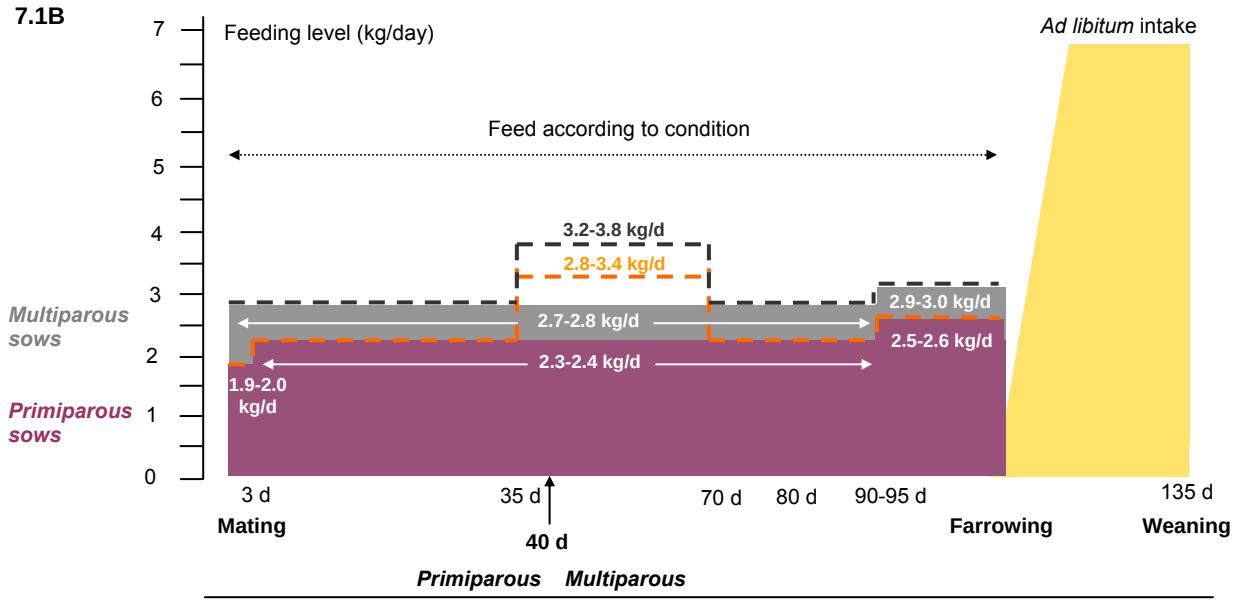
Estimated backfat target levels at farrowing from our results (Chapter 6) are in line with those reported in the literature (Close and Cole, 2003; Marco, 2004; Young et al., 2004; PIC, 2007). Under our experimental conditions, the maximum level of backfat thickness allowed at farrowing in order not to exceed 4 mm of backfat losses during the subsequent lactation suggested by De Rensis et al. (2005), is settled at around 20-21 mm of backfat. Additionally, it was estimated that 17 mm of backfat thickness at farrowing was the minimum needed in order to not fall above the 14 mm of backfat at weaning; these 14 mm of backfat at weaning are considered essential in terms of (re)productive efficiency (Tummaruk et al., 2001; Marco, 2004; Marco and Barceló, 2006).

In terms of loin depth, suggested levels at different times are scarce in the literature. From our results (see Chapter 5), levels of 56 to 57 mm on day 40 of gestation may be recommended in first parity and multiparous sows in order to assure a minimum stayability of three complete cycles within the herd. Our results also support the fact that setting optimum levels of loin depth is important in first parities (0 to 3 parities). If these levels are not achieved, feed supplementation might be a tool to increase protein accretion during gestation, thus permitting sows with lower lean reserves at mating to recover and express their maximum productive potential (see Chapter 4 and Section 7.3). Therefore, in figure 7.1B, we also suggest the "*optional"* possibility of supplementing primiparous sows during mid-pregnancy when their levels of loin depth at mating are lower than the recommended. Also, from our results it is advised that levels at around 60-65 mm of loin depth at farrowing lead to moderate loin depth losses during lactation without compromising reproductive performance (see Figure 6.2B in Chapter 6). Moreover, levels of 55 mm of loin depth at farrowing are the minimum required in order to assure zero mobilization during lactation and therefore, maintain loin depth levels at weaning.

