Environmental and genetic factors driving robustness in reproductive rabbit does

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Evaluator three

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Environmental and genetic factors driving robustness in reproductive rabbit does

Davi Savietto

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Supervisors

Dr. Juan José Pascual Amorós
Dr. Nicolas Charles Friggens

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List of Abbreviations

Abbreviation:	description
	GENOTYPES
V:	maternal rabbit line selected for litter size at weaning
V16:	generation 16 of line V
V36:	generation 36 of line V
LP:	maternal rabbit line founded for productive longevity
	ENVIRONMENTS
N:	normal room temperature (18 to 24°C)
H:	high room temperature (25 to 35°C)
C:	control diet (11.6 MJ DE/kg DM)
F:	fibrous diet (9.1 MJ DE/kg DM)
HC:	combination of high room temperature and diet C
NC:	combination of normal room temperature and diet C
NF:	combination of normal room temperature and diet F
	VARIABLES
ADF:	acid detergent fibre
ADFd:	apparent digestible coefficient of ADF
ADL:	acid detergent lignin
BA:	number of kits born alive
BHB:	β-OH-butyrate
CP:	crude protein
CPd:	apparent digestible coefficient of CP
CV:	coefficient of covariance
DE:	digestible energy

DEI:	digestible energy intake
DM:	dry matter
DMI:	dry matter intake
DMd:	apparent digestible coefficient of DM
DP:	digestible protein
DPI:	digestible protein intake
dpp:	days post-partum
GE:	gross energy
GEd:	apparent digestible coefficient of GE
HEM:	insoluble hemicellulose
HEMd:	apparent digestible coefficient of HEM
LW:	female rabbits live weight
LSW:	litter size at weaning
NEFA:	non-esterified fatty acids
NDF:	neutral detergent fibre
NDFd:	apparent digestible coefficient of NDF
OM:	organic matter
Omd:	apparent digestible coefficient of OM
PFT:	perirenal fat thickness
TB:	number of kits born in total

Abstract

Selection strategies to increase productive traits of farm animals have been effective and highly specialized breeds and strains were obtained. At the same time, the effort made to obtain extremely high producing animals was accompanied by undesired effects, such as a reduced ability to sustain production, reproduction and health; especially under constrained conditions. The perception that selection was degrading robustness, lead to selection strategies aiming to improve the ability of animals to perform in a wider range of environmental constrain. However, at the present moment, the physiological mechanisms allowing some farm animals to perform well in a wide range of environments, while others succumb, have not been described. The present thesis intended to address this question by describing the evolution of traits related to fitness, survival and to the adaptability to environmental constraints. Two maternal rabbit lines differing in their ability to face the environmental constraints, i.e. a 'specialist' and a 'generalist' maternal rabbit line were available. Additionally, two generations (20 generations apart) of the specialized line were simultaneously available. During the first two consecutive reproductive cycles, female rabbits were simultaneously subjected to three environmental conditions differing in the intensity and in the physiological constrain imposed. Digestive capacity, the acquisition of resources and the partitioning of resources into different functions (i.e. litter size, milk yield, growth, body reserves, etc.) were also assessed. Results showed a greater acquisition capacity of 'generalist' females in constrained conditions with respect to 'specialist' females. Moreover, the greater acquisition capacity was not accompanied by a reduction in the digestive efficiency, allowing the 'generalist' females a relative greater acquisition of digestible energy. The maintenance of reproductive performance by having a greater acquisition capacity, together with the avoidance of making an intensive use of body reserves were both related to the capacity of 'generalist' females to sustain reproduction in a wide range of environmental conditions. Twenty generations of selection exclusively for reproduction (specialized line), was not accompanied by a higher acquisition capacity, but by a change in the relative priority between the litter being nursed (actual) and the litter being gestate (future litter). In this sense, females from the actual generation of selection for litter size at weaning had a greater milk yield in the first week of lactation (period of great importance to kits survival), reducing it by the end of lactation. The present thesis also evidenced the importance of the environment where the animals are being selected in the evolution of the interplay between competing functions.

Resumen

Las estrategias de selección para mejorar los caracteres productivos de los animales de granja han sido efectivas, resultando en razas y líneas altamente especializadas. Al mismo tiempo, el esfuerzo realizado para obtener animales de alto rendimiento ha sido acompañado por efectos indeseables, como la reducción de la habilidad de los animales para sostener la producción, la reproducción y la salud en condiciones adversas. La percepción de que la selección está degradando la robustez, ha llevado a la búsqueda de estrategias de selección que visen mejorar la capacidad de los animales en afrontar una gran diversidad de condiciones ambientales. Sin embargo, hasta el presente momento, los mecanismos fisiológicos que permiten a algunos animales desempeñar su potencial productivo en una gran diversidad de ambientes mientras otros sucumben, no han sido descritos. El presente trabajo intenta abordar esta cuestión describiendo la evolución de los caracteres relacionados con la aptitud de transmisión de sus genes, la supervivencia y la capacidad de adaptación a los desafíos ambientales. Para ello dos líneas de conejos difiriendo en la capacidad de afrontar los desafíos ambientales fueron utilizadas. Además se disponía coetáneamente dos generaciones de selección (alejadas en 20 generaciones) de una línea altamente especializada. Durante los dos primeros ciclos reproductivos, las hembras fueren sujetas a tres condiciones ambientales difiriendo en la intensidad y en el tipo del desafío fisiológico aplicado. Parámetros como la capacidad digestiva, la capacidad de obtención de recursos y la partición de los recursos obtenidos entre las distintas funciones (ej. camada, producción de leche, crecimiento, manutención de las reservas corporales, etc.) fueron controlados. Los resultados han demostrado una mayor capacidad de obtención de recursos de las hembras de la línea robusta en condiciones de restricción en relación a las hembras de la línea especializada. Además, su mayor capacidad de ingestión no ha sido acompañada por una reducción en la eficacia digestiva, permitiendo a las hembras de la línea robusta ingerir, relativamente, una mayor cantidad de energía digestible. La capacidad de manutención del rendimiento reproductivo por tener una mayor obtención de recursos junto con la capacidad de evitar hacer uso intensivo de las reservas corporales fueron ambas relacionadas con la capacidad de las hembras robustas en mantener la reproducción en una amplia gama de condiciones ambientales. Veinte generaciones de selección basadas exclusivamente en un criterio reproductivo (línea especializada), no resultó en una mayor capacidad de obtención de recursos, pero si en un cambio de la prioridad relativa entre la camada actual (en lactancia) y la camada futura (en gestación). De esta forma, hembras de la generación actual de selección por tamaño de camada al destete tienen una mayor producción de leche en la primera semana de lactación (periodo de gran importancia para la supervivencia de la camada), reduciendo la misma al final de la lactación. En esta tesis también se evidencia la importancia del ambiente de selección de los animales en la evolución de la interacción entre funciones que compiten por los recursos.

Resum

Les estratègies de selecció per augmentar característiques productives dels animals de granja han estat eficaços i han obtingut races altament especialitzades. Alhora, l'esforç realitzat per obtenir animals extremadament productius va ser acompanyada per efectes no desitjats, com ara una disminució de la capacitat per mantindre la producció, la reproducció i la salut, especialment sota condicions restringides. La percepció que la selecció estava degradant la robustesa, condueix a emprendre estratègies de selecció amb l'objectiu de millorar la capacitat dels animals per a mantindre un bon rendiment en una varietat més àmplia d'ambients. No obstant això, els mecanismes fisiològics que permeten que uns animals de granja rendeixen bé en una àmplia varietat d'ambients, mentre que altres sucumbeixen sense èxit, no han sigut descrits fins el moment actual. La present tesi pretén abordar aquesta qüestió mitjançant la descripció de l'evolució de trets relacionats amb l'aptitud de transmissió dels seus gens, la supervivència i la capacitat d'adaptació dels animals front a diferents desafiaments ambientals. Per a aquest fi, es va disposar de dues línies maternals de conill, distingides per la seva capacitat per fer front a les limitacions ambientals: una línia materna "especialista" i una altra "generalista". Simultàniament, dues generacions (amb 20 generacions de diferència) de la línia especialitzada estaven també disponibles. Durant els dos primers cicles reproductius consecutius, les conilles es van sotmetre paral·lelament a tres condicions ambientals que difereixen en la intensitat i en la restricció fisiològica imposada. També es van avaluar les capacitats digestives, l'adquisició de recursos i el repartiment d'aquests per a diferents funcions (p.e., la producció de llet, el creixement, les reserves corporals, etc). Els resultats van mostrar una major capacitat d'adquisició en condicions limitades de les femelles "generalistes" comparant-les amb les femelles "especialistes". D'altra banda, la major capacitat d'adquisició no va anar lligada a una reducció en l'eficiència digestiva, permetent que les femelles "generalistes" una major adquisició relativa d'energia digestible. El manteniment de la funció reproductora a partir d'una major capacitat d'adquisició, juntament amb l'evitació de fer un ús intensiu de les reserves corporals, estaven tots dos relacionats amb la capacitat de les femelles "generalistes" per mantindre la reproducció en una àmplia varietat de condicions ambientals restringides. Vint generacions de selecció exclusivament basada en la reproducció (línia especialitzada), no va ser acompanyada per una capacitat d'adquisició superior, sinó per un canvi en la prioritat relativa entre la camada que està sent alimentada (l'actual) i la que està sent gestada (futura camada). En aquest sentit, les femelles de la generació actual de la selecció per major nombre de conillons al deslletament van tenir una major producció de llet a la primera setmana de la lactància (període de gran importància per a la supervivència dels conillons), que va anar reduint-se pel final de la lactància. Aquesta tesi també va posar de manifest la importància del medi ambient en què estan sent seleccionats els animals en l'evolució de la interacció entre les funcions que competeixen.

1

General Introduction

1.1 Few words on selection, evolution and robustness

Selection of farm animals to ameliorate productive traits (eggs, milk, meat, etc.) resulted in specialized breeds and strains (e.g. broilers: Havenstein et al., 2003a; Havenstein et al., 2003b; dairy cows: Hansen, 2000; pigs: Merks, 2000; hens: Poggenpoel et al., 1996; rabbits: García and Baselga, 2002), capable of high productive performances. The environment conditions (as feeding, veterinary control, housing systems, etc.) have been also improved, and both factors contributed to the results seeing today (good examples in Havenstein et al., 2003a and Havenstein et al., 2003b). Additionally, in order to control the environmental variance and favor the estimation of breeding values, selection normally occur in non-limiting and highly-controlled environmental conditions. But, is it reasonable to expect an adequate performance of high genetic merit animals in any environment? According to Hammond (1947), once the progress obtained is attributable to genetics, high genetic merit animals would perform well even in strange conditions. Falconer (1952), in change, stated that selection should be carried out under the conditions in which the animals will eventually be required to live, favoring their adaptation to the environment goodness and constraints. An argument corroborated two decades later by Lewontin (1974), who said: "*…the usual outcome of an analysis of variance in a particular population in a restricted range of environments is to underestimate severely the factors that occur over the whole range.*", in other words the fact that genotype and environment interacts (GxE) is underestimate.

The importance of considering the effects of GxE interaction can be seen in practice. For example, North-American Holstein-Friesian dairy cows, a breed intensively selected for milk yield in stall systems receiving concentrate-rich diets, sustained a high milk yield in a grassland system at the expense of a very intense use of body reserves in early lactation (Horan et al., 2005). A strategy related with reduced health and fertility (Roche et al., 2009). Many other examples exists, leading to the perception that selection exclusively on productive traits in specific conditions was accompanied by undesired effects (mainly in metabolic, reproductive and health traits; Rauw et al., 1998).

However, based on the arguments that the cost of providing an adequate environment is low compared to the cost of genetic improvement in itself, or that farmers who acquires high genetic merit animals do provide adequate conditions, many selection programs still underestimate the presence of GxE. But in reality, conditions observed in commercial farms not always correspond to the selection ones [see the example on pig production systems described by Dourmad et al. (2010)]. In any case, the expected consequence of such strategy is an increment in the environmental sensitivity because "*it does not make sense to have a very high fitness for an environment the individual rarely encounters*" (Strandberg, 2009). In this sense, selection strategies should consider the effect of GxE interaction.

To measure the effects of GxE interaction, it is mandatory to subject the population of study to a wide variety of environments, which in most case is impractical. Another difficulty is the method used to measure it. The re-ranking of animals or the changes in the population variance across environments are in itself measures of GxE (Bowman, 1972). Others, like the character state model, reaction norms, and covariance functions can be used to assess the environmental sensitivity (Kolmodin, 2003). However, these methods in themselves do not provide enough information in a dynamic and global perspective, which are essential in studies dealing with characterization of robust animals (Sauvant and Martin, 2010).

Evidences that genetic progress solely based on productive traits has a cost can be also regarded in the sense that resources allocated to one function are no longer available to another. This trade-off concept, extensively discussed in evolutionary biology (Stearns, 1992) and recently introduced to agricultural science (Beilharz et al., 1993), brings a wider perspective to unravel the reasons why single trait selection has been related to reduction on the fitness of farm animals. There are cases however, where a trade-off condition is not established (Reznick et al., 2000), by the simple fact that environment provides enough resources to be allocated into different functions (see in Figure 1.1 the model proposed by van Noordwijk and de Jong, 1986). In this sense, it could be argued that the environment is, together with the selection criteria, an important factor involved in the evolution of the negative effects of selection.

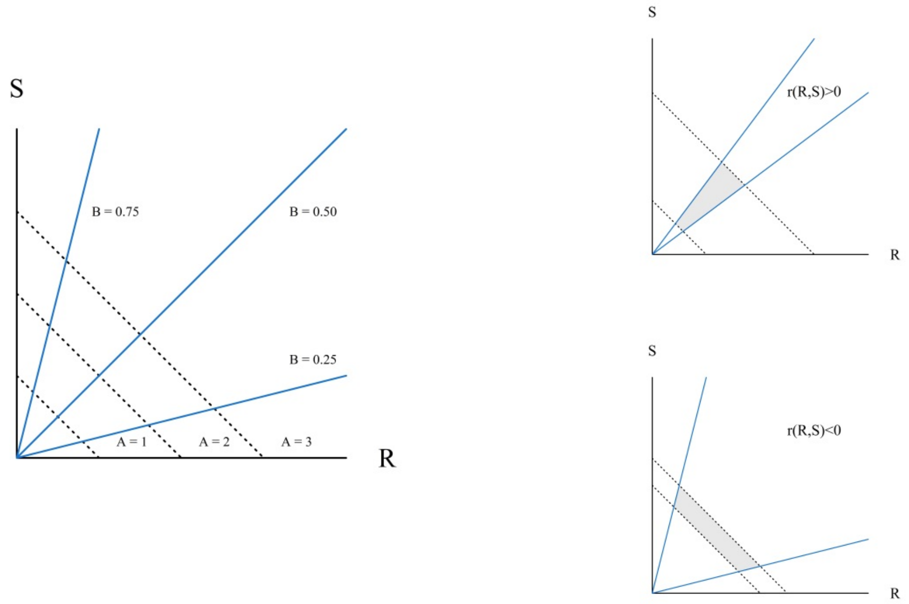

Figure 1.1 Model of van Noordwijk and de Jong (1998), representing the environmental limitations (A=1 to A=3) and the partition of resources between traits related to reproduction (R) and traits related to survival (S) (B=0.25 to B=0.75). Figure in the top right represents a condition where the environment provides enough resources to be allocate between the traits R and S. In bottom right, the environments provide few resources, forcing animals to allocate more resources to R or S, not both. A trade-off condition is established, and a negative correlation is observed.

The model proposed by van Noordwijk and de Jong, (1986) states that the trade-off between competing functions (e.g. reproduction and survival) may not suppose a reduction in the fitness when the environment provides enough resources to be allocated into competing functions. However, the allocation of resources is not only limited by the environmental abundances and/or constrains, it is also dependent on the individual acquisition capacity; a function of genotype. Additionally, even if the amount of resources obtained is not a function of the genotype, the partitioning of obtained resources could be different by the simple fact that the selection criteria may be favoring animals with a specific resources assignation pattern. Apart from the resources availability and the individual acquisition capacity, the environment may also limits the proportion of the obtained resources allocated into different functions. For example, animals may have to combat pathogens, or maintain body temperature in extreme conditions, being thus obligated to derive resources to survival. Therefore a tight relationship between the genotype and the environment is established (i.e. GxE interaction), affecting the evolution of the interplay between life functions (e.g. productive and functional traits). In fact, the model proposed by Friggens and van der Waaij (2009) shows the evolution of different resources allocation strategies in response to the selection criteria and the environmental condition. Similar results were observed in a simulation study (Douhard, 2013), evidencing a specialization to the selection condition. "*Selection may shape many characters toward the optimum for a specific environment* (Futuyama and Moreno, 1988)."

Specialization of farmed animals not only altered the ability of animal to function well in the environment they live (Rauw et al., 1998), it was also accompanied by a reduced ability to function well over a range of environments (e.g. climates, production systems or farms; Knap and Rauw, 2009; Veerkamp et al., 2009). In other words, specialization of farm animals is degrading robustness. Robustness is a complex concept. Knap (2005) for example, defined robustness as "*the ability to combine a high production potential with resilience to stressors, allowing to unproblematic expression of a high production potential in a wide variety of environmental conditions*". Strandberg (2009) used a more literal definition: "*the ability of an animal to function well in their*

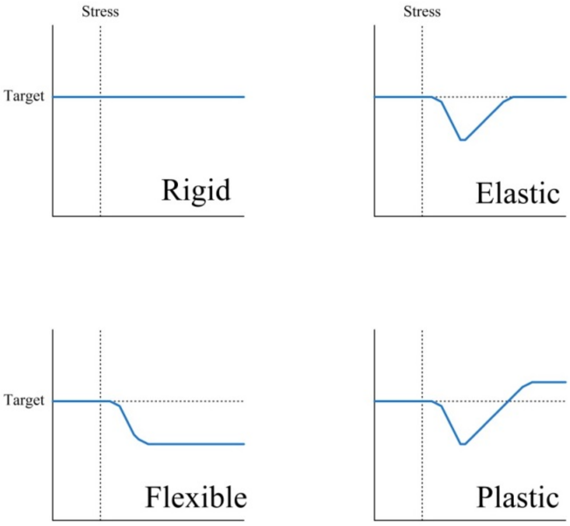

Figure 1.2 Different dynamic responses in a system where perturbation occurs. Rigid system does not deviated from the target value (i.e. fitness) when perturbation occurs. Elastic system deviates from the targed value recovering to the initial state. Flexible system adapt to the perturbation condition and the plastic system defines a new target value after recover from the perturbation (adapted from Sauvant and Martin, 2010).

own environment, being resilient to changes in the 'microenvironments' that it encounters (through life) as well as in a wide range of climates, production systems or herds". Sauvant and Martin (2010) describe many other definition of robustness used in a variety of disciplines, rather than biology, reaching the general conclusion that robustness should be regarded as a dynamic system (Figure 1.2). Friggens and van der Waaij (2009) also added that robustness is not a single trait definition, because it "*ignores that an animal (e.g. a cow) that sustain production (e.g. milk yield) by suppressing its immune function is clear less robust than an animal that maintains production by reducing growth rate*". From these definitions, robustness can be regarded as a life condition that allows farm animals to live and produce even when the environment deviates from its optimal.

In most farm species the environmental conditions are more or less constant. However, even in highly controlled production systems, variations in the environmental conditions exist (see the review of Dourmad et al., 2010 in pig productions systems). Robust animals are then strongly desirable. Because animals reaching long productive lives are deemed to be robust, under commercial conditions, productive longevity is a synonym of robustness. Strandberg and Sölkner, (1996), for example, indicated that the increment of herd health status, fertility, and thus the reduction in the voluntary culling were all beneficial traits for the inclusion of longevity in the selection index of dairy cows. Similarly, Engblom et al. (2007) observed an overall better reproductive performance and health status of sows culled at 'old ages'. And together all these observations reinforce the concept that robust animals not only represents an improvement in the overall productive performance, but also represents efficiency and health.

Life history theory (Stearns, 1992) states that survival is sacrificed by functions related to reproduction (Snell and King, 1977; Stearns, 1989; Kirkwood and Rose, 1991). Similar evidences have recently been reported in dairy cows (Pritchard et al., 2013) and sows (Hoge and Bates, 2011). There are cases, however, where such trade-off was not observed (rabbits: Piles et al., 2006; Sánchez et al., 2006). One explanation for such unexpected result could be attributable to the fact that under commercial conditions low producing animals are mainly culled early in life (Essl, 1998; Serenius and Stalder, 2006). Another argument could be based in abundance of the environment, allowing the animals to acquire and then assign an adequate amount of resources to both functions (Reznick et al., 2000). The later seem to fit better to the identification of the housing system as an important factor conditioning lifespan (dairy cows: Essl, 1998 *apud* Sölkner and Essl, 1989). In this sense, any selection strategy aiming to increase production without degrading health must consider the production conditions, where the animals will encounter before defining the selection conditions. Additionally the selection criteria should be widened to include robustness related traits. Multi-trait selection strategies together with the consideration of environmental sensitivity variance may be, for example, an alternative to reduce the negative effects of selection derived from the specialization process observed in farm animals (ten Napel et al., 2009).

1.2 Down the rabbit role

Long term selection experiments to increase litter size traits in maternal rabbit lines (García and Baselga, 2002; Garreau et al., 2004; Piles, et al., 2006) has also been effective. The obtained genetic progress on the number of kits born in total, born alive and weaned ranged from 0.08 to 0.09 kits per generation (Baselga, 2004). At the same time selection strategies to increase kit survival during lactation (Garreau et al., 2008) and the use of paternal lines to produce fast growing kits (Estany et al., 1992) increased the nutritional requirements of rabbit females (Quevedo et al., 2006a and Quevedo et al., 2006b), and impaired the adequacy of females to face constrained conditions (Pascual et al., 2013). In this sense, to avoid problems related with the adaptability to the environmental conditions (commercial rabbit farms are characterized by a great variability in the technology, reproductive and management practices adopted; Rosell, 2003 and Rosell et al., 2009) it is recommended to select animals in similar conditions to that they will be required to live (Baselga, 2004).

In rabbits, in opposition with resources allocation theory, there is no evidence to support that selection to increase litter size at weaning is degrading lifespan (Piles et al., 2006; Sánchez et al., 2006); at least under controlled and non-changing environmental conditions normally found in selection farms (Baselga personal communication). But at the same time, the replacement rate of female rabbits in commercial farms reached high values (i.e. around 10% per month; Ramón et al., 2004; Rosell and de la Fuente, 2009; Abadal et al., 2013). Under such conditions, female rabbits are mainly culled due to an inadequate reproductive performances (infertility, long parturition interval, low prolificacy, neglected litter, or high pre-weaning mortality) or pathology process (mainly respiratory and digestive troubles) (Rosell and de la Fuente, 2009). Additionally, female rabbits are normally subjected to a semi-intensive reproductive cycles (varying between 42 to 56 days), finding themselves in a condition where the gravid uterus competes with mammary gland for resources (Fortun-Lamothe et al., 1998). To face this condition female rabbits make use of body reserves (Xiccato et al., 1999; Costa et al., 2004; Pascual et al., 2013), and if they make an inadequate use of it (females becoming skinny or fatty), both reproductive and health traits are affected (Castellini et al., 2010) and then higher risks of being culled are expected (Theilgaard et al., 2006; Sánchez et al., 2012).

Summing up the fact that selection is attained under controlled non-limiting conditions, to the fact that management in commercial farms are highly variable, being female rabbits, in some conditions, unable to sustain both the reproductive performance and health, the high replacement rate found in commercial farms seems reasonable. In this sense, to improve female's lifespan in commercial rabbit farms without impairing productivity, Sánchez et al. (2008) founded a long and productive maternal rabbit line. Using high selection intensities (i.e. hyper-selection concept; Cifre et al., 1998) females from commercial farms of the Iberian Peninsula having extremely long (at least 25 parturitions) and productive (minimum average litter size of 7.5 live-born kits) lives were used to establish the long-lived productive rabbit line. Since its foundation, female rabbits from long-living and productive line has been characterized by having a late reproductive senescence (Theilgaard et al., 2007), a greater adaptability to maintain the productive level in the face of nutritional constrains (Theilgaard et al., 2009), a higher lymphocytes ($CD14^-$, $CD25^+$, and $CD45^+$) count (Ferrian et al., 2012), and a faster immune response to a lipopolysaccharide challenge (Ferrian et al., 2013) with respect to a maternal rabbit line solely selected for litter size at weaning (Estany et al., 1989).

