UNIVERSITAT POLITÈCNICA DE VALÈNCIA

REARING PROGRAMMES FOR BREEDING RABBITS. EFFECT OF EARLY DEVELOPMENT AND FEEDING ON FUTURE REPRODUCTIVE PERFORMANCE OF RABBIT FEMALES AND MALES.

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"A day without laughter is a day wasted"

Charles Chaplin

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Abstract

Nowadays, the greatest consumer demand on new production standards, that are also being applied in rabbit production, could be reducing farmers profitability. This is the reason that farmers main concern is to maximize the productivity of their reproductive animals, to remain sustainable their activity. In order to contribute to the rabbit farming sustainability, it should be crucial to have breeders, at the beginning of their reproductive life, with an adequate development to be able to express all their reproductive potential. For this reason, any improvement on the knowledge of both nutrition and management of the future rabbit breeders should be considered as crucial. However, main research efforts have been focused on animals during reproduction phase. The initial hypothesis of this thesis was that achieving an adequate early development during young rabbits' gestation, lactation, fattening and rearing, with the help of fitted feeding programmes in some of these periods, would be improved reproductive performance and lifespan of the future breeding rabbits. To evaluate this hypothesis, two types of trials, both in rabbit females from maternal lines selected for litter size at weaning and males from paternal lines selected for growth rate and intended on artificial insemination, were carried out. The first of these trials were mainly focused on the different growth patterns, from birth to the end of the rearing age, and their influence on rabbit's reproductive career and lifespan. In a second type of trials, the effect of the use of different diets or feeding programs during the rearing period in the performance in the short- medium-term was evaluated. The results obtained from the evaluation of the different growth patterns trials seem to indicate that, we could differentiate two parts on the early development according to their effects on reproductive performance and lifespan. To show a greater body development from birth to 63 days of life seems to have a positive influence on the maturity degree and productivity at the beginning of their reproductive life, as well as being able to improve their survival in the short-term, but it could be even negative for long-term lifespan. However, the development during the rearing period (from 63 days of life to the beginning of reproduction career) seems to have a higher impact on the productivity and lifespan of our rabbit breeders. Fatter rabbit females at first insemination had smaller litter sizes (born and weaned) and a higher risk of being culled (+13% per positive change in mm of perirenal fat) than lean ones ($P<0.05$). Also, rabbit males that showed a greater average daily gain during rearing period had a lower number and percentage of profitable ejaculates through it productive life (–10.1 and –4.9% per each 10 g of daily gain increase, respectively; $P<0.05$) and higher risk of death or culling (+16.2% per each increase in one standard deviation unit on live weight; $P<0.05$). These results could indicate that the key to carrying out an adequate development during the rearing period should allow the rabbits to reach an appropriate degree of physiological and reproductive

maturity to face the future productive challenges, avoiding an excessive fatness at the beginning of their productive life. The use of a feeding restriction in rabbit females, and especially the use of fibrous diets (from 8.5 to 10 MJ DE/ kg DM) lead a gradual and adequate body development at mating, with enough body reserves to face the beginning of reproduction life and improving their whole reproductive performance and lifespan. The success of these rearing feeding programs depends in the moment and duration of application of them and improved with the application of a nutritional flushing around first mating. In rabbit males, it was observed that an adequate rearing feeding program should adapt it to the male's intake capacity and their nutritional requirements rearing period, especially to their high DP needs at the beginning of rearing period or when their requirements were close to maintenance, to improve some semen parameters at the beginning of their reproduction life. In conclusion, both the adequate early development of future rabbit breeders, from their own gestation to the beginning of their reproductive life, and the use of appropriate rearing feeding programs could significantly improve their future reproductive performance, as well as their life expectancy, both in rabbit females and males.

Resumen

Actualmente, la cada vez mayor demanda de los consumidores de nuevos objetivos en los modelos de producción, también aplicados en la cunicultura, podría estar reduciendo el margen de rentabilidad de los ganaderos. Esta es la razón por la que la principal preocupación de ellos sea maximizar la productividad de sus animales reproductores, permitiendo que su actividad continúe siendo sostenible. Para contribuir a la sostenibilidad en la cría de conejos, debe ser crucial contar con reproductores, al comienzo de su vida reproductiva, con un desarrollo adecuado para poder expresar todo su potencial reproductivo. Por esta razón, cualquier mejora en el conocimiento tanto de la nutrición como del manejo de los futuros criadores de conejos debe considerarse como prioritario. Sin embargo, los principales esfuerzos en investigación se han centrado en estos animales durante la fase de reproducción propiamente dicha. La hipótesis inicial de esta tesis fue que alcanzar un desarrollo temprano adecuado durante la gestación, lactancia, engorde y recría, con la ayuda de programas ajustados de alimentación en algunos de estos períodos, mejoraría el rendimiento reproductivo y la vida útil de los futuros conejos reproductores. Para evaluar esta hipótesis, se llevaron a cabo dos tipos de ensayos, tanto en conejos hembra de líneas maternas seleccionadas para el tamaño de la camada al destete, como en machos de líneas paternas seleccionadas para la velocidad de crecimiento y destinadas a la inseminación artificial. El primero de estos ensayos se centró en los diferentes patrones de crecimiento, desde el nacimiento hasta el final de la fase de recría, y su influencia en la carrera reproductiva y la vida útil del conejo. En el segundo tipo de pruebas, se evaluó el efecto del uso de diferentes dietas o programas de alimentación durante el período de recría en la productividad a corto y medio plazo. Los resultados obtenidos de la evaluación de los diferentes ensayos sobre los patrones de crecimiento parecen indicar que podríamos diferenciar dos fases en el desarrollo temprano, de acuerdo con sus efectos sobre el rendimiento reproductivo y la vida útil. Mostrar un mayor desarrollo corporal desde el nacimiento hasta los 63 días de vida parece tener una influencia positiva en el grado de madurez y la productividad al comienzo de su vida reproductiva, además de poder mejorar su supervivencia a corto plazo, pero con posibles efectos negativos en la esperanza de vida a largo plazo. Sin embargo, el desarrollo durante el período de recría (desde los 63 días de vida hasta el comienzo de la carrera reproductiva) parece tener un mayor impacto en la productividad y la esperanza de vida de nuestros futuros conejos reproductores. Las hembras más engrasadas en la primera inseminación tuvieron tamaños de camada más pequeños (tanto nacidos como destetados) y un mayor riesgo de ser sacrificadas (+ 13% por cada aumento en un mm de grasa perirrenal) que las magras ($P<0.05$). Además, los machos que mostraron una mayor ganancia media diaria durante el período de recría tuvieron un número y porcentaje de eyaculados viables más bajos a lo largo de su vida

productiva (–10.1 y –4.9% por cada 10 g de aumento de ganancia media diaria, respectivamente; P<0.05) y mayor riesgo de muerte o sacrificio (+ 16.2% por cada aumento en una unidad de desviación estándar en el peso vivo; P<0.05). Estos resultados podrían indicar que la clave para llevar a cabo un desarrollo adecuado durante el período de recría debería basarse tanto en conseguir que los conejos alcancen un grado de madurez apropiado, tanto fisiológica como reproductivamente, para afrontar los futuros desafíos productivos, como evitar un excesivo engrasamiento al inicio de su vida productiva. En los ensayos destinados a evaluar los programas de alimentación durante la recría, el uso de una restricción alimentaria en hembras, y especialmente el uso de dietas fibrosas (de 8.5 a 10 MJ ED/kg MS) permiten un desarrollo corporal gradual y adecuado a la edad de cubrición, con suficientes reservas corporales para afrontar el comienzo de la vida reproductiva y mejorando tanto su rendimiento reproductivo como su vida útil. El éxito de estos programas de alimentación de recría depende del momento y la duración de la aplicación de estos, mejorándose con la aplicación de un cambio a un pienso enriquecido energéticamente alrededor del primer apareamiento. En los machos, se observó que un programa de alimentación durante la recría adecuado debería adaptarse a la capacidad de ingesta de estos y a sus necesidades nutricionales en períodos concretos de la recría, con especial atención al comienzo de la recría por sus altas necesidades en PD o cuando sus necesidades globales estén próximas al mantenimiento, consiguiendo así mejoras en algunos parámetros del semen al inicio de su vida reproductiva. En conclusión, tanto un desarrollo temprano adecuado desde la gestación hasta el comienzo de su vida reproductiva, como el uso de programas adecuados de alimentación durante la recría podrían mejorar significativamente el rendimiento reproductivo futuro, así como la esperanza de vida, tanto en conejos hembras como machos.

Resum

Actualment, la cada vegada major demanda dels consumidors de nous objectius en els models de producció, també aplicats a la producció de conills, podria estar reduint el marge de rendibilitat dels ramaders. Aquesta és la raó per la qual la principal preocupació d'ells siga maximitzar la productivitat dels seus animals reproductors, permetent que la seua activitat continue sent sostenible. Per a contribuir a la sostenibilitat en la cria de conills, ha de ser crucial comptar amb reproductors, al començament de la seua vida reproductiva, amb un desenvolupament adequat per a poder expressar tot el seu potencial reproductiu. Per aquesta raó, qualsevol millora en el coneixement tant de la nutrició com del maneig dels futurs criadors de conills ha de considerar-se com a prioritari. No obstant açò, els principals esforços en investigació s'han centrat en aquests animals durant la fase de reproducció pròpiament dita. La hipòtesi inicial d'aquesta tesi va ser que aconseguir un desenvolupament primerenc adequat durant la gestació, lactància, engreix i criança, amb l'ajuda de programes ajustats d'alimentació en alguns d'aquests períodes, milloraria el rendiment reproductiu i la vida útil dels futurs conills reproductors. Per a avaluar aquesta hipòtesi, es van dur a terme dos tipus d'assajos, tant en conills femella de línies maternes seleccionades per a la grandària de la ventrada al desllet, com en mascles de línies paternes seleccionades per a la velocitat de creixement i destinades a la inseminació artificial. El primer d'aquests assajos es va centrar en els diferents patrons de creixement, des del naixement fins al final de la fase de criança, i la seua influència en la carrera reproductiva i la vida útil del conill. En el segon tipus de proves, es va avaluar l'efecte de l'ús de diferents dietes o programes d'alimentació durant el període de criança, en la productivitat a curt i mig termini. Els resultats obtinguts de l'avaluació dels diferents assajos sobre els patrons de creixement semblen indicar que podríem diferenciar dues fases en el desenvolupament primerenc, d'acord amb els seus efectes sobre el rendiment reproductiu i la vida útil. Mostrar un major desenvolupament corporal des del naixement fins als 63 dies de vida sembla tenir una influència positiva en el grau de maduresa i la productivitat al començament de la seua vida reproductiva, a més de poder millorar la seua supervivència a curt termini, però amb possibles efectes negatius en l'esperança de vida a llarg termini. No obstant açò, el desenvolupament durant el període de criança (des dels 63 dies de vida fins al començament de la carrera reproductiva) sembla tenir un major impacte en la productivitat i l'esperança de vida dels nostres futurs conills reproductors. Les femelles més greixades en la primera inseminació van tenir grandàries de ventrada més xicotets (tant nascuts com deslletats) i un major risc de ser sacrificades (+ 13% per cada augment en un mm de greix perirenal) que les magres ($P<0.05$). A més, els mascles que van mostrar un major guany mitjà diari durant el període de criança van tenir un nombre i percentatge d'ejaculats viables més baixos al llarg de la seua vida productiva (–10.1 i –4.9% per cada 10

g d'augment de guany mitjà diari, respectivament; P<0.05) i major risc de mort o sacrifici (+ 16.2% per cada augment en una unitat de desviació estàndard en el pes viu; P<0.05). Aquests resultats podrien indicar que la clau per a dur a terme un desenvolupament adequat durant el període de criança hauria de basar-se tant a aconseguir que els conills aconseguisquen un grau de maduresa apropiat, tant fisiològic com reproductiu, per a afrontar els futurs desafiaments productius, com evitar un excessiu greixatge a l'inici de la seua vida productiva. En els assajos destinats a avaluar els programes d'alimentació durant la criança, l'ús d'una restricció alimentària en femelles, i especialment l'ús de dietes fibroses (de 8.5 a 10 MJ ED/kg MS) permeten un desenvolupament corporal gradual i adequat a l'edat de cobriment, amb suficients reserves corporals per a afrontar el començament de la vida reproductiva i millorant tant el seu rendiment reproductiu com la seua vida útil. L'èxit d'aquests programes d'alimentació de criança depèn del moment i la durada de l'aplicació d'aquests, millorant-se amb l'aplicació d'un canvi a un pinso enriquit energèticament al voltant del primer cobriment. En els mascles, es va observar que un programa d'alimentació durant la criança adequat hauria d'adaptar-se a la capacitat d'ingesta d'aquests i a les seues necessitats nutricionals en períodes concrets de la criança, amb especial atenció al començament de la criança per les seues altes necessitats en PD o quan les seues necessitats globals estiguen pròximes al manteniment, aconseguint així millores en alguns paràmetres del semen a l'inici de la seua vida reproductiva. En conclusió, tant un desenvolupament primerenc adequat des de la gestació fins al començament de la seua vida reproductiva, com l'ús de programes adequats d'alimentació durant la criança podrien millorar significativament el rendiment reproductiu futur, així com l'esperança de vida, tant en conills femelles com a mascles.

GENERAL INTRODUCTION

The importance of the journey

Nowadays, one of the biggest challenges on animal production and, especially in rabbit farming systems, is to remain sustainable, generating high quality products at the lowest possible cost and respecting the environment and animal welfare. However, in recent years, the margin to achieve this goal is getting smaller. In order to help the cuniculture in this challenge, the research must address and deepen the knowledge of those key points that may improve the productivity of our animals. Both animal nutrition and management are two of the fundamental instruments on which to rely, but initially, from the practical point of view, we have dedicated our efforts in the development of these instruments for the animals that are already in the productive phase. In fact, there are extensive reviews on reproductive rabbit nutrition such as those of Xiccato (1996), Pascual (2002) or Pascual *et al.* (2003). However, different works in different species suggest that reaching an adequate development at the beginning of their productive life could be key to both improve productivity in the short-medium term and persist it for a longer time. In this sense, many works consider body condition or fatness, weight or size and hormonal status as basics at the beginning of animal's productive life. However, the individual variability observed at birth and during early development in the different sexes and genetic lines used (Figure 0.1), it could make difficult to obtain an adequate model that can concrete the values to achieve in each moment to optimize rabbit production.

In the case of pig females, O'Downd *et al.* (1997) reported an improved fertility and longer lifespan among sows of a lean line accreting body reserves before and during pregnancy. In a fat maternal pig line, Tarrés *et al.* (2006) proposed the existence of an optimal body condition of gilts at first farrowing to reduce culling due to low productivity. Moreover, Jørgensen and Sørensen (1998) argued that a deviation from this optimum fatness level would impair sow longevity. This phenomenon seems to happen in rabbit females. Theilgaard *et al.* (2006) indicated a tendency to greater survival in the same population after 12 generations of selection by litter size at weaning, characterized with a higher body condition around the first artificial insemination (AI) in the females (Quevedo *et al.*, 2005 and 2006). Another indicator of reproductive maturity in rabbits is live weight. Rommers *et al.* (2002) observed how the heaviest rabbit females at 14.5 weeks of age were maintained throughout the reproductive period and improved litter size only in the first parity (8.9, 7.7, and 6.4 kits total born for the heavy, medium and small specimens, respectively). In another work, Rommers *et al.* (2004a) reported additional results on the relevance of the maturity level of young rabbits when they reach the first mating. Using another approach, Arias-Álvarez *et al.* (2009) proposed that a minimum circulating level of blood leptin should be attained to trigger puberty. If this level is not reached or low circulating levels are present (even in adult animal), reproduction function is temporally shut down until the critical level is recovered to initiate a new pregnancy (Moschos *et al.*, 2002).

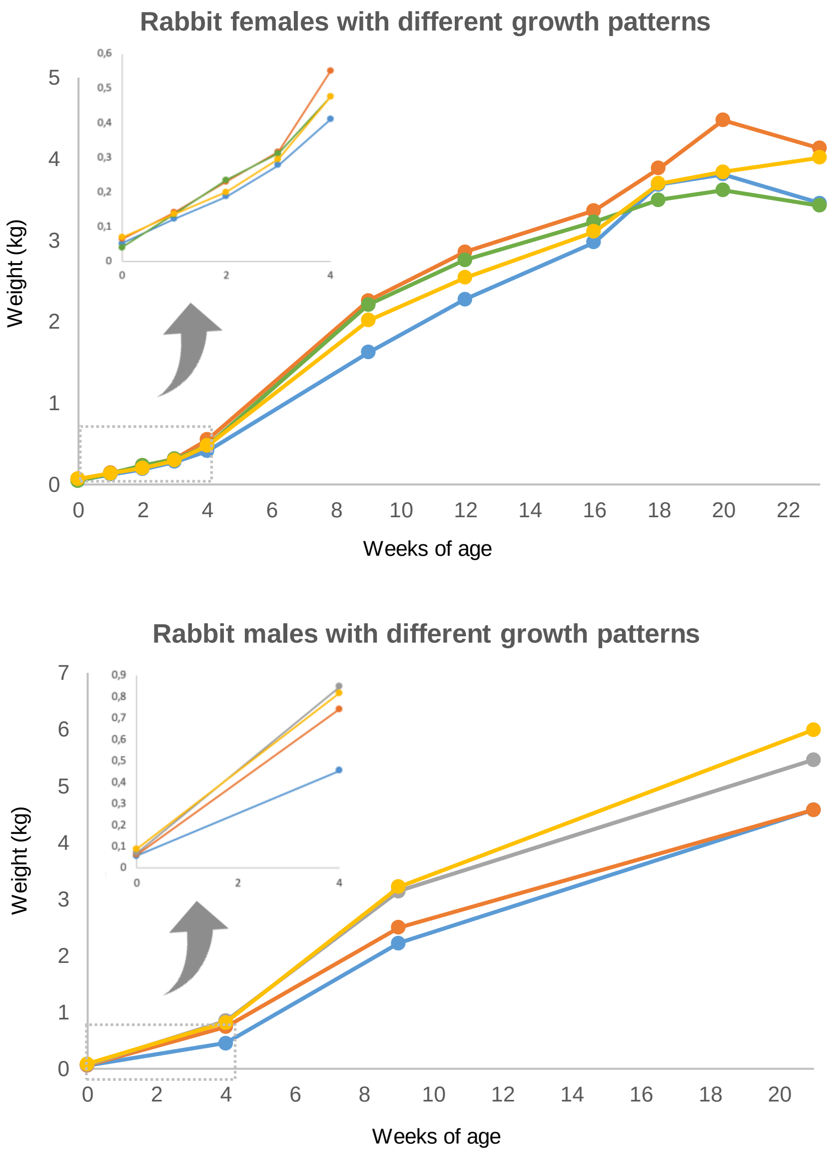

Figure 0.1 Different individual examples about the evolution of the weight from birth to the end of the rearing period of different maternal line females (top) and different males selected by growth rate (below). Built from real own data.

In the case of rabbit males, the majority of individuals reaching the adult age are raised for semen production. In human males, individuals with higher weight present a drop-in androgen levels and high levels of oestrogens (Hammoud *et al.*, 2008), and obesity affects the GnRH-LH-FSH pulses, altering Leydig and Sertoli cell functions, and in turn modifying sperm maturation (Bélanger *et al.*, 2002).

Based on the actual knowledge, the hypothesis that early factors influencing the body development of young rabbit females up to the maturity may also affect its future reproductive life merits further studies. In this sense, the evaluation and the understanding on how factors like birth weight, the feed received during the juvenile life and even the exposure to environmental factors are essential. The main hypothesis behind this affirmation concerns the knowledge that the reproductive potential of rabbits is expressed throughout the development of the newborn rabbit. Among these essential steps, we can highlight the foetal development and uterine nutrition, the development during the lactation phase, the development during the fattening and rearing periods. It seems reasonable to think that knowing each single step is important. If so, by adopting feeding and management practices the farmer may be able to modulate the development of its future reproducing animals, which may result in long living, health and productive breeders.

The preparation

The development of rabbits until their reproductive maturity could be divided into 4 phases. Foetal development, and lactation, fattening and rearing periods are conditioned by intrinsic and extrinsic factors. A deep knowledge of the effect of the extrinsic ones could help to understand and to act in the different phases of the development, to maximize the intrinsic potential of the future breeding animals.

The most influential factors in rabbit foetal development until the moment of birth and their possible consequences on future breeding rabbits are described below.

Foetal development

The survival of each of the implanted foetuses, such as an adequate birth weight, should be the two main objectives to obtain a good future breeding animal in this phase. However, from the physiological point of view, it should be noted that the development and multiplication of the primordial germ cells (10-26 d of gestation) and of the primordial follicles occurs during pregnancy (Fortun, 1994), in contrast to other species (primates and ruminants) where it is already established at birth (Marion and Gier, 1971; Hirshfield, 1991). Consequently, nutritional deficiencies during this phase could cause reproductive problems in adult rabbits.

Although the foetus does not have a direct role on the acquisition of resources during gestational phase, some factors may directly affect birth weight. Even though feeding and corporal condition of the mother may influence the quantity and quality of nutrients available to the foetuses, the availability of resources during gestation seems to be crucial at the end of the gestational period.

Foetal blood vessels

Several authors (Bruce and Abdul-Karim, 1973; Argente *et al.*, 2003) agrees that foetal size is associated with the size of the foetal placenta. In this sense, both foetal development and survival are directly related to the vascular blood supply reaching the placenta in rabbits (Hafez, 1965; Duncan, 1969; Bruce and Abdul-Karim, 1973; Argente *et al.*, 2003; Mocé *et al.*, 2004; Argente *et al.*, 2008). For this reason, number of blood vessels arriving at each implantation site is regarded as one of the most reliable estimators of foetal development. In this sense, Argente *et al.* (2003 and 2008) reported an increased weight of both foetuses and their foetal and maternal placentas when the number of blood vessels at each implantation site was relatively superior to the other sites. More precisely, at 25 days of gestation, implantation sites with a single blood vessel had lower foetal weight (–8%) and lighter placenta (–17 and –7% for foetal and maternal placenta, respectively) than implantation sites with four or more blood vessels (Argente *et al.*, 2003), but what determines the number of vessels and the nutrient flow to the foetus?

Foetal position and uterine space

In the uterine horn of the female rabbit, we could differentiate three positions that will determine the physical space for foetal develop and the nutrient supply. The best implantation sites for the foetuses are the two distal parts (ovarian and cervical extremes) of the uterine horn. Foetuses attached to these sites are heavier than foetuses located in the central part (Figure 0.2). According to Argente *et al.* (2003), the difference on foetus weight may reach 7% between the best and the worst implantation sites, due to a difference on the nutrient flow at these sites. In fact, foetuses and placentas located at the ovarian extremes benefits of a better irrigation, being better developed at birth than those at central positions (rabbits: Duncan, 1969, Lebas 1982, Poigner *et al* 2000a and Argente *et al.*, 2003 and 2008; mice: McLaren, 1965; pigs: Waldorf *et al.*, 1957, Perry and Rowell 1969 and Wise *et al.*, 1997). They also benefit of more physical space for growth (foetuses of extremes do not have competition by one of the sides) than foetus at the central positions, reaching birth better developed than foetus developing at extreme positions.

Litter size

In addition to the foetal position, another factor influencing the foetal development is the number of gestating embryos. Once the uterine space is limited, having more implanted embryos may condition the foetal development. A logical statement corroborated by different studies. Breuer and Claussen (1977) and Argente *et al.* (2003) found a negative correlation between offspring number and foetal weight. In another study, Argent *et al.* (2008) also observed a directly association between the number of implanted embryos and the foetal mortality, as well as a quadratic relationship between the number of implanted embryos and the uterine space per implantation site. In the light of these results, these authors argued that rabbit embryos require a minimum uterine space to attach, survive and develop.

Although the number of gestating embryos should not suppose a problem in a prolific specie like the rabbit, the selection of highly prolific females reduced both the space and the nutrient flow per developing embryo. The consequence of it is a reduced weight and size of newborn rabbits (Vicente *et al.*, 1995). One manner to counteract this side effect of selection for prolificacy is to select females with a greater uterine capacity and/or with more space uterine and more efficient in the acquisition and donation of resources to the foetuses. Garreau *et al.* (2008) proposed another strategy, select for a reduced birth weight variability. The objective is similar, reduce the embryo mortality, and obtain in the limit of what is biologically possible, offspring of better quality to be sold or to become future breeders.

Figure 0.2 Uterine horns of a rabbit female. Foetuses located in both extremes (ovarian and cervical extremes, top and bottom of the photo, respectively) are larger than foetuses located in the central part (Argente *et al.*, 2003). Photo courtesy of M.A. Santacreu**.**

The starting point

Birth weight is the first *in vivo* data easily accessible for quantifying how well the future breeder has been developed in the uterus and how promising its future reproductive life might be. Birth also represents the first moment on the rabbit life in which we can carry out individual actions to direct the resources to improve their post-natal development. Knowing the influences that birth weight may exert on the future reproductive performance of rabbits as breeders, open new perspectives like choosing the individuals better capable to deal with the variety of road bumps that an exigent reproductive life imposes on animals with different productive objectives. Although birth weight is the consequence of the factors discussed above during pregnancy, it is also affected by other factors such as the season, the order of parity, the most important is the litter size (Fortun *et al.,* 1993; Bolet *et al.,* 1996; Argente *et al.,* 1999). Litter size influence newborn growth from birth to weaning (Drummond *et al.*, 2000, Poigner *et al.*, 2000b) and impacts the reproductive performance of future females (Rommers *et al.,* 2001). The first advantage of knowing the birth weight is its relation to the individual survival probability. Offspring born in large litters are normally smaller than offspring born in small litters. They also have higher risks of dying. In fact, Argente *et al.* (1999) obtained a correlation of +0.30 between birth weight and lactation survival. Although these same authors observed a similar birth weight range between the group of survivors and death offspring, the found that a low birth weight is not a death penalty, as 70% of the smallest offspring survived to the weaning age. Pursuing a similar objective, Drummond *et al.* (2000) and Coureaud *et al.* (2007) proposed a minimum birth weight below which the chances of survival decreases. They set the limit at 43 and 48 g respectively. However, birth weight cannot be looked as an independent factor influencing rabbit offspring survival. Birth weight homogeneity among littermates is highly important (Bolet *et al.*, 1996, Argente *et al.*, 1999), being the offspring mortality higher in litters of heterogeneous birth weight greater than offspring mortality in litters with more homogeneous birth weights (rabbits: Poigner *et al.*, 2000b; pigs: Mesa *et al.*, 2006).

Another important aspect of having access to the birth weight is its correlation to weaning and adult weight. Argente *et al.* (1999) obtained a genetic correlation between birth weight and weaning weight of 0.87, and Szendrő *et al.* (2006) observed a delayed development up to the reproductive age among offspring born with a low birth weight. With a similar perspective, Poigner *et al.* (2000b) observed that females born with a heavier weight produced more offspring (+12.4%) for six consecutive reproductive cycles than females born with a reduced weight.

In the case of the influence of litter size or birth weight in future breeding males, the available information is scarce or almost non-existent.

The vision on this starting point would indicate that, the study of the effect of managing the size and homogeneity of the most prolific litters, such as adequate birth weight for the correct physiological and reproductive development could be useful tools for the productivity of the breeding rabbits.

The first steps

In the first steps of the journey towards maturity, there are two key periods: lactation (from birth to weaning) and the post-weaning period (period after weaning that stands out for the high daily growth). These periods have relevance in the future characteristics of rabbits, since much of the physiological (Deltoro and López, 1985) and reproductive (García-Tomás *et al.*, 2008) development is defined then. Furthermore, it must consider that the genetic selection criteria of the different lines will condition their development indirectly. In the three-way crossing scheme, the paternal and maternal lines are selected separately according to different criteria (daily gain after weaning and litter size, respectively), with different feeding and growth patterns (Feki *et al.*, 1996).

The long-term consequences of early development are well documented in some animals (Lindström, 1999), so we would have to delve deeper into knowledge during these phases in rabbits.

Lactation

During lactation (usually up to 28, 35 or 42 days of life), as it occurs during pregnancy, the acquisition of an adequate amount of resources may have consequences on the growth during this phase and in later ones, as well as on future reproductive ability. This amount of resources will depend on factors such as the quantity and quality of milk produced by the females, as well as the competitiveness among members of the litter. The quantity and quality of rabbit females' milk may be influenced by environmental conditions. In nature, one of the causes of a bad start in the offspring is due to a poor nutrient supply capacity of parents (Metcalfe and Monaghan, 2001), which may be caused by adverse environmental conditions. Moreover, regarding to the quantity and quality of milk that each kit swallows, the litter size and individual weight influence directly in the competitiveness among siblings for mothers' milk. In this sense, heavier newborns are usually located in the central positions of the litter, reducing heat loss and facilitating access to the mother's nipples (Bautista *et al.*, 2015) and also have more fat reserves (García-Torres *et al.*, 2015), acquiring an advantage over their siblings. Consequently, some authors consider that the greater development of rabbits during their life could be due to an improvement of the animal's competitiveness by the mother's milk (Szendrö, 1986; Poigner *et al.*, 2000a). Otherwise, kits modify their circadian rhythms when they do not ingest milk, altering the regulation of their body temperature and reduce the ability to compete with littermates for the milk (Jilge *et al.*, 2001). These results highlight the idea that milk intake during the first days of life is fundamental for the development and survival. Nevertheless, maternal lines selection criteria result in big litters and highly variable offspring size at birth (Hudson *et al.*, 2011). Some authors have been proposed alternatives to minimize these possible lactation mistakes. Sánchez *et al.* (2008) founded a rabbit line by productive longevity, where females are capable of adjusting their reproductive effort to environmental abundances and limitations (Savietto *et al.*, 2013). Other authors have proposed a selection canalized by birth weight, achieving a lower number of stillbirths, a better survival capacity during lactation and a larger litter size at weaning (Garreau *et al.*, 2008). Other measures to ensure the correct supply of nutrients during lactation, and to avoid a low weight during lactation, could be the litter homogenization, according to the birth weight, to promote the early development (Poigner *et al.*, 2000b) and ensuring the milk intake during the first hours of life (Coureaud *et al.*, 2007).

In the case of future males, a better lactation likely will indirectly promote the growth rate during fattening, so this phase should not be underestimated.

Lactating studies have focused on how to improve the quantity and quality of resources obtained by the rabbit female and obtain larger and heavier litters. Even though the selection of the future breeding female rabbits at the end of this lactation phase could indirectly favour the amount of resources acquired by the surviving rabbits, it has not been sufficiently evaluated the direct repercussions on young rabbits in adult stages.

The post-weaning

The post-weaning phase is characterized by transition from milk to solid feed, high energy and protein requirements and high growth rate. Since the 90s, growth of rabbits has been conditioned by rabbit epizootic enteropathy (ERE) and, despite being diagnosed (Licois *et al.*, 2005), a definitive solution has not yet been found. Any future effect of feeding program during the fattening phase should be considered. At the onset of the disease emergence, the use of antibiotics was an effective measure to prevent high mortality rates. At the same time, qualitative restriction strategies, by the use of different combinations of fibrous fractions, have been implemented that favour the development and digestive maturity (Gidenne *et al.*, 2002, Debray *et al.*, 2003, Gallois *et al.*, 2005, Romero *et al.*, 2009, Martínez-Vallespín *et al.*, 2011) or even an adequate colonization of the microbiota (Combes *et al.*, 2014). Another possibility to reduce ERE processes is quantitative feed restriction (Gidenne *et al.*, 2012). This quantitative restriction, like the qualitative one, modifies the amount of flow and/or the cecal microbial activity pattern (Gidenne and Feugier, 2009), without apparent consequences in growth (Gidenne *et al.*, 2009; Romero *et al.*, 2010), through to the compensatory growth or the *ad libitum* application of another type of feed, more digestible, at the end of fattening. Nowadays, many rabbit farmers extend the lactation period in order to immunologically protect the kits through mother's milk, make a more gradual transition to solid feed. Respect to the feeding programs during the fattening phase, some studies show changes in body composition and some organs, in function of age, type (quantitative or qualitative), duration and the level of restriction, which could have short or long-term consequences. About the influence of quantitative restriction on body fat content at the end of the fattening period, Ledin (1984) and Ouhayoun (1989) reported lower levels, while more recent studies reported higher levels (reviewed by Gidenne *et al.*, 2012). Additionally, in the case of liver development, Ledin (1984) and Tumova *et al.* (2007) observed a reduction during the restriction period, but greater at the end of the trial. Ouhayoun (1989) found a lower proportion of liver, in relation to carcass weight, after a severe restriction beyond 11th week of life. Because fat reserves (Pascual *et al.*, 2013; Savietto *et al.*, 2015) and liver metabolic function plays a central role in the metabolism and energy partitioning for reproduction and other vital functions (Wade & Schneider, 1992), correctly assessing of impacts of post-weaning restriction strategies on the energy reservoirs and organs related to the reproductive function are necessary.

Results from qualitative restriction strategies, such as increase the fibre and reduction on energy content of the diet, are quite similar to those observed with quantitative restriction (lower dissectible fat and liver, Pascual *et al.*, 2014).

Regarding the physiological peculiarities according to the sex, breeder rabbit males, used in three-way crossbreed scheme and selected for the greater growth during the fattening (Baselga, 2004), are characterized to have a greater development of the testicular volume during the first weeks of life (Iczkowoski *et al.*, 1991). In the same way, García-Tomas *et al.* (2008) studied the development of reproductive physiology of rabbit males selected for growth rate. They observed that the greatest development of the diameter of the seminiferous tubules, which coincided in turn with the appearance of the lumens, was from eight to 14 weeks of life. It would be interesting to know how the development in this phase could affect the performance qualities in the finalizer males. In fact, Lavara *et al.* (2008) observed a positive genetic correlation (+0.33) between the abnormality and the selection by growth rate in another meat line.

In multiparous rabbit females, there are not many works related to early development and the consequences in the reproductive phase. Poigner *et al.*, (2000b) observed that a worse early development, supposes a future reproductive disadvantage. It would be interesting to deepen the knowledge during this phase, since in other species (mice: Falconer, 1955 and 1960, Nelson and Robinson, 1976; sows: Van-der Steen, 1985 and Horn *et al.*, 1987) the results obtained are similar.

In summary, the large number of variables (age of onset, duration of the restricted period, level of restriction and type of recovery programmed after restriction), the influence in development patterns (growth, organs, tissues ...), and the scarce information, make difficult the evaluation of the effect on the post-weaning phase in the performance of future breeding animals.

Rearing period: the final stage

Rabbits passing the fattening period, around 70 days of age, are intended to be future breeding animals. As the genetics and the housing are, in most cases, constant variables, in the recent years, the majority studies have been mainly addressed to feeding and management programs aimed to prepare the future breeding animals to productive challenges in the short, medium and long term. The knowledge generated on feeding strategies for future females (Xiccato *et al.*, 1999, Rommers *et al.*, 2004a, Arias-Álvarez *et al.*, 2009, Rebollar *et al.*, 2011) have been considerable, because an adequate physiological development of future breeding females may have a major impact on farm productivity. In the case of future rabbit males, the lack of available information seems to be related to the general assumption that males need no more nutrients than what is required to sustain life and health to be reproductive effective.

