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Optimising robustness in rabbit paternal lines considering resource allocation and immune status

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“The future belongs to those who believe in the beauty of their dreams.”

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Abstract

Genetic selection in rabbit paternal lines has long been pursued to enhance growth performance. However, various studies have demonstrated that this strategy may adversely affect traits related to animal fitness, such as reproductive and health related traits. The central hypothesis of this thesis was that new lines obtained by the selection of elite animals and the introduction of robust genetics may be able to sustain production while safeguarding health and reproductive functions. Three consecutive experiments were conducted to test our hypothesis using a total of 319 female rabbits and 387 weaned rabbits. The animals belonged to three different genetic lines: R, RF, and RFLP. The R line is a paternal line selected for average daily gain during the growing period for 37 generations. The RF line was founded by selecting elite animals from the R line (average daily gain > 60g/d) selectively bred to create the RF line. Finally, genetics from a long-living maternal line (LP) was incorporated through backcrossing to enhance the RF line's general robustness and disease resistance, resulting in the RFLP line. Productive, reproductive and immunological data were collected from females from the first artificial insemination to the third parturition and from kits from birth to slaughter. Results suggested that re-founding with elite animals, as seen in the RF line, improved reproductive traits initially (fertility and litter growth and survival during the first cycle) but led to body wear-out and reduced fertility in subsequent cycles. However, introducing resilient genetics through backcrossing, as seen in the RFLP line, improved resource allocation, ensuring better adaptation to physiological changes and enhanced reproductive success (higher fertility, milk yield, litter survival and growth, as well as female lifespan). Growth and digestibility trials comparing the three genetic lines during the growing period showed that while slight changes were observed in nutrient utilisation and growth, overall performance remained similar among different lines. Immunological data suggested that: i. the selection of elite animals improved the immune status of both females and their litters at weaning (higher total and CD4⁺ lymphocytes) and ii. the additional introduction of robust genetics seemed to influence resource allocation strategies, resulting in distinct immune responses during parturition and weaning (higher CD4⁺ to CD8⁺ ratio in females). Furthermore, the positive correlations observed between specific leukocyte populations in female rabbits and corresponding populations in their offspring might indicate potential maternal contributions to the innate and adaptive immunity of the kits. Moreover, the RFLP line consistently exhibited lower mortality rates and sanitary

risk index (SRI), emphasising the potential benefits of incorporating robust genetics for improved health traits in growing rabbits. Results obtained in the present thesis suggest that the crossbreed strategy between a fast growing and a robust line could contribute to refining existing genetic lines without sacrificing growth potential.

Resumen

La selección genética en líneas paternas de conejos se ha buscado durante mucho tiempo para mejorar el rendimiento del crecimiento. Sin embargo, diversos estudios han demostrado que esta estrategia puede afectar negativamente a características relacionadas con el fitness animal, como la reproducción y la salud. La hipótesis central de esta tesis fue que las nuevas líneas obtenidas mediante la selección de animales de élite y la introducción de genética robusta pueden sostener la producción salvaguardando la salud y las funciones reproductivas. Se realizaron tres experimentos consecutivos para probar nuestra hipótesis utilizando un total de 319 conejas y 387 conejos destetados. Los animales pertenecían a tres líneas genéticas diferentes: R, RF y RFLP. La línea R es una línea paternal seleccionada por su ganancia diaria promedio durante el período de crecimiento durante 37 generaciones. La línea RF se fundó seleccionando animales de élite de la línea R (ganancia media diaria > 60 g/d) criados selectivamente para crear la línea RF. Finalmente, se incorporó genética de una línea maternal (LP) longeva mediante retrocruzamiento para mejorar la robustez general y la resistencia a enfermedades de la línea RF, lo que dio como resultado la línea RFLP. Se recolectaron datos productivos, reproductivos e inmunológicos de las hembras desde la primera inseminación artificial hasta el tercer parto y de los gazapos desde el nacimiento hasta el sacrificio. Los resultados sugirieron que la refundación con animales de élite, como se ve en la línea RF, mejoró los rasgos reproductivos inicialmente (fertilidad, crecimiento y supervivencia de la camada durante el primer ciclo), pero condujo al desgaste corporal y redujo la fertilidad en ciclos posteriores. Sin embargo, la introducción de genética resiliente a través del retrocruzamiento, como se ve en la línea RFLP, mejoró la asignación de recursos, aseguró una mejor adaptación a los cambios fisiológicos y mejoró el éxito reproductivo (mayor fertilidad, producción de leche, rendimiento de la camada y supervivencia tanto de las hembras como de la camada). Los ensayos de crecimiento y digestibilidad que compararon las tres líneas genéticas durante el período de crecimiento mostraron que, si bien se observaron ligeros cambios en la utilización de nutrientes y el crecimiento, el rendimiento general permaneció similar entre las diferentes líneas. Los datos inmunológicos sugirieron que: i. la selección de animales de élite mejoró el estado inmunológico tanto de las hembras como de sus camadas al destete (mayores linfocitos totales y CD4+) y ii. la introducción adicional de genética robusta pareció

influir en las estrategias de asignación de recursos, lo que resultó en distintas respuestas inmunes durante el parto y destete (mayor proporción de CD4+ a CD8+ en las conejas). Por otro lado, las correlaciones positivas observadas entre poblaciones de leucocitos específicas en conejas y las poblaciones correspondientes en sus crías podrían indicar posibles contribuciones maternas a la inmunidad innata y adaptativa de los gazapos. Además, la línea RFLP mostró consistentemente tasas de mortalidad y un índice de riesgo sanitario (SRI) más bajos, lo que enfatiza los beneficios potenciales de incorporar genética robusta para mejorar los rasgos de salud en los conejos en crecimiento. Los resultados obtenidos en la presente tesis sugieren que la estrategia de cruce entre una línea de rápido crecimiento y una línea robusta podría contribuir a refinar las líneas genéticas existentes sin sacrificar el potencial de crecimiento.

Resum

La selecció genètica en línies paternes de conill s'ha perseguit durant molt de temps per millorar el rendiment del creixement. No obstant això, diversos estudis han demostrat que aquesta estratègia pot afectar negativament els trets relacionats amb el fitness animal, com ara la reproducció i la salut. La hipòtesi central d'aquesta tesi era que les noves línies obtingudes mitjançant la selecció d'animals d'elit i la introducció de genètica robusta podrien ser capaços de mantenir la producció alhora que salvaguarden la salut i les funcions reproductives. Es van realitzar tres experiments consecutius per provar la nostra hipòtesi amb un total de 319 femelles i 387 conills deslletats. Els animals pertanyien a tres línies genètiques diferents: R, RF i RFLP. La línia R és una línia paterna seleccionada per obtenir un guany mitjà diari durant el període de creixement durant 37 generacions. La línia RF es va fundar seleccionant animals d'elit de la línia R (guany mitjà diari > 60 g/d) criats selectivament per crear la línia RF. Finalment, es va incorporar la genètica d'una línia materna de llarga vida (LP) mitjançant el retrocreuament per millorar la robustesa general i la resistència a les malalties de la línia RF, donant lloc a la línia RFLP. Es van recollir dades productives, reproductives i immunològiques de les femelles des de la primera inseminació artificial fins al tercer part i dels llogrons des del naixement fins a la matança. Els resultats van suggerir que la refundació amb animals d'elit, tal com es veu a la línia RF, va millorar inicialment els trets reproductius (fertilitat i creixement de la brossa i supervivència durant el primer cicle), però va provocar un desgast corporal i va reduir la fertilitat en cicles posteriors. No obstant això, la introducció de genètica resilient mitjançant el retrocreuament, tal com es veu a la línia RFLP, va millorar l'assignació de recursos, assegurant una millor adaptació als canvis fisiològics i un èxit reproductiu millorat (més fertilitat, rendiment de llet, rendiment de la llograda i supervivència tant femelles com de la llograda). Els assaigs de creixement i digestibilitat que van comparar les tres línies genètiques durant el període de creixement van demostrar que, tot i que es van observar lleugers canvis en la utilització i el creixement dels nutrients, el rendiment general es va mantenir similar entre les diferents línies. Les dades immunològiques suggereixen que: i. la selecció d'animals d'elit va millorar l'estat immunològic tant de les femelles com de les seves camades al deslletament (limfòcits CD4+ i totals més alts) ii. la introducció addicional de genètica robusta semblava influir en les estratègies d'assignació de recursos,

donant lloc a diferents respostes immunes durant el part i deslletament (índex CD4⁺ a CD8⁺ més elevat en les femelles). A més, les correlacions positives observades entre poblacions específiques de leucòcits en conills femelles i les poblacions corresponents de la seva descendència podrien indicar contribucions maternes potencials a la immunitat innata i adaptativa dels llogrons. A més, la línia RFLP va mostrar constantment taxes de mortalitat i índex de risc sanitari més baixes, posant èmfasi en els beneficis potencials d'incorporar una genètica robusta per millorar els trets de salut dels conills en creixement. Els resultats obtinguts en la present tesi suggereixen que la l'estratègia de retrocreuament entre una línia de ràpid creixement i una línia robusta podria contribuir a refinar les línies genètiques existents sense sacrificar el potencial de creixement.

GENERAL INTRODUCTION

In recent years, the exponential population growth and changing dietary preferences have led to increased demand for animal protein. Therefore, the agricultural sector has tried to improve productivity by selecting animals with advantageous traits such as growth rate, feed efficiency, and reproductive performance. However, the intense focus on productivity has come at a cost. While successful in increasing output, the genetic selection processes have led to unintended consequences, particularly concerning the fitness of these animals. Many livestock species have seen a decline in their ability to resist diseases and adapt to environmental stressors due to the narrow selection criteria focused solely on productivity.

This dissertation uses rabbits as an experimental model to evaluate specific strategies to improve a population's robustness and its impact on overall productive, reproductive and health traits. This work aims to reconcile the need for high productivity with maintaining animal health and welfare by reintroducing fitness traits into the breeding process.

In the following introduction, we will discuss the current state of knowledge in this field, followed by formulating a hypothesis and a series of objectives designed to test it.

Feeding the future: the role of technology and efficiency

According to the United Nations (2022), the world's population is expected to grow to 8.6 billion people by 2030 and 9.8 billion by 2050. As the global population continues to expand, the demand for animal protein is expected to rise significantly (Williams and Speller, 2016). Thus, higher outputs for livestock and crops will be necessary to meet the world's food demand in the future (Hume et al., 2011). Despite the impending pressures on food demand, past experiences indicate that much more significant increases in agriculture have been successful within comparable time frames (FAO, 2017). Notably, between 1961 and 2011, global agricultural output more than tripled, demonstrating the adaptability of agricultural systems to substantial growth (Alexandratos and Bruinsma, 2012). However, repeating past strategies may not be enough, given the current environmental conditions and resource constraints. From a technological point of view, these challenges can only be achieved by higher development and increased investments towards improved efficiency rather than relying solely on intensification with which inputs transform into outputs. This strategic shift is crucial for ensuring sustainability and meeting future food needs (FAO, 2017). In this context, biotechnological advancements have emerged as integral components of this approach (Van Vleck, 1981).

Addressing sustainability issues in meat production becomes essential within this technological advancement and efficiency framework. Animal-derived products offer a concentrated source of nutrients with a high biological value, which is essential for providing a healthy and balanced diet for the global population. Most sustainability concerns surrounding meat arise at the production level, necessitating continuous development of better technologies to mitigate these issues. Consequently, improvements in animal genetics, nutrition, and health hold significant potential for enhancing productivity and efficiency in meat production, reducing the utilisation of scarce natural resources per unit of output. Lastly, the meat production industry can transform grass and crop residues, which are unsuitable for human consumption, into food for humans, contributing to food security on a planet where natural resources are limited (Parlasca and Qaim, 2022).

Human dietary requirements: the role of meat and meat products

In contemporary diets, meat and meat products account for one-sixth of the daily energy intake for an adult in Europe, contributing significantly to protein, vitamin D, and iron intake at 40, 30 and 23%, respectively (Salter, 2018). Its protein content covers the nutritional requirements of humans since it is rich in all essential amino acids. In addition, the bioavailability of the iron content in meat is considerably higher than that of iron from plant-based sources.

Various studies from diverse geographical contexts support the nutritional benefits of incorporating meat into diets across all age groups. These studies emphasise that including meat in the diet significantly reduces the probability of nutritional deficiencies among children, adolescents, and adults (Headey et al., 2018; Parlasca and Qaim, 2022). Further research shows positive health associations with unprocessed red meat, like lower dementia risk, and with poultry, linked to reduced risk for some types of cancer (Parlasca and Qaim, 2022; Lippi et al., 2016). In addition, meat-eaters may have better bone health and a lower risk of specific fractures (Tong et al., 2020). Therefore, meat and meat products should not be discarded as they help prevent health issues and can also avoid malnutrition in nations with limited access to other highly nutritious foods (Salter, 2018).

The impact of animal product consumption on health and life expectancy is a topic of ongoing debate. A substantial body of research suggests that their consumption may have adverse effects (Singh et al., 2003). Indeed, the WHO (2015) classifies processed meats and red meat as products with a potential carcinogenic effect. However, more recent studies have questioned the robustness of the evidence that links red meat consumption to health issues, suggesting that the connection may not be as solid or conclusive as previously thought (Lescinsky et al., 2022). Studies such as the Oxford-EPIC Study (Key et al., 2003) and the 45-and-Up Study (Mihirshahi et al., 2017) show that the adverse effects of red meat on all-cause mortality become negligible in populations adhering to overall healthy lifestyles. However, in North America, where meat consumption is often associated with fast food chains and less healthy lifestyles, associations between meat consumption and health risks appear more pronounced (Leroy et al., 2022).

Moreover, in most studies, the introduction of animal products is confounded with an increase in excess energy and/or protein consumption. Furthermore, in epidemiological studies in humans, the reduction in consumption of these products is often associated with other behavioural changes, including a decrease in alcohol consumption, an increase in sporting activity and the adoption of low-calorie diets. Indeed, in a recent study that considered these potential confounding effects in 173 countries and territories (You et al., 2023), the authors observed an increase in life expectancy with meat consumption. These results may indicate that, provided it does not lead to an overconsumption of protein and energy, animal products of high nutritional value can improve our quality of life, health, and life expectancy.

However, it is essential to acknowledge the challenges associated with meat production. Meat production has a significant environmental impact, contributing to greenhouse gas emissions, deforestation, and water use (Salter, 2018). In addition, modern production systems rise ethical concerns regarding animal welfare on intensive farming (McClements, 2023). Addressing these controversies requires an approach that incorporates sustainable practices and considers the ethical implications of modern agriculture.

Rabbit meat as a sustainable protein source

Given the significant role of meat in meeting human dietary requirements, it is crucial to explore alternative nutritious and sustainable animal protein sources. One such alternative could be rabbit meat, which is highlighted for its potential as a sustainable protein source (Dalle Zotte and Cullere, 2024).

In a recent report by the European Commission, rabbits are presented as the second-best source of animal protein behind poultry in terms of converting proteins from cellulose-rich plant sources into food (EC-DGHFS 2018). Additionally, since rabbits are usually fed a high-fibre pelleted diet, avoiding selection among the different raw matters, and there is a possibility for higher inclusion of by-products, avoiding feed-food competition (Maertens, 2020). Furthermore, rabbits have different feeding habits than other mammals, including unique characteristics like caecotrophy related to a specific digestive system as a non-ruminant herbivore. As a result, the rabbit can

survive in various environmental conditions, given its ability to eat a wide range of feeds, including seeds and herbaceous plants (Gidenne et al., 2020).

Regarding reproduction, rabbits' high reproductive rate and short gestation period allow for fast population growth and high productivity (Lebas et al., 1997). Furthermore, these animals can turn 20% of their protein intake into meat, higher than pigs and cattle (16-18% and 8-12%, respectively) (Dalle Zotte, 2014). Its meat is a relatively low-cost protein source that can be raised on small land plots, making it an attractive option for small-scale farmers and rural communities (European Commission, 2018). In addition, rabbit meat has high nutritional and sensory properties. It is lean and high in protein with a tender texture and delicate flavour (Dalle Zotte, 2002). It is also high in nutrients such as selenium, zinc and iron, B vitamins, vitamin E and omega-3 fatty acids, which most adults lack (Dalle Zotte and Szendrő, 2011). These characteristics make rabbit production relevant in many countries where the majority of the population lives under the poverty line, particularly in Indonesia, where it initially began as a strategy to provide a protein source to a growing population predominantly fed a rice-based diet (Oseni and Lukefahr, 2014; Lukefahr et al., 2022).

Despite its potential, harsh environmental conditions (heat stress and inadequate housing systems) and insufficient feeding and management strategies remain critical constraints. However, since religious limitations regarding the consumption of rabbit meat are rare, in theory, a global market opportunity exists to be explored (Cullere and Dalle Zotte, 2018). Therefore, providing suitable genetic types adapted to adverse environments could be a vital aspect of the diffusion of rabbit production in many countries (Oseni and Lukefahr, 2014). However, Kaasschieter et al. (1992) point out a paradox: while the rising demand for animal products often leads to the introduction of exotic purebreds or crossbreeding, conserving local breeds is crucial since they have valuable traits like heat tolerance and disease resistance. Therefore, preserving these breeds could be essential to achieving sustainability in animal production (Kaasschieter et al., 1992).

Farm Animals by design: The role of genetics.

Concerning animal production, techniques such as artificial insemination, embryo transfer and genomic selection have substantially impacted animal breeding, improving productivity, reproduction, and selection programs (Das et al., 2022). In

contrast to these modern techniques, animal breeding traditionally focused on individual characteristics for practical and economic reasons (Muir and Craig, 1998). To better understand the progress in this area, identifying superior animals relies on phenotype and statistical models, such as best linear unbiased prediction (BLUP), which effectively separates genetic and environmental causes (Das et al., 2022). This approach has played a critical role in the evolution towards improving animal performance. Over time, this has led to a dilution of the requirements for maintenance per unit of product (milk or carcass weight), thus improving the feed conversion rate (Faverdin et al., 2022). As a result of these advancements, this paradigm shift has been especially pronounced in the last century, as genetic selection has become progressively more intensive to enhance desirable traits, resulting in remarkable improvements across various species (Hill, 2008). A few examples can be considered to illustrate this concept. Research by Havenstein (2006) conducted two trials in which modern broiler stocks (Arbor Acres in 1991 and Ross 308 in 2001) were compared with a control population. It was assessed how these broilers performed on modern diets and diets formulated to match 1957 specifications. The study found significant improvement in broiler growth rates over 44 years of genetic selection, such as increased body weight, meat yield and reduced fat content. In pigs, the number of litters needed to wean 1000 piglets decreased from 126 to 80 in 20 years. In addition, the percentage of total mortality (during farrowing and lactation) went from 41% to 31% (Knap et al., 2023).

Lastly, in rabbits, average daily gain (ADG) has improved from 0.45 to 1.23 g/d per generation due to the selection for growth rate (Piles and Blasco, 2003). Additionally, selection for improved litter size has also been successful in rabbits. A study by Ragab and Baselga (2011) comparing four genetic lines founded on different criteria but selected for litter size at weaning (A, V, H and LP) showed an improvement in prolificacy since their foundation for the four genetic lines. Moreover, when comparing the four lines, the authors found that their differences had decreased since their foundation (Ragab and Baselga, 2011). In a related topic, a study comparing two generations from the same genetic rabbit line (V16 vs V36) observed that the change in the allocation of resources to increase milk production during the first half of lactation led to a reduction in post-natal mortality. This change seems to have contributed to a higher litter size at weaning (Saviotto et al., 2014).

