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# INFLUENCES OF ENVIRONMENT ON THE DEVELOPMENT AND LIFETIME REPRODUCTIVE PERFORMANCE IN DOMESTIC RABBIT FEMALES

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Abstract: Environmental insults of different nature and intensity, such as fluctuation in the air temperature, which can affect access to food, its quality and diseases, are a reality in any livestock system. This is much more important when the insults occur in early life, conditioning the development and adult life of animals. In ecology, for instance, it is widely accepted that high quality offspring are more reactive against predators, occupy better territories and find more mates, resulting in longer lives and greater fitness. It is also a given that adults exposed to famine or disease as juveniles have shorter lives and produce fewer offspring. To determine whether the environment influences the development and lifetime reproductive performance of rabbit females, we designed an experiment combining two factors: nest and pubescent development. Nest development was measured by recording the average daily gain of 864 females during suckling and during their pubescent life (63 to 184 d old), and body development was conditioned by providing animals a high-energy control (C) or a fibre-rich (F) diet. However, in the course of the study, 191 of the 864 pubescent females were exposed to rabbit haemorrhagic disease (RHD). This unexpected environmental insult was considered as a third experimental factor influencing the reproductive performance of rabbit females. Contrary to expectation, fast suckling gain impairs reproductive lifespan, resulting in fewer newborn kits produced in a female lifetime. Although females on diet F lived 37 d longer than females on diet C, this difference was only perceived in their pubescent life. In addition, the exposure to RHD interacted with suckling gain (SG). Exposed females with a fast SG produced more kits as adults, but in the absence of the virus, high SG females produced fewer newborn kits. These results open new insights into the management of future breeders during nesting and pubescent life.

Key Words: *Oryctolagus cuniculus*, rabbit haemorrhagic disease, rearing diet, survival, reproduction, management.

## INTRODUCTION

In ecology, it is widely accepted that a bad start in life reduces fitness and lifespan (Lindström, 1999). For the domestic rabbit, birth weight appears to be positively correlated to maturity, fertility and prolificacy (Szendrö *et al.,* 2006), and all these parameters are impaired when a female is born with a body weight lower than 45 g. Another piece of information relates an excess of fat reserves around first insemination to a subsequent reduced fertility (Savietto *et al.,* 2016). This result reinforces the need to adjust rearing diets to individual nutritional requirements. To test the hypothesis that both fitness and lifespan are positively linked to an adequate body development in the pubescent stage, we designed a trial where rabbit females, of different birth weights, were evenly assigned to feeding on a high-energy or a high-fibre diet in their pubescent life (Martínez-Paredes *et al.,* 2018). As a result, we observed a different growth curve between females fed on these diets and a better survival and lifetime reproductive performance among the animals on the high-fibre diet.

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At the time, our farm was affected by an outbreak of the rabbit haemorrhagic disease (RHD), exposing 22.1% of our pubescent females to the RHD virus. So, to correctly account for the diet effect, we decide to not consider the exposed animals in our statistical analysis. However, there is an ongoing scientific debate on the relation between disease exposure in infancy and adult lifespan and fertility (Hayward *et al.,* 2016). Some argue that exposure to diseases selects robust infants, resulting in long living adults, while others describe no links between disease exposure and lifespan or fitness.

Based on the concept that a bad start in life condition fitness and lifespan, and given the ongoing debate concerning the influence or not of disease exposure on these parameters, we decided to use the information of our exposed rabbit females to test the hypothesis that disease exposure interacts with body development, affecting both the fitness and lifespan of domestic rabbits. In this sense, we used the available data to describe how nest and pubescent development may provide animals with additional resources to withstand a strong environmental insult. We expect that kits presenting a fast nest development, measured as daily weight gain during suckling, and fed on a high-energy diet (faster pubescent development), would result in individuals with a greater capacity to resist this disease, resulting in longer lives and better reproductive performance.

## MATERIALS AND METHODS

The ethics committee of Universitat Politècnica de València (UPV) approved all procedures involving the animals. The committee followed the recommendations on the protection and use of animals for experimentation described in Spanish Royal Decree 1201/2005.

### *Animals, farms, diets and experimental procedures*

Animals, farms, diets and experimental procedures can be consulted in detail at Martínez-Paredes *et al.* (2018). In brief, we followed the life of 864 newborn crossbreed rabbit females (line A×line V of UPV) from birth to death, culling or censoring age. Females were born between December 2008 (birth cohort 1) and October 2009 (birth cohort 14) in a selection nucleus and transferred to a commercial farm every 21 d. At birth, newborn females were individually weighed and identified with a subcutaneous glass microchip (8.5 mm×Ø 1.4; Felixcan Animal ID, Albacete, Spain) and the presence of abdominal milk spot was registered. All females were weighed again at weaning (38 d old) and before transfer to the commercial farm (at 63 d old). Until this point, the young females were managed in the same manner and received the same diets (a standard feed for lactation until weaning and then a fattening feed).