An adequate feeding program requires knowing the main management and feed requirements of our target animals to help to optimize their reproductive potential. In the case of females, selection for prolificacy has caused an increase of nutritional requirements, mainly due to the greater gestational and milk production effort, and consequently a more pronounced negative energy balance could appear due to the needs of the litter (Pascual *et al.*, 2013). Defining an adequate nutritional strategy that allows the future rabbit female to minimize and/or to prepare for the great effort of successive reproductive cycles, we would be able to continue to raise rabbits in such exigent conditions. In females, works focused on improving the voluntary feed intake during rearing were those that have obtained the best results (Pascual *et al.*, 2002, Rommers *et al.*, 2004b, Quevedo *et al.*, 2005, Rebollar *et al.*, 2011). In the case of the rabbit males, high-speed growth animals in the fattening period are used in three-way crossbreed scheme, which usually produce semen of lower quality than the males from maternal lines (Vicente *el al.*, 2000). Consequently, feeding programs addressed to improve both quantity and quality of sperm should be explored.

Rearing diets for future breeding rabbits should have three main objectives: an adequate meet of nutritional needs, assurance of welfare and health, and maximize both the reproductive performance of females in long-term as the semen production and quality of males. To achieve these goals, young rabbits must have an adequate physiological development (both digestive and reproductive), to optimize future resources utilization and have good body condition, which will allow them to face environmental and reproductive challenges.

Females

The main problems usually associated to the traditional rearing management (*ad libitum* supply of reproductive or fattening feeds) have been an increased risk of digestive problems in the first weeks of rearing period (Rommers *et al.*, 2004a), greater fatness, higher pre-natal mortality (Viudes-de-Castro *et al.*, 1991), lower intake at the start of lactation and reduced lifespan of rabbit females (Rosell, 2000). The alternatives proposed are two: i) feed restriction of current reproductive or fattening feeds or ii) *ad libitum* supply of fibre-enriched diets with less energy and protein than reproductive or fattening diets.

Some authors (Partridge, 1986; Maertens, 1992) proposed the use of feed restriction as an alternative to the traditional *ad libitum* rearing programs. However, several studies indicate that some rearing programs based on feed restriction could delay growth and sexual maturity at first matting (Rommers *et al.*, 2004b), impair fertility (Szendrő *et al.*, 2002, Rebollar *et al.*, 2011) and prolificacy (Rommers *et al.*, 2001 and 2002). Such strategy may also affect milk production (Nicodemus *et al.*, 2007). These handicaps could be the result of not having properly handled the restriction, because there are many variables (the application period, restriction level, genetic type…) that can influence on the program success. In fact, the results associated to feed restriction are very diverse (Table 0.1).

Respect to the age to start the feed restriction, it seems appropriate not to start the restriction before 12 weeks in order to ensure the proper development of the main physiological structures of the female around 10-12 weeks of age (Deltoro and Lopez, 1985). Pascual *et al.* (2002) delayed AI of young females restricted until an adequate development was reached (around 3 kg) to ensure good subsequence reproductive performance. More recently, Manal *et al.* (2010) observed higher feed intake and weight at the end of pregnancy, even greater weight of kits at parturition, when mature females (5 months old) were restricted during 15 to 20 d just after first mating. Respect to restriction level, Eiben *et al.* (2001), comparing young females fed 100, 95 (full-fasting one day in a week), 82 (9 h of daily access to feed) and 76% (fixed daily maintenance provision) of *ad libitum* feeding, only observed a delay in reaching the adequate body weight at first mating in the last two groups, without negative consequences on reproductive performance. Although there are not many studies with different genotypes, Matics *et al.* (2008) described a longer delay achieving the targeted weight (between 4.5 to 5 kg) when restriction was applied in females with a larger format (around 6 kg on adult age). Respect to the long-term effect of feed restriction during rearing, Martínez-Paredes (2008) did not find differences in

Table 0.1 *Results obtained in different studies for the effect of rearing feeding programs based on restriction (respect to ad libitum) on the performance of rabbit females at the beginning of reproduction.*

Authors	Restriction	Duration	Mating Age[1,2]	At Mating			At 1st Parturition			
				BW	F	BC	BW	FI	LS	LW
Rommers *et al.* (2001)	79% *ad libitum*	6 w of age to 1st parturition (flushing 5d)	17.5 *vs* 14.5	**ND**	**--**	↓	**--**	**--**	**--**	**--**
Eiben *et al.* (2001)	76% *ad libitum* (130-140g/d)	10 w of age to 1st parturition (flushing 4 d)	19.5	↓	**ND**					
	95% *ad libitum* (one day without feed)		18.1	↑	↑	**--**	↓	**--**	**--**	**--**
	82% *ad libitum* (feed *ad libitum* 9 hours/d)		18.7	↓	**ND**					
Bonanno *et al.* (2004)	75% *ad libitum*	11 w of age to 10d pre-AI	19 *vs* 16	↑	**ND**	**ND**	**--**	**--**	↑	**--**
Rommers *et al.* (2004b)	79% *ad libitum*	5 w of age to 1st parturition (flushing 5d)	17.5 *vs* 14.5 and 17.5	**ND(14.5w) ↓(17.5w)**	**ND**	**--**	**--**	↑	↑	**ND**
Matics *et al.* (2008)	130 g/d (medium genetic females)	11 w of age to 8 d pre-AI	19.5 *vs* 15.5	↑	↑	**--**	↑	**--**	**ND**	**ND**
	130 g/d (heavier genetic females)		19.5 *vs* 15.5	↑	↑↑					
Manal *et al.* (2010)	1.32 x recommendations Maertens (1993)	10 d after mating	21				↑	**ND**		**ND**
		15 d after mating		**ND**	**ND**	**ND**	↑↑	↑	**ND**	↑
		20 d after mating					↑↑	↑		↑
Rebollar *et al.* (2011)	150 g/d	11-16 w of age	17 *vs* 16	**ND**	↓	↓	**--**	**ND**	**ND**	**ND**

w: week; BW: Body weight; F: Fertility; BC: Body condition (leptin level, perirenal fat thickness…); FI: Feed intake until parturition; LS: Total litter size (or alive if controlled); LW: Total litter weight (or alive if controlled); --: unknown; ND: no differences; ↓: significative lower results for restriction vs *ad libitum* (fattening or lactation rabbit diets); ↑: significative higher results for restriction vs *ad libitum*. [1] restricted vs. *ad libitum* (if there was only a single data it was for restricted rabbits). [2] values expressed in weeks of age.

lifespan nor in reproduction parameters compared to other rearing feeding systems. In summary, the results seem to indicate that the restriction does not produce negative effects in the beginning of the reproductive life of the rabbit females, if they assure the sufficient physiological maturity at AI. However, more studies in long-term must be done to

Table 0.2 *Results obtained in different studies for the effect of rearing feeding programs based on fibrous ad libitum diets (respect to ad libitum or restricted concentrated diets) on the performance of rabbit females at the beginning of reproduction.*

Author/s	Fibrous diet vs. commercial diets[1] (%DM)	Duration	Mating Age	At Mating			At 1st Parturition			
				BW	**F**	**BC**	**BW**	**FI**	**LS**	**LW**
Nizza *et al.* (1997)	NDF: 48.9%; DE: 10.2 MJ/kg *vs*. 11.2 MJ/kg	50 d of age to 10 d post mating	--	**--**	**ND**	**--**	**--**	↑	**ND**	↑
Xiccato *et al.* (1999)	NDF: 40.8; DE: 9.5 MJ/kg *vs.* 10.7 MJ/kg	40 d of age to 1st parturition	19.5 w	**ND**	**ND**	**ND**	**ND**	↑	**ND**	**ND**
Pascual *et al.* (2002)	NDF: 44.4; DE: 8.0 MJ/kg *vs.* 11.0 MJ/kg	70 d of age to 1st parturition	3.2-3.5 kg of BW	**ND**	**--**	**--**	**ND**	↑	**ND**	**ND**
Quevedo *et al.* (2005)[2]	NDF: 36.4; DE: 7.3 MJ/kg *vs.* 10.8 MJ/kg	90 d of age to pre-parturition	19 w	↓	**ND**	**ND**	**ND**	↑	↓	**ND**
Verdelhan *et al.* (2005)[2]	CF: 30; DE: 7.2[4] MJ/kg *vs.* 10.5[4] MJ/kg	84 d of age to pre-parturition[5]	18-19 w	**ND**	**ND**	**--**	**ND**	**--**	**ND**	**--**
	CF: 30; DE: 7.2[4] MJ/kg 11.9[4] MJ/kg			↓					↑	
Arias-Álvarez *et al.* (2009)[3]	Low lignine (LAD: 4.9% DM; DE: 9.3 MJ/kg)	11 w of age to parturition	16 w	**ND**	↑	↑	**ND**	↓	**--**	**--**
	High lignine (LAD: 15.8% DM; DE: 11.3 MJ/kg)				↓	↓		↑		
Rebollar *et al.* (2011)	NDF: 50.5; DE: 10.0 MJ/kg *vs*. 12.6 MJ/kg	11 w of age to parturition	17 w *vs* 16 w	**ND**	**ND**	**ND**	**--**	↑	**ND**	**ND**

w: week; BW: Body weight; F: Fertility; BC: Body condition (leptin level, perirenal fat thickness…); FI: Feed intake until parturition; LS: Total litter size (or alive if it was controlled); LW: Total litter weight (or alive if it was controlled); --: unknowed; ND: no differences; ↓: significative lower results for fibrous vs concentrate *ad libitum* diets; ↑: significative higher results for fibrous vs concentrate *ad libitum* diets. [1] Fattening or lactation rabbit diets; [2] Trials comparing *ad libitum* fibre respect to restricted concentrate diets (140-150g/d); [3] Results only compared between treatments explained: low and high lignine; [4] DE calculated for 90% MS; [5] 7-d of same *ad libitum* diet before mating for commercial diets.

corroborate this result and understand the implications of this management system. Another alternative could be the *ad libitum* use of high-fibrous diets during rearing, as they could prevent over-fatting, improve the feed intake capacity before and after first parturition, contributing thus to reduce possible negative energy balances that mainly affects primiparous rabbit females (Table 0.2). The results obtained of this feed strategy seems to depend on factors such as the chemical composition of the diet, the management of the females, the genotype and the environmental conditions of the farm. As the success of this strategy is partially given by the greater development of the digestive tract of the female (Fernández-Carmona *et al.*, 1998), it would be advisable to begin the administration of fibrous diets before 12 weeks of age, when the development of digestive tract is almost complete (Deltoro and Lopez, 1985). For this reason, Pascual *et al.*, (2013) proposed the application of fibrous diets as early as 60 days of life, because if these diets are included later the expected benefits is limited, regardless of the dietary amount of fibre (360-500 g NDF per kg DM; Quevedo *et al.*, 2005; Verdelhan *et al.*, 2005; Pereda *et al.*, 2010).

Another important aspect of the high-fibrous diets is the quality of the fibre it contains. If the lignin levels are excessive (above 150 g of acid detergent lignin per kg of DM; ADL), puberty is delayed and fertility is impaired respect to normal lignin values (50 g ADL/kg DM; Arias-Alvarez *et al.*, 2009). However, intermediate values (93 g of ADL per kg DM) promote a higher feed intake of young females, resulting in young females capable to ingest enough energy to overcome gestation, lactation and growth, and at the same time being also capable to conceive again (Nicodemus *et al.*, 2007). The reduced DE content of high-fibrous diets does not seem to be a limiting factor, even when animals are subjected to fibrous diets containing 8 to 9 MJ of DE per kg DM. In fact, when female is feed with such low levels, they could reach an adequate live weight between 18 to 19 weeks of age (Pascual *et al.*, 2002).

Rabbit females should use the energy stored in their body to promote some vital functions. These uses of reserves differ among different animals and genetic types. Friggens (2003) suggests that mobilization could have negative consequences on reproduction when is far from its optimal level. In fact, a deviation in the use of reserves could affect functions as reproduction and even impair life, as observed with the non-esterified fatty acids (NEFA) in rabbit females. Rebollar *et al.* (2011) observed a lower concentration of NEFA in the blood of females at first parturition fed with a high-fibrous diet compared to those reared with a lactating diet. These authors further correlated the low NEFA values with a better fertility at 11 days post-partum, and with a lower mortality of females and its litter at birth.

Furthermore, the degree of maturity achieved at the first AI with these diets could also affect further acquisition and use of the resources during first lactation. Xiccato *et al.* (1999) observed that the administration of a high-fibrous diet during rearing, that lead to a lower body weight at mating (although no significative), increased feed intake of lactating rabbit does, which was addressed to contribute to reduce body condition losses instead of greater litter development. However, when high-fibrous diets lead to adequate body weight at first AI, the higher feed intake during lactation was mainly addressed to improve milk production (Pascual *et al.*, 2002). These studies would reinforce the idea that perhaps we should evaluate in long-term the relevance of achieving an appropriate physiological development at the beginning of reproduction.

Usually, reproductive and physiological differences observed between the different rearing feeding programs disappear from second parturition. In this sense, Martínez-Paredes (2008) did not find long-term differences associated to the use of high-fibrous rearing diets. However, some authors have described some long-term effects of high-fibrous rearing diets: Nizza *et al.* (1997) obtained greater and heavier number of weaned kits during the first 4 reproductive cycles; and Pascual *et al.* (2002) observed increased number of weaned kits, lower interval between parturition and longer lifespan of females. However, although lifespan depends of a large number of factors, one of the most important particularities of rabbit females characterized by higher lifespan is their lower dependence on fat mobilization to ensure reproduction (Savietto *et al.*, 2015), as body reserves are used as a safety factor. In fact, Theilgaard *et al.* (2006) observed that rabbit females with the low body condition or showing higher mobilizations had an increased risk of culling. Therefore, theoretically, rearing feeding programs allowing gradual provision of resources, proportional development of young breeding animals and proper mobilization at reproductive challenge events should provide breeding animals a lower risk of culling throughout their reproductive life.

In addition to the use of fibrous diets, other complementary and interesting ways open up for the improvement of young rabbit female's reproductive performance, such as the use of diets with a better balance in term of n-6/n-3 polyunsaturated fatty acids ratio (Maranesi *et al.*, 2018, Mattioli *et al.*, 2019)

Finally, some development indicators like body condition (Rebollar *et al.*, 2011) and some metabolites such as leptin (Nicodemus *et al.*, 2007, Rebollar *et al.*, 2008) seem to indicate that the young females should reach a minimum threshold to maximize fertility when any type of restriction is applied (qualitative or quantitative). These results would indicate that a hypothetic appropriate degree of maturity should be achieved at the first mating to ensure successful breeding. These indicators (different in function of the sex, genetic type, environmental conditions…) could help optimize the different feeding programs during the rearing period in the future.

Males

At the beginning, the low importance that has been assigned to the rabbit male in the rabbit production system has caused that the available information on feeding strategies that allows maximizing its reproductive characteristics is scarce and sometimes confusing.

Respect to growth during rearing period, rabbit males selected for growth rate from 28 to 63 days of age show an average daily gain of 40 g per day from 9 to 14 weeks of age, decreasing to 5 to 10 g per day from 14 to 24 weeks (the onset of reproductive life). This clearly denotes two growth phases characterized by different nutritional requirements (Pascual, 2002). Therefore, to reach young male requirements would be necessary to consider these phases to design a feeding program. Peculiarities such as the irregular feed intake after 14 weeks of life, as well as environment or management could have relevance in their future reproduction performance and should be deeply studied.

To regulate the feed intake, Luzi *et al.*, (1996) proposed a feed restriction (perhaps too strong, around 25% reduction of *ad libitum* intake) in young males from 15 weeks of age, with negative consequences on libido and sperm production. Recently, Pascual *et al.* (2015 and 2016) found that a moderate restriction (around 12%) may be useful to fit male needs and to provide a constant daily supply of nutrients, which lead to improve some sperm morphological characteristics, as well as the fertility of their pooled semen (+4%), especially during the seasons where this program allowed a higher daily feed intake stability.

A reduction on nutritional levels can produce a worsening on semen characteristics. Nizza *et al.* (2000) observed lower sperm production and motility on males fed with a 13% crude protein diet compared to those with 15 and 17%. However, an adjustment of feeding to daily needs has been proposed as useful to reduce problems associated to fatness (Maertens, 2010). In fact, Du Plessis *et al.* (2010) observed that obesity in men sometimes can be associated with an increase in abnormal spermatozoa and a high risk of fertility problems.

Finally, some amino acids and micronutrients related with semen production, sperm quality and reproductive physiology development in reproduction age should be considered on the future experiments on formulation of males rearing diets. Among others, include vitamins A, E, C and D3, zinc, L-arginine, L-carnitine and polyunsaturated fatty acids (PUFAs). Vitamins are directly or indirectly related with spermatogenesis, but only vitamin E, with antioxidant capacity, seems to improve semen of adult males when it was frozen or males were fed with diets enriched on PUFAs (Castellini, 2008). Zinc affects male reproductive organs through the pituitary-gonadal axis, and Mocé *et al.* (2000) observed that productive and qualitative characteristics of semen improved when diet was supplemented with 49 ppm of Zn. L-arginine deficiency may cause deterioration of the sperm metabolism, resulting in reduced motility and spermatogenesis (Holt *et al.*, 1944). Sperm accumulate L-carnitine in the distal part of the epididymis and its supplementation has been associated with improved survival and viability of sperm, as well as the total number of ejaculated spermatozoa (Jeulin and Lewin, 1996), although in rabbits the results are inconclusive. Finally, the phospholipid membrane of the sperm contains considerable quantities of long-chain PUFAs, which together with cholesterol, are responsible for changes in membrane fluidity and thus regulates the responsiveness of the acrosome and ovule-spermatozoa fusion (Roldan and Harrison, 1993; Apel-Paz *et al.*, 2003). Most of these PUFAs come from linoleic and alpha linoleic acid of the diet and the concentration could be easily modified. However, the results are inconclusive (Yamamoto *et al.*, 1999; Castellini *et al.*, 2005; Mourvaki *et al.*, 2010).

Hypotheses and objectives

The current knowledge indicates that the physiological and reproductive development of future reproductive rabbits would be modified by pre- and post-natal factors. In addition, feeding and management strategies, during the different phases of growth up to the adult age, could affects their future ability to acquire adequate resources. The initial hypothesis was that achieving an adequate early development during young rabbits' gestation, lactation, fattening and rearing, with the help of fitted feeding programmes in some of these periods, will improve reproductive performance and lifespan of the future breeding rabbits. Therefore, the main objective of this PhD Thesis was to find out the developing degree to be achieved at key points on early life and the best feeding strategies during the rearing, that will help us to propose the most adequate management and feeding programme to maximize the reproductive performance in a short and long-term, both for breeding rabbit females and males.

To reach this goal, we have planned different trials to evaluate the effects of the developmental variability and feeding strategies on the reproductive performance in short- and long-term, both in females and in males. During these trials have been monitored some parameters related with our objectives: i) evolution of body weight from parturition to the beginning of reproductive life; ii) acquisition and use of resources (feed intake, body condition, milk yield and blood metabolites) up to the first reproductive cycles; iii) rabbit performance in short and long term (litter or semen traits for rabbit females and males, respectively); iv) life expectancy in commercial conditions.

More concretely, the specific objectives −corresponding to the works reported in this PhD Thesis− are described below:

1. To determine which feeding programme during the rearing period could optimize the physiological and reproductive development of young rabbit females to maximize the reproductive performance at first parturition (Paper I) and up to the second parturition (Paper II).
2. To assess the influence of the early growth, as well as the feeding programme provided during the rearing, in the long-term reproductive performance and life expectancy of the rabbit females (Paper III).
3. To determine which is the feeding strategy to meet adequately the nutritional requirements during the rearing period in function of the season and to maximize seminal traits during the initial reproductive phase of the rabbit males (Paper IV).
4. To determine the influence of early growth of young males on the sperm production and quality during the testing and semen productive phases, as well as on their life expectancy (Paper V).

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PAPER I

Effects of feeding programme on the performance and energy balance of nulliparous rabbit does

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Abstract

A total of 190 rabbit females were used to evaluate five feeding programmes from 9 weeks of age to the first parturition: CAL, fed *ad libitum* with a control diet [C: 11.0 MJ digestible energy (DE) and 114 g digestible protein (DP)/kg dry matter (DM)] until first parturition; CR, fed *ad libitum* with C diet until 12 weeks of age and then C diet restricted (140 g/d) until first parturition; F, fed *ad libitum* with a low-energy, high-fibre diet [F: 8.7 MJ DE and 88 g DP/kg DM] until first parturition; FC, fed with F diet *ad libitum* until 16 weeks of age, and C diet *ad libitum* until first parturition; FCF, fed with F diet *ad libitum* until 16 weeks of age, then C diet *ad libitum* until 20 weeks and then F diet *ad libitum* until first parturition. The rabbits were artificially inseminated at 18 weeks of age. CAL group had a higher mortality rate compared with the other groups between 9 and 12 weeks of age (34 v. 3%; P<0.05) and during the last 3 weeks of first pregnancy (14 v. 3%; P<0.05). The CAL and FC females presented higher BW and perirenal fat thickness (PFT) than CR females at 11 days of pregnancy (+0.41 kg and +0.6 mm; P<0.05), with F females showing medium values. The type of feeding procedure did not affect the fertility rate of young females at first artificial insemination. Differences in BW disappeared at parturition, when only CAL females presented a greater PFT than CR and FC females (+0.3 mm; P<0.05). In comparison to FCF, CAL females had smaller and thinner live born litters (–2.5 kits and –139 g, respectively; P<0.05), with CR, F and FC females showing medium values. The low number of kits born alive for CAL females was because of their lesser total number of kits born (–1.7 kits; P<0.05) and the greater mortality of their litters at birth (+13.9 %; P<0.05) compared to FCF females. Non-esterified fatty acid was higher in the blood of females fed C diet (CAL and CR) than in others at partum day (on average +0.15 mmol/L; P<0.05). In conclusion, the *ad libitum* use of diets for lactating rabbit does throughout the rearing period could lead young rabbit females to present a higher risk of early death and smaller litter size at first parturition. Feed restriction or earlier use of suitably fibrous diets led females to achieve the critical BW and fat mass at first mating to ensure reproduction.

Key words: rabbit females, rearing, pubertal development, body condition, metabolic status.

Introduction

The negative effects of underfeeding on pubertal maturation have long been known in numerous species (Frisch, 1984). However, overfeeding during rearing period has been also related with lower reproductive performance in dairy heifers (Sejrsen *et al.*, 1982), pullets (Whitehead, 1988) and gilts (Klindt *et al.*, 1999). Young rabbit females fed *ad libitum* until first parturition usually suffer similar problems to those mentioned for other species (Maertens, 1992).

For this reason, in the last decade some works assessed the possible impact of different management and feeding plans for rearing period on female development and reproduction: feed restriction (Rommers *et al.*, 2004), BW at weaning or at the first artificial insemination (AI) (Rommers *et al.*, 2001a, 2001b, 2002), or the use of fibrous diets (Xiccato *et al.*, 1999; Pascual *et al.*, 2002; Quevedo *et al.*, 2005). However, some of these works have shown an antagonism between proper development and improvement of reproductive response. The earlier the introduction of the restriction programme and the lower the energy supply, the higher the voluntary feed intake of primiparous does with improved milk yield or reduced body reserves mobilisation during first lactation (Nizza *et al.*, 1997; Xiccato *et al.*, 1999; Pascual *et al.*, 2002), but reaching later or inadequate their pubertal maturation (Pascual *et al.*, 2002; Rommers *et al.*, 2004).

On the basis of this previous information, in this work we evaluated the effects on nulliparous rabbit does development of a diet for reproductive rabbit does provided during the rearing period and first pregnancy both *ad libitum* and restricted, compared with three different feeding programmes based on the use of a low energy-high fibre diet designed for young rabbit females and provided: i) until first parturition, ii) until two weeks before first mating and iii) until first parturition applying C diet from 16 to 20 weeks of age.

Material and methods

The experimental procedure was approved by the animal welfare ethics committee of the Universitat Politècnica de València (UPV) and carried out following the European Union (2003) recommendations on care and protection of animals used for experimental purposes and the advice for applied nutrition research in rabbits according to the European Group on Rabbit Nutrition (Fernández-Carmona *et al.*, 2005).

Diets

Ingredients and chemical composition of the experimental pelleted diets used in this trial are summarised in Table 1.1. A control diet (C), similar to a commercial diet for reproductive rabbit does [11.0 MJ digestible energy (DE) and 114 g digestible protein (DP) per kg dry matter (DM)], was formulated following main recommendations of De Blas and Mateos (2010). In addition, a low energy-high fibre diet (F) was also formulated [8.7 MJ DE and 88 g DP per kg DM], including some minor ingredients and supplements to partially correct obvious deficiencies in amino acids and minerals.

Table 1.1 *Ingredients and chemical composition of experimental diets*

	Diets	
	C	F
Ingredient (g/kg)		
Barley	312	78
Alfalfa hay	450	570
Sunflower meal	94	51
Soybean meal	85	-
Sugar beet pulp	-	152
Cereal straw	-	100
Soybean oil	30	10
HCl L-lysine, 780	2	3.9
DL-methionine, 990	-	0.85
L-threonine, 980	-	1.45
L-tryptophan, 980	1	1.5
L-Arginine, 990	-	4
Dicalcium phosphate	17	1.8
Monosodium phosphate	-	16.5
Salt	5	5
Vitamin-mineral premix[1]	4	4
Chemical composition (g/kg DM)		
Dry Matter (DM, g/kg)	899	900
Ash	90	103
Starch	205	63
Ether Extract	52	29
Crude Protein	179	146
Neutral Detergent Fibre	358	476
Acid Detergent Fibre	277	394
Acid Detergent Lignin	59	88
Gross Energy (MJ/kg DM)	18.24	18.67
Digestible energy (DE; MJ/kg DM)	11.03	8.72
Digestible protein (DP; g/kg DM)	114	88
DP/DE (g/MJ)	10.3	10.1

[1] Per kg of feed: Vitamin A: 8,375 IU; Vitamin D3: 750 IU; Vitamin E: 20 mg; Vitamin K3: 1 mg; Vitamin B1: 1 mg; Vitamin B2: 2 mg; Vitamin B6: 1 mg; Nicotinic acid: 20 mg; Choline chloride: 250 mg; Mg: 290 mg; Mn: 20 mg; Zn: 60 mg; I: 1.25 mg; Fe: 26 mg; Cu: 10 mg; Co: 0.7; Butyl hydroxylanysole+ethoxiquin: 4 mg.

Apparent digestibility coefficients of energy and CP were determined for each diet, using a total of 30 three-way crossbred rabbits, aged 42 days with an average BW of 1.32 (s.d. 0.07) kg according to Perez *et al.* (1995).

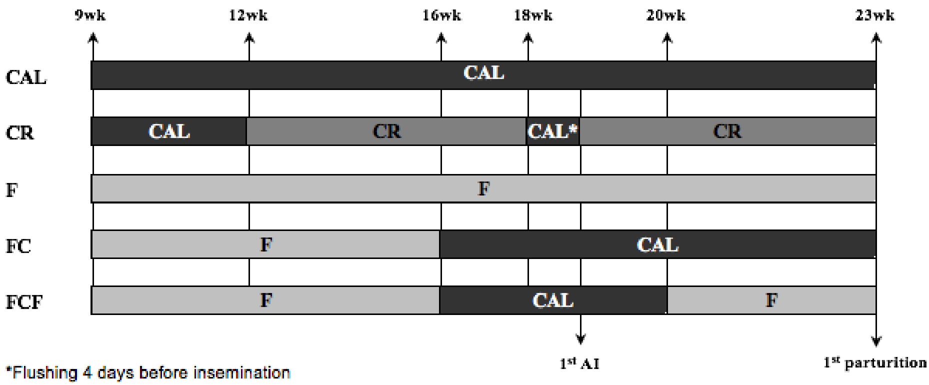
Chemical analysis of diets and faeces were performed following the Association of Official Analytical Chemist (AOAC) (1999) methods for DM, ash, ether extract, CP and crude fibre (934.01, 942.05, 920.39, 976.06 and 978.10, respectively). Ether extract was determined after acid hydrolysis. NDF, ADF and ADL were analysed sequentially (Van

Soest *et al.*, 1991) using a thermo-stable amylase (Thermamyl L120, Novo Nordisk, Gentofte, Denmark) pre-treatment and expressed exclusive of residual ash. Gross energy was determined by adiabatic bomb calorimetry (Gallenkamp Autobomb, Loughborough, UK) following the recommendation of EGRAN (2001).

Animals and experimental procedure

A total of 190 young rabbit does (line A from UPV, selected over 36 generations for litter size at weaning) were used from 9 weeks of age to first parturition. The animals were housed in a traditional building under controlled environmental conditions, with light alternating on a cycle of 16 h light and 8 h dark. The experiment was carried out from January to June 2007.

Until 9 weeks of age, young rabbit females were caged collectively, receiving the same commercial diet *ad libitum* (185 g crude fibre and 175 g CP per kg DM), and subsequently housed in individual cages with access to one of the experimental diets. Combining two diets and three different feeding schemes, five feeding programmes were formed (Figure 1.1).



*Flushing 4 days before insemination

CAL: C diet *ad libitum*; CR: C diet restricted at 140 g per day; F: F diet *ad libitum*

AI: Artificial Insemination

Figure 1.1 Diagram of the different feeding programmes carried out during rearing and first pregnancy for the 5 experimental groups (CAL, CR, F, FC and FCF).

At 9 weeks of age animals were randomly assigned to one of the experimental diets [C (n = 91) and F (n = 99)] to allow the ulterior definition of the experimental groups, considering the early culling of females to provide enough stock for each group. The number of animals at the end of the period controlled for each experimental group is shown in Table 1.2. The CAL group included females which received the C diet *ad libitum* until first parturition. The CR group included females which received the C diet *ad libitum* until 12 weeks of age and then 140 g per day until first parturition, with a 4-day flushing period (C diet *ad libitum*) around AI. The F group included females which received the F diet *ad libitum* until first parturition. The FC group included females that received F diet *ad libitum* until 16 weeks of age and then received C diet *ad libitum* until first parturition. FCF group included females that received F diet *ad libitum* until 16 weeks of age, then received the C diet *ad libitum* until 11 days of pregnancy and finally the F diet *ad libitum* until first parturition.

While animals from different experimental groups kept the same feeding programme, data were analysed and presented as a whole (CAL and CR until 12 weeks of age, F, FC and FCF until 16 weeks of age, and then FC and FCF until 11 days of pregnancy).

Does were artificially inseminated at the end of the 18th week of age. As of this date, successive AI were carried out every 21 days, as necessary and data were only used for effective AI. Average AI to get pregnant was 1.18 and maximum successive AI allowed were 3. Average and maximum age at first positive AI were 18.6 and 27 weeks, respectively. Average and maximum age at first parturition were 23.6 and 32 weeks, respectively. AI was done using polyspermic semen, supplying GnRH hormone by intramuscular injection. Pregnancy was tested by manual palpation at 11 days post-AI. After the 28th day of pregnancy, maternal cages were provided with a nest equipped for the litter. Not-pregnant animals were not included in the data assay.

The traits measured for all does were BW and food intake at 9, 12 and 16 weeks of age, effective AI, 11 days of pregnancy and parturition, as well as perirenal fat thickness (PFT) by ultrasounds at 9 and 12 weeks of age, effective AI, 11 days of pregnancy and parturition. Total and live litter size and weight at parturition were also recorded. From the same 12 rabbit does per group, blood samples were collected at 9 and 12 weeks of age, effective AI and parturition. On sampling day, feeders were closed at 0700 h and blood samples were taken from the central ear artery into EDTA-containing tubes from 1100 to 1300 h. Blood samples were centrifuged immediately after sampling (3000 × g, 4ºC and 10 minutes) and plasma was stored at −20ºC before being assayed for insulin, glucose, non-esterified fatty acids (NEFA), leptin, cortisol and tri-iodothyroxine (T3) concentrations. Controls at 9, 12 and 16 weeks of age and 11 days of pregnancy were done on Mondays, and those at AI on Friday.

Ultrasound measurements

The PFT of rabbit does was measured by ultrasound to evaluate body condition, as described by Pascual *et al.* (2000 and 2004). Images were obtained with an ultrasound unit (JustVision 200 ‘SSA-320A’ real-time machine; Toshiba) equipped with image analyser software to determine distances. Estimated body energy content (EBE; MJ/kg) was determined at AI and parturition from BW and PFT data as described by Pascual *et al.* (2004).

Hormone and metabolite assays

Plasma insulin concentrations were determined by the double antibody/PEG technique using porcine insulin radioimmunoassay (RIA) kit (Linco Research Inc., St Charles, MO, USA). The antiserum was guinea pig anti-porcine insulin, while both labelled antigen and standards used purified recombinant human insulin. Leptin concentrations were determined by double antibody RIA using the multi-species leptin kit (Linco Research Inc.) as previously reported (Brecchia *et al.* 2006). Total T3 was assayed by RIA according to the procedure provided by the manufacturer (Immunotech, Marseille, France). The assay sensitivity was 0.13 ng/mL, and the major analogues of T3 did not interfere with the assay. Plasma cortisol was assayed by RIA, using the CORT kit (ICN Biomedicals Inc., Costa Mesa, CA, USA). CORT assay sensitivity was 0.15 ng/mL. Dilution and recovery tests done on insulin, leptin, T3 and corticosterone using five different samples of rabbit plasma showed linearity.

Glucose was analysed by the glucose oxidase method using the Glucose Infinity kit from Sigma (Sigma Diagnostic Inc., St. Louis, MO, USA). NEFA concentrations were analysed using enzymatic colorimetric assay from Wako (Wako Chemicals GmbH, Neuss, Germany) as previously reported (Brecchia *et al.*, 2006).

Statistical Analysis

The model used to analyse performance, hormonal and metabolic data of young rabbit does during rearing and first gestation was a mixed model (PROC MIXED by SAS, Statistical Analysis System, 2002), in a repeated measure design that considered the variation between animals and covariation within them. Covariance structures were objectively compared using the most severe criteria (Schwarz Bayesian criterion), as suggested by Littell *et al.* (1998). The model included the feeding programme (CAL, CR, F, FC and FCF), the control day (9 and 12 weeks of age, effective AI, 11 days of pregnancy and parturition; data for week 16 was also included for consumption and BW), and their interaction as fixed effects. Random terms in the model included a permanent effect of each animal (p) and the error term (e), both assumed to have an average of zero, and variance σ^2_p and σ^2_e.

Different contrasts were computed to test the significance of the differences between treatments while animals of different experimental groups received the same feeding programme at 12 weeks [(CAL+CR)/2 *vs*. (F+FC+FCF)/3], at 16 weeks [CAL *vs*. CR *vs*. (F+FC+FCF)/3], at effective AI and 11 days of pregnancy [CAL *vs*. CR *vs*. F *vs*. (FC+FCF)/2] and parturition [CAL *vs*. CR *vs*. F *vs*. FC *vs*. FCF].

To analyse the litter data at first parturition, a fixed effects model (PROC GLM of SAS, 2002) was used that included only the feeding programme (CAL, CR, F, FC and FCF). Data concerning mortality of females during the rearing and first pregnancy were analysed according to a nonparametric procedure (PROC NPAR1WAY of SAS, 2002), using a chi-square test for mean separation.

Results

Animal Performance

As it can be seen in the Figure 1.2, a high mortality rate was observed for the animals receiving *ad libitum* the C diet (34%) between 9 and 12 weeks of age compared to those fed with the F diet (3%; P<0.05), probably due to an outbreak of epizootic rabbit enteropathy (ERE). Considering the age of the animals and the short period where ERE was detected, no treatment with antimicrobials was done to control the disease for a better comparison of the feeding programmes. Mortality was low and similar in groups under different feeding programmes from 12 weeks of age to 11 days of pregnancy. However, the CAL group again presented a significantly higher mortality (14%; P<0.05) compared to the other groups (on average 3%) during the last 3 weeks of pregnancy.