In the study of Theilgaard et al. (2007), a serendipitous discovery occurred. Due to a strong feed restriction occurring during summertime, long-living and productive females were able to sustain the reproductive performance by using its greater soma, while females solely selected for reproductive performance drastically reduced the litter size at birth and at weaning. However, until the present date, no experiments where designed to assess the mechanisms by which long-lived and productive females were able to sustain a high reproductive performance in the face of designed environmental constrains. Moreover, if selection for litter size at weaning did not degraded longevity (Piles et al., 2006; Sánchez et al., 2006), a trait clearly related with reproductive performances (Rosell and de la Fuente, 2009), why females of a line solely selected for litter size at weaning were unable to maintain prolificacy?

1.3 Objectives

The present thesis aims to unravel the physiological mechanisms by which females having a long productive lifespan or being specialized for reproduction use to interact with the environmental abundances and constrains, allowing them to attain their fitness. Moreover, the availability of two coetaneous generations (apart 20 generations) of the specialized female rabbits (exclusively selected for litter size at weaning), allowed the study on how selection shifts the way by which animals interact with environment to attain the selection criteria.

The thesis is subdivided in four articles. The first article evaluated the digestive efficiency of female rabbits of different genotypes in three environmental conditions differing in the intensity and type of the physiological constrains imposed. Article two presents the physiological strategies adopted by long living and productive females and the current generation of a line selected exclusively on litter size at weaning to counteract the negative effects caused by high temperatures or nutritional constrain. The third article presents the changes in the allocation of resources derived as result to 20 generations of selection exclusively for litter size at weaning. Finally, article four congregates the results of articles one, two and three in a discussion on how the particular strategies assumed by the different genetic types can be viewed as an evidence of how the environment of selection and the selection history can denote a specialist or generalist genetic type.

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2

Digestive efficiency in rabbit does according to environment and genetic type

Savietto D.*, Blas E.*, Cervera C.*, Baselga M*,
Friggens N.C.†‡, Larsen T.§ and Pascual J.J.*

**Institute for Science and Animal Technology, (ICTA). Universitat Politècnica de València. Camino de Vera s/n. 46022, Valencia, Spain.*

†UMR0791 Modélisation Systémique Appliquée aux Ruminants (MoSAR). Institute National de la Recherche Agronomique (INRA). 16 Rue Claude Bernard. 75231, Paris, France.

‡UMR0791 Modélisation Systémique Appliquée aux Ruminants (MoSAR). AgroParisTech. 16 Rue Claude Bernard. 75231, Paris, France.

§Department of Animal Science, Integrative Physiology. Aarhus University.
Blichers Allé 20. 8830, Tjele, Denmark.

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2.1 Abstract

Ninety lactating rabbit does from three different genetic types [two of a line differing 20 generations on selection for litter size at weaning (line **V16** and **V36**) and one of a line founded under reproductive longevity criteria and then selected by litter size at weaning (line **LP**)] were subject to three environmental conditions: **NC**, females housed under normal conditions (14 to 20°C) and fed with a control diet [333 g of neutral detergent fibre (**NDF**) per kg of dry matter (**DM**)]; **HC**, females housed under heat conditions (25 to 35°C) and fed with the control diet; or **NF**, females housed under normal conditions and fed with a fibrous diet (443 g NDF/kg). The apparent digestible coefficients of dry matter (**DMd**), organic matter, crude protein (**CPd**), gross energy, NDF (**NDFd**) and acid detergent fibre, as well as the daily intake of DM, digestible protein (**DP**) and digestible energy (**DE**), were determined (14 to 18 days post-partum). The environment affected all the variables analysed. In general, heat conditions reduced the daily feed intake (around -30.0%; $P<0.05$) and increased main apparent digestible coefficients (+4.5 points of percentage for DMd). In opposite, the use of a fibrous diet lead to lower DE intake (-217 kJ/d; $P<0.05$) and main apparent digestibility coefficients (-13.5 points of percentage for DMd). Females of line V, regardless of generation, showed lower daily DM intake (-19.2 g/d; $P<0.05$) and NDFd (-1.5 percentage points; $P<0.05$) than line LP. Interactions between genetic type and environment were found for daily DM intake, NDFd and CPd. When receiving fibrous diet, LP females showed a higher increment in daily DM intake (+65.6 g/d; $P<0.05$) than V36 females, compared to control. Under heat conditions, NDFd obtained for LP females were higher to those in normal conditions (+3.14 percentage points), while V females showed similar NDFd. In addition, the increase on CPd observed under heat conditions was higher for LP (+9.87 percentage points) and V36 (+8.74 percentage points) than V16 females (+3.84 percentage points). In conclusion, rabbit females from a line founded for reproductive longevity seem to show a higher flexibility on their digestive capacity under constrained conditions.

Key Words: rabbit does, heat stress, dietary energy, genetic selection, digestibility

2.2 Introduction

Selection for litter size at weaning (**LSW**) in reproductive rabbit has not only succeeded in improving the target trait, but also changes important physiological aspects in the animals. In this sense, Quevedo et al. (2005) described a possible better efficacy in the digestible energy use for foetal growth and even an improvement in feed intake capacity at early lactation (Quevedo et al., 2006), both changes directly related to the selection objective.

On the other hand, the foundation of a rabbit line by screening for females with very long reproductive career (Sánchez et al., 2008) resulted in animals with different capacity to manage the resources along their reproductive life. In fact, this strategy resulted in animals well adapted to manage the resources under adverse environmental conditions (i.e. high temperatures and feed restriction). They delayed reproductive senescence (Theilgaard et al., 2007) and improved their lifespan (Theilgaard et al., 2006; Sánchez et al., 2008), resulting in more robust animals (Theilgaard et al., 2009). All of these findings could be related to a better use of the energy resources available to cope with the reproduction effort, as hypothesized by Pascual et al. (2008).

The current knowledge shows that maternal rabbit lines founded under different criteria but which had been selected for the same trait (i.e. LSW) are different for the selected trait (Ragab and Baselga, 2011), and previous works demonstrated that they adopted different fitness strategies when the environmental conditions were not at all suitable.

Therefore, the aim of the present work was to evaluate how selection for LSW (comparing animals separated by 20 generations of selection) or the foundation for hyper-reproductive longevity criteria could have affected female digestive efficiency under normal and constrained environmental conditions (heat or nutritional challenges).

2.3 Material and methods

The experiment was designed and carried out according to the European Union recommendations on the protection of animals used for scientific purposes (European Union, 2010) and followed the guidelines for applied nutrition experiments in rabbits (Fernández-Carmona et al., 2005).

2.3.1 Animals

Ninety lactating rabbit females in first or second lactation were used to evaluate the apparent digestibility of animals belonging to three genetic types and kept under three different environmental conditions. The genetic types consisted of animals from a line founded under hyper-reproductive longevity criteria (see foundations details at Sánchez et al., 2008) and then selected for LSW over 6 generations (line **LP**), and animals from line **V**, selected for LSW (Estany et al., 1989) and belonging to generations 16 and 36 (**V16** and **V36**, respectively).

2.3.2 Diets

Two diets differing in energy, mainly modifying the forage content, were formulated and pelleted (Table 2.1). The control diet (**C**) was formulated following the recommendations of de Blas and Mateos (2010) for lactating rabbit does [having on average 11.7 MJ of digestible energy (**DE**), 120 g digestible protein (**DP**), 168 g acid detergent fibre (**ADF**) and 333 g neutral detergent fibre (**NDF**) per kg dry matter (**DM**)]. A second fibrous low energy diet (**F**) was designed to induce a nutritional challenge (to have on average 9 MJ DE, 105 g DP, 266 g ADF and 443 NDF per kg DM).

2.3.3 Experimental procedures

At parturition, the rabbit does were housed in individual metabolic cages and randomly allocated to one of the three different environments resulting from the combination of environmental temperature and the diet received. Two room temperatures were used: a climatic chamber set up to obtain heat conditions (**H**) by means of a daily sigmoid temperature curve with a range from 25 to 35°C (see description by García-Diego et al., 2011) and a normal conditions room (**N**) with a temperature range between 14 and 20°C. All animals housed at H received diet C while half of the animals placed at N were fed on diet C and half on diet F. As a result, the tested environments were **HC** (heat conditions with diet C), **NC** (normal conditions with diet C) or **NF** (normal conditions with diet F). Table 2.2 summarises the experimental groups in terms of genetic type, environmental temperature and parturition order. Diets and water were provided ad libitum throughout the experiment, the daylight scheme was a 16 h light and 8 h dark period and the animals were artificially inseminated at day 11 post-partum.

Litters were standardised to 9 and 10 kits at first and second parturition, respectively, to compare the digestive efficiency at similar productive effort. Rabbit does were transferred daily to the production cage to suckle their respective litters. At 14 days of lactation, similar live weight were observed for the different animal types (on average 3,837 ± 51 g), although females housed under heat conditions were slightly lighter (-197 ± 73 g; $P<0.05$). Feed intake and faecal collections were recorded daily just after the milk production measurement (using the does double weight method) from the 14th to 18th day of lactation. This period was selected to perform the digestibility trial, as it corresponds to the period of maximum milk yield in rabbit does (Casado et al., 2006) and is characterised by a regular females' feed intake.

The chemical analyses of diets and faeces for DM, crude protein (**CP**), ADF, acid detergent lignin (**ADL**) and ash followed the AOAC methods (934.01, 976.05, 973.18 and 942.05, respectively; AOAC, 2000). NDF was analysed by the method described by Mertens (2002) and the gross energy (**GE**) was determined in adiabatic bomb as recommended by EGRAN (2001).

Table 2.1 Ingredients and chemical compositions of experimental diets

Ingredients (g/kg)	Control diet	Fibre diet
Barley grain	315	0
Wheat bran	50	100
Sunflower meal (30% CP)	100	52
Soybean meal (44 % CP)	101	0
Alfalfa hay	370	660
Sugar beet pulp	0	138
Soybean oil	40	10
L - Lysine HCl	0.45	2.8
DL - Methionine	0.95	2.6
L - Threonine	0.6	1.6
Arginine	0	0.8
Cycostat 66G®[1]	1	1
Bacipremix 50®[2]	2	2
Calcium carbonate	1	0
Dicalcium phosphate	10	0
Monosodium phosphate	0	21.2
Sodium chloride	4	4
Vitamin/mineral mixture[3]	4	4
Chemical composition (g/kg DM)		
Dry matter (DM; g/kg)	951	938
Organic matter	917	881
Neutral detergent fibre	333	443
Acid detergent fibre	168	266
Acid detergent lignin	25	44
Crude protein (CP)	175	162
Gross energy (MJ/kg DM)	18.69	18.31

[1] Alpharma, Antwerp (Belgium): provides 66 ppm of robenidine. [2] Andreés Pintaluba SA, Reus (Spain): provides 100 ppm of zinc bacitracin. [3] Contains (g/kg): thiamine, 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; cyanobalamin 0.0006; choline chloride, 100; $MgSO_4$ H_2O, 7.5; ZnO, 30; $FeSO_4$ $7H_2O$, 20; $CuSO_4$ $5H_2O$, 3; KI, 0.5; $CoCl_2$ $6H_2O$, 0.2; Na_2SeO_3, 0.03.

Table 2.2 Number of animals according to environment condition and genetic type by parity order

Environment[1]		HC			NC			NF	
Genetic type[2]	LP	V16	V36	LP	V16	V36	LP	V16	V36
Parturition order									
First	5	6	6	5	6	6	6	5	5
Second	4	5	5	5	3	4	5	5	4
Total	10	11	11	10	9	10	11	10	9

[1] Environment: HC, heat conditions (25 to 35°C) and diet C (on av. 11.6 MJ digestible energy/kg dry matter); NC, normal conditions (14 to 20°C) and diet C; and NF, normal conditions and diet F (on av. 9.1 MJ digestible energy/kg dry matter).
[2] Genetic type: LP, line constituted for longevity-productive criteria; V16 and V36, line V selected by litter size at weaning during 16 or 36 generations.

2.3.4 Statistical analysis

Data on the apparent digestibility coefficients (**d**) of DM, organic matter (**OM**), CP, GE, NDF, ADF and insoluble hemicelluloses (**HEM** = NDF - ADF), as well as the daily intake of DM (**DMI**), DP (**DPI**) and DE (**DEI**), were analysed using the GLM procedure of SAS software (SAS Institute, Cary, NC). The model included as fixed effects the genetic type ($\mathbf{G}_i$: 3 levels), the environment ($\mathbf{E}_j$: 3 levels) and their interactions. Although the trial was not designed to study the parturition order effect ($\mathbf{PO}_k$: 2 levels), it was treated as a fixed effect, increasing the hypothesis test exigency. No interactions and no inference were performed with respect to PO_k. The model used was:

$$Y_{ijk} = G_i + E_j + PO_k + G_i \times E_j + e_{ijk} \qquad (2.1)$$

To test the significances differences within genetic type or environment, different contrasts of interest were computed as follow:

$$LP - V = \frac{(LPHC+LPNC+LPNF)}{3} - \left\{\frac{\left[\frac{(V16HC+V16NC+V16NF)}{3} + \frac{(V36HC+V36NC+V36NF)}{3}\right]}{2}\right\} \qquad (2.2)$$

$$LP - V16 = \frac{(LPHC+LPNC+LPNF)}{3} - \frac{(V16HC+V16NC+V16NF)}{3} \qquad (2.3)$$

$$LP - V36 = \frac{(LPHC+LPNC+LPNF)}{3} - \frac{(V36HC+V36NC+V36NF)}{3} \qquad (2.4)$$

$$V16 - V36 = \frac{(V16HC+V16NC+V16NF)}{3} - \frac{(V36HC+V36NC+V36NF)}{3} \qquad (2.5)$$

$$HC - NC = \frac{(LPHC+V16HC+V36HC)}{3} - \frac{(LPNC+V16NC+V36NC)}{3} \qquad (2.6)$$

$$NF - NC = \frac{(LPNF+V16NF+V36NF)}{3} - \frac{(LPNC+V16NC+V36NC)}{3} \qquad (2.7)$$

Finally, for variables where $G_i \times E_j$ interaction was declared significant, the means comparisons were performed using a t-test.

2.4 Results and discussion

The environment affected all the variables studied (Table 2.3). Animals submitted to the heat challenge (HC) showed a lower DMI, DPI and DEI (34.3, 24.3 and 29.6%, respectively; $P<0.05$), while their main apparent digestibility coefficients (DMd, OMd, CPd, GEd and ADFd) increased (from 4 to 7 percentage points; $P<0.05$) in comparison to animals kept at NC. As recently reviewed by Cervera and Fernández-Carmona (2010), high temperatures usually depress feed intake of rabbits and consequently increase the apparent digestibility coefficients of main nutrients. A decrease in the amount of feed eaten normally leads to a lower passage rate, hence the ingested feed is exposed to the action of digestive enzymes for a longer period, thereby increasing digestibility of nutrients (Carabaño et al., 2010).

The only nutrient whose apparent digestibility coefficient not increased at high environmental temperature was insoluble hemicellulose (HEM: 40.6% and 42.4% for HC and NC groups, respectively; $P<0.05$). This discordant response is difficult to explain from our results. In any case, Gidenne et al. (2002), and Gidenne and Feugier (2009) described similar bacterial fibrolytic activity independently of the feed intake level, suggesting that digestibility of some cell-wall constituents is not substrate-dependent. Moreover, Hannah et al. (1990) observed that NDF digestibility was also similar in sheep housed at 27 to 34°C.

On the other hand, animals at NF showed higher DMI and DPI (17.3% and 6.6%, respectively; $P<0.05$) and a reduction in the digestibility (DMd, OMd, CPd, GEd and NDFd; $P<0.05$) except for ADFd, which was higher (3.6 percentage points; $P<0.05$) compared to those at NC. The dry matter intake, clearly increased by the use of a high fibre low energy diet during lactation, agrees with the literature (Fernández-Carmona et al., 1995; Fernández-Carmona et al., 2003; Quevedo et al., 2006; Nicodemus et al., 2010), where the response on DMI was related to the dietary DE content. On average, DMI of lactating rabbit does increased about 8.0% per each MJ of DE decreased in the diet. In spite of this, the DE content of the diet F (about 9.0 MJ/kg DM) did not allow females to compensate the DE intake completely (5.9% lower compared to control; $P<0.05$).

The reduction of DMd, OMd and GEd (13.0 to 14.0%) in diet F mainly reflects the change in carbohydrate composition, higher fibre and lower starch than diet C, as well as the effect of increased DMI. However, NDFd and ADFd were less affected by the dietary change. The design of a high fibre diet by changing the forage content also involved a change in the fibre sources (Table 2.1) and, consequently, in the nature of fibre constituents, and this also affected the digestibility of fibre fractions (Gidenne et al., 2010). Moreover, the higher addition of unsaturated oil in diet C could have affected its fibre digestibility (Fernández et al., 1994).

Regarding the genetic type, a higher DMI was observed for LP in comparison to V16 animals (+26.2 g DM/d; $P<0.05$) whereas compared to V36 animals it was similar (+3.9%). Similarly, DEI was higher for LP than for V16 animals (3,345.8 ± 77.9 and 3,096.2 ± 78.1 kJ/d, respectively; $P<0.05$). Moreover, the NDFd was higher (1.4%; $P<0.05$) for LP than for V36 animals (+1.8%, $P<0.05$). As observed by Pascual et al. (2008) and Theilgaard et al. (2009), females from LP line showed a higher feed intake (19.21 g DM/d) during lactation period than the V line. However, the difference between V16 and V36 (-14.0 g DM/d) was not statistically significant.

Interactions between genetic type and environment were observed for DMI, NDFd and CPd. Dry matter intake of LP animals was significantly higher compared to V16 and V36 animals, but only when fed with diet F (+47.9 ± 16.6 and +57.4 ± 17.1 g DM/d, respectively; $P<0.05$) (Figure 2.1A). Furthermore, the higher NDFd observed for LP animals was mainly due to the increase observed under heat challenge (HC) relative to normal room temperature (NC) (+3.1 ± 1.3 percentage points; $P<0.05$), which was not observed for V animals (Figure 2.1B).

In the current study, only the NDFd was higher for the LP line, relative to line V. However, Pascual et al. (2008) observed better digestibility of DM, OM and GE in favour of line V. This difference in the NDFd in the present study did not seem to be a direct response to differences in feed intake, because the highest NDFd occurred in animals submitted to a heat challenge (Table 2.3), where feed intake was similar among genetic types (208.0 ± 12.7, 194.2 ± 11.5 and 217.6 ± 11.5 g DM/d for LP, V16 and V36, respectively). In fact, the reduction in DMI of line LP at HC to similar levels of line V could explain these results, because the passage rate would also decrease, promoting an increase in fibre digestibility. Generations 16 and 36 of line V presented similar raw results for this variable, but a possible different pattern in restricted environments (especially at high temperatures) should be confirmed.

Table 2.3 Apparent digestible coefficients [dry matter (DMd), organic matter (OMd), crude protein (CPd), gross energy (GEd), neutral detergent fibre (NDFd), acid detergent fibre (ADFd) and insoluble hemicellulose (HEMd)] and daily intake [dry matter (DM), digestible protein (DP) and digestible energy (DE)] according to environment and genetic type of females on lactation (from 14 to 18 d)

Environment[1]	HC			NC			NF			Contrasts[3]					
Genetic Type[2]	LP	V16	V36	LP	V16	V36	LP	V16	V36	LP-V	LP-V16	LP-V36	V16-V36	HC-NC	NF-NC
Apparent digestibility coefficients (%)															
DMd	67.85^{d}	65.64^{c}	66.50^{cd}	61.90^{b}	63.03^{b}	61.52^{b}	48.25^{a}	49.02^{a}	48.67^{a}	0.27 (0.45)	0.10 (0.51)	0.44 (0.51)	0.33 (0.51)	4.51* (0.51)	-13.51* (0.52)
OMd	68.37^{d}	66.47^{c}	67.16^{cd}	62.95^{b}	64.08^{b}	62.74^{b}	48.90^{a}	49.58^{a}	49.40^{a}	0.17 (0.43)	-0.03 (0.50)	0.30 (0.50)	0.27 (0.50)	4.07* (0.50)	-13.96* (0.50)
CPd[4]	77.35^{d}	74.73^{d}	75.57^{d}	67.48^{b}	70.89^{c}	66.83^{b}	63.29^{a}	64.83^{ab}	65.10^{ab}	-0.28 (0.81)	-0.78 (0.93)	0.21 (0.94)	0.99 (0.94)	7.48* (0.93)	-3.99* (0.95)
GEd	67.80^{c}	66.03^{c}	66.52^{c}	62.33^{b}	63.29^{b}	61.74^{b}	48.92^{a}	49.84^{a}	49.83^{a}	0.14 (0.49)	-0.04 (0.56)	0.32 (0.56)	0.36 (0.56)	4.32* (0.56)	-12.93* (0.57)
NDFd[4]	31.50^{d}	28.01^{c}	27.43^{bc}	28.36^{c}	28.55^{c}	26.59^{abc}	24.56^{a}	24.40^{a}	25.03^{ab}	1.47* (0.65)	1.15 (0.75)	1.79* (0.75)	0.64 (0.75)	1.15 (0.76)	-3.17* (0.76)
ADFd	21.15^{d}	18.90^{cd}	16.89^{bc}	13.48^{a}	14.34^{ab}	13.89^{ab}	17.33^{c}	17.50^{c}	17.72^{c}	0.78 (0.77)	0.40 (0.88)	1.15 (0.90)	0.75 (0.89)	5.08 (0.90*)	3.61* (0.90)
HEMd	43.36^{d}	38.44^{bc}	39.47^{c}	43.55^{d}	43.11^{d}	41.24^{cd}	35.40^{a}	34.72^{a}	36.00^{ab}	1.94* (0.73)	2.01* (0.84)	1.86* (0.85)	-0.15 (0.84)	-2.21* (0.85)	-7.26* (0.85)
Daily intake															
DM (g/d)[4]	208.1^{a}	194.2^{a}	217.6^{a}	316.3^{b}	299.5^{b}	327.5^{bc}	415.4^{e}	367.5^{d}	358.0^{dc}	19.2* (8.5)	-26.2* (9.9)	12.2 (9.9)	-14.0 (9.9)	-107.8* (9.9)	-65.9* (9.9)
DP (g/d)	28.16^{a}	25.30^{a}	28.73^{a}	37.11^{b}	37.13^{b}	38.27^{b}	42.56^{c}	38.65^{bc}	38.84^{bc}	1.46 (1.1)	2.25 (1.2)	0.67 (1.2)	-1.58 (1.2)	-10.11* (1.2)	2.51* (1.2)
DE (kJ/d)	2,642^{a}	2,393^{a}	2,706^{a}	3,675^{cd}	3,541^{bcd}	3,778^{d}	3,720^{d}	3,355^{bc}	3,267^{b}	172.6 (95)	249.6* (110)	95.6 (110)	-154.0 (110)	-1,084.7* (110)	-217.4* (111)

[1] Environment: HC, heat conditions (25 to 35°C) and diet C (on av. 11.7 MJ digestible energy/kg dry matter); NC, normal conditions (14 to 20°C) and diet C; and NF, normal conditions and diet F (on av. 9 MJ digestible energy/kg dry matter). [2] Genetic type: LP, line constituted for longevity-productive criteria; V16 and V36, line V selected by litter size at weaning during 16 or 36 generations. [3] Contrast within genetic type or environment were calculated on complete data; e.g. LP-V = (LPHC+LPNC+LPNF)/3-{[(V16HC+V16NC+V16NF)/3 + (V36HC+V36NC+V36NF)/3]/2}. [3] Contrasts (standard error) followed by * are significant at $P<0.05$. [4] Significant interaction ($P<0.05$) between environment and genetic type. $^{a-d}$ Means not sharing common superscripts, in rows, are different at $P<0.05$.