On their arrival at the commercial farm, we assigned 431 females to a high-energy control diet (C) and 433 females to a high-fibre diet (F). Diet C contained 184 g of crude protein (CP), 381 g of neutral detergent fibre (NDF) and 11.8 MJ of digestible energy (DE) per kg of dry matter (DM). Diet F contained 134 g of CP, 436 g of NDF and 10.0 MJ of DE/kg of DM. Females on diet C received this diet from 63 d of age until the end of the experiment, while females on diet F changed to diet C at 157 d of age (first artificial insemination; AI). The table of ingredients and chemical composition of experimental diets are available at Martínez-Paredes *et al.* (2018).

Young females were weighed every 25 d, resulting in a total of five measures per female that had at least one litter. At first AI, perirenal fat thickness (PFT) was recorded as an indicator of body condition, using the ultrasound method described by Pascual *et al.* (2004). Throughout reproductive life, we checked the litter size at birth (number of newborn and stillborn kits) and at weaning (35 d). Litters were standardised to eight or nine kits at first parturition and to a maximum of 11 in the subsequent cycles. Females followed a theoretical reproductive rhythm of 49 d (AI 18 d after parturition). In each reproductive cycle, non-pregnant females were re-inseminated 21 d after the scheduled AI date.

Females were removed from the herd if culled or dead and the data concerning the 47 females still alive at the end of the study (November 2011) was treated as a censored record. Rabbit females were culled owing to low fertility (failure to conceive after three consecutive AI attempts), low productivity (fewer than seven kits weaned in three consecutive parities) or health disorders (sore hocks, mastitis, abortions or low body condition). Deaths occurred naturally throughout the experimental period for no particular reason, except between February and April 2009,

when an outbreak of RHD ravaged 60% of all females of birth cohorts 1, 2 and 3. The exposure to RHD was of limited duration. Once the problem was identified and communicated, the remaining adults and young animals were all vaccinated. When animals from the fourth birth cohort arrived at the commercial farm, the problem was under control. We estimate that the RHD virus was present in the farm, in its most virulent phase, for at least 70 d from the introduction of animals of the first birth cohort.

### *Population demography*

Throughout the experiment we observed the following population demography: from the initial 864 females transferred to the commercial farm at 63 d, 561 reached the first AI age and 522 produced at least one litter. For females exposed to RHD, representing the 191 rabbit females from birth cohorts 1 to 3 transferred to the commercial farm at 63 d (96 on diet C and 95 on diet F), only 66 animals reached the first AI age and 62 produced at least one litter (28 received C diet and 34 F diet). For females not exposed to the virus, representing 673 rabbits from birth cohorts 4 to 14, 31% never conceived (125 on diet C and 87 on diet F) and 461 females had at least one litter (210 on diet C and 250 on diet F), 41% had between one and five litters (129 on diet C and 145 on diet F), 23% had between six and 10 litters (44 on diet C and 63 on diet F), and only 31 females had 11 litters or more (6 on diet C and 5 on diet F).

### *Body development measures at different life stages*

*During nest development (0 to 38 d old).* We used the birth and weaning weight measurements to calculate the suckling gain (SG) of each rabbit female, a variable assumed to be a proxy of nest development. SG was described by Equation 1:

$$Suckling\ gain_i = (Weaning\ weight_i - Birth\ weight_i)/38 \quad (1)$$

In summary, SG had an average of 22.4 g/d, with a median of 22.3 g/d.

*During pubescent life (63 to 188 d old).* In this period, we individually weighed all rabbit females at 63, 88, 113, 138, 163 and 188 d of age (4 d after first parturition), a few days after the animals entered the commercial farm. Live weight measures were conditioned by the technical visits to the farm every 25 d. For instance, females on diet C reached 4.0 kg of live weight 43 d before females on diet F, this being the main effect of the different regimes on the body development of our animals. Considering the observed differences, the diet effect was assumed as a proxy of pubescent development.

### *Reproductive life measurements*

Reproductive life started at first parturition (184 d old) and ended at culling, death or the end of the trial (censored records). In this period and for each female, we measured the number of AI attempts, the number of litters produced and the prolificacy (number of live and weaned kits). In order to impose a similar lactation burden, we set litter size to eight kits for primiparous females and between 9 and 11 for multiparous females, the most frequent litter size being equal to 10 kits. The farmer did not take into account the weight of the individual kits to perform adoptions. However, he was advised to perform a visual inspection to judge the viability of each individual kit. Kits judged as non-viable were discarded, the same criteria being adopted for all litters.

### *Lifespan definitions*

To study the impacts of exposure to the RHD virus on the life of animals before and after the onset of their reproductive career, we worked with two lifespan definitions: chronologic and reproductive.