Finally, it must be warned that the suggested feeding pattern and also suggested body reserves levels in this chapter should be taken as merely recommended. To generalize among different herds is not easy and sometimes hazardous since, as it was stated in Chapter 1, differences among genetics, ultrasonic devices used and handling procedures can be sometimes more relevant on body reserves than differences in the feeding pattern *per se*.



BW, kg (prim./multip.)	120/250		
BCS	3	3.5	3
BF, mm	>15	18-20	14-16
LD, mm (parity 1)	56	60	56



BW, kg (prim./multip.)	120/250				
BCS		3.5	3.5	3.5	3
BF, mm		16-17	19	17-20	14-16
LD, mm		56-57	56-57	60-65 (55 min)	55 min

- – – Optional for primiparous sows during their three first parities, in order to optimize piglet weight at birth in a long term or when loin depth level is lower than 55 mm at first mating
- – – Multiparous sows with low body reserves on day 40 of gestation (≤15 mm of BF)

Figure 7.1 Sow feed intake pattern during gestation suggested according to different sources (Close and Cole, 2003; Marco, 2004; Young et al., 2004, 7.1A). Sow feed intake pattern during gestation suggested under our experimental conditions (7.1B). Body condition score (BCS), ultrasonic backfat (BF, P2) and ultrasonic loin depth (LD, P2) levels are suggested. Estimated feed energy content of 12.1 -12.6 MJ ME/kg.

Chapter 8

Conclusions

Conclusions

The results presented in this PhD dissertation allow us to conclude that, under our experimental conditions, an extra feed allowance of + 50 % and + 75 % to primiparous and multiparous sows, respectively, during mid-gestation (from day 45 to 85 of gestation):

Regarding the progeny,

1. Does not lead to consistent differences on postnatal growth performance neither in lactation nor in nursery and growing-finishing periods, between treatment groups.

2. Results in differences in meat quality traits at slaughter. Pigs born from supplemented mothers showed less acid (higher pH at 24 hours postmortem) and darker (lower lightness at 24 hours postmortem) meats.

3. Brings about changes in muscle fibre morphological characteristics. Contrary to what we expected, feed supplementation during mid-pregnancy resulted in a lower number of muscle fibres with larger mean areas in the *longissimus thoracis* muscle compared to control pigs. This diminution in the number of muscle fibres in the pigs born from supplemented sows proceeded with invariable estimated embryonic secondary to primary ratio.

4. Leads to a decrease in the number and percentage of fast glycolytic muscle fibres (Type IIB) in the *longissimus thoracis* muscle, consistently with the lower total number of fibres and the effects found in meat quality characteristics. Overall, the number of type IIB fibres resulted positively correlated with meat lightness (L*; r = 0.32, $P < 0.05$) and negatively correlated with pH after 24 hours postmortem (r = - 0.30, $P < 0.05$) in the *longissimus thoracis* muscle.

Regarding sows,

5. When applied during three consecutive cycles, this feeding strategy allowed sows to accumulate backfat thickness at the end of the three cycles, whereas restricted feeding did not. In terms of body lean and lipid reserves, increasing feeding allowance promotes lean and fat accretion in primiparous sows, and lipid accretion in multiparous sows after three cycles of feed supplementation.

6. Results in higher litter and piglet weights at birth in gilts after three cycles of feed supplementation (+ 3.3 kg/litter and + 0.300 kg/piglet in cycle 3, $P < 0.05$), probably due

to the enhanced lean body content when supplementation starts early in life. But, no such improvement in productivity is found in fully mature animals.

7. In multiparous sows in good-high condition on day 45 of gestation, this feeding strategy shows detrimental effects on lactation performance, increasing the risk of suffering from mamitis-metritis-agalactia syndrome.

Also, from the exploratory analysis of the associations between sow body condition at different times within the reproductive cycle and productive-reproductive efficiency, and the study of the accuracy of body condition score (BCS) in predicting body reserves, the following conclusions are drawn:

8. An interaction between sow body reserves during gestation and their subsequent mobilisation during lactation is evident. Sow backfat, but also loin depth levels at farrowing are positively correlated with their subsequent losses during lactation. But, higher sow backfat and loin depth levels at farrowing also guarantee higher levels at weaning.