Nevertheless, the application of genetic selection also presents its share of disadvantages, often rooted in unintended consequences. For instance, the selection of animals for improved performances has inadvertently led to fewer breeds per species and a decreased genetic variability in highly selected populations, leading to a decreased long-term adaptive potential (Sherf, 2000; Danchin-Burge et al., 2012). As a result of reduced genetic diversity, these animals exhibit a lower resource management capacity and reduced resilience to environmental variability and new stressors (Colditz and Hine, 2016; Destoumieux-Garzón et al., 2020). Therefore, animals in a population that has undergone intensive genetic selection appear to be more susceptible to behavioural, physiological, and immunological issues (Rauw et al. 1998). For instance, selecting solely for milk yield might inadvertently reduce traits related to the cow's ability to adapt to its environment, overall health, and welfare (Brito et al., 2021). In purebred zebu cattle, selection for milk yield has affected heat stress tolerance, which is one of the main characteristics of these animals (Santana Jr et al., 2015). In rabbits, selection for high prolificacy has reduced kits' birth weight and survival (Argente et al., 2008).

Moreover, intensive selection for growth rate in paternal rabbit lines has led to abnormal sexual behaviour, such as low libido and low sperm production (Pascual et al., 2004). Additionally, heavier individuals often exhibit hormonal imbalances, characterised by reduced androgen levels and elevated oestrogen levels, which can disrupt reproductive functions (Hammoud et al., 2008). Obesity intensifies these issues by disrupting hormonal balance, affecting sperm maturation and seminal parameters (Bélanger et al., 2002; Hammoud et al., 2008). In addition, research by Martínez-Paredes et al. (2018) found that young female rabbits exhibiting faster growth during the rearing period experienced poorer reproductive performances than those with slower growth rates. These rabbits, reaching their maximum weight 31 days sooner during rearing, showed decreased performance in their first parturition, with an average of 1.3 fewer liveborn kits.

These examples above illustrate the direct effects of selective breeding and highlight the broader implications of resource allocation in livestock robustness.

Livestock Robustness: The Role of Resource Allocation

Resource allocation involves distributing a finite amount of resources among several activities or structures (Friggens et al., 2017). This process includes allocating metabolisable energy among physiological functions, including growth, gestation, lactation, maintenance, and energy reserves. Genetic factors and physiological stages influence the priorities of this distribution (Puillet et al., 2016). However, trade-offs are inevitable since resources allocated to one purpose are unavailable for others (Rauw, 2012).

In animals, resource-demanding physiological processes exhibit trade-offs due to limited resources, feed intake, and digestive capacity (Rauw and Gomez-Raya, 2015). Consequently, an animal cannot maximise all physiological functions simultaneously (Stearns, 1992). How resources are allocated within the body can further restrict the animal's ability to manage multiple demands, leading to genotype × environment interactions (Rauw and Gomez-Raya, 2015). For instance, animals genetically selected for high production may divert resources from other essential processes, leaving fewer resources for coping with additional demands such as fighting off diseases or handling stress (Rauw, 2009). In rabbits, this allocation shift often leads to a negative energy balance during periods like early lactation or overlapping lactation/gestation, where high energy demands lead to excessive mobilisation of body reserves (Pascual et al., 2003). As a result, this could cause reproductive and health issues, such as reduced fertility and increased disease susceptibility (Puillet et al., 2016).

Thus, the expected benefits of high production, such as the dilution effect where maintenance costs are spread over higher outputs, may be counterbalanced by a decline in productive lifespan due to poor health and fertility (Puillet et al., 2016). Therefore, these animals might struggle to maintain overall health and robustness while prioritising high production. Consequently, animals cannot fully express every biological function, suggesting that robustness to all environmental perturbations is unrealistic (Friggens et al., 2017). This inherent limitation emphasises the need for a more integrated animal breeding and management approach. Genetic selection should not solely focus on maximising production traits but also consider traits that enhance fitness. For instance, selecting for better immune function and stress resilience can

help animals cope with environmental challenges more effectively, ultimately leading to more balanced and sustainable production systems (Rauw, 2009).

Adopting a more integrated approach could enhance the sustainability of livestock production systems, ensuring that animals can thrive while meeting production goals (Friggens et al., 2017; Rauw and Gomez-Raya, 2015).

Paternal rabbit lines

The implications of resource allocation are particularly evident in the breeding practices of paternal rabbit lines.

Paternal rabbit lines are usually selected for ADG during the fattening period or by weight at slaughter by individual selection (Baselga, 2004). These lines contribute to the three-way crossbreeding scheme where crossbred females from maternal lines selected for reproductive traits are paired with males from a paternal line chosen exclusively for growth criteria. This strategy provides a large number of young rabbits with rapid growth and high feed efficiency. Consequently, using paternal lines in rabbit production has significantly improved growth performance and feed conversion ratio (FCR) since rabbits reach heavier weights in less time (Baselga, 2004).

However, despite the advantages of the selection for growth rate, concerns arise regarding the reproductive performance of paternal lines. The R line, a paternal line developed by the Universitat Politècnica de València, will be used as an example. Originated from the fusion of two paternal lines, one was established in 1976 with California rabbits bred by Valencian farmers, while the other was founded in 1981 with rabbits from specialised paternal lines; the R line underwent individual selection based on post-weaning daily gain (Estany et al., 1992; Nagar et al., 2020). Nonetheless, various studies indicate that this paternal line exhibits poorer reproductive performance, such as a decreased maternal instinct and a lower litter size than maternal lines (Gómez et al., 1999). Additionally, García-Quirós et al. (2014) found that animals from the R line presented greater sensitivity to immunological challenges, potentially attributed to lower innate and adaptative immune cells (García-Quirós et al., 2014; Naturil-Alfonso et al., 2016). Sánchez et al. (2012) further observed a higher

incidence of mastitis and ulcerative pododermatitis in the R line compared to three different genetic lines.

The concerns mentioned above represent a considerable obstacle to the profitability of rabbit farming. A study by Rosell and De la Fuente (2009) showed that the prevalence of illness or reproductive complications eliminates two-thirds of female rabbits. This high culling rate and the current practice of high replacement by low productivity level lead to a considerable replacement rate, often resulting in the introduction of animals from other farms (Nagar et al., 2020), which can inadvertently lead to the spread of diseases within the population. Consequently, a decline in reproductive traits and high mortality could negatively impact selection pressure. As the pool of available individuals for selection decreases due to mortality and illness-related culling, the number of rabbits with the desired traits becomes increasingly scarce, posing challenges for effective breeding and genetic improvement programs.

Considering these obstacles, there is a pressing need for animals with robust productivity traits and the ability to balance production, health, and reproductive efficacy effectively (Rauw, 2009; García-Quirós et al., 2014). Addressing these concerns is essential to ensure the long-term sustainability and profitability of rabbit farming.

The Robust Path to Sustainability

According to Knap (2008), sustainable breeding aims to balance traits that contribute to animal production and those that make animals more adaptable to their environment. The goal is to ensure that as genetic selection is carried out to improve production traits, it does not inadvertently make animals more vulnerable to environmental stressors or less adaptable to changing conditions. Instead, it aims to achieve a harmonious equilibrium between improved productivity and increased environmental sensitivity or robustness. Robustness is a complex concept studied and defined by many authors. Knap (2005) defines robustness as the ability to express high production potential in different environmental conditions due to a combination of high production potential and resilience.

In line with this vision, contemporary livestock selection programs are prioritising functional traits and integrating robustness and adaptability characteristics alongside productivity (Berghof et al., 2019). This tactical change mirrors Knap's notion of robustness by ensuring animals maintain high production potential across varying environments. Furthermore, including functional diversity within these selection programs enhances resilience by expanding the array of traits available, thus increasing responses to disturbances (Messier et al., 2019). Resilience, in contrast to robustness, refers to the ability of an organism to recover from disturbances or stressors (Friggens et al., 2022). Consequently, species are better equipped to compensate for lost functions during disturbance events (Destoumieux-Garzón et al., 2021).

The LP maternal rabbit line exemplifies the integration of robustness and adaptability, aligning with the principles of sustainable breeding. The LP line, founded between 2002 and 2003, exemplifies specialised breeding efforts to maximise reproductive longevity. Selected through a rigorous process, only a fraction of females (two to five per thousand) were chosen based on strict criteria, requiring a minimum of 25 parturitions with at least 7.5 born alive kits each. After identifying these females through screening across Iberian Peninsula farms, a breeding program started at Universitat Politècnica de València. The newly founded line, LP, was bred to enhance litter size at weaning, mirroring conditions applied to other maternal lines developed at the university (Sánchez et al., 2008). These animals show an extended productive lifespan, a constant reproductive effort, and better innate immune responses (Ferrián et al., 2012; García-Quirós et al., 2014; Savietto et al., 2015; Penades et al., 2018).

One potential solution to reduce problems in paternal lines would be selecting “elite” animals from current paternal populations, with the aim of achieving a balance between reproduction and growth rate. Additionally, the introduction of genetic robustness, defined as the ability to withstand environmental challenges without compromising reproductive capacity (Pascual et al., 2013; Friggens et al., 2017), could result in resilient animals with high reproductive capability (García-Quirós et al., 2014).

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OBJECTIVES

Justification

Rabbits have emerged as a particularly effective model for exploring genetic selection models due to their low generation interval and efficient feed conversion. However, the success of genetic selection in rabbits, as in other livestock species, has sometimes come with unintended consequences, such as decreased resistance to diseases or environmental stressors. In contrast, recent advancements in selection criteria have demonstrated that prioritising genetic robustness alongside productivity traits can yield more robust animal populations. We expect to reconcile high productivity with enhanced health traits by introducing fitness traits into breeding programs.

This dissertation proposes the evaluation of experimental paternal stocks reconstituted by means of selecting more efficient animals and incorporating fitness traits. This approach aims to address the limitations inherent in traditional breeding methods. Our work employs an experimental model with genetic material provided by the Universitat Politècnica de València and intends to assess how this process may impact the original genetic type. This thesis aimed to test whether populations obtained through this process have modified the fitness related traits of reproducing rabbit does.

Hypothesis and objectives

Our hypothesis originates from acknowledging that while successful in increasing productivity, conventional breeding methods have often neglected the broader implications of animal welfare and robustness.

We hypothesised that obtaining a population that maintains production could be possible without compromising health and reproduction. Therefore, our approach aims to balance functional and growth traits. By testing an experimental model using paternal lines through carefully selecting more efficient animals (RF) and integrating genetics with enhanced robustness, we believe that we can effectively address the challenges associated with paternal lines, ensuring improved outcomes in both performance and robustness.

For this dissertation, three specific objectives were defined and explored in three consecutive experiments.

1. To understand the patterns of resource acquisition and allocation among fitness traits of rabbit females of three genetic types (solely selected for growth, founded from elite animals or selected from elite animals and backcrossed with a long-lived and productive line).
2. To assess feed efficiency and health status of growing rabbits from the genetic types mentioned above.
3. To characterise and compare immune profiles of primiparous rabbit females of these three diverging genetic types.

CHAPTER I

Reproductive performance of rabbit females from three paternal lines with a different potential for growth rate and resilience

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Abstract

A total of 197 nulliparous rabbits (from three paternal lines) were used to test potential strategies to overcome the consequences on reproduction associated with the selection for high growth rate. The R line was selected for growth rate during the growing period for 37 generations, the RF line was founded through a high selection intensity of elite animals of the R line, and the RFLP line, which was obtained by backcrossing RF animals with the LP line (a long-lived productive maternal line, characterised by high resilience). Body weight, perirenal fat thickness, fertility, daily feed intake, milk yield and blood metabolites of females were controlled from 1st artificial insemination to 3rd parturition. Litter size, litter weight, individual weight and feed ingestion of kits were controlled from birth to weaning. Our results show that RF females were significantly lighter than R and RFLP females throughout the trial (-5.0%; $P < 0.05$). Furthermore, RF animals had a higher fertility rate than RFLP females, at first cycle (+10.5 percentage points; $P < 0.05$). However, RFLP had a higher fertility rate than RF females at second cycle (+21.5 percentage points; $P < 0.01$). On average, RFLP females had higher perirenal fat thickness than R females at parturition (+3.0%; $P < 0.05$) and higher daily feed intake than of R and RF females during gestation and late lactation (+9.7% and +8.7%, respectively; $P < 0.05$). RFLP females produced more milk than R and RF females in the two first lactations (+18.5%; $P < 0.001$). In the first three parturitions, R females delivered fewer kits born alive (-1.7 kits than RF and RFLP; $P < 0.05$). In addition, R females' blood had a higher concentration of glutamine and glutamate than RFLP (+24% and +22.7%, respectively; $P < 0.05$). RFLP litters were heavier than both R and RF litters throughout lactation. However, R kits were heavier at birth than RF and RFLP (+7.9 %). Results suggest that the foundation of a paternal line using elite animals could generate females with better early reproductive performance. In addition, backcrossing the RF line with a maternal LP line resulted in a genetic line whose females had a different resource allocation strategy to foster reproduction during the studied period.

Keywords: reproduction, genetic, resources allocation, foundation, robustness.

Introduction

Deciding the selection objective is perhaps one of the most important steps in animal breeding, as it determines the course of action to achieve the best results. Genetic selection has come a long way, from focusing primarily on economic traits to searching for more balanced, productive, and healthy animals in the last decades (Berghof et al., 2019). Genetic selection for growth rate has been successful in non-ruminant animals (Havenstein et al., 2003; Suzuki et al. 2005). In rabbits, main selection programs show that selection for growth rate has allowed an improvement in average daily gain (ADG) of 0.45 to 1.23 g/d per generation of selection (Estany et al., 1992; Piles and Blasco, 2003), but some authors indicate the genetic progress has been lower than expected in recent years (Blasco et al., 2018).

Paternal lines selected for ADG during the growing period play a key role in rabbit production. These lines are used as a terminal crossing to achieve the final commercial lines reared for meat production (Naturil-Alfonso et al., 2017). However, low reproductive performances have been reported in relation to selection for growth rate (Roberts, 1979). Studies concerning rabbit females showed inadequate responses to ovulation and exacerbated losses during embryonic implantation in a paternal line (Naturil-Alfonso et al., 2015). In addition, rabbit males from a paternal line displayed abnormal sexual behaviour, such as low libido and sperm count (Pascual et al., 2004).

Highly specialised rabbits prioritise the functions for which they were selected (Savietto et al., 2015), reducing the resources available for other functions. For example, innate and adaptive immune cell count seems to be negatively influenced by selection for productive criteria, being lower in lines selected by growth rate in both pigs (Clapperton et al., 2008) and rabbits (García-Quirós et al., 2014).

Lower milk production and extended longevity have been associated with higher resilience in dairy cattle (Pope et al., 2020). Moreover, nutrient partitioning changes associated with selection for ADG can also affect the offspring's resilience. In addition, a higher incidence of digestive disorders, such as epizootic enteropathy, was described in a paternal line than in a line founded for productive longevity (García-Quirós et al., 2014).

Genetic selection exclusively addressed to increase production would not be sustainable in the future because of its negative impact on animals' functionality (García-Quirós et al., 2014). So, a question arises, can resilience traits be introduced in breeding programs without compromising genetic progress? Bergohf et al. (2019) suggested that the inclusion of resilience in breeding programs favours health and simplifies the health management of animals. Since resilient animals are less sensitive, this strategy allows the farmer to manage large groups of animals better.

We evaluated two strategies to mitigate the potential negative effects of the selection for growth rate in a paternal rabbit line: firstly, the foundation of a new line from a population of elite animals from the original paternal line (high growth and reproduction characteristics); and secondly, the creation of another population by backcrossing this elite paternal line with a long-lived productive maternal rabbit line. This work aimed to investigate how these newly founded lines for rabbit females acquire and allocate resources and its consequences on their reproductive performance.

Material and methods

The experimental procedure was approved by the Animal Welfare Ethics Committee of the Universitat Politècnica de València (UPV; code: 015/VSC/PEA/00061) and carried out following the recommendations of the European Group on Rabbit Nutrition (Fernández-Carmona et al., 2005) and the Spanish Royal Decree 53/2013 on the protection of animals used for scientific purposes.

Animals

A total of 197 rabbit females from 3 paternal lines were used for this experiment. The animals belonged to lines R (n=65), RF (n=63) and RFLP (n=69), developed by the Animal Breeding Group of the Universitat Politècnica de València in Spain. The R line is a paternal line selected by growth rate during the growing period for 37 generations (Estany et al., 1992). The RF line was founded by selecting a population of elite R animals from two genetic selection centres, which had a high growth rate during the growing period (more than 60 g/d; ADG of the final population: 61.94 g/d). These animals were subjected to an open nucleus breeding system. Lastly, the RFLP line was created by backcrossing the males of the RF line with females of the maternal line LP, generating 7/8 RF and 1/8 LP animals. The LP line was founded by productive

longevity criteria (minimum of 25 parturitions and 7.5 kits born alive; Sánchez et al., 2008). Animals from the LP line were found to be less affected when exposed to environmental changes and stress, suggesting these animals are more resilient than other rabbit lines (Saviotto et al., 2015; Arnau-Bonachera et al., 2018b).

Animals' housing, diets and management

This trial was carried out from January 2020 to January 2021. The animals were kept in a controlled environment subjected to a photoperiod of 16 hours of light and 8 hours of darkness, with mechanical ventilation and cooling panels to maintain the farm's temperature between 15 to 22°C. The rabbit females were housed in individual wired cages for reproductive rabbit females (W 500 × L 700 × H 320 mm) from the start of the trial, at 9 weeks of age, until the end of the trial at the third parturition.

All animals were fed two different commercial diets throughout the experiment. The first diet for growing rabbits (158 g crude protein, 25 g ether extract and 231 g crude fibre per kg of feed; Cunivita, Nanta S.A.) was provided from 9 weeks until the first parturition. From this moment to the end of the trial, the second diet for reproductive rabbit females was provided (193 g crude protein, 40 g ether extract and 169 g crude fibre per kg of feed; Cunilactal, Nanta S.A.). The animals had free access to feed and water throughout the experimental period.

The first artificial insemination (AI) of young rabbit females was performed when the females reached 20 weeks of age, and the subsequent AI was performed 11 days postpartum. Pregnancy was tested by manual palpation at 11 days post-AI. Non-pregnant rabbits were inseminated again 21 days after the previous AI, until a maximum of 3 attempts when females were culled for reproductive reasons. After the 28th day of pregnancy, maternal cages equipped with an external nest box were provided for the litter (W 400 × L 220 × H 380 mm).

At parturition, the number of kits per litter was standardised within line to 6 for the 1st lactation and to 8 from the 2nd lactation onwards. For this adjustment, nursing females from the same genetic lines and synchronised to experimental females were used. On the 18th day post-partum (dpp), the litters were separated from the mothers to separately control feed intake as well as milk yield until weaning. The kits were fed the same diet for young rabbits described above. Litters were weaned at 28 dpp.

Females's body weight (BW), feed intake and perirenal fat thickness (PFT; using ultrasounds following Pascual et al., 2000 and 2004) were monitored at first AI, and then at each parturition, 18 dpp and weaning. Daily milk production was registered five days per week (from Monday to Friday) using the doe weight-suckle-weight method, always at 8:00 a.m, during the first two lactations. To prevent free nursing, the nest boxes were closed between nursings from parturition to 18 dpp. From 18 dpp to weaning, litters were housed in a wired cage (W 500 × L 700 × H 320 mm) close to their mother to control milk production of the female and feed consumption of the litter.

Females' fertility, defined as the number of parities per insemination (Martínez-Paredes et al., 2018), was also registered. Litter size and weight were controlled at birth (total, alive and standardised), 18 dpp and weaning. Litter feed intake was controlled from 18 dpp until weaning. The mortality of mothers and kits was recorded daily.