*Chronologic lifespan*, defined as the number of days between birth date and the date of death, culling or censoring of a female.

*Reproductive lifespan*, defined as the number of days between the date of the first AI (153 d old) and the date of death, culling or censoring.

The trial ended on October, 6th 2011. At that time, only 47 rabbit females, from all birth cohorts, were still alive at the commercial farm. Therefore, we treated the data on these 47 rabbit females as a censored record, representing 5.4% of the initial rabbit population.

### *Lifetime reproductive performance traits*

The cumulative number of newborn kits and the cumulative number of weaned kits a female produced in its life were our measures of lifetime reproductive success.

We also checked for the number of viable litters each female produced, the number of AI attempts and the fertility rate (calculated as the number of litters over the number of AI attempts).

### *Statistical analysis*

All statistical analyses were performed using R language (R Core Team, 2018).

*Cox proportional hazard models used to analyse chronologic and reproductive life.* Our first statistical model related the chronologic and reproductive lifespans to the exposure to RHD. Birth cohort entered the model as a non-independent cluster variable, and we used the Efron method, as recommended by Hertz-Picciotto and Rockhill (1997), to account for tie events (i.e. events happening at the same time). This model is given by Equation 2:

$$h(t)_i = \exp_i^{\mu+\beta\cdot \mathrm{RHD}} + e_i \quad (2)$$

Model (2) revealed a different hazard function for females exposed compared to females not exposed to the RHD. For this reason, and to correctly model the possible influence of the diet received during the pubescent stage on both chronologic and reproductive lifespan, we included the exposure to the RHD virus as a stratification variable of the baseline hazard function. We also included the birth cohort as a cluster variable and used the Efron method to handle tie events. Model (3) is given by:

$$h(t)_i = RHD(t) \cdot \exp_i^{\mu+\beta\cdot Diet} + e_i \quad (3)$$

The last Cox proportional hazard regression model we designed included SG as a covariate influencing the chronologic and reproductive lifespans. Model (4), an extension of model (3), is represented by:

$$h(t)_i = RHD(t) \cdot \exp_i^{\mu+\beta_1\cdot \mathrm{Diet}+\beta_2\cdot \mathrm{SG}} + e_i \quad (4)$$

*Lifetime reproductive performance traits.* The cumulative number of AI attempts is a counting variable, better described by a Poisson distribution with lambda equal to 6.8. To analyse this variable correctly, we used a generalised linear model with a log-link function. The model initially included the diet, the RHD exposure, SG and their two-way interactions. We then performed a stepwise process to select the relevant descriptive variables (based on the Akaike Information Criterion, AIC). The final model used to analyse the cumulative number of AI attempts is in equation (5):

$$AI\ attempts_i = \exp_i^{\mu+\beta_1\cdot \mathrm{Diet} + \beta_2\cdot \mathrm{RHD} + \beta_3\cdot \mathrm{SG} + \beta_4\cdot \mathrm{Diet:RHD} + \beta_5\cdot \mathrm{Diet:SG}} + e_i \quad (5)$$

The cumulative number of viable litters also followed a Poisson distribution with lambda equal to 4.8. The stepwise process retained all variables initially included in the model, but since the interaction between RHD and SG was not significant, we dropped this effect from our final model (6). The AIC for the initial and the final model were 3332.4 and 3332.6, respectively. Therefore, retained model for the cumulative number of litters produced was:

$$Number\ of\ litters_i = \exp_i^{\mu+\beta_1\cdot \mathrm{Diet} + \beta_2\cdot \mathrm{RHD} + \beta_3\cdot \mathrm{SG} + \beta_4\cdot \mathrm{Diet:RHD} + \beta_5\cdot \mathrm{Diet:SG}} + e_i \quad (6)$$

The cumulative number of newborn kits (NB) and kits weaned (WND) are both count variables following a Poisson distribution. For NB, the lambda value was 51.3 and for WND it was 43.6. The general linear model (7) we used to analyse these variables was:

$$NB_i\ ;\ WND_i = \exp_i^{\mu+\beta_1\cdot \mathrm{Diet} + \beta_2\cdot \mathrm{RHD} + \beta_3\cdot \mathrm{SG} + \beta_4\cdot \mathrm{Diet:RHD} + \beta_5\cdot \mathrm{Diet:SG} + \beta_6\cdot \mathrm{RHD:SG}} + e_i \quad (7)$$

For equations (2) to (7), the following mathematical abbreviations refers to: $h(t)$: hazard function at time $t$; exp: exponential function; $\mu$: intercept of regression models; $\beta$: regression coefficients of regression models; $e$: error term of regression models.