Finally, Figure 2.1C shows the genetic type by environment interaction for CPd as changes due to heat and nutritional challenges (HC and NF, respectively) compared to NC. Heat challenge led LP animals to an increase of CPd significantly higher than that observed for V16 animals (9.9 ± 1.6 *vs.* 3.8 ± 1.6 percentage points, respectively; P<0.05), with V36 animals showing an intermediate increment (8.7 ± 1.6). However, in NF environment, V16 showed a greater reduction on CPd (-6.1 ± 1.7 percentage points; P<0.05), while V36 females did not seem to be affected when fed with diet F (-1.7 ± 1.7 percentage points) compared to NC environment. Females from line LP showed an intermediate reduction in CPd with diet F (-4.2 ± 1.6%) compared to diet C. The genetic type fixed effect per se did not affect the CPd but the environment did (+7.5% under heat challenge and -4.0% for a high fibre-low energy diet). However, there was a significant genetic type by environmental interaction. Generation 16 of line V presented a high CPd at NC compared to generation 36 and line LP (+6.6%; P<0.05). However, under the constrained environment, this difference disappeared, which suggests that line LP and generation 36 of line V adjusted the CPd better in unfavourable conditions. Theilgaard et al. (2007) and Theilgaard et al. (2009) described the flexibility of LP line, especially in constrained environments and later in productive life. In summary, the LP line seems to have a greater ability to obtain resources when the environment is not suitable, which is manifested in its higher DMI, when fed a high fibre low-energy diet (NF), in the better digestibility of fibre under heat stress (HC), and the advantage in CPd (both under HC and NF environment). All of this suggests that the foundational criteria applied to line LP also selected animals with a greater digestive flexibility.

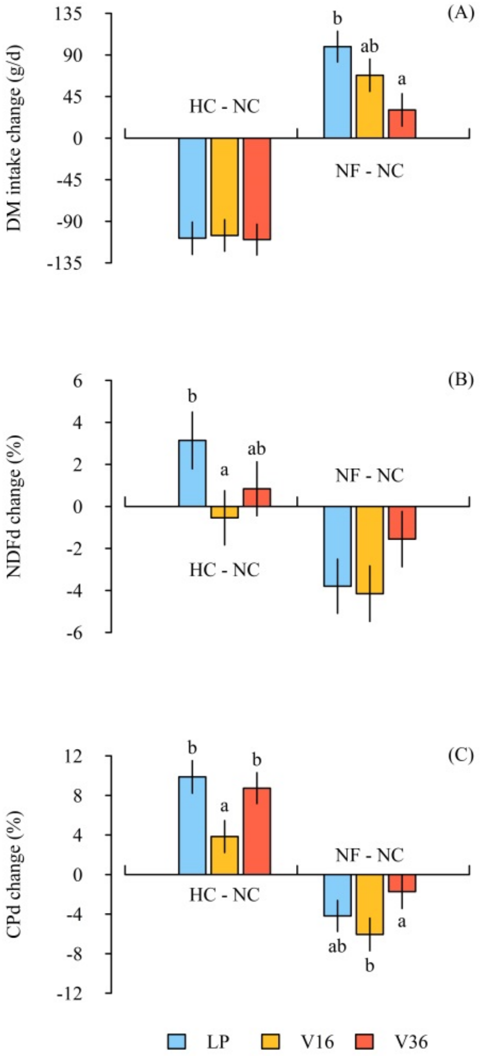

Figure 2.1 Change on (A) dry matter intake, (B) apparent digestibility coefficients of neutral detergent fibre and (C) crude protein caused by the heat or nutritional challenges respect to the control conditions for LP, V16, and V36 females. Error bars represent the standard errors. a-b: means for the different genetic types on same environment not sharing letters were significant different (P<0.05).

On the other hand, digestive efficiency of females did not seem to be affected after 20 generations of selection for litter size at weaning. Although females more selected for this criteria could have increased their ability to obtain resources at the beginning of lactation, as observed by Quevedo et al. (2006), and which could be also guessed from our results, digestive efficiency for main nutrients was not changed to any great extent. However, the higher DEI reduction of more selected animals under a nutritionally constrained environment (NF) could be related to a higher sensitivity to that environment, which must be studied in further works.

2.5 Conclusions

Both heat and the nutritional challenges affected the feed intake (reduction and increasing, respectively) and consequently the apparent digestible coefficients (improved and decreased, respectively). The selection over 20 generations for litter size at weaning was not a factor improving the feed intake capacity or the digestibility of the animals, but did improve the flexibility under constrained conditions. The foundation criteria for long-productive animals followed by a selection for litter size at weaning was effective, improving the feed intake capacity without reducing the digestive capacity and flexibility under constrained conditions. These patterns seem to contribute to a longer productive lifespan.

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3

Environmental sensitivity differs between rabbit lines selected for reproductive intensity and longevity

Savietto D.*, Cervera C.*, Blas E.*, Baselga M*, Larsen T.§, Friggens N.C.†‡, and Pascual J.J.*

**Institute for Science and Animal Technology, (ICTA). Universitat Politècnica de València.Camino de Vera s/n. 46022, Valencia, Spain.*

§*Department of Animal Science, Integrative Physiology. Aarhus University. Blichers Allé 20. 8830, Tjele, Denmark.*

†*UMR0791 Modélisation Systémique Appliquée aux Ruminants (MoSAR). Institute National de la Recherche Agronomique (INRA). 16 Rue Claude Bernard. 75231, Paris, France.*

‡*UMR0791 Modélisation Systémique Appliquée aux Ruminants (MoSAR). AgroParisTech. 16 Rue Claude Bernard. 75231, Paris, France.*

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3.1 Abstract

To better understand the mechanisms that allow some animals to sustain their productive effort in harsh environmental conditions, rabbit does from two selection lines (**LP** and **V**) were housed in normal (**NC**), nutritional (**NF**) or heat (**HC**) challenging environmental conditions from first to third partum. The LP line (n = 85) was founded on reproductive longevity criteria by selecting females from commercial farms that had a minimum of 25 partum with more than 7.5 kits born alive per parity. Line V (n = 79) was constituted from four specialised maternal lines into a composite synthetic line and then selected by litter size at weaning for 36 generations. Females in NC and NF environments were housed at normal room temperature (18 to 24°C) and fed with control (11.6 MJ DE/kg DM) or low-energy diets (9.1 MJ DE/kg DM). HC females were housed at high room temperatures (25 to 35°C) and fed the control diet. Females on HC and NF ingested 11.5% and 6.0% less DE than NC females, respectively (P<0.05). These differences between environments occurred in both lines, with the differences being higher for LP than V does (+6.0%; P<0.05). Milk yield responses followed those of energy intake, also being higher for LP does (+21.3 g/d; P<0.05). The environmental conditions did not affect the perirenal fat thickness (**PFT**), but a genotype by environment interaction was observed. On NC and HC the PFT was higher for line V (+0.23 and +0.35 mm, respectively; P<0.05) than for LP does but this was not the case for NF (-0.01 mm). Moreover, the PFT evolution was different between them. In the NC environment, LP females used the accreted PFT in late lactation (-0.29 mm) while V females did not (-0.08 mm). Conversely, in the HC environment LP females showed a flat PFT evolution in late lactation, while V females accumulated PFT. In the NF environment, LP and V females had a similar PFT evolution. There was also a litter size reduction for V does of -2.59 kits total born on HC and -1.78 kits total born on NF, whereas this was not observed for LP females. The results for LP females indicate direct use of DE ingested for reproduction with little PFT change, while V females actively use the PFT reserves in reproduction.

Key words: rabbit does, genotype environment interaction, adaptability, reproductive efficiency, longevity

3.2 Implications

Selection programs considering longevity and functional traits can contribute to the health status and fertility of the herd. In rabbits, a maternal line established by selecting females with a long productive lifespan has been characterised by a greater reproductive robustness to environmental changes compared to females from a line founded and selected exclusively for reproductive traits. This study highlights the role of body reserves in underpinning the reproductive stability of these robust females. It also helps understand why they have a longer productive lifespan.

3.3 Introduction

In meat producing rabbits, the high replacement rate of females (e.g. >120%; Ramón et al., 2004) does not seem to be directly related to selection for reproductive intensity (Piles et al., 2006). Indeed, negligible genetic correlations between longevity and litter size traits were found in a line selected for litter size at weaning (Sánchez et al., 2006). However, Rosell (2003) and Rosell et al. (2009) have described discrete and seasonal factors (e.g. human and/or environmental) associated with an increment in sanitary risks; especially high in young reproductive does (Rosell and de La Fuente, 2009). If the selection for reproductive intensity does not reduce longevity, but at the same time the environmental factors increases the sanitary risks, and thus the replacement rate, the selection for reproductive intensity may have adverse effects on other life functions related to robustness.

Strandberg and Sölkner (1996) indicated that the increment of herd health status, fertility, and thus the reduction in voluntary culling were all beneficial traits for inclusion of longevity in the selection index for dairy cows. Similarly, Engblom et al. (2007) observed an overall better reproductive performance and health status of sows culled at 'old ages'. Such information suggests that animals with long productive lifespans were those that were more able to adapt to a wide range of changing environmental factors (e.g. housing features, climatic conditions, and diet) while maintaining adequate productive levels, i.e. they are more robust.

This seems to be the case of a maternal rabbit line, called LP, founded by selecting females of extremely long longevity and a minimum acceptable litter size from a large set of Spanish and Portuguese commercial farms (Sánchez et al., 2008). Females from line LP were characterised by having an extended lifespan (Sánchez et al., 2008), a later reproductive senescence (Theilgaard et al., 2007) and a greater adaptability to maintain productive level in the face of nutritional constraints (Theilgaard et al., 2009) compared to a maternal line exclusively founded and subsequently selected for reproductive intensity (line V; Estany et al., 1989). The advantages of LP females were dependent on specific conditions (environmental restriction or high reproductive effort) and seem to be related to the use of their greater soma (i.e. live weight and body reserves) (Theilgaard et al., 2007 and Theilgaard et al., 2009).

However, it is also known that rabbit lines selected for growth rate, characterised by a high feed intake, growth rate and adult live weight (Estany et al., 1992) but also by a reduced reproductive performance (Mehaisen et al., 2004; Vicente et al., 2012), showed an elevated disease incidence despite having a higher body condition score (Sánchez et al., 2012). Therefore, robustness in rabbit females seems not to be based solely on their greater body reserves or on their ability to obtain resources (greater intake capacity due to larger size). It seems likely that the way in which such animals allocate their resources to different life functions, i.e. their control of nutrient partition, is a major factor in their robustness (Friggens et al., 2012). In this context, it is noteworthy that Ferrian et al. (2013) observed a better immunological response of LP rabbit females to a lipopolysaccharide challenge.

The above cited evidence suggests that the LP line may be classified as robust (Theilgaard et al., 2007 and Theilgaard et al., 2009) but it is not clear how these animals alter their nutrient partitioning in response to environmental constraints. Therefore, the present work was designed to: 1) characterise in restricted environments the changes in resource acquisition and allocation of females from a line recently founded on the basis of hyper-longevity, compared to a line founded and selected over 36 generations for litter size, 2) quantify the consequences on productive performance, and 3) thus improve our understanding of the possible implications of selection on reproductive intensity for robustness and lifespan.

3.4 Material and methods

The experimental proceedings were approved by the Universitat Politècnica de València ethical committee on protection of animals used in experimentation and other scientific purpose, as established by Royal Decree 1201/2005 (BOE, 2005).

3.4.1 Animals

Rabbit females from two lines differing in foundational criteria and selection degree for litter size at weaning were compared. A total of 164 females started the trial, 85 from line **LP** and 79 from line **V**. The LP line was founded on reproductive longevity criteria by selecting females from commercial farms (indistinctively of its origin; i.e. purebred or crossbreed of synthetic lines) that had a minimum of 25 partum with more than 7.5 kits born alive per partum (more details of LP line constitution are given in Sánchez et al., 2008) and then selected by litter size at weaning for the subsequent six generations. Line V was constituted from four specialised maternal lines into a composite synthetic line and then selected by litter size at weaning (Estany et al., 1989) for 36 generations. Both lines are used to produce crossbreed females destined to commercial farms.

3.4.2 Environments

To evaluate the animal response to environmental changes, three environmental conditions differing in room temperature and/or diet quality were set up. The control environment (**NC**) was the combination of normal room temperatures [**N**: 95% confidence intervals of min. (17.7 to 19.3°C) and max. (23.0, 24.7°C) daily registered temperatures] and a control diet (**C**), similar to commercial diets formulated to cover the requirements of reproductive rabbit does [11.6 MJ digestible energy (**DE**)/kg of dry matter (**DM**), 126 g digestible protein (**DP**)/kg DM and 168 g of acid detergent fibre (**ADF**)/kg DM]. The heat challenging environment (**HC**) was achieved by the combination of a high temperature room (**H**: following a daily sinusoidal variation of 25 to 35°C) and diet C. Detailed information on the design and operating system of the climatic chamber can be found in García-Diego et al. (2011). Finally, the nutritional challenging environment (**NF**) was created by combining normal room temperatures (**N**) with a low-energy fibrous diet (**F**), formulated to achieve 9.1 MJ DE/kg DM, 104 g DP/kg DM and 266 g ADF/kg DM, which is clearly below the recommendations for lactating rabbit does (de Blas and Mateos, 2010). Ingredients, chemical composition and apparent digestible coefficients of the experimental diets used in the different environments and the digestible energy intake were calculated from digestibility obtained in each environment, as described in chapter two (i.e. Savietto et al., 2012). Therefore, the DE content of diets consumed by LP and V females were 12.67 and 12.43 at HC, 11.65 and 11.54 at NC and 8.95 and 9.12 MJ DE/kg DM at NF, respectively.

3.4.3 Experimental procedures

Rabbit females were raised from birth until 63 days of age, according to a standardised management schedule described by Ragab and Baselga (2011), at which time they were transferred to the experimental farm. During the rearing period (from 63 days old to first partum) animals were subject to standard management with a commercial rearing diet (crude protein = 15.3%, ether extract = 2.5%, and crude fibre = 23.1% of fresh matter) supplied ad libitum and a daylight scheme of 16 h light and 8 h dark. Young females were first artificially inseminated at 125 days old, reaching the first partum with an average live weight of 3,636 ± 294 g (mean ± s.d.). At first partum, females from lines LP and V were randomly assigned to one of the three environments (NC, HC and NF) in a 2 x 3 factorial design (LPNC = 31, LPHC = 26, LPNF = 28, VNC = 29, VHC = 25, and VNF = 25 rabbit does). Because the availability of animals was limited by the selection nucleus the initial number of females differed. During the experimental period, which lasted from first partum until the third partum, female rabbits followed a programmed reproductive interval of 42 days, being inseminated at 11 days post-partum (**dpp**). Non-pregnant females were re-inseminated 21 days later and so on, until a maximum of three consecutive failures, when they were culled for infertility. The total number of kits born and born alive was recorded at each partum. Litters were standardised at birth to 9 kits in first lactation and to 10 kits in second lactation. Subsequently, dead kits were not replaced. Kits were 28 days old when weaned.

In both lactations, the female's live weight was measured at 0, 7, 14, 21 and 28 dpp, while perirenal fat thickness (**PFT**) was measured at 0, 14 and 28 dpp using the ultrasound method described by Pascual et al. (2004). Milk yield was measured 4 days per week during four weeks. In the first three weeks, females were weighted before having access to the nest box and just after nurse their kits (i.e. weigh-suckle-weigh method). In week four the kits were too big to be confined to the nest space. Females were then placed in new cages, being daily transfer to nurse their kits. Due to a limited number of cages at HC environment this practice was not possible; females and their kits shared a common space, making impossible to control the milk yield. Dry matter intake was monitored weekly during both lactation and weaning to partum intervals.

3.4.4 Blood plasma parameters

Blood samples were collected from the central artery of the ear using tubes with EDTA after a minimum fasting period of 3 h on 0, 14 and 28 dpp. Samples were immediately centrifuged (3,000 g during 10 min at 4°C), and plasma was separated and frozen at -40°C until further analysis. Samples from 12 females per group [2 lines (LP and V) x 3 treatments (NC, HC and NF)] with complete records (for each partum, artificial insemination and weaning time) were analysed for total T_3, leptin, non-esterified fat acids (**NEFA**), β-OH-butyrate, lactate and glucose. Total T_3 was analysed using the Beckman Coulter "Total T_3 RIA KIT" (IM1699-IM3287) (Immunotech AS, Prague, Czech Republic), according to the manufacturer's guidelines. Intra-assay coefficient of variation (**CV**) was 7.1% and inter-assay CV was 7.5%. Leptin was analysed by Multi-species Leptin assays (RIA, XL-85K) (Millipore Corporation, Billerica, MA, USA), according to the manufacturer's guidelines. Intra- and inter-assay CV were 9.1% and 9.3%, respectively. NEFA were determined using the NEFA C ACS-ACOD assay method (Wako Chemicals GmbH, Neuss, Germany). β-OH-butyrate was determined as an increase in absorbance at 340 nm due to the production of NADH, at slightly alkaline pH in the presence of β-OH-butyrate dehydrogenase. Sample blanks were included and the method involved oxamic acid in the media to inhibit lactate dehydrogenase as proposed by Harano et al. (1985). Glucose and lactate were determined according to standard procedures (Siemens Diagnostics® Clinical Methods for ADVIA 1650). Analyses of NEFA, β-OH-butyrate, lactate and glucose were performed using an auto-analyser, ADVIA 1650® Chemistry

System (Siemens Medical Solutions, Tarrytown, NY 10591, USA); in all instances the intra- and inter-assay CV was below 2.0% and 4.0%, respectively.

3.4.5. Statistical analysis

A mixed model with a repeated measure design (mixed procedure of SAS; SAS Institute Cary, NC) was used to analyse performance, hormonal and metabolic data of rabbit does until third partum. The model considered the variation between animals and the co-variation within them. The covariance structure was modelled using the spatial power function, after objectively comparing among other covariance structures as suggested by Littell et al. (1998). The spatial power covariance function is a direct generalisation of first order auto-regressive covariance function with the advantage of considering different lag time between repeated measures (i.e. measures on the same individual are continuous). This covariance function is flexible, because for equally time-spaced measurements, the covariance structure is equal to fit a first order auto-regressive covariance function. The model used to analyse reproductive performance (Table 3.2) included the line (LP and V), the environment (NC, HC and NF), partum (first, second and third) and their interactions. The model used to analyse performance traits (Table 3.3) and blood plasma parameters (Table 3.4) included the line, the environment, the reproductive cycle (first and second), and their interactions. This model also included measurement day (different in function of the variable studied; see experimental procedure) and its interactions with line and environment as fixed effects. Finally, the evolution of DE intake, milk yield and PFT was analysed considering the line, the environment, the lactation week and their interactions. All models included the permanent effect of animal $[\boldsymbol{p} \sim \boldsymbol{N}(0, \sigma_p^2)]$ and the error term $[\boldsymbol{e} \sim \boldsymbol{N}(0, \sigma_e^2)]$ as a random terms. The models for intake (both DM and DE) and milk yield included the average litter size during lactation as a covariate. Serum concentrations of total T_3, leptin, NEFA, β-OH-butyrate and lactate did not follow a normal distribution; hence a $\log_{10}$ transformation was applied before analysis. Variables were presented as least square means, and different contrasts were computed to test the effect of the environmental challenge and of the line at each reproductive cycle as follow:

$$\mathrm{HC} - \mathrm{NC} = \frac{(\mathrm{LPHC+VHC})}{2} - \frac{(\mathrm{LPNC+VNC})}{2} \tag{3.1}$$

$$\mathrm{NF} - \mathrm{NC} = \frac{(\mathrm{LPNF+VNF})}{2} - \frac{(\mathrm{LPNC+VNC})}{2} \tag{3.2}$$

$$\mathrm{LP} - \mathrm{V} = \frac{(\mathrm{LPNC+LPNC+LPNF})}{3} - \frac{(\mathrm{VNC+VHC+VNF})}{3} \tag{3.3}$$

3.5 Results

The number of females housed and the number of does reaching the second and third partum are present in Table 3.1. From the 164 females initially housed, 135 completed the experiment. In the normal temperature room, 11 females fed with diet C (five LP and six V) and five with diet F (four LP and one V) did not finish the experiment. In the HC environment, 13 females (five LP and eight V) failed to reach the third partum. Among these, seven LP (three, one, and three at HC, NC, and NF, respectively) and two V females at NC were culled, and another seven LP (three, three, and one) and 13 V females (eight, four, and one at HC, NC, and NF, respectively) died.

Table 3.1 Summary statistics for number of females housed, number of inseminations, conception rate, average interval between weaning to parturition and average interval between parturitions for LP and V rabbit females housed at heat (HC), normal (NC) or nutritional (NF) challenging conditions

Environment[1]	HC		NC		NF		Parameter
Line[2]	LP	V	LP	V	LP	V	
Number of females housed at							*Total*
First partum	31	29	26	25	28	25	164
Second partum	28	22	24	21	25	25	145
Third partum	26	21	21	19	19	24	135
Number of inseminations at							
First partum	37	35	28	27	30	28	185
Second partum	46	42	38	33	41	44	244
Third partum	35	34	32	26	37	38	202
Conception rate at (%)							*Average*
First partum	83.8	82.9	92.9	92.6	93.3	89.3	89.1
Second partum	60.9	52.4	63.2	63.6	61.0	56.8	59.6
Third partum	74.3	61.8	65.6	73.1	64.9	63.2	67.1
Average interval between (days)							*Std. Dev.*
1st weaning and 2nd partum	27.5	34.3	27.5	26.0	27.7	30.8	13.6
2nd weaning and 3rd partum	22.0	28.7	25.1	23.1	26.1	28.5	12.6
Average interval between (days)							
First and second partum	55.5	62.2	55.5	54.0	55.7	58.8	13.6
Second and third partum	50.0	56.7	53.2	51.1	54.1	56.5	12.6

[1]Environment: HC, heat condition (25 to 35°C) and diet C (11.6 MJ DE/ kg DM); NC, normal conditions (18 to 24 °C) and diet C; and NF, normal condition and diet F (9.1 MJ DE/ kg DM). [2]Line: LP, founded on reproductive-longevity criteria and selected for litter size at weaning for six generations; and line V, founded and selected for litter size at weaning for 36 generations.

Independently on the environmental conditions, the conception rate, the weaning to partum interval, as well as the partum to partum interval was similar between LP and V females (Table 3.4). The overall conception rates at first, second and third partum were 89.1, 59.6 and 67.1%, respectively. The overall interval between first weaning and second partum was 29.0 ± 13.6 d (mean ± s.d.), and between second weaning and third partum was 25.6 ± 12.6 d. The overall interval between first and second partum was 57.2 ± 13.6 d and between second and third partum was 53.6 ± 12.6 d.

3.5.1 Performance traits

The average number of kits total born and born alive is presented in Table 3.2. At first partum, just before the random allocation of rabbit females to different environments, the average number of total born tended to be higher for V than LP litters (mean difference ± std. error of difference: +0.80 ± 0.47 kits; $P<0.10$). At second partum, regardless of the environment, no significant differences between LP and V were observed neither in the number of total born nor in the number of born alive. However, V litters had a higher average number of stillborn (+0.74 ± 0.36 kits; $P<0.05$) than LP.

Table 3.2 The effect of environment and genetic line on reproductive performance of rabbit does at first, second and third partum

Environment[1]	HC		NC		NF		Contrasts[3]		
Line[2]	LP	V	LP	V	LP	V	HC-NC	NF-NC	LP-V
Number of kits total born per partum									
First	9.52	10.66	9.15	10.60	9.68	9.48	0.21 (0.57)	-0.30 (0.59)	-0.80 (0.47)
Second	10.07	10.18	10.38	11.14	10.16	10.12	-0.63 (0.62)	-0.62 (0.62)	-0.28 (0.50)
Third	10.08^{ab}	9.14^{a}	9.86^{a}	11.74^{b}	9.58^{a}	9.96^{a}	-1.19† (0.65)	-1.03† (0.64)	-0.44 (0.52)
Number of kits born alive per partum									
First	8.94	9.62	8.88	9.44	8.86	8.88	0.12 (0.67)	0.29 (0.69)	-0.42 (0.55)
Second	9.29	8.14	9.46	9.86	9.72	9.08	-0.95 (0.72)	0.26 (0.72)	0.46 (0.58)
Third	8.56^{b}	6.19^{a}	9.48^{bc}	10.68^{c}	8.96^{bc}	8.13^{ab}	-2.71** (0.76)	1.54* (0.75)	0.66 (0.61)

[1] Environment: HC: high room temperature (25 to 35 °C) and diet C (11.6 MJ of digestible energy per kg of dry matter); NC: normal room temperature (18 to 24 °C) and diet C; and NF: normal room temperature and diet F (9.1 of digestible energy per kg of dry matter). [2] Line LP, founded on reproductive-longevity criteria and then selected for litter size at weaning during 6 generations; and line V, founded on litter size at weaning and then selected during 36 generations. [3] Contrasts (standard error) followed by **, *, and † are significant at $P<0.01$, $P<0.05$, and $P<0.10$, respectively. $^{a-c}$ Values within a row with different superscripts differ significantly at $P<0.05$.