9. Under our experimental conditions, sow parity influences piglet weight at farrowing and at weaning (day 18 of lactation), more than sow body reserves at farrowing. In this way, first parity sows show lighter pigs at farrowing and at weaning, but also greater weaning to oestrus intervals.

10. Sow backfat losses during gestation are positively correlated with litter growth rates during lactation, and negatively correlated with weaning to oestrus interval. Therefore, lactation performance and reproductive success measured in terms of weaning to oestrus interval depend less on the achievement of a recommended backfat depth at weaning, than on the maintenance of body condition during lactation.

11. Body condition score alone is not an accurate method to predict sow backfat reserves and on which to base a feeding program, at least in gilts and sows with medium backfat levels (16-21 mm). The body condition scoring method is subjected to sow body weight and loin depth levels variation, additionally to backfat levels.

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Annex 1

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How many muscle samples are required to obtain reliable estimations of muscle fibre characteristics from pig *longissimus* muscle?

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Abstract

In order to investigate the reliability of muscle fibre trait estimations of pig *longissimus* muscle and to derive the minimum number of samples required per muscle cross-section and animal, intraclass correlation coefficients (ICC, $\hat{\vartheta}$) were obtained by one-way analysis of variance. From each of 23 market weight pigs five samples, evenly distributed over the muscle cross-sectional area at the 12th/13th rib level, were taken and analyzed for various muscle fibre traits. The number of samples required per muscle cross-section was found to be different between selected fibre traits, ranging from a minimum of three (for number of muscle fibres) to a maximum of five or more (for mean fibre area, fibre type composition and relative area occupied by each fibre type). These findings should be taken as a recommendation, but their usefulness will depend upon the goal and conditions of future experiments.

Keywords: Muscle fibre; *Longissimus* muscle; Pig; Method; Intraclass correlation coefficient

1. Introduction

Methodologically, muscle fibre counting and typing is a very tedious and time consuming task that has a series of technical problems (Rehfeldt, Fiedler, & Stickland, 2004). In addition, some subjectivity of measurements has to be taken into account, in particular regarding fibre type classification (Henckel, Ducro, Oksbjerg, & Hassing, 1998). Fibre counting from histological cross-sections is still the technique most widely used (Fiedler & Branscheid, 1998). In livestock species, such as sheep, cattle and pig, the impracticability of preparing and counting entire cross-sections of large muscles necessitates a sampling procedure based on subsampling techniques. Thus, the myofibre trait estimations will be subjected to a high level of error (Bee, 2004). The number of fibres and their mean area obtained from small samples are then extrapolated to the whole muscle cross-section, but their reproducible and accurate measurement remains a challenge.

Both fibre type proportions and fibre cross-sectional area may vary considerably within mammalian skeletal muscles (Oksbjerg, Gondret, & Vestergaard, 2004). Although muscles generally contain a mixture of myofibres, we can distinguish fast and slow or glycolytic and oxidative muscles based on the dominating fibre type within the muscles. The so-called mixed muscles mostly exhibit higher proportions of oxidative or type I fibres in their deep, near-bone parts compared with their superficial parts as shown for the *semitendinosus* and *longissimus* muscles (Fiedler, Ender, Wicke, & Lengerken, 1998; Lefaucheur, Edom, Ecolan, & Butler-Browne, 1995). Also differences along the longitudinal extension have been reported for pig (Fiedler et al., 1998) and rabbit (Vigneron, Bacou, & Ashmore, 1976) *longissimus* muscle. Consequently,

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within-muscle variations have to be considered in muscle fibre analyses.

There are only very few reports in the literature providing information about the minimum number of fibres that should be measured in order to minimize the error in the estimation of muscle fibre traits (for young pigs: White, Cattaneo, & Dauncey, 2000; for lambs: Greenwood, Hunt, Hermanson, & Bell, 2000). Regarding adult pigs, there is no agreement about how many samples per animal should be taken, which ranges from one (Bee, 2004; Dwyer, Stickland, & Fletcher, 1994; Gondret et al., 2005; White et al., 2000) to a maximum of five (Nissen, Danielsen, Jorgensen, & Oksbjerg, 2003; Pedersen et al., 2001). Likewise, there is no consensus about the optimal sampling locations (midbelly of the muscle or evenly distributed) or the number of fibres that should be counted per sample, which varies from 200 to 300 (Gondret et al., 2005; Nissen et al., 2003; Pedersen et al., 2001) over 500 (Fiedler et al., 2005; Gentry, McGlone, Miller, & Blanton, 2004; White et al., 2000) up to 1000 (Lefaucheur, Ecolan, Barzic, Marion, & Le Dividich, 2003). Some authors use different numbers of fibres per sample depending on the trait studied (Gentry et al., 2004; Gondret et al., 2005; Lefaucheur et al., 2003).