## RESULTS

### *Chronologic and reproductive life according to RHD virus exposure*

Survival curves for chronologic and reproductive live depending on the exposure to RHD are shown in Figure 1. Of the 864 females transferred to the commercial farm, we observed 817 events (death or culling). The average chronologic lifespan was 332±8.0 d and the time when 50.0% of females left the herd (median) was 255 d. On average, females not exposed to RHD virus lived 125 d more than exposed females ($P<0.05$). The median lifespans of exposed and non-exposed females were 176 and 283 d, respectively. The hazard ratio obtained was 1.81 times higher for females exposed to RHD compared to those females not exposed, with most mortalities affecting the exposed animals between ages 84 and 162 d (Figure 1, left); before first parturition.

Lifespan females producing at least one litter was 458±10 d on average. For this variable, hazard ratios did not differ between exposed and non-exposed females ($P=0.65$). The median age for exposed females was 377 d, while for non-exposed ones it was 405 d (Figure 1, right). On average, RHD exposure reduced the reproductive lifespan by only 16 d.

### *Chronologic and reproductive life according to RHD exposure and diet*

Chronologic and reproductive life depending on the RHD exposure and diet are in Figure 2. The overall hazard ratio for chronologic life among females fed on diet F was 0.85 ($P=0.02$), representing a reduction of 15% in the relative risk of being culled or dying compared to females fed with diet C. This reduced risk represented a chronologic life of 37 d longer for females fed on diet F. When analysing the diet effect for females exposed to the RHD, the group fed on diet F lived 31 d more than females fed on diet C (250 *vs.* 219 d, respectively). For non-exposed females, those fed on diet F lived, on average, 38 d more than those fed on diet C (385 *vs.* 347 d, respectively). Therefore, the median chronologic lifespan of exposed females fed on diet C was 140 d, while exposed females fed on diet F had a median chronologic life of 162 d (Figure 2, left). For non-exposed females, the median chronologic lifespan of rabbits fed on diet C and F was 280 and 306 d, respectively.

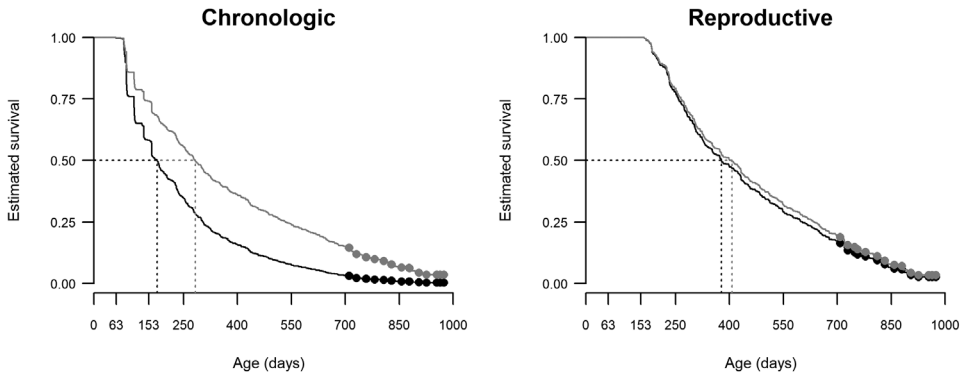


Figure 1: Estimated survival curves for chronologic (left) and reproductive (right) life definitions for females exposed or not exposed to rabbit haemorrhagic disease. For chronologic life, 191 of 864 females were exposed to this virus. For reproductive life, 66 of the 191 exposed females reached insemination age. Dotted lines represent the age at which 50% of animals left the herd; the median survival time. — Exposed, — Not exposed.

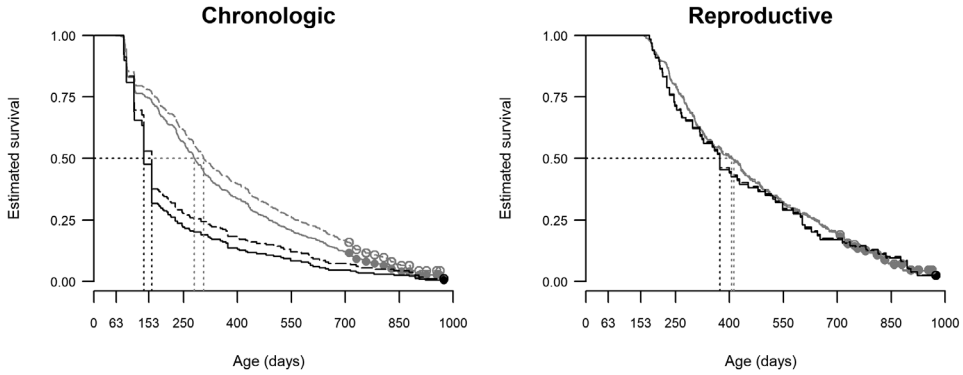


Figure 2: Estimated survival curves for chronologic (left) and reproductive (right) life definitions for females exposed to rabbit haemorrhagic disease or not and fed with the control (C) or fibrous (F) diets. For chronologic life, 191 of 864 females were exposed to the virus. For reproductive life, 66 of the 191 exposed females reached insemination age. Dotted lines represent the age at which 50% of animals left the herd; the median survival time. — Exposed C, – – Exposed F, — Not exposed C, – – Not exposed F.

