

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). BW, 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.

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Implications

Paternal lines are a key element of three-way crossbreed schemes used in rabbit production farms. Selected for feed efficiency-related traits, like growth rate and/or residual feed intake, their reproductive and health traits have been neglected, resulting in low fertility rates, small litter sizes and high juvenile loss. The refoundation and the crossbreed strategy are well-known strategies to counteract these undesirable selection effects

on a single trait. This work demonstrates the interest in such strategies to improve paternal lines' reproductive and health traits, both in the short and the middle term.

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

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been successful in non-ruminant animals (Havenstein et al., 2003; Suzuki et al. 2005). In rabbits, main selection programmes show that selection for growth rate has allowed an improvement in average daily gain (ADG) of 0.45–1.23 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 (Saviotto 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 the 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 (Poppe 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 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 programmes without compromising genetic progress? Berghof et al. (2019) suggested that the inclusion of resilience in breeding programmes 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. Preliminary results have been previously presented in a meeting (Peixoto-Gonçalves et al., 2022).

Material and methods

Animals

A total of 197 rabbit females from three 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; Saviotto et al., 2015). 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 commercial 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, according to the ethical statement's details. 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 and 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 CP, 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 CP, 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 three 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 synchronised to experimental females were used. On the 18th day postpartum (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 BW, feed intake and perirenal fat thickness (PFT; using ultrasounds following Pascual et al., 2000; 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 0800 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 0700 h, and blood samples were taken starting at 1100 h. The samples were collected from the central ear artery into tubes containing ethylenediaminetetraacetic acid. Blood samples were centrifuged immediately after sampling (3 000g, 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 (three levels, R, RF and RFLP), the season within the year (four levels), the parity order (three levels 1, 2 and 3), the overlapping of lactation with the previous or posterior gestation (two levels, yes or not) and their interactions as fixed effects.

For plasma traits, the model included the genetic type (three levels, R, RF and RFLP), the season within the year (four levels), the control day (eight 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 (two 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. 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 show 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

Table 1
Effect of genetic type (R, RF and RFLP) on the reproductive performance of rabbit females from first mating to third parturition.

Item	Genetic type (GT)			P-value				
	R	RF	RFLP	GT	PO	GT × PO	O	GT × O
No. females at first mating	65	63	69					
BW (g)								
First mating	5 578 ± 71 ^b	5 328 ± 71 ^a	5 586 ± 72 ^b	0.016				
Parturition	5 555 ± 74 ^b	5 237 ± 67 ^a	5 570 ± 64 ^b	0.001	0.000	0.037	0.006	0.026
18 dpp	5 950 ± 85 ^b	5 645 ± 75 ^a	5 914 ± 78 ^b	0.007	0.177	0.880	0.070	0.685
28 dpp	5 951 ± 87 ^b	5 694 ± 77 ^a	5 995 ± 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
0-18 dpp	304 ± 9	312 ± 8	322 ± 8	0.263	0.000	0.439	0.129	0.971
18-28 dpp	342 ± 11 ^a	341 ± 10 ^a	371 ± 10 ^b	0.042	0.000	0.002	0.130	0.136
Milk yield (g/d)								
1-18 dpp	132 ± 6 ^a	139 ± 5 ^a	158 ± 5 ^b	0.002	0.000	0.466	0.001	0.106
18-28 dpp	148 ± 8 ^a	153 ± 7 ^a	182 ± 7 ^b	0.000	0.000	0.001	0.170	0.399
Fertility (%)								
1st cycle	86.7 ^{ab}	95.4 ^b	85.0 ^a	0.041				
2nd cycle	70.9 ^{ab}	62.8 ^a	83.3 ^b	0.005				
3rd cycle	67.4	73.1	75.0	0.400				

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

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

third parturition (Fig. 1). This effect was observed independently of the overlapping of gestation with the previous lactation.

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 (Fig. 2). In general, genetic type did not affect the body condition of females (Table 1), but RFLP females had a higher PFT than R females at parturition (on av. +3.0% for the three parturitions; $P < 0.05$).

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 (Fig. 3, left).

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 (Fig. 3 right). Fertility was also affected by the genetic type (Table 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 Fig. 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. As shown