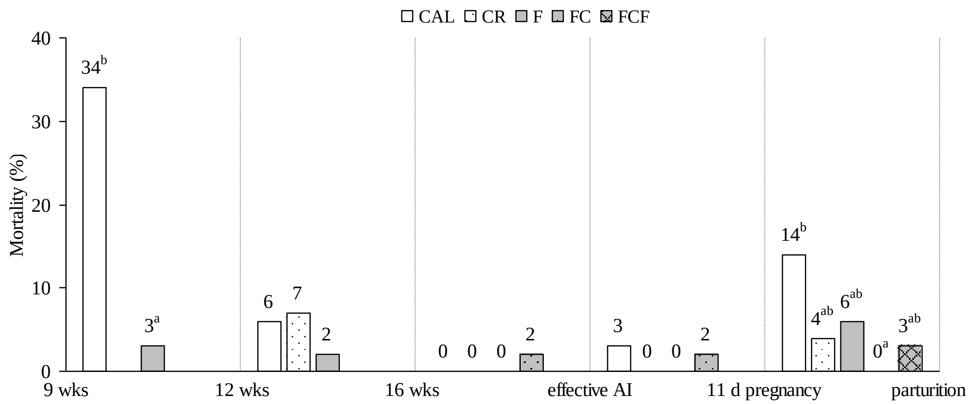


Figure 1.2 Percentage of does dead during the rearing and first pregnancy (from 9 week of age to first parturition) with the different feeding programmes (abbreviations as in Table 1.2). Bars within a period not sharing any superscript are significantly different at P<0.05. AI: Artificial Insemination**.**

Daily intake, BW and PFT of young rabbit does during rearing and pregnancy are presented in Table 1.2 and Figure 1.3. The BW and PFT at 9 weeks of age was 1.97±0.03 (standard error) kg and 6.9 ± 0.1 mm, respectively. Females *ad libitum* fed with C diet (CAL) showed significantly higher DE and DP intake between 9 and 12 weeks of age (+89 kJ and +1.1 g per day, respectively; $P<0.05$) and BW at week 12 (+0.11 kg; $P<0.05$) than those with F diet. From 12 to 16 weeks, DE and DP intake were similar for CAL and F females (on average 841 kJ and 8.6 g per day, respectively), but significantly lower for those restricted (CR; 699 kJ and 7.2 g per day; $P<0.05$). Thus, BW at week 16 was significantly higher for

Table 1.2 *Daily dry matter (g DM per kg metabolic weight ($BW^{0.75}$)) and digestible energy (kJ DE per kg $BW^{0.75}$) intake, and body weight (BW; g) of young rabbit does during rearing and first pregnancy (mean ± standard error).*

	Feeding programme[1]				
	CAL		F		
9-12 wk	(n=60)		(n=96)		
DM intake	82.44^{a} ±1.21		94.05^{b} ±0.94		
DE intake	909.3^{b} ±12.3		819.7^{a} ±9.5		
BW at 12 wk	2771^{b} ±32		2664^{a} ±25		
	CAL	CR	F		
12-16 wk	(n=30)	(n=26)	(n=94)		
DM intake	75.46^{b} ±1.62	63.34^{a} ±1.72	97.43^{c} ±0.91		
DE intake	832.3^{b} ±16.3	698.6^{a} ±12.3	849.0^{b} ±9.2		
BW at 16 wk	3689^{c} ±40	3241^{a} ±42	3466^{b} ±23		
	CAL	CR	F	FC	
16 wk- effective AI	(n=30)	(n=26)	(n=31)	(n=62)	
DM intake	68.58^{b} ±1.50	63.17^{a} ±1.59	90.54^{d} ±1.5	84.28^{c} ±1.03	
DE intake	756.4^{b} ±15.6	696.7^{a} ±16.5	789.0^{b} ±15.5	929.5^{c} ±10.6	
BW at effective AI	4077^{b} ±39	3798^{a} ±41	3818^{a} ±38	3989^{b} ±27	
Effective AI - 11 d pregnancy	(n=29)	(n=26)	(n=31)	(n=61)	
DM intake	66.14^{b} ±1.5	61.78^{a} ±1.59	91.09^{d} ±1.48	75.61^{c} ±1.03	
DE intake	729.5^{b} ±15.6	681.3^{a} ±16.5	793.9^{c} ±15.3	833.9^{d} ±10.6	
BW at 11 d pregnancy	4344^{c} ±39	3933^{a} ±41	4143^{b} ±38	4343^{c} ±27	
	CAL	CR	F	FC	FCF
11 d pregnancy - parturition	(n=25)	(n=25)	(n=29)	(n=31)	(n=29)
DM intake	48.68^{a} ±1.68	53.13^{b} ±1.61	73.18^{d} ±1.5	56.42^{b} ±1.47	61.35^{c} ±1.55
DE intake	536.8^{a} ±17.2	585.9^{b} ±16.5	637.8^{c} ±15.3	622.3^{bc} ±15.0	586.5^{b} ±15.9
BW at parturition	4056 ±56	3994 ±56	4001 ±52	4064 ±52	4065 ±53

[1] Feeding programme: CAL group received the C diet *ad libitum* until 1st partum; CR group received the C diet *ad libitum* until 12 wk and then, 140 g per day until 1st partum; F group received the F diet *ad libitum* until 1st partum; FC and FCF group received F diet *ad libitum* until 16 wk and then, FC group received the C diet *ad libitum* until 1st partum and FCF group the C diet *ad libitum* until 20 wk and then the F diet *ad libitum* until 1st partum; AI: Artificial Insemination; ^{a,b,c,d} Means within a row not sharing any superscript are significantly different at $P<0.05$.

CAL than for F group (3.69 and 3.47 kg, respectively; P<0.05), and higher for both than for CR (3.24 kg; P<0.05). From 16 weeks of age to 11 days of pregnancy, DE and DP intake of F group was even higher (on average 792 kJ and 8.0 g per day, respectively) than that observed for CAL group (742 kJ and 76 g per day; P<0.05) and higher for both than for CR group (690 kJ and 7.1 g per kg per day; P<0.05). In fact, F females going on to *ad libitum* C diet at 16 weeks (FC) showed the highest intake values (on average 883 kJ and 9.1 g per day; P<0.05). In consequence, CAL and FC females at 11 days of pregnancy presented higher BW and PFT (4.34 kg and 7.3 mm, respectively) than CR females (3.93 and 6.7 mm; P<0.05), with F females showing medium values (4.14 kg and 7.1 mm).

The type of feeding programme did not affect the fertility rate of young females at first AI (85.2, 84.0, 89.7 and 85.0% for CAL, CR, F and FC females, respectively).

During the last 3 weeks of pregnancy, F and FC females presented higher DE and DP intake (on av. 630 kJ and 6.5 g per day, respectively) than CR and FCF females (on av. 586 kJ and 6.0 g per day; P<0.05), with CAL females showing the lowest intake values (537 kJ and 5.5 g per day; P<0.05). Thus, differences in BW between feeding programmes disappeared at parturition (Table 1.2), while only CAL females presented a greater PFT than CR and FC females (6.4 *vs*. 6.1 mm, respectively; P<0.05).

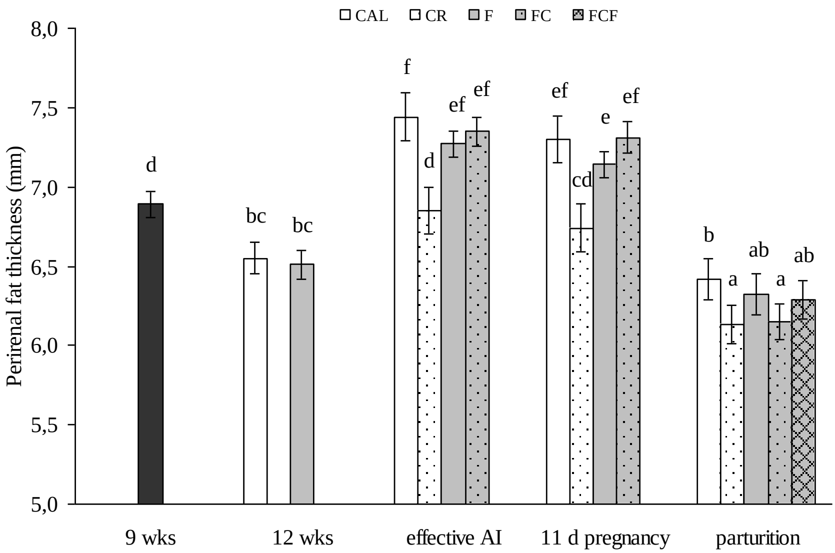


Figure 1.3 Perirenal fat thickness evolution of young rabbit does during rearing and first pregnancy (9 wk of age to first parturition) with the different feeding programmes (abbreviations as in Table 1.2). Data at 9 weeks of age are presented as a whole. Bars not sharing any superscript are significantly different at P<0.05.

Table 1.3 shows the effect of the feeding programme adopted during rabbit doe rearing on litter traits at the first parturition. The lower number of kits born could be caused by the use of pure genetic lines, which have been frequently described as more sensible to the environment. In comparison to the CAL group, FCF females had larger (7.7 *vs*. 5.2 kits; P<0.05) and heavier live born litters (419 *vs*. 280 g; P<0.05), with CR, F and FC females showing medium values (on av. 6.1 kits and 349 g). The small number of kits born alive at first parturition to CAL females was due to their lower number of total kits born (6.6 *vs*. 8.3 kits P<0.05) and the greater mortality of their litters at birth (20.6 *vs*. 6.7 %; P<0.05) compared to FCF.

Table 1.3 *Litter size and weight at first parturition (mean ± standard error).*

	Feeding programme[1]				
	CAL	CR	F	FC	FCF
Litter size at parturition					
Total	6.6^{a} ±0.6	6.8^{ab} ±0.6	6.9^{ab} ±0.6	7.0^{ab} ±0.6	8.3^{b} ±0.6
Alive	5.2^{a} ±0.7	6.2^{ab} ±0.7	6.0^{ab} ±0.6	6.1^{ab} ±0.6	7.7^{b} ±0.6
Mortality at birth	20.6^{c}	8.3^{ab}	12.1^{ab}	12.4^{b}	6.7^{a}
Litter weight at parturition (g)					
Total	340^{a} ±25.4	391^{ab} ±25.4	406^{ab} ±23.6	368^{a} ±23.2	448^{b} ±24.5
Alive	280^{a} ±31.6	355^{abc} ±31.6	369^{bc} ±29.3	323^{ab} ±28.8	419^{b} ±30.4

[1] Feeding programme: Abbreviations as in Table 1.2; ^{a,b} Means within a row not sharing any superscript are significantly different at P<0.05.

Metabolic and hormonal parameters

The plasma profiles of insulin, glucose, NEFA, leptin, cortisol and T3 during rearing and first pregnancy in the different feeding programmes are shown in Figure 1.4. An increase in circulating insulin concentrations was observed with advancing age in all the groups, although it decreased at parturition (Figure 1.4a). CAL group animals presented lower mean plasma insulin concentration than F females at effective AI (−19.5 µUI/mL; P<0.05).

Both glucose and NEFA plasma concentrations showed the highest values at 9 weeks of age and dropped thereafter. Glucose concentration in plasma was opposite to insulin, being lower for CAL (−31.4 mg/dL; P<0.05) than for F females at 12 weeks of age (Figure 1.4b). At partition day, glucose was lower in CAL, CR and FCF than F and FC females (on av. −20.7 mg/dL; P<0.05). Although NEFA levels were similar for all the groups at effective AI (Figure 1.4c), females receiving the C diet (CAL, CR and FC) presented the

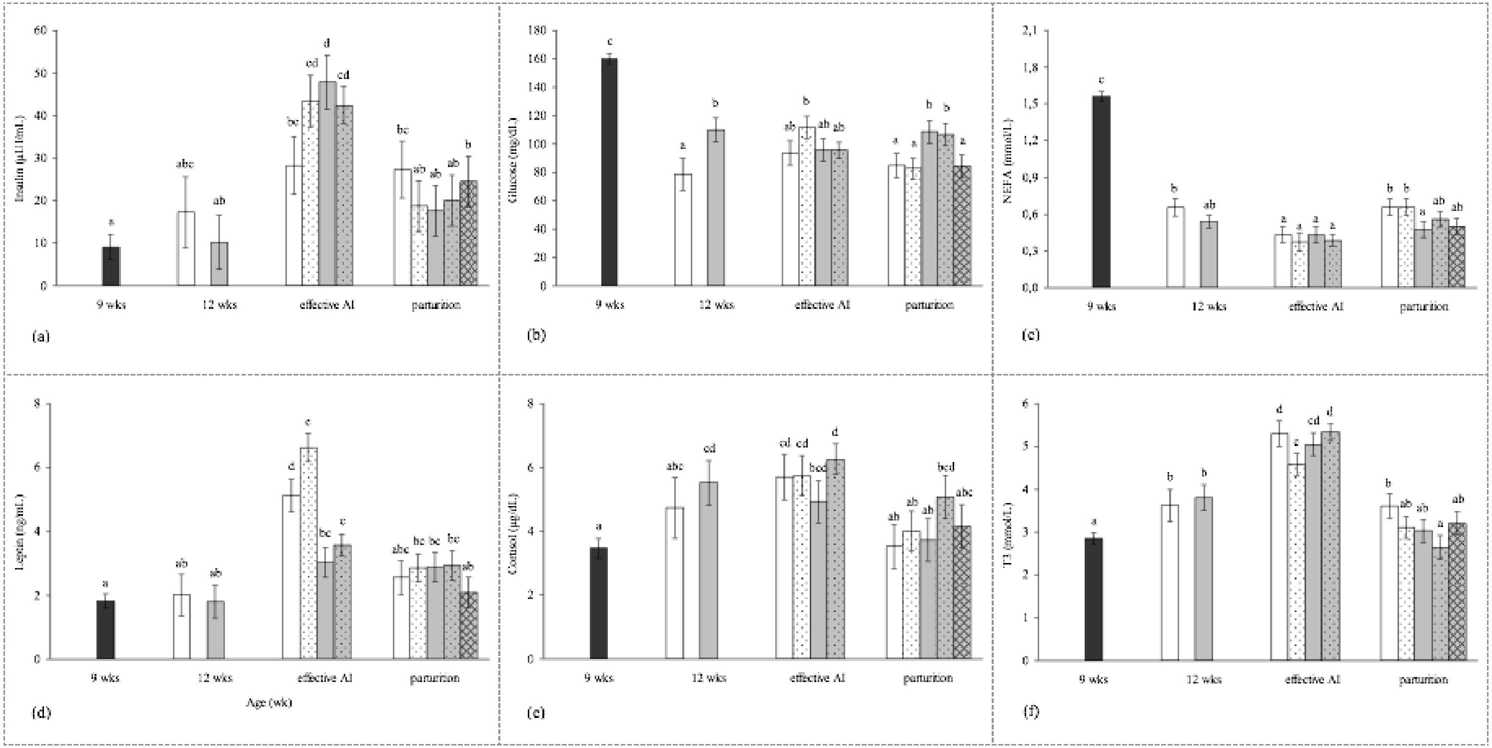


Figure 1.4 Evolution of blood plasma (a) insulin, (b) glucose, (c) non-esterified fatty acids (NEFA), (d) leptin, (e) cortisol and (f) tri-iodothyroxine (T3) concentrations in young rabbit does during rearing and first pregnancy (9th week of age to first parturition) with the different feeding programmes (abbreviations as in Table 1.2). The caption of these figures is the same as in figure 1.3. Data at 9th week of age are presented as a whole. Bars not sharing any superscript are significantly different at P<0.05.

highest NEFA values in plasma at parturition, only being significantly higher in CAL and CR compared to F females (on av. +0.18 mmol/L; P<0.05).

Leptin levels were similar for all groups at 12 weeks of age and at partum day (Figure 1.4d). An increase in plasma leptin concentration was observed at 18 weeks, especially in CR females (6.6 ng/mL; P<0.05), where plasma had higher leptin levels than CAL (5.1 ng/mL), and both F and FC females (on average 3.3 ng/mL; P<0.05). Plasma cortisol increased from 9 weeks of age to effective AI, although it decreased at parturition (Figure 1.4e). No significant differences between feeding programmes on cortisol in plasma were observed throughout the experiment. Plasma concentrations of T3 at 12 weeks of age were similar for all the groups (Figure 1.4f). Females given C diet *ad libitum* at effective AI (CAL and FC) had higher levels of plasma T3 than CR females (on av. 0.75 mmol/L; P<0.05). However, CAL females showed higher T3 levels than FC females at parturition (−0.96 mmol/L; P<0.05).

Discussion

No previous work evaluating the use of rearing diets described the high mortality rate observed in the present work from 9 to 12 weeks of age when young females were fed the control diet. This fact seems to be related to ERE incidence when no medicated diets are used. Under these conditions, insufficient level or inadequate quality of dietary fibre can increase the risk of digestive disorders in young rabbits (Gidenne, 1997; Gidenne and Garcia, 2006). In the current work, although higher soluble fibre was expected for the F diet (from alfalfa and beet pulp), both diets were designed to meet fibre recommendations to prevent digestive problems from 9 to 12 weeks of age (ADL>50, ADF>190 and NDF-ADF>80 g/kg). However, a recent review (Blas and Gidenne, 2010) highlighted that, even if requirements proposed to prevent digestive disorders are met, replacing starch with insoluble or soluble fibre reduces mortality rate, especially in the context of ERE, probably because of favourable changes induced in the caecal environment (Martínez-Vallespin *et al.*, 2011)

Young rabbit female needs from 9 to 12 weeks of age (approx. 1.52 MJ per day, considering their mean live weight and daily gain; Xiccato and Trocino, 2010) were met with both C and F diets (1.74 and 1.54 MJ per day, respectively). Although the lower DE intake led females receiving the F diet to reach 12 weeks with a smaller BW, as in a previous work (Pascual *et al.*, 2002), the main metabolic and hormonal parameters here examined were not greatly affected. Rebollar *et al.* (2011) also found similar concentrations of leptin (2.8 ng/mL) and NEFA (0.22 mmol/L) in the blood of young females at 11 weeks of age when comparing *ad libitum* supplying of control and fibre-rich diets. However, when higher feed restriction is asserted (even below animal needs 1.03 MJ per day; Rommers *et al.*, 2004), the blood levels of glucose, leptin, insulin, and T3 of young females (from 6 to 12 weeks of age) were clearly reduced.

As a consequence of feed intake restriction from 12 weeks of age to effective AI, CR females reached effective AI age with a delay in their development, showing lower BW and PFT than those fed *ad libitum*. These results agree with those reported in previous works where feed restriction reduced BW as well as body fat and protein content of young rabbit females at first AI (Rommers *et al.*, 2001b, 2004), and even caused a delay in the effectiveness of this AI (Rebollar *et al.*, 2011). In the present work, CR females presented a slight reduction of T3 blood levels at effective AI together with an unexpected higher concentration of leptin compared to those with free access to the control diet. Several studies have shown that fasting reduces leptin, mainly synthesised and secreted by adipocytes, circulating in blood at levels proportional to body fat stores in humans (Weigle *et al.*, 1997), gilts (Barb *et al.*, 2001), ruminants (Chilliard *et al.*, 2000), and also in rabbits (Rommers *et al.*, 2004; Brecchia *et al.*, 2006; Rebollar *et al.*, 2011). However, the mechanisms whereby feeding restriction affects circulating leptin levels are still unclear, and different responses were observed depending on type and length of fasting and blood sampling protocols. In this respect, Brecchia *et al.* (2006) described higher leptin levels in the plasma of 48-h fasted than in 24-h fasted does. In any case, it might be considered that CR females were subject to a 4-day flushing period prior to AI, where animals had free access to the C diet, which could have conditioned the plasma metabolic profile for these days.

On the other hand, females with free access to the F diet were able to compensate for the lower nutritive dietary concentration with a greater feed intake from 12 weeks of age to effective AI. Thus, they achieved DE and DP intakes similar to those of rabbits receiving the C diet *ad libitum* and, consequently, reduced their gaps in BW and PFT at effective AI. In fact, these differences disappeared when females of F group had free access to C diet as of 16 weeks of age. Pascual *et al.* (2002) described how young rabbit females fed with a low-energy diet (8 MJ DE/kg DM) from 10 weeks of age, had a greater DE intake than those fed with a restriction of 150 g per day of a standard diet (11 MJ DE/kg DM) during late rearing. The greater feed intake, however, did not compensate earlier differences in BW and these rabbits achieved first AI 10 days later. However, a later introduction of a very low-energy diet at 13 weeks of age (7.3 MJ DE/kg DM; Quevedo *et al.*, 2005) or the use of moderate-energy diets (9.5 MJ DE/kg DM; Xiccato *et al.*, 1999) enabled young rabbit females to achieve first mating at an adequate age and BW.

In this sense, although the use of a low-energy diet during the rearing period led females to reach first mating with lower energy body reserves (Figure 1.5) and lower blood leptin levels than those fed with a conventional diet for reproductive does, no consequence on fertility at first AI was reported. It is well-known that nutrient restriction may delay the onset of puberty, leading to the hypothesis that a critical soma must be achieved before puberty can occur (Frisch, 1980). Furthermore, although fertility rate is affected by many factors, Arias-Álvarez *et al.* (2009) recently proposed that reaching the permissive leptin threshold should be necessary for pubertal reproductive activity and may be associated with inhibition of reproduction if the critical soma is insufficient to trigger gestation (Moschos *et al.*, 2002). In fact, when the relationship between fertility and blood leptin levels of young rabbit females around first insemination is drawn (Figure 1.6), the hypothesis of a leptin threshold for initiation of puberty and reproductive success which is not improved by additional provision of this hormone seems to be confirmed. Consequently, these results could reveal that in terms of *ad libitum* feeding during rearing, both feed restriction and earlier use of a low-energy diet (8.7 MJ/kg DM) could lead females to achieve the critical BW and fat mass at first AI to ensure reproduction, in spite of their lower fatness and leptin content in blood.

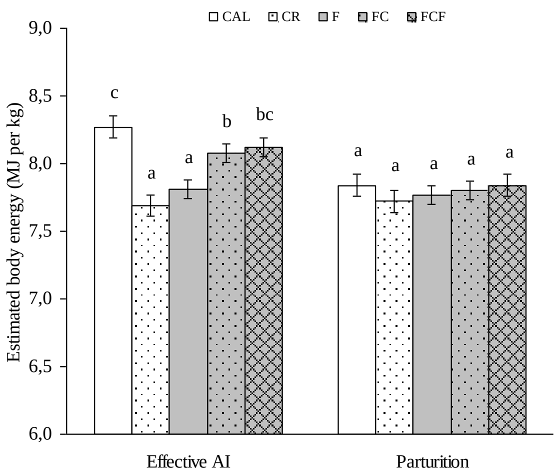


Figure 1.5 Estimated energy content of young rabbit does at effective artificial insemination (AI) and parturition days with the different feeding programmes (abbreviations as in Table 1.2). Bars not sharing any superscript are significantly different at P<0.05**.**

After the first AI, although young females receiving the C diet *ad libitum* maintained a greater consumption than those restricted until 11 days of pregnancy, the fatness accumulated by CAL females throughout rearing allowed them to reduce their feed intake as pregnancy progressed, allowing CR females to diminish the differences in BW, PFT, and EBE observed up to this point with the CAL group during late pregnancy. In a previous work (Rommers *et al.*, 2004), where development between young females fed *ad libitum* and early restricted (restriction: from 5 to 10 weeks of age; recovery: 10 to 17.5 weeks of age) was compared, although compensatory growth of the restricted group was also observed during pregnancy, the early differences achieved in BW of females were maintained throughout the 3 reproductive cycles controlled by the authors. In gilts, where feed restriction of young females has been studied extensively, most works (Sørensen *et al.*, 1998; Klindt *et al.*, 1999 and 2001b) show that moderate feed restriction during the rearing period helps females avoid excessive fatness, while more intense restriction (earlier and/or stronger) leads to smaller development and sometimes even to lower reproductive performance. Therefore, these results seem to confirm the effectiveness of moderate restrictive feeding in preventing excessive fatness in young females, although the starting age and restriction level should be controlled to avoid an inadequate pre-pubertal body development.

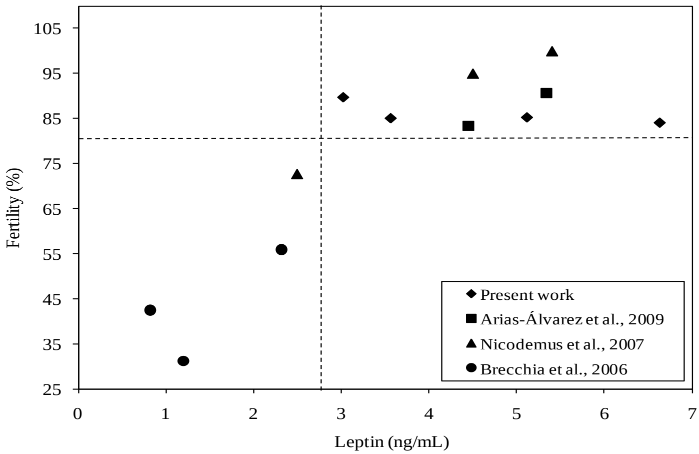


Figure 1.6 Relationship between leptin levels in the blood of young rabbit does at first mating (16-18 wks of age) and the fertility observed during the first reproductive cycle. Data obtained from the present results and three previous works of the literature.

A practical alternative to restriction could be the use of fibrous diets. Several works found in the literature showed that the use of fibrous diets during rearing led nulliparous rabbit females to a greater DE intake after first mating, independently of their previous growth rate during development. Even so, when very low-energy fibrous diets are used (<8.5 MJ DE/kg DM), females are not able to compensate the previous developmental delay (Pascual *et al.*, 2002; Quevedo *et al.*, 2005). However, when females have the chance of receiving a low-energy fibrous diet (approx. 9 MJ DE/kg DM), they reach the first parturition with a development and BW similar to those of rabbit does fed *ad libitum* a diet for reproductive does (>10.5 MJ DE/kg DM), but with a lower fatness (Xiccato *et al.*, 1999;

Rebollar *et al.*, 2011). In the present work, and independently of the fibrous feeding systems used (F, FC or FCF), females reached first parturition in an intermediate developmental situation to that observed in females fed with the C diet *ad libitum* or restricted. Similar results were also obtained by Rebollar *et al.* (2011), where the use of a fibrous diet (9.4 MJ DE/kg DM) from 11 weeks of age to first parturition led young rabbit females to reach the end of first pregnancy with body energy and protein content halfway between *ad libitum* and restricted administration of a control diet (11.6 MJ DE/kg DM). The use of a fibrous diet with 8.5 to 9.5 MJ DE/kg DM should therefore allow young rabbit females to reach first parturition in an adequate state of development, avoiding excessive fatness without the need for feeding restriction.

In fact, the possible negative effects of excessive fatness could be behind the problems detected around first parturition in the CAL group. Compared to the other feeding systems evaluated here, females fed the C diet *ad libitum* during rearing showed the lowest DE intake and the highest body energy mobilisation recorded during late pregnancy. In fact, the plasma of these females at partum day was characterised by higher NEFA and lower glucose levels. The aforementioned profile is frequently related to pregnancy toxaemia risk (Martenink and Herdt, 1988; Bezille, 1995; Rosell, 2000), and could explain the higher mortality in late pregnancy for the females of this group and the smaller size of their litters at first birth caused by both lower total litter size and higher mortality at birth. Rommers *et al.* (2002) also observed that heavier young females at first AI (more than 4 kg BW) had a higher percentage of stillborn at first parturition (13.4%) than smaller females (5%). In gilts, Klindt *et al.* (2001a and 2001b) related an excessive energy intake during rearing with a lower number of corpora lutea and live embryos per gilt, and also observed a tendency towards the reduction of litter size at first birth (−0.8 piglets born) and the increase of gilts removed until this time (+13%). In this sense, the highest prolificacy and the lowest mortality at birth were recorded for females given the F diet with a flushing of 4 weeks with the C diet applied around first mating. In a recent revision, Theau-Clement (2007) concluded that feed flushing after nutritive restriction could improve the reproduction performance, at least at the beginning of the reproductive career.

From the results of the present work it could be concluded that the *ad libitum* uses of diets formulated to cover the needs of lactating rabbit does for the whole rearing period could lead young rabbit females to present a higher risk of early death and smaller litter size at first parturition. As an alternative, either feed restriction or earlier use of an adequate fibrous diet could lead females to achieve the critical BW and fat mass at first AI to ensure reproduction. However, under these feeding programmes for young females, the starting age and nutritive level of the fibrous diet should be controlled to avoid an inadequate prepubertal development. In fact, the introduction of a flushing around first AI when fibrous diets were used led to avoid possible retard on young females' development and the best reproductive results at the first parturition.

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PAPER II

Consequences of rearing feeding programme on the performance of rabbit females from first to second parturition

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Abstract

To evaluated how rearing programmes could affected resources allocation and reproductive performance of primiparous rabbit females, a total of 118 rabbit females were used to evaluate the effects of five rearing feeding programmes on their performance from first to second parturition: CAL, fed *ad libitum* C diet (11.0 MJ digestible energy (DE), 114 g digestible protein (DP) and 358 g neutral detergent fibre (NDF)/kg DM) until first parturition; CR, fed *ad libitum* with C diet until 12 weeks of age and then C diet restricted (140 g/day) until first parturition; F, fed *ad libitum* with F diet (8.7 MJ DE, 88 g DP and 476 NDF/kg DM) until first parturition; FC, fed with F diet *ad libitum* until 16 weeks of age, and C diet *ad libitum* until first parturition; FCF, fed with F diet *ad libitum* until 16 weeks of age, then C diet *ad libitum* until 20 weeks and then F diet *ad libitum* until first parturition. From first parturition, C diet was *ad libitum* offered to all the experimental groups until second parturition. CAL females presented lower feed intake than females of F, FC and FCF groups in the first week of lactation (on av. –16.6%; P<0.05). During first lactation, the perirenal fat thickness change in CAL females was not different from zero (+0.02 mm), while in the other four groups it increased (on av. +0.44 mm; P<0.05). Plasma of females fed with F diet during rearing (F, FC and FCF) had lower non-esterified fatty acids content than those exclusively fed with C diet (–0.088 and –0.072 mmol/L compared to CAL and CR, respectively; P<0.05). FCF litters had higher weight than F litters at day 21 of lactation (+247 g; P<0.05), but FCF litter had significantly lower weight than FC litters at weaning (+170 g; P<0.05). CR females had the shortest average interval between the first and second parturition (49 days) and FCF females the longest (+ 9 days compared to CR; P<0.05). At second parturition, liveborn litters of F females were larger and heavier than litters of FCF females (+2.22 kits and +138 g; P<0.05), probably due to the lower mortality at birth of F litters (–16.5 percentage points; P<0.05). In conclusion, rearing females on fibrous diets seems to increase the ability of primiparous rabbit females to obtain resources, especially at the onset of lactation.

Keywords: Oryctolagus cuniculus, rearing programmes, fibrous diet, body condition, metabolic status, resources allocation.

Introduction

In a previous work (Martínez-Paredes *et al.*, 2012), we were able to confirm that the *ad libitum* use of energetic reproduction diets during rearing had negative effects on young rabbit females until first parturition, such as higher risk of digestive troubles (Rommers *et al.,* 2004) and gestational toxaemia (Viudes-de-Castro *et al.*, 1991 and Rosell, 2000), smaller litter size at first parturition, probably due to a misuse of the available resources (both feed and body reserves), and inappropriate physiological development.

On the other hand, we verified that alternatives, such as restriction and some programmes based on high-fibre diets, allowed them to reach an adequate degree of maturity, without prejudice to the rabbit female or the first litter, when an adequate flushing was applied around first artificial insemination (AI), as well as a greater uptake of resources during pregnancy (Pascual *et al.*, 2002 and Manal *et al.*, 2010). However, these improvements would have less impact if the benefits do not remain in the medium and long term, improving the further reproductive performance of rabbit females (feed intake, milk yield, litter size, survival...). Nonetheless, the number of works that have attempted to elucidate the effects of the restriction or use of fibrous diets on subsequent reproductive performance are few and present variable results. Rebollar *et al.* (2011) did not register improvements in feed intake during the first lactation when young rabbit females were restricted during rearing. Other works also failed to show improvements in the feed intake of primiparous lactating females when fibrous diets were used during rearing (Quevedo *et al.*, 2005; Verdelhan *et al.*, 2005). However, another of these works did report an improvement in feed intake capacity, which was addressed to recovery of reserves (Xiccato el al., 1999) or to milk yield promotion (Pascual *et al.*, 2002). In the long term, some works (Nizza *et al.* 1997; Martínez-Paredes *et al.*, 2018) have observed slight improvements in litter performance at birth or during lactation in females reared on a fibrous diet.

For a better understanding of the consequences that these rearing feeding programmes can have on the future reproductive capacity of our rabbit females, it is essential to assess the changes entailed by their implementation on the ability to obtain resources and their partition among the different vital functions of the females. To this end, the aim of the present work was to evaluate how five different feeding rearing programmes used in a previous work (Martínez-Paredes *et al.*, 2012) could have affected resources allocation and reproductive performance of rabbit females from first to second parturition.

Material and methods

All experimental procedures were approved by the Animal Welfare Ethics Committee of the UPV, which follows Spanish Royal Decree 1201/2005 on the protection and use of animals for scientific purposes and carried out following the advice for applied nutrition research in rabbits according to the European Group on Rabbit Nutrition (Fernández-Carmona *et al.*, 2005).

Composition of experimental diets

Two experimental diets were formulated and pelleted. A control diet (C), similar to a commercial diet for reproductive rabbit does [11.0 MJ digestible energy (DE), 114 g digestible protein (DP) and 358 g neutral detergent fibre (NDF)/kg (DM)], was formulated following the main nutritional recommendations of De Blas and Mateos (2010). In addition, a low-energy high-fibre diet (F) was also formulated (8.7 MJ DE, 88 g DP and 476 g NDF/kg DM). Details of ingredients and chemical composition of both diets can be seen in Table 2.1. Methods for chemical analysis and in vivo determination of DE and DP of both diets can be consulted in Martínez-Paredes *et al.* (2012).