At third partum, V litters showed a higher number of total born (+1.88 ± 0.94 kits; $P<0.05$) than LP when housed in NC, whereas the difference was not significant for the number of born alive. In this parity, the constrained environments (HC and NF) did not affect the total number of total born and born alive for the LP line, relative to NC. However, for the V line there was a significant reduction in litter size in the constrained environments in terms of total born (-2.59 ± 0.94 and -1.78 ± 0.92 kits for HC and NF; P<0.05) and born alive (-4.49 ± 1.11 and -2.56 ± 1.08 kits for HC and NF; P<0.05), relative to NC.

In general, the HC environment limited the intake of DM (-21.0%; P<0.01) and DE (-11.5%; P<0.01), and reduced milk yield (-15.0%; P<0.01) compared to NC. However, the negative effect of high environmental temperatures on live weight was seen only in second lactation (-4.0%; P<0.01). For females on NF, although DM intake increased (+16.5%; P<0.01) during the first cycle, a lower DE intake was recorded (-8.9%; P<0.01), impairing both milk yield (-11.0%; P<0.01) and PFT (-2.7%; P<0.05) compared to NC. During the second reproductive cycle, females on NF also presented a higher DM intake (+24.0%; P<0.01) than those in NC, resulting in no significant differences between them in DE intake (-3.4%). However, milk yield (-16.0%; P<0.01) and live weight (-2.0%; P<0.10) of females housed in NF were lower compared to NC. LP females were characterised by higher feed intake and milk yield than V both during the first (5.0% and 10.0%; P<0.10) and especially the second reproductive cycle (7.0% and 13.0%; P<0.05). In spite of their greater live weight in the first cycle (2.0%; P<0.05), LP does showed a lower average PFT than V females in both reproductive cycles (2.0% at first and 3.0% at second). The main values of the productive traits of lactating rabbit does housed in different environments during the first and second reproductive cycles are in Table 3.3.

Figure 3.1 shows the evolution of DE intake of the LP and V females in the different environments. Although there were some minor differences, the intake curves for LP and V lines were similar in each of the lactations in NC, HC and NF environments but with the DE intake of the LP line being systematically higher than that of line V in NC (+114.2 ± 64.5 kJ/d; P<0.10), HC (+199.2 ± 60.1 kJ/d; P<0.01) and NF (+219.7 ± 61.4 kJ/d; P<0.01).

The evolution of milk yield for LP and V females in the different environments is shown in Figure 3.2. In NC, LP females always yielded more milk than V, significant at weeks one (+27.7 ± 9.5 g/d) and four (+25.2 ± 9.5 g/d) of first lactation and three (+26.1 ± 10.4 g/d) and four (+35.2 ± 10.4 g/d) of the second lactation. The higher milk yield of LP females was also observed in the second lactation on HC (on average +31.8 g/d; P<0.05), although it was similar for both lines during first lactation. On NF, LP females also yielded more milk than V, being significantly different at mid-lactation of the first (29.5 g/d; P<0.01) and second cycle (33.12 g/d; P<0.01). The milk yield differences observed between LP and V females during lactations followed the DE intake pattern.

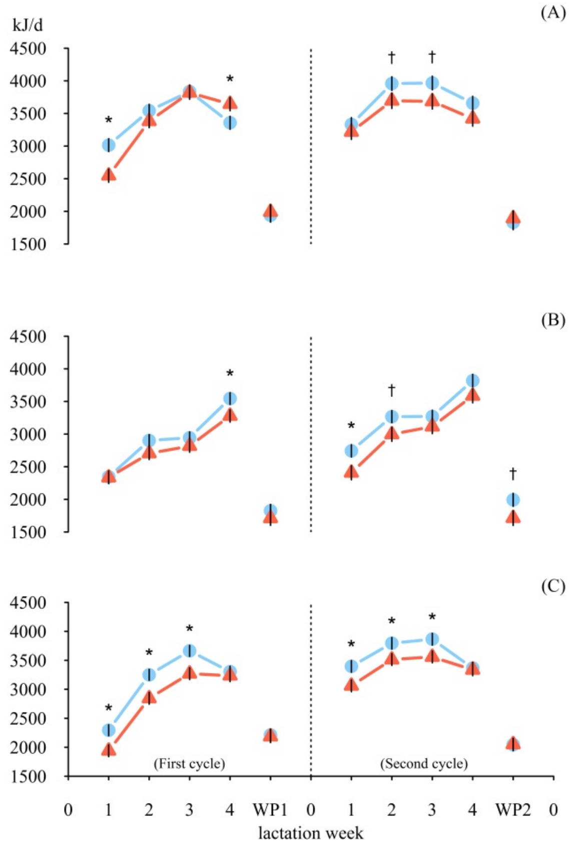

Figure 3.1 Digestible energy intake (kJ/d) of LP (blue) and V (red) female rabbits housed in: (A) normal [normal room temperature (18°C to 24°C) and fed with diet C (11.6 MJ DE/kg DM)], (B) heat [high room temperature (25°C to 35°C) and diet C] or (C) nutritional [normal room temperature and fed with diet F (9.1 MJ DE/kg DM)] challenging conditions. Vertical bars represent the standard errors of means. WP represents the weaning to partum interval. *P<0.05 and †P<0.10.

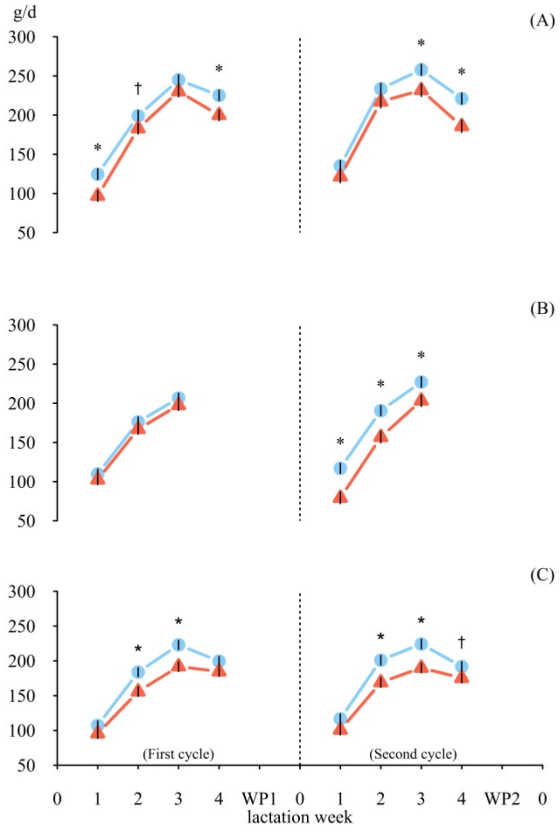

Figure 3.2 Milk yield (g/d) of LP (blue) and V (red) female rabbits housed in: (A) normal, (B) heat or (C) nutritional challenging conditions. Vertical bars represent the standard errors of means. WP represents the weaning to partum interval.*P<0.05 and †P<0.10.

Table 3.3 The effect of environment and genetic line on average dry matter (DM) and digestible energy (DE) intakes, milk yield, live weight and perirenal fat thickness (PFT) of rabbit does during first and second reproductive cycles.

Environment[1]	HC		NC		NF		Contrasts[3]		
Line[2]	LP	V	LP	V	LP	V	HC-NC	NF-NC	LP-V
First reproductive cycle (from first to second partum)									
DM intake (g/d)	207.4^{a}	204.0^{a}	253.4^{b}	250.9^{b}	308.5^{d}	279.2^{c}	-46.5** (8.2)	41.7** (8.3)	11.8† (6.7)
DE intake(kJ/d)[4]	2,626^{ab}	2,534^{a}	2,957^{c}	2,901^{c}	2,773^{bc}	2,563^{ab}	-349** (88)	-261* (90)	119† (72)
Milk yield (g/d)	163.0^{ab}	153.7^{a}	188.6^{c}	168.9^{bc}	170.2^{bc}	146.7^{a}	-20.3** (7.0)	-20.2** (7.2)	17.5** (5.8)
Live weight (g)	3,769^{ab}	3,783^{ab}	3,837^{b}	3,738^{ab}	3,828^{b}	3,688^{a}	-11 (45)	-30 (46)	75* (37)
PFT (mm)	6.72^{a}	6.97^{bc}	6.88^{abc}	7.06^{c}	6.77^{ab}	6.80^{ab}	-0.13 (0.08)	-0.19* (0.08)	-0.15* (0.07)
Second reproductive cycle (from first to second partum)									
DM intake (g/d)	238.0^{a}	221.0^{a}	287.3^{b}	273.2^{b}	364.0^{d}	333.1^{c}	-50.7** (8.9)	68.3** (8.7)	20.7** (7.1)
DE intake (kJ/d)[4]	3,018^{b}	2,751^{a}	3,349^{c}	3,153^{bc}	3,251^{c}	3,031^{b}	-366** (96)	-110 (94)	228* (76)
Milk yield (g/d)	175.8^{b}	145.7^{a}	207.1^{c}	188.7^{bc}	178.9^{b}	152.1^{a}	-37.1** (7.8)	-32.4** (7.7)	25.1** (6.2)
Live weight (g)	3,763^{a}	3,897^{b}	3,971^{b}	3,982^{b}	3,911^{b}	3,865^{ab}	-147* (47)	-89† (47)	-33 (38)
PFT (mm)	6.75^{a}	7.27^{c}	6.96^{ab}	7.17^{bc}	7.05^{bc}	6.94^{ab}	-0.05 (0.09)	-0.07 (0.09)	-0.21** (0.08)

[1]Environment: HC: high room temperature (25 to 35 °C) and diet C (11.6 MJ of digestible energy per kg of dry matter); NC: normal room temperature (18 to 24 °C) and diet C; and NF: normal room temperature and diet F (9.1 of digestible energy per kg of dry matter). [2]Line LP, founded on reproductive-longevity criteria and then selected for litter size at weaning during 6 generations; and line V, founded on litter size at weaning and then selected during 36 generations. [3]Contrasts (standard error) followed by **, *, and † are significant at P<0.01, P<0.05, and P<0.10. [4]Digestible energy concentration (kJ/g DM): HCLP = 12.67, HCV = 12.43, NCLP = 11.65, NCV = 11.54, NFLP = 8.95, and NFV = 9.12. $^{a-d}$ Values within a row with different superscripts differ significantly at P<0.05.

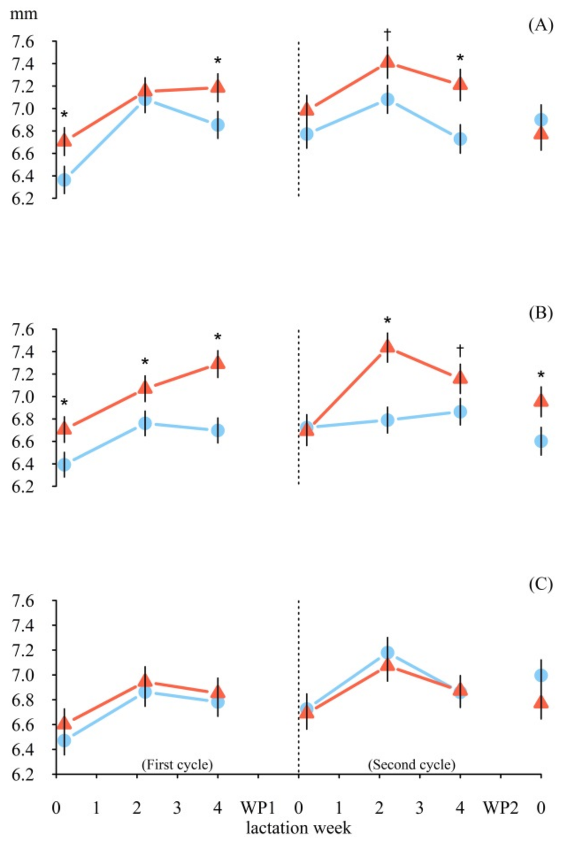

Figure 3.3 Perirenal fat thickness (mm) of LP (blue) and V (red) female rabbits housed in: (A) normal, (B) heat, or (C) nutritional challenging conditions. Vertical bars represent the standard errors of means. WP represents the weaning to partum interval. *P<0.05 and †P<0.10.

The evolution of PFT is presented in Figure 3.3. Independently of the environment, line or reproductive cycle, an accretion phase was observed during the first 2 weeks of lactation, whereas the evolution from this point to weaning, most frequently a mobilisation of PFT, was more dependent on DE intake and milk yield. Females of line V had greater PFT than LP females in the NC environment (+0.23 ± 0.09 mm; P<0.01). This difference was accentuated in the HC environment (+0.35 ± 0.08 mm; P<0.01), whereas in the NF environment the difference was reduced and became non-significant (0.01 ± 0.08 mm).

3.5.2 Blood plasma parameters

The effect of environment and line on the concentrations of serum parameters is presented in Table 3.4. No significant differences between lines were observed for any monitored plasma parameters of females. Only the lactate concentration of LP and V females differed during the second reproductive cycle on NF (2.61 and 3.40 mM, respectively; P<0.05). Although no significant differences were observed between LP and V does on HC, they showed a different response to this environment in respect to NC. While LP does on HC reduced both lactate (at first cycle) and total T_3 (at second cycle), line V increased the levels of β-OH-butyrate and NEFA (only at second cycle). In a similar way, each line showed distinct responses to the NF environment respect to NC. Thus, LP females in NF had low levels of leptin in the first cycle, and low levels of NEFA in both cycles. In contrast, the response of V females to the NF environment was a reduction in lactate concentration during first cycle. Moreover, both lines responded to the NF environment by increasing the β-OH-butyrate levels.

Table 3.4 Effect of environment and genetic line on serum concentration of total T$_3$, leptin, non-esterified fatty acids (NEFA), β-OH-butyrate, lactate and glucose of rabbit does during first and second reproductive cycle. Parity order effect and parity order within environment and line not shown.

Environment[1]	HC		NC		NF		Contrasts[3]		
Line2	LP	V	LP	V	LP	V	HC-NC	NF-NC	LP-V
First reproductive cycle (from first to second partum)									
Total T$_3$ (log$_{10}$ nM)	0.229	0.233	0.299	0.262	0.244	0.271	-0.049† (0.027)	-0.022 (0.027)	0.002 (0.022)
Leptin (log$_{10}$ ng/ml)	0.230^{b}	0.249^{b}	0.276^{b}	0.230^{b}	0.080^{a}	0.158^{ab}	-0.014 (0.044)	-0.134** (0.044)	-0.017 (0.036)
NEFA (log$_{10}$ μ.ekv/l)	2.55^{ab}	2.52^{ab}	2.58^{b}	2.56^{ab}	2.50^{a}	2.57^{ab}	-0.041 (0.030)	-0.044 (0.030)	-0.009 (0.025)
β-OH-butyrate[4] (log$_{10}$ mM)	1.88^{a}	1.90^{a}	1.98^{ab}	1.81^{a}	2.17^{bc}	2.30^{c}	-0.007 (0.074)	0.340** (0.074)	0.008 (0.061)
Lactate (log$_{10}$ mM)	0.514^{ab}	0.557^{abc}	0.631^{c}	0.614^{bc}	0.545^{abc}	0.509^{a}	-0.087* (0.036)	-0.095* (0.036)	0.004 (0.030)
Glucose (mM)	6.16^{ab}	5.85^{a}	6.13^{ab}	5.87^{a}	6.22^{b}	6.08^{ab}	0.005 (0.125)	0.152 (0.125)	0.233* (0.102)
Second reproductive cycle (from second to third partum)									
Total T$_3$ (log$_{10}$ nM)	0.210^{a}	0.253^{ab}	0.302^{b}	0.272^{ab}	0.263^{ab}	0.252^{ab}	-0.055* (0.027)	-0.029 (0.027)	-0.001 (0.022)
Leptin (log$_{10}$ ng/ml)	0.189	0.259	0.274	0.228	0.153	0.191	-0.027 (0.044)	-0.079† (0.044)	-0.021 (0.036)
NEFA (log$_{10}$ μ.ekv/l)	2.57^{bc}	2.62^{c}	2.57^{bc}	2.53^{ab}	2.46^{a}	2.52^{ab}	0.053† (0.030)	-0.055† (0.030)	-0.023 (0.025)
β-OH-butyrate[4] (log$_{10}$ mM)	1.90^{bc}	1.94^{bc}	1.76^{ab}	1.64^{a}	2.04^{c}	2.09^{c}	0.220** (0.075)	-0.367** (0.075)	0.011 (0.061)
Lactate (log$_{10}$ mM)	0.454^{ab}	0.526^{bc}	0.496^{abc}	0.562^{c}	0.416^{a}	0.531^{bc}	-0.039 (0.036)	0.055 (0.037)	-0.085** (0.030)
Glucose (mM)	6.10	5.97	6.10	5.77	5.81	5.87	0.010 (0.125)	0.101 (0.125)	0.133 (0.102)

[1] Environment: HC: high room temperature (25 to 35 °C) and diet C (11.6 MJ of digestible energy per kg of dry matter); NC: normal room temperature (18 to 24 °C) and diet C; and NF: normal room temperature and diet F (9.1 of digestible energy per kg of dry matter). [2] Line LP, founded on reproductive-longevity criteria and then selected for litter size at weaning during 6 generations; and line V, founded on litter size at weaning and then selected during 36 generations. [3] Contrasts (standard error) followed by **, *, and † are significant at $P<0.01$, $P<0.05$, and $P<0.10$. [4] To back transform the β-OH-butyrate to normal scale apply $[(10^{X} + 5) / 1000]$, where x is the tabulated value. $^{a-c}$ Values within a row with different superscripts differ significantly at $P<0.05$.

3.6 Discussion

The designed environments aimed to produce different physiological constraints on female rabbits. The direct consequence of this was observed in the DE intake. Does subject to high temperatures (HC) had a DE intake reduction of -12.0% and -11.0% in the first two reproductive cycles, whereas the bulk feed generated by the fibrous diet (NF) resulted in a DE intake reduction of -9.0% and -3.0%, in first and second reproductive cycle, respectively. These responses confirm, on the one hand, the rabbit's capacity to avoid excessive heat load by reducing the feed intake when exposed to high temperatures (Cervera and Fernández-Carmona, 2010), and on the other hand to partially compensate for a low-dietary-energy density by increasing the feed intake on feeds with an energy content below 9.0 MJ DE/kg DM (Fernández-Carmona et al., 2003). It is also important to note that does were allocated to harsh environments just after first partum, a period of great energy demand because of milk production and the need to recover body reserves (Xiccato et al., 1999; Pascual et al., 2002). Thus, in the first lactation, the constrained does have to cope with the same litter size as non-constrained does (litters were standardised at partum). The situation in second lactation is different because the harsh environments were also applied during that pregnancy.

3.6.1 Genetic differences in NC environment

In the present study (i.e. with relatively few animals), just before the allocation of animals to different environments (i.e. after first partum), V does did not have significantly bigger litters (+0.80 kits total born and +0.44 kits born alive) than the LP does. However, the V does produced significantly larger litters at third partum (+1.88 kits total born and +1.20 kits born alive) in unconstrained conditions (NC). These values are in agreement with a large-scale study (>200 does per line; Sánchez et al., 2008) that reported higher total born (+0.74 kits), born alive (+0.77 kits) and weaned kits (+0.54) during the first three partum for V does as compared with LP does. The main differences between LP and V does in the NC environment were observed in the evolution of DE intake, milk yield and PFT. In both lactations, LP seemed to adapt the DE intake and milk yield to the litter requirements, avoiding the accumulation of PFT. Line V, in contrast, seemed to adjust the DE intake and milk yield to ensure a higher PFT at weaning. The effort of V does to accrete more PFT than LP does was also observed by Theilgaard et al. (2009), but cannot be clearly elucidated from Theilgaard et al. (2007). However, Theilgaard et al. (2007) observed a tendency of V line to sustain the PFT level across parities, whereas the LP line was more flexible. In addition, Theilgaard et al. (2007 and 2009) reported higher live weight of LP does compared with V does. In the present study, LP and V does had similar live weight. A likely reason for this difference between studies is the difference in the reproductive rhythm adopted in each one. When the reproductive rhythm was less intense (insemination at 25 or 30 dpp), the initial live weight differences between lines were maintained (Theilgaard et al., 2007 and 2009), whereas when it was more intense (insemination at four or 11 dpp) the live weight differences disappeared (Theilgaard et al., 2009).

3.6.2 Genetic differences in HC environment

The reductions in DE intake observed during the whole experimental period for does from lines LP and V were -12.0% and -18.0%, respectively. This was less than the DE intake reduction of -35.0% reported by Fernández-Carmona et al. (2003) in crossbreed rabbit does housed at a constant high temperature (30°C). The lower DE intake restriction observed in the present study may be related to the climatic chamber programme, which was set up to produce a daily sigmoid temperature curve from 25°C to 35°C, and thus allowed the does to concentrate meals in periods of reduced temperature (25°C), alleviating the effects of heat stress. During the first lactation, the main differences between LP and V does in DE intake and milk-yield profiles were reduced relative to the differences between LP and V does in the NC environment. LP does adjusted their milk production and PFT use to the level of DE obtained, whereas does from line V generally showed a linear PFT accretion pattern during the whole lactation. This difference persisted even in the fourth week of lactation in HC, despite the LP does having a greater DE intake.

However, despite the above-mentioned differences, both LP and V does had a similar number of kits total born and born alive at second partum in HC environment. Thus, it seems that even under constrained conditions, the V line was able to sustain the reproductive effort by privileging body reserve accretion in the second part of lactation, ensuring thus a high reproductive performance in the subsequent partum. The LP does had achieved similar results by increasing the DE intake. The ability of LP does to increase the DE intake under high temperatures may be associated with a reduced metabolic rate evidenced by a reduction in the total T_3 (-0.081 $\log_{10}$ nM; $P<0.05$) and lactate (-0.079 $\log_{10}$ ng/ml; $P<0.05$) levels at HC compared with NC. Moreover, a reduced metabolic rate in the HC as compared with the NC environment may explain the different responses of these two lines. The different strategies used by the LP and V females to ensure an adequate reproductive level in the first lactation was confirmed at the second reproductive cycle. Does from the LP line used the greater intake to ensure both milk yield and litter size at third partum without increasing the PFT levels, and also appeared to reduce the metabolic rate compared with the LP does in NC (i.e. lower total T_3). The V does seem to base their reproductive success on ensuring they had ample body reserves (PFT). However, after an intense accretion of PFT reserves during the first half of the second lactation, a mobilisation period was established ending at third partum. Indeed, compared with NC, the V does in HC showed higher levels of NEFA and β OH-butyrate. High NEFA serum concentration may be related to a reduction in the number of born alive (-2.1 kits in does submitted to fasting until 2 h before insemination; Brecchia et al., 2006). The intense PFT mobilisation of V does throughout the gestational period impaired their reproductive performance (-4.5 kits born alive compared with NC), probably as a consequence of subclinical ketosis.

3.6.3 Genetic differences in NF environment

Does eating a low-energy high-fibre diet could not fully compensate for the decrease in DE feed content, despite increasing their DM intake. Therefore, a lower milk yield and PFT was observed during the first lactation, compared with does in NC. However, there was an adaptation to this diet in terms of DM intake capacity, so that the NF does increased the intake by 26.6 g/day between the first and second reproductive cycles. This almost allowed DE intake compensation (-3.4% compared with NC) but not for milk yield (-16.4% compared with NC), because of the lower efficiency for milk yield of the DE ingested coming from high-fibre diet (Fernández-Carmona et al., 1995). Does in NF had a similar milk yield in both lactations, whereas the milk yield at NC increased in the second lactation (+19.2 g/day).