Diet in combination with RHD exposure had no influence on reproductive lifespan (Figure 2, right). The diet did not influence the median age of rabbits exposed to RHD, both medians being equal to 373 d. For non-exposed females, the median reproductive lifespan of animals receiving diets C and F was 406 and 412 d, respectively.

### *Chronologic and reproductive life according to RHD exposure, diet and suckling gain*

Chronologic and reproductive lifespan depending on RHD exposure, diet and SG are shown in Figure 3. Suckling gain has no influence on either chronologic (Figure 3, left) or reproductive life (Figure 3, right; *P*=0.10). Although not statistically significant, we observed an overall increment in the relative hazard risk of death or culling of 1 percentage point per g of increment on the SG, an effect observed in both exposed and non-exposed females.

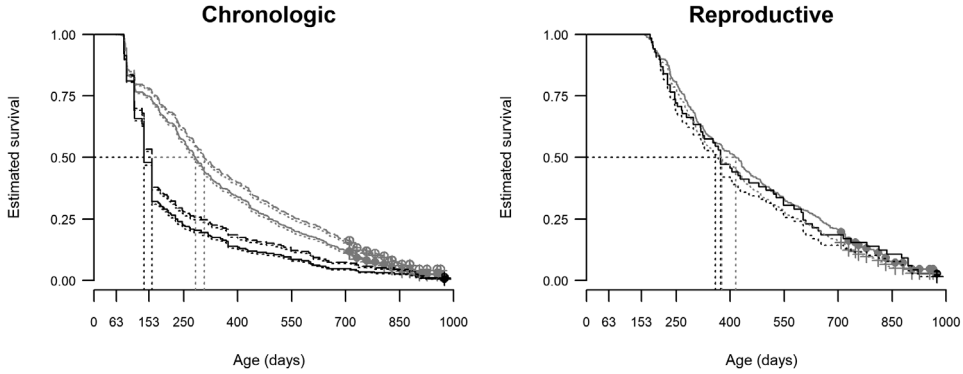


Figure 3: Estimated survival curves for chronologic (left) and reproductive (right) life definitions for females exposed exposed to rabbit haemorrhagic disease or not, fed with the control (C) or fibrous (F) diet and presenting suckling gains of 20 or 30 g/d. For chronologic life, diet effect was significant (P = 0.02), but SG had no impact on chronologic life. For reproductive life, diet had no impact, but SG appears to influence it. In this sense and for a better visualisation of survival curves for reproductive life, we did not plot curves for diet F. Dotted lines represent the age at which 50% of animals left the herd; the median survival time. — Exposed C 20, ••••• Exposed C 30, — Not exposed C 20, ····· Not exposed C 30.

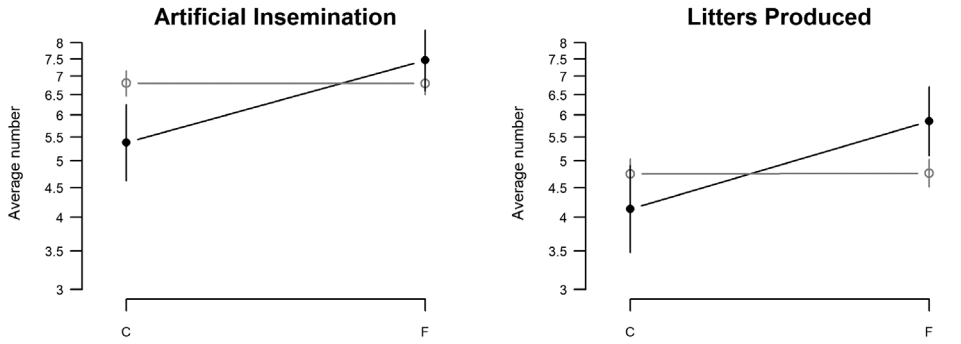


Figure 4: Interaction between diet and exposure to rabbit haemorrhagic disease for the cumulative number of artificial insemination (left) and litters produced (right). C for control diet and F for the fibrous diet. Results are averaged over the levels of SG (μ=29.98 g/d). Vertical bars around means represent the 95% confidence interval. Plotted values are in a log scale. Values appearing in the y-axis are labels representing the corresponding original value on the log scale. — Exposed, — Not exposed.