Animals and experimental procedure

In the present work, 118 rabbit females (line A of the Universitat Politècnica de València; UPV), which achieved the first parturition in a previous work (Martínez-Paredes *et al.*, 2012), were controlled from first to second parturition. In this previous work, 190 young rabbit females were subjected to five different feeding programmes from 9 weeks of age to first parturition (Figure 2.1). In brief, C group was fed C diet *ad libitum* until first parturition; CR

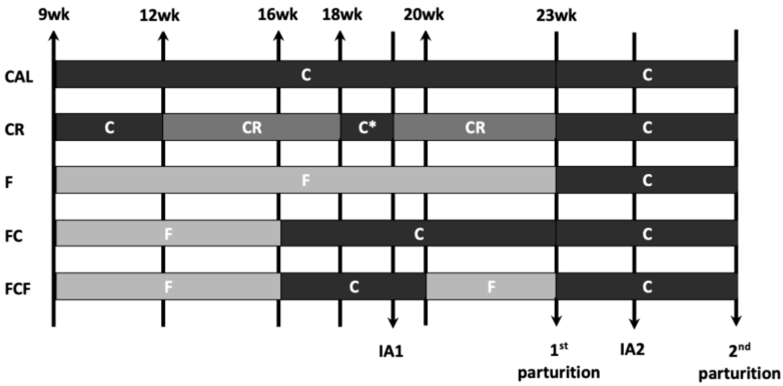

Figure 2.1 Diagram of the different feeding programmes carried out by the rabbit females from rearing to the second parturition for the 5 experimental groups. CAL group received the C diet *ad libitum* until first parturition, CR group received the C diet *ad libitum* until 12 weeks and then, 140 g/day until first parturition, F group received the F diet *ad libitum* until first parturition, FC and FCF group received F diet *ad libitum* until 16 weeks and then, FC group received the C diet *ad libitum* until first parturition and FCF group the C diet *ad libitum* until 20 weeks and then the F diet *ad libitum* until first parturition. * flushing 4 days before artificial insemination.AI1: effective 1st artificial insemination; AI2: effective 2nd artificial insemination; wk: weeks of age.

group was fed C diet *ad libitum* until 12 weeks of age and then 140 g/day until first parturition, with a 4-day *ad libitum* flushing period around the first AI; F group was fed F diet *ad libitum* until first parturition; FC group was fed F diet until 16 weeks of age and then C diet until first parturition, both *ad libitum*; and FCF group was fed F diet until 16 weeks of age, then C diet until 11 days of pregnancy, and finally F diet until first parturition, all of them *ad libitum*. Animals were housed in a traditional building under controlled environmental conditions, with light alternating in a cycle of 16 h light and 8 h dark. For more details of management and results with the different feeding programmes throughout the rearing period, see Martínez-Paredes *et al.* (2012).

At first parturition, litters were standardised to nine kits and all groups were *ad libitum* fed on C diet until second parturition. Rabbit females were AI at 11 days after the first parturition and successive AIs were carried out every 21 days, as necessary. Artificial insemination was performed using polyspermic semen (line R of UPV), supplying gonadotropin-releasing hormone (GnRH) hormone by intramuscular injection. Pregnancy was tested by manual palpation at 11 days after AI. Litter was weaned at 28 days of age. At the 28th day of pregnancy, a nest equipped for the litter was provided.

The traits measured for all females were body weight and feed intake, weekly during the first lactation and at second parturition, as well as perirenal fat thickness (PFT) by ultrasound at first parturition, AI, weaning and second parturition. Daily milk production was measured using the weight(doe)-suckle-weight(doe) method. To prevent free nursing, nest boxes were closed between nursings from first parturition to 21 days of age. From this moment to weaning, litters were housed in a cage close to their mother to control milk production of the female and feed consumption of the litter. Two milk samples were collected on days 4 and 21 of the first lactation from 12 rabbit females per group, following the methodology described by Pascual *et al.* (1999). Litter size and weight were controlled at first parturition after standardisation and weekly until first weaning. Mortality was recorded daily. The interval from first to second parturition of rabbit females and the total and live size and weight of litters at second parturition were recorded. From the same 12 rabbit females per group, blood samples were collected at first parturition, AI, weaning and second parturition. On sampling day, feeders were closed at 0700 h and blood samples were taken from the central ear artery into ethylenediaminetetra-acetic acid containing tubes from 1100 to 1300 h. Blood samples were centrifuged immediately after sampling (3 000 g, 4ºC and 10 min) and plasma was stored at –20ºC before being assayed for insulin, glucose, non-esterified fatty acids (NEFA), leptin, cortisol and tri-iodothyroxine (T3) concentrations.

Table 2.1 *Ingredients and chemical composition of experimental diets for rabbit females.*

	Diet C	Diet F
Ingredient (g/kg)		
Barley	312	78
Alfalfa hay	450	570
Sunflower meal	94	51
Soybean meal	85	-
Sugar beet pulp	-	152
Cereal straw	-	100
Soybean oil	30	10
HCl L-lysine, 780	2	3.9
DL-methionine, 990	-	0.85
L-threonine, 980	-	1.45
L-tryptophan, 980	1	1.5
L-Arginine, 990	-	4
Dicalcium phosphate	17	1.8
Monosodium phosphate	-	16.5
Salt	5	5
Vitamin-mineral mixture[1]	4	4
Chemical composition (g/kg DM)		
Dry Matter (DM, g/kg)	899	900
Ash	90	103
Starch	205	63
Ether Extract	52	29
Crude Protein	179	146
Neutral Detergent Fibre	358	476
Acid Detergent Fibre	277	394
Acid Detergent Lignin	59	88
Gross Energy (MJ/kg DM)	18.24	18.67
Digestible Energy (DE; MJ/kg DM)[2]	11.03	8.72
Digestible Protein (DP; g/kg DM)[2]	114	88
DP/DE (g/MJ)	10.3	10.1

[1] Per Kg of feed: Vitamin A: 8 375 IU; Vitamin D3: 750 IU; Vitamin E: 20 mg; Vitamin K3: 1 mg; Vitamin B1: 1 mg; Vitamin B2: 2 mg; Vitamin B6: 1 mg; Nicotinic acid: 20 mg; Choline chloride: 250 mg; Mg: 290 mg; Mn: 20 mg; Zn: 60 mg; I: 1.25 mg; Fe: 26 mg; Cu: 10 mg; Co: 0.7; Butyl hydroxylanysole+ethoxiquin: 4 mg.

[2] In vivo determination of DE and DP was performed in Martínez-Paredes *et al.* (2012).

Ultrasound measurements

The PFT of females was measured to evaluate body condition, as described by Pascual *et al.* (2000 and 2004). Images were obtained with an ultrasound unit (JustVision 200 'SSA-320A' real-time machine; Toshiba) equipped with image analyser software to determine thickness measurements.

Hormone and metabolite assays

Plasma insulin concentrations were determined by the double antibody/ polyethylene glycol technique using porcine insulin radioimmunoassay (RIA) kit (Linco Research Inc., St Charles, MO, USA). The antiserum was guinea pig anti-porcine insulin, while both labelled antigen and standards used purified recombinant human insulin. Glucose was analysed by the glucose oxidase method using the Glucose Infinity kit from Sigma (Sigma Diagnostic Inc., St. Louis, MO, USA). NEFA concentrations were analysed using enzymatic colorimetric assay from Wako (Wako Chemicals GmbH, Neuss, Germany) as previously reported (Brecchia *et al.*, 2006). Leptin concentrations were determined by double antibody RIA using the multi-species leptin kit (Linco Research Inc.) as previously reported (Brecchia *et al.* 2006). Plasma cortisol was assayed by RIA, using the CORT kit (ICN Biomedicals Inc., Costa Mesa, CA, USA). CORT assay sensitivity was 0.15 ng/mL. Finally, total T3 was assayed by RIA according to the procedure provided by the manufacturer (Immunotech, Marseille, France). The assay sensitivity was 0.13 ng/mL, and the major analogues of T3 did not interfere with the assay. Dilution and recovery tests performed on insulin, leptin, T3 and corticosterone using five different samples of rabbit plasma showed linearity.

Milk chemical composition

Milk samples were analysed for total solids, ash, protein and energy. Total solids and ash contents of milk were obtained using the Association of Official Analytical Chemist (1999) methods. Milk protein content was calculated by the Kjeldahl method according to FIL Standard: 20B (Federation Internationale de Lacterie, 1993). Adiabatic bomb calorimetry method was used to determine the energy content of lyophilised milk.

Statistical analysis

The model used to analyse performance, hormonal and metabolic data and milk composition of rabbit females from first to second parturition and litter weight throughout first lactation was a mixed model (PROC MIXED by SAS, Statistical Analysis System, 2002), in a repeated measure design that considered the variation between animals and covariation within them. Covariance structures were objectively compared using the Schwarz Bayesian criterion, as suggested by Littell *et al.* (1998). The model included the feeding programme (CAL, CR, F, FC and FCF), the overlapping between lactation and gestation (yes and no), the time (control levels for each trait) and their interaction as fixed effects. Random terms in the model included a permanent effect of each animal (p) and the error term (e), both assumed to have an average of zero, and variance σ^2_p and σ^2_e.

To analyse the solid feed intake of litter during last week of first lactation, interval between first weaning to second parturition and litter data at second parturition, a general linear model was used (PROC GLM of SAS, 2002) that included the feeding programme (CAL, CR, F, FC and FCF) and the overlap between lactation and gestation (yes and no).

Different contrasts were computed to test the significance of the differences between treatments, CAL vs. CR, CAL vs. Fs and CR vs. Fs, Fs being 1/3[F+FC+FCF].

Results

No significant differences among rearing feeding programmes for the evolution of females' body weight were observed from first to second parturition (on av. 4 100±59 g). Figure 2.2 shows the evolution of the rabbit females' feed intake from first to second parturition depending on the rearing feeding programme received. CAL group females presented significantly lower feed intake than females from groups F, FC and FCF during the first week of lactation (on av. −38.8 g DM/d; P<0.05). In addition, FCF females showed significantly higher feed intake compared to the rest of the groups during this first week (+65.9, +42.3, +29.5 and +36.5 g DM/d compared to CAL, CR, F and FC, respectively; P<0.05). From this moment to second parturition, differences in daily feed intake among groups disappeared, with the exception of F group, which showed the lowest values at the second week of lactation (on av. −29.4 g DM/d; P<0.05). In the whole period, FCF females had a significantly higher feed intake than CAL females (+19.7±7.4 g DM/d; P=0.0088).

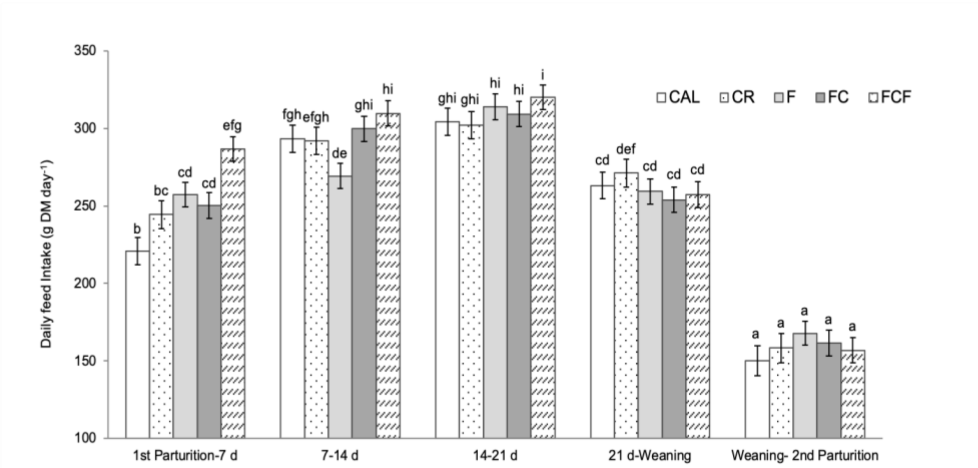

Figure 2.2 Daily feed intake of rabbit females from first to second parturition according to the rearing feeding programme (abbreviations as in Figure 2.1). All the animals, independently of the rearing programme, were fed with the same feed (diet C) from first to second parturition. Bars not sharing any superscript are significantly different at P<0.05.

Figure 2.3 shows the PFT change in rabbit females throughout the first lactation and from first to second parturition. During first lactation, the PFT change in CAL group was not significantly different from zero (+0.02 mm PFT), while the other four groups increased PFT (on av. +0.44 mm; P<0.05). In fact, the PFT increase in CR during lactation was significantly higher in CAL females (+0.55 mm of PFT; P<0.05). From first to second parturition, CAL females showed a significantly different PFT change compared to FC females (–0.24 and +0.29 mm, respectively; P<0.05), while the other four groups kept PFT between parturitions.

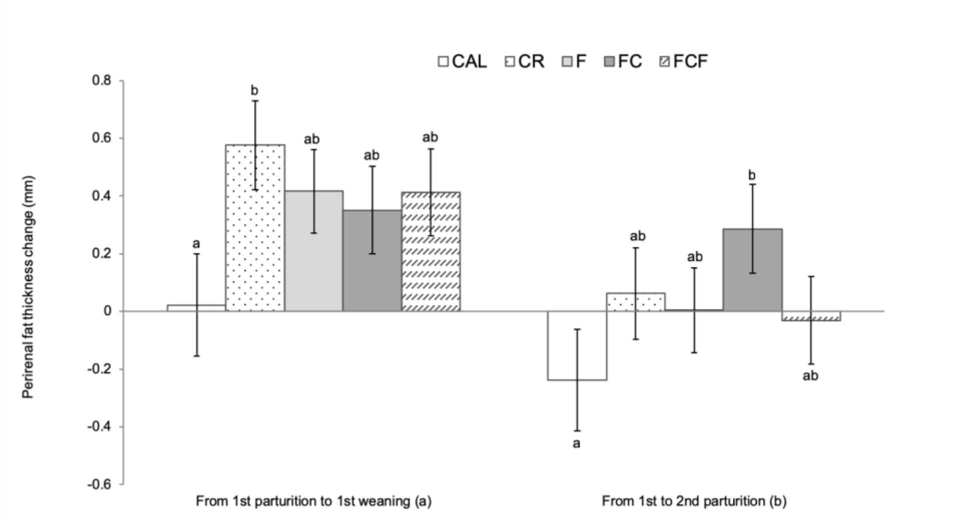

Figure 2.3 Perirenal fat thickness changes of rabbit females during whole lactation and from first to second parturition according to the rearing feeding programme (abbreviations as in Figure 2.1). Bars not sharing any superscript are significantly different at P<0.05.

Females' milk yield during first lactation is shown in Table 2.2. On average, FCF females produced more milk than CAL and F females (+10 and +13 g/d, respectively; P<0.05). Weekly, FC and FCF females yielded more milk than F females at the second week (+22 g/d; P<0.05) and FCF females to CR and F females at the third week (on av. +18 g/d; P<0.05). Milk composition at days 4 and 21 of first lactation is also presented in Table 2.2. Milk from CR females had more total solids (+4.6 and +2.7 g/100g at days 4 and 21, respectively; P<0.05) and lower ash contents (–0.22 g/100g at day 21; P<0.05) than the milk of the other four groups. At day 4 of lactation, F females produced less milk protein than FC and FCF (on av. –2.25 g/d; P<0.05) and less milk energy than FC (–0.21 MJ/d; P<0.05). However, at day 21 of lactation, milk of CR females had higher energy and protein content than FCF milk (+1.0 g/100g and +1.37 MJ/kg, respectively; P<0.05).

Average content of blood plasma parameters in the rabbit females from first to second parturition is shown in Table 2.3. Interaction between rearing feeding programme and time was not significant for any blood plasma trait. There were no significant differences in the insulin, leptin and cortisol content among the experimental groups (on av. 16.03 μUI insulin/mL, 2.95 ng leptin/mL and 4.6 μg cortisol/dL). Plasma of FC blood had higher glucose than CAL and FCF (+19.0 and +16.3 mg/dL, respectively; P<0.05). Plasma of females fed with F diet during rearing (F, FC and FCF) had lower NEFA content than those

Table 2.2 *Average milk yield and composition of rabbit females at first lactation according to rearing feeding programme.*

		Rearing feeding programme[1]							Contrasts[2]		
		CAL	CR	F	FC	FCF	SEM	P-value	CAL–CR	CAL–Fs	CR–Fs
No. of females		18	23	25	26	26					
Milk yield:		172^{a}	174^{ab}	169^{a}	176^{ab}	182^{b}	5	0.0018	-2.4 ± 4.4	-4.3 ± 3.5	-1.9 ± 3.5
	1st week	119	121	122	127	131	6	0.1512	-3 ± 9	-8 ± 7	-5 ± 7
	2nd week	185^{ab}	183^{ab}	170^{a}	192^{b}	192^{b}	6	0.0092	2 ± 9	1 ± 7	-1 ± 7
	3rd week	208^{ab}	204^{a}	206^{a}	212^{ab}	223^{b}	6	0.0221	4 ± 9	-6 ± 7	-10 ± 7
	4th week	175	187	178	175	181	6	0.1443	-13 ± 9	-4 ± 7	9 ± 7
Day of lactation											
Day 4	No. of females	12	12	11	11	12					
	Total solids (g/100g)	31.9^{a}	36.4^{b}	32.7^{ab}	31.6^{a}	31.0^{a}	1.5	0.0185	-4.5 ±2.1*	0.2 ±1.6	4.6 ±1.9*
	Ash (g/100g)	1.65^{a}	1.73^{ab}	1.71^{ab}	1.68^{a}	1.85^{b}	0.07	0.0186	-0.08 ±0.11	-0.10 ±0.07	-0.02 ±0.10
	Protein (g/100g)	10.7	10.7	10.9	10.6	11.1	0.3	0.1816	0.0 ±0.4	-0.2 ±0.3	-0.2 ±0.4
	Protein (g/d)	13.2^{ab}	12.9^{ab}	12.1^{a}	14.3^{b}	14.4^{b}	0.8	0.0383	0.4 ±1.1	-0.4 ±0.8	-0.7 ±1.0
	Energy (MJ/kg)	8.92	8.93	9.33	9.01	9.02	0.45	0.4664	0.02 ±0.76	-0.21 ±0.45	-0.19 ±0.70
	Energy (MJ/d)	1.09^{ab}	1.12^{ab}	1.00^{a}	1.21^{b}	1.16^{ab}	0.07	0.0171	-0.03 ±0.11	-0.04 ±0.06	-0.01 ±0.10
Day 21	No. of females	12	12	11	11	13					
	Total solids (g/100g)	28.3^{a}	32.1^{b}	30.4^{ab}	30.1^{ab}	28.7^{a}	0.09	0.0056	-3.7 ±1.3*	-1.4 ±0.9	2.3 ±1.1*
	Ash (g/100g)	2.12^{b}	1.86^{a}	2.07^{b}	2.04^{b}	2.07^{b}	0.05	0.0013	0.26 ±0.08*	0.06 ±0.06	-0.20 ±0.07*
	Protein (g/100g)	10.6^{ab}	11.1^{b}	10.8^{ab}	10.4^{ab}	10.1^{a}	0.3	0.0435	-0.4 ±0.5	0.2 ±0.4	0.6 ±0.4
	Protein (g/d)	21.8	19.9	21.5	21.3	20.7	0.9	0.1798	1.9 ±1.4	0.7 ±1.1	-1.3 ±1.2
	Energy (MJ/kg)	8.52^{ab}	9.47^{b}	8.77^{ab}	8.71^{ab}	8.10^{a}	0.36	0.0141	-0.95 ±0.54	-0.01 ±0.39	0.94 ±0.47*
	Energy (MJ/d)	1.75	1.71	1.74	1.77	1.66	0.09	0.3112	0.05 ±0.12	0.03 ±0.09	-0.01 ±0.11

[1] Rearing feeding programme: CAL group received the C diet *ad libitum* until first parturition; CR group received the C diet *ad libitum* until 12 weeks and then, 140 g/day until first parturition; F group received the F diet *ad libitum* until first parturition; FC and FCF group received F diet *ad libitum* until 16 weeks and then, FC group received the C diet *ad libitum* until first parturition and FCF group the C diet *ad libitum* until 20 weeks and then the F diet *ad libitum* until first parturition.

[2] Fs: 1/3[F+FC+FCF]; mean±standard error. * Contrast significant at P<0.05. SEM: Pooled standard error of the means.

Table 2.3 *Average blood plasma insulin, glucose, non-esterified fatty acids (NEFA), leptin, cortisol and tri-iodothyroxine (T3) concentrations in rabbit females from first to second parturition according to rearing feeding programme.*

	Rearing feeding programme[1]							Contrasts[2]		
	CAL	CR	F	FC	FCF	SEM	P-value	CAL–CR	CAL–Fs	CR–Fs
No. of females	12	12	12	12	12					
Insulin (μUI/mL)	15.67	18.29	14.82	15.92	15.46	2.67	0.3616	–2.62 ± 3.78	0.27 ± 3.02	2.89 ± 3.14
Glucose (mg/dL)	90.8[a]	93.9[ab]	95.0[ab]	109.8[b]	93.5[a]	5.5	0.0191	–3.1 ± 7.8	–8.6 ± 6.3	–5.5 ± 6.5
NEFA (mmol/L)	0.653[c]	0.637[bc]	0.515[a]	0.590[b]	0.590[b]	0.024	0.0001	0.015 ± 0.034	0.088 ± 0.027*	0.072 ± 0.028*
Leptin (ng/mL)	3.05	3.24	2.78	2.87	2.79	0.25	0.2007	–0.19 ± 0.36	0.24 ± 0.28	0.43 ± 0.30
Cortisol (μg/dL)	4.31	4.61	4.59	4.47	4.82	0.32	0.2510	–0.30 ± 0.45	–0.31 ± 0.36	–0.01 ± 0.37
T3 (mmol/L)	2.81[b]	2.81[b]	2.56[ab]	2.40[a]	2.87[b]	0.11	0.0061	0.00 ± 0.16	0.20 ± 0.13	0.20 ± 0.13

[1] As defined in Table 2.2.
[2] Fs: 1/3[F+FC+FCF]; mean±standard error. * Contrast significant at P<0.05.
SEM: Pooled standard error of the means.
[a,b,c] Means within a row not sharing any superscript are significantly different at P<0.05.

Table 2.4 *Average weight, mortality and solid feed intake of rabbit litters in the first lactation according to rearing feeding programme.*

	Rearing feeding programme[1]							Contrasts[2]		
	CAL	CR	F	FC	FCF	SEM	P-value	CAL–CR	CAL–Fs	CR–Fs
No. of Litters	18	23	25	26	26					
Litter Weight (g) at:										
1st day of life[3]	531	534	538	536	512	51	0.7153	-4 ± 77	2 ± 64	5 ± 59
7th day of life	1 132	1 144	1 173	1 180	1 218	74	0.4182	-12 ± 107	-58 ± 88	-46 ± 85
14th day of life	1 924	1 963	1 871	1 967	2 034	74	0.1181	-39 ± 107	-33 ± 89	5 ± 86
21st day of life	2 657[ab]	2 686[ab]	2 553[a]	2 748[ab]	2 800[b]	75	0.0191	-29 ± 107	-44 ± 89	-15 ± 86
28th day of life (weaning)	4 466[ab]	4 456[ab]	4 441[ab]	4 489[b]	4 319[a]	52	0.0203	9 ± 78	49 ± 66	40 ± 60
Mortality (%)	5.1	7.3	4.5	4.2	5.9		0.6267[4]			
Feed intake from 21st to 28th days of life (g/day)	69.0	69.8	81.1	71.0	81.1	5.2	0.0718	-0.9 ± 7.7	-9.3 ± 6	-8.4 ± 6.1

[1] As defined in Table 2.2.
[2] Fs: 1/3[F+FC+FCF]; mean±standard error.
SEM: Pooled standard error of the means.
[3] Litter size standardised at nine pups.
[4] Probability of Chi-Square.
[a,b] Means within a row not sharing any superscript are significantly different at P<0.05.

Table 2.5 *Average reproductive performance of rabbit females at second parturition according to rearing feeding programme.*

	Rearing feeding programme[1]							Contrasts[2]		
	CAL	CR	F	FC	FCF	SEM	P-value	CAL–CR	CAL–Fs	CR–Fs
No. of females	18	23	25	26	26					
Interval 1st to 2nd parturition (days)	52.53[ab]	49.22[a]	57.52[ab]	51.52[ab]	58.04[b]	3.22	0.0429	3.31 ± 4.72	-3.17 ± 4.01	-6.48 ± 3.57
Litter size at birth:										
Total born	10.63	10.75	10.35	9.39	9.52	0.62	0.1334	-0.13 ± 0.97	0.87 ± 0.78	1.00 ± 0.75
Born alive	7.58[ab]	7.44[ab]	9.30[b]	7.69[ab]	7.08[a]	0.82	0.0389	0.15 ± 1.28	-0.44 ± 1.02	-0.58 ± 0.99
Mortality at birth (%)[3]	26.75[ab]	31.91[b]	11.07[a]	16.25[ab]	27.52[b]	6.12	0.0328	-5.17 ± 9.58	8.03 ± 7.66	13.20 ± 7.40
Litter weight at birth (g):										
Total born	566	577	555	539	536	31	0.1762	-11 ± 47	27 ± 38	39 ± 36
Born alive	419[ab]	408[ab]	515[b]	448[ab]	377[a]	43	0.0155	11 ± 67	-28 ± 54	-39 ± 52
Individual weight at birth (g):										
Total born	56.87	54.94	54.34	60.39	56.34	2.78	0.0803	1.94 ± 4.31	-0.30 ± 3.46	-2.23 ± 3.34
Born alive	57.59	55.16	55.92	61.31	57.66	2.97	0.1314	2.42 ± 4.81	-0.91 ± 3.59	-3.33 ± 3.88

[1] As defined in Table 2.2.
[2] Fs= 1/3(F+FC+FCF); mean±standard error.
SEM: Pooled standard error of the means.
[3] Interaction feeding programme x overlapping degree was significant at P<0.01.
[a,b] Means within a row not sharing any superscript are significantly different at P<0.05.

with C diet (−0.088 and −0.072 mmol/L compared to CAL and CR, respectively; P<0.05). Particularly, NEFA content was the lowest in F females and the highest in CAL females (P<0.05). Finally, plasma T3 content of the CAL, CR and FCF blood were significantly higher than for FC (on av. +0.43 mmol/L; P <0.05).

Table 2.4 shows the performance traits of litters during the first lactation. No significant differences were observed in litter mortality. After litter size standardisation at birth, no significant differences in litter weight at 1^{st}, 7^{th} and 14^{th} day of lactation were observed. However, FCF litters had significantly higher weight than F litters at day 21 of lactation (+247 g; P<0.05). On the contrary, the FCF litter had significantly lower weight than FC litters at weaning (+170 g; P<0.05). No significant differences among groups were observed for litter feed intake during the last week of lactation.

Finally, the reproductive performance of rabbit females at second parturition according to rearing feeding programme is described in Table 2.5. CR females had the shortest interval between the first and second parturition (49 days), significantly different from that obtained for FCF females (−9 days; P<0.05). F females had a significantly higher number of kits born alive at second parturition compared to FCF females (+2.22 kits; P<0.05), probably due to the lower mortality at birth of F litters compared to FCF (−16.5 percentage points; P<0.05), but also compared to CR (−20.8 percentage points; P <0.05). Consequently, F litters had a significantly higher liveborn weight at second parturition than FCF litters (+138 g; P <0.05).

Discussion

The interest of specific rearing feeding programmes mainly lies in providing adequate resources to correctly cover the females' requirements (maintenance, growth and gestation), avoiding possible deficits or excesses (Pascual *et al.*, 2013). A good rearing programme choice should promote an adequate physiological and reproductive development of the females, which should allow a good start to their reproductive life (Martínez-Paredes *et al.*, 2012); but it should also improve the way they obtain and use the available resources, which could have positive effects on their reproductive capacity and lifespan (Martínez-Paredes *et al.*, 2018). In our previous work (Martínez-Paredes *et al.*, 2012), we described the effects of these same rearing programmes on the development of young rabbit females up to the first parturition. In that study, we observed that programmes based on feed restriction or fibrous diets reduced the risk of early death in females associated to digestive disorders and led to achieving an adequate weight and fat mass at first AI, a reserve that was further used to ensure reproduction. On this basis, the present work was focused on how these rearing programmes could also have modified the way females acquire and use the resources available during their first reproductive cycle.

In order to better understand the effects observed from first to second parturition depending on the feeding programme applied during rearing, we decided to discuss each of the feeding programmes separately, to achieve a better view of the evolution of the rabbit females, with results from the previous work (Martínez-Paredes *et al.*, 2012) as starting point.

In the previous work, CAL females were characterised by an overweight at the first AI and a smaller litter size at first parturition. As in previous works (Nizza *et al.* 1997; Pascual *et al.*, 2002), we observed that females' *ad libitum* fed with a non-fibrous diet showed significantly lower feed intake during the first lactation, especially during the first weeks. Excessive overweight during the first gestation has been associated with a reduction in feed intake late in pregnancy, which seems to be maintained at least during the onset of the first lactation (Pascual *et al.*, 2002 and the present work), as differences disappeared thereafter. As a consequence of their reduced ability to obtain resources, CAL females showed the lowest milk output and PFT recovery during first lactation. Blood metabolites confirmed this acquisition and use pattern, with CAL females showing both the lowest glucose and the highest NEFA and T3 concentrations in plasma, in agreement with previous works (Savietto *et al.*, 2014; Arnau-Bonachera *et al.*, 2018). Although the reduced resources acquisition in first lactation did not affect the reproductive performance of the CAL females at second parturition, the use of this rearing programme may lead primiparous females to suffer a higher negative balance in their body condition, with their possible associated risks in the long term (Pascual *et al.*, 2013).

During rearing, CR females accomplished their performance goals, achieving an adequate energy feed intake and body reserves balance, without affecting fertility and litter size at first parturition. In the present work, restriction during the rearing period allowed CR females to show a good body balance during first lactation, which resulted in a reduction in the interval between parturitions. Moreover, we reported no relevant differences in the ability to acquire resources or to use them to produce milk yield when compared to CAL females. Similarly, Bonanno *et al.* (2004) did not find differences in milk yield between females restricted and *ad libitum* fed during the rearing period. In fact, the plasma metabolites profile was similar to that of the CAL group, characterised by low glucose and high NEFA and T3 levels compared to Fs groups. As is well known, rich starch diets promote insulin sensitivity, and consequently glucose infusion rate (Daly *et al.*, 1997). However, the shortest interval between parturitions had negative consequences on the body reserves recovery time, which could also explain the high levels of NEFAs and T3 in CR females. These levels denote a greater mobilisation of the acquired reserves, which may be behind the high mortality at birth observed among the litters of CR females at the second parturition.

In our previous paper, F diet allowed young females to increase their intake capacity already during the rearing period, without any noticeable negative consequence on the reproductive outcomes at first parturition. As a consequence of these effects, most works (Nizza *et al.* 1997; Xiccato *et al.*, 1999; Pascual *et al.*, 2002) have observed an increase in feed intake during first lactation when females were fed with high-fibre diets, compared to commercial diets given *ad libitum*, during the rearing. In the present work, F females only showed higher feed intake during the first week of lactation compared to CAL females, but quite similar to CR females during the first lactation. In any case, receiving a poor diet (rich in fibre and low in starch) throughout rearing may have induced physiological changes in how females may address the acquired resources to the different life functions. Friggens *et al.* (2011) proposed that the nutritional environment may slightly affect gene expression and thus genetically driven partition of nutrients to the different life functions. Therefore, although the F and CR females showed similar resources acquisition and body condition during first lactation, the metabolism of the F females seems to be less dependent on the body reserves to ensure reproduction (lower NEFA levels to CAL and CR groups). In fact, the discrete lower feed intake observed at the second week of lactation in F females, and their possible tendency to safeguard reserves, had as consequences both low milk delivery and low effectiveness in the insemination at that week. Perhaps the females' safeguarding of reserves could also be behind the larger litter size and lower mortality at second parturition of F litters. In fact, Martínez-Paredes *et al.* (2018) described long-term reduced numbers of stillborn and offspring that died during lactation in females fed with a F diet during rearing.

In our previous work, F females that were changed to C diet at two weeks before first AI (FC) showed higher energy intake from that moment onwards and, as a consequence, higher body reserves than F females at the first AI, but similar performance at the first parturition. This feeding programme allowed FC females to show similar feeding and body reserves patterns during the first lactation to that obtained with the F programme, as well as to undergo a similar homeorhetic change to safeguard their body reserves. However, earlier introduction of C diet could have led to additional changes in the females' metabolism and improved adaption to the reproductive feed. This fact can be shown by the promotion of milk metabolism (higher plasma glucose level, milk energy and protein delivery and litter performance) compared to maintenance (reduced T3 level) from similar available resources, especially at the onset of lactation. This preferential use of the energy intake for milk may explain why the litter performance observed at the second parturition for F females was not achieved by the FC females.

Finally, in our previous work, F females fed with a flushing with C diet around first AI (16 to 20 weeks of age; FCF) had the best performance litter traits at first parturition. As a consequence of the larger litter size at birth and/or the adequate feeding management during rearing period, FCF females did achieve one of the main goals proposed for these programmes, an increase in the ingestion capacity during the first lactation (Pascual *et al.*, 2013). FCF females showed the highest feed intake observed during the first lactation, even compared to F females during the first two weeks. Although PFT evolution and plasma energy metabolites were not much different from that observed for the other F groups, the higher feed intake observed in FCF was directly addressed to a clear increase in milk yield and litter growth until the third week of lactation. However, diverting the acquired energy mainly to lactation came with some costs, such as a longer interval between parturitions and the lowest number of kits born alive at the second parturition. In this sense, some previous works have also observed that the use of F diets during rearing has been associated with an increased feed intake and milk yield during lactation of both primiparous and multiparous females (Nizza *et al.*,1997), but no negative effects on litter performance at birth have been described in the long term (Nizza *et al.*,1997; Pascual *et al.*, 2002; Martínez-Paredes *et al.*, 2018).

Conclusions

The results of the present work have confirmed that the possible overweight at the end of the rearing period when young rabbit females *ad libitum* fed with reproductive commercial diets seems to have negative consequences until the second parturition. This *ad libitum* programme decreases primiparous females' ability to obtain resources and leads them to suffer possible negative body balances. The restriction of these reproductive diets during rearing to avoid the cited overweight, although it did not increase the ability of primiparous females to obtain resources, led females to a better energy balance. As an alternative, three different rearing programmes based on the use of a high-fibre low-energy diet have been proposed. We have confirmed the usefulness of these fibrous programmes to increase the ability of primiparous females to obtain resources, especially at the onset of their first lactation and when a previous flushing was applied around first insemination. In addition, the use of these low-energy rearing diets seems to provoke homeorhetic and metabolic changes in females' resources use, which enables females to be less dependent on their body reserves for reproduction. In this way, the additional feeding intake was mainly addressed to milk yield, and although the greater lactational effort could affect next litter size at birth, other works have confirmed that fibrous rearing programmes do not seem to have effects on reproduction in the long term.

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Paper III

Early development and reproductive lifespan of rabbit females: implications of growth rate, rearing diet and body condition at first mating.

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Abstract

Factors influencing early development such as birth weight, nest competition, and the diet received during rearing have been proposed as elements conditioning the future reproductive performance of European rabbit (Oryctolagus cuniculus) females. To evaluate their effects, we followed the life of 1 513 females from birth to time of death, culling or censoring (animals alive at a fixed date). Between 0 and 63 days of age 353 females died. From the remaining 1 160 females, 864 were chosen based on their birth weight to be transferred from the selection to the production farm. At this farm, 431 females received the control diet (184 g of CP, 381 g of NDF and 11.8 MJ of DE per kg DM), while the other 433 received the fibrous diet (134 g of CP, 436 g of NDF and 10.0 MJ of DE per kg DM). Throughout the rearing period, we checked for the individual live weight and body condition (perirenal fat thickness) at first artificial insemination. Reproductive lifespan was defined as the number of days between the first parturition and the time of death, culling or censoring. Birth weight affected the survival of newborn females during lactation and the presence of a milk spot at birth (related to nest competition) increased the survivability of newborns weighing less than 45 g (P<0.001). Rearing di*et* altered the growth curve of females and their body condition at first insemination. The diet also altered the relative risk of death during the rearing period, which was lower among females fed on the fibrous diet (–12.5 %; P<0.001). Therefore, a higher number of females fed with this diet reached their reproductive life, directly affecting the productivity measured per housed female. Fatter females at first insemination had smaller litter sizes and a higher risk of being culled than lean ones (P<0.05). In general, the fibrous diet reduced the risk of leaving the herd at early rearing, and both birth weight and perirenal fat thickness affected female's reproductive lifespan. An excess of fat (positive change in one unit of perirenal fat) at their first insemination represented an increased the risk of death or elimination of 13 %.