Furthermore, the response to the NF environment seemed to have a genetic component. This was evident in the reproductive performance observed in NF. The higher feed intake capacity of LP does allowed an ingestion of +219.7 kJ of DE/day more than V does in NF throughout the experimental period. This higher DE intake allowed the LP does to sustain the number of kits total born and born alive and an adequate milk production to nurse the current litter, without affecting the development of the future one. In fact, the LP does in NF and V does in NC yielded a similar amount of milk, especially during the first reproductive cycle. In contrast, the inability of V does to acquire resources in the NF environment was clearly seen by a lower milk yield (-22.1 g/day; $P<0.05$) and PFT (-0.26 mm; $P<0.05$) compared with NC, with the latter perhaps negatively affecting the number of total born (-0.98 and -1.78 kits at second and third partum, respectively). As the reproductive success of V line seemed to be based on the accretion and use of fat reserves, their low PFT values on NF led to a clear reduction in the number of total born at second and, especially, third partum. This also affected the number of born alive at second and third parity (-0.78 and -2.55 kits, respectively).

3.6.4 Environmental sensitivity reasons

Theilgaard et al. (2007) made two main observations, which, together with the present results, give a new insight to better understand and describe the relationship between body reserves, reproduction and survival. They first observed that the V does maintained their litter size during a stress period but not after it, and contrary to LP does, they showed a greater PFT mobilisation. Therefore, the hypothesis that reproduction in V does depends more on the use of body reserves than that in LP does that sustain it by increasing intake and maintaining PFT seems plausible.

The variations that occur in the environments experienced by rabbits on commercial farms have been described (Rosell et al., 2009; Rosell and de La Fuente, 2009). These variations were present in the commercial rabbit farms of Portugal and Spain where female rabbits used to establish the LP line came from (Sánchez et al., 2008). This may explain the greater robustness (Theilgaard et al., 2007 and 2009) and the extended reproductive lifespan of LP does (Sánchez et al., 2008). This study emphasises the role of body reserve usage in providing rabbits with adaptive capacity and are in agreement with previous findings. Theilgaard et al. (2007) and Sánchez et al. (2012) showed that any deviation from an adequate body condition increases the health risks in rabbit does. Ferrian et al. (2013) also observed that the maintenance of body reserves under an immunological challenge with lipopolysaccharide provided an advantage for LP does compared with V does. It should be noted that it is not just the level of body reserves but also their rate of usage that is important. In this context, Rosell and de La Fuente (2009) reported the greatest mortality risk to be at the end of gestation, the period of greatest mobilisation of body reserves (Quevedo et al., 2005 and 2006). Therefore, the ability of LP does to sustain reproduction in the different environments without presenting great deviations in PFT levels and its ability to use reserves at the onset of constraints (Theilgaard et al., 2007) seems to be a safeguarding factor to ensure both reproduction and longevity.

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4

Different resource allocation strategies result from selection for litter size at weaning in rabbit does

Savietto D.*, Cervera C.*, Ródenas L.*, Martínez-Paredes E.*, Baselga M*, García-Diego F.§, Larsen T.¥, Friggens N.C.†‡, and Pascual J.J.*

**Institute for Science and Animal Technology (ICTA). Universitat Politècnica de València.*
Camino de Vera s/n. 46022, Valencia, Spain.

§Department of Applied Physics (U.D. Agrónomos). Universitat Politècnica de València.
Camino de Vera s/n. 46022, Valencia, Spain.

¥Department of Animal Science, Integrative Physiology. Aarhus University.
Blichers Allé 20. 8830, Tjele, Denmark.

†UMR0791 Modélisation Systémique Appliquée aux Ruminants (MoSAR). Institute National de la Recherche Agronomique (INRA). 16 Rue Claude Bernard. 75231, Paris, France.

‡UMR0791 Modélisation Systémique Appliquée aux Ruminants (MoSAR). AgroParisTech.
16 Rue Claude Bernard. 75231, Paris, France.

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4.1 Abstract

This study examined the effect of long-term selection of a maternal rabbit line, solely for a reproductive criterion, on the ability of female rabbits to deal with constrained environmental conditions. Female rabbits from generations 16 and 36 (n = 72 and 79, respectively) of a line founded and selected to increase litter size at weaning were compared simultaneously. Female rabbits were subjected to normal (**NC**), nutritional (**NF**) or heat (**HC**) challenging conditions from first to third parturition. Animals in NC and NF were housed at normal room temperatures (18 to 24°C) and respectively fed with control (11.6 MJ DE/kg DM, 126 g DP/kg DM, and 168 g of ADF/kg DM) or low-energy fibrous diets (9.1 MJ DE/kg DM, 104 g DP/kg DM and 266 g ADF/kg DM), whereas those housed in HC were subjected to high room temperatures (25 to 35°C) and the control diet. The litter size was lower for female rabbits housed in both NF and HC environments, but the extent and timing where this reduction took place differed between generations. In challenging conditions (NF and HC), the average reduction in the reproductive performance of female rabbits from generation 16, compared with NC, was -2.26 ($P<0.05$) and -0.51 kits born alive at second and third parturition, respectively. However, under these challenging conditions, the reproductive performance of female rabbits from generation 36 was less affected at second parturition (-1.25 kits born alive), but showed a greater reduction at the third parturition (-3.53 kits born alive; $P<0.05$) compared with NC. The results also showed differences between generations in digestible energy intake, milk yield and accretion, and use of body reserves throughout lactation in NC, HC and NF, which together indicate that there were different resource allocation strategies in the animals from the different generations. Selection to increase litter size at weaning led to increased reproductive robustness at the onset of an environmental constraint, but failure to sustain the reproductive liability when the challenge was maintained in the long term. This response could be directly related to the short-term environmental fluctuations (less severe) that frequently occur in the environment where this line has been selected.

Key words: rabbit does, selection for litter size, robustness, heat stress, feeding restriction

4.2 Implications

Selection for litter size at weaning has successfully increased the reproductive performance of female rabbits, but the question arises as to whether this has altered resource allocation to other traits. When the environment provides sufficient resources, selection can improve individual traits without penalties in other traits, that is, without changing resource allocation. However, farms are frequently subjected to occasional challenges (feed quality, heat stress, pathogens, etc.), and when resources become limited, a preferential allocation of resources to selected traits (growth, reproduction, etc.) is expected, reducing the ability of animals to respond to other demands (such as coping with disease, stress, etc.). Therefore, a better understanding of the effect of selection to increase litter size at weaning on resource allocation strategies under challenging conditions could help to maintain an adequate productive level of rabbit farms in the long term.

4.3 Introduction

Maximum profitability in maternal rabbit lines is achieved when a female rabbit has high reproductive longevity and high litter size. This basic concept of maximum fitness is frequently applied in rabbit selection programmes, which mainly concentrate their efforts on increasing litter size with no special focus on longevity (Estany et al., 1989; García and Baselga, 2002; Piles et al., 2006a). Attention to studying longevity is recent (Garreau et al., 2001; Sánchez et al., 2006; Piles et al., 2006b), arising from the high replacement rates observed in commercial farms (Ramon et al., 2004).

Some authors (Sánchez et al., 2006; Theilgaard et al., 2006; Piles et al., 2006b) found no evidence of antagonism between selection to increase litter size at weaning and lifespan, in relatively abundant conditions. However, it has been suggested that selection for litter size could increase the reproductive sensitivity when environmental conditions limit the availability of resources (Theilgaard et al., 2007). In fact, reproduction is enhanced during spring and early summer under natural conditions, which means that the rabbits reproduce with high grassland quality and comfortable temperatures, but when the conditions are not suitable reproduction declines. Domestic breeds are also predisposed to express their genetic potential under adequate environmental conditions, reducing their reproductive performance when exposed to high temperature or low-energy diets (Fernández-Carmona et al., 1995 and 2003), which could lead to higher culling rates.

However, it may be that different physiological responses are induced, depending on the nature of the environmental constraint. For example, low-energy diets usually lead to a restriction in nutrient supply, whereas high temperatures would mainly bring about a constriction of metabolic rate (and thereby a negative feedback on intake) (Cervera and

Fernández-Carmona, 2010). In addition, the challenge length may also be considered, as during the normal selection process these animals have been faced with short-term challenges (such as the seasonal hot period; Baselga, 2004). There is evidence that when selection to increase a trait is carried out under adequate environmental conditions, the environmental sensitivity could also increase (Falconer, 1990).

The present work aimed to add knowledge on how long-term selection for reproduction could have affected the time-course sensitivity of female rabbits to different environmental challenges. For this task, we compared the reproductive and physiological responses of female rabbits from the same line, but differing by 20 generations of selection to increase litter size at weaning, when subjected to three different environmental conditions (normal, heat and nutritional challenge) during two reproductive cycles.

4.4 Material and methods

The experimental procedures were approved by the Universitat Politècnica de València ethic committee on protection and use of animals for experimentation and other scientific purposes, as set forth by Royal Decree 1201/2005 (BOE, 2005).

4.4.1 Animals

Female rabbits belonging to a line constituted from four specialised maternal lines and then selected to increase litter size at weaning (line **V**; Estany et al., 1989) over 16 or 36 generations, hereinafter referred as **V16** and **V36**, were compared simultaneously. Line V has a population size of around 120 female rabbits and 25 bucks and because the selection programme has no control population, a representative sample of each generation (each male contributing with two straws) has been cryopreserved as embryos, from which generation 16 was reconstituted. The parents of V16 female rabbits used in this study, stored as frozen embryos, were thawed and transferred to female rabbits of another line, also selected for litter size at weaning. After one generation without selection, to avoid the environmental maternal effect, 72 adult V16 female rabbits were obtained to be simultaneously compared with 79 female rabbits of generation 36. Detailed information concerning the cryopreservation and embryo transfer techniques used in this study are available in Vicente et al. (1999) and Besenfelder and Brem (1993), respectively.

4.4.2 Environments

Three environmental conditions were set up, differing in the room temperature and/or the diet provided. The normal environment (**NC**) was achieved by combining housing at normal room temperature (**N:** traditional building equipped only with a cooling system, registering a daily temperature variation from 18°C to 24°C) with a control diet (**C**) similar to commercial diets formulated to cover the requirements of reproductive rabbit does (11.6 MJ digestible energy (**DE**)/kg dry matter (**DM**), 126 g digestible protein (**DP**)/kg DM and 168 g acid detergent fibre (**ADF**)/kg DM). The heat environment (**HC**) was created by the combination of a high temperature room (**H:** climatic chamber set to achieve a daily sinusoidal temperature variation from 25°C to 35°C) with diet C. Detailed information on the design and operating system of climatic chamber can be found in a study by García-Diego et al. (2011). Finally, an environmental restriction because of feed quality (**NF**) was produced by combining N housing with a low-energy fibrous diet (**F:** 9.1 MJ DE/kg DM, 104 g DP/kg DM and 266 g ADF/kg DM), following recommendations for lactating rabbit does (Nicodemus et al., 2010). Ingredients and chemical composition of the experimental diets and the apparent digestible coefficients for each generation and environment are given are available in chapter two (Savietto et al., 2012). As a reference, the calculated DE content of diets in N housing was 11.7 MJ DE/kg DM for diet C and 9.1 MJ DE/kg DM for diet F. In H housing, the DE content of diet C was 12.5 MJ DE/kg DM. These values were used to calculate the DE intake of female rabbits.

4.4.3 Experimental procedures

Young female rabbits were reared following the management scheme proposed by Ragab and Baselga (2011) from birth to 63 days of age, and then transferred to the experimental farm. From 63 days to first parturition, animals were fed with a commercial diet (CP = 15.3 g/kg DM, ether extract = 2.5 g/kg DM, and crude fibre = 23.1 g/kg DM) supplied *ad libitum* and daily exposed to 16 h of light. Female rabbits were first artificially inseminated at 125 days of age, reaching the first parturition with an average live weight (**LW**) of 3583 ± 240 g (mean ± s.d.). At first parturition, female rabbits from both generations (V16 and V36) were randomly assigned to one of the three environments (HC, NC, NF) in a 2 × 3 factorial design. The number of animals differed owing to the availability of young female rabbits from the selection nucleus (V16HC = 31, V16NC = 22, V16NF = 19, V36HC = 29, V36NC = 25 and V36NF = 25). During the experimental period, which lasted until third parturition, female rabbits followed a programmed reproductive interval of 42 days, with insemination at 11 days post-parturition (**dpp**). Non-pregnant female rabbits were re-inseminated 21 days later and so on, up to a maximum of three consecutive failures, whereupon they were culled because of infertility. The total number of kits born (**TB**) and kits born alive (**BA**) was recorded at each parturition. Litter size was standardised at birth to nine kits in the first lactation and 10 in the second. Subsequently, dead kits were not replaced. The number of weaned kits was recorded at 28 dpp.

In both lactations, the female rabbit's LW was measured at 0, 7, 14, 21 and 28 dpp, and perirenal fat thickness (**PFT**) at 0, 14 and 28 dpp, using the ultrasound method described by Pascual et al. (2004). Dry matter intake was monitored weekly during both lactations and during the weaning to parturition intervals. Milk yield was measured four days per week during four weeks. In the first three weeks, female rabbits were weighed before having access to the nest box and just after nursing their kits (i.e. weigh-suckle-weigh method). At week four, the kits were too big to be confined to the nest space. The female rabbits were then placed in new cages, being transferred once per day to nurse their kits. Owing to a limited number of cages in the climatic chamber (HC environment) this practice was not possible; hence, female rabbits and their kits shared a common space, making it impossible to measure milk yield. Thus, no milk yield was available at week four at HC, and the DM intake corresponded to the joint female-litter consumption.

4.4.4 Blood plasma parameters

Blood samples were collected from the central artery of the ear using tubes with EDTA after a minimum fasting period of 3 h at 0, 14 and 28 dpp. Samples were immediately centrifuged (3,000 g during 10 min at 4°C) and plasma was separated and frozen at -40°C until further analysis. Samples from 12 female rabbits per group (two generations × three environments) with complete records were analysed for total T_3, leptin, non-esterified fatty acids (**NEFA**), β-OH-butyrate (**BHB**), glucose and lactate. Total T_3 was analysed using the Beckman Coulter 'Total T3 RIA KIT' (IM1699-IM3287) (Immunotech AS, Prague, Czech Republic), according to the manufacturer's guidelines. The intra-assay coefficient of variance (**CV**) was 7.1% and the inter-assay CV was 7.5%. Leptin was analysed by Multi-species Leptin assays (RIA, XL-85K) (Millipore Corporation, Billerica, MA, USA), following the manufacturer's guidelines. Intra- and inter-assay CVs were 9.1% and 9.3%, respectively. NEFAs were determined using the NEFA C ACS-ACOD assay method (Wako Chemicals GmbH, Neuss, Germany). BHB was determined as an increase in absorbance at 340 nm owing to the production of NADH, at slightly alkaline pH in the presence of BHB dehydrogenase. Sample blanks were included and the method involved oxamic acid in the media to inhibit lactate dehydrogenase, as proposed by Harano et al. (1985). Glucose and lactate were determined according to standard procedures (Siemens Diagnostics® Clinical Methods for ADVIA 1650). Analyses of NEFA, BHB, glucose and lactate were performed using an auto-analyser, ADVIA 1650® Chemistry System (Siemens Medical Solutions, Tarrytown, NY, USA); the intra-assay CV in all instances was below 2.0%, whereas the inter-assay CV was below 4.0%.

4.4.5 Statistical analysis

A mixed model (SAS Institute Inc., Cary, NC, USA), with a repeated measure design, was used to analyse performance, hormonal and metabolic data of rabbit does until third parturition. The model considered the variation between animals and the co-variation within them. The covariance structure was estimated using the spatial power function, after objectively comparing among other covariance structures as suggested by Littell et al. (1998). The spatial power function is a direct generalisation of first-order auto-regressive covariance function for equally time-spaced data, with the advantage of accounting for different lag times between two measurements. The model used to analyse reproductive performance (Table 4.1) included the generation (V16 and V36), environment (NC, HC and NF), parturition (first, second and third) and their interactions. The model used to analyse other performance traits (Table 4.2) and blood plasma parameters (Table 4.3) included the generation, environment, reproductive cycle (two levels: first (all measures between first and second parturition; only second parturition included) and second (all measures between second and third parturition; only third parturition included) and their interactions. This model also included measurement day (different depending on the variable studied; see experimental procedure) and its interactions with generation and environment as fixed effects. Finally, differences in the evolution of DE intake, milk yield and PFT with time within lactation (Figures 4.1 to 4.3) were analysed considering the generation, environment, lactation week and their interactions. All models included the random effect of animal $[\boldsymbol{p} \sim \boldsymbol{N}\,(0, \sigma_{p}^{2})]$. The models for intake and milk yield included the average litter size during lactation as a covariate. Plasma concentrations of total T_3, leptin, NEFA, BHB and lactate did not follow a normal distribution; hence, $\log_{10}$ transformation was applied before analysis. Variables were presented as least square means followed by their standard errors, and different contrasts were computed to test the effect of the environmental challenge and selection for litter size at weaning in each reproductive cycle as follow:

$$\mathrm{HC} - \mathrm{NC} = \frac{(\mathrm{V16HC} + \mathrm{V36HC})}{2} - \frac{(\mathrm{V16NC} + \mathrm{V36NC})}{2} \tag{4.1}$$

$$\mathrm{NF} - \mathrm{NC} = \frac{(\mathrm{V16NF} + \mathrm{V36NF})}{2} - \frac{(\mathrm{V16NC} + \mathrm{V36NC})}{2} \tag{4.2}$$

$$\mathrm{V16} - \mathrm{V36} = \frac{(\mathrm{V16HC} + \mathrm{V16NC} + \mathrm{V16NF})}{3} - \frac{(\mathrm{V36HC} + \mathrm{V36NC} + \mathrm{V36NF})}{3} \tag{4.3}$$

4.5 Results

From the 151 female rabbits with which the experiment was started, 120 reached the third parturition. In HC, 16 female rabbits (eight from each generation) did not finish, with death around parturition being the main failure reason. Under N room temperature, 11 female rabbits receiving diet C (five from V16 and six from V36) and four in diet F (three from V16 and one from V36) did not reach the third parturition.

4.5.1 Performance traits

The average TB and BA per parturition are shown in Table 4.1. At first parturition, just before random allocation of female rabbits to the different environments, higher numbers of TB (+0.85 ± 0.49 kits; P=0.09) and BA (+0.90 ± 0.60; P=0.13) were observed for V36 litters compared with V16. Subsequently, the HC or NF environment led to an average reduction in TB (-2.03 ± 0.49 and -2.01 ± 0.51, respectively; P<0.05) and BA (-2.31 ± 0.60 and -1.48 ± 0.61, respectively; P<0.05), compared with NC. Compared with NC, the HC environment caused a greater litter size reduction in V16 (-2.33 ± 0.85 TB and -2.35 ± 1.02 BA; P<0.05) than in V36 (-0.96 ± 0.91 TB and -1.72 ± 1.09 BA) at second parturition. However, V36 litters were more affected (-4.49 ± 1.13 BA; P<0.05) than V16 (-0.72 ± 1.16 BA) at third parturition. Similarly, the NF environment led to a greater litter size reduction in V16 (-3.39 ± 0.94 TB and -2.16 ± 1.12 BA; P<0.05) than in V36 (-1.03 ± 0.89 TB and -0.77 ± 1.04 BA) at second parturition, and V36 litters were more affected (-2.56 ± 1.10 BA; P<0.05) than V16 -0.29 ± 1.25 BA) at the third. Litter size at weaning is also shown in Table 4.1, and no differences were observed between generation 16 and 36 in the HC, NC and NF environments, with the exception of first weaning where female rabbits of both generations in HC weaned fewer kits with respect to NC and NF.

The effect of environment and generation on average productive traits of female rabbits during first and second lactation is presented in Table 4.2. Female rabbits in HC had lower intake (on average -18.5% of DM and -14.0% of DE; P<0.05) than those in NC, leading to lower milk yield (-9.0 and -20.0% in first and second lactation, respectively; P<0.05) and a lower average LW in second lactation (4.0%; P<0.05). During first lactation, although female rabbits in NF had slightly higher DM intake (+12.0%; P<0.01) than those in NC, this did not allow DE intake compensation (-12.0%; P<0.01), and the average milk yield (-8.0%; P=0.10) and PFT were also lower (-5.0%; P<0.01). However, digestible energy intake compensation in NF did (approximately) occur in second lactation, and although milk yield (-15.0%; P<0.05) and LW (4.0%; P<0.05) were even more affected (compared with NC) than in first lactation, PFT differences (-2.0%; P<0.10) were lower than those observed in the first lactation.

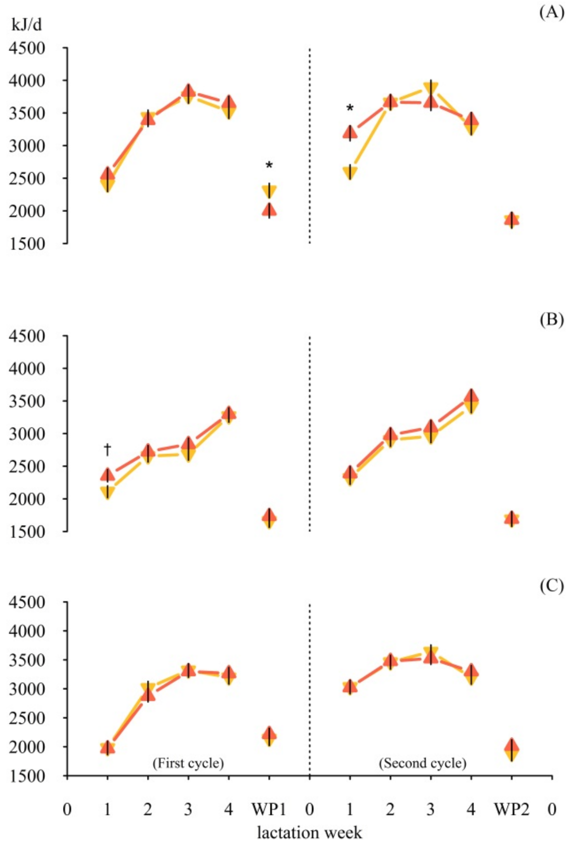
Table 4.1 The effect of environment and generation of selection for litter size at weaning on reproductive performance of females rabbit at first, second and third parturition

Environment[1]	HC		NC		NF		Contrasts[3]		
Generation[2]	16	36	16	36	16	36	HC-NC	NF-NC	16-36
Number of kits total born per partum									
First	9.26	10.66	9.77	10.60	9.16	9.48	-0.23 (0.59)	-0.87 (0.63)	-0.85† (0.49)
Second	9.54^{ab}	10.18^{ac}	11.86^{c}	11.14^{bc}	8.47^{a}	10.12^{ab}	-1.65** (0.63)	-2.21** (0.65)	-0.52 (0.52)
Third	9.95^{a}	9.14^{a}	12.24^{c}	11.74^{bc}	10.19^{ab}	9.96^{a}	-2.44** (0.68)	-1.91** (0.70)	-0.51 (0.56)
Number of kits born alive per partum									
First	8.55	9.62	8.64	9.44	8.05	8.88	-0.05 (0.70)	-0.57** (0.76)	-0.90 (0.59)
Second	7.96^{a}	8.14^{a}	10.32^{b}	9.86^{ab}	8.16^{a}	9.08^{ab}	-2.04** (0.75)	-1.47† (0.78)	-0.21 (0.62)
Third	8.64^{bc}	6.19^{a}	9.35^{bc}	10.68^{c}	9.06^{bc}	8.13^{ab}	-2.61** (0.81)	-1.43† (0.83)	+0.68 (0.66)
Number of kits weaned per lactation									
First	7.45^{a}	7.28^{a}	8.00^{ab}	8.24^{b}	7.74^{ab}	7.56^{ab}	-0.76** (0.26)	-0.47† (0.28)	+0.04 (0.22)
Second	8.35^{a}	8.77^{ab}	9.18^{b}	8.79^{ab}	9.53^{b}	9.28^{b}	-0.43 (0.29)	+0.41 (0.29)	+0.07 (0.23)

[1] Environment: HC: high room temperature (25 to 35°C) and diet C (11.6 MJ DE kg/DM), NC: normal room temperature (18 to 24°C) and diet C, and NF: normal room temperature and diet F (9.1 MJ DE kg/DM). [2] Generations of selection to increase litter size at weaning. [3] Contrasts (standard error) followed by ** and † are significant at P<0.01 and P<0.10, respectively.

$^{a-c}$ Values within a row with different superscripts differ significantly at P<0.05.