The aim of this study was to determine the repeatability for different muscle fibre characteristics within a defined cross-section of the pig *longissimus* muscle, in order to derive the minimum number of samples per animal needed and, thereby, to minimize the level of error in the estimations of these traits.

2. Material and methods

2.1. Sampling procedure

This experiment received approval from the Animal Protocol Review Committee of the Universitat Autònoma de Barcelona. Twenty-three (Landrace × Large White) × Duroc pigs were slaughtered at an average weight of 104 ± 1 kg. After slaughter, the thoracic part of the *longissimus* muscle (*M. longissimus thoracis*) was removed from the carcass and the section between the last rib and the interface between 12th and 13th ribs was sliced and kept at 4 °C for 24 h. After that, five cube-shaped (1 cm^3 approx.) muscle samples were taken evenly distributed over the cross-sectional area of the muscle. The number of five samples per pig was chosen, as this is the highest number of samples analyzed per animal, which has been reported in the literature. The cubes were cut parallel to the longitudinal myofibre axis, placed on a piece of cork, embedded in OCT embedding medium (Tissue-Tek®, Zoeterwoude, Netherlands) and talcum powder, snap frozen in liquid nitrogen and stored in a −80 °C ultracold freezer until further processing for histochemical analysis. The adjacent cranial slice in the *longissimus thoracis* (until interface 11th–12th ribs) was frozen immediately postmortem and defrosted after three days in order to be photographed and to measure the muscle cross-sectional area. For that purpose, a computer-assisted image analysis system (MIP 4, Digital Image System, S.A., Barcelona, Spain) was used.

2.2. Histochemistry and microscopy

Transverse serial sections (10 μm) were cut in a cryostat (Leica CM 1900, Nussloch, Germany) at −15 °C, placed on three silane-treated microscope slides and allowed to thaw and dry at room temperature for 1–2 h. The sections were then stained for myosin adenosine triphosphatase (m-ATPase) activity after alkaline (pH 10.3) and acid (pH 4.40 and pH 4.45) preincubation, using a modification of the method described by Latorre et al. (1993). Sections stained for m-ATPase reaction after alkaline preincubation served to determine the number of muscle fibres/mm^2 and the areas of fibre types I, IIA and IIB using the computer-assisted image analysis system MIP 4. Results were validated using the acid-preincubated sections. In a low number of samples odd fibres appeared, which were intermediate between IIA and IIB fibres; these were categorized as IIB fibres. All measurements were made by the same person in order to reduce subjective variability to a minimum.

For each sample, measurements were made on at least 300 fibres (that means at least 1500 fibres per animal). The number of 300 fibres per sample was chosen because it is one of the most commonly used number in the literature. Then, these data were used to calculate the percentage of fibres type I, IIA and IIB, the mean area of each fibre type and the relative area occupied by each fibre type (%), the latter was calculated from the sums of the individual fibre areas of each type. Also, the total number of fibres was estimated as (total number of fibres counted * muscle cross-sectional area)/the area occupied by the fibres counted.

2.3. Statistical analyses

The recorded data was subjected to summary statistics for obtaining means and standard errors (SE). In order to evaluate the intra-animal variability, the coefficient of variation (CV) within the animal was calculated for each trait. To evaluate the repeatability of the measures within the animal, intraclass correlation coefficients (ICC) were obtained from one-way analysis of variance according to Rasch (1983). The ICC ($\hat{\vartheta}$) was calculated as follows:

$$\hat{\vartheta} = \frac{\hat{\sigma}_a^2}{\hat{\sigma}_a^2 + \hat{\sigma}^2} \quad (1)$$

where $\hat{\sigma}_a^2$ and $\hat{\sigma}^2$ are the variance between animals and the variance within the animal, respectively. The variance components ($\hat{\sigma}_a^2$ and $\hat{\sigma}^2$) were calculated from a one-way *ANOVA* table that was obtained using the GLM procedure of SAS (SAS System® Software Release 9.1; SAS Institute Inc., Cary, NC 27513, USA) with animal as the main factor.