Blood sampling and plasma chemical analysis

Blood samples were collected from females at first AI, parturition, 18 dpp and weaning until the third parturition, until obtaining the complete sample sets of 16 females from genetic type to be analysed. This subpopulation had quite similar fertility rates throughout the trial to the population constituted by rabbit does that reached the third parturition. Thus, it can be considered a good representative. On sampling day, feeders were closed at 07:00 h, and blood samples were taken starting at 11:00. The samples were collected from the central ear artery into tubes containing ethylenediaminetetraacetic acid. Blood samples were centrifuged immediately after sampling (3000 × g, 4°C and 10 min). The plasma samples were stored at -40°C until further analysis for glucose, non-esterified fatty acids (NEFA), β -OH-butyrate, leptin, glutamine, and glutamate.

Glucose was determined by the hexokinase method using an ADVIA 1800 (Siemens Diagnostics® Clinical Methods for Tarrytown, NY, USA). Non-esterified fatty acids were determined using the NEFA C ACS-ACOD assay method (Wako Chemicals GmbH, Neuss, Germany). The concentration of β -OH-butyrate was determined according to Harano et al. (1985) as an increase in absorbance at 340 nm due to NADH production at slightly alkaline pH in the presence of β -OH-butyrate dehydrogenase. For this technique, sample blanks were included, and oxamic acid was used in the media to inhibit lactate dehydrogenase. To determine glucose, NEFA,

β -OH-butyrate an auto-analyser, ADVIA 1800® Chemistry 53 System was used (Siemens Medical Solutions, Tarrytown, NY 10591, USA). The intra- and inter-assay CV were consistently below 2.0% and 4.0%, respectively. According to the manufacturer's guidelines, leptin was analysed by Multi-Species Leptin assays (RIA, XL-85K) (Millipore Corporation, Billerica, MA, USA). Intra- and inter-assay CV were 9.1% and 9.3%, respectively. Analyses of L-glutamine and L-glutamate were performed according to Larsen and Fernández (2017).

Statistical analysis

Data corresponding to female rabbits (BW, daily feed intake, PFT and milk yield) and litter traits (litter size, litter weight and kit weight and intake) were analysed with a model that included the genetic type (3 levels, R, RF and RFLP), the season within the year (4 levels), the parity order (3 levels 1, 2 and 3), the overlapping of lactation with the previous or posterior gestation (2 levels, yes or not) and their interactions as fixed effects.

For plasma traits, the model included the genetic type (3 levels, R, RF and RFLP), the season within the year (4 levels), the control day (8 levels, 1st AI, 1st parturition, 1st 18 dpp, 1st weaning, 2nd parturition, 2nd 18 dpp, 2nd weaning and 3rd parturition) and the overlapping with the previous or posterior cycle (2 levels, yes or not) and their interactions as fixed effects. The triple interaction among genetic type, parity order and overlapping were not significant for any of the traits analysed.

Performance and plasma traits were analysed using a mixed model (PROC MIXED by Statistical Analysis System (SAS), 2002) in a repeated measure design. Random terms in the model included a permanent effect of each animal (p) and the error term (e), both assumed to have an average of 0 and variance σ_p^2 and σ_e^2 . A t-test was used for the comparison of the least-square means. Fertility percentages were analysed using a chi-square test to determine the effect of the genetic type by a CATMOD procedure of SAS (2002). Significant differences were declared at $P < 0.05$.

Results

Reproductive performance of females

The effect of the genetic type on the reproductive performance of rabbit females from the first AI to the third parturition is presented in Table 1.1. The possible effect of parity order, overlapping with the previous and following reproductive cycle and their interactions with the genetic type, is also shown. Overall data shows pregnant females had higher BW than non-pregnant females at weaning (+322 g; $P < 0.001$; data not in tables) and BW at parturition increased with the parity order ($P < 0.001$). As regards the genetic type, RF females were significantly lighter than R and RFLP females throughout the trial (on av. -4.6, -5.9, -4.8 and -4.7% at first mating, parturition, 18 and 28 dpp, respectively; $P < 0.05$).

However, although RF and RFLP females reached their adult weight at the second parturition, R females' BW still increased until the third parturition (Figure 1.1). This effect was observed independently of the overlapping of gestation with the previous lactation.

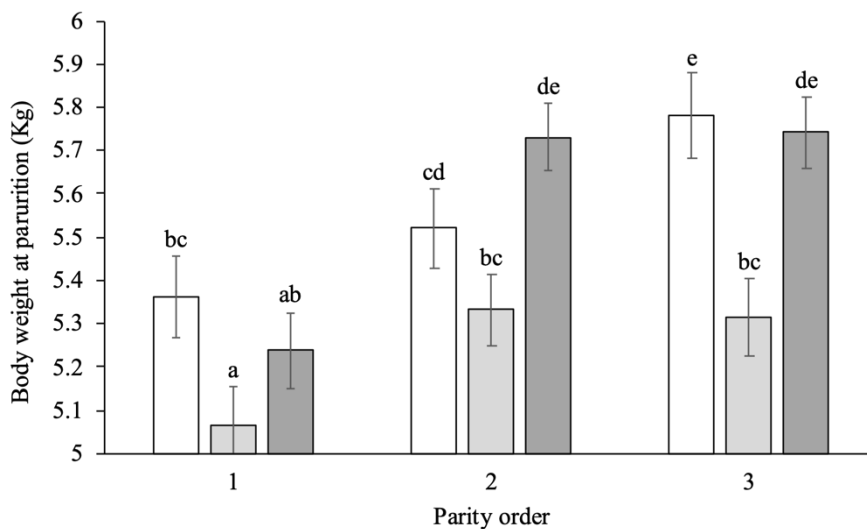


Figure 1. Effect of genetic type (R □, RF ■ and RFLP ■) and parity order on the body weight of rabbit females at parturition. ^{a,b,c,d,e} Means not sharing letter were significantly different at $P < 0.05$.

Table 1.1. Effect of genetic type (R, RF and RFLP) on the reproductive performance of rabbit females from first mating to third parturition.

	Genetic type (GT)				P-value			
	R	RF	RFLP	GT	PO	GT×PO	O	GT×O
No. females at first mating	65	63	69					
Body weight (g):								
First mating	5578 ± 71 ^b	5328 ± 71 ^a	5586 ± 72 ^b	0.016				
Parturition	5555 ± 74 ^b	5237 ± 67 ^a	5570 ± 64 ^b	0.001	0.000	0.037	0.006	0.026
18 dpp	5950 ± 85 ^b	5645 ± 75 ^a	5914 ± 78 ^b	0.007	0.177	0.880	0.070	0.685
28 dpp	5951 ± 87 ^b	5694 ± 77 ^a	5995 ± 80 ^b	0.009	0.553	0.338	0.000	0.462
Perirenal fat thickness (mm):								
First mating	8.87 ± 0.09	8.80 ± 0.09	8.74 ± 0.09	0.601				
Parturition	8.35 ± 0.09 ^a	8.45 ± 0.08 ^{ab}	8.60 ± 0.08 ^b	0.091	0.017	0.303	0.053	0.790
18 dpp	8.71 ± 0.10	8.79 ± 0.09	8.81 ± 0.09	0.715	0.652	0.390	0.644	0.090
28 dpp	8.80 ± 0.1	8.86 ± 0.09	8.95 ± 0.09	0.448	0.064	0.524	0.910	0.211
Daily feed intake (g DM/d):								
First gestation	198 ± 4 ^b	186 ± 4 ^a	211 ± 4 ^c	0.000	0.000	0.847	0.630	0.166
0 to 18 dpp	304 ± 9	312 ± 8	322 ± 8	0.263	0.000	0.439	0.129	0.971
18 to 28 dpp	342 ± 11 ^a	341 ± 10 ^a	371 ± 10 ^b	0.042	0.000	0.002	0.130	0.136
Milk yield (g/d):								
1 to 18 dpp	132 ± 6 ^a	139 ± 5 ^a	158 ± 5 ^b	0.002	0.000	0.466	0.001	0.106
18 to 28 dpp	148 ± 8 ^a	153 ± 7 ^a	182 ± 7 ^b	0.000	0.000	0.001	0.170	0.399
Fertility (%):								
1 st cycle	86.7 ^{ab}	95.4 ^b	85.0 ^a	0.041				
2 nd cycle	70.9 ^{ab}	62.8 ^a	83.3 ^b	0.005				
3 rd cycle	67.4	73.1	75.0	0.400				

PO= parity order; O= overlapping with the previous and following reproductive cycle; dpp= days post parturition.

^{a-c} Means not sharing letter were significantly different at P<0.05.

Regarding the body condition, genetic type did not affect PFT at first mating. However, R females had higher PFT at first mating in spring and summer than in winter, while this trait was not affected by the season in RF and RFLP females (Figure 1.2). In general, genetic type did not affect the body condition of females (Table 1.1), but RFLP females had a higher PFT than R females at parturition (on av. +3.0% for the three parturitions; $P < 0.05$).

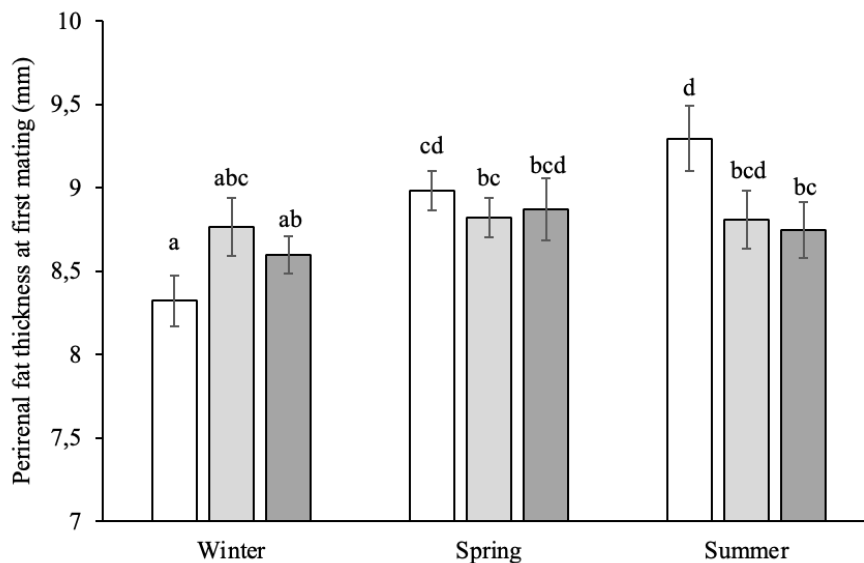


Figure 1.2. Effect of genetic type (R □, RF ■ and RFLP ■) and season on the perirenal fat thickness of rabbit females at first mating. ^{a,b,c,d} Means not sharing letter were significantly different at $P < 0.05$. No. of females: winter (20 R, 15 RF and 37 RFLP); spring (33 R, 33 RF and 16 RFLP); summer (12 R, 15 RF and 16 RLP).

Daily feed intake (DFI) during the first gestation of RFLP females was higher than that of R and RF females (+6.3 and 13.3%, respectively; $P < 0.001$), whereas it was lower in RF than in R females (-6.2%; $P < 0.001$). From 18 to 28 dpp, RFLP females had a higher daily feed intake compared with the other genetic types (on av. +8.7%; $P < 0.05$). This difference was mainly due to their higher feed intake during the second lactation (Figure 1.3, left).

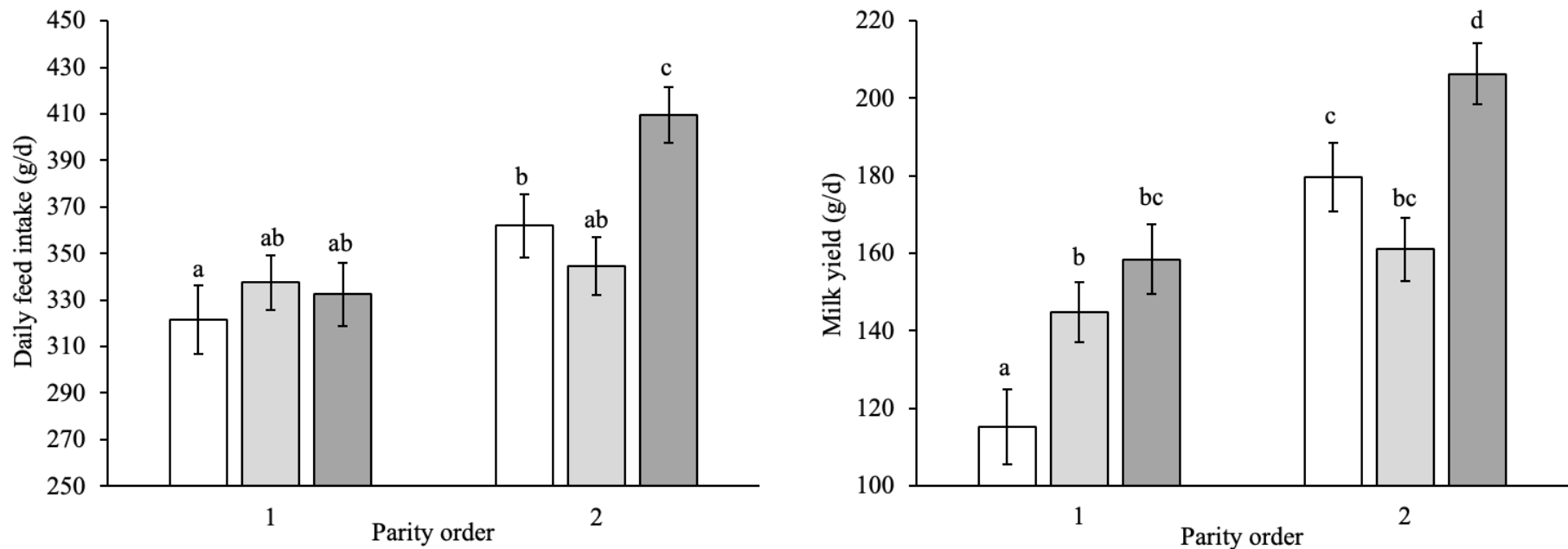


Figure 1.3. Effect of genetic type (R □, RF ■ and RFLP ■) and parity order on the daily feed intake (left figure) and milk yield (right figure) of rabbit females from 18 to 28 days postpartum. ^{a,b,c,d} Means not sharing letter were significantly different at P<0.05.

Regarding milk production, RFLP females yielded more milk than other genetic types during the whole lactation (on av. +18.5%; $P < 0.001$). However, at late lactation, RF and RFLP yielded more milk than R females during the first cycle, while RFLP yielded more than RF and R females during the second cycle (Figure 1.3 right). Fertility was also affected by the genetic type (Table 1.1). RF females had higher fertility than RFLP females in the first cycle (+10.5 percentage points; $P < 0.05$). On the contrary, RFLP females had higher fertility than RF females in the second cycle (+21.5 percentage points; $P < 0.01$).

Overall, the genetic type did not affect the concentration of most of the blood energetic measured metabolites (glucose, NEFA, BOHB and leptin). However, as shown in Figure 1.4, RFLP females had a higher leptin level at the first parturition (+38.3% compared to RF $P < 0.05$) and the lowest leptin level at the first weaning (-17.2% compared to R $P < 0.05$) than the other genetic types.

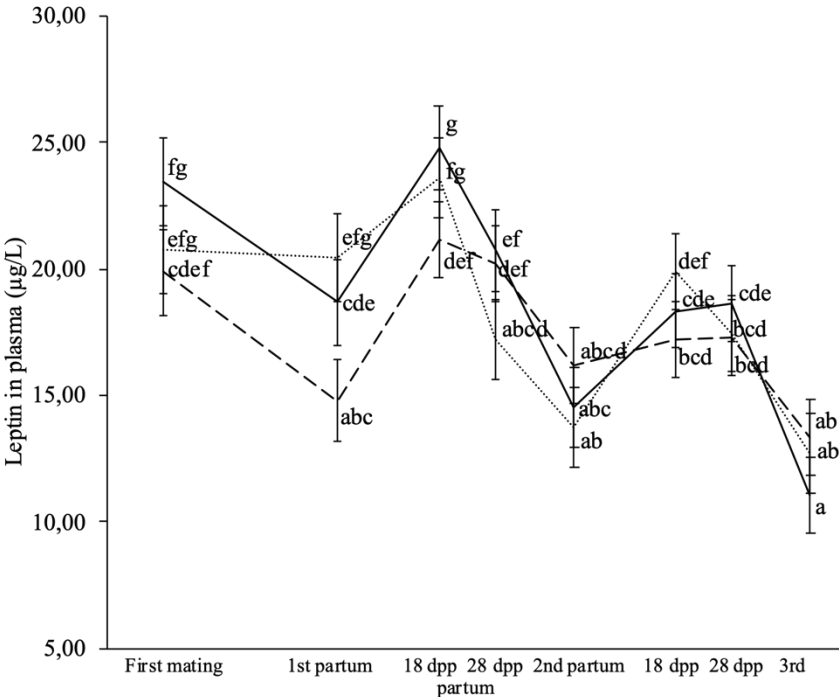


Figure 1.4. Effect of genetic type [R (—), RF (---) and RFLP (·····)] and physiological state on plasma concentration of leptin from the first mating to the third parturition. ^{a,b,c,d,e,f,g} Means not sharing letter were significantly different at $P < 0.05$.

As shown in Table 1.2, the concentration of glutamine in the blood of R females was significantly higher than that of RF and RFLP females throughout the trial (on av. +27.5%: $P < 0.001$), especially at the first parturition (Figure 1.5, left).

Table 1.2. Effect of genetic type (R, RF and RFLP) on the blood metabolites concentration in rabbit females from first mating to third parturition (n=384).

	Genetic type (GT)			P-value
	R	RF	RFLP	
No. females	16	16	16	
Glucose (mM)	8.81 ± 0.22	8.37 ± 0.22	8.75 ± 0.22	0.304
NEFA (µekv/L)	589 ± 24	556 ± 24	574 ± 25	0.627
BOHB (log ₁₀ mM)	-1.07 ± 0.04	-1.14 ± 0.04	-1.16 ± 0.04	0.314
Leptin (µg/L)*	18.8 ± 0.8	17.5 ± 0.8	18.2 ± 0.8	0.503
Glutamine (µM)*	103 ± 3 ^b	84 ± 3 ^a	78 ± 3 ^a	0.000
Glutamate (µM)*	633 ± 14 ^c	462 ± 14 ^a	489 ± 14 ^b	0.000

*Interaction genetic type with the sampling day ($P < 0.05$).

NEFA= non-esterified fatty acids; BOHB= beta-hydroxy butyrate.

^{a-c} Means not sharing letter were significantly different at $P < 0.05$.

In addition, females from genetic line R had +37.0 and +29.4% higher blood glutamate than RF and RFLP, respectively ($P < 0.001$), and RFLP females had +6% more blood glutamate than RF ($P < 0.001$). The blood glutamate concentration in RFLP females was rather stable throughout the trial, while it was affected by time in R and RF females (Figure 1.5, right).

Litter traits

According to Table 1.3, R females delivered fewer kits born alive (on av. -1.7 kits) and total born (on av. -1.3) than RF and RFLP. On the other hand, RF and RFLP females that overlapped pregnancy with the previous lactation delivered more kits born in total than those without overlapping (+2.77 and +1.52, respectively; $P < 0.05$; data not in tables). At 18 dpp, litter size from RFLP females was larger compared with R and RF litters (on av. +0.53; $P < 0.05$) due to the lower survival of R and RF kits at the first and second lactations, respectively (Figure 1.6, left). On average, females whose gestation overlapped with the previous lactation had more kits born alive (+1.86 kits; $P < 0.001$).