Keywords: Oryctolagus cuniculus; birth weight; nutrition; reproduction; survival;

Introduction

In Spain, two out of three captive rabbit females starting their productive life are culled due to disease (mainly respiratory, enteric or alterations in the reproductive organs) or low reproductive performance (e.g. consecutive infertile mattings, large parturition intervals or few liveborn offspring), with renewal rates from 7 to 12 % per month commonly being observed (Rosell and de la Fuente, 2009). Similar values were reported in France, Italy and the Netherlands. According to Rosell and de la Fuente (2009), young females (until third parity) are at high risk of death or culling, as well as females in late pregnancy (between 25 and 33 days). In addition, the voluntary feed intake of primiparous rabbit females could be insufficient to meet the nutrient requirements for milk synthesis and foetal development, establishing a nutritional competition between the mammary gland and the gravid uterus (Fortun-Lamothe, 1999). This condition may be detrimental to the young female's body development (Xiccato *et al.*, 1995) and to their future reproductive life. In this sense, feeding and management strategies favouring at the same time the correct development of the females' body and feed intake capacity should favour their ability to overcome the reproductive and environmental stress and prolong their reproductive lifespan.

Among the pre- and post-natal factors influencing growth and development of the adult rabbit female, birth weight had far greater importance than the size of the litter in which it was raised (Hardman *et al.*, 1970; Poigner *et al.*, 2000). Birth weight was also described as an important variable conditioning the body weight at first insemination (Szendrö *et al.*, 2006), but to our knowledge studies on rearing strategies aiming to increase reproductive lifespan are still lacking. On the other hand, some feeding strategies, such as restricted feeding of young females, have been proposed to ensure correct body development, avoid excessive body fatness upon the first parturition and improve reproductive performance (Rommers *et al.*, 2004a and 2004b). However, feed restriction may increase labour and depending on the feed formulation, undesirable restriction of nutrients rather than energy may occur. One alternative to feed restriction is the use of fibrous diets. Among the reported advantages related with the use of fibrous diets during rearing is an increased survival rate (Martínez-Paredes *et al.*, 2012), improved ovarian and embryo quality (Arias-Álvarez *et al.*, 2009), reduction of kitten mortality at birth (Xiccato *et al.*, 1999; Pascual *et al.*, 2002a; Martínez-Paredes *et al.*, 2012), an increase in the feed intake of young females in the first reproductive cycle (Xiccato *et al.*, 1999; Pascual *et al.*, 2002a; Quevedo *et al.*, 2005), a reduction in the energy deficit during first lactation (Xiccato *et al.*, 1999), and an increase in milk yield and kittens' weight (Pascual *et al.*, 2002a).

In addition, there is evidence that an optimal body condition at the beginning of reproductive life could improve fertility and maximize lifespan in captive gilts (O'Downd *et al.*, 1997; Tarrés *et al.*, 2006) and rabbits (Savietto *et al.*, 2016) and wild rabbits (Wells *et al.*, 2016). Others have reported that excessive fatness at the onset of the reproductive life increases the risk of pregnancy toxaemia around first parturition (Martínez-Paredes *et al.*, 2012) and favours milk production at the expense of the recovery of the fat reserves during lactation (Pascual *et al.*, 2002b). For this reason, we believe that an adequate development allowing young captive rabbit females to reach their first mating with an appropriate level of fat reserves may favour their future reproductive performance and expand their reproductive lifespan.

Therefore, we evaluated the influence of early developmental factors such as birth weight and growth rate, and the diet used during the rearing period on the reproductive performance and lifespan of rabbit females.

Material and methods

All experimental procedures were approved by the Animal Welfare Ethics Committee of the Universitat Politècnica de València who follow the Spanish Royal Decree 1201/2005 on the protection and use of animals for scientific purposes.

Diets

Two diets were designed to feed the young females during the rearing period. The control diet (C) was similar to a commercial diet for reproductive rabbit females and contained 184 g of CP, 381 g of NDF and 11.8 MJ of DE per kg of DM; following the recommendations of De Blas and Mateos (2010). The experimental diet, characterized by its high fibre content (F), contained 134 g of CP, 436 g of NDF and 10.0 MJ DE per kg of DM; following the recommendations of Pascual *et al.* (2013). Ingredients and chemical composition of the diets are listed in Table 3.1.

Animals and feeding schedule

Data on 1 513 newborn crossbreed females of two strains of Universitat Politècnica de València (UPV) Line A × Line V, born from 120 females between December 2008 and October 2009 (14 birth groups), were controlled at birth in a selection farm (Fabara, Zaragoza, Spain). From these 1 513 newborn females, a total of 1 227 survived to weaning (38 days old) and 1 160 reached the slaughter age (63 days). Until this moment, the young females were managed in the same manner and received the same diets (a standard feed for lactation until weaning and then a fattening feed). At 63 days, 864 young females were selected by their birth weight, in order to obtain a similar proportion of light (<55 g; n = 286), medium (from 55 to 65 g; n = 275) and heavy females (>65 g; n = 303) to be transferred to a production farm (Valderrobres, Teruel, Spain).

Table 3.1 *Ingredients and chemical composition of the experimental rearing diets for young rabbit females.*

Ingredients & chemical composition (g per kg of DM)	Control diet (C)	Fibrous diet (F)
Alfalfa meal	290	412
Wheat bran	300	181
Beet pulp	87.4	156
Cereal straw	-	151
Sunflower meal	160	50.0
Soybean meal	27.7	-
Barley	71.8	20.0
Soybean oil	16.9	10.0
Sugarcane molasses	20.0	-
L - Lysine HCL	1.5	-
Methionine OH	1.0	-
L - Threonine	-	1.7
Calcium carbonate	12.7	-
Dicalcium phosphate	-	6.3
Sodium chloride	4.0	5.0
Vitamin and mineral premix[1]	2.0	2.0
Cycostat 66G®[2]	5.0	5.0
Dry matter (DM; g per kg)	905	910
Ash	80	114
Crude protein	184	134
Ether extract	50	26
Neutral detergent fibre	381	436
Acid detergent fibre	195	234
Acid detergent lignin	24	41
Digestible energy (MJ per kg of DM)[3]	11.8	10.0

[1] Vitamin and mineral premix (g per kg): thiamine, 0.25; riboflavin, 1.5; calcium pantothenate, 5.0; pyridoxine, 0.1; nicotinic acid, 12.5; retinol, 2.0; cholecalciferol, 0.1; α-tocopherol, 15.0; phytylmenaquinone, 0.5; cyanobalamin, 0.0006; choline chloride, 100.0; MgSO4 H2O, 7.5; ZnO, 30; FeSO4 7H2O, 20.0; CuSO4, 5 H2O, 3.0; KI, 0.5; CoCl2 6 H2O, 0.2; Na2SeO3, 0.03. 2 Cycostat 66G® Alpharma (Antwerp, Belgium): 66 g of robenidine HCL per kg of product. [3] Digestible Energy (MJ per Kg DM): Estimated using the equation proposed by Villamide *et al.* (2009): DE=16.43–0.0191·Acid Detergent Fibre–0.0208·Ash+0.0148·Ether extract.

Half of the selected females were then assigned to diet C (n = 431) and the other half to diet F (n = 433), blocking by their birth weight within birth batch. They had *ad libitum* access to the diets throughout the rearing period (from 63 d to first parturition). At the production farm, young females housed between December 2008 and February 2009 were exposed to an outbreak of rabbit haemorrhagic disease. From the 191 females exposed, a total of 134 (diet C = 69 and diet F = 65) died before delivering their first parturition. To avoid the natural selection effect caused by exposure to the virus, we did not consider the data on the 191 females exposed to it. From the remaining 673 young females (diet C = 335 and diet F = 338), 461 had at least one litter (diet C = 210 and diet F = 251). At first parturition, all females received diet C until time of death, culling or censoring.

Experimental procedure

At birth, newborn females were individually weighed and identified with a subcutaneous glass microchip (8.5 mm × Ø 1.4; Felixcan Animal ID, Albacete, Spain) and the presence of abdominal milk spot was registered. All females were weighed again at 38 and 63 days old. At the production farm, young females were weighed every 25 days, resulting in a total of 5 measures per female that had at least one litter. At first artificial insemination (157 days old), perirenal fat thickness (PFT) was recorded as an indicator of body condition using the ultrasound method described by Pascual *et al.* (2004).

Throughout reproductive life, litter size at birth (number liveborn and stillborn) and at weaning (35 days) was recorded. Litters were standardized to eight or nine kittens at first parturition and to a maximum of 11 in the subsequent ones. Females followed a theoretical reproductive rhythm of 49 days (inseminated at 18 days' post-parturition). In each reproductive cycle, non-pregnant females were re-inseminated 21 days after the scheduled post-partum insemination, until a maximum of two consecutive negative attempts before being culled due to low fertility. Rabbit females were also culled owing to low productivity (less than seven kittens weaned in three consecutive parities) or health disorders (sore hocks, mastitis, abortions or low body condition). Data of females alive at the end of the experiment (November 2011) were treated as a censored record.

Statistical analysis

Statistical analyses were performed with R software (R Core Team, 2016).

The probability of newborn females reaching weaning (0, dead; 1, alive; n = 1 513) was analysed using a logistic regression model (glm function with binomial link). The model [1] included the female's birth weight $(\mathrm{birth_i})$, the presence or not of a milk spot $(1 \text{ or } 0;\ \mathrm{milk_i})$ and their interaction as covariates:

$$[1]\ \mathrm{Logit(sucess\ rate)_i} = \beta_0 + \beta_1 \cdot \mathrm{birth_i} + \beta_2 \cdot \mathrm{milk_i} + \beta_3 \cdot (\mathrm{birth} \times \mathrm{milk})_i + \varepsilon_i$$

The error term of model [1] was assumed to follow a Bernoulli distribution $\varepsilon \sim B(n,p)$.

Reproductive performance (RP: the cumulative number of liveborn, stillborn, dead during lactation and weaned kittens) were analysed using a linear mixed-effect model (LMM) including the rearing diet ($\mathrm{diet_i}$) and the covariates birth weight ($\mathrm{birth_i}$) and PFT at insemination ($\mathrm{PFT_i}$) as fixed effect and the birth batch ($1|\beta_{0\cdot\mathrm{batch}}$) as a random intercept. The error term was assumed to follow a Poisson distribution $\varepsilon \sim P(\lambda,\lambda)$. Two models were used: model [2.1] to analyse the data on females with at least one litter and model [2.2] to analyse the data on all females housed at the production farm (a zero was assigned to the RP variables of females not reaching the productive life).

$$[2.1]\ \mathrm{RP_i} = (1|\beta_{0\cdot\mathrm{batch}}) + \beta_1 \cdot \mathrm{birth_i} + \beta_2 \cdot \mathrm{PFT_i} + \beta_3 \cdot \mathrm{diet_i} + \varepsilon_i$$

$$[2.2]\ \mathrm{RP\ _i} = (1|\beta_{0\cdot\mathrm{batch}}) + \beta_1 \cdot \mathrm{birth_i} + \beta_2 \cdot \mathrm{diet_i} + \varepsilon_i$$

Fertility, defined as the number of parities per insemination (only considering females having at least one litter; n = 461), was also analysed using a LMM.

$$[3.1]\ \mathrm{Fertility_i} = (1|\beta_{0\cdot\mathrm{batch}}) + \beta_1 \cdot \mathrm{birth_i} + \beta_2 \cdot \mathrm{PFT_i} + \beta_3 \cdot \mathrm{diet_i} + \varepsilon_i$$

Number of inseminations performed on females that never conceived (34 females) was also analysed using a LMM. Model [3.2] was similar to model [3.1]:

$$[3.2]\ \mathrm{Inseminations_i} = (1|\beta_{0\cdot\mathrm{batch}}) + \beta_1 \cdot \mathrm{birth_i} + \beta_2 \cdot \mathrm{PFT_i} + \beta_3 \cdot \mathrm{diet_i} + \varepsilon_i$$

The error term of models [3.1] and [3.2] were assumed to follow a normal distribution $\varepsilon \sim N(0,\sigma^2)$.

Survival ability of females (between 63 days until natural death, culling or censoring) was analysed using the Cox proportional hazard regression model. To test the effect of rearing diet, model [4.1] included the diet as a stratification variable of the baseline hazard function, the year season (defined in function of the entering date at production farm: 1 = Apr to Jun/2009; 2 = Jul to Aug/2009; 3 = Sep to Nov/2009; 4 = Dec/2009) as a non-independent variable ($\mathrm{cluster_i}$) and birth weight was considered as a covariate ($\mathrm{birth_i}$):

$$[4.1]\ \mathrm{h_i(t)} = \mathrm{h_0(t)} \cdot \exp(\beta_1 \cdot \mathrm{birth_i} + \beta_2 \cdot \mathrm{cluster_i}) + \varepsilon_i$$

To study the influence of PFT at first insemination on the survival of rabbit females, model [4.2] included the year season as baseline hazard function, the diet as a non-independent covariate ($\mathrm{cluster_j}$) and the PFT as covariate:

$$[4.2]\ \mathrm{h_i(t)} = \mathrm{h_0(t)} \cdot \exp(\beta_1 \cdot \mathrm{PFT_i} + \beta_2 \cdot \mathrm{cluster_i}) + \varepsilon_i$$

Model diagnosis for proportional hazards, influential observations and non-linearity was performed following the recommendations of Fox (2002).

Female body growth was fitted using the nonlinear Weibull growth model:

$$[5.0]\ \text{Live weight} = \alpha - \beta \cdot e^{(-e^{K} \cdot \text{Age}^{\delta})}$$

where (α) is the upper asymptote, (β) the growth range, $(-e^{K})$ the growth rate and (δ) how growth slows down with age. These parameters were estimated using the nonlinear (weighted) least square method. Two Weibull growth models were fit, one for diet C and one for diet F. Estimated parameters for diet C and diet F were assumed to be different if their 95 % confidence interval did not overlap.

Results

Survival during lactation

From the 285 females died before weaning, 66.7 % had not suckled at birth (absence of milk spot). The lack of milk spot significantly increased mortality of newborns weighing less than 45 g at birth (P<0.05; Figure 3.1). Among newborn females that had not suckled at birth, an increment of 1 g on birth weight represented a relative increment of 10.2 % in the odds of surviving to weaning. However, among newborn females that suckled at birth, an increment of 1 g on birth weight represented only 4.7 %.

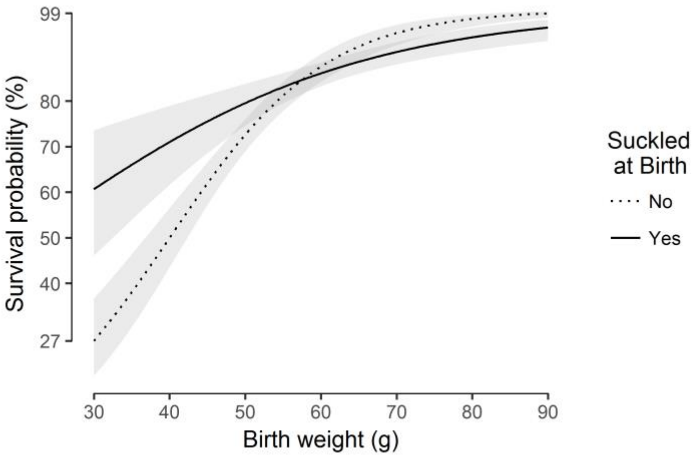

Figure 3.1 Predicted probability (%) of newborn rabbit female survive to weaning age (38 days) depending on its birth weight and whether it had suckled milk or not just after birth. Shaded areas represent the 95% confidence interval.

Development during rearing

Growth curves of young females from birth to the age of first parturition are shown in Figure 3.2 (left panel). Almost linear growth was observed between 0 and 63 days (R^2=0.95), where females gained on average 27.8 g per day.

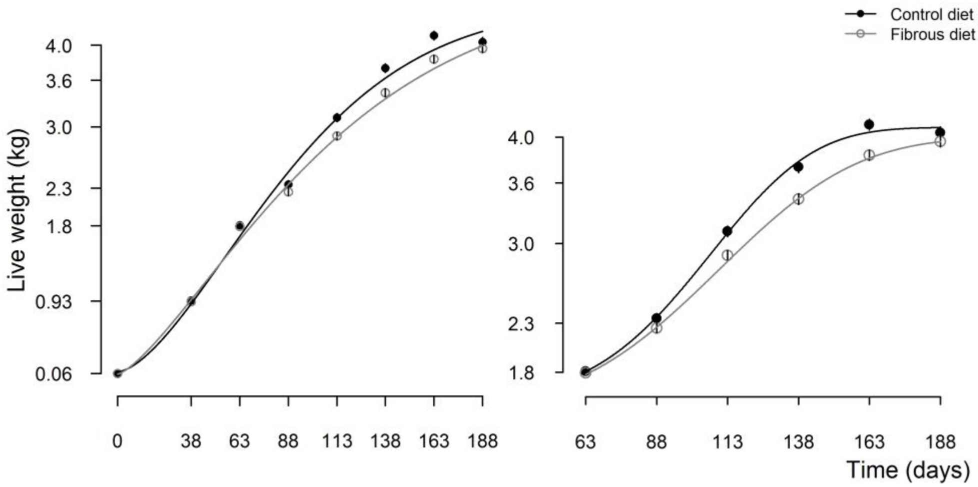

Figure 3.2 Weibull growth curves for young rabbit females fed with control or fibrous diet from 63 to 188 days. Points represent the average live weight and the vertical bars around means represent the 95 % confidence interval (no overlapping bars are significantly different). Left panel shows growth curves from 0 to 188 days. Right panel shows growth curves from 63 to 188 days old.

From 63 days, the period when young females where fed with the C or F diet, different growth patterns were observed (right panel), especially between 88 and 163 days (P<0.05). Females had grown faster with diet C, reaching their maximum weight 6 days before the first insemination age, whereas females on diet F reached this weight approximately 37 days later (P<0.05). Parameters of the fitted Weibull growth models confirmed the differences in live weight observed between diet C and F (Table 3.2). Model parameters for diet C (from 63 to 188 days) had higher influence on the upper asymptote (α) and growth rate ($-e^{K}$) than those for F diet. In addition, we found no influence in the growth range (β) or the growth slope of (δ) for the diet effect, as their 95% confidence interval did not overlap.

Measures of PFT were available on 494 females alive at the insemination age (diet C = 226 and diet F = 268). At first insemination, the PFT range varied from 4.1 to 8.1 mm. Its average was 6.28 mm and a SD of 0.54 mm. For females fed with diet C, the PFT range was 4.7 to 8.1 mm with an average of 6.40 mm and a SD of 0.56 mm. For females on diet F, PFT ranged from 4.1 to 7.7 mm, with an average of 6.19 mm and a SD of 0.50 mm. At first insemination, females fed with diet F had, on average, −0.20 ± 0.04 mm of PFT (P <0.05) and −271.8 ± 32.6 g of body weight (P<0.05) less than females fed with diet C (Table 3.3).

Table 3.2 *Parameters of the Weibull growth curves of rabbit females fed with control (C) or fibrous (F) diet during their rearing period. SE is the standard error of each estimated parameter.*

		Weibull growth curve parameters[1]							
Growth period	Rearing diet	Alpha (α)	SE	Beta (β)	SE	Kappa (K)	SE	Delta (δ)	SE
From birth to 188 days	Diet C	4 500	56	4 419	65	-7.4	0.15	1.6	0.04
	Diet F	4 695	96	4 642	96	-6.5	0.12	1.4	0.03
From 63 to 188 days	Diet C	4 115	23	2 479	64	-18.9	1.07	4.0	0.22
	Diet F	4 025	31	2 461	74	-15.8	0.88	3.3	0.18

[1] Weibull growth model [$Live\ weight\ =\ \alpha - \beta \cdot e^{(-e^{K} \cdot \mathrm{Age}^{\delta})}$], where alpha ($\alpha$) is the upper asymptote, beta (β) the growth range, kappa ($-e^{K}$) the growth rate and delta (δ) how growth slows down with age.

Table 3.3 *Least square means and SE for insemination weight, perirenal fat thickness and fertility rate of rabbit females fed with control (C) or fibrous (F) diet during their rearing period.*

	Rearing diet				Contrast of means
Variable	Diet C	SE	Diet F	SE	C – F
Insemination weight (g)	4 131	47	3 859	46	271.8*
Perirenal fat thickness (mm)	6.4	0.1	6.2	0.1	0.20*
Fertility rate (%)[1]	85.8	1.2	83.5	1.1	2.3

Contrast of means followed by a star (*) are significant different from zero at P <0.05 (*t*-test).[1] Fertility rate was defined as the number of parities per insemination through a female reproductive life. No difference was observed in the number of inseminations spend for females that never conceived (Diet C: n = 16; 1.5 ± 0.3 inseminations. Diet F: n = 18; 1.7 ± 0.3 inseminations).

Reproductive performance

Cumulative number of liveborn, stillborn, weaned and dead kittens according to the rearing diet are shown in Table 3.4. Among females who had at least one litter, the diet effect had no influence, except for the high number of stillborn (+22.8 %; P<0.001) and death kittens during lactation (17.6 % higher; P<0.001) for litters of females fed with diet C. When we considered all females housed at the production farm, the cumulative number of liveborn and weaned kittens was higher for females fed on diet F (on average +6.1 liveborn and +4.8 weaned kittens; P<0.001) compared to those fed with diet C.

Table 3.4 *Cumulative number of liveborn, stillborn, weaned, death kittens during lactation and their SEM according to the diet females received during their rearing period [control (C) or fibrous (F) diet].*

Population[1]	Success Reproduction					Whole Population				
Rearing diet	Diet C (*n* = 210)	SE	Diet F (*n* = 251)	SE	P-value	Diet C (*n* = 335)	SE	Diet F (*n* = 338)	SE	P-value
Liveborn	50.4	1.56	50.4	1.54	0.983	31.3	1.63	37.4	1.94	<.001
Stillborn	5.76	0.49	4.69	0.40	<.001	3.65	0.25	3.46	0.24	0.172
Weaned	39.5	1.34	39.6	1.33	0.890	24.5	1.29	29.3	1.53	<.001
Death[2]	5.71	0.40	4.86	0.34	<.001	3.57	0.22	3.57	0.22	0.962

[1] Success Reproduction: females having at least one partum; Whole Population: females house at the production farm. [2] Offspring that died during lactation.

Regardless of the rearing diet (Table 3.5), the covariate birth weight only affects the number of weaned kittens of females reaching the reproductive life. Each unit of increment of birth weight value increased the number of weaned kittens by 0.5. In the case of the covariate PFT, it negatively affected the number of liveborn and weaned kittens (P<0.001). Each unit of increment in the PFT value reduced the cumulative number of liveborn and weaned by 3.2 and 3.0 kittens, respectively.

Table 3.5 *Estimated regression coefficients for model 2.1 and 2.2 used to evaluate the effect of birth weight, perirenal fat thickness (PFT) and the fibrous diet (F) rabbit females received during rearing on reproductive traits.*

Population[1]	Success Reproduction (Model [2.1])				Whole Population (Model [2.2])		
Regression Coefficients[2]	Intercept	Birth weight	PFT	Diet F[3]	Intercept	Birth weight	Diet F[3]
Born alive	3.92**	0.009	-0.066**	-0.000	3.44**	-0.005	0.176**
Stillborn	1.75**	-0.002*	0.027	0.206**	1.29**	0.004	-0.055
Weaned	3.67**	0.014*	-0.078**	0.002	3.20**	-0.006	0.179**
Death[3]	1.74**	-0.005	0.011	-0.162**	1.27**	0.009	-0.002

[1] Success Reproduction: females having at least one partum; Whole Population: females housed at the production farm. [2] Regression coefficients followed by two stars (**) significantly differed from zero at *P*<0.001 and those followed by a one star (*) differed from zero at *P*<0.05. [3] Diet effect was coded as 0 for diet C and 1 for diet F. Birth weight and PFT covariates were standardized to perform the multiple regression.

From the 673 females entering the production farm, 34 females reaching the insemination age never conceived (diet C = 16 and diet F = 18). For those females fed with diet C, the average number of artificial insemination attempts was 1.5 ± 0.3. The value for females fed with diet F was similar (1.7 ± 0.3). Among females having at least one litter, the number of parities over the number of artificial inseminations was not influenced by the diet (diet C = 85.8 ± 1.2 % *vs*. diet F = 83.5 ± 1.1 %; Table 3.3). No effect of the covariates birth weight or PFT was observed.

Productive lifespan

The proportion of females fed with diet C or F according to their maximum parity order is in Figure 3.3. From the 673 females transferred to the production farm (diet C = 335 and diet F = 338), 31 % never conceived (diet C = 125 and diet F = 87). From the 461 females having at least one litter (diet C = 210 and F = 251), 274 females (41 %) had between one and five litters, from which 129 (47 %) were fed with diet C and 145 (53 %) were fed with diet F. Females having between six and ten litters represented 23 % of the data, from which 41 % received diet C and 59 % the diet F. Only 31 females had 11 litters or more (55 % on diet C and 45 % on diet F).

The overall relative hazard risk of culling or death for females fed with diet F was 13 % lower than females fed with diet C ($P<0.05$). From Figure 3.4 (left panel), which represents the percentage of live females throughout the experimental period according to the rearing diets, two drops in the percentage of live females can be distinguished, one around day 91 and another around day 120. Higher losses were observed for females on diet C than females on diet F.

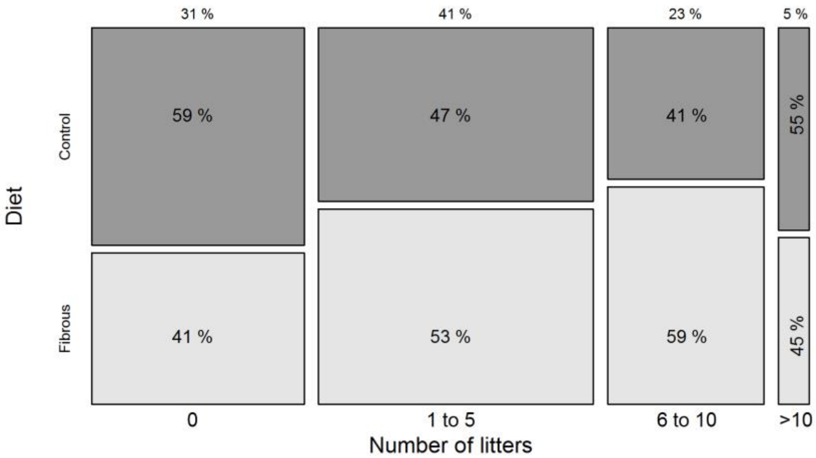

Figure 3.3 Proportion of rabbit females fed with control or fibrous diet according to their maximum number of litters (0 = never conceived; 1 to 5 = females having at least 1, 2, 3, 4 or 5 litters; 6 to 9 = females having at least 6, 7, 8, 9 or 10 litters; > 10 = females having at least 11, 12, 13, 14 or 15 litters).

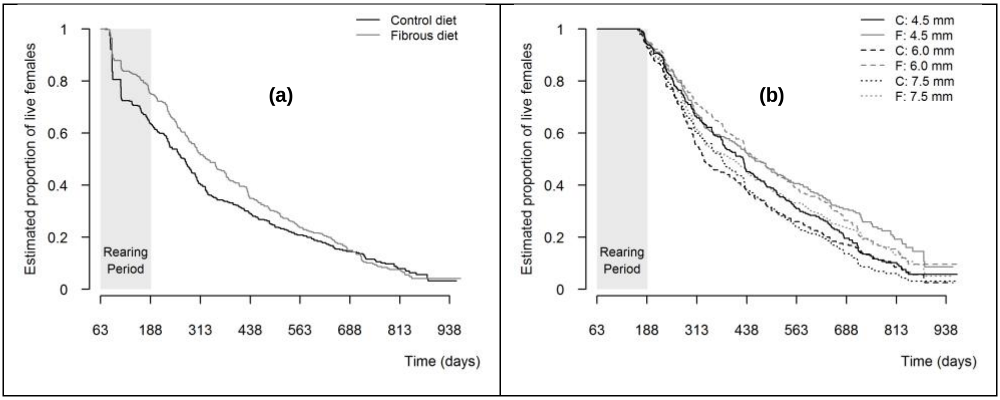

Figure 3.4 (a) Estimated proportion of live rabbit females fed with the control (C) or fibrous (F) diet during rearing period. (b) Estimated proportion of live females considering the diet and three hypothetical levels of perirenal fat thickness: 4.5, 6.0 and 7.5 mm.

Independently of the diet, survival analysis showed that per unit of augmentation in the PFT, the overall relative hazard risk of culling or death incremented by 13 % (P <0.05). However, no difference in the relative hazard risk was observed between females reared with diet C or F (13 % *vs*. 14 %) during their reproductive life. Figure 3.4b exemplifies the effect of PFT on the proportion of females alive throughout the experimental period for three hypothetical PFT levels: 4.5, 6.0, and 7.5 mm. No diet differences were observed within these proposed PFT levels, the females with low PFT levels at insemination being the ones at lower risk of death or culling.

Discussion

Survival during lactation

Many factors which take place early in life may condition survival and reproductive performance of captive rabbit females. Our results revealed a greater chance of survival as birth weight increases, in agreement with Argente *et al.* (1999), who described a positive correlation (+0.30) between birth weight and offspring survival in the first week of life. In fact, some authors proposed a minimum birth weight below which the chances of survival decrease (Argente *et al.*, 1999: 50 g, Drummond *et al.*, 2000: 43 g, Coureaud *et al.*, 2007: 48 g, present work: 45 g). In addition, heavier newborns are usually located in the central positions of the litter huddle, a position that reduces the heat loss and facilitates access to the mother's teats (Bautista *et al.*, 2015). Heavier newborns also have more fat reserves (García-Torres *et al.*, 2015), supporting the relevance of birth weight in newborn survival.

Among the smallest newborn of a litter, milk intake is crucial to survival (Argente *et al.*, 1999; Coureaud *et al.*, 2000). The inability to suckle not only hampers correct development, it also alters the circadian rhythm, impairs the regulation of body temperature and reduces the capacity to compete with the well-nourished littermates for milk (Jilge *et al.*, 2001). All these factors have an impact on survival. In this sense, our results provide evidence that small newborns that had access to milk in the first hours after birth almost doubled their chances of survival when compared to those that had not suckled.

Development during rearing

Adequate growth during the rearing period is crucial to ensure proper physical and physiological development of future reproductive females. In this phase, the diet must provide all the nutrients to cover the maintenance requirements, promote lean growth and ensure an adequate level of body fatness that is essential for their future reproductive career. Energy requirements for this period vary from 1.0 to 1.3 MJ per day depending on the age and size of females (Xiccato and Trocino, 2010). However, when young females are fed with feeds designed for lactating does, their daily energy intake is much higher than required and this overconsumption affects their reproductive performance (Martínez-Paredes *et al.* 2012).

To avoid overfeeding, the *ad libitum* use of fibrous diets was proposed as an attempt to modulate the energy intake of young females (Pascual *et al.*, 2013). Here we observed the impact of this strategy on the growth pattern of young females during their rearing period. Those fed with diet F showed a smoother growth pattern, with a lower asymptote that did not impair live weight at first parturition (similar to females on diet C). The observed pattern agrees with the proposed goal: stimulate a gradual growth, avoid reaching insemination age with an excess of fat reserves and at the same time allow females to achieve their first parturition with an adequate maturity (no differences in fertility rate and live weight at first parturition were observed). Martínez-Paredes *et al.* (2012) reported similar results when comparing diets of similar characteristics.

Reproductive performance

Physiological status and management practices adopted during the pubertal life of rabbit females, such as the birth weight, body fatness at first insemination and the diet used, may influence their future reproductive performance in terms of fertility and prolificacy. Concerning birth weight, Poigner *et al.* (2000) observed that heavy newborn females (between 63 and 70 g) reached maturity more rapidly and delivered more offspring in their first parturition than females born with a lower weight (between 39 and 43 g). Likewise, our heavy newborn females (>57 g) had significantly higher body fatness at first insemination (+0.18 mm of PFT) and bigger litter sizes at first parturition (+0.89 kittens) than lean ones (<57 g). However, no influence of birth weight on the lifetime reproductive performance of females as adults was observed in either study. Poigner *et al.* (2000) observed a longer reproductive life of females born with a lighter weight (22.0% reached the sixth parturition) compared to heavier females (only 15.5%). Therefore, regardless of whether females were inseminated at the same age or at the same weight, heavy newborns reached reproduction with a greater amount of body reserves, which they invest in delivering more offspring at the beginning of their reproductive life; a fact that may have a long-term cost.

Pascual *et al.* (2002a) reported a better reproductive performance when young females were fed with fibrous diets. In fact, the diets compared in the present study only influence the number of stillborn (+1.07 for diet C with respect to diet F; P<0.001) and weaned (+0.85 for diet C; P<0.001) kittens of females having at least one litter. However, when we considered all females housed, those fed with diet F had 6.1 liveborn and 4.8 weaned offspring more than those fed with diet C, a result directly related to the higher number of females fed with diet F that reached reproductive life. This result may be related to a protective effect of the diet F as reported by Martínez-Paredes *et al.* (2012), who observed a better survival rate among females fed on a fibrous diet. In their study, the fibrous diet reduced both the incidence of digestive troubles and pregnancy toxaemia before first parturition.

It is widely accepted that larger animals have better fertility because they have more resources to invest in reproduction. Here, although females fed with diet C had more weight and fat reserves at insemination age than females fed with diet F, their fertility was similar. After marginalizing the effect of the diet, we observed that females with higher PFT values at first insemination had fewer liveborn and weaned kittens when compared to females that reached the insemination age with lower PFT values. The worse reproductive performance of "fat" young females may be related, first to an increased number of pre-natal losses during gestation (Vicente *et al.*, 2012), or to an increased culling risk among females with excessive PFT (Theilgaard *et al.*, 2006; Martínez-Paredes *et al.*, 2012).

Productive lifespan

Rearing diet. Fibrous diet is designed to promote adequate body development, favour future reproductive life and reduce the associated risk of death and culling, mainly related to the energy burden of overlapping lactation and gestation, especially in their first two reproductive cycles. It mainly works by increasing the female's intake capacity (Xiccato *et al.*, 1999; Pascual *et al.*, 2002a; Quevedo *et al.*, 2005) and by reducing the risks related to the mobilization of body reserves around parturition (Xiccato *et al.*, 1999; Martínez-Paredes *et al.*, 2012). Instead of extending their reproductive career, the F diet reduced the risks associated with the new environment. In the first two-months after being transferred to the production farm, 30 % of females fed with diet C died, while the values for females fed with diet F were well below 20 %; a difference maintained until the third parturition.

The protective effect of diet F may be related to: (1) the energy restriction hypothesis or (2) to the benefits of fibre in itself. In the present study, although we were unable to measure the degree of energy restriction promoted by diet F, females on this diet reached the insemination age weighing 250 g less than females on diet C. Energy restriction is known to extend lifespan from yeast to humans by reducing the incidence of both infectious and chronic diseases (Fontana *et al.*, 2010). However, the time and level of restriction are extremely important in defining the influence, if any, of energy restriction on extending life (Ross, 1972). Alternatively, Gidenne (2015) submitted an extensive review demonstrating the importance of fibrous diets in the digestive health of rabbits after weaning.