From the ANOVA table we obtained: $MS_{ANIMAL} = \hat{\sigma}^2 + n\hat{\sigma}^2_a$; $MS_{RES} = \hat{\sigma}^2$, where MS_{ANIMAL} is the mean square of the pig effect, MS_{RES} is the mean square of the residual effect and n is the number of samples analyzed per animal and muscle ($n = 5$). The resulting ICC shows the repeatability of measurements in the case that only one sample is analyzed. The ICC $\hat{\vartheta}_a$ was then estimated in the case that more than one sample per animal ($n = 2$–5) is included in the analyses:

$$\hat{\vartheta}_a = \frac{\hat{\sigma}^2_a}{\hat{\sigma}^2_a + \frac{\hat{\sigma}^2}{n}} \quad (2)$$

3. Results and discussion

Mean (±SE) and mean intra-animal coefficients of variation (CV ± SE_{CV}) for different *longissimus* muscle fibre traits based on five samples from each of the 23 pigs are presented in Table 1. The results reflect the typical fast twitch glycolytic muscle fibre distribution of the *longissimus* muscle (e.g. Lefaucheur et al., 2003) with the type IIB fibres as the major fibre type compared with the type I and IIA fibres. Similarly to other studies, in the *longissimus* muscle from adult pigs (Henckel et al., 1998; Lefaucheur et al., 1991), the mean fibre area increased in the order type IIA, type I and type IIB. However, some studies revealed that type I fibres were smaller (Rehfeldt, Fiedler, & Wegner, 1987) or almost equal (Fiedler et al., 2001; Rehfeldt & Ender, 1993) in size to type IIA fibres. This inconsistency may result from breed differences or other factors (e.g. Fiedler et al., 2001).

Table 1
Means and mean intra-animal coefficients of variation (CV) of *longissimus* muscle fibre traits studied in 23 (Landrace × Large White) × Duroc pigs[a]

Muscle fibre traits	Mean ± SE[b]	Mean CV (%) ± SE_{CV}[c]
Number of animals (n)	23	23
Number of muscle fibres (no./mm²)		
Total	286.1 ± 9.25	11.3 ± 0.89
Type I	23.8 ± 1.40	26.2 ± 2.09
Type IIA	17.3 ± 1.59	38.8 ± 2.86
Type IIB	245.0 ± 8.11	12.3 ± 0.91
Fibre type composition (%)		
Type I	8.4 ± 0.44	25.8 ± 2.18
Type IIA	6.0 ± 0.48	37.4 ± 2.85
Type IIB	85.6 ± 0.61	3.1 ± 0.27
Estimated total number of muscle fibres (×10³)[d]		
Total	1989.1 ± 73.8	11.3 ± 0.89
Type I	165.4 ± 9.6	26.2 ± 2.09
Type IIA	122.5 ± 11.9	38.8 ± 2.86
Type IIB	1701.2 ± 62.4	12.3 ± 0.91
Mean fibre area (μm²)		
Total	3588.4 ± 105.1	11.3 ± 0.96
Type I	3418.2 ± 103.4	12.0 ± 1.05
Type IIA	1827.9 ± 71.0	21.2 ± 2.20
Type IIB	3736.1 ± 115.3	12.2 ± 0.95
Relative area occupied (%)[e]		
Type I	7.9 ± 0.36	23.8 ± 1.93
Type IIA	3.2 ± 0.29	42.4 ± 3.04
Type IIB	88.9 ± 0.45	2.4 ± 0.20

[a] Five samples from the 12th/13th rib level cross-section of the *longissimus thoracis* muscle were analyzed per animal.
[b] Mean ± standard error (SE) for each trait was obtained averaging the means of the 23 animals.
[c] CV: intra-animal coefficient of variation ± standard error (SE_{CV}) was calculated for the five samples of each animal and averaging the values of the 23 animals.
[d] Estimated total number of muscle fibres was calculated as (total number of fibres counted × muscle cross-sectional area)/the area occupied by the fibres counted.
[e] Relative area occupied was calculated as the sum of the individual fibre areas of each type divided by the total area counted (sum of the areas of all fibres counted) multiplied by 100.