Selection to increase litter size at weaning during 20 generations did not significantly affect the average productive traits, but the time course of these traits was different for each generation depending on the environment (Figures 4.1 to 4.3). In the NC environment, V36 female rabbits had lower DE intake during the first weaning to parturition interval (-311.5 ± 155.4 kJ/d; P<0.05) but higher during the first week of the second lactation (+591.7 ± 157.4 kJ/d; P<0.05) than V16 (Figure 4.1). These differences between generations disappeared when they were subjected to the HC and NF environments.



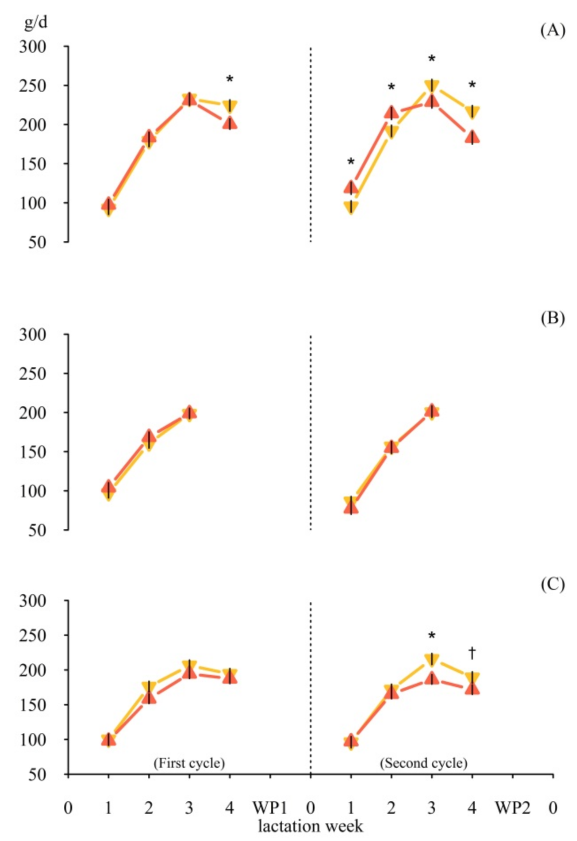


Figure 4.1 Digestible energy intake (kJ/d) of V16 (gold) and V36 (red) female rabbits housed in: (A) normal [normal room temperature (18 to 24°C) and fed with diet C (11.6 MJ DE/kg DM)], (B) heat [high room temperature (25 to 35°C) and diet C] or (C) nutritional [normal room temperature and fed with diet F (9.1 MJ DE/kg DM)] challenging conditions. Vertical bars represent the standard errors of means. WP represents the weaning to partum interval. *P<0.05 and †P<0.10.

Figure 4.2 Milk yield (g/d) of V16 (gold) and V36 (red) female rabbits housed in: (A) normal, (B) heat or (C) nutritional challenging conditions. Vertical bars represent the standard errors of means. WP represents the weaning to partum interval.*P<0.05 and †P<0.10.

When in NC, V36 female rabbits had higher milk yield in the first half of the second lactation (+22.9 g/d; P<0.05) and lower milk yield at the end of both lactations (on average -26.4 g/d in week four; P<0.05), compared with V16 does. This higher initial milk yield of V36 does was not observed in the HC or NF environments, owing to a higher milk yield reduction for V36 (on average -50.5 and -35.1 g/d at HC and NF, respectively) than for V16 (-22.1 and -10.4 g/d) under these constrained conditions. Milk yield of V16 female rabbits was higher than that observed for V36 during the last two weeks of the second lactation in NF (+23.4 g/d; P<0.05).

Table 4.2 The effect of environment and generation of selection for litter size at weaning on average dry matter (DM) and digestible energy (DE) intake, milk yield, live weight, perirenal fat thickness (PFT), and weaning to parturition interval of rabbit does at first and second reproductive cycles. Survival rate of female rabbits is also presented

Environment[1]	HC		NC		NF			Contrasts[3]	
Generation[2]	16	36	16	36	16	36	HC-NC	NF-NC	16-36
Survival rate (%)	74.2^{a}	72.4^{a}	76.0^{ab}	76.0^{ab}	84.2^{ab}	96.0^{b}	-2.7	14.9†	-3.7
First reproductive cycle (from first to second parturition)									
DM intake (g/d)	178.7^{a}	191.6^{a}	243.3^{b}	245.4^{bc}	271.7^{c}	271.7^{c}	-59.2** (8.3)	+27.3** (8.9)	-3.6 (6.9)
DE intake (kJ/d)	2,200^{a}	2,377^{ab}	2,901^{c}	2,808^{c}	2,492^{b}	2,494^{b}	-566** (90)	-361** (96)	-29 (75)
Milk yield (g/d)	151.6	155.6	167.5	169.6	159.8	150.2	-15.0* (7.6)	-13.6† (8.3)	+1.1 (6.4)
Live weight (g)	3,687^{ab}	3,780^{ab}	3,794^{b}	3,734^{ab}	3,644^{a}	3,683^{ab}	-30 (48)	-100† (52)	-24 (41)
PFT (mm)	6.98^{bc}	6.96^{bc}	7.05^{c}	7.05^{c}	6.67^{a}	6.79^{ab}	-0.09 (0.09)	-0.32** (0.09)	-0.03 (0.07)
Weaning to partum interval (d)	32.8^{ab}	34.3^{b}	30.1^{ab}	26.0^{a}	34.8^{b}	30.8^{ab}	+5.4† (2.8)	+4.7† (2.9)	2.2 (2.3)
Second reproductive cycle (from second to third parturition)									
DM intake (g/d)	200.3^{a}	203.8^{a}	253.7^{b}	265.5^{b}	329.9^{c}	324.6^{c}	-57.6** (9.0)	+67.7** (9.2)	-3.4 (7.3)
DE intake (kJ/d)	2,475^{a}	2,538^{a}	3,005^{b}	3,065^{b}	2,992^{b}	2,947^{b}	-529** (98)	-65.6 (100)	-27 (79)
Milk yield (g/d)	146.1^{a}	143.0^{a}	177.3^{bc}	184.7^{c}	159.2^{ab}	147.6^{a}	-36.4** (8.3)	-27.6** (8.5)	+2.4 (6.8)
Live weight (g)	3,750^{a}	3,895^{bc}	3,992^{c}	3,977^{c}	3,803^{ab}	3,861^{abc}	-162** (50)	-152** (53)	-62 (52)
PFT (mm)	7.17^{ab}	7.28^{b}	7.22^{b}	7.17^{ab}	7.11^{ab}	6.95^{a}	+0.03 (0.09)	-0.17† (0.10)	+0.03 (0.08)
Weaning to partum interval (d)	24.4	28.7	22.6	23.1	30.3	28.5	+3.7 (3.0)	+6.5* (3.1)	-1.0 (2.5)

[1]Environment: HC: high room temperature (25 to 35°C) and diet C (11.6 MJ DE kg/DM), NC: normal room temperature (18 to 24°C) and diet C, and NF: normal room temperature and diet F (9.1 MJ DE kg/DM). [2]Generations of selection to increase litter size at weaning. [3]Contrasts (standard error) followed by **, *, and † are significant at P<0.01, P<0.05, and P<0.10, respectively. $^{a-c}$ Values within a row with different superscripts differ significantly at P<0.05.

In general, the evolution of PFT during lactation had an accretion phase until 14 dpp and a mobilisation phase from this point to weaning (Figure 4.3). In NC, both V16 and V36 female rabbits had increased PFT (on average +0.533 ± 0.139 mm; $P<0.05$) in the first part of lactation one. In the second part of lactation one, V16 mobilised PFT (-0.348 ± 0.160 mm; $P<0.05$) but V36 did not (0.032 ± 0.152 mm), resulting in similar PFT between V16 and V36 female rabbits at weaning. No generational differences were observed in second lactation. However, whereas V36 significantly reduced their PFT (-0.444 ± 0.164 mm; $P<0.05$) from second weaning to third parturition, V16 did not (-0.117 ± 0.166 mm): a result not influenced by the weaning to parturition interval (V16NC = 22.6 d and V36NC = 23.1 d). During first lactation on HC, V36 female rabbits showed a cumulative PFT accretion of +0.584 ± 0.158 mm in late lactation, which resulted in higher PFT values at weaning (+0.363 ± 0.166 mm) compared with V16. Subsequently, V16 and V36 female rabbits had respectively low (-0.167 ± 0.133; $P=0.21$) and high (-0.596 ± 0.148 mm; $P<0.05$) PFT mobilisations in the first weaning to parturition interval (V16HC = 32.8 d and V36HC = 34.3 d). When housed in NF, V16 mobilised in late lactation two (-0.742 ± 0.172 mm; $P<0.05$) the PFT accreted during early lactation (+0.763 ± 0.152 mm; $P<0.05$), but no significant mobilisation was observed for V36 female rabbits in this period (-0.200 ± 0.150 mm).

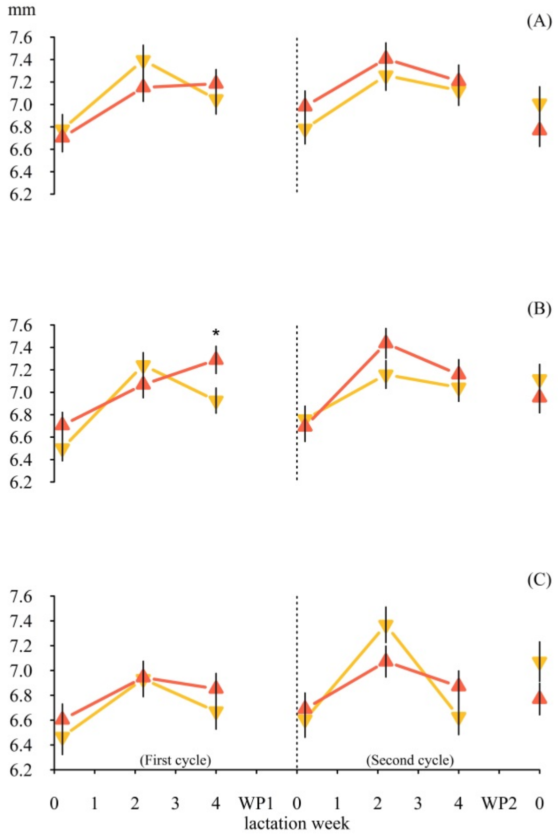


Figure 4.3 Perirenal fat thickness (mm) of V16 (gold) and V36 (red) female rabbits housed in: (A) normal, (B) heat, or (C) nutritional challenging conditions. Vertical bars represent the standard errors of means. WP represents the weaning to partum interval. $^*P<0.05$.

4.5.2 Blood plasma parameters

At first parturition, before random allocation of female rabbits to treatments, plasma concentrations of leptin (1.74 ng/ml), NEFA (506.4 µekv/l), BHB (0.268 mM), glucose (6.76 mM) and lactate (4.70 mM) were similar in both generations, whereas total T_3 was slightly lower for V16 than V36 (1.67 and 1.97 nM, respectively; $P<0.10$). Blood plasma parameters in the first and second reproductive cycle are in Table 4.3. In NC, V16 had a higher plasma concentration of glucose (+11.0%; $P<0.05$) during the first reproductive cycle and higher BHB (+13.0%; $P<0.05$) during the second cycle than V36. However, when housed in NF, V36 had a higher total T_3 level (+42.0%; $P<0.05$) during the first reproductive cycle than V16. During the first reproductive cycle, and always compared with NC, V16 female rabbits showed significantly lower total T_3 (-33.0%) and glucose levels (-9.0%) in HC, and lower total T_3 (-36.0%), leptin (-76.0%), NEFA (-4.0%) and higher BHB (+14.0%) levels in NF. During the second reproductive cycle, V16 female rabbits in NF also had significantly lower leptin (-61.0%) and NEFA (-3.0%) levels, but higher BHB (+16.0%) and lactate (+22.0%), compared with NC. Housing V36 female rabbits in HC resulted in significantly higher NEFA (+4.0%) and BHB (+18.0%) levels during the second reproductive cycle compared with NC, whereas V36 in NF had a reduced lactate (-17.0%) in the first cycle and higher BHB (+27.0%) level in both reproductive cycles.

4.6 Discussion

The main aim of this study was to investigate the effect of long-term selection to increase litter size at weaning (i.e. a reproductive trait) on the female rabbit's capacity to adapt to environmental challenges. The generational increment owing to selection for litter size at weaning in both TB and BA reported at first parturition was +0.045 kits. Despite the differences in the litter size at birth between the present study and that reported by García and Baselga (2002) for the same line (+0.10 kits TB and 0.095 kits BA), both studies reflected the different genetic potentials of V16 and V36 female rabbits.

The environmental challenges were intentionally chosen to affect reproductive performance using different physiological constraints. The high temperature environment was intended to create a constrained environment by limiting the heat loss capacity, whereas the use of a low energy diet limited the energy intake. As a result, overall DE intake reductions of 418 kJ/d and 210 kJ/d were respectively caused by HC and NF environments, compared with NC. Therefore, the planned environmental constraints were sufficient to reduce reproductive performance of rabbit does (Table 4.1) without noticeably impairing the health status of female rabbits (Table 4.2).

In general, the results suggest that selection to increase litter size at weaning had changed the way in which the female rabbits interact with environment. To aid interpretation, it should be noted that litter traits at first parturition represent differences purely because of genetic selection, as animals were allocated to different environments just after the first parturition, whereas differences in the second and third parturition reflect the effect of genotype-environment interaction. Observed performance differences after the first parturition were the result of differences in adaptative capacity of V16 and V36 female rabbits to the different environmental constraints, with results in the first lactation indicating the effect of the constraint being applied in lactation only, whereas during the second reproductive cycle the effects were the result of the constraint being applied both in that lactation and the preceding gestation. As no differences between V16 and V36 within environments were found in the percentage of female rabbits completing the first and second cycle nor in the LW at the end of first lactation, results concerning the second lactation and the third parturition can be interpreted as the response of selection to a long-term environmental constraint.

4.6.1 Effect of selection for reproduction (NC)

Twenty generations of selection for litter size at weaning changed the way in which female rabbits manage the available resources. Selection to increase litter size at weaning was achieved by improving the BA, without impairing kit survival during lactation. The higher DE intake and milk yield observed for V36 in the first part of second lactation, a pattern also described by Quevedo et al. (2006) in adult crossbred female rabbits from more advanced generations of selection (for increased litter size at weaning) supports the hypothesis that higher litter size at weaning was also achieved by a reduction in post-natal kit mortality. In this context, Coureaud et al. (2007) observed no mortality during the first week in kits that ingested at least 7 g of milk on the first 24 h. However, there were important differences in lactational performance between the generations. Whereas V16 female rabbits had higher milk yield in late lactation and used their PFT accumulated during the early lactation, it was observed that V36 female rabbits reduced milk yield, without reducing DE intake, to maintain their PFT reserves. Consequently, V36 female rabbits had a lower DE intake after first weaning, although they had a greater reproductive performance at second parturition. The greater milk yield of V36 than V16 female rabbits in early lactation may be a response to a change in the relative priorities between the current and future litter, as proposed by Friggens (2003). Therefore, selection for litter size at weaning induced a change in resource allocation (i.e. reduction of milk yield, preserving PFT with a higher DE intake in late lactation) to properly balance resources between current and future litters, and thereby enhancing the female rabbit's fitness.

Table 4.3 The effect of environment and generation of selection for litter size at weaning on plasma concentrations of total T$_3$, leptin, non-esterified fatty acids (NEFA), β-OH-butyrate (BHB), lactate and glucose of rabbit does during first and second reproductive cycle

Environment[1]	HC		NC		NF		Contrasts[3]		
Generation[2]	16	36	16	36	16	36	HC-NC	NF-NC	16-36
First reproductive cycle (from first to second partum)									
Total T$_3$ (log$_{10}$ nM)	0.201^{ab}	0.233^{ac}	0.298^{c}	0.262^{ac}	0.191^{a}	0.271^{bc}	-0.063* (0.027)	-0.049† (0.027)	-0.025 (0.022)
Leptin (log$_{10}$ ng/ml)	0.269^{bc}	0.249^{bc}	0.290^{c}	0.226^{bc}	0.071^{a}	0.156^{ab}	-0.001 (0.042)	-0.145** (0.044)	-0.001 (0.035)
NEFA (log$_{10}$ µekv/l)	2.56^{ab}	2.52^{a}	2.60^{b}	2.57^{ab}	2.49^{a}	2.57^{ab}	-0.04 (0.03)	-0.05† (0.03)	-0.01 (0.02)
BHB[4] (log$_{10}$ mM)	1.84^{a}	1.90^{a}	2.00^{a}	1.81^{a}	2.29^{b}	2.30^{b}	-0.04 (0.07)	+0.39** (0.07)	+0.04 (0.06)
Lactate (log$_{10}$ mM)	0.538^{ab}	0.557^{ab}	0.582^{ab}	0.614^{b}	0.518^{ab}	0.508^{a}	-0.051 (0.036)	-0.085** (0.036)	-0.014 (0.029)
Glucose (mM)	5.88^{a}	5.85^{a}	6.49^{c}	5.87^{a}	6.28^{bc}	6.08^{ab}	-0.315* (0.135)	+0.002 (0.134)	+0.276** (0.110)
Second reproductive cycle (from second to third partum)									
Total T$_3$ (log$_{10}$ nM)	0.239	0.253	0.263	0.272	0.239	0.252	-0.022 (0.027)	-0.023 (0.027)	-0.012 (0.022)
Leptin (log$_{10}$ ng/ml)	0.249^{ac}	0.258^{bc}	0.333^{c}	0.234^{ac}	0.130^{a}	0.194^{ab}	-0.030 (0.043)	-0.122** (0.44)	+0.009 (0.036)
NEFA (log$_{10}$ µekv/l)	2.61^{c}	2.62^{c}	2.57^{bc}	2.53^{ab}	2.49^{a}	2.52^{ab}	+0.07* (0.03)	-0.046 (0.03)	+0.01 (0.02)
BHB[4] (log$_{10}$ mM)	1.87^{b}	1.94^{bc}	1.86^{b}	1.64^{a}	2.16^{d}	2.09^{cd}	+0.15* (0.07)	+0.37** (0.08)	+0.07 (0.06)
Lactate (log$_{10}$ mM)	0.470^{ab}	0.527^{ab}	0.559^{b}	0.562^{b}	0.434^{a}	0.532^{ab}	-0.062† (0.036)	-0.078* (0.036)	-0.052† (0.029)
Glucose (mM)	5.72	5.96	5.85	5.77	5.97	5.86	+0.032 (0.135)	+0.109 (0.137)	-0.018 (0.111)

[1]Environment: HC: high room temperature (25 to 35°C) and diet C (11.6 MJ DE kg/DM), NC: normal room temperature (18 to 24°C) and diet C, and NF: normal room temperature and diet F (9.1 MJ DE kg/DM). [2]Generations of selection to increase litter size at weaning. [3]Contrasts (standard error) followed by **, *, and † are significant at P<0.01, P<0.05, and P<0.10, respectively. [4]To back transform data of BHB apply ((10^{x} −5)/1000), where X is the tabulated BHB value. $^{a-d}$ Values within a row with different superscripts differ significantly at P<0.05.

4.6.2 Response to heat stress restriction (HC)

The drop in feed intake at high temperatures (i.e. sows: Quiniou et al., 2000; ruminants: Morand-Fehr and Doreau, 2001; rabbits: Fernández-Carmona et al., 2003) is a well-known physiological adaptation to prevent an increment in body core temperature above the normal state, and can be considered a metabolic restriction. Female rabbits of generation 16 had a quicker response to heat challenge than V36. They reduced both the DE intake and the plasma concentrations of glucose and total T_3 (both positively correlated with energy intake; Dauncey, 1990; Rommers et al., 2004; Brecchia et al., 2006), in the first reproductive cycle, with respect to NC female rabbits. In contrast, in the first reproductive cycle, V36 female rabbits did not reduce glucose and total T_3 concentrations, and maintained a higher PFT at weaning. This different adaptation between V16 and V36 female rabbits to the HC environment could be associated with their different reproductive performances at second parturition, where V16 female rabbits had higher reduction in TB and BA than V36 rabbits. These results suggest that female rabbits coming from more recent generations of selection for reproduction conserved, in the short term, their reproductive effort after being exposed to high temperatures. The negative effect of HC environment on V16 female rabbits in the second lactation was similar to that observed in the previous one. However, symptoms of exhaustion from prolonged heat exposure appeared in the V36 female rabbits; the reduction in DE intake and milk yield in early lactation and the high levels of plasma NEFA and BHB of V36 female rabbits owing to HC were greater in the second reproductive cycle. The low plasma NEFAs (positively correlated to mobilisation of body reserves Brecchia et al., 2006) reflected the absence of body reserve accretion – fuel for reproduction – by V36 female rabbits from second insemination to third parturition. This clearly affected their reproductive performance at third parturition.

Moreover, the high number of stillborn observed for V36 litters at third parity suggests a possible relationship between PFT mobilisation rate in late pregnancy and prenatal survival. Indeed, Martínez-Paredes et al. (2012) described an increment in the number of stillborn at birth (+44.8%) when primiparous female rabbits showed a high PFT mobilisation during late pregnancy. When short-term environmental constraint occurs, V36 female rabbits maintained an adequate balance of resources between the current and the future litters to ensure their higher fitness. However, this strategy could not be maintained when the environmental constraint was prolonged in the long term.

4.6.3 Response to dietary energy restriction (NF)

Similar apparent digestibility of gross energy with diets C and F for V16 and V36 female rabbits was reported in chapter two (Savietto et al., 2012). Therefore, the planned DE intake restriction was mainly because of feed quality and its effects on intake capacity. In NF, both V16 and V36 does reduced DE intake (-186.7 ± 67.4 and -222.0 ± 63.2 kJ/d, respectively) compared with NC. Similarly, Quevedo et al. (2006) observed a greater reduction in DE intake (-319.7 kJ/d) when dietary energy was reduced in 1.8 MJ DE/kg DM. Moreover, from both studies it can be deduced that the greater ability of these female rabbits from advanced generations to obtain additional resources is limited by feed quality.

As energy acquisition in NF was similar for both generations, no differences in the evolution of milk yield, LW and PFT were expected. During the first reproductive cycle, this expectation was confirmed. However, in the second lactation, V16 female rabbits showed greater PFT accretion in early (+0.76 ± 0.15 mm) and mobilisation in late lactation (-0.74 ± 0.17 mm). They also maintained a higher milk yield in late lactation. In contrast, V36 does reduced milk yield, safeguarding PFT, a pattern also observed during the first reproductive cycle, which is compatible with an increased priority for the future litter, relative to V16. In fact, although high plasma BHB levels were found in both generations on NF, indicating PFT mobilisation in late lactation, leptin levels – an indicator of long-term body condition and fitness levels – were only significantly reduced in V16 animals. The physiological response of V16 to a poor quality feed was also reflected in a litter size reduction at second parturition. Brecchia et al. (2006) reported a similar reduction (-2.1 kits born alive; P=0.27) in female rabbits with lower total T_3, leptin and NEFA plasma concentrations. In contrast, in the same period, V36 female rabbits appeared to sustain their reproductive level.

However, prolonged exposition to the NF environment limited the expression of the genetic potential of V36 female rabbits at third parturition, whereas the lower potential of V16 female rabbits was still expressed in this poorer environment. As hypothesised, when the environmental conditions deviate from normal, female rabbits from advanced generations of selection for increased litter size at weaning prioritised the selected trait. However, when the constrained environmental conditions did not improve, their genetic potential was limited (as observed in NF) or even reduced (HC). This response could be related to the environmental conditions in which the V line has been selected (i.e. where occasional and seasonal fluctuations in the environmental conditions normally occur). In fact, the initial studies with this line (Estany et al., 1989) also noted the importance of environmental fluctuations on the selection results.

4.7 Conclusions

Selection to increase litter size at weaning during 20 generations has changed female rabbits' capacity to obtain and partition available resources to promote litter size at birth and milk yield in early lactation to ensure the selected trait. In addition, the more selected female rabbits showed higher reproductive robustness at the onset of an environmental constraint, prioritising body condition (HC) or reducing milk yield (NF) to ensure litter size at birth, which could not be sustained in the long term. This response could be related to the environment in which these female rabbits have been selected, reflecting the importance of the selection environment on female robustness.

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5

Reproductive robustness differs between generalist and specialist maternal rabbit lines: the role of acquisition and allocation of resources

Savietto D.*, Friggens N.C.†‡ and Pascual J.J.*

**Institute for Science and Animal Technology (ICTA). Universitat Politècnica de València.*
Camino de Vera s/n. 46022, Valencia, Spain.