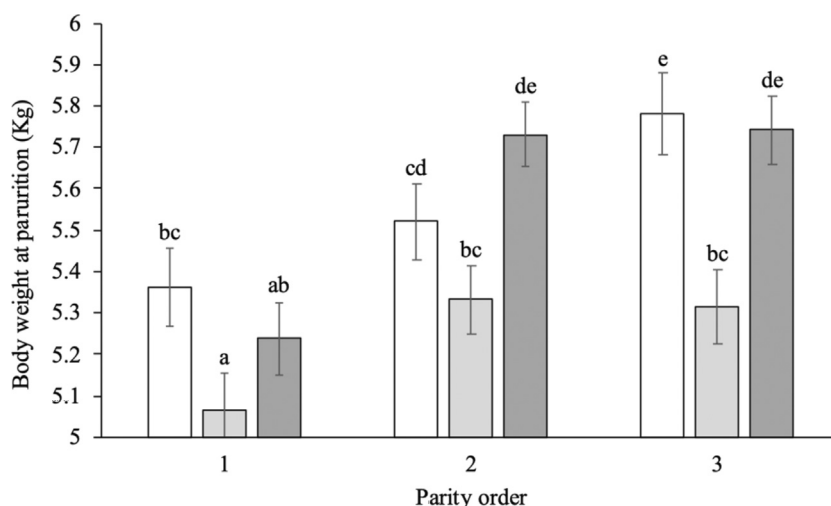


Fig. 1. Effect of genetic type (R □, RF ■ and RFLP ■) and parity order on the BW of rabbit females at parturition. ^{a-e}Means not sharing a common letter are significantly different at $P < 0.05$.

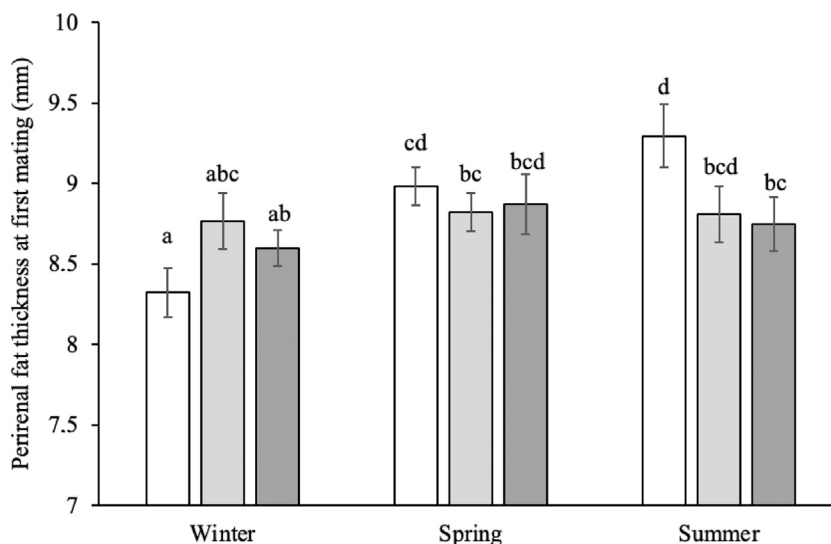


Fig. 2. Effect of genetic type (R □, RF ■ and RFLP ■) and season on the perirenal fat thickness of rabbit females at first mating. ^{a-d}Means not sharing a common letter are significantly different at $P < 0.05$. No. of females: winter (20 R, 15 RF and 37 RFLP); spring (33 R, 33 RF and 16 RFLP); summer (12 R, 15 RF and 16 RLP).

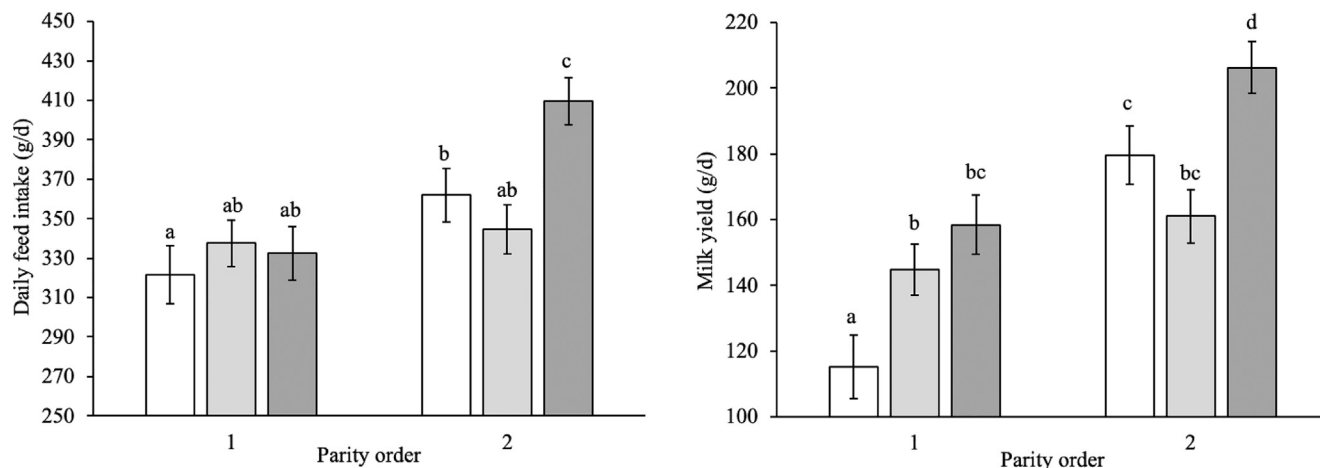


Fig. 3. Effect of genetic type (R □, RF ■ and RFLP ■) and parity order on the daily feed intake (left figure) and milk yield (right figure) of rabbit females from 18 to 28 days postpartum. ^{a-c}Means not sharing a common letter are significantly different at $P < 0.05$.