The reasons why the protective effect of diet F was not extended throughout reproductive life is not clear. Although we could not check, it is possible that the intended increment in the intake capacity during the first two reproductive cycles did not occur. In fact, our diet F contained about 1.0 MJ of DE per kg of DM more than other diets that effectively increased the feed intake capacity of young females. We were obliged to make this choice because our diets were manufactured by a feed factory, which ran into some technical limitations when attempting to include more fibre in the diet F. Alternatively, the absence of a continued positive effect of diet F later in life could be related to a loss of microbial diversity when the females on diet F started to consume diet C, a condition related to an increased risk of several diseases (Tambutini *et al.*, 2016). Sonnenburg *et al.* (2016) observed that microbial diversity can be stimulated by increasing the fibre content of the diet, but it can be lost very quickly.

Body fatness at first mating. Starting reproductive life with 'optimal' development and an adequate level of reserves is expected to improve fertility and lifespan. In contrast, an inadequate development or inappropriate levels of body reserves increase the risk of death and culling (Theilgaard *et al.*, 2006) and impair fertility (Savietto *et al.*, 2016). Among the many factors associated with the high risk of death observed around parturition (Rosell and de la Fuente, 2009), an excess of fat at first effective mating followed by a high pre-partum mobilization of reserves, lower DE intake, high non-esterified fatty acids and low glucose levels are all related to pregnancy toxaemia (Martínez-Paredes *et al.*, 2012). In this context, our results support the hypothesis that when an adequate level of body reserves is attained, both reproduction and lifespan are reinforced. Although we could not specify whether the extended lifespan is a result of reducing the risks related to the mobilization patterns of PFT (Savietto *et al.*, 2016), our results indicate that PFT at first mating are a good predictor of the future lifetime reproductive performance of rabbit females. Although we should be cautious before recommending a target level of PFT over which females would be at higher risk of being culled, for this particular population we observed that PFT values at first mating above 6.0 mm should be avoided (Figure 3.4b). So, it is advisable to control the body condition of young rabbit does to avoid excessive fatness at first mating. In gilts, for example, having an adequate body condition at first mating appears to improve fertility (O'Downd *et al.*, 1997) and lengthen their lives (Tarrés *et al.*, 2006). In adult captive rabbits' deviation from the adequate levels reduces lifespan (Theilgaard *et al.*, 2006).

Conclusions

The results of this study have allowed us an evaluation of the main early life traits that most affect reproduction and lifespan of rabbit females. Regarding reproduction, although a higher birth weight might have a positive effect on litter size in the first cycle, it does not appear to be important in the long term. However, although the use of the diet F in the pubertal phase did not influence the lifetime reproductive performance, it reduced the PFT of young females at their first mating. And the fatness level did improve the reproductive performance (+2.9 total weaned kittens for each unit of decrease in PFT). Regarding lifespan, the different traits evaluated had an effect on survival that was delimited to specific moments of the animal's life. Thus, having a greater birth weight only increased survival expectancy during lactation; being suckling especially important among light newborns. Diet F only had a positive effect on the survival of young females during the period it was applied. However, from this moment, the PFT at first mating was the factor that most influenced rabbit female's life expectancy.

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PAPER IV

Rearing management of rabbit males selected by high growth rate: the effect of diet and season on semen characteristics

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Abstract

A total of 66 young males were used to evaluate the effect of low (L), medium (M) and high (H) dietary concentrations received during the rearing seasons (autumn and spring) on the performance and main semen characteristics of males for AI selected by high growth rate. Males reared during the spring season presented a significantly higher weight at weaning than those reared during autumn season ($P<0.001$), and these differences were maintained until the end of the trial. Requirements of males were easily covered as a general rule. In the autumn group, males were unable to intake the digestible protein recommended only during their 3rd month of life, especially with low concentrate diets ($P<0.05$). H males showed higher semen concentration and production during autumn season, while L males showed higher semen concentration and production than M males during spring season, H group showing intermediate values ($P<0.001$). Males reared during spring season showed significantly higher values for sperm concentration ($P<0.01$) and production ($P<0.01$). H males presented a lower percentage of spermatozoa with cytoplasmatic droplets than L group ($P<0.05$) and the lowest values for sperm abnormalities during autumn season, while L group presented higher values for percentage abnormalities, especially during the last month controlled ($P<0.05$). As a general rule, main motility parameters controlled were not affected by the rearing diet received nor the season. These results seem to indicate that the management of rabbit males during the growing and rearing periods seem to significantly affect their subsequent performance and semen production.

Keywords: rabbit males, rearing, semen characteristics, dietary energy, season.

Introduction

Rabbit males have traditionally been located between females for management reasons and consequently have usually been fed with diets for reproductive rabbit does. However, as a consequence of artificial insemination (AI) development, specific farms for males have appeared in recent years, the use of diets designed from specific characteristics, goal and requirements of males now being possible.

AI has been valuable for rabbit producers because males selected on the basis of desired traits of rapid and efficient growth could be used to inseminate large population of females. However, these animals selected by high growth rate usually present a relatively low sperm count (200-600 $\times$ 10^6), the number of inseminations per week being reduced to 10 or 20 (main authors advise around 16-30 $\times$ 10^6 spermatozoa per insemination). Several studies have thus been carried out to increase the number of doses and quality of sperm produced per male, reducing the number of sperm in insemination doses (Viudes de Castro and Vicente, 1997) or improving male management (Theau-Clément *et al.*, 1995; Rebollar and Alvariño, 1997; Mocé *et al.*, 2000).

On the other hand, sperm production and spermatozoa characteristics could also be modified by nutritional changes, and a significant number of works have been carried out to study the effect of energy (Papadomichelakis *et al.*, 2000), protein (Luzi *et al.*, 1996; Scapinello *et al.*, 1997; Nizza *et al.*, 2000; Papadomichelakis *et al.*, 2000) and different micronutrient contents (El-Masry *et al.*, 1994; Castellini *et al.*, 1999; Lavara *et al.*, 2000; Mocé *et al.*, 2000; Yousef *et al.*, 2003) of diet on the main sperm characteristics of males destined to AI, recently reviewed by Pascual (2002).

However, there is a lack of information about the adequate nutrition of animals selected by high growth rate, and especially during their rearing period (from weaning to semen collection beginning). Considering the high growth rate and requirements of these animals, an adequate nutrition during this period could be critical for a good reproductive performance.

The aim of the present work was to evaluate the effect of dietary concentration received during the rearing season on the performance and main semen characteristics of males selected for AI by high growth rate.

Material and Methods

The experimental procedures of the present work were fully agreed with the standards for use of animals established by the Ethical Committee of the Polytechnic University of Valencia.

Diets

The main ingredients and chemical analyses of experimental diets are summarised in Table 4.1. Three pelleted diets were formulated with different digestible energy (DE) content to study the effect of the dietary concentration received during the rearing time on the seminal parameters of males selected by high growth rate. The medium diet (M) was similar to a typical commercial diet for reproductive rabbit does, with 12.3 MJ kg^{-1} dry matter (DM) and 129 g of digestible protein (DP) kg^{-1} DM. From this, a low energy diet (L), with 10.9 MJ DE kg^{-1} DM and 112 g DP kg^{-1} DM, and high energy diet (H), with 13.5 MJ DE kg^{-1} DM and 146 g DP kg^{-1} DM, were formulated by partially substituting the alfalfa hay and wheat bran content for barley grain, animal fat and soyabean meal. The DP/DE ratio was similar for the three diets (10.3, 10.4 and 10.8 g DP per MJ DE for L, M and H diets, respectively), but acid detergent fibre (ADF) decreased as DE content of diet increased (198, 174 and 131 g ADF kg^{-1} DM for L, M and H diets, respectively).

Animals received a commercial diet for reproductive rabbit does (C) during semen collection period. This diet presented a similar composition to that described for M diet with 12.4 MJ DE kg^{-1} DM and 129 g DP kg^{-1} DM.

Apparent digestibility coefficients of energy and protein were determined using the experimental males when they were 16 weeks of age with a mean live weight of 3985 ± 47 g for L, M and H diets and when they were 31 weeks of age with a mean live weight of 4967 ± 47 g for C diet. The rabbits were housed in metabolism cages and feed and water were offered *ad libitum* during the experimental period. Following an adaptation period of 7 days the faeces collection period was 4 days, as described by Pérez *et al.* (1995).

Pelleted diets were manufactured and analysed twice during the experimental period.

Chemical analysis of diets and faeces were performed following the methods of the Association of Official Analytical Chemists (AOAC) (1991) for DM, ash, crude protein (CP), ether extract (EE) and crude fibre (CF), and Van Soest *et al.* (1991) for fibre fractions, with a thermostable amylase pre-treatment. Gross energy (GE) was determined by adiabatic bomb calorimetry following the recommendation of the EGRAN (2001).

Animals and experimental procedures

A total of 66 young males from line R of the Animal Breeding farm of Animal Science Department (DCA) of Polytechnic University of Valencia (UPV) were used in this experiment. Line R is characterised by a selection criterion based on the growth rate from weaning to slaughter (28-63 days of age; Estany *et al.*, 1992), actually showing a growth rate around 50 g per day.

Table 4.1 *Main ingredients (g kg^{-1}) and chemical composition (g/kg dry matter) of diets.*

	Diets			
	L	M	H	C[5]
Ingredients				
Alfalfa hay	680	500	380	
Wheat bran	150	100	50	
Barley grain	99	250	320	
Soya-bean meal (440 g CP per kg)	29	106	90	
Soya-bean grain			113	
Animal fat[1]	5	10	15	
Calcium hydrogen phosphate	10	15	11	
Monosodium phosphate	15	9	12	
Sodium chloride	4	4	4	
Lysine	2	1		
DL-methionine	2	2	2	
Arginine	1			
Vitamin/mineral mixture[2]	2	2	2	
Vitamin E	0.1	0.1	0.1	
BHT[3]	0.1	0.1	0.1	
Robenidine[4]	0.8	0.8	0.8	
Chemical composition				
Dry matter (DM; g kg^{-1})	912	913	914.7	912
Ash	116	105	91.4	91
Ether extract (EE)	30	36	45.1	33
Crude fibre (CF)	180	136	132.6	165
Neutral detergent fibre (NDF)	340	309	250.4	352
Acid detergent fibre (ADF)	198	174	130.8	184
Lignin detergent fibre (ADL)	28	24	13.6	31
Crude protein (CP)	171	179	194.7	184
Digestible Protein (DP)	112	129	146	129
Digestible Energy (DE; MJ kg^{-1} DM)	10.9	12.3	13.5	12.4
DE/DP (kJ g^{-1})	97.3	95.3	92.5	96.1

[1] 650 g kg^{-1} lard, 250 g kg^{-1} tallow and 100 g kg^{-1} poultry fat; [2] Contains (g kg^{-1}): thiamin, 0.25; riboflavin, 1.5; calcium pantothenate, 5; pyridoxine, 0.1; nicotinic acid, 12.5; retinol, 2; cholecalciferol, 0.1; α-tocopherol, 15; phytylmenaquinone, 0.5; cyanocobalamin 0.006; choline chloride, 100; $MgSO_4 \cdot H_2O$, 7.5; ZnO, 30; $FeSO_4 \cdot 7H_2O$, 20; $CuSO_4 \cdot 5H_2O$, 3; KI, 0.5; $CoCl_2 \cdot 6H_2O$, 0.2; Na_2SeO_3, 0.03; [3] BHT (butylhydroxytoluene): antioxidant; [4] Robenidine: coccidiostatic; [5] Cunicebial CD-14 (Nutreco): commercial diet for reproductive rabbit does (ingredients are not available).

Only litters with at least three siblings (males) were used in the present experiment. Until Spanish commercial slaughter date (63 days) animals were collectively housed in the Animal Breeding farm of UPV and fed the same commercial diet for growing rabbits. From this moment, each sibling was assigned to a different dietary treatment in order to avoid kinship effects and housed in individual cages of males for AI in the Animal Nutrition farm of

UPV with light alternating on a cycle of 16 light hours and 8 dark hours, and under controlled environmental conditions (cooling).

Rearing diets (L, M and H) were offered *ad libitum* to males until first semen collection at 24th week of age (usual management for males destined to AI). From this moment and until the end of the trial, all animals received the same diet (C), also offered *ad libitum*.

Animals were studied during two different seasons of the year (autumn and spring). For autumn group, a total of 36 males fattened during August-September [mean temperature (Tªm) 23.6ºC] period was controlled during rearing (October to December; Tªm 16.5ºC) and semen collection periods (December to February; Tªm 13.0ºC). Spring group (30 males) were fattened during January-February period (Tªm 13.0ºC), and then controlled during rearing (March to May; Tªm 18.0ºC) and semen collection periods (May to July; Tªm 21.0ºC).

Individual live weight and feed intake was controlled weekly throughout the trial. Perirenal fat thickness of males (PFT) was measured monthly by ultrasound to evaluate the body condition, as described by Pascual *et al.* (2000). Fur was removed from the thoracic and lumbar vertebrae area by shearing to improve image retrieval. Animals were situated in an immobilising box (150 × 370 mm) during ultrasound measurement and ultrasound gel (Eko Gel) was applied to the scanning area. The probe was always placed in the same position to obtain a repeatable transversal section of perirenal fat at 3 cm ahead of the 2nd to 3rd lumbar vertebrae. Images were obtained with an ultrasound unit (JustVision real-time machine; Toshiba) equipped with a 5.0 to 8.0MHz sector probe, and PFT was measured with the image processing system of the equipment.

Semen collection and evaluation

From the 24th week of age, data from 14 and 12 weeks of sexual activity were recorded for autumn and spring groups, respectively. Two ejaculates per male were collected each week (obtained at the same day with a lag of 30 minutes) using an artificial vagina and samples were maintained at room temperature and analysed within 1 hour. Semen samples were analysed in the Laboratory of Reproduction Biotechnology of UPV. Semen volume was measured in a graduated conical tube. A sperm sample was fixed with a solution of glutaraldehyde 0.25% in Dulbecco's Phosphate Buffered Saline (Pursel and Johnson, 1974) to measure concentration and perform the morphological evaluation. Sperm concentration was calculated by a Thoma-Zeiss cell chamber (final dilution: 1:50), and the proportion of sperm with normal apical ridge (NAR), abnormal sperm morphologically and cytoplasmatic droplets were estimated at a magnification of 400x with a differential interference contrast microscope (Nomarski contrast).

Kinetic parameters of semen samples were estimated by CASA (Computer-Assisted Sperm Analysis). Aliquots (10 µl) of semen diluted 1:50 with Tris- citric acid-glucose extender (300 mOsm kg^{-1}; pH 6.8) were laid over a pre-warmed slide at 37ºC and 4 fields (2 drops × 2 fields) or 2 fields (2 drops × 1 field) were analysed by CASA (SCA 4.0, Microptic, Spain) using setup parameters previously established for better discriminating granules from static cells. Recorded sperm parameters were: total motile sperm percentage (MOT), percentage of medium and fast sperm (MED, HIG) and good motile sperm percentage (M+H), curvilineal, straight line and average path velocity (VCL, VSL and VAP, respectively; µm sec-1), linearity (LIN = [VSL/VCL] × 100) and wobble (WOB = [VAP/VCL] × 100), amplitude of lateral head displacement (ALH; µm), angularity index (AI; %) and beat cross frequency (BCF; Hz).

Statistical analysis

Statistical analyses of data were carried out with SAS (Statistical Analysis System Institute, 1990). Data were analysed using a mixed procedure (PROC MIXED) and according to a repeated measures design that considers the variation between animals and covariation within them. Covariance structures of mixed procedure were objectively compared using the most severe criteria (Schwarz Bayesian criterion), as suggested by Littell *et al.* (1998).

For the analysis of live weight, feed intake and PFT evolution of males and for the motility parameters of their semen, a model considering the fixed effect of the rearing diet, the year season and their interaction was used. For the analysis of quantitative and qualitative semen parameters of males, the model used considered the fixed effects of the diets, the year season, the semen collection month and their interactions, the number of ejaculates recovered weekly being included as a covariate.

Results

Performance traits

The effects of the rearing diet and rearing season on the live weight, feed intake and PFT evolution of males are presented in Table 4.2. The type of diet used during the rearing period had no effect on the live weight of males, showing similar values for the different diets in both seasons and during the whole experimental period. However, rearing season did have a clear effect upon the live weight of males. Males controlled during the spring season presented a significantly higher weight at weaning (+88 g; $P<0.001$) and at slaughter date (+316 g; P<0.001) than those reared during autumn season, and these differences were maintained at the beginning of semen collection (+261 g; P<0.001) and until the end of the trial (Figure 4.1).

Males showed a significant lower feed intake as DE content of diet increased from 9th to 14th week of age, but DE and DP intake of animals was higher as DE content increased (P<0.10 and P<0.05, respectively). Feed intake regulation in accordance with DE content of diets was maintained until the beginning of semen collection, but contrary to that observed during the first month after slaughter date, animals receiving H diet showed on

Table 4.2 *Effect of the energy rearing diet and the rearing season on the live weight and food intake evolution of males[1].*

	Autumn season			Spring season				Signification[2]	
	L	M	H	L	M	H	s.e.	D	S
Live weight (g):									
at 4 weeks of age (weaning)	538^a	511^a	491^a	613^b	612^b	580^{ab}	33		***
at 9 weeks of age	2148^a	2134^a	2054^a	2398^b	2466^b	2422^b	60		***
at 24 weeks of age	4375^a	4548^{ab}	4495^{ab}	4911^c	4796^{bc}	5091^c	135		***
at 32 weeks of age	4816	4856	4832	5123	5136	5071	150		*
Feed intake (g DM kg^{-1} LW$^{0.75}$ d^{-1}):									
9 to 14 weeks of age	71.5^c	65.9^b	59.3^a	82.5^d	72.4^c	65.0^b	1.7	***	***
15 to 24 weeks of age	66.6^{bc}	59.8^b	50.1^a	68.6^c	57.7^b	49.6^a	1.3	***	
25 to 28 weeks of age	53.1^c	53.7^c	48.9^{bc}	51.5^c	44.7^{bc}	41.4^a	2.3	*	***
29 to 37 weeks of age	50.4^b	47.9^b	45.8^b	37.2^a	39.7^a	36.9^a	2.5		***
Energy intake (kJ DE kg^{-1} LW$^{0.75}$ d^{-1}):									
9 to 14 weeks of age	779^a	811^b	800^b	899^c	890^c	877^c	9		***
15 to 24 weeks of age	725^b	735^b	677^a	858^d	832^d	806^c	11	***	*
25 to 28 weeks of age	659^b	666^b	606^b	639^b	554^{ab}	513^a	29	*	**
29 to 37 weeks of age	625^c	594^{bc}	568^b	462^a	492^a	458^a	22	*	***
Protein intake (g DP kg^{-1} LW$^{0.75}$ d^{-1}):									
9 to 14 weeks of age	8.00^a	8.51^{ab}	8.67^b	9.24^c	9.34^c	9.49^c	0.21	*	***
15 to 24 weeks of age	7.45^b	7.70^b	7.32^{ab}	7.66^b	7.44^a	7.23^a	0.16	*	
25 to 28 weeks of age	6.85^c	6.92^c	6.31^{bc}	6.65^c	5.77^{ab}	5.34^a	0.30	*	***
29 to 37 weeks of age	6.50^b	6.18^b	5.91^b	4.80^a	5.13^a	4.77^a	0.31		***
Perirenal fat thickness (mm):									
at 9 weeks of age	7.78	7.61	7.59	7.23	7.63	7.45	0.20		
at 24 weeks of age	10.40^b	10.70^b	10.30^{ab}	9.64^a	9.82^a	9.86^a	0.19		**
at 32 weeks of age	10.30	10.11	9.91	10.09	9.84	10.14	0.22		

Rearing diets: low (L), medium (M) and high (H) energy content; [a,b,c,d] Means for diets within a row with different superscripts are significantly different (P<0.05); * P<0.05; *** P<0.001; [1] Animals were weaned at 28 days of age (4 week), fatted in collective cages until 63 days of age (commercial Spanish slaughter date) and then caged in individual cages. Semen collection begins at 24 weeks of age; [2] Signification: Effect of diet (D), season (S). None D×S interactions were observed.

average a lower DE and DP intake (P<0.001 and P<0.05, respectively). As all the animals received the same diet from 24th week of age, differences between dietary treatments in feed intake clearly decreased from this moment on, although DE and DP intake values were in average terms lower for the animals that received H diet during rearing time (a mean of −60 kJ DE kg^{-1} metabolic weight ($LW^{0.75}$) day^{-1} and –0.6 g DP kg^{-1} $LW^{0.75}$ day^{-1}).

Rearing season had a clear greater effect on the feed intake of the animals than dietary treatment throughout the trial. Initially, animals rearing during the spring season presented a higher feed intake than those reared during autumn (+7.8 g kg^{-1} $LW^{0.75}$ day^{-1}; P<0.001), but as heat season came closer the feed intake of the animals clearly decreased,

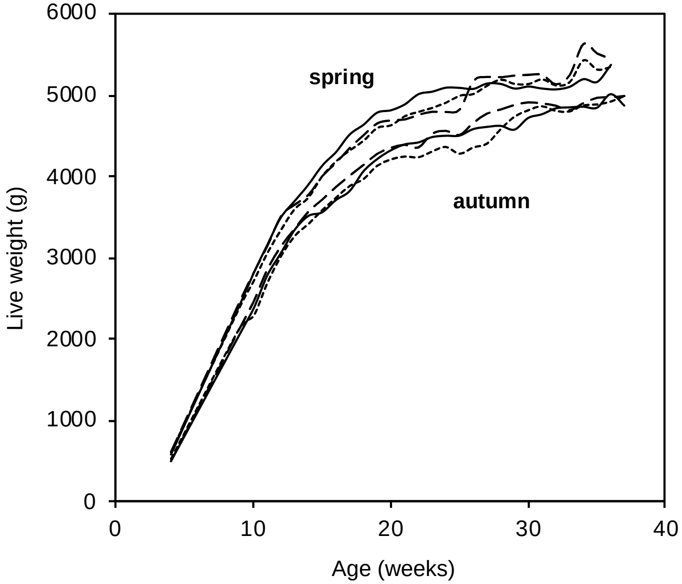

Figure 4.1 Evolution of live weight of males for the different rearing diets (L · · · ·, M – – – and H ——) and for the different year season (autumn and spring) analysed between weaning (4th week) to 38th week of age.

with males in spring group showing a lower feed intake than autumn males during the semen collection period, especially in the last two months (−10.1 g kg^{-1} $LW^{0.75}$ day^{-1}; P<0.001).

As can be seen in Figure 4.2, energy and protein requirements of males were easily covered as general rule. In the autumn group, males were unable to intake the recommended DP only during their 3rd month of life (9.1 g PD kg^{-1} $LW^{0.75}$ day^{-1}), especially with low concentrate diets (8.0, 8.5 and 8.7 g DP kg^{-1} $LW^{0.75}$ day^{-1} for L, M and H diets, respectively; P<0.05). In the spring group, DE energy requirements (490 MJ DE kg^{-1} $LW^{0.75}$ day^{-1}) were not covered during the last two months controlled independently of dietary treatment (a mean of 470 MJ DE kg^{-1} $LW^{0.75}$ day^{-1}).

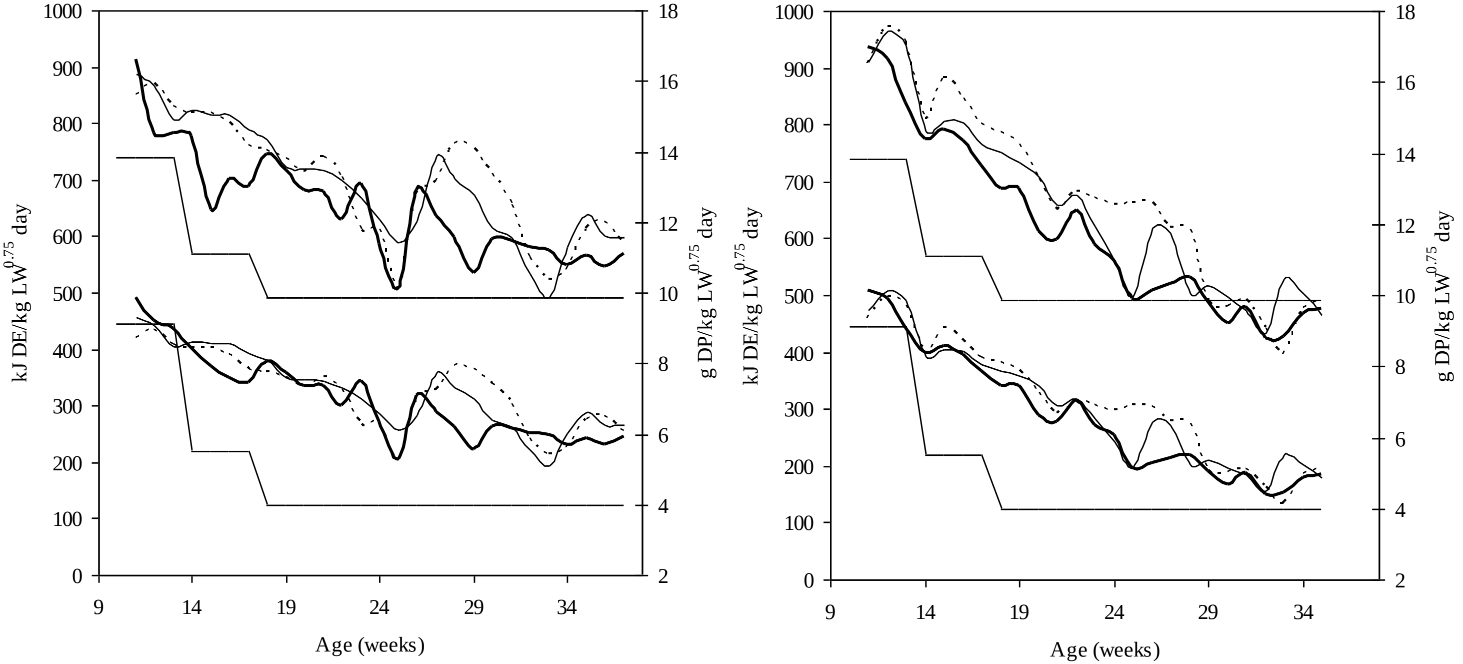

Figure 4.2 Evolution of digestible energy (DE) and protein (DP) intake of males with the different rearing diets (L · · · ·, M —— and H ▬▬) and for the different year season (autumn and spring) analysed between post commercial slaughter date (9th weeks) to 38th week of age.

Although the PFT of males increased from 7.5 to 10.1 mm during rearing period (being maintained from 6th month of age), type of diet did not affect the PFT of males throughout the experiment. Season only affected the PFT value of males at the beginning of semen collection, being higher for animals reared during the autumn season (10.5 and 9.8 mm for autumn and spring groups, respectively; $P<0.01$), although these differences disappeared at 32 weeks of age.

Seminal parameters

From the 66 males used in the present trial, a total of 586 semen samples were obtained from only 53 animals (13 males did not give samples because of death, illness or non-mount behaviour). On the other hand, only 38 animals (13, 12 and 13 for L, M and H diets, respectively) presented an adequate reproductive behaviour, assiduously providing semen samples.

Table 4.3 *Effect of the energy rearing diet and the month of mating on some quantitative semen parameters of males reared during autumn and spring seasons.*

	Autumn season					Spring season				
	Mean	**L**	**M**	**H**	**s.e.**	**Mean**	**L**	**M**	**H**	**s.e.**
% animals able to produce semen		55	55	64			64	55	55	
No of ejaculate (n=586)										
1st month	1.32^{A}	1.47^{b}	1.04^{a}	1.44^{b}	0.05	1.44^{A}	1.54^{b}	1.26^{a}	1.51^{b}	0.05
2nd month	1.70^{B}	1.84^{b}	1.50^{a}	1.77^{b}	0.05	1.83^{B}	1.91	1.75	1.82	0.05
3rd month	1.86^{C}	1.97^{b}	1.65^{a}	1.98^{b}	0.05	1.70^{B}	1.59	1.79	1.73	0.07
s.e.	0.05					0.06				
Mean		1.76^{b}	1.40^{a}	1.73^{b}	0.11		1.68	1.60	1.684	0.11
Volume (ml/ejaculate) (n=391)[1]										
1st month	1.02^{B}	0.99	1.09	0.99	0.06	0.80^{A}	0.80	0.72	0.89	0.06
2nd month	0.87^{AB}	0.78	1.01	0.83	0.05	0.89^{AB}	0.90	0.77	1.02	0.05
3rd month	0.71^{B}	0.78	0.61	0.74	0.06	0.98^{B}	0.90	0.94	1.13	0.06
s.e.	0.06					0.06				
Mean		0.85	0.90	0.85	0.07		0.86	0.81	1.01	0.10
Spermatozoa × 10^{6}/ml (n=380)[1]										
1st month	189.5^{B}	213.1^{ab}	104.9^{a}	250.6^{b}	22.7	283.2^{B}	416.6^{b}	120.8^{a}	312.1^{b}	39.6
2nd month	130.4^{A}	167.0	86.6	137.7	20.2	169.3^{A}	258.0^{b}	94.0^{a}	156.1^{ab}	32.0
3rd month	134.0^{A}	139.3	123.3	139.5	22.4	166.8^{A}	307.0^{b}	83.9^{a}	109.3^{ab}	45.9
s.e.	21.8					40.0				
Mean		173.1^{ab}	104.9^{a}	175.9^{b}	24.3		327.3^{b}	99.6^{a}	192.5^{ab}	50.0
Spermatozoa × 10^{6}/ejaculate (n=411)										
1st month	206.0^{B}	171.1^{a}	202.5^{ab}	259.3^{b}	19.6	240.5^{B}	377.9^{b}	93.7^{a}	250.0^{ab}	32.5
2nd month	116.4^{A}	121.6	123.4	108.1	18.6	154.7^{A}	266.2^{b}	84.6^{a}	113.4^{a}	25.7
3rd month	97.9^{A}	76.2	120.2	107.5	18.9	152.2^{A}	260.7	92.1	103.7	37.6
s.e.	19.0					32.0				
Mean		123.5	148.9	158.3	27.6		301.6^{b}	90.1^{a}	155.7^{ab}	52.2

Rearing diets: low (L), medium (M) and high (H) energy content; Volume and concentration (spermatozoa×10^{6}/ml) data were calculated from non-dirty ejaculates (without urine or blood); Production (spermatozoa×10^{6}/ejaculate) data were calculated from total ejaculates; ^{a,b,c} Means for diets within a row with different superscripts are significantly different ($P<0.05$); ^{A,B,C} Means for months within a column with different superscripts are significantly different ($P<0.05$); [1]covariate (no of non-dirty ejaculates) affected significantly $P<0.05$

Table 4.3 shows the effect of the diets received during the rearing time and the mount month on main quantitative seminal parameters controlled during autumn and spring seasons. The number of weekly ejaculates recovered per male significantly increased with the mount month (+0.40 ejaculates from the 1^{st} to the 2^{nd}-3^{rd} months; P<0.001), and males from M group tended to show lower values, especially during autumn season (–0.35 ejaculates per male; P<0.05).

Mean volume of the ejaculates decreased with the mount month during autumn (–0.31 ml per ejaculate from 1^{st} to 3^{rd} month; P<0.01) but increased during spring season (+0.18 ml per ejaculate from 1^{st} to 3^{rd} month; P<0.05). Type of diet received during rearing season had no effect on the mean volume of the ejaculates.

Mean concentration of semen was higher in the first collecting month than in the following months during both seasons (+85 × 10^6 spermatozoa per ml; P<0.001) and consequently semen production showed a similar tendency (+90 × 10^6 spermatozoa per ejaculate for first month; P<0.001). With respect to the effect of rearing diet, H males showed higher semen concentration and production during autumn season, mainly due to their greater values during the 1st mount month (+88 × 10^6 spermatozoa per ejaculate; P<0.05). However, L males showed higher semen concentration and production than M males during spring season, with H group showing intermediate values (+180 × 10^6 spermatozoa per ejaculate; P<0.001).

The mean number of ejaculates and volume of semen were similar for both seasons (1.6 ejaculates and 0.88 ml/ejaculate, respectively). However, males reared during spring season showed significantly higher values for sperm concentration (+55 × 10^6 spermatozoa per ml; P<0.01) and production (+42 × 10^6 spermatozoa per ejaculate; P<0.01).

Table 4.4 shows the results concerning main qualitative semen parameters of males in line with the rearing diet, mount month and rearing season. The lowest values for normal apical ridge were obtained at 2^{nd} and 3^{rd} months during autumn and spring seasons respectively (P<0.05), not being significantly affected by rearing diet received by males (although H group presented higher values during autumn season).

The mount month did not affect the percentage of sperm abnormalities, or the proportion of spermatozoa with cytoplasmatic droplets. However, H males presented a lower percentage of spermatozoa with cytoplasmatic droplets than L group (–6.6%; P<0.05) as well as the lowest values for sperm abnormalities during autumn season. Differences between dietary treatments disappeared during spring season, although L group presented higher values for abnormalities percentage, especially during the last month controlled (+6.8%; P<0.05).