From the CV results, it becomes clearly apparent that type IIA fibres exhibit the highest intra-animal variability for all muscle fibre traits studied, ranging from 21% to 42%. Also Henckel et al. (1998) reported that type IIA fibres had the highest variability in relative distribution and relative area occupied, when different histochemical methods were combined and compared. Type I fibre traits, showed CV values from 12% to 26% and type IIB fibres exhibited the lowest CV values, ranging from 2% to 12%. This high inconsistency for IIA fibres may result from their relatively low number and size and from their intermediate character between type I and IIB (Brooke & Kaiser, 1970), which makes classification sometimes difficult. However, fibre type classification of ATPase-stained sections has been found to be less subjective than that of oxidative or combined stainings (Henckel et al., 1998). It must be emphasized that the variance within the animal mainly results from the biological variability within the muscle cross-section and less from an insufficient reliability and repeatability of the measurements themselves. Regional differences within mixed muscle cross-sections have been found to be much higher than the differences between repeated measurements (Fiedler et al., 1998; Rehfeldt, 1981).

From the data obtained ($n = 23$ pigs, five samples/pig), the ICC was calculated ($\hat{\vartheta}$). From this basic value we further estimated the ICC values in the cases of increased number of samples ($\hat{\vartheta}_2$ to $\hat{\vartheta}_5$; Table 2). The ICC measures the correlation between observations within a group and represents the percentage of the overall variance due to differences between the animals for each muscle fibre trait. Thus, the difference from 1.0 represents the proportion of the variance, which is mainly caused by differences among the samples within the muscle cross-section. We consider ICCs $\geqslant 0.8$ as sufficiently high. According to Bogduk et al. (cited by Will, 2004) ICCs of 0.8–0.99 reflect an excellent conformity of measurements within biological subjects (good conformity for 0.6–0.79) and thus, assure an acceptable repeatability and precision of the estimations. Consequently, for our experimental conditions the analysis of one or two samples (300 muscle fibres each) is not sufficient to give a reliable estimation for the muscle fibre traits of the whole *longissimus* muscle cross-sectional area at the

Table 2
Intraclass correlation coefficients (ICC, $\tilde{\vartheta}$ and $\tilde{\vartheta}_n$) for various pig *longissimus* muscle fibre traits in dependence of the number (n) of samples per muscle cross-sectional area at 12th/13th rib level

Muscle fibre traits[a]	$\tilde{\vartheta}$	$\tilde{\vartheta}_n$			
		$\tilde{\vartheta}_2$	$\tilde{\vartheta}_3$	$\tilde{\vartheta}_4$	$\tilde{\vartheta}_5$
Number of muscle fibres (no./mm²)					
Total	0.57	0.72	0.80	0.84	0.87
Type I	0.42	0.59	0.69	0.74	0.78
Type IIA	0.49	0.66	0.74	0.80	0.83
Type IIB	0.53	0.69	0.77	0.82	0.85
Fibre type composition (%)					
Type I	0.38	0.55	0.65	0.71	0.75
Type IIA	0.44	0.61	0.70	0.76	0.80
Type IIB	0.46	0.63	0.72	0.78	0.81
Estimated total number of muscle fibres					
Total	0.65	0.78	0.85	0.88	0.90
Type I	0.44	0.61	0.70	0.76	0.80
Type IIA	0.50	0.67	0.75	0.80	0.83
Type IIB	0.60	0.75	0.82	0.86	0.88
Mean fibre area (μm²)					
Total	0.52	0.68	0.76	0.81	0.84
Type I	0.49	0.66	0.74	0.79	0.83
Type IIA	0.34	0.51	0.61	0.67	0.72
Type IIB	0.51	0.68	0.76	0.81	0.84
Relative area occupied (%)					
Type I	0.31	0.48	0.58	0.64	0.69
Type IIA	0.47	0.64	0.73	0.78	0.82
Type IIB	0.41	0.58	0.67	0.73	0.78

The standard deviations of $\tilde{\vartheta}$ and $\tilde{\vartheta}_n$ range from 0.04 to 0.10.
[a] Explanation of traits: see Table 1.

selected 12/13th rib level ($\tilde{\vartheta} < 0.8$, see Table 2). The highest ICCs were obtained for the total number of muscle fibres (number of muscle fibres/mm^2 and estimated total number of fibres), and three samples are requested to obtain reasonable estimations. Overall, the use of five samples per animal results in ICC values near to 0.8 in most of the muscle fibre traits studied.