†UMR0791 Modélisation Systémique Appliquée aux Ruminants (MoSAR). Institute National de la Recherche Agronomique (INRA). 16 Rue Claude Bernard. 75231, Paris, France.

‡UMR0791 Modélisation Systémique Appliquée aux Ruminants (MoSAR). AgroParisTech.
16 Rue Claude Bernard. 75231, Paris, France.

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5.1 Abstract

Farm animals are normally selected under high controlled non-limiting conditions to favour the expression of their genetic potential. Selection strategies also focus in a single trait, favouring the most ‘specialized’ animals. Theoretically, if the environment provides enough resources, the selection strategy should not give raise to changes in the interplay between life function, such as reproduction and survival. However, highly ‘specialized’ farm animals may be required to live in conditions alien to the selection condition. The consequence is a degraded ability of ‘specialized’ animals to sustain reproduction, production, and health, resulting in a reduced lifespan. This study was designed to address this issue using maternal rabbit lines. A line highly specialized with respect to reproductive intensity (called **V**), and a generalist line founded from females having a long reproductive life (called **LP**) were used to study the strategies of acquisition and use the available resources adopted by these lines when housed on different environments. In addition, two generations of the specialized line, generations 16 and 36 were simultaneously available. This allowed better understanding on how selection criteria in a specific environment changed the interplay between functions related to reproduction and survival. We evidenced that under constrained conditions, line LP had a greater acquisition capacity than line V, avoiding the risks related to making and intensive use of body reserves. Twenty generations of selection for litter size at weaning, however, were not accompanied by an increment in the acquisition capacity. The two generations of ‘specialized’ line differed in the partitioning of resources.

Key words: female rabbits, selection strategies, litter size, longevity, environment, robustness, trade-off.

5.2 Introduction

In farmed livestock, robustness (as defined by Knap, 2005) represents the ability of a genotype to maintain a good production level whilst continuing to maintain all its other life functions in a wide variety of environmental conditions (i.e. food quality, temperature, pathogen load, management, etc.). From this definition robust animals may be considered as 'generalists'. However, the intensive selection of farm animals to increase productive traits has resulted in specialized breeds and strains (broilers: Havenstein et al., 2003a; Havenstein et al., 2003b; dairy cows: Hansen, 2000; pigs: Merks, 2000; hens: Poggenpoel et al., 1996; rabbits: Estany et al., 1992; García and Baselga, 2002).

North-American Holstein-Friesian dairy cows, an example of a high specialized breed for milk yield, prioritize milk production (Horan et al., 2005) at the expense of fertility (Lucy, 2001). Other examples of undesired effects in response to selection have been described for different species (pigs: Knap and Rauw, 2009; Dourmad et al., 2010; poultry: Siegel et al., 2010; Felver-Gant, 2012; rabbits: Vicente et al., 2012; Lavara et al., 2012), leading to the general perception that selection is degrading the ability of animals to simultaneously sustain production, reproduction and health (Rauw et al., 1998; Pascual et al., 2013). Nevertheless, artificial selection of high producing animals does not necessarily entail the emergence of negative effects (dairy cows: Begley et al., 2009; rabbits: Theilgaard et al., 2007; Sánchez et al., 2008), and breeds and strains capable of sustaining production, fertility and health over different environments (i.e. 'generalist') can be obtained. This is of special interest because it indicates that it is possible to select animals that are able to balance production, reproduction and health. However, there is currently insufficient information on the consequences of selecting 'generalist' farm animals with respect to their performances across environments, especially constrained ones.

Theoretically, if animals are selected in non-limiting conditions, responses to selection can be attained without the need to produce changes in the interplay between life functions, but under limited condition this interplay is re-defined (van Noordwijk and De Jong, 1986; Reznick et al., 2000). However, this does not a priori provides insights into the consequences of selecting high specialized animals in relatively good environments on ability to cope with poor environments. The present study was designed to address this issue using maternal rabbit lines: a line highly specialized with respect to reproduction intensity (i.e. litter size at weaning), and a more generalist line founded for reproductive longevity. In addition, two generations of the specialized line, generations 16 and 36 were simultaneously available. These lines have previously been shown to differ in their ability to maintain litter size in the face of constraints (Theilgaard et al., 2007; Theilgaard et al., 2009), and also in their strategies used to attain the selection criteria [e.g. usage of body reserves (Chapter 3: Savietto et al., 2013), and shape of lactation curve (Chapter 4: Savietto et al., 2014)].

To evaluate the acquisition capacity and the resource allocation strategies resulting from the different selection background of these different lines, three environmental conditions were set up. The aim of this study was to describe the ability of these maternal rabbit lines to acquire (feed intake) and allocate (litter size, milk production and body condition) the resources available in markedly different environments.

5.3 Material and methods

5.3.1 Animals and their history of selection

The selection history concerns the two maternal rabbit lines used in the present study: line V (specialist maternal rabbit line) and line LP (generalist maternal rabbit line).

Line V, was established at Universitat Politècnica de Valencia in 1981 by crossing the progeny of four specialized maternal rabbit lines. After three generations of random mating, avoiding mating close relatives, selection to increase the number of kits weaned per litter started (Estany et al., 1989). Over generations, the effective population size has been kept to 120 females and 25 males. A high number of males have been used to keep inbreeding at low levels. In each generation, at least one male offspring per sire was kept, and mating of relatives sharing a grand-parent avoided. Selection has been also conducted in non-overlapping generations of nine months. In each generation, young females were weaned at 28 days old, and those reaching the age of 4.5 months matted. After parturition, mating was attempted on day 11, in an attempt to reach a reproductive cycle of 42 days, and only after three consecutive failures, females were culled due to infertility. To preserve the genetic material, the Universitat Politècnica de València rabbit selection programme has a cryopreserved control population. Every two or three generations of selection, embryos from a representative sample of the best matings (each sire contributing with two or more straws of embryos) are recovered and vitrified. Line V recently reached the generation 36.

Since selection began, no substantial environmental improvement in the selection of line V occurred (Baselga personal communication). Animals are housed in flat deck indoor cages, with free access to water and commercial pelleted diets [minimum of 15 g of crude protein per kg of dry matter (**DM**), 15 g of crude fibre per kg of DM, and 10.2 MJ of digestible energy (**DE**) per kg of DM]. The photoperiod is set to provide 16 h of light and 8 h of dark, and the room temperature regulated to keep temperatures between 10°C and 28°C. Rooms are cleaned and disinfected every week and the animals are vaccinated against rabbit haemorrhagic diseases and myxomatosis. Sick animals are also culled (e.g.

due to respiratory disorders, pasteurellosis, sore hocks, etc.). No animals are culled for low productivity.

Line LP, was established between 2002 and 2003 by applying a very high selection intensity (i.e. 2 to 5 females per thousand were selected) to obtain females having a long productive lifespan (i.e. at least 25 parturitions averaging a minimum of 7.5 kits born alive per parturition). To identify the long-living and productive females, three screening steps were realized in commercial rabbit farms all over the Iberian Peninsula. In the first screening 15 females were identified and transferred to the university facilities. They were inseminated with semen from males of generation 27 of line V (the current generation in 2002). Fifteen ½ LP and ½ V males were obtained from 12 females. These males were used to inseminate a new set of 15 females selected in a second screening, generating a total 17 ¾ LP and ¼ V males. These males where then used to inseminate a final group of 32 females from a final screening. A total 32 males and 42 females (⅞ LP and ⅛ V) were obtained from 30 females, constituting the generation zero of line LP. From this moment, line LP was selected to increase the litter size at weaning (now on generation six) under similar conditions of those of line V. The direct consequences of the multi-trait criteria used to select the founders of line LP, regardless of the environmental conditions, resulted in animals having a long productive lifespan (35 days more than line V; Sánchez et al., 2008), a constant reproductive effort through life (the maximum reproductive performance of line V is reached at parity four; Theilgaard et al., 2007), and a better innate immune response in constrained conditions (Ferrian et al., 2012 and Ferrian et al., 2013).

To evaluate the specialization process derived from a long-term selection, solely on a reproductive criterion, the generations 16 and 36 of line V were used (hereinafter referred as **V16** and **V36**). The parents of V16 females used in this study, stored as vitrified embryos, where thawed and transferred to females of another line, also selected for litter size at weaning (line A; Estany et al., 1989; Baselga, 2002). After one generation without selection, to avoid the environmental maternal effect, 72 young V16 females were finally obtained to be simultaneously compared with 79 females of generation 36. Detailed information concerning the cryopreservation and embryo transfer techniques used in the present study are available in Vicente et al. (1999) and Besenfelder and Brem (1993), respectively.

5.3.2 Environmental conditions

To evaluate the acquisition capacity and the resources allocation strategy derived from the foundational criteria and history of selection, three environmental conditions were set up. Environments differed in room temperature and/or diet quality. The control environment (**NC**) was the combination of normal (**N**) room temperatures (daily variation from 18°C to 24°C) and a control (**C**) diet [formulated to achieve 11.6 MJ of DE per kg of

DM, 126 g of digestible protein per kg of DM, and 169 g of acid detergent fibre per kg of DM]. Heat environment (**HC**) was obtained combining high (**H**) room temperatures (climatic chamber designed to perform a daily sinusoidal temperature curve from 25°C to 35°C; detailed specifications available in García-Diego et al., 2011) and diet C. Finally, the nutritionally constrained environment (**NF**) was achieved combining the normal room temperature with a low-energy fibrous (**F**) diet (formulated to achieve 9.1 MJ of DE per kg of DM, 104 g of digestible protein per kg of DM, and 266 g of acid detergent fibre per kg of DM). Detailed composition of diets is available in Chapter 2 (Savietto et al., 2012).

Housing facilities (cages, feeders, drinkers, nest box, etc. and their display) were identical in all environments. The photoperiod (16 h of light and 8 h of dark), and the reproductive management was also identical.

5.3.3 Experimental procedures

At first parturition LP, V16 and V36 females were random allocated to one of the three environments (NC, HC, or NF) in a 3 × 3 factorial design. From the 236 females housed (LPNC = 26, LPHC = 31, LPNF = 28, V16NC = 22, V16HC = 31, V16NF =19, V36NC = 25, V36HC = 29, and V36NF = 25) a total of 191 reached the third parturition (LPNC = 21, LPHC = 26, LPNF = 24, V16NC = 17, V16HC = 23, V16NF = 16, V36NC = 19, V36HC = 21, and V36NF = 24). The initial number of animals housed in NC, HC, and NF differed according to the availability of animals in the selection nucleus.

Females were monitored until the day of third parturition, being subjected to a semi-intensive reproductive rhythm of 42 days. They were inseminated on day 11 post-parturition and their litters weaned on day 28. Females not conceiving on day 11 were re-inseminated 21 days later, and so on, until a maximum of three attempts, after which non-pregnant animals were culled for low fertility.

Litter size (number of kits born in total and born alive: n) and litter weight (g of kit born in total and born alive) was controlled at birth. Litters were them standardized to nine kits in first and to 10 kits in second lactation. Subsequently, dead kits were not replaced, and both litter size and weight were controlled at weaning. Milk yield (g/d) was measured four days a week during the first-three weeks of lactation by weighing females before and after nursing their kits. Dry matter intake was measured weekly in the first three-weeks of lactation, and during the weaning to parturition interval (variable depending on the females' real reproductive rhythm). Females' digestible energy intake (MJ/d) was calculated from the dry matter intake and the apparent digestible coefficients of gross energy obtained in a digestibility trail for LP, V16, and V36 females in NC, HC, or NF environment (values available in Chapter 2; Savietto et al., 2012).

Female body condition was assessed by measuring live weight (at 0, 7, 14, 21 and 28 days post-parturition) and perirenal fat thickness (at 0, 14 and 28 days post-parturition). Perirenal fat thickness (mm) was measured by ultrasonography, following the technique described by Pascual et al. (2004). Energy retained in the litter (stillborn and liveborn kits), in the milk and in the female's body (i.e. as perirenal fat depots) was calculated assuming: 3.4 MJ per kg of kits produced (Fortun-Lamothe and Lebas, 1996), 8.5 MJ per kg of milk produced (Xiccato and Trocino, 2010), and 37.6 MJ per kg of perirenal fat tissue (g of perirenal fat tissue were calculated from the equation developed by Pascual et al., 2004).

5.3.4 Data management and statistical analyses

To avoid influences related to non-controlled factors that may affect the acquisition and/or the allocation of resources of the different genetic types, data of females not reaching the third parturition was discarded. From the 236 females housed 45 did not reaches the third parturition. Culling reasons were low fertility (LPHC = 2, LPNF = 1, V16NC = 1, V16NF = 1 and V36NC = 1), retained foetus (LPNC = 1 and V36NC = 1) or diseases (pasteurellosis: LPNF = 2, V16HC = 2, V16NC = 2, and V16NF = 2, or colibacillosis: V16HC = 1), in total 17 females. Another 28 females were found death (LPHC = 3, LPNC = 3, LPNF = 2, V16HC = 5, V16NC = 2, V36HC = 8, V36NC = 4, and V36NF = 1).

Prolificacy of LP, V16 and V36 females was assessed, in each environment, as the cumulative number of kits produced during the second and third parturition. As litters were standardized at birth the cumulative number and weight of kits weaned represented, to some extent, female's maternal ability. The acquisition capacity was measured as the total intake of dry matter and digestible energy during the experimental period (i.e. first three-weeks of each lactation first plus the real weaning to parturition intervals of each female). Female live weight and perirenal fat thickness were represented as the average values measured in the first-two reproductive cycle (i.e. between first and second parturition and between second and third parturition).

Statistical analyses were performed using the general linear model function of R software (R Core Team, 2012) and the least square means were computed using the lsmeans package (Lenth, 2013). The model used to analyse the number of kits born in total, the number kits born alive and the number and individual weight of kits weaned included the environment (**E**: HC, NC and NF), the genetic type (**G**: LP, V16 and V36) and their interaction:

$$Y_{ij} = E_i+G_j + (E_i \times G_j)+e_{ij} \qquad (5.1)$$

The model used to analyse the individual kit weight (born alive and born in total) also included the number of kits born in total (**KT**$_k$) as a covariate:

$$Y_{ijk} = E_i+G_j + (E_i \times G_j)+KT_k + e_{ijk} \quad (5.2)$$

The model used to analyse the intakes of dry matter and digestible energy, the live weight, and PFT included the parturition order (**PO**$_k$: first or second) as a fixed effect:

$$Y_{ijk} = E_i+G_j + (E_i \times G_j)+PO_k+e_{ijk} \quad (5.3)$$

Finally, the model used to analyse milk yield incorporate the average number of kits during lactation (**KL**$_k$) as a covariate:

$$Y_{ijk} = E_i+G_j + (E_i \times G_j)+ KL_k + e_{ijk} \quad (5.4)$$

To evaluate the overall environmental sensitivity depending on foundational criteria or history of selection, data was presented as reaction norms. Data concerning the percentage of energy retained into milk, litter (stillborn and born alive kits) and perirenal fat depots was presented in a radial plot, allowing the comparisons of the different allocation strategies used by the different genetic types in the face of different environmental constrains.

5.4 Results

5.4.1 Resource acquisition capacity

Average daily DM and DE intakes of LP, V16 and V36 females in normal (NC), heat (HC), and nutritional (NF) challenging environments are shown in Figure 5.1A and 5.1B, respectively. As expected, the NF environment was associated with an increased DM intake but a limited intake of DE, whilst the HC environment depressed both DM and DE intakes. The performance results revealed different responses of LP, V16, and V36 females to constrained conditions. Females from line LP showed a greater acquisition capacity (as evidenced by energy intake; $P<0.05$) than both V16 and V36 females in HC and NF. Intake of DM did not significantly differ between LP, V16 and V36 females in NC. However, reduction of DM intake due to HC housing was significantly lower in LP females, which were able to ingest on average 0.27 MJ of DE/day (d) more than V females ($P<0.05$). In the NF environment, LP females had a higher acquisition capacity of DM than V16 and V36 females (on average +33.9 g DM/d; $P<0.05$), which allowed LP females to show a similar DE to that observed in NC. The energetic deficit due to exposure to the NF environment was higher for V16 (-0.28 MJ DE/d compared to V16 in NC; $P<0.05$) than for V36 females (-0.19 MJ DE/d compared to V36 females in NC; $P=0.17$).

5.4.2 Resource allocation strategies

At first parturition, LP, V16, and V36 females had 9.3, 9.5, and 10.3 kits born in total, from which 8.6, 8.4, and 9.3 kits were born alive, respectively. There were no statistically significant differences between lines (pooled standard error of means was 0.57 for kits born in total and 0.68 for kits born alive).

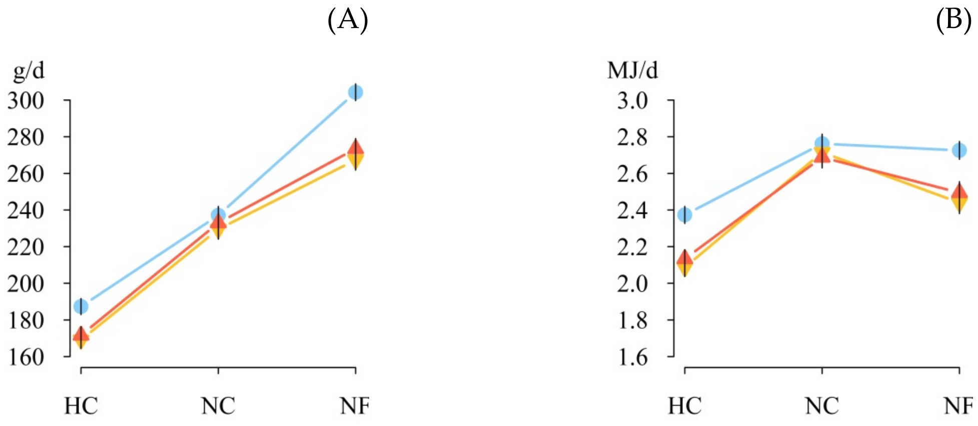

Figure 5.1 Resources acquisition capacity of LP, V16, and V36 females housed in different challenging conditions. Average daily dry matter (g/d) and digestible energy (MJ/d) intakes from first to third parturition are respectively shown in panels A and B. Blue: LP, Gold: V16, and Red: V36. Heat (HC), normal (NC) and nutritional (NF) challenging conditions. Vertical lines represent the standard error of mean.

Constrained conditions did not affect the reproductive performance of LP females (Figure 5.2 A and 5.2 B), which had a similar litter size at birth in the normal and constrained environments (e.g. 10.4, 9.9, 9.9 kits born in total per parturition at NC, HC, and NF). However, harsh environmental conditions constrained reproductive performance of V females, with different response for generations 16 and 36. In constrained conditions, V16 females showed a great reduction in the number of kits born in total (-2.6 and -3.2 kits per parturition in HC and NF respectively; P<0.01) compared to NC, although the reduction in the number of live born kits was less pronounced (-1.6 and -1.7 kits per parturition in HC and NF, respectively; P=0.67). In contrast, females of generation 36 sustained more the number of kits born in total (-2.0 and -1.4 kits per parturition in HC and NF, respectively; P=0.35) than the kits born alive per parturition in HC (-3.4 kits; P=0.003) and NF (-2.1 kits; P=0.24). After litter standardization at birth, no differences in the number of kits weaned was observed as a function of the genetic type or environmental constraint (on average 9.5 kits per parturition; Figure 5.2 C).

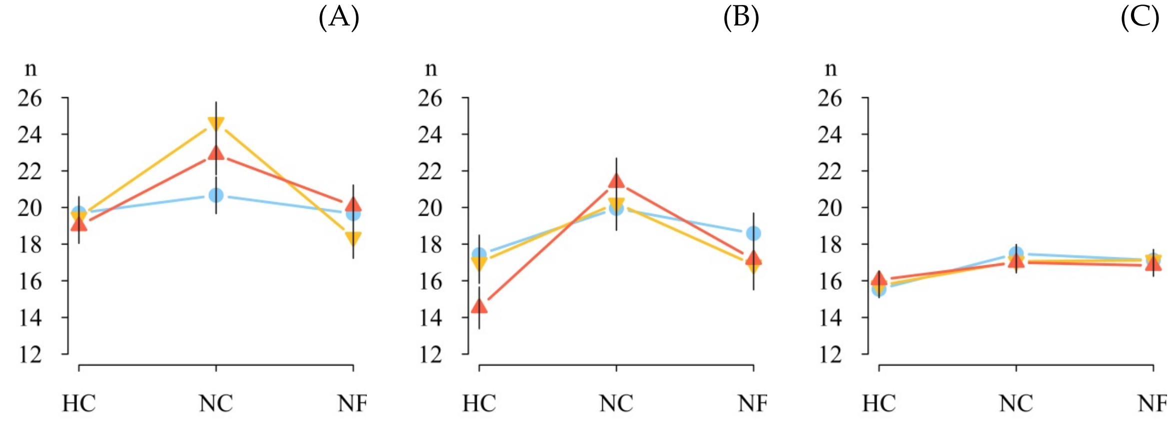

Figure 5.2 Litter sizes of LP, V16, and V36 females housed in different challenging conditions. Cumulative number of kits born in total and kits born alive in the second and third parturition are respectively shown in panels A and B. Cumulative number of kits weaned in the first two-lactations is also shown (panel C). At birth adoptions were set up to 9 kits at first and to 10 kits at second lactation (actual value: 18.9 ± 0.7 kits per female; mean ± s.d.) and dead kits were not replaced. Blue: LP, Gold: V16, and Red: V36. Heat (HC), normal (NC) and nutritional (NF) challenging conditions. Vertical lines represent the standard error of mean.

Reaction norms for average individual weight of kits at birth and weaning are show in Figure 5.3. Long lived and productive females gave birth to lighter kits (born in total and alive) under constrained conditions than in normal conditions (on average -6.9 and -4.7 g; $P<0.05$). The individual weight at birth of V16 kits did not significantly differ in the three environmental conditions. However, although the weight of V36 kits at birth did not differ on NF with respect to NC, they were significantly lighter on HC (-6.0 and -6.5 g for kits born in total and alive, respectively; $P<0.05$). Across environments, the weight of kits weaned by LP females was on average 26.7 g heavier than those weaned by V16 and V36 females (Figure 5.3 C). The drop in the weight of kits weaned due to HC and NF conditions seems to have been less pronounced in LP and V16 kits than that observed with V36 females.

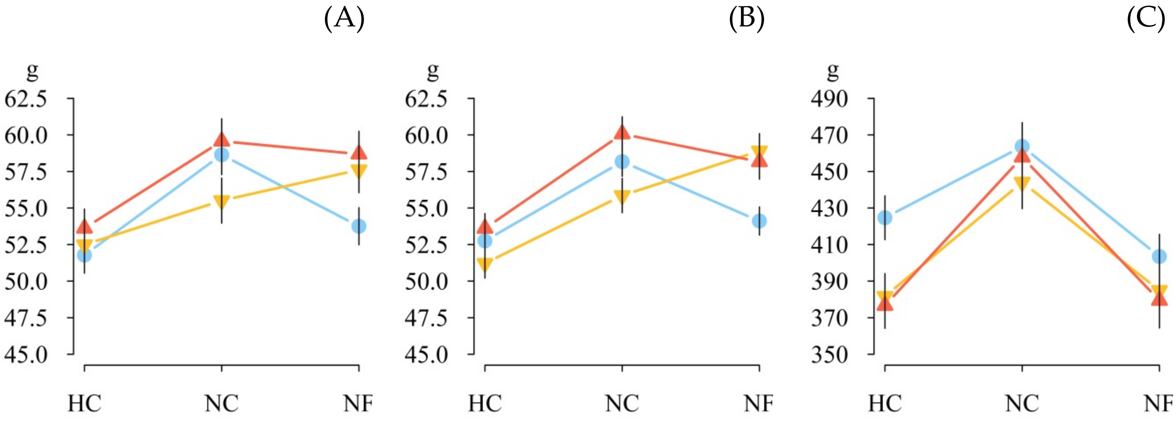

Figure 5.3 Litter weights of LP, V16, and V36 females housed in different challenging conditions. Average individual weights (g) of kits born in total, kits born alive, and kits weaned are respectively shown in panels A, B, and C. Blue: LP, Gold: V16, and Red: V36. Heat (HC), normal (NC) and nutritional (NF) challenging conditions. Vertical lines represent the standard error of mean.