Table 4.4 *Effect of the energy rearing diet and the month of mating on some qualitative semen parameters of males reared during autumn and spring seasons (n=407).*

	Autumn season					Spring season				
	Mean	**L**	**M**	**H**	**s.e.**	**Mean**	**L**	**M**	**H**	**s.e.**
Acrosoma integrity (%)										
1st month	85.67^{AB}	80.25	81.30	83.46	1.95	85.29^{B}	85.93	86.46	83.50	0.88
2nd month	80.74^{A}	70.46^{a}	74.56^{a}	88.20^{b}	1.71	84.42^{B}	84.68	84.77	83.81	0.70
3rd month	86.11^{B}	81.10	90.69	86.53	1.89	81.64^{A}	80.68	83.09	81.17	1.04
s.e.	1.76					0.87				
Mean		80.27	82.18	86.06	4.63		83.76	84.77	82.82	1.11
Sperm abnormalities (%)										
1st month	6.23	5.13	7.60	5.98	0.83	7.24	9.73	5.67	6.31	0.77
2nd month	7.35	7.66	8.30	6.09	0.78	7.72	9.47	6.64	7.05	0.62
3rd month	6.08	5.82^{ab}	8.25^{b}	4.18^{a}	0.81	9.30	13.80^{b}	8.11^{a}	6.00^{a}	0.91
s.e.	6.55					0.77				
Mean		6.21	8.05	5.41	2.03		11.00	6.81	6.45	2.10
Protoplasmatic drop (%)										
1st month	16.58	22.01^{b}	14.81^{ab}	12.92^{a}	1.54	13.20	13.42	11.28	14.90	0.95
2nd month	15.66	16.67	17.26	13.06	1.45	10.87	12.07	9.83	10.69	0.76
3rd month	15.30	17.23^{b}	18.64^{b}	10.03^{a}	1.50	11.62	12.74	10.26	11.86	1.11
s.e.	2.03					0.94				
Mean		18.64^{b}	16.90^{ab}	12.00^{a}	2.55		12.75	10.46	12.49	2.33

Rearing diets: low (L), medium (M) and high (H) energy content; ^{a,b,c} Means for diets within a row with different superscripts are significantly different (P<0.05); ^{A,B,C} Means for months within a column with different superscripts are significantly different (P<0.05)

Table 4.5 *Effect of the energy rearing diet and the rearing season on semen motility parameters.*

	Autumn season			Spring season				Signification[3]		
	L	**M**	**H**	**L**	**M**	**H**	**s.e.**	**D**	**S**	**D×S**
Motility parameters:										
Total motility (MOT; %)	47.73^{b}	37.38^{ab}	44.39^{b}	36.97^{ab}	47.01^{b}	30.57^{a}	3.00			**
Medium motility (MED;%)	6.71	5.75	5.59	5.34	6.57	4.48	0.82			
High motility (HIG;%)	10.86^{ab}	5.21^{a}	9.23^{ab}	5.22^{a}	14.26^{b}	6.01^{a}	1.39			***
Good motility (M+H; %)[1]	17.57^{ab}	10.96^{a}	14.82^{ab}	10.57^{a}	20.82^{b}	10.49^{a}	1.92			**
Rate parameters:										
Track speed (TS; ∝m/sec)	43.85^{b}	35.10^{a}	42.60^{b}	38.83^{ab}	46.52^{b}	40.64^{ab}	1.79			**
Progressive speed (PrS; ∝m/sec)	20.37^{b}	15.45^{ab}	17.66^{ab}	14.44^{a}	19.79^{ab}	16.38^{ab}	1.40			*
Path speed (PaS; ∝m/sec)	24.91^{b}	19.73^{ab}	22.81^{ab}	19.30^{a}	24.98^{b}	21.47^{ab}	1.35			*
Linearity index (LIN; %)[2]	46.18	49.93	43.34	40.53	44.02	41.28	2.35		+	
Oscillation index (OSC; %)	58.59	61.94	57.25	54.73	56.79	55.22	1.70		+	
Angularity and beaten parameters:										
Amplitude of lateral head displacement (ALH; ∝m)	1.77	1.59	1.91	1.91	2.09	1.92	0.12			
Angularity index (AI; %)	28.58	24.78	28.59	27.96	30.35	31.58	1.61			
Beat cross frequency (BCF; Hz)	8.23	7.52	10.96	10.02	9.53	9.85	0.72			

Rearing diets: low (L), medium (M) and high (H) energy content; a,b,c Means for diets within a row with different superscripts are significantly different (P<0.05); + P<0.10; * P<0.05; ** P<0.01; *** P<0.001; 1 Good motility = medium motility + high motility; 2 Linearity (LIN = [Pr/TS] ×100); 3 Signification: Effect of diet (D), season (S) and the interaction (D×S).

Finally, the effect of rearing diet on semen motility parameters of males reared during autumn and spring seasons is shown in Table 4.5. Dietary treatment did not affect any rearing, semen from M group presented higher MOT, HIG and M+H values ($P<0.01$), while of the motility parameters controlled for the males reared during autumn season and only one rate parameter was lower for M group (–8.1 µm sec^{-1} for VCL; $P<0.05$). In the case of spring semen from L group showed the lowest values VSL and VAP ($P<0.05$), but the other motility parameters controlled were not affected by the rearing diet received.

Discussion

Conversion rate seems to be the most adequate criterion, from an economical point of view, to improve rabbit meat lines (Baselga and Blasco, 1989). However, growth rate is usually the selected criterion due to the management problems of a program based on the conversion rate of the animals. The growth rate of males selected by this criterion, and consequently their nutritional requirements (Pascual, 2002), have clearly changed in recent years, from 40-45 g day^{-1} to 50-55 g day^{-1} during the growing period (28 to 63 days of age). In fact, our animals presented a mean growth rate of 50 g day^{-1} during the growing period, changing to 42 g day^{-1} during the 3rd month of life and falling thereafter to values close to 0-10 g day^{-1}.

Following the recommendations of Parigi-Bini and Xiccato (1998) and Fraga (1998), the energy and protein requirements of males selected by growth rate could be calculated as Figure 4.2. As a general rule, DE and DP requirements were easily covered throughout the trial with the different dietary treatments and independently of the rearing season. However, although nutrient requirements of males seem to be well met in males reared during spring, DP requirements will be only covered with H diet from 9th to 13th week of age on males fattened during summer (9 g DP kg^{-1} LW$^{0.75}$ day^{-1}). This protein deficit could affect the performance of males receiving the L and M diets and could perhaps explain the slightly better results obtained for H animals only for autumn group on the main seminal parameters controlled (semen production, sperm abnormalities, cytoplasmatic droplets).

Many authors have observed a lower feed intake and growth rate of rabbits fattened under hot conditions (Pascual *et al.,* 2002). In the present work, autumn reared males already presented a lower live weight at the beginning of the dietary treatment than spring reared males, and the differences were maintained until the end of the experiment. In addition, spring reared males showed a higher feed intake during the rearing period than autumn reared males, although it decreased during the semen collection period due to the proximity of summer.

In general, sperm volumes were slightly higher in spring than in autumn, as has previously been reported in other works (Egea and Roy, 1992; Alvariño, 2000). At the same time, concentrations obtained in spring were in general higher than those obtained in autumn, which was very pronounced for the group of males that had been fed with the low energy diet during the rearing period. Several authors have observed higher concentrations and productions in semen collected during spring than after the hot season (Mocé *et al.,* 2000). With regard to the quantitative seminal parameters, it was observed that the group from spring season which had been fed on the high energy diet presented higher volumes of semen than those fed with the low energy or the control diets. Louis *et al.* (1994) reported that boars fed on low energy diets had reduced libido and semen volume and sperm output than those fed with high energy diets. In our work, males fed the low energy diet presented higher concentration and, as a consequence, higher production than male rabbits fed with high energy and control diets. However, in the group of males whose semen was obtained from October to December (autumn season), the volume values were higher in the control group and the concentrations values were higher in the groups fed with H and L energy diets, obtaining a higher sperm production in the group of males fed on high energy diets.

Therefore, future reproductive males fattened during summer season usually present a worse performance during the growing period than those fattened during winter season, which could affect the subsequent performance. This lower performance and higher temperature suffered 2 months after could be the reasons for the worse sperm production results obtained for autumn group and the positive effect observed when highly concentrated diets were used during rearing. However, this type of diets should not have any advantage in the case of animals well developed and with higher intake ability, the use of a moderate energy diet perhaps being more adequate in order to avoid an excessive fatness and an adequate performance of future males for AI. Intensive feeding of young bulls can result in lowered reproductive performance probably due to an excessive fat deposition around the testis, which would hinder their correct thermoregulation, leading to a reduction in sperm production (for a review, see Brown, 1994).

On the other hand, and as observed by Pascual (2002), although rabbit males selected by growth rate are able to cover the energy requirements with a diet similar to commercial feeds (10.5 MJ ED per kg) during the growing period, protein requirements were difficult to meet with this type of feed (107 g PD per kg) especially when feed intake was lower than 150 g per day (hot seasons).

As for the morphological evaluation of sperm, the results observed in the present work were similar to those reported by other authors working with males from the same rabbit line (85% observed by Vicente and Viudes de Castro, 1996). Percentage of spermatozoa with cytoplasmatic droplets was higher than that reported by other authors working with the adult males from the same line (11%; Mocé *et al.,* 2000) which is probably due to the youth of the animals (since ejaculates from young males frequently have a high percent of sperm with droplets; Barth and Oko, 1989).

Motility rate observed in this experiment was lower than motility rate reported by other authors when working with fresh semen (usually higher than 70% of motile sperm, (Vicente and Viudes de Castro, 1996; Castellini and Lattaioli, 1999; Roca *et al.*, 2000). This could have been due to the high dilution rate performed in this work (dilution 1:50) to measure the motility parameters with the CASA system; Castellini *et al.* (2000) observed a pronounced reduction in motility parameters from rabbit spermatozoa when seminal plasma had been diluted more than 30-fold. With respect to the other motility parameters measured in this work, they are similar to those reported by Viudes de Castro *et al.* (1999) working with sperm from the same line, but clearly lower than those reported by other authors using the same CASA system (Castellini *et al.*, 2000 and 2002)

In any case, the results of the present work seem to indicate that the management of males during the growing and rearing periods (especially the season) seems to significantly affect their subsequent performance and semen production. The development of early management programs that should consider the adequate feeding program according to the housing (environmental) conditions of males is therefore advisable.

Conclusion

In conclusion, males receiving high energy rearing diets during autumn had a higher concentration and sperm production since those receiving low energy rearing diets during spring had a higher quantitative production. In general, males reared during spring had a higher weight at weaning, concentration and sperm production as a lower percentage of spermatozoa with cytoplasmatic droplets and abnormalities. As general rule, main motility parameters were not affected by the rearing diet nor the season.

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PAPER V

Effect of early development on semen parameters and lifespan of rabbit males selected by high growth rate

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Abstract

Life history theory suggests that different dynamics of body development may influence survival and future reproductive performance of organisms. The present work studied how these dynamics could influence on seminal traits and lifespan of rabbit males selected for growth rate and intended to AI. To achieve this goal, a total of 550 rabbit males were controlled from birth, evaluated both during the testing phase (four consecutive weeks after reaching 147 days of life) and during the productive phase (377 of them from the end of the testing phase until 2 years of life). In order to obtain individuals with different body development dynamics, we pre-select males based on their live weight (LW) at 0, 28, 63 and 147 days and on their average daily gain (ADG) between each period (0-28, 28-63 and 63-147 days). Libido and main seminal traits (semen's volume, motility, concentration, and production, as well as spermatozoa normal apical ridge and abnormalities) were controlled during the testing phase. Afterward, semen's volume, motility and concentration were controlled during the productive phase, as well as the length of the male life, calculated as the number of days a rabbit was present at the farm between age 147 to the day of death, culling or censoring; set to 2 years of life). The birth weight, the ADG between 0-28 days and between 28-63 days to be positively related to some seminal parameters measured during the testing phase (semen's volume, concentration, production and motility; P<0.05), while the ADG between 63-147 days was negative related to the seminal productivity throughout the productive life of males (an increment of 10 g per day on ADG reduced a 4.9% the number of profitable ejaculates; P<0.05). In addition, a higher growth between 0-28 and between 63-147 days increased the risk of death or culling of males during the productive phase (P<0.05). In conclusion, an adequate body development early in life seems to have a positive effect on rabbit male's sexual maturity degree with which they begin their reproductive life, but reaching the reproduction onset with an excessive weight can reduce their reproductive performance and lifespan.

Keywords: Oryctolagus cuniculus, body development, growth rate, reproduction, lifespan, semen quality.

Introduction

In the last decades, the use of the artificial insemination (AI) in rabbits has promoted an organizational improvement in the management of the commercial farms, as well as the appearance of specific centres of males addressed to the AI. These AI centres mainly breed rabbit males from genetic lines selected for growth rate at fattening, which are used as breeding male in the three-way crossing scheme. This scheme allows an effective dissemination of the genetic material to optimise commercial farms productivity.

Despite the impact of these males on farm profitability, the scientific information regarding the proper feeding and raising management of male rabbits is still scarce. Nowadays we have information on the influence of the nutritional requirements [both in rearing (Pascual *et al.*, 2004 and 2016) and productive periods (Castellini, 2008)] and the physiological development of males (Lavara *et al.*, 2003; García-Tomás *et al.*, 2008) on their fertility. We also have some estimation of the genetic parameters for seminal traits (Lavara *et al.*, 2011 and 2012), but we lack information on how the management practices in the early developmental age may influence the future reproductive life.

Furthermore, rabbit males (especially those selected for growth rate) have some reproductive peculiarities which could be improved on (late reproductive onset, moderate both sexual libido and sperm production, ...), justifying a better understanding of the main factors that could affect their reproductive performance.

Some of the most important milestones concerning the reproductive physiological development of male rabbits happens early in life, especially during fattening and rearing periods (García-Tomás *et al.*, 2008). In addition, the higher nutritional requirements of males selected for growth rate during early growth development, until 14^{th} weeks of life, are more difficult to be covered than in other genetic lines with lower nutritional requirements (Pascual, 2002, Marín-García *et al.*, 2018). In this sense, we hypothesize that the reproductive success of rabbit males (especially those from lines selected for growth rate) are influenced by the different development states achieved at key phases of their developmental life (i.e. birth, weaning, and end of both juvenile and pubescent life).

If we look at other animal species, although selection for growth rate is positively correlated to the size of reproductive organs, this selection criteria is negatively correlated to seminal quality (in mice, Eisen and Johnson, 1981; in pigs, Johnson *et al.*, 1994; in bulls, Kealey *et al.*, 2006). In the case of breeding rabbits, there are few studies that refer to the influence of body development of juveniles on the subsequent reproductive performance of individuals as adults. Poigner *et al.* (2000) observed that heavier rabbit females at birth showed later larger litter size at their first parturition (+12.4%), while Rommers *et al.* (2002)

reported a better reproductive performance (first cycle) among heavier rabbit female at first mating; probably due to their greater maturity degree when first matted at a fixed age (14.5 weeks old). Recently, Martínez-Paredes *et al.* (2018) observed that thick rabbit females at the beginning of their reproductive life had smaller litter size and higher risk of being culled compared to the lean ones. In rabbit males, Brun *et al.* (2006), using two divergent lines of males selected for growth rate, observed that some reproductive traits were related to live weight. Lavara *et al.* (2012) observed the existence of negative genetic correlations between different seminal traits, as normal apical ridge (NAR) and motility, and the average daily gain of males selected for growth rate. Altogether these results indicate that an adequate body development of rabbit males through lactation, the fattening and the rearing periods may impact their future reproductive performance.

For this reason, the present work aims to evaluate the effect that early development, from birth to the end of rearing period, could have on the seminal performance of rabbit males selected for growth rate addressed to AI, both in the training phase and throughout their reproductive life, as well as in their lifespan.

Material and Methods

All experimental procedures were approved by the Animal Welfare Ethics Committee of the Universitat Politècnica de València (UPV) following the Spanish Royal Decree 1201/2005 on the protection and use of animals for scientific purposes.

Animals and housing

We checked the live weight (LW) of 550 male rabbits from the R line of UPV (selected for growth rate from 28 to 63 days of age) at 0, 28, 63 and 147 of life. In the four weeks following the 147d of age, we evaluated the libido and some seminal parameters (once a week) on 550 males, pre-selected based on their live weight and average daily gain (ADG) variability on each weight control. After this testing phase, we selected 377 males to be followed to as potential reproduction male for 2 years of life.

To obtain the experimental males, 179 reproductive rabbit females from the R line, housed at a selection centre (El Adil Redondo S.L., Carrizo de la Ribera, León, Spain) were used. R females were artificially inseminated during five consecutive reproductive cycles, using the semen from 55 R males from a different genetic origin. All the rabbit females were inseminated at the same time (single batch), with a period between insemination of 42 days. Inseminations were carried out between the months of February and September.

Experimental procedure

Litters were sexed at birth in order to identify the males. A total of 1945 males were individually weighed at birth with a precision balance (±0.01 g). Litter size (total and alive) and the visual presence of milk in the stomach (milk spot) was also recorded. To identify the males born, a 2 × 12 mm glass-chip (EI1001, Felixcan S.A., Spain) was injected (subcutaneous) in the back of the animals, between both scapulae. We used a needle disinfected in iodine and a syringe with a plunger impeller. As main of the rabbit offspring had milked just after birth, visually checked by the presence of a milk spot, we corrected live weight (LW) at birth from such animals by applying the following equation:

$$MS\ (g) = -9.497 + 0.239 \cdot OLWB + 0.303 \cdot LSB$$

The estimated milk spot (MS) weight was a function of the offspring live weight at birth (OLWB) with a milk spot and the litter size at birth (LSB). This equation was obtained in a short trial performed on 120 offspring of the same line R, weighing both the mother and their offspring before and after milking at day two after birth.

At 28 days old, the 1159 surviving male rabbits were weighed and re-identified with an ink tattoo on the left ear. Litters were weaned at 36 days, transferring the mother to an adjoining shed and leaving the weaned rabbits in the same cage in which they were born.

Subsequently, the 1025 surviving males were weighed at 63 days of age. At that time, a selection of the males that would be transferred to the AI centres was done. Selection was made considering the individual LW recorded and the average daily gain (ADG) calculated in the period 28-63 days. Males were chosen in each batch to obtain a population (604 males) that gathered the maximum possible variability for the LW and ADG (Table 5.1) in the different growth periods until this moment (at birth, between 0-28 days and between 28-63 days of life; corresponding to the lactation and the fattening period, respectively). Once selected at 63 days of age, males were sent in an equitable manner to two different locations (maintaining the variability profile in both ones). Half of them were sent to the AI centre A (Adil Redondo, Carrizo de la Ribera, León, Spain) and the other half to the AI centre Z (Zapiños, Abegondo, A Coruña, Spain).

Males were reared until the 147 days of age (from 63 to 147 days), and the 550 males that reached this age were weighed for the last time. During the next four weeks (testing phase), the main seminal traits were controlled to determine the potential of each male to be used as semen producers at the AI centres. For each male, one ejaculate per week was collected with the help of an artificial vagina, and semen' volume, motility, concentration and production, as well as spermatozoa's normal apical ridge (NAR) and abnormalities, were determined.

From 175 days up to a maximum of 2 years of life, 377 males were selected from the initial 550 to be maintained at the AI centres. In this period, seminal traits like volume, motility and concentration were individually recorded twice a week. Productive life represented the number of days a male was present at the AI centre from 175 days old to its death, culling or censoring (set to 2 years).

All the males were fed with the same feeding program during trial. During lactation, young males ate the same diets as their mothers, with 16.8% crude protein (CP) and 14.1% crude fibre (CF) from 0 to 30 days of age, and with 15.0% CP and 18.4 CF from 30 to 36 days of age (pre-weaning). Once the males were weaned, the litter received a fattening diet with 15.8% CP and 14.7 CF until 63 days of age. From this moment until the 147 days of age, the males were fed with a rearing diet (15.0% CP and 17.5 CF). Finally, during the productive phase, the diet offered had 16.5% CP and 13.0% CF.

Semen evaluation

Testing phase (from 147 to 175 days of life)

Collected ejaculates with urine, or any irregular aspect were discarded, and the rest (successful attempts) were observed firstly if they contained gel (immediately discarded to avoid spermatozoa agglutination). Subsequently, the volume, the concentration and the sperm production were recorded according to the methods described by Lavara *et al.* (2011). In brief, they are based on the visual evaluation of ejaculation in a graduated tube followed by the sperm count in a counting chamber (Thoma-Zeiss cell) under a microscope (phase contrast, 40× increase). Visual sperm motility was evaluated under a microscope by a trained technician (percentage of spermatozoa showing motility), after dilution of the complete ejaculate to 1/5 in a tris-citric-acid-glucose buffer. Motility of the ejaculate was presented as a binomial variable: 0 for samples with percentage of motile spermatozoa below 65% or 1, for samples with a percentage of motile spermatozoa above 65%.

NAR and percentage of abnormal spermatozoa was evaluated after their coloration with eosin-nigrosin mix. This procedure was similar to that described by García-Tomás *et al.* (2006), in at least 100 spermatozoa. The evaluation of the abnormal forms was evaluated according to the criteria described by Barth and Oko (1989) including the spermatozoa without flagella.

Production phase (from 175 days to 2 years of life)

Firstly, the aspect of each extracted ejaculate was evaluated, discarding those with a yellowish colour and/or presence of urine, paste, gel, precipitates or blood. After that, the volume of the ejaculate was measured in a graduated tube (± 0.1 mL) and visual motility and concentration in a microscope after a 1:5 dilution with a tris-citric-glucose diluent were evaluated, both by the same trained technician in each AI centre. Total collection attempts in the productive life was defined as the number of times a male rabbit was taken to semen collection. Ejaculates were classified as profitable when they presented adequate traits for its use as commercial semen (a normal aspect, an adequate visual motility above 65% and concentration). For each individual, the percentage of profitable ejaculates was calculated in relation to the total collection attempts.

Statistical analysis

Libido "mating attempts" of young male rabbits during the testing phase in function of the body development traits

To evaluate the libido of young male rabbits, we implemented a series of multinomial logistic regression models. Models contained two fixed effects, the batch (1 to 5) and the destination farm (A or Z) and a standardized co-variable related to the body development (LW at 0, 28, 63 and 147 days of life, and ADG for the periods 0-28, 28-63 and 63-147). We used the R-software (version 3.5.0) and the "*multinom*" function of the package "*nnet*".

Seminal characteristics of young male rabbits during the testing phase in function of the body development traits

To evaluate the seminal characteristics (volume, concentration, production, motility, NAR, and spermatozoa morphological normality) of young male rabbits, we implemented a series of linear mixed models. All models included the AI centre (A or Z), the batch (1 to 5), and the week of testing (1 to 4) as a fixed effect, the body development traits (LW at 0, 28, 63 and 147 days old, and ADG for the periods 0-28, 28-63 and 63-147) as a covariate, and the permanent effect of each rabbit female (*p*) and the error term (*e*) as random effects. Random effects were assumed to have an average of zero and a variance of σ_p^2 for permanent, and σ_e^2 for the error term. We model the variance-covariance among animals by using a compound symmetric structure for the variance-covariance matrix of the residuals. The asymmetrical distribution of the original data led to the logarithmic transformation of data from concentration and production ($\log_{10}$ 10^6spermatozoa per mL or ejaculate, respectively). As motility during the testing phase was recorded as a binomial variable (0, motile sperm below 65%; 1 motile sperm above 65%), data were analysed under the GLIMMIX procedures of SAS (2002) for binomial distributions. Solution provided odds values about the increase of relative probability to be classified as 1 (motile sperm above 65%) per unit of proposed LW or ADG change. Finally, as NAR data presented a Poisson distribution, data were analysed with six GLIMMIX procedures for Poisson distributions.

Finally, semen production and motility data during the testing phase were also analysed with the same procedures described above, including the effect of the growth variables as main effect (4 quartile classes) instead as a covariate, for a better presentation of the obtained results.

Seminal parameters of male rabbits (as adults) during the production phase in function of the body development traits

For each of the variables average volume, average motility and profitable ejaculates (both number and percentage) controlled during the productive phase, the data were analysed with six GLM procedures of SAS (2002), each of them included as fixed effects the location, the batch, and as a covariate one of the growth variables to be evaluated. In addition, profitable ejaculates data during the productive phase were also analysed with the same GLM procedures described above. However, for an easy interpretation of results, the body development variables were set as a fixed effect with four levels, representing the four quantiles (quantile limits are in Table 5.1).

Productive lifespan of rabbit males (as adults) during the productive phase in function of the body development traits

Lifespan during the productive phase in function of the body development traits (LW at 0, 28, 63 and 147 days old, and ADG for the periods 0-28, 28-63 and 63-147) was analysed using a series of Cox proportional hazard ratio models (one model for each developmental trait). We first tested for the influence of the batch (1 to 5) or AI centre the animal lived in (A or Z) on the productive lifespan. As neither of these variables influence the productive life, they were not included in the models used to assess the influence of the body development traits on the productive lifespan of adult male rabbits. As for the multinomial model and to avoid the scale effect, each development trait was standardized before being included in the model. Hazard ratios, p-values and the log likelihood ratio test for the model significance of each body developmental trait is in Table 5.5. Kaplan-Meier estimations of survivorship (percentage of live animals) in function of the LW at 28 and at 147 days (the developmental traits explaining the variability in the survival of male rabbits during their productive life) are in Figure 5.4.

Results

Means, standard deviations, coefficients of variation (CV), quartiles, minimum and maximum values of LW, ADG and main traits controlled during the testing and productive phases are listed in Table 5.1. Based on this natural variability, we could correctly select individuals having a different dynamic of body development to test the hypothesis that the

Table 5.1 *Main descriptive values of population used in the experiment (550 males until testing phase and 377 males for production phase).*

Variables	Mean	SD	Min	Lower Q	Median	Upper Q	Max	CV
LW and ADG (g)								
LW 0 days	65.0	11.1	34.3	57.8	64.9	72.1	102.0	17.15
LW 28 days	765	157	318	662	764	870	1240	20.53
LW 63 days	2785	319	1696	2586	2800	3012	3640	11.45
LW 147 days	4909	530	3095	4580	4905	5260	6495	10.79
ADG 0-28 days	25.01	5.43	9.75	21.45	25.00	28.62	41.16	21.72
ADG 28-63 days	57.69	6.92	30.51	53.09	58.17	62.91	73.89	11.99
ADG 63-147 days	25.28	5.74	8.60	21.64	25.15	28.76	42.85	22.69
Semen traits during testing phase[1]								
Ejaculates (n)	2.68	1.43	0.00	2.00	3.00	4.00	4.00	53.29
Average Volume (mL)	0.60	0.28	0.00	0.40	0.58	0.75	1.77	46.49
Average Motility (%)[1]	42.01	26.42	0.00	23.17	45.00	67.50	82.00	62.89
Average concentration (spz×10^6/mL)	123.8	100.9	0.0	50.9	102.0	165.8	817.5	81.51
Average production (spz×10^6/ejaculate)	79.4	76.2	0.0	22.2	57.5	110.7	530.4	96.04
Average NAR (%)	89.5	15.5	19.9	90.1	96.0	98.0	100.0	17.25
Average spz abnormalities (%)	34.50	20.20	1.56	17.80	30.01	48.14	90.10	58.56
Traits during production phase[2]								
Productive life (days)	351.2	198.2	7.0	155.0	395.0	546.0	550.0	56.42
Collection attempts (n)	88.1	53.1	2.0	38.0	88.0	144.0	182.0	60.25
Extracted ejaculate (n)	83.6	52.3	1.0	36.0	83.0	138.0	159.0	62.62
Average Volume (mL)	0.87	0.20	0.00	0.76	0.86	1.00	1.38	22.60
Average Motility (%)[3]	76.00	7.57	0.00	75.00	77.00	78.00	82.00	9.96
Profitable ejaculates (n)	67.8	50.1	0.0	22.0	58.0	113.0	155.0	73.92
Profitable ejaculates (%)	69.9	22.5	0.0	54.7	74.6	90.3	100.0	32.16

SD: standard deviation; Min: minimum value; Q: quartile; Max: maximum value; CV: coefficient of variation; LW live weight; ADG: average daily gain; spz: spermatozoa; NAR: normal apical ridge; Profitable ejaculates ejaculates of normal appearance with an adequate visual motility and concentration (percentage of the tota collection attempts).

[1] Testing phase: from 147 to 175 days old; [2] Production phase: from 175 to 2 years old (maximum);

[3] Visual motilities done by the same technician at each centre.

early body development pattern influence lifespan and the seminal characteristics of male rabbits. Concerning the body development traits, the observed CV varied from 11 to 23%. For seminal characteristics (production and quality) the CV range from 17 to 96% during the testing phase and from 10 to 74% in the productive phase. This variability allows us to correlate the possible differences in the seminal traits to the different growth patterns. The same is applicable for productive lifespan on the productive phase; the productive lifespan range from 7 to 550 days on production.

Libido "mating attempts" of young male rabbits during the testing phase in function of the body development traits are in Table 5.2. Having a different LW or ADG, in all the controlled times, did not affect the number of successful IA attempts, evaluated as the relative risk of changing from zero to one, two, three or four successful attempts.

Table 5.2 *Relative risks ratio, of changing from zero mating attempts to one, two, three or four successful attempts, per each unit of increase in the standardized variables related to the early development.*

Standardized variable[1]	Number of realized attempts					P-values		
	Zero[2]	One	Two	Three	Four	One	Two	Three
LW 0 days	1.00	0.852	0.979	0.840	0.927	0.431	0.905	0.221
LW 28 days	1.00	1.182	1.277	1.140	1.180	0.417	0.184	0.386
LW 63 days	1.00	1.184	1.444	1.266	1.195	0.417	0.052	0.119
LW 147 days	1.00	1.349	1.394	1.354	1.184	0.238	0.131	0.103
ADG 0-28 days	1.00	1.207	1.294	1.165	1.198	0.360	0.161	0.314
ADG 28-63 days	1.00	1.125	1.437	1.281	1.146	0.590	0.074	0.117
ADG 63-147 days	1.00	1.227	1.101	1.109	1.023	0.398	0.634	0.547

[1]LW = live weight and ADG = average daily gain. [2] Zero represents the reference level to which the pairwise com were performed.

Relationship between body development traits and seminal parameters during the testing phase are in Table 5.3. LW at 0, 28 and 63 days old, as well as the ADG between the periods 0-28 and 28-63 influenced the semen characteristics. NAR was influenced by no developmental traits.

In summary, the increment (per each 10 g) on the LW at 0 days resulted in a higher volume, concentration, production and spermatozoa motility (+0.02 mL, $+1.1\times10^6$ spermatozoa/mL, $+1.1\times10^6$ spermatozoa/ejaculate and a odds ratio of 1.13, being the likelihood of observing ejaculates with an adequate motility (>65%) 13% superior, respectively; $P<0.05$). In fact, semen production linearly increased with LW at 0 days of male rabbits (Figure 5.1a) and motility was significantly lower for the males weighing less than 65 g at birth (Figure 5.2a).

An increase in ADG in the period 0-28 days of 10 g represented an improvement on semen concentration, production and motility of $+1.3\times10^6$ spermatozoa/mL, $+1.4\times10^6$

Table 5.3 *Linear effect of early growth traits[1] on semen parameters during the testing phase (estimate ± standard error).*

Semen parameters		LW at birth[2] (×10g)	LW at weaning (×100g)	LW at rearing onset (×1000g)	LW at rearing end (×1000g)	ADG lactation (×10g)	ADG fattening (×10g)	ADG rearing (×10g)
Volume (mL)		0.019 ± 0.009*	0.013 ± 0.007	0.069 ±0.036	0.057 ±0.024*	0.037 ±0.021	0.024 ±0.017	0.020 ±0.023
Concentration	(log_{10} 10^6spz/mL)	0.038 ± 0.015*	0.043 ± 0.012***	0.210 ±0.061***	0.046 ±0.042	0.123 ±0.036***	0.071 ±0.030*	-0.048 ±0.039
	(10^6spz/mL)	1.091	1.104	1.622	1.112	1.327	1.178	0.895
Production	(log_{10} 10^6spz/ejac)	0.051 ± 0.018**	0.055 ± 0.015***	0.286 ±0.072***	0.092 ±0.050	0.158 ±0.043***	0.102 ±0.036**	-0.038 ±0.046
	(10^6spz/ejac)	1.125	1.135	1.932	1.236	1.439	1.265	0.916
Motility	(ln odds)[3]	0.123 ± 0.059*	0.155 ± 0.049**	0.870 ±0.248***	0.238 ±0.157	0.447 ±0.142**	0.321 ±0.122**	0.000 ±0.146
	(odds)[3]	1.131	1.168	2.387	1.269	1.564	1.379	1.000
NAR (%)		0.000 ± 0.023	-0.001 ± 0.019	0.003 ±0.095	-0.003 ±0.063	-0.002 ±0.056	0.003 ±0.046	-0.006 ±0.058
Spz abnormalities (%)		-0.003 ± 0.004	-0.003 ± 0.004	-0.024 ±0.018	0.001 ±0.012	-0.007 ±0.011	-0.012 ±0.009	0.010 ±0.011

[1] In brackets appears the change on each growth trait needed to obtain the estimated change for the different semen parameter.
[2] Standardized weight at birth (corrected by milk spot presence).
[3] Motility of the ejaculate presented as a binomial variable: 0, motile sperm below 65%; 1, motile sperm above 65%. Odds: increase of relative probability to be classified as 1 (motile sperm above 65%) per unit of proposed LW or ADG change (see [a]).
LW: live weight; ADG: average daily gain; NAR: normal apical ridge; spz: spermatozoa; ejac: ejaculate.
Weaning (28 days old); Rearing onset (63 days old); Rearing end (147 days old).
*P<0.05; ** P<0.01; *** P<0.001.

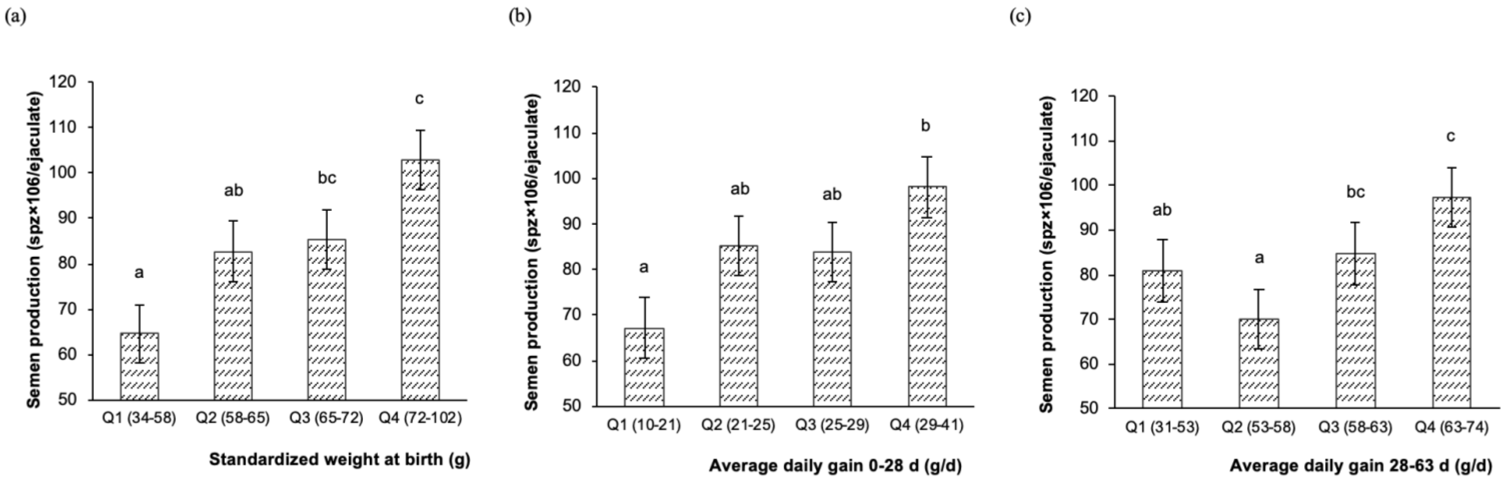

Figure 5.1 Average semen production (spermatozoa $\times$ 10^6/ejaculate) according to the classification by quartiles of the population for: (a) standardized weight at birth (corrected in function of milk spot presence), (b) average daily gain from 0 to 28 days of age and (c) average daily gain from 28 to 63 days of age.