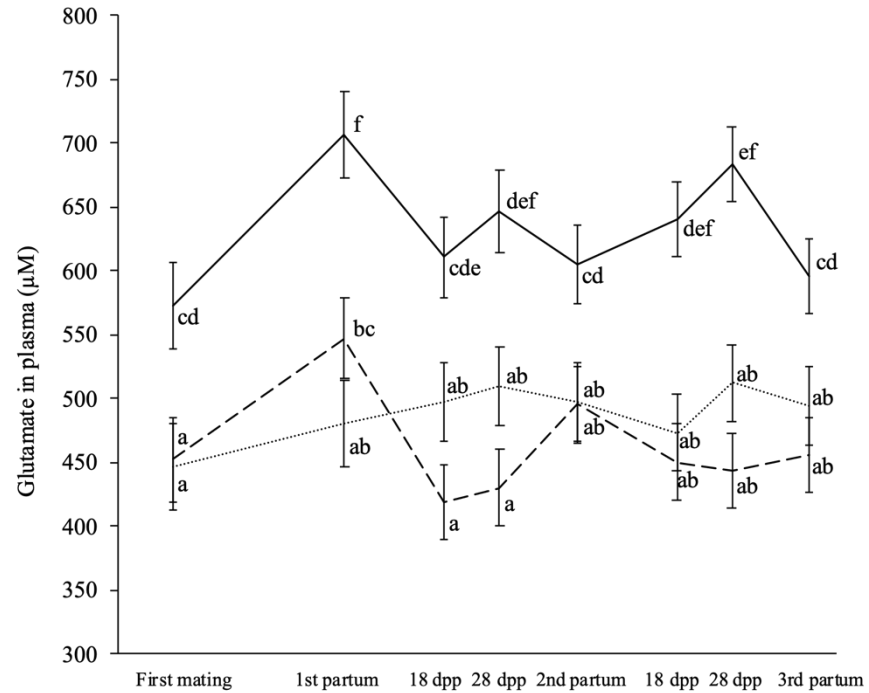
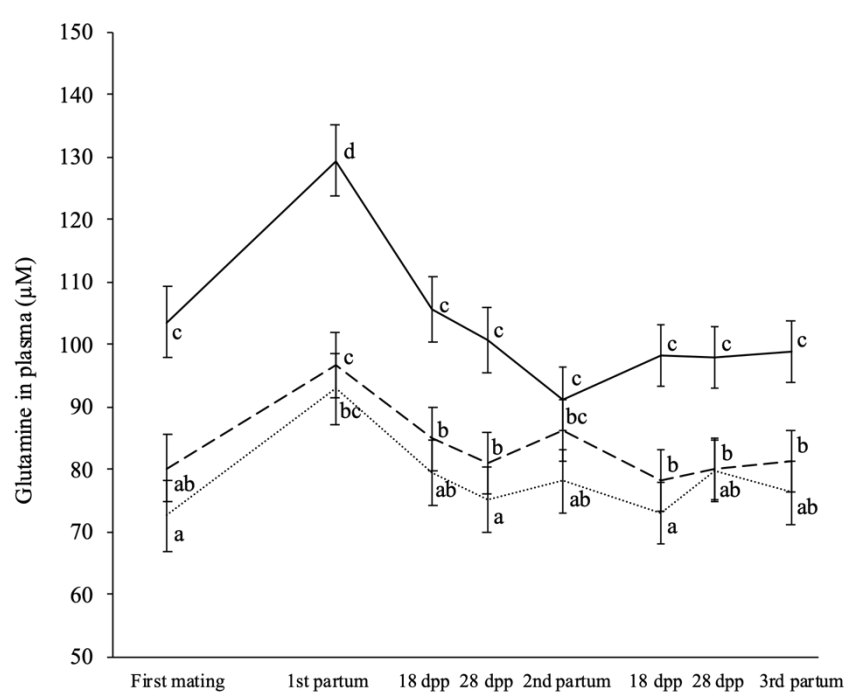


Figure 1.5. Effect of genetic type [R (—), RF (---) and RFLP (·····)] and sampling day on plasma concentration of glutamine (left figure) and glutamate (right figure) from the first mating to the third parturition.

Litters from R females were significantly lighter than RFLP ones (-23.6% alive at birth, -12.2% total at birth, -25.5% at 18 dpp and -27.8% at 28 dpp; $P < 0.05$). However, the weight of R litters standardised at birth was higher (+4.2% than RF litters and +5% than RFLP; $P < 0.05$) due to the higher weight of R kits at birth (on av. +7.9% concerning RF and RFLP). As shown in Table 1.3 and Figure 1.6 (right), kits of RFLP and RF lines were heavier than those from the R line during the first lactation (on av. +21.4 and +18.2% at 18 and 28 dpp, respectively; $P < 0.05$), while kits of RFLP were heavier than those from R and RFLP lines at 28 dpp of the second lactation (on av. +8.7%; $P < 0.05$). Due to the larger size and heavier weight of their kits, RFLP litters were heavier than both R and RF litters throughout lactation (+18.0 and 27.9% with respect to RF and R litters, respectively; $P < 0.001$). Concerning the kits' intake from days 18 to 28 postpartum, RFLP kits had a higher feed intake than R and RF kits (on av. +46.8%; $P < 0.001$).

Discussion

Effect of genetic type on females' reproductive performance

When comparing genetic types, RF females had lower average BW (5% lighter) than R and RFLP females. RF animals belong to a population founded by selecting animals with a high growth rate, but the population obtained was also characterised by a better reproductive performance. Selection for growth rate can increase the BW of young rabbit females (Marín-García, 2019), and genetic lines with a high reproductive capacity are lighter than those with a high growth rate (Arnau-Bonachera et al. 2017). Therefore, it is possible that a higher allocation of resources to reproduction could have affected the adult BW of the RF females.

On the other hand, R and RFLP females had a similar BW throughout the experiment. Although the RFLP line has 1/8 LP origin, which could hypothetically limit females' growth compared to a paternal line, Theilgaard et al. (2007, 2009) reported that LP females had a higher BW than other maternal lines. This characteristic and other heterotic effects could have reduced the likely effect on females' BW caused by the backcrossing of the R line with this maternal line.

Table 1.3. Effect of genetic type (R, RF and RFLP) on litter traits during lactation until third parturition.

	Genetic type (GT)			GT	PO	P-value		
	R	RF	RFLP			GT×PO	O	GT×O
No. of litters at first parturition	48	57	58					
Litter size:								
Alive at birth	5.16 ± 0.44 ^a	6.77 ± 0.38 ^b	6.98 ± 0.35 ^b	0.003	0.923	0.119	0.000	0.046
Total at birth	7.40 ± 0.36 ^a	8.54 ± 0.32 ^b	8.77 ± 0.29 ^b	0.011	0.619	0.247	0.007	0.023
Standardised at birth	6.67 ± 0.19	6.98 ± 0.16	7.25 ± 0.15	0.057	0.000	0.054	0.003	0.120
18 dpp	6.01 ± 0.18 ^a	6.08 ± 0.16 ^a	6.58 ± 0.16 ^b	0.017	0.000	0.033	0.075	0.183
28 dpp	5.88 ± 0.21	5.99 ± 0.19	6.38 ± 0.19	0.115	0.000	0.019	0.132	0.425
Litter weight (g):								
Alive at birth	333 ± 26 ^a	400 ± 22 ^{ab}	412 ± 21 ^b	0.048	0.226	0.106	0.000	0.107
Total at birth	453 ± 21 ^a	488 ± 18 ^{ab}	508 ± 17 ^b	0.119	0.122	0.059	0.001	0.024
Standardised at birth	454 ± 8 ^b	436 ± 8 ^a	433 ± 7 ^a	0.041	0.000	0.069	0.302	0.067
18 dpp	1660 ± 82 ^a	1838 ± 75 ^a	2083 ± 76 ^b	0.000	0.000	0.009	0.357	0.326
28 dpp	2776 ± 140 ^a	3008 ± 125 ^a	3550 ± 129 ^b	0.000	0.000	0.001	0.407	0.277
Kit weight (g):								
Alive at birth	65.0 ± 1.4 ^b	60.1 ± 1.2 ^a	60.4 ± 1.1 ^a	0.019	0.000	0.933	0.888	0.481
Total at birth	63.9 ± 1.4 ^b	58.8 ± 1.2 ^a	59.6 ± 1.1 ^a	0.014	0.000	0.441	0.910	0.814
Standardised at birth	64.1 ± 1.2	61.8 ± 1.1	61.6 ± 1.1	0.132	0.000	0.151	0.357	0.156
18 dpp	272 ± 9 ^a	303 ± 8 ^b	317 ± 9 ^b	0.001	0.012	0.041	0.739	0.895
28 dpp	471 ± 16 ^a	508 ± 12 ^a	558 ± 15 ^b	0.000	0.497	0.105	0.574	0.622
Kit intake 18 to 28 dpp (g dry matter/d)	3.66 ± 0.41 ^a	3.93 ± 0.35 ^a	5.57 ± 0.37 ^b	0.001	0.156	0.097	0.847	0.877

^{a,b} Means not sharing letter were significantly different at P<0.05.

PO= parity order; O= overlapping with the previous and following reproductive cycle; dpp= days post parturition.

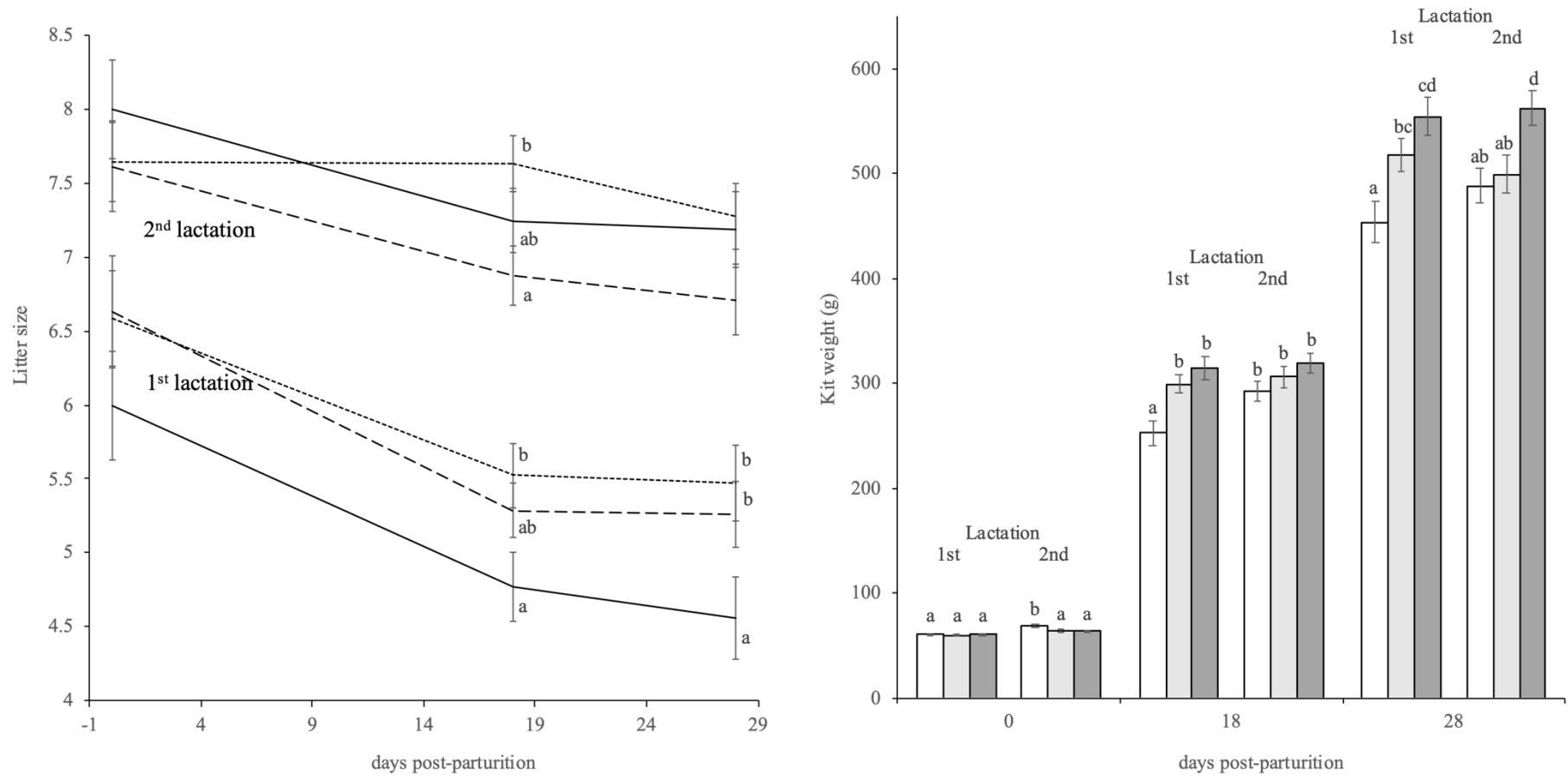


Figure 1.6. Effect of genetic type [R (— and □), RF (--- and ■) and RFLP (····· and ■)] and day post parturition on the litter size (left figure) and individual kit weight (right figure) during the first two lactation. Litter size was standardised to 6 and 8 at the first and second parity, respectively. ^{a,b,c,d} Means not sharing letter within lactation and day postpartum were significantly different at P<0.05

The genetic type had little impact on body condition. However, RFLP females had the most stable PFT values, with minimal loss of body condition at parturition. This different response may be due to LP genetics, whose patterns for acquisition and allocation of resources are more adapted to the changing needs depending on the physiological state (Savietto et al., 2015; Arnau-Bonachera et al., 2018a). According to Theilgaard et al. (2006), PFT can be a valuable indicator of how animals prioritise the distribution of their resources, and body condition is essential to maintain rabbit females' life functions while assuring the survival of their litter. In addition, Martínez-Paredes et al. (2018) described PFT at first mating as the most important trait that guarantees females' reproductive longevity. These findings support the hypothesis that the availability of a more stable body condition of RFLP females throughout the reproductive cycles could indicate a better ability to face environmental constraints.

On the other hand, the body condition of R females' at first mating fluctuates more depending on the season than the other lines. In addition, R females had the lowest PFT at parturition. A lower body condition around parturition could compromise R females' fertility during the following cycles (Savietto et al., 2016). The mobilisation of resources around parturition is a natural reproductive strategy for rabbit females, but the distribution of resources among the different vital functions might change depending on the selection criteria (Pascual et al., 2013). In fact, selected animals prioritise the selected criteria through a resource allocation change, which could influence animals' health and reproduction (Penadés et al., 2018; Arnau-Bonachera et al., 2018b).

RFLP females had higher DFI at gestation and late lactation. Multiple studies with LP females had that these animals have a higher DFI than the average maternal genetic lines (Savietto et al., 2015). In addition, Arnau-Bonachera et al. (2018a) demonstrated that among three different genetic lines of rabbit (lines H, LP and R) LP females had the highest feed intake per metabolic weight, even compared with R females. This fact could indicate that the inclusion of LP genes has increased the ability of RFLP to acquire resources.

Furthermore, RFLP females had a higher milk yield than R and RF throughout the experiment. This could indicate that RFLP females can obtain enough resources to ensure an adequate body condition at the beginning of lactation (higher intake and

body condition). Thus, they can provide their litter with more resources during lactation than R and RF females. On the contrary, the lower capacity to obtain resources and the lower body condition at parturition of R females would negatively impact their reproduction. According to our results, R and RF females seem to prioritise the recovery of their body condition during lactation over the requirements of their offspring, which could explain why milk yield is lower than expected for their metabolic weight (Arnau-Bonachera et al., 2018a).

During the first cycle, RF females presented the highest fertility of the three genetic types and a similar milk yield to RFLP females. This could be a consequence of RF females' foundation criterion for elite animals. Lines selected for growth rate rely on their body reserves to cope with the demands of lactation (Martínez-Paredes et al., 2022). Perhaps a high reproductive effort during the first reproductive cycle (fertility, litter size and milk yield), not linked to a greater ability to obtain resources (feed intake and body condition), could explain the drop in the reproductive performance traits of RF females in subsequent cycles (fertility and milk production). This fact could indicate that during the foundation of the new populations (RF and RFLP), the priorities for resources allocation could have changed with respect to the R line.

At first parity, females from the RFLP line had higher plasma leptin levels than RF females and PFT than R females. Probably, while R and RF females sustained more foetal growth by mobilising their reserves, the higher feed intake of the RFLP females during the first gestation helped them meet this demand, achieving the first parturition with higher PFT or leptin levels. Leptin is a key adipocyte hormone in regulating energetic homeostasis, which can interact with the reproductive axis at multiple sites (Moschos et al., 2002). A decrease in plasma leptin levels is usually associated with a reduced reproduction ability and promotion of body reserve accretion at the expense of milk yield, as observed by R and RF females at the beginning of the first lactation (PFT and leptin level recovery and reduced milk yield and fertility).

On the other hand, R line females presented higher plasma levels of both glutamine and glutamate than the other two genetic lines. Glutamine is one of the most abundant free amino acids in the body and the most abundant in blood plasma (Windmueller, 1982). Moreover, glutamine concentrations stimulate both the production of growth hormones and the inhibition of catabolic hormones, allowing cell growth and protein

deposition (Xi et al., 2011). However, although glutamine comprises only about 6% of the amino acids in muscle protein, it accounts for about 25% of the amino acids released during a protein breakdown (Chang and Goldberg, 1978). The breakdown of muscle protein cannot solely supply enough glutamate to synthesise this amount of glutamine. However, there is an additional conversion of other amino acids (aspartic acid, asparagine, glutamic acid, isoleucine and valine) into glutamate in rabbits (Leweling et al., 1989). For this reason, glutamine and glutamate plasma concentrations could be suitable biomarkers of protein mobilisation in rabbit females. A potential protein shortage during the first reproductive cycle could explain why young rabbit females of the R line do not reach adult BW until the third parturition (Figure 1.1), unlike in the RF and RFLP lines that reach their adult BW by the second parturition.

Effect of genetic type on the performance of the litter

The foundation of the new lines, RF and RFLP, successfully increased litter size at birth, both total and alive. In the case of RF females, the improvement of females' prolificacy (+31%) seems to be related to the constitution of this line from elite animals. In previous trials in our laboratory (Marín-García, 2019), we observed that selection for growth rate during the fattening period does not seem to affect the litter size of rabbit females negatively. These results indicate that the R line's reproductive limitations could be related to the foundation criteria used. Therefore, hyper-selection strategies within population may be useful to improve the performance traits in rabbit paternal genetic lines, although the inbreeding risk could be increased. In the case of the RFLP line, the improvement in prolificacy observed (+35%) may be due to different reasons. Firstly, since the base of RFLP genetics is the RF line, with which it does not show differences in prolificacy. Secondly, RFLP was generated by backcrossing of RF line with a maternal line (LP) selected for litter size. However, resources allocation strategy of the LP line, which makes it less susceptible to environmental challenges, allows this genetic type to maintain a larger number of kits born alive in the long term, even exceeding the values obtained with genetic lines hyper-selected for this criterion (Arnau-Bonachera et al., 2018b). Finally, a heterotic effect cannot be discarded.

On the contrary, R kits were the heaviest at birth (+8%). This could be due to its genetics, as the individual weight of kits from line R is usually higher than other genetic types (Arnau-Bonachera et al., 2018b). However, this could also in part be related to a

higher nutrient availability during their gestation due to the smaller litter size (Argente et al., 2003). A larger uterine space allows more blood vessels to reach each implant site. With a better blood supply, the foetuses have a lower probability of mortality and higher weight (Argente et al., 2003, 2008).