In fact, only three traits (the percentage of type I fibres, the mean area of type IIA fibres and the relative area occupied by type I fibres) showed ICC values lower than 0.8 at the level of five samples ($\tilde{\vartheta}_5 = 0.75, 0.72$ and 0.69, respectively). Therefore, in these three cases, even more than five samples should be used to obtain reliable results. When ICC is calculated for more than five samples (Eq. (2)), we obtained that seven samples in the case of type I fibre percentages, nine samples for the type I fibre relative area and eight samples for the type IIA fibre mean area are required to obtain an ICC of approximately 0.8.

The differences in ICCs obtained between the various muscle fibre traits, could justify the use of different numbers of samples per animal depending on the muscle fibre trait studied. Thus, in contrast to the techniques used in other studies (e.g. Gentry et al., 2004; Gondret et al., 2005), our results suggest that the determination of fibre type composition requires higher numbers of samples and/or fibres per animal than the determination of mean fibre areas.

Our results aimed to characterize muscle fibre traits of the entire *longissimus* muscle cross-section at the 12th–13th rib, since the five samples analyzed in this study were taken evenly distributed over the cross-sectional area. But, alternatively, other methods of estimation seem possible. Intraclass correlation coefficients of about 0.9 have been estimated for most of the muscle fibre traits in whole *rectus femoris* muscle sections from laboratory mice, when two sequential sections from the same sample, taken at the same location have been analyzed (Rehfeldt, 1981). Also, when two or three adjacent samples (cubes) from the superficial part of the pig *longissimus* muscle (taken by biopsy on the live animal) have been compared, almost identical fibre type distributions were measured, but the repeatability for fibre size and fibre number per unit area was lower ($\tilde{\vartheta} = 0.6$–0.7) (Fiedler et al., 1998; Schoppmeyer, 2003). Therefore, as an alternative, only one or two samples could be taken at a precisely defined point of the muscle, for example in the superficial position in the case of *longissimus* muscle biopsy. However, due to the reported differences that exist among the fibre type composition or even fibre sizes in their near-bone, deep sites compared with their superficial parts (Fiedler et al., 1998; Lefaucheur et al., 1995) these results would not be representative for the whole muscle cross-section. On the other hand, only slaughtered, but not live animals can be subjected to detailed analyses of whole muscles.

In conclusion, the results obtained suggest that, when samples are taken evenly distributed in the pig *longissimus thoracis* muscle cross-sectional area, in general, five samples per animal and muscle are recommended to obtain acceptable estimations of muscle fibre characteristics. However, the number of samples per animal required can be lower (3–4) or higher (>5) for selected muscle fibre traits. Thus, these results can help to adjust the number of samples used to obtain representative and more reliable estimations of different muscle fibre traits.

Restrictively, our results refer to the specific pig cross, the weight class and the anatomical site and level of *longissimus* muscle used in this study. Problems with the repeatability of the estimation of muscle fibre traits will analogously be apparent for different experimental conditions (species, age, muscle, etc.). Another important conclusion is that these problems are not restricted to the measurements of histological muscle fibre traits. It is well recognized that skeletal muscle fibre types differ not only in structural but also in a lot of functional and biochemical properties (e.g. Lefaucheur et al., 1995; Picard, Lefaucheur, Berri, & Duclos, 2002). Thus, they exhibit different amounts of substrates and metabolites (e.g. glycogen, lipid), enzyme activities (e.g. enzymes of the glycolytic and oxidative metabolism) and structural proteins (e.g. myosin heavy chains). Consequently, sampling procedures that are aimed at various kinds of functional (e.g. enzyme activities) and expression (e.g. mRNA, proteins) analyses of skeletal muscle likewise require studies on the repeatability of measurements. Thus, the results obtained from this

study can serve as a suggestion for future research on skeletal muscle tissue, but similar approaches should be carried out in each case in dependence of the specific goal and conditions of each experiment.

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