### *Lifetime reproductive performance*

*Interaction of RHD exposure and rearing diet.* The cumulative number of AI and litters produced in a female's lifetime according to the exposure to the RHD virus and the diet it received during its pubescent life are shown in Figure 4. While the average number of AI attempts for females exposed to the virus receiving the C diet was 5.4, exposed females on diet F were AI 7.4 times ($P<0.01$). For the rabbit female population not exposed to the virus, the average number of AI attempts of females on diet C was similar to that of females on diet F (6.81 and 6.80 AI attempts, respectively; Figure 4, left). The cumulative number of litters produced followed a similar pattern (Figure 4, right). Rabbit females exposed to RHD fed on diet C had, on average, 4.1 litters, while those on diet F produced 5.8 litters ($P=0.03$). Although the diet received in pubescent life did not influence the number of litters produced by non-exposed females, when focusing on females receiving the F diet, females exposed to RHD produced 1.2 litters more than females not exposed (5.9 and 4.7 litters respectively; $P=0.03$). These results do not seem to be influenced by differences in the fertility rate (number of litters produced over the number of AI attempts). Fertility rates of females exposed to the RHD virus on diet C and F were 74 and 77%, respectively. Values for non-exposed females on diet C and F were 67 and 66% respectively.

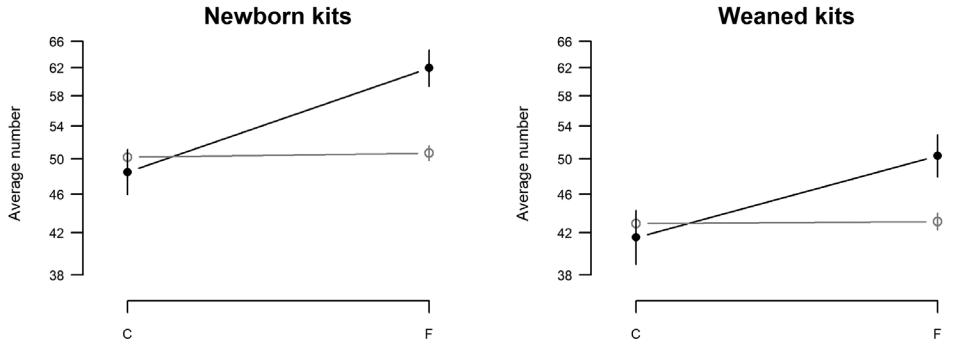


Figure 5: Interaction between diet and the exposure to rabbit haemorrhagic disease for the cumulative number of newborn (left) and weaned kits (right). C for control diet and F for the fibrous diet. Results are averaged over the levels of SG (μ = 29.98 g/d). Vertical bars around means represent the 95% confidence interval. Plotted values are in a log scale. Values appearing in the y-axis are labels representing the corresponding original value on the log scale. — Exposed, — Not exposed.

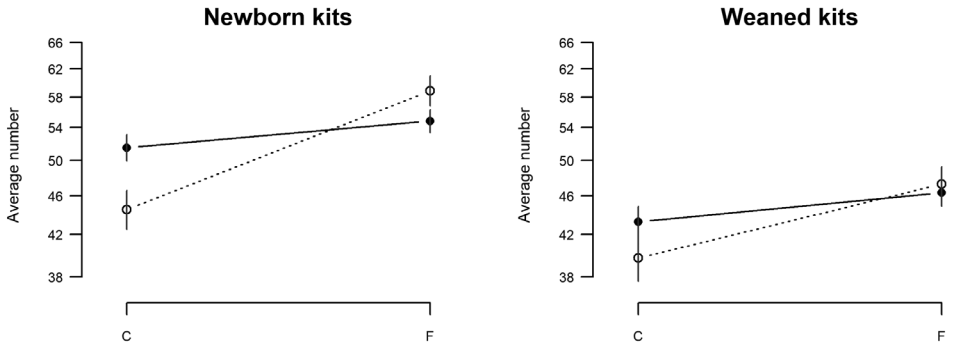


Figure 6: Interaction between diet and suckling gain (SG; g/d) for the cumulative number of newborn (left) and weaned kits (right). C for control diet and F for fibrous diet. Results are averaged over the levels of exposure to rabbit haemorrhagic disease. Vertical bars around means represent the 95% confidence interval. Plotted values are in a log scale. Values appearing in the y-axis are labels representing the corresponding original value on the log scale. — SG=20 g/d, •••• SG=30 g/d.

The cumulative number of newborn and weaned kits according to the exposure to RHD and the diet are in Figure 5 (left and right, respectively). Females reared on diet C and females reared on diet F not being exposed to the RHD virus produced a similar cumulative number of alive kits at birth, around 50.6 kits, on average. However, when exposed to the RHD virus, females on diet C produced an average of 48.4 viable kits, while those on diet F produced 61.9 ($P$<0.01). Focusing on exposed and non-exposed females fed on diet F, the difference between these groups represented 11.2 kits in favour of exposed females ($P$<0.01). We observed a similar pattern in the cumulative number of weaned kits. Among exposed and non-exposed females reared on diet F, females exposed to RHD weaned, on average, 7.2 kits more than non-exposed females ($P$<0.01; Figure 5, right).