Our results suggest that the LP genes could provide RFLP females with the acquisition capacity, being the additional nutrient surplus diverted to milk yield. This was obtained with a BW similar to the R line, and an intermediate and constant level of body reserves. This acquisition and allocation strategy positively influences their overall fertility limiting the loss of offspring. RFLP kits have shown higher litter size, growth values, and feed intake throughout lactation than other genetic types. Considering that environmental conditions and the individual characteristics of the mother have been reported to have a high impact on kits' milk intake, this could substantially condition their life span (Saviotto et al., 2015). According to Martínez-Paredes et al. (2018), kits that could suckle within the first few hours after being born doubled their size and chances of survival. In addition, the quantity of milk ingested at early lactation is associated with lower mortality of the kits, a more mature digestive tract, and a higher feed intake before weaning (Szendrő et al., 2002; Arnau-Bonachera et al., 2017).

RF kits had similar survival and growth to that obtained in the RFLP line during the first cycle. However, the limited ability of RF females to obtain and allocate additional resources compared with RFLP females could indicate that RF females were unable to maintain and balance a high reproductive rate together with a high litter survival and development as observed in the RFLP females during the second lactation.

Conclusions

When only productive criteria are considered in the foundation of paternal rabbit lines, their rabbit females could have reproductive and robustness limitations that may even affect genetic progress in the selection for growth rate (reduced effective population size reduces the selection intensity). The refoundation of paternal rabbit lines from "elite" animals within population, such as the RF line, significantly improves the reproductive traits of rabbit females and the growth and survival of their litter during the first reproductive cycle. However, this effort without additional resources seems to generate body wear out in females, leading to a drop in reproductive success in the following cycles. In any case, this effect can be reduced by introducing resilient

genetics. The RFLP line, obtained by backcrossing the RF line with the robust LP line, changed the females' ability to acquire and allocate the available resources, being more suited to cope with the changing needs regarding their physiological state, maintaining an adequate body condition to ensure the future offspring and providing enough milk to the current litter during the whole trial. For this reason, it could be concluded that the refoundation of paternal rabbit lines, with elite animals within the population and the crossbreed strategy here used, can be an effective strategy to obtain animals with an adequate growth rate and fertility traits. These strategies contribute to improving the future genetic progress in rabbit production.

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CHAPTER II

Growth Performance Of Three Paternal Rabbit Lines With Different Potential For Growth Rate And Resilience

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Abstract

This experiment aimed to compare the growth performance, digestive efficiency and health status of three paternal lines for growing rabbits. The R line was selected by growth rate during the growing period for 37 generations; the RF line was founded by selecting a population of elite R animals (average daily gain >60 g/d); and the RFLP line was founded by backcrossing males from the RF line with females from the LP maternal line. A total of 387 weaned rabbits were used to evaluate growing performance from weaning until 63 d of age in individual cages, in three batches. Additionally, 33 animals were used to determine nutrient digestive efficiency in a digestibility trial. Body weight and feed intake were controlled at weaning (28 d), 46 and 63 d of age. Mortality and morbidity were also monitored daily. During the digestibility trial, feed intake and faeces excretion were controlled daily. Results showed no significant effects of genetic type on body weight, daily feed intake and feed conversion ratio throughout the experiment. However, RF animals had a higher average daily gain from 28 to 46 d of age (+4.4%; $P < 0.05$) compared with R animals, but lower from 46 to 63 d of age (-4.3%; $P < 0.05$). Regarding digestive efficiency, RF and RFLP lines showed slightly higher faecal digestibility for dry matter and gross energy of the diet compared with the R line (+1.3 percentage points; $P < 0.05$). Mortality was higher in animals from R and RF lines compared with RFLP (on av. 25.40 vs. 14.06%; $P < 0.05$). Our results suggest that the introduction of resilient genetics could be a suitable strategy to improve the digestive health of paternal lines without affecting significantly their growth performance.

Key Words: growth rate, digestibility, mortality, genetics, rabbit.

Introduction

Rabbit meat production has significantly evolved over the past few decades thanks to pertinent advances in genetic selection, reproductive management and feeding systems (Pascual, 2010). Since feed conversion ratio (FCR) and litter size are the main productive traits determining farm profitability, genetic improvement programmes should consider both paternal and maternal lines (Cartuche *et al.*, 2014). Three-way crossings are the basis for breeding programmes in commercial farms. The paternal line is usually selected for average daily gain (ADG) to provide terminal males, while two maternal lines are selected for reproductive traits and crossed to generate a commercial doe. Theoretically, this scheme provides numerous litters with heavier kits (Blasco *et al.*, 2018).

Using paternal lines selected for high growth rate has widely improved growth performance in rabbit production. Consequently, growing rabbits reach a heavier weight in less time, which is associated with improved FCR (Baselga, 2004). However, the genetic selection of only specialist criteria could be related to reproductive, physiological and immunological disorders (Rauw *et al.*, 1998). Therefore, obtaining productive and functional animals in a commercial environment may be a more sustainable option in the long term (Arnau-Bonachera *et al.*, 2018).

The performance of the R line can be used as an example to illustrate the reproductive and immunological problems associated with this genetic selection process. The R line is a paternal line selected for ADG during the growing period (Estany *et al.*, 1992). In the last generations, these animals have shown reproductive limitations such as inadequate responses to ovulation, foetal losses and reduced litter size (Vicente *et al.*, 2012; Naturil-Alfonso *et al.*, 2015). Such a decline in reproductive traits can affect the selection pressure, as fewer individuals are available to select, and even fewer have the desired characteristics. In addition, animals selected for ADG have shown a high prevalence of digestive disorders such as epizootic rabbit enteropathy, which results in high mortalities and increases the need for antimicrobial treatments (Garcia-Quiros *et al.*, 2014).

Within this framework, two strategies were considered by our research team in order to explore how to improve growth performance and reduce the risk of digestive disorders: (1) selecting elite animals from the R line (ADG>60 g/d) for the foundation

of an elite line called RF. (2) The introduction of resilient genetics is achieved by backcrossing these elite animals with a long-living maternal line, the LP line, thus creating the RFLP line. In a previous work, the authors studied how these new paternal lines acquire and allocate resources and their effects on the reproductive performance of female rabbits (Peixoto-Goncalves *et al.*, 2023). While RF females showed enhanced reproductive traits in the first cycle, RFLP females' reproductive performance was more stable over time and showed an increased capacity to tackle the demands of lactation and gestation while maintaining an adequate body condition. However, to date, there is no information on the impact of these genotypes on growing rabbits' growth performance and incidence of digestive disorders.

Therefore, this study aimed to evaluate how genetic type affects growing rabbits' growth performance, digestive efficiency and health status.

Material and methods

The experimental procedure was approved by the Animal Welfare Ethics Committee of the Universitat Politècnica de València (UPV; code: 015/VSC/PEA/00061) and conducted following the recommendations of the European Group on Rabbit Nutrition (Fernandez-Carmona *et al.*, 2005) and the Spanish Royal Decree 53/2013 on the protection of animals used for scientific purposes.

Animals

A total of 384 weaned rabbits (28 d of age) from 3 paternal lines were used for this experiment. The animals belonged to lines R (n=125, 26 litters), RF (n=131, 27 litters) and RFLP (n=128, 27 litters), developed by the Animal Breeding and Genetics Group of the UPV in Spain. Animals came from 70 different females, from 80 litters. The R line was a paternal line selected for growth rate during the growing period (post-weaning) for 37 generations (Estany *et al.*, 1992). The RF line was founded by selecting a population of elite R animals from two genetic selection centres, which had a high growth rate during the growing period (ADG from 28 to 63 d of life of the final population: 61.94 g/d). Finally, the RFLP line was created by backcrossing the males of the RF line with females of the maternal line LP, generating 7/8 RF and 1/8 LP animals as in other previous foundations per hyper-selection (Cifre *et al.*, 1998;

Sanchez et al., 2008). The LP line was founded by productive longevity criteria (minimum of 25 parturitions and 7.5 kits born alive; Sanchez et al., 2008; Savietto et al., 2015).

Experimental procedure

The trial was conducted in three batches of 128 animals from September 2020 to March 2021 at the experimental facilities of the Universitat Politècnica de València. Throughout the trial, the animals were kept in a controlled environment with a photoperiod of 16 h of light and 8 h of darkness, with mechanical ventilation and cooling panels. All animals were fed a commercial growing rabbit pelleted diet (161 g crude protein, 401 g neutral detergent fibre, 218 g acid detergent fibre, 36 g acid detergent lignin per kg of feed; Cunitiva Fibra, Nanta, S.A) throughout the experimental period. The experiment lasted 35 d (from 28 to 63 d of age). At the start, weaned animals were weighed and housed in individual wired cages. Water was given *ad libitum* throughout the experiment. Feed was restricted following Gidenne *et al.* (2009) for the first 18 d, progressively increasing the offered amount until 46 d of age (35 g the first 4 d, 55 g 4 d, 70 g 3 d, 80 g 3 d and 90 g the last 4 d post-weaning). On day 46 of age, all animals were weighed again and fed *ad libitum* until the end of the experiment. This strategy was adopted to minimise digestive distress. Individual body weight (BW) was registered at 28, 46 and 63 d of age. Daily feed intake (DFI) was controlled in each period. Morbidity (apathy and diarrhoea) and mortality were monitored daily. In addition, animals with a severe disturbance in feed intake (under 2.5 the standard deviation of the mean) or growth (weight loss in 7 d) were considered morbid (Bennegadi *et al.*, 2000). Additionally, a digestibility trial was performed according to Perez *et al.* (1995). A total of 33 animals (49 d of age) were used in the digestibility trial (11 animals from each genetic line) from 21 different rabbit females. After a 7-day adaptation period, feeders were weighed and the cages were prepared for faeces collection at 56 d of age. In these cages, metal cones were fitted with a metal grid to retain the faeces and separate them from the urine. Feed intake was controlled and faeces were collected daily for four days and stored individually in labelled bags. The faeces were stored at -20°C until further analysis. Then they were dehydrated in an oven for 48 h at 80°C and weighed. Any feed pellet present was removed and weighed to correct dry matter intake and avoid overestimating the nutrient excretion. The dehydrated faeces and feed were ground in a 0.5 mm mesh mill before analysis.

Chemical analyses

Feed and faeces were analysed in duplicate using AOAC methods (AOAC, 2023). Dry matter (DM) was determined following AOAC official method 934.01 and crude protein (CP) following method 990.03 (Dumas method, CN628 Elemental Analyzer, LECO, St. Joseph, USA). Gross energy (GE) was determined using an adiabatic bomb calorimeter (Gallenkamp Autobomb, Loughborough, RU). Additionally, the feed was analysed for fibre fractions. Neutral detergent fibre, acid detergent fibre and acid detergent lignin were sequentially determined following the method of Van Soest *et al.* (1991), using pre-treatment with thermostable α -amylase and the nylon bag technique (Ankom, Macedon, USA).

Statistical analyses

Performance traits (BW, ADG, DFI and FCR) were analysed using a MIXED model by SAS (Statistical Analysis System) for repeated measures, considering the lack of data homoscedasticity. The model included the genetic type, age, their interaction and the batch as fixed effects and the animals as a permanent effect. Apparent faecal digestibility coefficient data were analysed using a GLM model from SAS, including the genetic type as a fixed effect. Orthogonal contrasts of interest were also obtained by comparing R with the new lines [$R - \frac{1}{2}(RF+RFLP)$]. The effect of genetic type on the health traits was analysed using logistic regression by the GENMOD procedure of SAS, considering a binomial distribution (logit scale). Significant differences were declared at $P < 0.05$.

Results

Table 2.1 shows the effect of genetic type on rabbits' growth performance. In general, genetic type did not affect BW, ADG (global av. 49.2 g/d), DFI (global av. 93.17 g/d) and FCR (global av. 1.96) throughout the growing period. However, rabbits from the RF line showed higher ADG from 28 and 46 d of age ($+1.1 \pm 0.52$ g/d; $P = 0.0221$) than the R line rabbits. There were no statistically significant differences in ADG between rabbits in RF and RFLP lines.

Table 2.1. Effect of genetic type (R, RF and RLP) on the productive performance (mean \pm standard error) of growing rabbits from 28 to 63 days of age (n=271).

	Genetic type (GT)			P-value
	R	RF	RFLP	
From 28 to 46 days:				
Body weight at 28	603 \pm 12	607 \pm 11	622 \pm 11	0.4204
ADG 28 to 46	27.3 ^a \pm 0.4	28.4 ^b \pm 0.4	27.5 ^{ab} \pm 0.3	0.0487
DFI 28 to 46	55.7 \pm 0.26	55.4 \pm 0.26	55.7 \pm 0.24	0.5635
FCR 28 to 46	2.09 \pm 0.05	2.00 \pm 0.05	2.03 \pm 0.04	0.3338
From 46 to 63 days:				
Body weight at 46	1094 \pm 11	1118 \pm 10	1116 \pm 10	0.1865
ADG 46 to 63	72.2 \pm 1.06	69.2 \pm 1.05	70.8 \pm 0.98	0.1379
DFI 46 to 63	130 \pm 4.94	127 \pm 4.89	135 \pm 4.51	0.4787
FCR 46 to 63	1.85 \pm 0.04	1.87 \pm 0.04	1.90 \pm 0.04	0.6627
Body weight at 63	2321 \pm 24.1	2294 \pm 23.8	2319 \pm 22	0.6552
From 28 to 63 days:				
ADG 28 to 63	49.7 \pm 0.52	48.8 \pm 0.51	49.1 \pm 0.47	0.3685
DFI 28 to 63	92.8 \pm 2.48	91.3 \pm 2.45	95.4 \pm 2.56	0.4883
FCR 28 to 63	1.97 \pm 0.02	1.93 \pm 0.02	1.97 \pm 0.02	0.1180

ADG: Average Daily Gain; DFI: Daily Feed Intake; FCR: Feed Conversion Ratio

^{a,b} Means not sharing superscript were significantly different at $P < 0.05$.

Table 2.2 shows the effect of the genetic type on the apparent nutrient faecal digestibility coefficients in growing rabbits' diets from 56 to 63 d of age. The genetic type did not affect the faecal digestibility coefficient of DM (av. 54.5%), CP (av. 66.9%) and GE (54.2%). However, when comparing the original R line with the new RF and RFLP lines through contrast analysis, the new ones had higher digestibility coefficients for DM and GE (+1.30 \pm 0.55 and +1.29 \pm 0.56 percentage points, respectively; $P < 0.05$).

Table 2.2 Effect of genetic type (R, RF and RLP) on the faecal digestibility coefficient (mean \pm standard error) of growing rabbits from 56 to 63 days of age (n=31).

	Genetic type (GT)			P-value
	R	RF	RFLP	
No. animals	10	11	10	
Feed intake (g DM/d)	193 \pm 8	182 \pm 7	180 \pm 8	0.4285
Faecal coefficients:				
Dry Matter	53.6 ^a \pm 0.5	54.9 ^b \pm 0.5	54.9 ^b \pm 0.5	0.0776
Crude Protein	66.5 \pm 0.9	67.5 \pm 0.8	66.7 \pm 0.9	0.6653
Gross Energy	53.4 ^a \pm 0.4	54.6 ^b \pm 0.4	54.7 ^b \pm 0.4	0.0907

^{a,b} Orthogonal contrast analysis RF and RFLP had higher digestibility coefficients for DM and GE (+1.30 \pm 0.55 and +1.29 \pm 0.56 percentage points, respectively; $P < 0.05$).

Figure 2.1 shows the evolution of the cumulated mortality rates throughout the growing period, and Table 2.3 shows the effect of the genetic type on the health traits monitored (mortality, morbidity and sanitary risk index SRI).

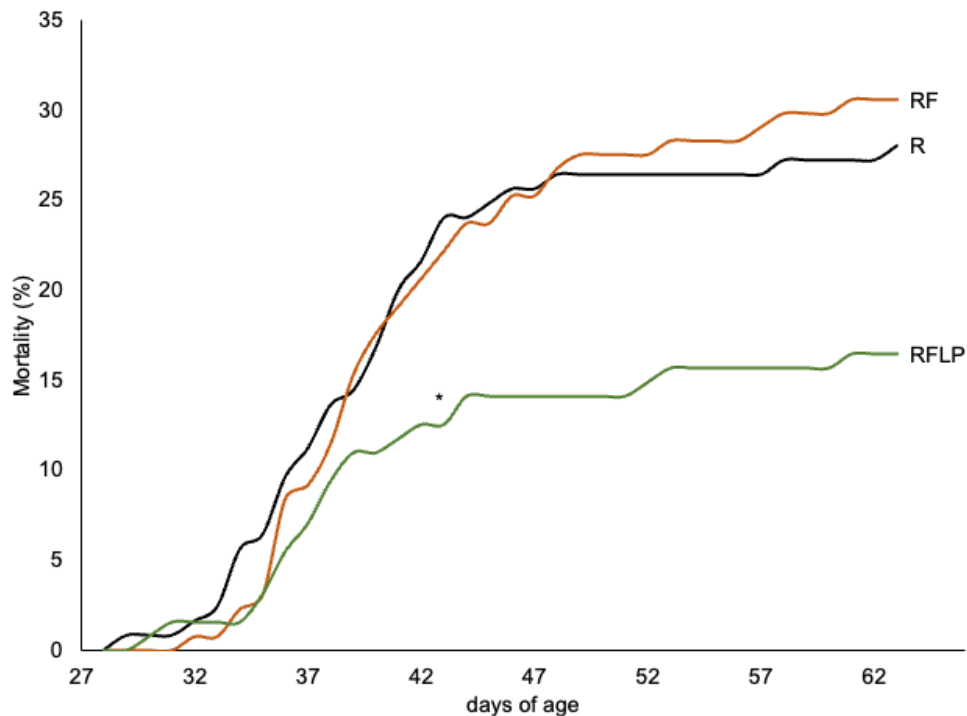


Figure 2.1. Cumulated mortality (%) throughout the growing period by genetic type (— R, — RF and — RFLP). * Day of age in which cumulated mortality differences become significant ($P < 0.05$).

Overall, global mortality throughout the experiment was 25%, highest during the first period post-weaning (28 to 46 d of age) and decreasing thereafter. All dead or morbid animals showed signs compatible with epizootic rabbit enteropathy (ERE). Animals from R and RF lines had higher mortality rates from 28 to 46 d of age than those from the RFLP line (on av. +11.3 percentage points; $P < 0.05$). Although there was no significant difference between genetic types in mortality from 46 to 63 d, global mortality of the R and RF animals was higher than for RFLP animals (on av. +12.9 percentage points; $P < 0.05$). As shown in Figure 2.1, the differences in cumulated mortality become significant between RFLP and the other genetic types from 43 d of age. On the other hand, the genetic type did not significantly affect animals' morbidity throughout the trial. Therefore, the sanitary risk index (SRI) showed a similar evolution to the mortality rate.

The R and RF lines showed a higher SRI than the RFLP line from 28 to 46 d of age (on av. +12.2 percentage points; $P < 0.05$) and during the global period (on av. +13.3 percentage points; $P < 0.05$).

Table 2.3. Effect of the genetic type (R, RF, RFLP) on the animals' health status from 28 to 63 days of age.

		Genetic Type (GT)			
		R	RF	RFLP	P-value
Mortality:	28-46 d	25.60 ^b	25.19 ^b	14.06 ^a	0.0394
	46-63 d	3.23	7.14	2.73	0.2424
	Global	28.00 ^b	30.53 ^b	16.41 ^a	0.0204
Animals at 28 d		125	128	131	
Animals at 46 d		93	98	110	
Animals at 63 d		90	91	107	
Morbidity:	28-46 d	2.40	0.76	0.78	0.4193
	46-63 d	1.08	0.00	0.91	0.6086
	Global	3.20	0.76	1.56	0.1757
SRI:	28-46 d	28.00 ^b	26.00 ^b	14.80 ^a	0.0268
	46-63 d	4.30	7.14	3.64	0.4775
	Global	31.20 ^b	31.30 ^b	17.97 ^a	0.0216

^{a,b} Means not sharing superscript were significantly different at $P < 0.05$.