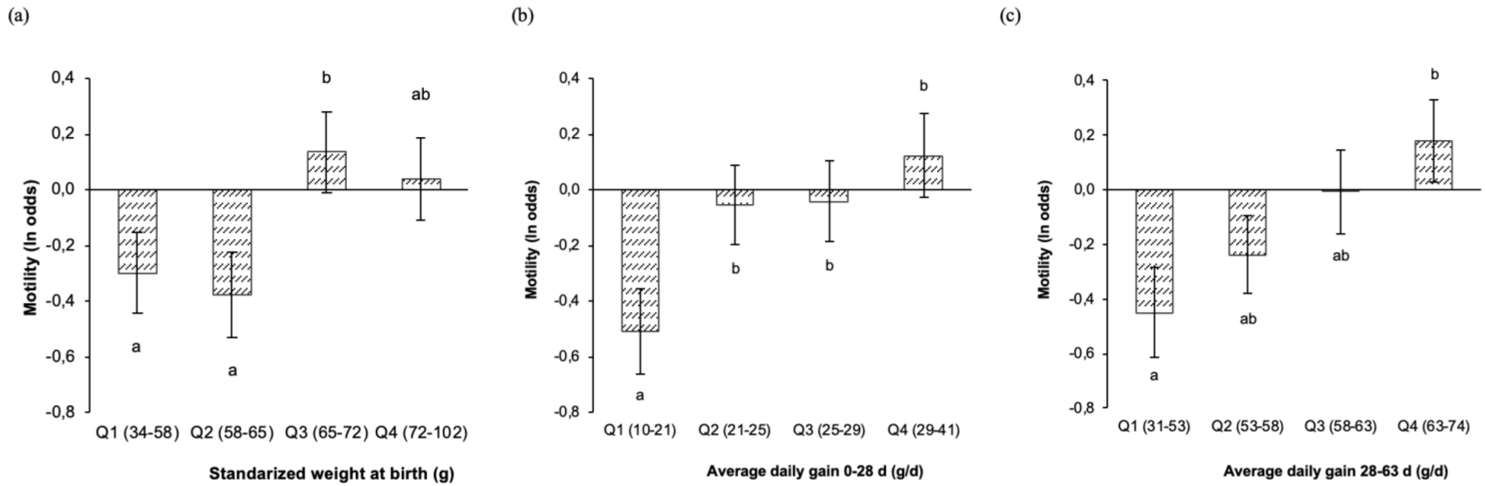

Figure 5.2 Average motility (ln odds) according to the classification by quartiles of the population for: (a) standardized weight at birth (corrected by milk spot presence), (b) average daily gain from 0 to 28 days of age and (c) average daily gain from 28 to 63 days of age. Motility of the ejaculate was presented as a binomial variable: 0, motile sperm below 65%; 1, motile sperm above 65%. Odds: increase of relative probability to be classified as 1 (motile sperm above 65%).

Table 5.4 *Linear effect of early growth traits[1] on semen parameters during the productive phase (estimate ± standard error).*

Semen parameters	LW at birth[2] (×10g)	LW at weaning (×100g)	LW at rearing onset (×1000g)	LW at rearing end (×1000g)	ADG lactation (×10g)	ADG fattening (×10g)	ADG rearing (×10g)
Average volume (mL)	0.000 ±0.007	0.000 ±0.005	-0.008 ±0.026	-0.030 ±0.018	0.000 ±0.015	-0.005 ±0.012	-0.028 ±0.016
Average motility (%)	0.029 ±0.102	0.101 ±0.077	0.492 ±0.370	0.119 ±0.243	0.298 ±0.226	0.168 ±0.177	-0.198 ±0.219
Profitable ejaculates (n)	-2.036 ±2.115	-2.314 ±1.590	-3.261 ±7.709	-10.945 ±5.219	-6.584 ±4.607	1.399 ±3.682	-10.057 ±4.699*
Profitable ejaculates (%)	-0.208 ±0.788	-0.804 ±0.592	-2.911 ±2.867	-5.829 ±1.898**	-2.374 ±1.715	-0.672 ±1.371	-4.856 ±1.713**

1 In brackets appears the change on each growth trait needed to obtain the estimated change at the different semen parameter.
2 Standardized weight at birth (corrected by milk spot presence).
LW: live weight; Weaning (28 days old); Rearing onset (63 days old); Rearing end (147 days old).
Average volume: average value of ejaculates with normal aspect; Average motility: average value of ejaculates with normal aspect.
Profitable ejaculates: ejaculates of normal appearance with an adequate visual motility and concentration (percentage of the total number of attempts to extract semen.).
*P<0.05; ** P<0.01.

spermatozoa/ejaculate and a likelihood of +56% of having an adequate spermatozoa motility (>65%), respectively; P<0.05. The same pattern was observed for the ADG between 28-63 days and for the measures of LW at 28 and 63 days. In fact, both semen production (Figure 5.1b) and motility (Figure 5.2b) linearly increased with the ADG of males in the period 0-28 days; males showing an ADG between 0-28 days below 21 g per day had a diminished semen production and motility in the testing phase.

For the ADG in the period 28-63 the improvements on semen concentration, production and motility per each 10 g of increment was $+1.2\times10^6$ spermatozoa/mL, $+1.3\times10^6$ spermatozoa/ejaculate and a likelihood 38% superior of having an adequate motility, respectively; P<0.05). In this case, semen production (Figure 5.1c) and motility (Figure 5.2c) seem to achieve the highest values for those males showing an ADG in the period 28-63 greater than 63 g/d. At the end of rearing period (147 day) an increase in LW of 1000 g only improved influenced the semen volume (+0.06 mL; P<0.05), although not relevant in practice.

Concerning the influence of LW and ADG on the volume, motility and the number and the percentage of profitable ejaculates (Table 5.4), only the number and the percentage of profitable ejaculates were influenced. In this sense, an increment of LW at 147 days of 1 kg represented a reduction of 6 points of percentage in the percentage of profitable ejaculates (P<0.05), while animals having gaining, on average, 10 g per day more in the period 63-147 had a lower number and percentage of profitable ejaculates (–10.1 and –4.9%, respectively; P<0.05) through it productive life. In fact, rabbit males that showed a

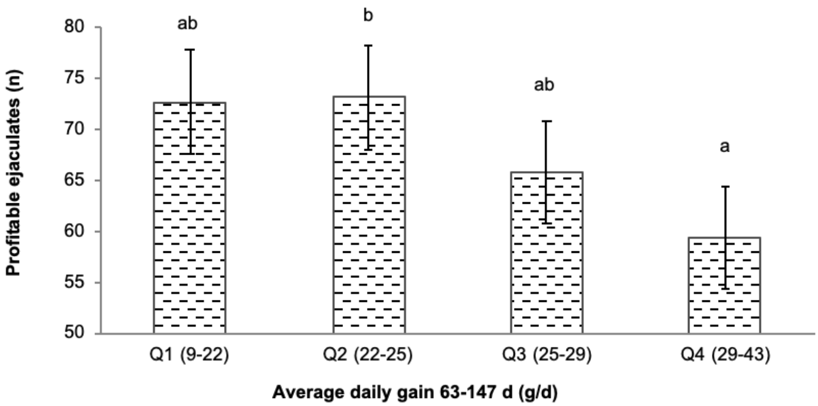

Figure 5.3 Number of profitable ejaculates (ejaculates of normal appearance with an adequate visual motility and concentration) according to the classification by quartiles of the average daily gain from 63 to 147 days of age.

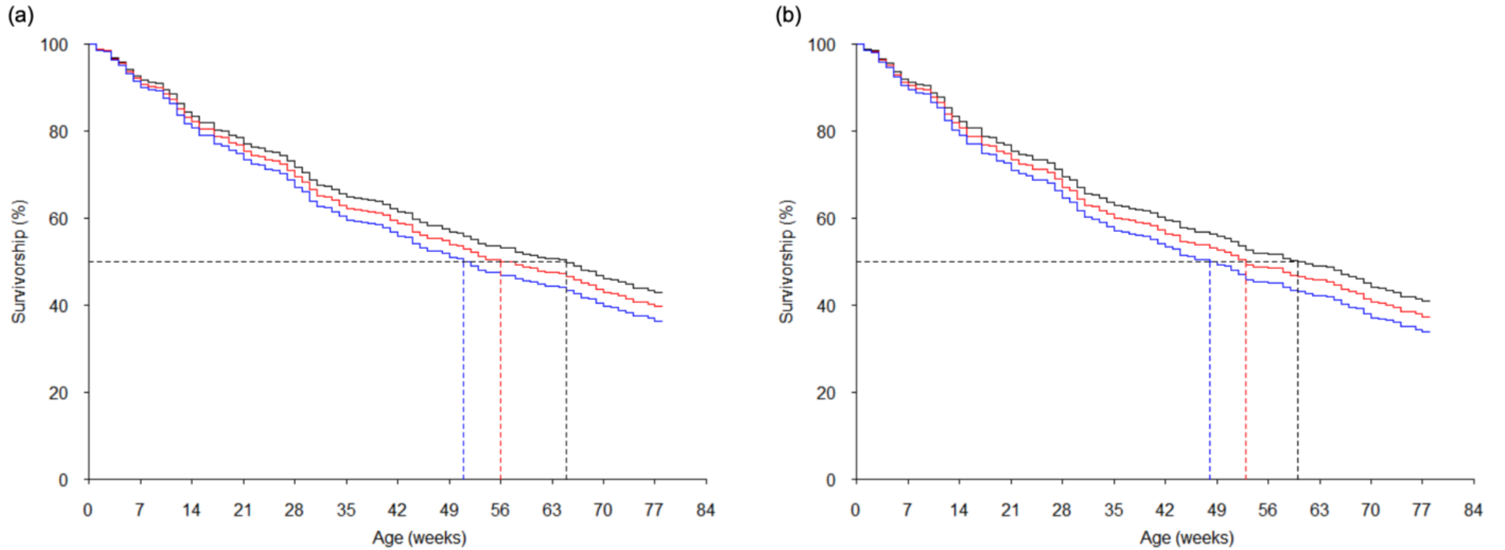

Figure 5.4 Survivorship estimation (percentage of live animals) across time (age in weeks) in function of the live weight in different moments of development: a) theoretical males with different live weight at weaning (black: 650 g; red: 750 g; blue: 850 g) and vertical segments represent the median survival time: 65, 56 and 51 weeks, respectively; b) theoretical males with different live weight at the end of the rearing period (black: 4600 g; red: 4900 g; blue: 5200 g) and vertical segments represent the median survival time: 60, 53 and 48 weeks, respectively.

growth rate above 29 g/d had the smallest number of profitable ejaculates during the productive phase (Figure 5.3).

Finally, Figure 5.4 and Table 5.5 show the influence of early development traits on the hazard ratio of death or culling during the productive phase. We observed that an increase in one standard deviation unit on LW at 28 or at 147 days, as well as for the ADG in the periods 0-28 and 63-147, all increases the risk of leaving the herd by +14.6, +17.5, +14.6 and +16.2 %, respectively ($P<0.05$). These values return a median survival life for theoretical males weighing 650, 750 and 850 g at 28 days old of 65, 56 and 51 weeks of age, respectively (Figure 5.4a), and for those weighting 4.6, 4.9 and 5.2 Kg at rearing end would be 60, 53, and 48 weeks of age, respectively (Figure 5.4b).

Table 5.5 *Influence of the early development variables (standardized variables) on the hazard ratio of death or culling of males during its productive life. Values above one represents an increment on the hazard ratio and therefore a reduction on the productive life.*

Standardized variable[1]	Hazard ratio	95% Confidence interval	P-value	Likelihood ratio test[2]
LW 0 days	1.067	[0.93-1.22]	0.34	0.30
LW 28 days	1.146	[1.00-1.31]	0.04	0.04
LW 63 days	1.039	[0.91-1.19]	0.57	0.60
LW 147 days	1.175	[1.02-1.35]	0.03	0.03
ADG 0-28 days	1.146	[1.00-1.31]	0.04	0.04
ADG 28-63 days	0.965	[0.84-1.10]	0.59	0.60
ADG 63-147 days	1.162	[1.02-1.33]	0.03	0.03

[1]LW = live weight and ADG = average daily gain. [2] P-values of likelihood ratio test for the null hypothesis that all covariates of the implemented model are equal to zero.

Discussion

To make an adequate evaluation of the possible effect of the different development degree of the males at different moments of their early life, it was essential to build a population of rabbit males that had a wide variability in the growth traits (Table 5.1). CV values obtained in this work show a wide range for the different growth variables controlled (from 11 to 23%). In fact, the standard deviation observed with 550 males (6.9 g/d) for ADG during the fattening period was similar to that obtained for a population of 12 908 rabbit males of this same genetic line (6.9 g/d; Lavara *et al.* (2012). In addition, mean values of growth traits were very close to the median values and there was, in general, a good equidistance between quartiles in both sides, denoting symmetry in data distribution. These results seem to confirm that the needed population structure was achieved. On the other hand, the average LW and ADG values obtained in this work were slightly higher than those obtained in other studies with selected males from this same genetic line (Pascual *et al.* 2004 and Lavara *et al.* 2012 and 2015). This fact could be related to different climatic and environmental conditions, as well as differences on the generation of selection in the animals of the different trials.

Regarding the semen traits, data variability was very high both in the testing and in the production phases (with CV values up to 96%). The same general conclusion was reached by Lavara *et al.* (2012) for the semen traits in rabbit males of R line (with CV values up to 78%). These authors, using R rabbit males after the testing phase, presented similar values to those obtained in our work for NAR during the testing phase and semen motility during the production phase, but lower for the percentage of abnormal spermatozoa (17.0 vs. 34.5 %) and higher motility (65.0 vs. 42.0 %) than our males in the testing phase. Moreover, Pascual *et al.* (2004) observed similar results on motility and NAR values, but lower number of ejaculates and abnormal spermatozoa, as well as higher semen volume, concentration and production during testing phase (–1.3, –27,8%, +0.31mL, +113 $\times$ 10^6 spermatozoa per mL and +144 $\times 10^6$ spermatozoa per ejaculate, respectively). Differences between trials could be due to the different environmental conditions, maturity degree of the rabbit males used and the use of different assessment techniques in the case of motility (CASA vs. visual).

The testing phase is usually used by the AI centres to determine which rabbit males will be chosen for semen production. Libido is a key behavioural trait in the election of the rabbit males to be destined to AI. The results of the present work have showed that early growth traits did not significant affect the number of realized attempts of males during the testing phase. However, higher ADG during the fattening period seems to be associated with increased probability of a higher number of attempts ($P<0.10$). Pascual *et al.* (2004) observed that the R rabbit males that adequately cover their nutritional requirements during the different phases of early development, also showed a high number of ejaculates during their testing phase (+0.40 and +0.28 ejaculates for males reared on autumn and spring seasons, respectively). These results could indicate that a proper growth during the first steps of the life could contribute to achieve sooner an adequate maturity degree, with a better libido, at the beginning of the reproductive life, although further studies would be needed to confirm it.

Corroboration of existence of a relationship among body development traits and the seminal parameters, both in the short- and long-term, would allow to anticipate and improve the choice of the most adequate rabbit males to be destined to AI. To have a higher LW at birth had a slightly positive effect on some seminal parameters at the testing phase (volume, concentration, production and motility), especially for those weighing over 65 g. Fortun (1994) observed that the development and multiplication of the primordial germ cells and of the primordial follicles already occurs during own gestation. In rabbit females, Poigner *et al.* (2000) observed that females born with a heavier weight produced more offspring as adults than lighter ones. However, the positive linear effect of a heavier weight at birth at early reproductive live of rabbit males seem to disappear after during the production period. Therefore, an adequate foetal nutrition and development could contribute to a better semen performance at early reproductive life, but without positive effects in long terms, so it does not seem to be one of the most key development traits in defining future male performance.

Growth during the first two months of life had a strong influence on some seminal traits (motility and semen concentration and production) during testing phase. Rabbit males having patterns of low growth (up to 21 and 53 g per day during lactation and fattening periods, respectively) showed clearly lower semen production with a worse motility at early reproductive life. These results may be due to the fact that important changes in physiological (Deltoro and López, 1985) and reproductive (seminiferous tubules; García-Tomás *et al.*, 2008) development take place during lactation and fattening, and probably lighter males did not reach the testing phase with an enough mature reproductive degree. Pascual and Pla (2007), comparing R rabbits differing in 16 generations of selection for growth rate during the fattening, observed that less selected animals (with lower growth rate both during lactation and fattening period) showed a lower physiological development at the end of the fattening period (–13% liver, –8% kidneys and –7% dissectible fat). In addition, rabbit males showing a low growth until 63 days of life may be due to not being properly covered their requirements. Pascual *et al.* (2004) observed worse semen traits during the testing phase in those rabbit males that did not correctly cover their nutritional needs from 9 to 14 weeks of life. However, the negative effects of a lower growth during the first 63 days of age on semen performance disappeared in the production phase. These results seem to indicate that an early underdevelopment could be delaying the reproductive capacity of young males, so it would be advisable management programs (standardized litter size during lactation, specific diets during fattening,…) that will ensure the necessary provision of nutrients to the males during the first two months of life.

Both ADG during rearing period and LW at rearing end had not relevant effects on semen traits during the testing phase. However, greater growth during the rearing period and heavier weight at the beginning of the reproductive life had negative effects on the number and percentage of profitable ejaculates obtained throughout the productive life of males. These negative linear effects associated to the rearing growth could be related with a non-appropriate feeding programmes adjustment during this period. During the rearing period, when using commercial diets provided *ad libitum*, rabbit males from parental lines tend to over-consume (Pascual et al, 2004), which may lead them to a level of fatness higher than that desirable for the beginning of the reproductive phase. Recently, Pascual *et al.* (2015 and 2016) found that when fitting the nutritional requirements and providing a constant daily supply of nutrients, to rabbit males selected for growth rate, improvements of some sperm morphological characteristics and of the fertility rate of their semen are achievable. In males, Du Plessis *et al.* (2010) also observed that overweight sometimes could be associated with an increase in abnormal spermatozoa and a high risk of fertility problems.

Finally, lifespan improvement of breeding rabbits should be one of the objectives of rabbit production, since it is usually associated with an improvement in both the farm efficiency and the welfare and health of the animals. In the rabbit males used for AI, reaching a higher life expectancy is mainly due to their better adaptability to the farm environmental conditions and to their adequate semen production. In our work, higher growths during lactation and rearing periods were associated with a higher risk to death or culling during the production phase of rabbit males. A hypothesis for this higher risk could be related to get and sustain an excessive level of fatness on rabbit males during the production phase. During lactation, the total number and size of adipocytes are defined, favoured by energy intake coming from fat (Snajder *et al.*, 2018). Rabbit milk has a high fat content respect other mammal (Pascual *et al.*, 1999) and, consequently, young rabbit males showing higher ADG would suck higher fat quantity from mother's milk. A higher number of adipocytes would help the excessive deposition of fat when the nutrition requirements are no fitted in long-term. In fact, the energy requirements for growth and maintenance during the rearing period are scarce, and an excessive energy intake could happen easily, that would increase the risk of rabbit males of achieving the beginning of their reproductive life with an overfatting status. In rabbits, Maertens (2010) proposed to fit the feeding level to daily requirements to reduce the troubles associated to fatness. Recently, some works (Martínez-Paredes *et al.* 2012 and 2018) have confirmed that non-adequate body condition around the first mating had clear negative effects on the future reproduction performance and lifespan of young rabbit females. Therefore, it seems that an overfatting status at the beginning of reproduction life could lead rabbit males to an increased risk of suffering health troubles and/or reducing their libido or semen’s production or quality, which could increase their risk of death and culling.

Conclusion

From these results, it could be concluded that early development of the rabbit males, selected by growth rate during fattening and intended to AI, influences their semen production and quality in the short- and long-term as well as their lifespan. A greater growth development of the rabbit males until the end of their fattening period seems to have a positive effect on their sexual maturity degree with which they begin their reproductive life, improving aspects such as libido, spermatozoa motility and seminal production, but no in the long-term. However, the rearing period seems to be key to have long and productive reproductive life. Reaching the reproduction onset with an excessive weight seems to reduce the reproductive performance and lifespan of rabbit males. Therefore, fitting the feeding requirements during the final phase of growth, when the energy needs for growth are reduced, could be an adequate management recommendation for the young reproductive rabbit males from paternal lines.

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General Discussion

The main aim of this PhD Thesis was to evaluate the possible long-term influence of the different patterns on early growth development and of the feeding management provided during the rearing period, in order to maximize the performance of the breeding rabbits. To achieve our goal, two types of trials, both in rabbit females and males, were carried out. The first of them was mainly focused on the different growth patterns from birth to the end of the rearing age and their influence on rabbit performance in the long-term, and the second one, on the use of different diets or feeding programs during the rearing period and their effect in the short- medium-term. Both types of trials allowed us to know which phases of animals' development seem to be key in their future performance and lifespan, which physiological indicators we could use to achieve an adequate physiological and reproductive development, and the most appropriate feeding programmes to achieve reproductive success, both in rabbit females and males.

The importance of a right body development

From birth to rearing onset

LW at birth is the first information we have about our animals. The possibility of improving the productivity of our future breeders by making decisions from the first day of life is feasible. Firstly, the existence of a minimum threshold of LW at birth that increase the probability of survival of future breeders has been corroborated in this thesis. After reviewing the literature (Argente *et al.*, 1999, Drummond *et al.*, 2000, Coureaud *et al.*, 2007), we could propose a minimum LW at birth between 45-50 g to increase significantly the survival of rabbit females. Also, we have shown that ensuring the intake of milk during the first hours of life, had a high positive impact on the probability of survival of those kits with lower weight to that threshold proposed, so having litter sizes that ensure milk intake could maximize the survival of the future breeders. On the other hand, the LW at birth also had an effect on short-term reproductive performance, both in rabbit females and males. In females of a maternal rabbit line, we observed that heavy newborn females (>57 g) had significantly higher body fatness at first insemination (+0.18 mm of PFT) and bigger litter sizes at first parturition (+0.89 kits) than lean ones (<57 g), similar results as obtained by Poigner *et al.* (2000), with no influence in the long-term. In males from a growth rabbit line, we had a linear slightly positive effect of LW at birth on some seminal parameters (volume, concentration, production and motility) at the beginning of reproductive life, especially for those kits weighing over 65 g, but also without effects in long-term. These results seem to indicate that, independently of genetics, an adequate body size at birth seems to be relevant to define early survival of the animals and to favour their productivity at the beginning of reproductive life.

Similarly, higher ADG during lactation and fattening periods were related with higher early reproductive performance in rabbit males. During the first 70 days of life, there is a great development of both the digestive tract and reproductive organs (Deltoro and López, 1985; García-Tomás *et al.*, 2008). An adequate growth during these early stages of development would predispose young animals to be better as breeders. In this thesis, we have observed that there was a positive linear relationship between growth rate of males during lactation and fattening with some of the seminal traits (motility and semen concentration and production) controlled in their first weeks as breeders. This effect was mainly due to those animals with low growth rates that probably are not adequately covering the higher nutritional requirements during these early phases, delaying their maturity. However, according to the results obtained, the hypothesis that an adequate development would improve the performance as breeding rabbits was only confirmed during the beginning of the reproductive life. In fact, excessive growth during the lactation phase has been related to an increased risk of death during the reproductive phase of the males, which could indicate that an excessive growth during these phases would not be adequate either.

In summary, the weight at birth and growth traits until the rearing onset seem to have influence on the maturity degree and productivity at the beginning of the reproductive life of future rabbit breeders, as well as being able to condition their survival positively in short-term, but also even negatively long-term.

Development during the rearing period

The trials carried out in this thesis have confirmed that an adequate growth development during the rearing period has a crucial role in the preparation of future breeders. However, as the reproductive goals of rabbit females and males are different, we should achieve different growth patterns for each of them. In rabbit females, they must face with a cyclical effort to achieve the possible highest productivity, by optimising fertility, kits weaned and lifespan. We observed that, for improving their reproductive performance, females must show a gradual growth pattern from rearing onset to parturition, avoid reaching artificial insemination (AI) age with an excess of fat reserves and at the same time allow females to achieve their first parturition with an adequate maturity level. In our works, we obtained worse results both in the first parturition (on av. –1.3 kittens alive and –86.5 gr of litter weight) and in the whole productive period (–6.1 liveborn and –4.8 weaned kittens) for those rabbit females showing with higher growth patterns during the rearing period. In addition, the rabbit females having high PFT values at the first artificial insemination also had lower reproductive performance at the first parturition and in whole productive period (–3.2 liveborn and –3.0 weaned kittens for each unit of increment in the PFT value). This worse reproductive performance of “fat” young females may be related to an increased number of pre-natal losses during gestation (Vicente *et al.*, 2012), and/or to an increased culling risk among females with excessive PFT (Theilgaard *et al.*, 2006). About the risk of death or culling of females during the rearing period, high PFT at first mating followed by a high pre-partum mobilization of reserves, low pre-partum DE intake, high non-esterified fatty acids and low glucose levels in blood at first parturition are all factors associated with pregnancy toxaemia. Complementary, rabbit females that had a high PFT at the first AI, showed a PFT evolution pattern different to the rest of the females in the second parturition, not being able to recover the body condition that they had at the moment of the first parturition. Although there was not any difference in their reproductive performance on the second parturition, this fact could be one of the causes of the greater risk of mortality of the females that followed this body condition evolution.

In rabbit males, their energy requirements situation is practically of maintenance during rearing period, having as major milestones to maintain a regular feed intake without greasing excessively (Pascual *et al.,* 2016). Contrary to what was observed during the growth in the previous periods, LW at rearing end and ADG during rearing period had no effects on semen traits at the beginning of production phase. However, they did have a strong negative influence on its semen performance (around −5-6% of profitable ejaculates per each increment of 10g on ADG) throughout the productive phase as well as on the lifespan (on av. +16,9% of risk of death or culling per each increase in one standard deviation) of the rabbit males. In both cases, they could be related with non-adequate fitting adjustment of the nutritional requirements and, consequently, an excessive LW and/or level of fatness during last stages of rearing period, remaining even for the rest of their productive life. Similar problems on semen traits and in fertility have been observed for obesity human males by Du Plessis *et al.* (2010).

In summary, we have observed that an adequate management of physiological development during rearing period is the best option to maximize both the productivity and lifespan of our breeders, highlighting that the handling must be differentiated between females and male rabbits.

Knowing the influence of the different phases of early development on the reproductivity performance and lifespan, we can propose the goals to be achieved at each phase by future rabbit breeders. From birth to the onset of the rearing period, we must ensure that the animal growth potential is not delayed ensuring a good reproductive start. During the rearing period, the animal's growth pattern should be progressive, allowing the achievement of an adequate maturity degree at the beginning of its productive life, avoiding an excessive fatness (Martens 2010), to favour long-term reproductive performance and lifespan. In addition, we have observed that *in vivo* tools to evaluate body condition, such as perirenal fat thickness (PFT) by ultrasounds or the determination of some metabolites in blood plasma (leptin, NEFAs), could be useful to predict lifespan or to adjust growth patterns in the future. In any case, the best tool we have to modify the growth pattern of rabbits are feeding programs, especially during the rearing period where the adjustment of nutrients to the requirements should be crucial to prepare future breeders to subsequent productive challenges.

Rearing feeding management

In rabbit females, we have evaluated different rearing feeding programs that cover most of the proposed systems in the last decades (reproduction diet, reproduction diet restricted and fibrous diet; Pascual *et al.*, 2013), as well as some modification of them (combinations of reproduction and fibrous diets), to evaluate which program we should be recommended to be applied, considering effects both in the short and in the long-term. Regarding fertility, there were no relevant differences among the different feeding programs evaluated during the first parturition. As first AI was done at 18 weeks, it allowed females on feeding programs providing lower energy until AI (reproduction restricted and fibrous diets) to achieve an adequate maturity degree to ensure reproductive success. As proposed by Árias-Álvarez *et al.* (2009), the reproductive success of young rabbit females at the beginning of the reproductive life could be very related to reaching a minimum threshold level of energy at the first AI (around 2.8 ng leptin/mL in blood plasma), as it can be seen in Figure 1.6 of Paper I in this thesis.

However, as we have previously proposed, it is necessary a gradual body development during this period, and the exclusive use of an *ad libitum* reproduction diet lead young rabbit females to reach a higher body energy and perirenal fat reserves. This fact has been related with a higher mortality around parturition (toxaemia), a lower DE intake during late pregnancy and the onset of the first lactation (Pascual *et al.*, 2002) and, consequently, a greater mobilization of reserves at first parturition (confirmed by NEFAs and PFT levels), as well as a smaller litter size at first parturition and a worse recovery of resources at the second parturition. In addition, the trial performed in long-term confirmed that the faster growth during the rearing and the bigger size of the fat reserves at the AI of females fed *ad libitum* with a reproduction diet respect to those with a fibrous diet, leading to worse results in the reproductive performance at the long-term, especially due to the greater mortality of females during rearing period. An earlier AI could partially correct the excessive body reserves of rabbit females fed *ad libitum* with reproduction diets during the rearing period, but it could likewise produce consequences on the rabbit female performance and lifespan (Pascual *et al.*, 2013).

As it was presented in the introduction of this thesis, the results found in the literature, in which any type of feed restriction has been used during the rearing period (energy restricted or fibrous diet), are very variable. The main aim of practising a feeding restriction during the rearing is avoiding the possible negative consequences on body development, reproductive performance and lifespan associated to the *ad libitum* programs with high energy diets. The success of restriction will depend to the moment of start the restriction, its duration and the energy level of the diet chosen, as well as of the use of techniques of flushing around the AI. In our females fed with the reproduction diet restricted, the energy intake was below the recommendations for their age (Xiccato and Trocino, 2010), causing a delay of body development, but reached first AI age a desirable lower PFT level than those fed *ad libitum* with this diet. However, the application of a flushing around the first AI, or choosing the age to be AI in function of the body development, was enough to ensure an adequate fertility with restricted feeding programs. Also, the reproductive performance in the short-term were intermediate respect the other feeding treatments evaluated.

In the trials of this thesis, some feeding programs based on the use of fibrous diets during the rearing period allowed females to achieve an adequate and more gradual body development and a greater feed intake capacity during this phase (as in previous works: Xiccato *et al.*, 1999, Pascual *et al.*, 2002 and Quevedo *et al.*, 2005), as well as a lower fatness at AI moment and mobilization around parturition (as in Xiccato *et al.*, 1999) respect to those females fed *ad libitum* with reproduction diets, but without a delay in the body development as observed in those with a quantitative restriction of the reproduction diet. Moreover, the fibrous diets' females achieved the better reproductive performance results, both in short and in long-term, and lifespan respect to the females with other feeding programs.

It is not easy to define which is the most appropriate fibrous feeding program for the rearing. As mentioned above, although it will depend on several factors, this thesis confirms that in spite the range for the DE content of fibrous diets can be very variable (literature: from 8.5 to 9.5 MJ DE/ kg DM; in this thesis: 8.7 and 10 MJ DE/ kg DM) main of them allowed to achieve good performance results. In this thesis, independently of the fibrous program, these females had similar results for many of the traits evaluated (fertility, birth weight, blood metabolites, PFT evolution), although the highest feed intake was obtained for the females that only consumed the fibrous diet from the beginning of the rearing period until the first parturition, as expected by the lowest DE offered. However, the application of a flushing around the first AI allowed females with fibrous diets to achieve the best results for litter at the first parturition, feed intake during the beginning of first lactation, and milk yield and litter growth during the first lactation, without relevant changes in females' resources use. On the contrary, this feeding program that had the worst performance results in the second parturition (mortality at parturition, size and weight of the alive litter at parturition) respect to the fibrous feeding program without flushing. This could be related to their greater wear during their first reproductive cycle. These results, together with the better management of the body reserves of these females, could indicate that the use of fibrous diets allows females to adapt better to the particularities of the first parturitions, where the nutritional requirements are more difficultly covered, and that could affect the survival of the females in the short and long-term, as observed in this thesis.

According to the results obtained on the influence of body development during the rearing period on rabbit males intended to AI, a proper feeding strategy during this phase should be that to adjust the nutrients intake, covering their nutritional requirements throughout the rearing period but avoiding the excessive fatness. The results of this thesis have provided interesting results that could improve the future feeding programs of the rabbit males intended to the AI. Firstly, the feed intake of these animals with low-energy level diets 10.9 MJ DE and 112 g DP/kg DM) is more than sufficient to reach the energy requirements during the whole rearing and production phases, although not the protein ones, which would suggest that a higher DP/DE ratio, adjusted in essential amino acids, are needed when these low-energy diets are used until 14 weeks of age. However, a remarkable feature observed was the low regularity on the daily feed consumption of the rabbit males for the different diets. It suggests that either the males have very irregular consumption *per se*, or that we should design diets that favour a more regular consumption (perhaps less energetic and more fibrous diets; Pascual *et al.*, 2016). Anyway, although animals try to regulate their energy intake according to the composition of the diet, those rabbit males that consumed a greater amount of feed during the rearing, continued to eat more amount of feed when all the animals received the same diet from the onset of productive life. Future rearing feeding programs, if fibrous diets will be used during the rearing of males selected by growth rate, it would be necessary to ponder not to change to a high-energy diet later, in order to avoid and excessive fattening of them during the reproductive life. Regarding the evolution of body condition, our studies also showed that, from 10.9 to 13.5 MJ DE/kg DM, PFT values of males were similar, and that PFT values established at the beginning of the productive life remain constant during the following months. This fact seems to indicate that if we want to modulate body condition of rabbit males, we need other nutritional strategies during the rearing period. Unfortunately, the number of works using different feed strategies in rabbit males are scarce. In agreement with our results, Pascual *et al.* (2015 and 2016) found that a moderate restriction (around 12%) may be a solution to provide a constant daily supply of nutrients, improving some sperm morphological characteristics, as well as the fertility of their pooled semen (+4%), especially during the seasons where this program allowed a higher daily feed intake stability. In summary, a combination of an adequate environmental conditions and feeding programs, that lead a constant daily supply of nutrients and minimize the overfatting of rabbit males during the rearing period, must be the future goals of the research on the feeding of rabbit males selected for growth rate and intended to AI.

The rearing feeding programs evaluated in this thesis, both females and males, have seem to indicate that, the key to designing an adequate rearing feeding program, is to achieve a correct regulation in the acquisition of the necessary resources to face the future productive challenges, without deviating in excess of the optimal ranges defined for each type of animal, and avoiding to put in risk both their reproductive performance and their lifespan.

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CONCLUSIONS

Firstly, this thesis has allowed a complete evaluation of how the different patterns on early body development could affect reproduction performance and lifespan of rabbit breeders. In rabbit females, we can conclude that a heavier weight at born seems to give females advantage on their early survival and on the litter size they have at the beginning of their reproduction career, but advantages seems to disappear in the long-term. In addition, our trials showed that females' reproductive performance and lifespan were directly influenced by their growth pattern during rearing period and, especially when this diverts young rabbit females from an adequate body condition at the first mating age. In rabbit males selected for growth rate during fattening and intended to semen production, a greater body development up to the end of the fattening period (63 days of life) seems to have a positive effect on their reproductive performance at the beginning of reproductive career, but without influence in the long-term. However, an excessive growth during rearing period had negative consequences both on males' reproductive performance and lifespan.

We can also conclude that the rearing feeding programs used in the trials of this thesis have influenced on the physiological and reproductive development of the young rabbit breeders, regardless of their sex or genetic lines. In rabbit females inseminated at 18 weeks of age, feeding restriction, and especially the use of fibrous diets (from 8.5 to 10 MJ DE/ kg DM), seems the best feeding strategy to promote their future reproductive performance and lifespan. Fibrous diets must be applied from and during enough time to reach an adequate physiological and sexual maturity at first mating, improving their effects with the application of a flushing period around first AI. In rabbit males selected by growth rate and intended to AI, the rearing feeding program needs to be adapted to environmental conditions, because it could have consequences on the regulation of the feed intake. In addition, this program must be fitted the changing nutritional requirements of young males, especially on the DP at the beginning of rearing period.

From the results of this thesis it can be concluded that, an adequate management of the development of the future rabbit breeders, from their own gestation to the beginning of their reproductive life, and the use of proper rearing feeding programs may allow farmer to improve the future reproductive performance, as well as their life expectancy, both in their rabbit females and males.

As future perspective, the use of management procedures, with the help pf indicators of body status (such as leptin and perirenal fat thickness, live weight…) and adapted feeding programs, could help us to better model our future rabbit breeders, in function of their genetic type, the environmental conditions and productive characteristics in our farms.