*Suckling gain interaction with both diet* (Figure 6) *and exposure to the RHD virus* (Figure 7). For rabbits fed on diet C, suckling gain negatively influenced the average cumulative number of newborn kits. By modelling females fed on diet C with an SG of 20 g/d or 30, we respectively obtained an estimated average cumulative number of 51.5 and 44.5 newborn kits ($P$<0.01). However, when fed on diet F, a female with an SG of 20 g/d would produce 54.8 kits at birth, while females with an SG of 30 g/d would produce 58.9 kits ($P$<0.01). In this sense, and regardless of the exposure to RHD, high suckling females are favoured when reared on diet F. We observed a similar pattern for the cumulative number of weaned kits. A females reared on the C diet with an SG of 20 g/d would wean 43.2 offspring,

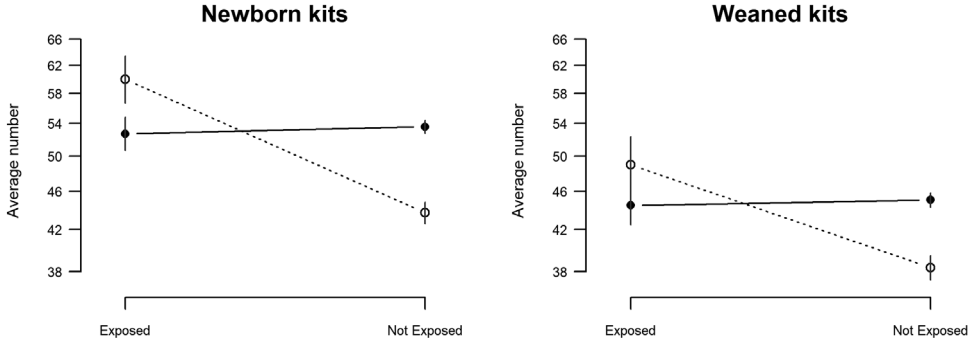


Figure 7: Interaction between exposure to rabbit haemorrhagic disease and suckling gain (SG; g/d) for the cumulative number of newborn (left) and weaned kits (right). Results are averaged over the levels of diet. Vertical bars around means represent the 95% confidence interval. Plotted values are in a log scale. Values appearing in the y-axis are labels representing the corresponding original value on the log scale. — SG=20 g/d, •••• SG=30 g/d.

while a female on the same diet with an SG of 30 g/d would wean 39.7 ($P<0.05$). However, when reared on the F diet, the number of kits weaned in its lifetime would be similar between a female with an SG of 20 g/d and a female with an SG of 30.

The interaction between SG and RHD exposure is shown in Figure 7. A female with a suckling gain of 20 g/d would produce a similar number of viable kits at birth independently of the exposure to RHD (52.7 and 53.6 for exposed and non-exposed, respectively). However, when we modelled the RHD exposure in females with an SG of 30 g/d, the exposed females would produce 60.0 kits at birth, while non-exposed ones would produce 43.7 kits at birth ($P<0.001$). We observed a similar pattern for the cumulative number of weaned kits. Although not statistically significant, high SG females seem to wean more kits when exposed to RHD (44.5 weaned kits for females with an SG of 20 g/d against 49.0 kits for females with an SG of 30 g/d; $P=0.06$).

## DISCUSSION

When conceiving this study, our main aim was to describe how the nest (SG) and pubescent development (the diet effect; see growth curves for females fed on diet C and F at Martínez-Paredes *et al.,* 2018) influence the lifetime reproductive performance of rabbit females. Nonetheless, the outbreak of RHD, which affected 22.1% of the herd, represented a unique opportunity to describe how the nest and pubescent development may provide animals with additional resources to withstand a strong environmental insult. In this sense, our main hypothesis stated that kits presenting a fast nest development, measured as daily weight gain during suckling, and fed on diet C (faster pubescent development), would result in individuals with a greater capacity to confront this disease, resulting in longer lives and better reproductive performance.

RHD exposure represented a strong environmental insult. It ravaged 60% of the exposed population and interacted with both the nest and pubescent development. In brief, a fast nest development and smooth pubescent development (diet F) favoured females exposed to RHD. Conversely, in the absence of this environmental insult, females with fast nest development fed on diet C had the worst lifetime reproductive performance. This result is contrary to the predictions of life history trait theory: well-developed individuals living under unrestricted conditions are expected to produce more offspring than under-developed individuals or those living under constrained conditions (Stearns, 1992).