SRI: Sanitary Risk Index.

Discussion

The main objective of this work was to evaluate whether the foundation of two new genetic lines through the selection of elite-growth animals and the potential introduction of robust genetics could contribute to solving growth and health limitations in growing rabbits. Thus, it would be expected that the new genetic lines would improve the growth performance, digestive efficiency and intestinal health of growing rabbits.

Feed was restricted for 18 days after weaning to avoid excessive digestive disorders. Feed restriction for the first 2-3 weeks after weaning is frequently used to decrease the incidence of digestive disorders (Di Meo *et al.*, 2007). This system was preferred to the inclusion of antibiotics, as antibiotics could mask the comparison between lines on digestive disorders sensitivity. Animals under such conditions might not express their full growth potential (Blasco *et al.*, 2018). In a previous study, Marin-Garcia *et al.* (2020) observed that R animals fed *ad libitum* during the whole growing period (with no feed restriction) presented an ADG of 64.73 g/d, a DFI of 154 g/d and BW at 63 days of 2930 g. Compared to our results, feed restriction could have reduced these traits (–23,

–40% and –21%, respectively). Since the experiment took place in Spain, where the growing period is until 63 days of life, applying this restriction during the first half of the growing period does not allow compensatory growth, unlike in other countries, where the end of the growing period occurs after 70 days of life. Consequently, this could have affected our results.

Regarding the RF line, these animals showed a significantly higher ADG than the R line from 28 to 46 days of age, similar to that of RFLP. As the RF line was selected for a very high growth rate (at least 60 g/d), and selection under restricted feed intake improves resource allocation for growth (Drouilhet *et al.*, 2016; Peixoto Goncalves *et al.*, 2023), this could indicate that the selection of elite animals could improve the growth rate during the initial phase of the growing period under restriction conditions. However, the slight delay of R animals was significantly compensated during the second half of the growing period, when all the animals were fed *ad libitum*. Compensatory growth after restriction has been widely described in growing rabbits (Gidenne *et al.*, 2012), with no significant differences in BW between R and RF animals at the end of the growing period. Thus, overall, the foundation of a new line by introducing elite animals for growth did not improve the ADG of growing rabbits.

Regarding the RFLP line, despite RFLP animals having 1/8 LP maternal origin, which could potentially affect the animals' growth performance, there were no significant differences among the genetic types in the body weight at 63 d or in global ADG, DFI and FCR. Garcia-Quiros *et al.* (2014) observed that, although R line animals have a higher ADG during the growing period than those of the LP line (+20%), the latter have a higher ADG than other maternal lines (+6%). This seems to be due to the larger adult size of this maternal line. In fact, Theilgaard *et al.* (2007) observed that the mean weight of LP rabbits was 4% higher than that of rabbits from another maternal line (V line). Consequently, the introduction of robust genetics through the backcrossing procedure proposed in this study did not penalise the growth potential during the growing period of the R paternal line.

When comparing digestive efficiency, animals from the newly founded lines (RF and RFLP) showed a slightly improved ability to utilise nutrients in the diet than those of the R line. Previous digestibility trials comparing genetic types have obtained contradictory results. Savietto *et al.* (2012) observed a higher digestibility of neutral

detergent fibre and hemicelluloses in the diet of rabbit females from the LP line compared to the V line. However, Pascual *et al.* (2008) observed a higher digestibility of acid detergent fibre in the diet of growing rabbits from the V line with respect to the LP line. The better utilisation of the diet observed with the RFLP line could have contributed to the better resource utilisation for reproduction observed in the work of Peixoto-Goncalves *et al.* (2023) with this line.

In terms of health status, almost all dead or sick animals presented signs of digestive distress, such as diarrhoea under the cage and dirt in the perineal area. According to Rosell and De la Fuente (2009), digestive disorders are some of rabbit farms' most common causes of mortality. To reduce post-weaning mortality, Gidenne *et al.* (2009) recommended a reduction of 20% of the feed intake for 20 days. The feed was restricted for the first 18 days of the growing period as a preventive strategy and to avoid using antimicrobials. However, the mortality rates were still abnormally high throughout the experiment. When comparing the three genetic lines, the R and RF lines had significantly higher mortality rates and, consequently, higher SRI than RFLP animals. These results agree with a previous study by Garcia-Quiros *et al.* (2014), in which growing rabbits from the R line had a higher mortality rate than those from the LP line (16.5 vs. 9.5%, respectively). The results could be due to the introduction of LP genetics. These animals are more robust than other commercial lines. Past studies with reproductive rabbit females found that the LP animals could overcome adverse environmental conditions and cope better with immunological challenges than other maternal lines. Females of the LP line were less affected during the hyper-acute phase induced by an intravenous lipopolysaccharide infusion (Ferrian *et al.*, 2013). Furthermore, in a previous experiment with these same lines, it was observed that suckling kits from the RFLP line had a higher survival during the first 18 days of life (+9%) and higher feed intake than those from the other genetic lines (+47%) from 18 to 28 days of age, achieving higher weaning BW (Peixoto-Goncalves *et al.*, 2023). Although the initial weight was similar in this study, greater maturity and previous intake of RFLP kits could also explain our results, as a higher DFI during this transitional time promotes better growth and lower mortality during the growing period (Pascual *et al.*, 2001).

Conclusions

The foundation of a new line with elite animals did not seem to have significantly improved the growth performance of growing rabbits. Although slight specific changes in the use of nutrients or growth were observed, the final growth performance was similar for the different lines. In addition, including LP maternal genetics through backcrossing did not penalise growth performance traits but improved the resistance to digestive disorders. These results can be helpful when creating new genetic lines or refining the characteristics of current genetic lines, overcoming the potential side effects of selecting animals only for growth criteria.

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CHAPTER III

Immunological studies on new rabbit paternal lines with a different potential for growth rate and resilience. Pathways towards healthier animals.

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Abstract

This study evaluated leukocyte percentage and immune parameters in female rabbits and their kits from three genetic lines (R, RF, and RFLP) during their first reproductive cycle. The R line was selected for growth rate during the growing period for 37 generations, the RF line was founded through a high selection intensity of elite animals of the R line, and the RFLP line, which was obtained by backcrossing RF animals with the LP line (a long-lived productive maternal line, characterised by high resilience). In the case of rabbit females, total lymphocyte percentage in blood drastically decreased from the first AI to weaning (-15.8 percentage points; $p < 0.001$); granulocyte percentage varied significantly among stages, peaking at weaning (12.4 percentage points higher than AI and 4.4 higher than parturition; $p < 0.01$); the CD4+/CD8+ ratio increased by 53.4% from the first AI to weaning ($p < 0.001$); and haptoglobin concentration doubled from the first AI to weaning ($p < 0.001$). RF and RFLP females had significantly higher total lymphocyte percentages in the blood (+6.4 and +4.6 percentage points, respectively; $p < 0.05$) than R females, while R females had lower CD4+ percentages and CD4+/CD8+ ratio than the RLFP line (-3.4 percentage points and -24.4%, respectively; $p < 0.05$). However, the R females showed higher monocyte and granulocyte percentages than RF and RFLP (on average +0.9 and +4.1 percentage points, respectively; $p < 0.05$). At weaning, kits from the R line had lower T-lymphocytes and CD4+ percentage in blood than the RF line (-8.1 and -6.1 percentage points, respectively; $p < 0.05$) and lower CD25+ percentage and phagocytosis of heterophils than RFLP (-0.8 and -1.2 percentage points, respectively; $p < 0.05$). The population of R females was culled more often than RFLP from the first parturition onwards (-24.0 percentage points at the second parturition; $P < 0.05$). In addition, associations between the leukocyte populations in females and kits at weaning were observed. The results suggest that the foundation of a paternal line using elite animals (RF) and backcrossing them with animals from a robust line resulted in a genetic line (RFLP) with improved immune status and stayability. Understanding these findings is crucial for rabbit breeding and management strategies, impacting rabbit health, disease resistance, and reproductive success.

Keywords: immunology, genetic lines, flow cytometry, rabbit, stayability.

Introduction

Traditionally, animal breeding has focused on selecting traits such as growth rate and milk production, with little consideration for immune function. Despite the apparent benefits of high production efficiency, animals in a population that has undergone selective breeding for this trait may also be predisposed to various behavioural, physiological, and immunological challenges (Rauw et al., 1998). For example, in chickens, the intensive selection for production and feed efficiency seems to have come at the expense of immunity, as evidenced by low adaptive immune responses and high vulnerability to illness (Zerjal et al., 2021). The intensive selection for high milk yield in dairy cattle could affect heat stress tolerance, negatively impacting females' health and milk production (Santana Jr et al., 2015). Young rabbits from paternal lines selected for productive traits showed a higher incidence of digestive disorders during the growing period than those from a more robust maternal line (García-Quirós et al., 2014). In addition, females from a paternal line displayed lower immune adaptability to the reproductive challenges than females selected for high robustness (Penadés et al., 2018).

A previous study (Wilkie and Mallard, 1999) discusses the potential benefits of breeding animals for high immune response to improve overall animal health and reduce the use of antibiotics and other treatments. The authors argue that breeding for more robust immune responses could provide a more sustainable and effective long-term solution to antimicrobial resistance. In addition, a recent study using females from a paternal rabbit line (the R line) demonstrated that selective breeding for increased growth rate resulted in altered immune cell populations and impaired immune responses to bacterial infection (Moreno-Grua et al., 2023). These findings suggest the existence of a potential trade-off between growth performance and immune competence.

Our research group considered two strategies to improve rabbit health and reproduction and reduce antimicrobial dependence in a paternal line selected for average daily gain (ADG). The R paternal line served as the control group, representing the baseline genetic background and two new genetic lines were created from these animals. The first genetic line (RF) was formed by selectively breeding elite animals from the R line based on their high growth rate, specifically selecting

individuals with an ADG higher than 60 g/d. This approach aimed to establish a new genetic line with enhanced growth performance. On the other hand, to improve the RF line's overall robustness and disease resistance, we introduced genetics from a long-living maternal line (LP) through backcrossing, creating the second genetic line (RFLP). Previous results suggest that the RF line produced females with better reproductive performance during the first reproductive cycle, and the reproductive performance was more consistent throughout their reproductive life with RFLP females (Peixoto-Gonçalves et al., 2023a). Furthermore, it was observed that young growing rabbits from the RFLP line showed lower mortality during the growth period (Peixoto-Gonçalves et al., 2023b). We hypothesise that the founding process carried out with the new genetic lines has improved their animals' immune status. To verify this hypothesis, in this study, we aim to identify possible differences in the composition and functionality of the immune system between the R, RF and RFLP lines by examining immune cell populations and haematological profiles of rabbit females and their kits.

Materials and methods

The experimental procedure was approved by the Animal Welfare Ethics Committee of the Universitat Politècnica de València (UPV; code: 015/VSC/PEA/00061) and carried out following the recommendations of the European Group on Rabbit Nutrition (Fernández-Carmona et al., 2005) and the Spanish Royal Decree 53/2013 on the protection of animals used for scientific purposes.

Animals and experimental procedure

A total of 122 rabbit females from 3 paternal lines were used for this experiment. The animals belonged to lines R (n=40), RF (n=37) and RFLP (n=45), developed by the Animal Breeding Group of the Universitat Politècnica de València in Spain. The R line was selected by growth rate during the growing period for 37 generations (Estany et al., 1992). The RF line was founded by selecting a population of elite R animals from different genetic selection centres. The RFLP line was created by backcrossing the RF line with the maternal line LP, generating animals that were 7/8 RF and 1/8 RFLP. The LP line was founded by productive longevity criteria (Sánchez et al., 2008). For further details of the genetic lines, see Peixoto-Gonçalves et al. (2023a).

The animals were kept in a controlled environment with a conventional ventilation system and a photoperiod of 16 hours of light and 8 hours of darkness. The rabbit females were randomly selected at 9 weeks of age and housed in individual cages for reproductive rabbit females (700 × 500 × 320 mm) until the end of the trial at first weaning.

The rabbit females were fed *ad libitum* with two different commercial diets throughout the experiment. Until the first parturition, the females were fed a diet for growing rabbits (158 g crude protein, 25 g ether extract and 231 g crude fibre per kg of feed) and then a diet for reproductive rabbit females (193 g crude protein, 40 g ether extract and 169 g crude fibre per kg of feed). The animals were artificially inseminated (AI) at 20 weeks of age. Gestation diagnosis was performed by manual palpation at 11 days post-AI. Non-pregnant females were re-inseminated 21 days later until a maximum of 3 attempts. After the 28th day of gestation, an external nest equipped for the litter was attached (220 × 400 × 380 mm). At parturition, the number of kits per litter was standardised to 6. Litter size and weight were controlled at birth (total, alive and standardised) and weaning. The litters were weaned at 28 days post-partum (dpp). The mortality of the mothers and kits was recorded daily.

Blood samples from rabbit females were collected at first AI, first parturition, and first weaning. In addition, blood samples were collected from three kits per litter at first weaning. These last were mixed and processed as a single sample. For the analysis of haptoglobin and immunophenotype, tubes with EDTA were used, and for phagocytosis studies, acid-citrate dextrose-containing tubes. Blood samples were collected approximately at the same time (9:00 am to 10:00 am) to prevent diurnal variations in haematological parameters.

Immunophenotypic characterisation of peripheral blood by flow cytometry

One millilitre of blood was lysed with 45 mL of ammonium chloride lysing solution to prepare the samples. The resulting leukocyte pellet was resuspended in 1 mL of Dulbecco's Phosphate-Buffered Saline (Sigma-Aldrich®) (DPBS). Each animal's cell suspension was then divided into six sample tubes. Primary monoclonal antibodies (Table 3.1) were added to the cell suspension according to the manufacturer's instructions and incubated for 25 minutes at room temperature. The samples were washed, and secondary antibodies (Rat anti-mouse IgG2ak or IgG2bk Phycoerythrin

[Nordic-MUbio] and Goat anti-mouse IgM: R-Phycoerythrin [Biorad]) were added and incubated for 25 minutes at room temperature. The samples were then rewashed, and 1 mL of DPBS was added before analysis on the flow cytometer. The leukocyte (WBC) suspensions were analysed using a CytoFLEX S flow cytometer (Beckman Coulter, Indianapolis, IN, USA). The "lymphogate" setup used the expression of the common leukocyte antigens CD14 and CD45, as previously described (Jeklova et al., 2007; Guerrero et al., 2011) and was used to obtain the total percentage of lymphocytes, monocytes, and granulocytes. From the total percentage of lymphocytes, the percentages of lymphocyte subsets were calculated as described by Guerrero et al. (2011).

Table 3.1. Monoclonal antibodies used for flow cytometry

Monoclonal antibody	Iso.	Spec.	Cell labelling	Clone	Ref.	Comp.
Mouse anti-rabbit T lymphocytes: FITC ¹	IgG1	CD5	T cell	KEN-5	Kotani et al., (1993a)	Abd Serotec
Mouse anti-rabbit α -pan B	IgM	IgM	B cell	MRB143A	Davis and Hamilton, (2008)	VMRD Inc.
Mouse anti-rabbit CD4	IgG2a	CD4	T cell subset	KEN-4	Kotani et al., (1993a)	Abd Serotec
Mouse anti-rabbit α -CD8	IgG2a	CD8	T cell subset	ISC27A	Davis and Hamilton, (2008)	VMRD Inc.
Mouse anti-rabbit CD25	IgG2b	CD25	Activated T cells	KEI-ALPHA1	Kotani et al., (1993b)	Abd Serotec
Mouse anti-human CD14: FITC	IgG2a	CD14	Monocytes and granulocytes	TüK4	Jacobsen et al., (1993)	Abd Serotec
Mouse anti-rabbit α -CD45	IgM	CD45	All leukocytes	ISC76A	Davis and Hamilton, (2008)	VMRD Inc.

Iso: Isotype; Spec: Specificity; Ref: References; Comp: Company

¹ Clon KEN-5 recognises rabbit T lymphocytes and immunoprecipitates. This antibody recognises rabbit CD5, but does not bind to rabbit CD5 transfectants. Known rabbit CD5 antibodies also show binding to most B lymphocytes, which are not labelled by this clone (information obtained from datasheet).

Determination of haptoglobin

For the analysis of haptoglobin, the blood samples were centrifuged (3000 × g, 4°C and 10 min), the plasma was separated, and the specimens were stored at -40°C for posterior analysis. Haptoglobin was determined due to its binding ability to haemoglobin (Phase™, Tridelta Developments, Wicklow, Ireland). For its

determination, an auto-analyser, ADVIA 1800® Chemistry 53 System was used (Siemens Medical Solutions, Tarrytown, NY 10591, USA). The intra and inter-assay CV were consistently below 2.0 and 4.0%, respectively.

Purification of Polymorphonuclear Leukocytes (PMN) and macrophages

The purification of PMN (also named heterophils) and macrophages was performed according to previously described protocols (Penadés et al., 2020; Yamane and Leung, 2016; Siemsen et al., 2014). Rabbit blood was collected with acid-citrate dextrose, and Hetastarch (Sigma-Aldrich) was added to separate PMNs by causing erythrocyte sedimentation. Neutrophils were isolated using a gradient with Histopaque 1077 (Sigma-Aldrich). For monocytes, peripheral blood mononuclear cells (PBMCs) were isolated with Lymphoprep (Palex, Madrid, Spain). After the density gradient separation, PBMCs were washed and cultured in RPMI-1640 medium with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (Thermo Fisher, Massachusetts, USA). Non-adherent cells were removed after 2 hours of incubation. Macrophages were polarised in vitro by culturing adherent cells for 6 days in RPMI-1640 medium with 10% FBS, 1% penicillin/streptomycin, and recombinant human GM-CSF. Cell count and viability were determined using a haemocytometer and trypan blue.

Phagocytosis of PMN and macrophages

To assess the phagocytosis of macrophages and PMN, fluorescent yellow-green latex beads with a mean particle size of 2.0 µm (Sigma-Aldrich®) were used. An inoculum of beads was prepared by adding 10 times more latex beads than cells (10^6 cells/mL). For both protocols, three replicates and a negative control (without beads) were performed for each animal. Animals with less than 5% cell confluence in the wells were excluded from the study. Phagocytosis was allowed to proceed for 30 minutes at 37°C before being stopped with ice. For the phagocytosis of macrophages, cells were collected from the wells using porcine pancreas Trypsin solution (1x) (Sigma-Aldrich®) and then marked with the monoclonal antibody CD11b-PE (Thermo Fisher®) according to the manufacturer's instructions. For the phagocytosis of PMN the cells were then marked with the monoclonal antibody CD11b-PE (Thermo Fisher®) according to the manufacturer's instructions and fixed with paraformaldehyde (4%). Finally, the cells were resuspended in 1 mL of DPBS and transferred to cytometer tubes for analysis using a CytoFLEX S flow cytometer (Beckman Coulter, Indianapolis,

IN, USA). All cells marked with the CD11b-PE antibody were considered part of the granulocyte, or macrophage population. Depending on the protocol, cells that had phagocytosed were identified by FITC fluorescence of the latex beads, while cells that had not phagocytosed did not show this fluorescence. The kits' blood samples were not used to determine the phagocytosis of macrophages.