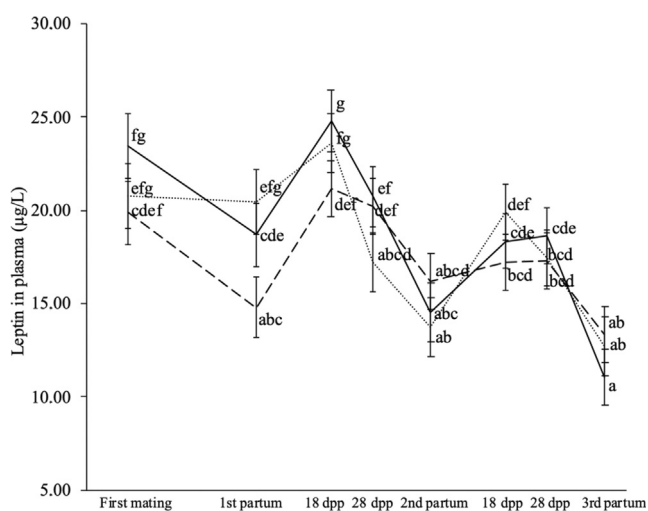


Fig. 4. Effect of genetic type [R (—), RF (---) and RFLP (.....)] and physiological state on plasma concentration of leptin of rabbit females from the first mating to the third parturition. ^{a-g}Means not sharing a common letter are significantly different at $P < 0.05$. dpp = days postparturition.

in [Table 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 ([Fig. 5](#), left). 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 ([Fig. 5](#), right).

Litter traits

According to [Table 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 ([Fig. 6](#), left). On average, females whose

gestation overlapped with the previous lactation had fewer kits born alive (-1.86 kits; $P < 0.001$).

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 3](#) and [Fig. 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 reproductive performance 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. \(2007 and 2009\)](#) 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.

The genetic type had little impact on body condition. However, RFLP females had the most stable PFT values, with minimal loss of

Table 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).

Item	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

NEFA = non-esterified fatty acids; BOHB = beta-hydroxybutyrate.

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

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

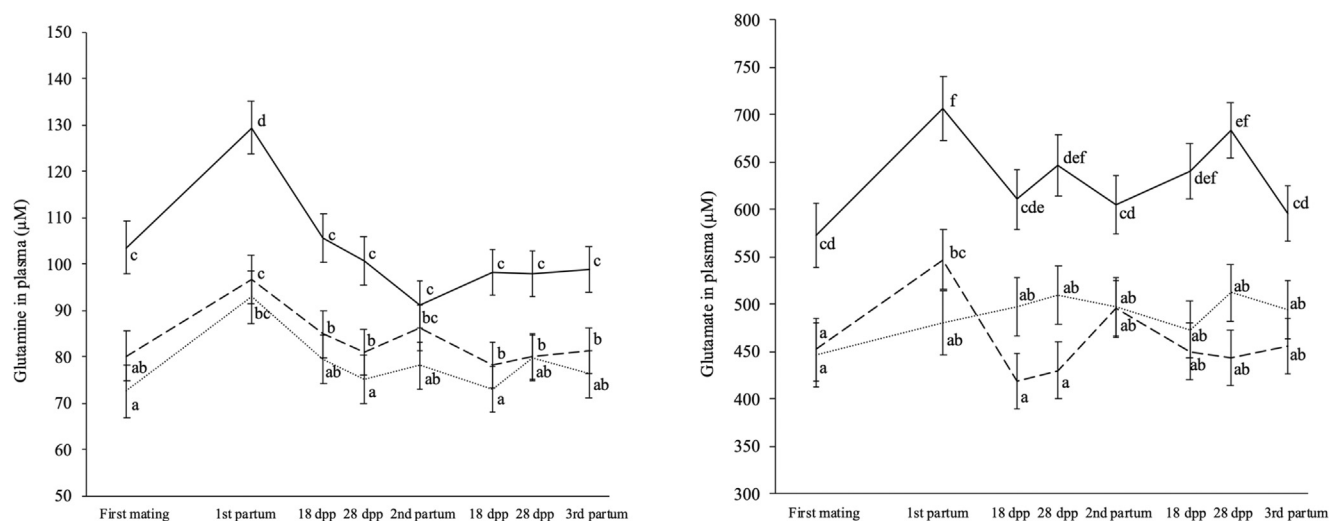


Fig. 5. Effect of genetic type [R (—), RF (---) and RFLP (····)] and sampling day on plasma concentration of glutamine (left figure) and glutamate (right figure) of rabbit females from the first mating to the third parturition. ^{a-f}Means not sharing a common letter within lactation and day postpartum are significantly different at P < 0.05.