Our results also revealed that, for the domestic rabbit, a fast nest development is only favourable when the environmental conditions are constrained (RHD or high-fibre diet). We also observed that a slow nest development resulted in a similar lifetime reproductive performance in both favourable (diet C and no RHD) and constrained environmental condition (diet F and RHD). This unexpected result shows that nest development interacts with the environment experienced during the pubescent development, affecting the robustness and fitness of domestic rabbit females as adults. In this sense, SG, our proxy of nest development, should be regarded as a fitness indicator.

The remaining question is why fast nest developing females performed worse as adults, especially when raised under unrestricted environmental conditions (diet C, no RHD). One possible explanation may be the practice of mating young females at a fixed age. For instance, (McNamara and Houston, 1996) predicted that females should attain a "critical level" of body development before starting breeding, and when the social and environmental conditions prevent females reaching this level, reproduction should wait. Here, the majority of our females reached the "critical level" of body development (measured as live weight and PFT; see Martínez-Paredes *et al.,* 2018) when mated for the first time at 153 d of age (only 34 of the 522 females inseminated when 153 d old never conceived and fertility rate at first AI was high, resulting in a small variation on the ages at first parturition, from 182 to 186 d). In this sense, and knowing that small newborn need more time to reach adult weight (Szendrö *et al.,* 2006), we argue that our fast SG females have reached the "critical level" of body development before low SG females, using the additional time to accumulate reserves. In fact, females with an SG above 22.3 g/d (the median value of our rabbit population) reached the AI age (153 d) with +0.38 mm of PFT more than females with an SG below this value (results not shown: 6.12 *vs.* 6.50 mm, respectively $P<0.01$). Actually, when young rabbit females reach the end of first pregnancy with too many fat reserves, they mobilise a greater amount of it around parturition, and quickly recover the reserves used at the onset of lactation. The result of this assignation of energy to reserve storage, while lactating, is a subsequent

low fertility (Savietto *et al.,* 2016) and a higher risk of culling (Theilgaard *et al.,* 2006). Likewise, high effort for reproduction represents fewer nutrients being diverted to maintain a functional immune system (Lochmiller and Deerenberg, 2000). Altogether, these observations indicate that fast SG coupled with unrestricted raising conditions and mating at a fixed age impair lifetime reproductive performance of domestic rabbit females.

The last question refers to the best lifetime reproductive performance observed among females exposed to RHD that received diet F between 63 and 184 d of age. Although few exposed animals reached maturity (28 fed on diet C and 34 fed on diet F), which in itself limits the discussion of the possible events leading to our observations, the best performance of the most restricted females falls outside of what the life history theory predicts, as health is traded for reproduction (Lochmiller and Deerenberg, 2000). When the survival prospects are low due to age and diseases, females tend to invest more in reproduction at the expense of their own survival (Clutton-Brock, 1984; Part *et al.,* 1992). We argue that this trade-off between health and survival for reproduction, referred to as "terminal investment", may also occur when animals are exposed to a restricted environment during their pubescent life. In this sense, it is reasonable to say that exposed females fed on diet F forecast bad future conditions and short lives after surviving RHD, resulting in high investments in reproduction.

Finally, SG also played an important role in the better reproductive outcome among RHD exposed females. Faster development during suckling favoured females that survived the RHD exposure to reach maturity with a better body condition than survivors that had a low SG. Our results indicate that in farms where the risk of an outbreak of RHD is high, it is preferable to keep females with a high SG as breeders, but in the absence of the RHD virus, it is better to keep females with a lower SG for this purpose. This developmental-environmental interaction seems to agree with the theoretical prediction of van Noordwijk and de Jong (1986), which states that more shall be given by those who have more.

## CONCLUSIONS

Altogether, these results open at least four new perspectives on how to prepare domestic rabbit females to become breeders. First of all, suckling gain, our proxy of nest development, seems to be a fair indicator of future lifetime reproductive performance. Second, the worst fitness observed among high SG kits raised in unrestricted conditions appears to be related to the practice of mating domestic rabbits at a fixed age. In this sense, mating should be, if practically needed, fixed to a given development degree rather than at a fixed age. A precise measure of the body development degree is required, together with individual monitoring of how the animal develops. Flexible strategies may be also developed to take into account the normal variability of body development. In summary, respecting the body development variety is advisable. Third, pubescent females fed with a fibrous diet presented a lower risk of mortality and increased their chronologic lifespan, but not their reproductive life. In this sense, further studies are required to extend this effect to reproductive life. Perhaps the use of fibrous diets during the reproductive stage is advisable. Fourth, the better lifetime reproductive performance of females exposed to RHD, especially among those with a fast SG, seems to be an example of a terminal reproductive investment. If the terminal reproductive investment really occurred in response to RHD, the immune system memory would act as a biological indicator of the environmental conditions to be faced by the animals. In that case, an experiment to test the relation between the immune response and the reproductive effort of the animals would be advisable.

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