Statistical analysis

When asymmetrical distributions were found, the original data were transformed by logarithmic transformation. The effect of the physiological state on the leukocyte percentage in females was analysed using a MIXED model of SAS (Statistical Analysis System) for repeated measures. The model included the genetic type, the physiological state, their interaction, and the batch as fixed effects and the animals as a permanent effect. The leukocyte percentage of kits at weaning was analysed using a GLM model from SAS, only including the genetic type as a fixed effect. Pearson's correlation coefficients (ρ) were obtained using PROC CORR of SAS to test the relationship between the females' and kits' leukocyte percentages. Significant differences were declared at $P < 0.05$.

Results

Table 3.2 shows the leukocyte percentage of the females' blood from the first artificial insemination to the first weaning. The effect of the physiological state was significant for all the measured parameters ($P < 0.001$). Total lymphocyte percentage drastically dropped from the first AI to weaning (-15.8 percentage points). Similarly, the percentage for T-lymphocytes, CD4⁺, CD8⁺, and CD25⁺ also decreased from the first AI to weaning (-7.7, -3.5, -4.1 and -1.4 percentage points, respectively). The percentage of B-lymphocytes varied across the reproductive cycle, showing an increase from first AI to parturition (+3.2 percentage points) and a subsequent decrease from parturition to weaning (-6.2 percentage points). In contrast, the monocyte percentage reached its lowest value at parturition, followed by an increase at weaning (+1.5 percentage points; $p < 0.001$). The granulocyte percentage significantly differed among the three moments, progressively reaching the highest value at weaning (12.4 percentage points higher than at AI and 4.4 higher than at partum). The CD4⁺/CD8⁺ ratio increased by 53.41% from the first AI to weaning. The

haptoglobin concentration was duplicated from the first AI to weaning. The phagocytosis of macrophages significantly decreased from first AI to weaning (on av. -17.6 percentage points; $P < 0.001$). However, the phagocytosis of heterophils increased from first AI to weaning (on av. +6.3 percentage points; $p < 0.05$).

The effect of the genetic type on the leukocyte percentage in the blood of females was significant only in some of the evaluated parameters. The RF and RFLP lines showed a higher total lymphocyte percentage than the R line (+6.4 and +4.6 percentage points; $p < 0.05$). The counts for $CD4^+$ and $CD4^+/CD8^+$ ratio for the R line were significantly lower than the RLFP line (-3.4 percentage points and -24.4%, respectively; $p < 0.05$). However, the monocyte and granulocyte percentage values were significantly higher for the R line than for both RF and RFLP lines (on av. +0.9 and +4.1 percentage points, respectively; $p < 0.05$). From all parameters, the monocyte percentage was the only one that showed an interaction between the genetic type and physiological state. As shown in Figure 3.1, there are no significant differences among the three genetic types at first AI. However, the R line had a higher monocyte percentage than both RF and RFLP at first parturition (on av. +1.3 percentage points; $p < 0.05$) and higher than RF at first weaning (+2.1 percentage points; $p < 0.05$). In general, there were no significant differences either in the leukocyte percentage or the phagocytosis percentage between RF and RFLP females. Regarding the concentration of haptoglobin in the plasma of rabbit females, there are no significant differences among the three genetic lines during the first reproductive cycle.

Table 3.3 shows the leukocyte percentage of the kits' blood at first weaning. T-lymphocytes and $CD4^+$ percentage for the R kits were significantly lower than the RF kits (-8.1 and -6.1 percentage points, respectively; $p < 0.05$). In addition, kits from the R line showed the lowest values for the $CD25^+$ percentage (on av. -0.8 percentage points; $p < 0.05$). Regarding the percentage of phagocytosis of heterophils, kits of the R line had significantly lower values than the RFLP line (-1.2 percentage points; $p < 0.05$) while RF kits showed intermediate values.

Table 3.2. Effect of genetic type and physiological state on the leucocyte percentage in the blood of rabbit females.

	Genetic type				Physiological State			P-value		
	R	RF	RFLP	SEM	AI	P	W	SEM	GT	S
n	40	37	45		122	122	122			
Total lymphocytes (%)	33.47 ^a	39.89 ^b	38.05 ^b	1.598	42.70 ^b	41.79 ^b	26.92 ^a	1.531	0.016	<0.0001
B -Lymphocytes (%)	17.00	16.45	15.55	1.089	16.27 ^a	19.46 ^b	13.28 ^a	1.059	0.625	0.0007
T-Lymphocytes (%)	35.40	36.76	37.56	1.166	39.84 ^b	37.72 ^b	32.16 ^a	1.048	0.404	<0.0001
CD4 ⁺ (%)	24.26 ^a	25.97 ^{a,b}	27.67 ^b	0.922	26.67 ^b	28.08 ^b	23.16 ^a	0.830	0.032	0.0002
CD8 ⁺ (%)	8.178	8.304	7.599	0.428	10.48 ^b	7.197 ^a	6.406 ^a	0.360	0.455	<0.0001
CD25 ⁺ (%)	3.902	4.055	3.462	0.265	4.691 ^b	3.449 ^a	3.280 ^a	0.275	0.262	0.0007
Monocytes ¹ (%)	5.535 ^b	4.553 ^a	4.636 ^a	0.277	5.208 ^b	4.078 ^a	5.538 ^b	0.256	0.023	0.0004
Granulocytes (%)	32.26 ^b	28.03 ^a	28.35 ^a	1.285	23.95 ^a	28.35 ^b	36.35 ^c	1.233	0.038	<0.0001
CD4 ⁺ /CD8 ⁺	3.313 ^a	3.717 ^{a,b}	4.385 ^b	0.243	2.756 ^a	4.436 ^b	4.228 ^b	0.218	0.007	<0.0001
Haptoglobin (log ₁₀ 10 ⁹ /L)	-0.478	-0.441	-0.456	0.058	-0.701 ^a	-0.687 ^a	-0.399 ^b	0.081	0.899	<0.0001
Phagocytosis (%)										
Macrophages	67.88	67.13	62.82	2.30	77.73 ^b	60.10 ^a	60.01 ^a	2.33	0.256	<0.0001
Heterophils	43.84	43.98	44.21	1.85	39.77 ^a	46.20 ^b	46.05 ^b	2.09	0.797	0.119

n: number of animals; GT: genetic type; S: physiological state; AI: artificial insemination; P: partum; W: weaning; ^{a,b} The means in a row within an effect not sharing a superscript were significantly different ($P < 0.05$); SEM: Pooled standard error of means; ¹ Interaction GT*S

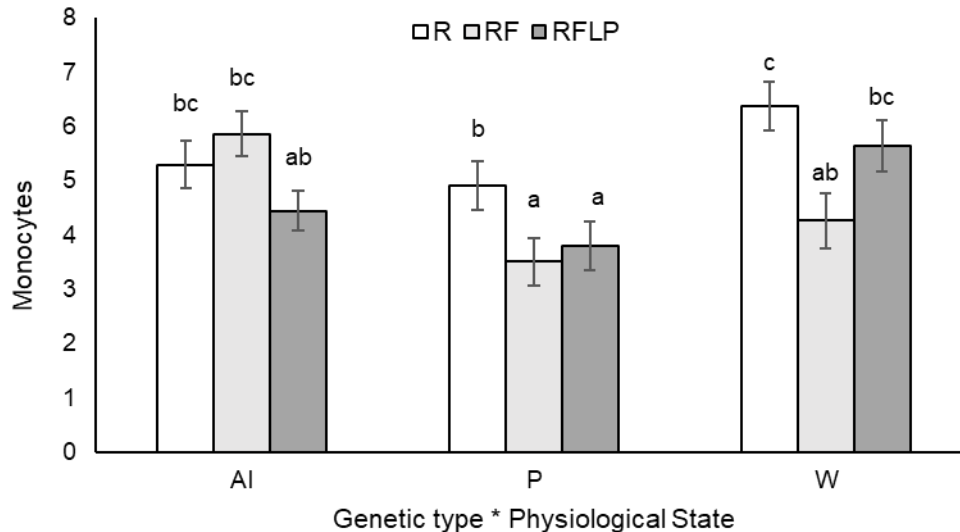


Figure 3.1. Effect of genetic type (R, RF and RFLP) on the percentage of monocytes of rabbit females from the first artificial insemination to weaning. Means not sharing superscript were significantly different ($P < 0.05$). AI: artificial insemination; P: parturition; W: weaning

Table 3.3. Effect of the genetic type on the percentage of leukocytes in weaned rabbits.

	Genetic type			SEM	P-value
	R	RF	RFLP		
No. of litters	19	15	18		
Total lymphocytes (%)	1.278	1.528	1.043	0.144	0.082
B-Lymphocytes (%)	4.912	4.946	5.057	0.570	0.982
T-Lymphocytes (%)	15.50 ^a	23.64 ^b	20.54 ^{ab}	2.176	0.036
CD4 ⁺ (%)	9.882 ^a	15.98 ^b	13.76 ^{ab}	1.458	0.016
CD8 ⁺ (%)	4.904	7.049	6.174	0.685	0.096
CD25 ⁺ (%)	0.736 ^a	1.420 ^b	1.502 ^b	0.198	0.012
Monocytes (%)	3.589	3.483	3.839	0.419	0.835
Granulocytes (%)	14.05	15.92	17.81	2.442	0.534
CD4 ⁺ /CD8 ⁺	2.191	2.360	2.288	0.135	0.679
Phagocytosis (%)					
Heterophils	1.812 ^a	2.874 ^{ab}	3.010 ^b	0.454	0.121

R: paternal line selected by growth rate; RF: new paternal line founded by selecting elite animals from the R line; RFLP: new paternal line founded by backcrossing the lines RF and a maternal line called LP; n: number of records; SEM: Pooled standard error of means; GT: genetic type; ^{a,b} Means in a row not sharing superscript were significantly different ($P < 0.05$);

Figure 3.2 shows the females' survival from the first AI to the second parturition. At the first parturition, the proportion of females from the R line significantly differs from the RF line (-16.0 percentage points; $P < 0.05$). At second parturition, the proportion of the

R line population was lower than the RFLP (-24.0 percentage points; $P < 0.05$), with RF females having intermediate values. The proportion of females from the R line showed the highest decline among the three lines (53.7 percentage points).

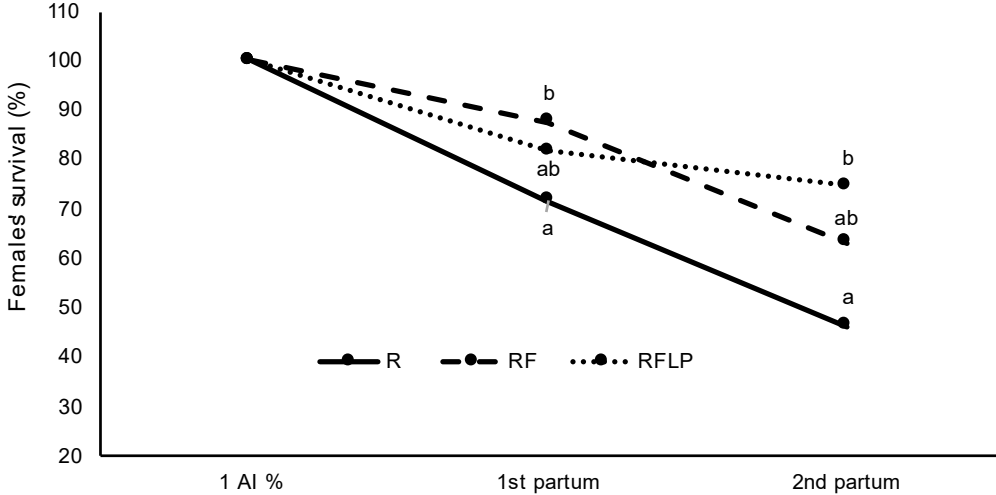


Figure 3.2. Females' survival from the first AI to the second parturition. Means in a physiological stage not sharing superscript were significantly different ($P < 0.05$).

Table 3.4 presents Pearson correlation coefficients demonstrating associations between the percentage of leukocytes in females and their kits at weaning. Precisely, an increase in the percentage of total lymphocytes in females corresponds to higher percentages of total Lymphocytes, T-lymphocytes, $CD4^+$, and $CD8^+$ in their kits (+0.339, +0.370, +0.409 and +0.305, respectively; $P < 0.05$). Similarly, populations of granulocytes, $CD8^+$ and $CD4^+/CD8^+$ of females and their kits were also correlated at weaning (+0.402, +0.297 and +0.412, respectively; $P < 0.05$). Furthermore, higher percentages of T-lymphocytes and $CD4^+$ in females are associated with increased $CD25^+$ in their kits (+0.334 and +0.295, respectively; $P < 0.05$).

Table 3.4. Pearson coefficients for the correlations between leukocyte populations of females and kits at weaning (n=52).

Kits	Females								
	Total Lymphocytes	Macrophages	Granulocytes	B-lymphocytes	T-lymphocytes	CD4 ⁺	CD8 ⁺	CD25 ⁺	CD4 ⁺ /CD8 ⁺
Total lymphocytes	0.339*	-0.138	-0.325	0.029	-0.009	-0.104	0.070	-0.089	-0.147
Macrophages	0.072	0.154	0.214	0.156	0.192	0.128	0.049	0.121	0.062
Granulocytes	-0.096	0.113	0.402**	0.069	-0.059	-0.184	0.099	0.214	-0.152
B-lymphocytes	0.053	-0.098	0.060	0.153	-0.026	-0.096	0.131	0.123	-0.203
T-lymphocytes	0.370**	0.071	0.038	0.031	0.212	0.005	0.206	0.177	-0.149
CD4 ⁺	0.409**	0.049	-0.015	0.023	0.217	0.031	0.180	0.179	-0.097
CD8 ⁺	0.305*	0.099	0.046	0.025	0.214	-0.035	0.297*	0.133	-0.277
CD25 ⁺	0.220	0.050	0.087	0.008	0.334*	0.295*	-0.055	0.238	0.219
CD4 ⁺ /CD8 ⁺	0.222	-0.020	-0.242	-0.038	-0.129	0.021	-0.323	0.003	0.412**

n: number of records; * P<0.05; ** P<0.01

Discussion

Haematological studies shed light on aspects such as the immune system's activation level, the short- and long-term effects of poor husbandry practices, potential illnesses, and genetic predispositions (Seibel et al., 2021). In rabbits, haematological and biochemical parameters are influenced by stress, age, sex, breed, and circadian rhythms (Melillo, 2007). Since the immune system of rabbits might subsequently respond to multiple factors throughout their lives, the first AI is the ideal moment to evaluate the immune system in relation to its genetic type (Pascual et al., 2013). Therefore, the sample taken at first AI was assumed as a control to compare the changes throughout the animals' lives.

In female rabbits, first weaning and second parturition are critical moments with high culling risks (Rosell and De la Fuente, 2009). At first weaning, females overlap lactation and gestation requirements, causing high demands and probably generating higher immunological stress in R females (Penades et al., 2018). In a recent study, comparing two different generations of the R line, it was observed that the concentration of total lymphocytes and the main lymphocyte populations (T-lymphocytes, CD4⁺, CD8⁺ and CD25⁺) decreased from first AI to weaning (Moreno-Grúa et al., 2023). Similarly, our results show a decline in the percentage of total lymphocytes and the main lymphocyte populations at weaning. Since adaptive immunity is suppressed during gestation to prevent fetus rejection, a decline in the percentage of the main leukocyte populations is expected (Warning et al., 2011). Also, physiological stress related to a high resource requirement between competing functions (growth, lactation and gestation), may induce general leukopenia with lymphopenia and an increase in granulocytes (Melillo, 2007). Adult rabbit females that have longer lactation period (later weaning) have lower total lymphocyte counts and lymphocyte subtypes than females that are weaned earlier (Guerrero et al., 2011). Furthermore, an increase in granulocytes with a lower number of lymphocytes is related to an increase in immunological stress and is considered a biological indicator that predicts future health issues in animals (Penadés et al., 2020). Declining lymphocyte populations directly impact the adaptive responses of animals to illnesses (Silva et al., 2020). These cells initiate, maintain, and control the immune response (Snyder, 2012). Therefore, a pattern of recurrent infection appears when there is a deficit of T-cells (Popovich et al., 2010).

Regarding phagocytic activity, a stress-related decrease has been described (Hine et al., 2019; Galapero et al., 2015). In this work, the decrease in macrophage activity from the first AI to weaning might also be an indicator of stress at this particular moment. However, the rise in granulocytes and heterophil activity observed in this work might suggest some improvement regarding innate immunity (Jaedicke et al., 2009). An increase in the innate immune response could be an upregulation strategy to compensate for the lower adaptive immune function during pregnancy. (Warning et al., 2011).

Monocytes function as cell phagocytes defending against bacterial infections and are found in the circulatory system for short periods (Davis et al. 2008). In humans, peripheral blood monocytes migrate out of circulation into areas of injury and help reduce inflammation and tissue restoration. During birth, for example, monocytes migrate to the cervix, myometrium, and endometrium (Ghattas et al., 2013; Tang et al., 2015). In general, the three genetic types showed a lower monocyte percentage at parturition. The lower monocyte percentage in the peripheral blood of adult RF and RFLP females, especially at parturition, could mean a migration of monocytes from the bloodstream to reproductive tissues. This migration could indicate an active tissue repair and inflammation regulation from these lines. This could indicate a change in resource allocation of the new lines, allowing them to exhibit a distinct immune response during the stress of parturition. Contrarily, the RF line exhibited a lower monocyte percentage at weaning than the R and RFLP lines. For the RFLP line, the monocyte percentage significantly increased from the first AI to weaning. However, Moreno-Grúa et al. (2023) found the lowest monocyte count at first AI.

The concentration of haptoglobin rises from the first AI to weaning. Haptoglobin is an acute-phase protein that modulates the immune response, such as minimising cellular damage during inflammation. A rise in haptoglobin concentration is a non-specific response related to inflammation that can be used indirectly to know the health status of animals (Oliviero et al., 1987; Naryzhny and Legina, 2021). The increase in this parameter could be due to the body's effort to cope with all physiological and environmental changes and exposure to pathogens.

When comparing genetic types, R females had a lower percentage of total lymphocytes than RF and RFLP. In addition, the CD4+ percentage and CD4+/CD8+

ratio for R females were also lower than for the RFLP females. Since the CD4⁺/CD8⁺ ratio predicts immune regeneration, a lower ratio suggests that the immune system is less equipped to recover from infections (Tinago et al., 2014). In this particular study, the R line shows the lowest ratio, the RF shows intermediate values, and the RFLP line presents the highest values. The lower leukocyte population could account for the significant decrease in the percentage of females from the R line, which became significantly lower than that of the RFLP line by the second parturition. Kits from the R line also showed a lower percentage of T-lymphocytes, CD4⁺, CD25⁺ and percentage of phagocytosis of heterophils. However, it is worth noting that the R line had higher monocyte and granulocyte percentages in adult females than both RF and RFLP lines. These results align with a previous study comparing the R line with two maternal lines, the long-living robust LP line and the hyper-prolific H line (Penadés et al., 2018). In that study, the authors found that the R line generally had lower leukocyte counts than monocytes and granulocytes when compared with the LP line (Penadés et al., 2018).

Furthermore, in a study using two different generations from the R line, the authors suggested that low leukocyte count could be a characteristic pattern exhibited by this genetic type (Moreno-Grúa et al., 2023). Therefore, due to the similarity of the results obtained in the present study, a low leukocyte percentage would indicate the normal health status for the R line and could reflect a strategy of immune tolerance. In this case, immune tolerance could be less energetically expensive and damaging to the host than a strong immune response (Schneider and Ayres, 2008). In contrast, a high leukocyte percentage of the R line could be a sign of infection or disease.