Table 3
Effect of genetic type (R, RF and RFLP) on rabbit litter traits during lactation until third parturition.

Item	Genetic type (GT)			P-value				
	R	RF	RFLP	GT	PO	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	1 660 ± 82 ^a	1 838 ± 75 ^a	2 083 ± 76 ^b	0.000	0.000	0.009	0.357	0.326
28 dpp	2 776 ± 140 ^a	3 008 ± 125 ^a	3 550 ± 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 18–28 dpp (g DM/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

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

^{a,b} Means not sharing at P < 0.05.

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

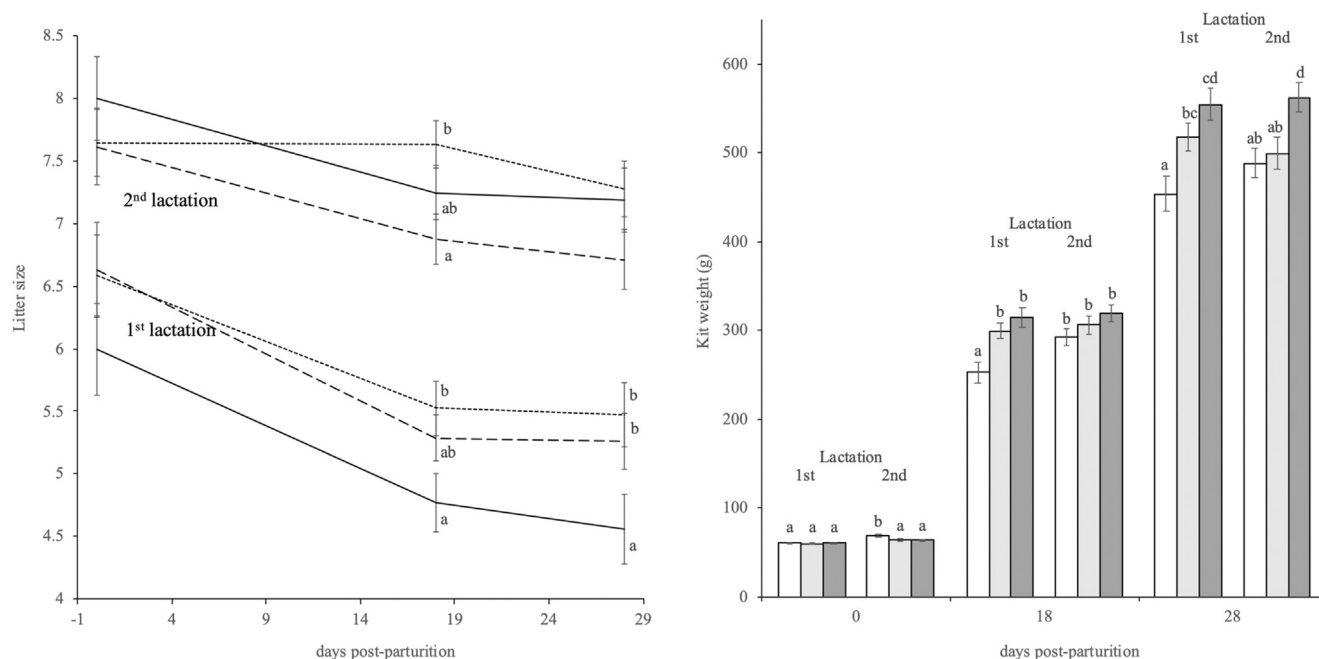


Fig. 6. Effect of genetic type [R (— and □), RF (--- and ●) and RFLP (···· and ▲)] and day postparturition on the rabbit litter size (left figure) and individual kit weight (right figure) during the first two lactations. Litter size was standardised to 6 and 8 at the first and second parity, respectively. ^{a-d}Means not sharing a common letter within lactation and day postparturition are significantly different at $P < 0.05$.

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 (Penades 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 resource 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 hor-

mones, 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 (Chan and Glodberg, 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 are not reaching adult BW until the third parturition (Fig. 1), unlike in the RF and RFLP lines that reach their adult BW by the second parturition.

Effect of genetic type on 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 the backcrossing of RF line with a maternal line (LP) selected for litter size. However, resource 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 and 2008).

Our results suggest that the LP genes provided 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 diges-

tive 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 cross-breed 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.

Ethics approval

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.

Data and model availability

The data that support these findings have not been deposited in an official repository, but they are available from the authors upon request.

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Declaration of interests

None.

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