Average females' live weight, perirenal fat thickness (PFT) and milk yield are shown in Figure 5.4.

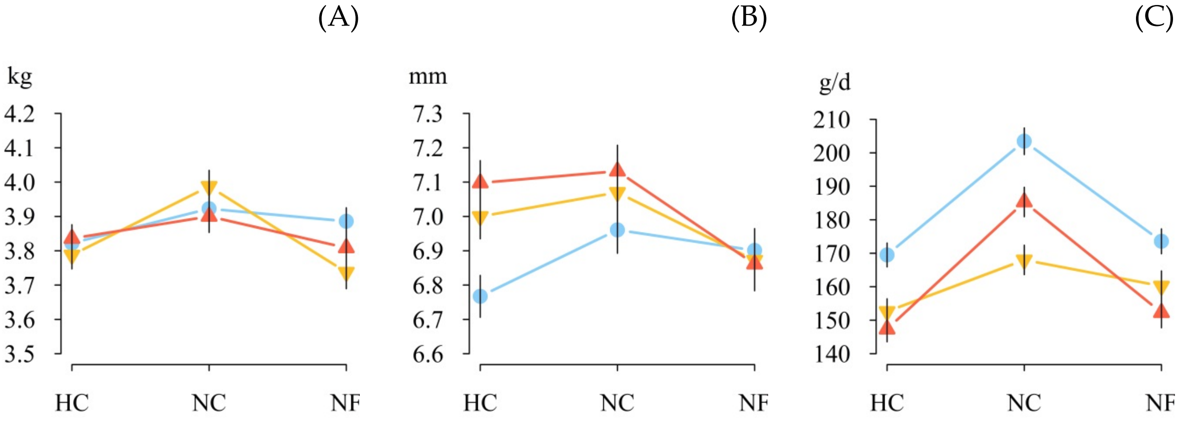

Figure 5.4 Body condition and milk yield of LP, V16, and V36 females housed in different environments. Average live weight (kg), perirenal fat thickness (mm), and daily milk yield (g/d in the first three weeks) of the first two-reproductive cycles are respectively shown in panels A, B, and C. Blue: LP, Gold: V16, and Red: V36. Heat (HC), normal (NC) and nutritional (NF) challenging conditions. Vertical lines represent the standard error of mean.

Only V16 females had a significant decrease in live weight under constrained conditions (HC - NC = -200 g, and NF - NC = -250 g; P<0.05). Perirenal fat thickness response to the different environments varied for the different genetic types. Although the PFT of the different genetic types did not significantly differ between NC and NF, it was the lowest for LP on HC (-0.28 mm; P<0.05). Independently of the environment, LP females produced more milk than V16 and V36 females (on average +21.1 g/d). Constrained conditions decreased milk yield of both LP (HC - NC = -34.0 g/d and NF - NC = -29.9 g/d; P<0.001), and V36 females (HC - NC = -38.0 g/d and NF - NC = -33.1 g/d; P<0.001). In contrast, milk yield of V16 females did not differ significantly across environments (168.0, 152.6, and 160.2 g/d on NC, HC and NF, respectively).

5.5 Discussion

5.5.1 Context of selection and the origin of maternal rabbit lines

The contexts in which the founder females of the LP line arose, as well as the selection criteria and the environmental conditions where the line V was selected, are important to understand the distinct resources acquisition capacities and allocation strategies used by these lines to attain their fitness. It is also important to note that generally, under commercial conditions, healthiness and prolificacy (i.e. fertility, litter size and maternal ability) are the main factors conditioning female fitness and lifespan.

Line LP was established by selecting females from different commercial farms of the Iberian Peninsula having at least 25 parturitions with a minimum number of 7.5 kits born alive in each one (Sánchez et al., 2008), resulting in females with a great robustness (Theilgaard et al., 2007; Theilgaard et al., 2009; Ferrian et al., 2012; Savietto et al., 2013). This robustness arises as a consequence of the seasonal and punctual fluctuations in the environmental conditions that occur in commercial farms. In order to avoid culling and thereby to have a long-productive lifespan, females must have a good long-term adaptive capacity to adequately face these environmental constraints across many parities. In the present study, LP females also demonstrated a greater reproductive stability in the face of constraints (i.e. they maintained litter size), leading to the perception of LP females as generalists animals.

Line V was founded 30 years ago from four specialized maternal rabbit lines (Estany et al., 1989) and, ever since, has been selected to increase the litter size at weaning (now in generation 36) in a context where the intergenerational change (9 months) has limited the females' productive life, and the timespan over which they had to demonstrate their genetic potential. Additionally, the interval of 42 days between parturitions used (females are mated on day 11 post-parturition) with litters being weaned on day 28 post-parturition, places selection pressure on females to adequately nurse the current litter while the future litter is developing in utero. In this sense, V females have been selected to cope with the short-term nutritional stress involved in weaning the greatest number of kits possible. Therefore, in the course of selection, V females should have adopted specific strategies to be selected. The present study found differences in patterns of usage of resources to sustain litter size at weaning between the two generations of line V (differing by 20 generations of selection), evidencing a specialization process.

5.5.2 Resources acquisition capacity

The constrained environmental conditions in the HC and NF environments were designed to limit the energy intake of rabbit females. Hot temperatures impair intake; even when animals are fed with a high energy density diet they were unable to meet their daily requirements (Cervera and Fernández-Carmona, 2010). High-fibrous low energy diets act differently; females increase intake in an attempt to satisfy their daily energy requirements but are physically limited (Fernández-Carmona et al., 2003). The designed constraints worked as expected.

As hypothesized, the acquisition capacity of rabbit females differed in function of their foundational criteria or history of selection. Females of line LP had a greater acquisition capacity than females of line V (independent of generation), but only in high demanding conditions (HC and NF). This pattern was already observed by Theilgaard et al. (2009) for LP and V females. Conversely, twenty generation of selection to increase litter size at weaning was not accompanied by an overall increment in the female's acquisition capacity. In selection programmes with crossbreed rabbit females it was also found that overall feed intake was not increased with (Costa et al. 2004), although Quevedo et al. (2005) and Savietto et al. (2014) observed a different time-trend in the intake of crossbreed and pure rabbit females from recent generations of selection; they had a higher intake in early lactation. This is a strategy which, combined with an adequate management of body reserves, seems to be appropriate to face the hot summers of Mediterranean areas, where line V is being select. Moreover, constrained conditions did not alter the intake pattern of V16 and V36 females. Savietto et al. (2014), studying the time trend for DE intake of V16 and V36 females, also observed a similar acquisition pattern of these generations in both HC and NF. Therefore, twenty generations of selection for litter size at weaning did not increase the environmental sensitivity of rabbit females with respect to acquisition capacity.

The higher acquisition capacity of LP females under challenging conditions seems to be the main strategy used by the founder females of this line to achieve long and productive lifespan. In commercial farms, long productive lifespan is only attained by females sustaining prolificacy (adequate litter size at birth and at weaning) over different reproductive cycles. In commercial farms,

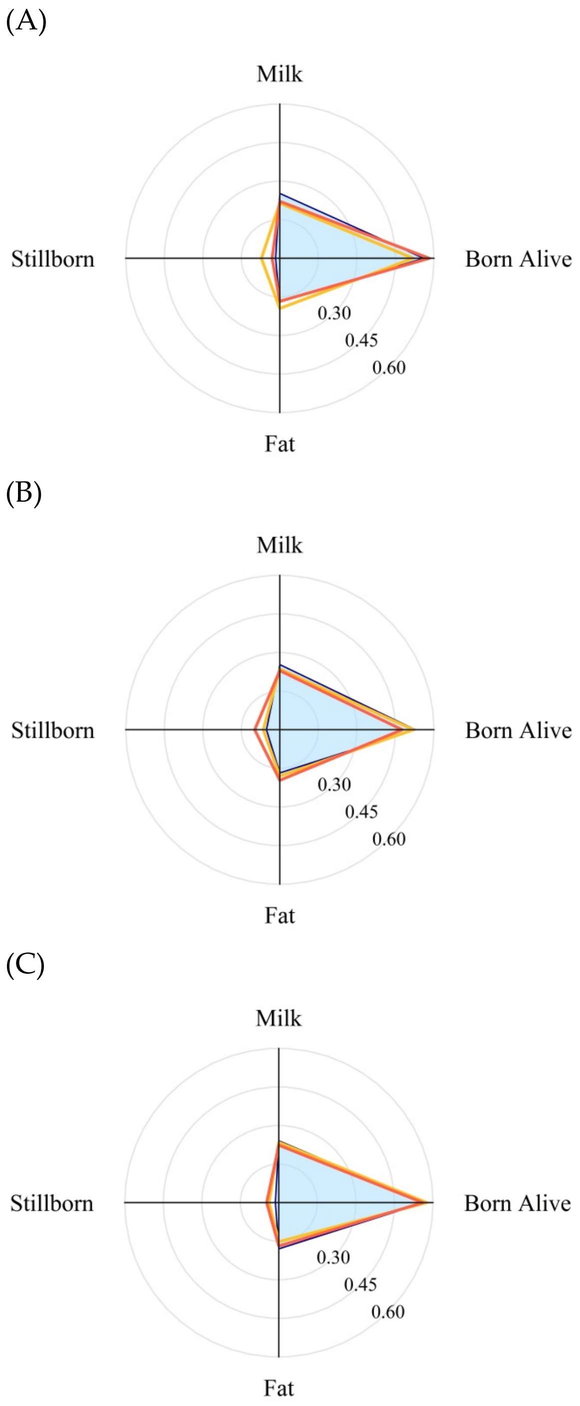

Figure 5.5 Radial plots showing the percentage of energy retained in milk, litter and perirenal fat depot. Data of milk, litter and perirenal fat depot of LP (blue area), V16 (gold lines), and V36 (red lines) females housed in NC, HC, or NF conditions are respectively shown in panels A, B, and C. Energy content of milk was calculated assuming 8.5 MJ per kg of milk (Xiccato and Trocino, 2010). Energy content per kg of kit was assumed to be 3.4 MJ (Fortun-Lamothe and Lebas, 1996), and the energy content per kg of perirenal fat weigh was assumed to be 37.6 MJ. Amount of perirenal fat (kg) was obtained from the equation developed by Pascual et al. (2004).

food is freely available throughout the females' productive life. However, to meet their needs and adequately face any possible environmental constraints, females can develop different strategies. Accretion of body reserves is one possibility whereby females accumulate fat in non-limiting conditions to make use of it when the acquisition of resources is limited (e.g. hot seasons, poor food quality or even pathogens). This is a well-known strategy used by different farmed livestock to alleviate period of nutritional stress (e.g. cows: Friggens et al., 2004; rabbits: Pascual et al., 2013). However, this strategy does not seem the most adequate to maximize fitness, as in the course of their productive lives, rabbit females may encounter expected but also unexpected constrains of different intensities, duration, and even frequency. Additionally, under conditions of unlimited food allowance, females may gain fat. Carrying excess body fat is costly, in both evolutionary (Friggens, 2003) and metabolic terms (Turer and Scherer, 2012); indeed in rabbit females excess body fat was found to be associated with an increased risk of being culled (Theilgaard et al., 2006; Sánchez et al., 2012). In this sense, the high acquisition capacity observed by LP females seem to be a more reasonable strategy to alleviate the negative effects of limiting conditions, avoiding the risk of making use of reserves, and thus favouring a long productive lifespan. However, for this acquisition strategy to be effective it needs to be associated with an adapted assignation of resources between competing life functions.

5.5.3 Resource allocation strategies

Neither the different foundational criteria of LP and V females (i.e. LP *vs.* V36) nor the history of selection of line V (i.e. V16 *vs.* V36) altered the females' acquisition capacity in normal conditions. However, the allocation of obtained resources differed. Figure 5.5A represents the percentage of energy retained in different life functions in the course of two consecutive reproductive cycles on NC. Long-living and productive females did not expend resources; they assigned a greater proportion of the obtained resources to milk (very important to ensure survival and development of the new-born kits; Coureaud et al., 2000), and accreted an adequate level of body reserves, but avoiding the risks related to excess body fatness (Friggens, 2003). They also invested a very low amount of energy in stillborn kits, an interesting strategy to avoid risks related to late gestational loss (Rosell and de la Fuente, 2009).

After twenty generations of selection to increase litter size at weaning, V36 females were able to assign 0.155 MJ per parturition more than V16 females to produce viable kits (born alive), reducing the expenses to non-viable kits (stillborn). García and Baselga (2002) already reported an increased number of kits born alive in response to selection in line V, and Quevedo et al. (2005) observed a greater efficiency on the use of DE for foetal growth. Selection to increase litter size at weaning was also accompanied by higher milk yield in early lactation (Quevedo et al., 2005; Savietto et al., 2014), a critical period for kits survival

(Coureaud et al., 2000). All of this was achieved with no negative impact on body fat reserves (Quevedo et al., 2005; Theilgaard et al., 2006) through lactation (Savietto et al., 2014). However, there is evidence of a trade-off between the pregnant uterus and the mammary gland when rabbit females are concurrently pregnant and lactating (Fortun-Lamothe et al., 1999). In a selection context where fitness is attained by females weaning big litters in a short interval, selection of line V seemed to favour females rapidly changing the priority between the current (kits being nursed) and the future litter (foetus development). Additionally, when the selection environment limits the time females have to show their genetic potential (selection method is a family index in a repeatability model; Estany et al., 1989), promoting different strategies to assign the obtained resources, the maintenance of reproduction was more dependent on the accretion and use of body reserves (Savietto et al., 2014).

An interesting aspect of the present study is that the differences in adaptive capacities that the different lines have acquired, and which contribute greatly to their robustness, were only revealed by comparing them in different environments. Understanding the strategy adopted by LP females to achieve a long productive lifespan was only possible by evaluating females' responses in constrained conditions. Similarly the emergence of environmental sensibility in response to selection, an indicative of specialization, was only observable in limiting conditions. The different life history-strategy of LP with respect to V36 females was highlighted at HC. It had previously been shown that LP females made use of body reserves to maintain their reproductive performance in the face of constraints (Theilgaard et al., 2007). However, in the present work, it was found that LP females also made use of their greater acquisition capacity to sustain reproductive performance. Intriguingly, this increased acquisition was coupled with an adjustment in the allocation of these resources such that there was a limitation in investment in litter size. Even though they acquired more DE than V36 females, LP females did not invest the energy surplus in the litter (see Figure 5.5B). They seemed to avoid the follow-on risks related to intense foetal growth, partitioning relatively less energy to their litters than V36 females. Instead, the greater intake capacity was partitioned to milk yield, ensuring kits survival, without reserving additional resources to maintenance the fat level to similar values observed at NC. For LP females, it would appear that fat reserves are a safety factor, rather than a necessary energy surplus to ensure reproduction (Savietto et al., 2013).

The advantage obtained by V36 females in response to selection for litter size after 20 generation was observed under constrained conditions, especially in the HC environment. Females of generation 36 reduced their milk yield and safeguarded body condition (i.e. live weight and perirenal fat thickness), resulting in a small reduction in the number of kits born in total (-1.9 kits per parturition, with respect to NC). However, a greater reduction in the number of kits born alive occurred (-3.4 kits per parturition, with respect to NC). In contrast, in constrained environments, females from generation 16 sustained milk yield to similar levels as NC, increasing the relative partition to the current litter. They also reduced the gestational effort (i.e. -2.6 kits born in total per parturition, with respect to NC) adjusting the available resources to give birth to viable kits (see Figure 5.5B). Contrary to what was observed in HC, V36 females in NF partitioned the energy not destined to milk production to gestation to ensure a small litter size reduction (-1.4 kits born in total per parturition, respect to NC) and more viable kits (+1.3 kits born alive per parturition), with respect to HC (Figure 5.5C).

The present work provided evidence of changes in the acquisition capacity and allocation of obtained resources into different life function as a result of different selection criteria in two maternal rabbit lines. Line LP was founded by selecting females having a long-productive lifespan from commercial farms (Sánchez et al., 2008) where culling and early mortality are the main factors limiting lifespan. Long-productive life is, thus, an indirect indicator of successful adaptation to variations to variable environmental conditions (see other examples at Kirkwood, 2000 and Kirkwood, 2005). Reproduction has a cost (Snell and King, 1977) leading to the consensus on the existence of a trade-off between reproduction and lifespan (Stearns, 1989). Trade-offs, however, are also dependent on the environmental conditions (Stearns, 1989; Reznick, 2000), and then the appearance of organisms able to obtain and partition resources into competing functions depends on the interaction of genotypes and environments (e.g. Reznick, 2000). The present work demonstrates such ability in LP females. They did not prioritize a single function, but had an increased acquisition capacity, allocating resources to sustain prolificacy without increasing the risks related to the excess usage of body reserves. In this case, fat reserves are a safety factor.

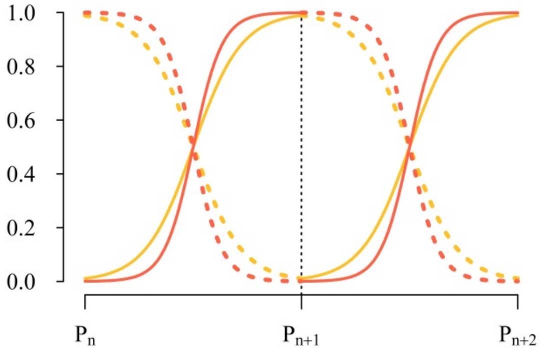

Figure 5.6 Relative priorities between the current and the future litter of rabbit females. Conceptual scheme representing the changes in the relative priorities between the litter being nursed (dotted lines) and the litter being gestate (solid lines) for V16 (gold) and V36 (red) females. Figure represents two consecutive cycles. Twenty generations of selection for litter size at weaning resulted in females rapidly changing the priority between the current and the future litter.

Selection to increase litter size at weaning during 20 generation did not favour a greater acquisition capacity of female rabbits, but the selection criteria were attained by female rabbits proportionally assigning more resources to favour litter size at weaning. The main reason for not favouring a greater acquisition capacity seems to be related to the change in priorities between the current and the future litter, as suggested by Friggens

(2003). The limited time females had to express their genetic potential, together with the evidence available in the literature relating to the competition between the gravid uterus and the mammary gland (Fortun-Lamothe et al., 1999), the greater litter size at birth with a relative higher milk yield in early and low milk late lactation (Quevedo et al., 2005; Savietto et al., 2014), and the greater dependence of V36 females on fat reserves to ensure reproduction (Savietto et al., 2014) support this idea. A schematic representation of our hypothesis on why V36 favoured the assignation of resources to functions related to the rapid change in priority between litters, but not a greater acquisition, is shown in Figure 5.6. Additional research on how time limitations affect the temporal pattern of allocation of resources (in non-limiting conditions) between life function is required.

5.6 Acknowledgements

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General Conclusions

The present thesis enlightened the physiological mechanisms by which female rabbits, differing in the foundational criteria or in their history of selection, fit to the environmental abundances and constrains in order to maximize their fitness and attain to the selection criteria.

With respect to the digestive capacity, the constitution of a line by selecting female rabbits having a long and productive lifespan resulted in animals with greater capacity to acquire resources in constrained conditions. Although, higher intakes are normally associated with a reduction in the apparent digestible coefficients, the digestive efficiency of LP females was not impaired. Twenty generation of selection to increase litter size at weaning was not accompanied by an increment in the intake capacity, but females from recent generation of line V showed an improved digestive flexibility under constrained conditions.

The main difference observed between LP and V females with respect to the maintenance of their reproductive fitness were based in the body reserves. At the onset of constrained condition females of line V sustained the reproductive effort by privileging body reserve accretion in the second part of lactation, ensuring thus a high reproductive performance in the subsequent partum. This strategy, however, was accompanied by an exhaustion process, resulting in low reproductive performances in the long therm. LP females in change, sustained reproductive effort by having a relatively higher intake of digestible energy that were diverted to milk yield and litter size avoiding an excessive accretion of fat reserves. The direct consequence of this management of resources was a stable body condition throughout the experimental period. In this sense, it can be argued that for rabbit females of line V, reproduction is dependent on the accretion and use of body reserves, while for LP females it is a safety factor.

Twenty generations of selection for litter size at weaning changed the way in which female rabbits manage the available resources. Under normal conditions, females from recent generation of selection yielded more milk in the first and less milk in the last week of lactation. This rapidly change in priority between the actual (kits being nursed) and the future litter (kits being gestate), has been favored by the selection criteria because females weaning more numerous litters are more likely to be selected. Females from actual generation of line V also presented a greater reproductive robustness at the onset of environmental constraints, reflecting a greater adaptability to the seasonal variations that normally occur in the environment where this line is being selected. However, if the environmental constrain persists, the strategy of favoring reproduction in the short term without taking into account the future conditions resulted in an exhaustion of females with respect to their reproductive performances.

From the present results, line LP can be characterized as a 'generalist' maternal rabbit line, capable of stable reproductive perform over a range of environments differing in the intensity and type of the physiological constraints. This line was also characterized by high efficiency in the acquisition and use of resources to sustain reproduction, reducing the risks related with the excessive use of body reserves. Line V can be characterized as a 'specialized' maternal line capable of high reproductive performances. This line is well adapted to the conditions where it is being selected, failing to perform well in constrained conditions of long duration.

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Perspectives

The present thesis highlights the potential of combining multi-trait selection criteria with the environmental conditions to establish a population of robust animals. Additionally, the environment where the animals are being selected clearly drives the interplay between functions. If the objective of a selection program is to improve the overall fitness of animals without impairing productivity, the strategy used to establish line LP is feasible. Selection to improve fertility in high producing dairy cows, selection to reduce metabolic problems of fast growing broilers, or to reduce leg problems in lean sows, should consider this methodology. Information and genetic material to do so are available. Other selection strategies, like selecting animals in a context were the availability and quality of resources are expected to be more and more irregular (global warming) should be also studied. In rabbits, one possible strategy to increase acquisition capacity could by understanding the physiological mechanisms involved in the role of microbiota. This also implies how the evolution of selection schemes may be driven in the near future.

In rabbits, there are no evidences that selection exclusively for reproduction impair lifespan or robustness. For this reason, since its foundation line LP is now being selected to increase litter size at weaning in the same conditions where line V has being selected. At the present, line LP reached the generation six of selection for litter size at weaning. However, the experiments determining the relationship between lifespan and litter size, where all performed in populations of rabbits highly selected for reproductive criteria, where very few animals are expected to have a low reproductive performance. Since the selection programme of rabbits for meat, developed at the Universitat Politècnica de València, counts with modern cryopreservation techniques to preserve the genetic material, embryos of generation three and seven of line LP are available for future comparisons with more advanced generations of selection of line LP. This comparison is, for example, of great importance in the field of evolutionary biology where the existence of a trade-off between reproduction and lifespan is extensively accepted. Another important result that can be highlighted with this comparison is the specialization process that line LP could suffer in the course of selection in a specific environment. In other words, does selection for litter size at weaning will degrade the robustness of LP females? If not, there is another argument to apply high intensities of selection to generate robust and high producing population of farm animals.

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Curriculum vitae

Davi Savietto was born on 28th of September 1985 in Jundiaí, SP, Brazil. Studied Animal Science at Faculdade de Zootecnia e Engenharia de Alimentos da Universidade de São Paulo (Pirassununga, SP, Brazil), obtaining the degree of BSc. on 22th December 2008. He moved to Valencia, Spain in August 2009 to start his graduate studies in Animal Breeding and Reproductive Biotechnology, reaching the degree of MSc. in 2013. During these years he worked in two different projects. The first project, funded by the European Commission, Framework 7, aimed to mitigate the greenhouse gases emissions by increasing the feed efficiency of ruminants (focus on dairy cattle). In the development of this project, Davi Savietto was engaged in a multidisciplinary research group, working together with researchers from The Irish Agriculture and Food Development Authority (TEAGASC, Fermoy, Co. Cork, Ireland) and the French Institute for Agricultural Science (INRA - MoSAR, Paris, France). The second project, and the main body of the present thesis, aimed to unravel the genetic and environmental factors driving robustness and longevity in reproductive female rabbits. This project was financed by the Spanish Ministry of Education and Science, and involved researchers from Aarhus University, (Tjele, Denmark) and the French Institute for Agronomic Research (INRA AgroPariTech UMR 791 - MoSAR, Paris, France). The present thesis and other scientific publications are the end product of this multidisciplinary network.

List of Publications

Research articles

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Conferences contribution

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