Although R animals show lower leukocyte counts than other genetic types, it might not necessarily translate to a worse immune status in a basal state (García-Quirós et al., 2014; Moreno-Grúa et al., 2023). However, when compared to other genetic types, with LP, for example, R animals are considered less robust (García-Quirós et al., 2014). We hypothesise that creating the RFLP line by introducing LP genetics might have changed the acquisition and allocation strategy related to the immune status (Peixoto-Goncalves et al., 2023a). The patterns previously observed showed that LP animals do not prioritise a specific life function. Instead, their enhanced capacity to acquire and allocate resources allows more effective distribution among different life functions and inhibits an excess mobilisation of body reserves (Saviotto et al., 2015). Furthermore, a different study comparing the LP line with a line selected for litter size (V), found that

LP animals could cope better under a heat stress challenge (Ferrian et al., 2012). These strategies might have positively influenced the RFLP line's ability to face environmental constraints and initiate an immune response.

The positive correlation between the percentages of some leukocyte populations of females and kits at weaning suggests a potential maternal contribution to the innate and adaptative immunity of the offspring. These results align with a previous study in pigs, where most immune traits studied were moderately to highly heritable (Clapperton et al., 2008). In this study we have observed that about 41 percent of the variability of the CD4⁺/CD8⁺ ratio in weaned rabbits could be explained by this same ratio in their mothers. Considering that this ratio is a biomarker of the immune system's ability to recover from infections (Tinago et al., 2014), this could explain why RFLP females with higher CD4⁺/CD8⁺ ratios have litters with higher survival rates after weaning (Peixoto-Gonçalves et al., 2023b). These results offer a promising perspective on using immune status in rabbit females to improve overall farm health (Pascual et al., 2013).

Conclusions

The results have demonstrated that the leukocyte populations fluctuate during the productive cycle of female rabbits. Our findings indicate that these oscillations differ depending on the genetic type and moment of the reproductive cycle. However, the interaction between genetic type and physiological state does not appear to alter most white blood cell populations significantly. Previous work showed that the introduction of LP genetics in the RFLP line appears to alter resource acquisition and allocation strategies, and this study seems to show that it potentially enhances the line's ability to face environmental constraints and produce an effective immune response. Furthermore, female rabbits' immune profiles may impact their kits' immune status, highlighting potential maternal-offspring interactions influencing immune parameters.

Finally, our results suggest that crossbreeding paternal rabbit lines with a maternal line selected for longevity and productive criteria could improve health traits in rabbit lines.

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GENERAL DISCUSSION

The primary objectives of our research were to evaluate the impact of the generation of two new genetic types on reproductive performance, growth rate, and immune responses in female rabbits and their growing rabbits. In this final discussion, we address the multifaceted implications of our findings across three distinct experiments.

While selecting for growth traits can provide significant benefits regarding weight gain and feed efficiency, it can also introduce trade-offs, particularly in reproductive health and immune function. These trade-offs may impact breeding programs' sustainability and productivity, emphasising the importance of an approach considering both short- and long-term viability.

This discussion focuses on two primary comparisons: R vs. RF and R vs. RFLP. We will discuss differences in reproductive performance, body weight, health, and immune function in female rabbits. Additionally, we will explore growing rabbits' growth performance, digestive efficiency, and health status. By comparing these aspects, we highlight the complexities and potential challenges of selective breeding strategies prioritising specific traits.

R vs RF

Female rabbits

Figure 4.1 presents a radial representation of the principal characteristics of the R and RF lines, both of the females and their litters.

Selection for a high growth rate generates females with higher BW, while females selected for reproductive performance are usually lighter (Arnau-Bonachera et al., 2018; Marín-García, 2019). However, in this case, despite selecting elite animals with high growth rates, the foundation of the RF line generated females with slightly lower BW than the R line. Interestingly, these animals showed better early reproductive performance, such as higher fertility and milk yield, than R females. In addition, over the first three parturitions, R females delivered fewer kits born alive compared to RF females (-1.6 kits; $P < 0.05$). R females' higher adult body weight might have led to less mature animals than RF females (Gondret et al., 2005). These results suggest that the intense selection for elite animals may have inadvertently enhanced reproductive performance in the early stages of reproductive life. Therefore, it might be

possible that RF females allocate more resources towards reproductive functions at the expense of achieving a higher adult weight (Arнау-Bonachera et al., 2018). However, this initial high reproductive effort is not sustained in subsequent cycles, where the decline in performance may be attributed to their lower capacity for resource acquisition.

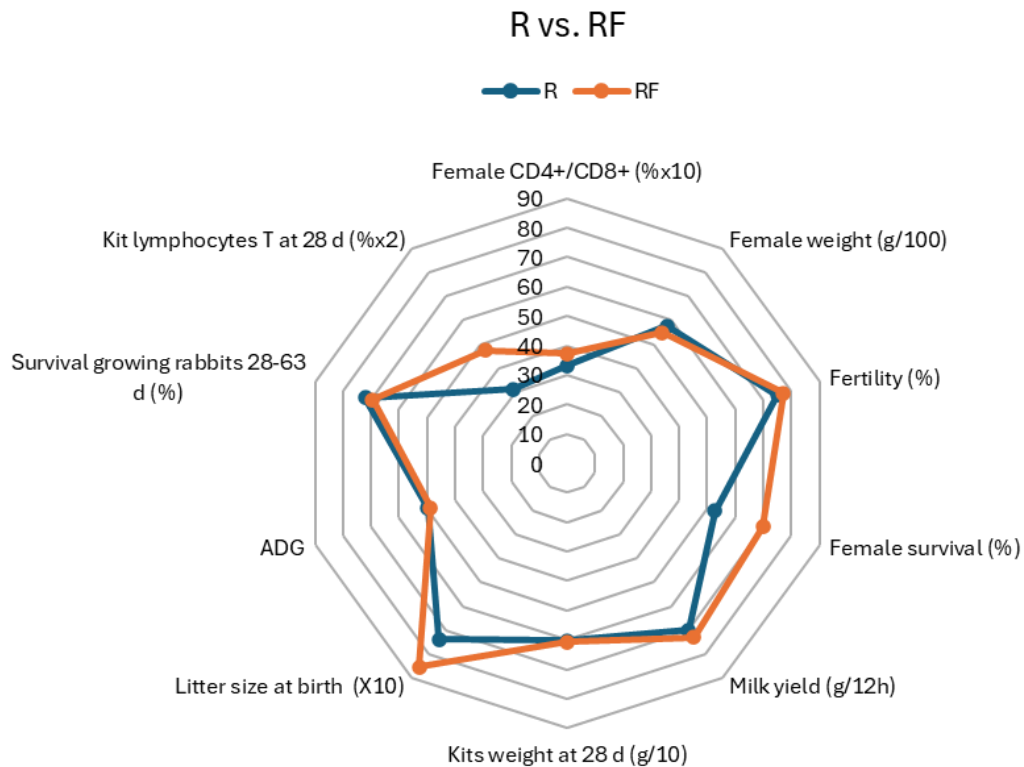


Figure 4.1. Radial plot comparing the main characteristics of R and RF lines.

Regarding the health status of the female rabbits, immunological studies revealed that RF females had significantly higher total lymphocyte percentages than R females (+6.42 percentage points; $P < 0.05$). In contrast, R females had higher monocyte and granulocyte percentages (+0.98 pp; +4.23 pp respectively; $P < 0.05$), which may indicate a heightened inflammatory or stress response (Penadés et al., 2020). Furthermore, RF females exhibited higher survival rates than R females during the first and second cycles. The increased culling rate among R females suggests lower reproductive longevity and resilience, likely due to the long-term selection pressure for growth at the expense of reproductive and health traits (Rauw et al., 1998). These

differences in immune profiles suggest that the RF line, selected for elite growth traits, also benefited from improved immune parameters, potentially contributing to their better reproductive performance, higher survival and overall health during the early reproductive stages.

Growing rabbits

The foundation of a new line by selecting elite animals did not improve growth parameters. Although RF animals showed a higher ADG than the R line from 28 to 46 days with restricted feed intake, overall, there were no significant differences between the two at the end of the growing period. However, RF rabbits showed slightly higher faecal digestibility for dry matter and gross energy than R rabbits (+1.3 percentage points; $P < 0.05$). These results suggest that selecting elite growth traits in the RF line might have conferred advantages in nutrient utilisation, which is crucial for optimising growth performance and overall health.

Analysis of immune function parameters revealed that RF rabbits had higher T-lymphocyte, CD4⁺ and CD25⁺ percentages. Since T-lymphocytes regulate the adaptive immune response, the results suggest that the selection for elite individuals might have generated a population with superior adaptive immunity (Snyder, 2012). However, there were no significant differences between R and RF when comparing mortality rates.

An overview of the radical scheme in Figure 4.1 shows that there are no major differences between the R and RF populations. Only, it seems to stand out that the RF population has a better immune status, which could be behind the higher survival of its females during the reproductive phase.

R vs RFLP

Female rabbits

Figure 4.2 presents a radial representation of the principal characteristics of the R and RFLP lines, both of the females and their litters.

Despite the introduction of maternal genetics, there were no significant differences in BW between R and RFLP females. According to Theilgaard et al. (2007, 2009), LP

females had higher BW than other maternal lines, which could have, along with heterotic effects, reduced the impact on body weight caused by backcrossing the R line with this maternal line.

RFLP females showed higher perirenal fat thickness at parturition (+3.0%; $P < 0.05$) and increased daily feed intake during gestation and late lactation (+9.7% and +8.7%, respectively; $P < 0.05$) than R females. These results suggest a different energy acquisition strategy in RFLP females, possibly to support their enhanced reproductive output and milk production (Saviotto et al., 2015). Previous studies showed that LP females were less affected by periods of severe feed restriction due to their ability to mobilise resources (Sánchez et al., 2008). The higher fat reserves and greater feed intake could also indicate better nutritional status and energy management in the RFLP line, facilitating improved reproductive performance (Pascual et al., 2013).

Regarding immune function, RFLP females had significantly higher total lymphocyte percentages and CD4+/CD8+ ratios, while R females exhibited higher monocyte and granulocyte percentages. Notably, a rise in granulocytes alongside a decrease in lymphocytes signals increased immunological stress and is regarded as a biological indicator of potential future health issues in animals (Penadés et al., 2020). Consequently, R females were more frequently culled than RFLP females from the first parturition onwards, indicating lower reproductive longevity and robustness.

The higher culling rate in R females could be attributed to the long-term selection pressure for growth, which may compromise reproductive health and longevity. In contrast, incorporating LP genetics seems to enhance reproductive longevity and reduce culling rates, emphasising the importance of integrating health and resilience traits into breeding programs. A previous study comparing the LP line with another maternal line (V) found no differences in survival between the lines when exposed to favourable environmental conditions. However, when subjected to environmental challenges, LP animals showed higher survival due to their robustness (Sanchez et al., 2008).

These immune profile differences suggest that the RFLP line benefits from the resilience traits of the LP line, resulting in better immune function and overall health.

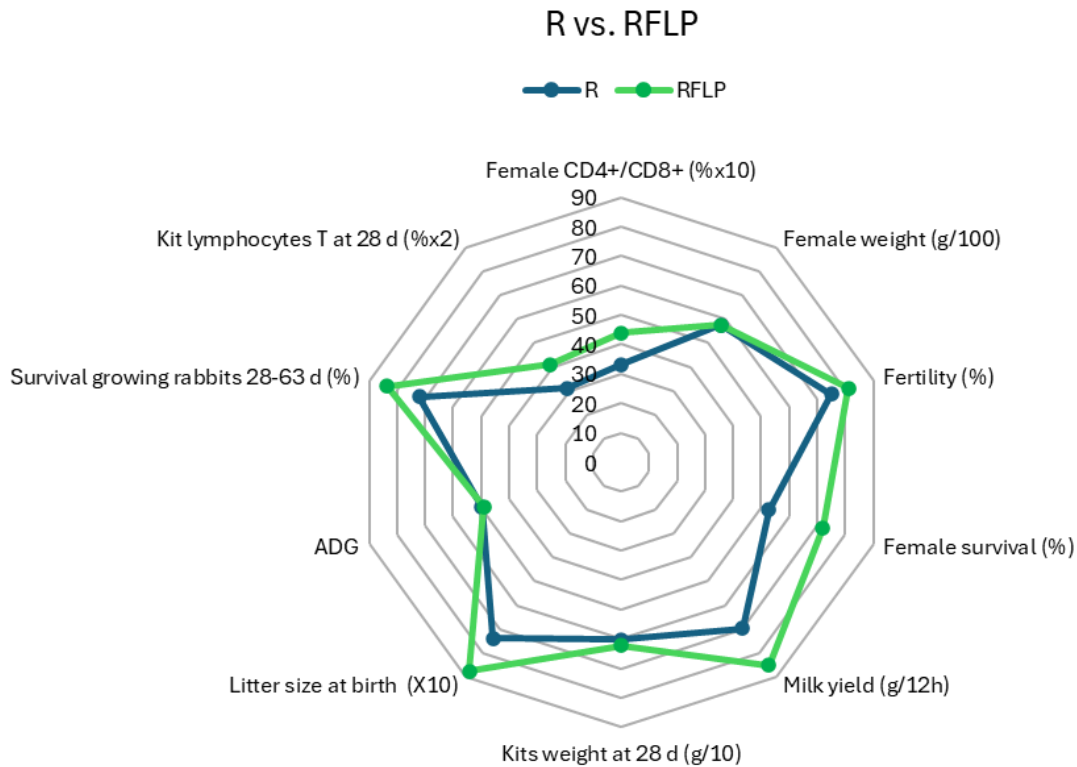


Figure 4.2. Radial plot comparing the main characteristics of R and RFLP lines.

Growing rabbits

R and RFLP rabbits did not show significant differences in body weight, daily feed intake, and feed conversion ratio throughout the experiment, indicating that the introduction of LP genetics did not adversely affect the growth performance of the RFLP line. However, the digestive efficiency of dry matter and gross energy was slightly higher in RFLP rabbits compared to R rabbits (+1.3 percentage points; $P < 0.05$). This improvement in digestive efficiency could be attributed to the genetic contribution of the LP line, although previous studies comparing the LP line with another maternal line (V) showed contrary results (Pascual et al., 2008).

Analysis of immune function parameters in growing rabbits showed that R kits had lower T-lymphocytes and CD4+ percentages than RFLP kits and lower CD25+ percentages. The lower percentage of adaptive immune cells in R rabbits is consistent with previous studies comparing the R line with the LP line and a third line selected for litter size (García-Quirós et al., 2014; Penadés et al., 2018). Furthermore, when

comparing different generations from the R line, Moreno-Grúa et al. (2023) suggested that a lower leukocyte count could be a characteristic of the R line. However, mortality rates were significantly lower in RFLP rabbits than in R rabbits (14.1 and 25.4%, respectively; $P < 0.05$), indicating better health and robustness in the RFLP line. This lower mortality rate could be linked to the nutrient surplus provided by the better utilisation of the diet and the improved immune function observed in RFLP females, as the heritability of most immune cells is moderate to high (Claperton et al., 2008). In addition, previous studies showed a maternal effect on the incidence of digestive disorders (Quevedo et al., 2003). A quick overview of the radical scheme shown in Figure 4.2 shows that the introduction of robust genetics in line R has resulted in notable improvements in numerous parameters. Without relevant alterations in growth and liveweight traits, the observed changes in resource allocation have led to a substantial enhancement in reproductive parameters and the health of both females and their litters. This illustrates the potential benefits of employing this strategy to enhance resilience in specialised lines.

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CONCLUSIONS

The findings of this research demonstrate that the breeding practices employed to develop the two genetic types generated from a rabbit paternal line have significantly impacted the reproductive capacity, growth performance, immune status and overall health of the resulting populations. Furthermore, it demonstrates the necessity of encompassing both productive and resilience-related traits for the sustainability of some genetic selection programmes.

Traditional foundation of paternal rabbit lines solely based on productive criteria might inadvertently introduce reproductive and robustness limitations in rabbit females. The re-foundation strategy, exemplified by the RF line, shows significant improvements in reproductive traits and litter survival during the first reproductive cycle. However, challenges appeared in subsequent cycles, emphasising the need for additional resources. As seen in the RFLP line, the introduction of robust LP genetics through backcrossing demonstrated enhanced resource acquisition and allocation, addressing physiological demands and ensuring reproductive success and litter survival.

Although minor alterations were noted in nutrient utilisation and growth, overall performance remained similar among different genetic lines during the growing period. The inclusion of robust genetics did not compromise growth traits, emphasising the feasibility of integrating resilient genetic traits without sacrificing growth potential. Furthermore, incorporating LP maternal genetics proved advantageous by enhancing resistance to digestive disorders. These findings emphasise the importance of considering factors beyond growth criteria when creating or refining genetic lines, mitigating potential side effects and promoting overall animal health.

Genetic types and physiological states influenced leukocyte populations, with the RFLP line exhibiting altered resource strategies, particularly in immune responses. Maternal-foetal interactions highlighted the potential impact of female rabbits' immune profiles on their kits. The study proposes that reestablishing paternal rabbit lines effectively enhances rabbit health, contributing to future genetic progress in rabbit farming.

These findings suggest that strategies based on the introduction of robust genetics can contribute to refining existing genetic lines.

Short Curriculum Vitae

I hold a Bachelor's and Master's degree in Engenharia Zootécnica from the Universidade de Trás-os-Montes e Alto Douro in Vila Real, Portugal. I have been a PhD candidate since 2019, funded by the Santiago Grisolia scholarship. As a PhD candidate, I took courses on animal experimentation and nutrition. My academic journey included a research stay at the Università degli Studi di Napoli Federico II in Naples, where I had the privilege of working on projects that involved researching sustainable feed systems and assessing their feasibility for commercial implementation. Currently, I am a professor at CEU Cardenal Herrera, where I teach subjects related to animal nutrition and production.

I possess a C1 level proficiency in both English and Spanish.

List of publications

Research articles

Peixoto-Gonçalves, C., Martínez-Paredes, E., Ródenas, L., Corpa, J.M., Blas, E., Cambra-López, M., Pascual, J.J. (2023). Growth performance of three paternal rabbit lines with different potentials for growth rate and resilience. *World Rabbit Science*, 31: 221-228.

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Science outreach articles

Ramón-Moragues, A., Peixoto-Gonçalves, C., Martínez-Paredes, E., Villagrà, A. (2023). Efecto de las técnicas de manejo como alternativa al uso de antibióticos sobre parámetros de estrés y producción en conejos de cebo. *Boletín de Cunicultura*, 210: 28-32.

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Conference contributions

Peixoto-Gonçalves, C., Martínez-Paredes E., Moreno-Grúa, E., Martínez-Seijas, C., Viana, D., Arnau-Bonachera, A., Ródenas, L., Cambra-López, M., Blas, E., , Pascual, J.J., Corpa, J.M. (2024). Fundación De Nuevas Líneas Paternales: Estudio Del Sistema Inmunitario. 48 Symposium de Cunicultura ASESCU. Córdoba, España.

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Peixoto-Gonçalves, C., Martínez-Paredes, E., Ródenas, L., Blas, E., Cambra-López, M., Pascual, J.J. (2022). Rendimiento reproductivo de las conejas de líneas paternales: reconciliando crecimiento y resiliencia. 46 Symposium de Cunicultura ASESCU. Pineda de Mar, España. Pp. 80-84

Rámon-Moragues, A., Carulla-Pascual, P., **Gonçalves, C.**, Escribano, D., Torres-Lozano, R., Romero-Rueda, T., Martínez-Paredes, E., Villagrà-García, A. (2022). Efecto de diferentes técnicas de manejo como alternativa al uso de antibióticos sobre parámetros de estrés en conejos de cebo. 46 Symposium de Cunicultura ASESCU. Pineda de Mar, España. Pp. 123-127

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Awards

IV Premio para jóvenes investigadores ADESCU-Andrés Pintaluba

Awarded on 01/06/2022