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

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Manuscript

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

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Abstract

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

Keywords: *Oryctolagus cuniculus*; rearing diet; birth weight; reproduction; lifespan;

Implications

The full reproductive potential of rabbit females (fertility, prolificacy and lifespan) relies on the combination of genes possessed by a particular animal. However, the expression of this potential is conditioned by the developmental factors that appear early in life, such as competition for nutrients and space in the uterus, the competition among littermates for heat and milk and the amount and quality of the feed available during the pubertal phase. Here, we describe the influence of a series of components related to the early development of reproductive rabbit females that may influence their future reproductive life, to help us develop more appropriate management systems.

Introduction

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

Among the pre- and post-natal factors influencing growth and development of the adult rabbit female, birth weight had far greater importance than the size of the litter in which it was raised (Hardman *et al.*, 1970; Poigner *et al.*, 2000). Birth weight was also described as an important variable conditioning the body weight at first insemination (Szendrö *et al.*, 2006), but to our knowledge studies on rearing strategies aiming to increase reproductive lifespan are still lacking.

On the other hand, some feeding strategies, such as restricted feeding of young females, have been proposed to ensure correct body development, avoid excessive body fatness upon the first parturition and improve reproductive performance (Rommers *et al.*, 2004a; Rommers *et al.*, 2004b). However, feed restriction may increase labour and depending on the feed formulation, undesirable restriction of nutrients rather than energy may occur. One alternative to feed restriction is the use of fibrous diets. Among the reported advantages related with the use of fibrous diets during rearing is an increased survival rate (Martínez-Paredes *et al.*, 2012), improved ovarian and embryo quality (Arias-Álvarez *et al.*, 2009), reduction of kitten mortality at birth (Xiccato *et al.*, 1999; Pascual *et al.*, 2002a; Martínez-Paredes *et al.*, 2012), an increase in the feed intake of young females in the first reproductive cycle (Xiccato *et al.*, 1999; Pascual *et al.*, 2002a; Quevedo *et al.*, 2005), a reduction in the energy deficit during first lactation (Xiccato *et al.*, 1999), and an increase in milk yield and kittens' weight (Pascual *et al.*, 2002a).

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

Material and methods

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

Diets

Two diets were designed to feed the young females during the rearing period. The control diet (**C**) was similar to a commercial diet for reproductive rabbit females and contained 184 g of CP, 381 g of NDF and 11.8 MJ of DE per kg of DM; following the recommendations of De Blas and Mateos (2010). The experimental diet, characterized by its high fibre content (**F**), contained 134 g of CP, 436 g of NDF and 10.0 MJ DE per kg of DM; following the recommendations of Pascual *et al.* (2013). Ingredients and chemical composition of the diets are listed in Table 1.

Animals and feeding schedule

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

Half of the selected females were then assigned to diet C (n = 431) and the other half to diet F (n = 433), blocking by their birth weight within birth batch. They had *ad libitum* access to the diets throughout the rearing period (from 63 d to first parturition).

At the production farm, young females housed between December 2008 and February 2009 were exposed to an outbreak of rabbit haemorrhagic disease. From the 191 females exposed, a total of 134 (diet C = 69 and diet F = 65) died before delivering their first parturition. To avoid the natural selection effect caused by exposure to the virus, we did not consider the data on the 191 females exposed to it. From the remaining 673 young females (diet C = 335 and diet F = 338), 461 had at least one litter (diet C = 210 and diet F = 251). At first parturition, all females received diet C until time of death, culling or censoring.

Experimental procedure

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

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

Statistical analysis

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Modelling female growth

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

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

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

Results

Survival during lactation

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

Development during rearing

Growth curves of young females from birth to the age of first parturition are shown in Figure 2 (left panel). Almost linear growth was observed between 0 and 63 days ($R^2 = 0.95$), where females gained on average 27.8 g per day. From 63 days, the period when young females where fed with the C or F diet, different growth patterns were observed (right panel), especially between 88 and 163 days (*P*<0.05). Females had grown faster with diet C, reaching their maximum weight 6 days before the first insemination age, whereas females on diet F reached this weight approximately 37 days later (*P*<0.05). Parameters of the fitted Weibull growth models confirmed the differences in live weight observed between diet C and F (Supplementary Table S1). Model parameters for diet C (from 63 to 188 days) had higher influence on the upper asymptote (α) and growth rate ($-e^{K}$) than those for F diet. In addition, we found no influence in the growth range (β) or the growth slope of (δ) for the diet effect, as their 95% confidence interval did not overlap.

Measures of PFT were available on 494 females alive at the insemination age (diet C = 226 and diet F = 268). At first insemination, the PFT range varied from 4.1 to 8.1 mm. Its average was 6.28 mm and a SD of 0.54 mm. For females fed with diet C, the PFT range was 4.7 to 8.1 mm with an average of 6.40 mm and a SD of

0.56 mm. For females on diet F, PFT ranged from 4.1 to 7.7 mm, with an average of 6.19 mm and a SD of 0.50 mm. At first insemination, females fed with diet F had, on average, −0.20 ± 0.04 mm of PFT (*P* <0.05) and −271.8 ± 32.6 g of body weight (*P*<0.05) less than females fed with diet C (Table 2).

Reproductive performance

Cumulative number of liveborn, stillborn, weaned and dead kittens according to the rearing diet are shown in Table 3. Among females who had at least one litter, the diet effect had no influence, except for the high number of stillborn (+22.8 %; *P*<0.001) and death kittens during lactation (17.6 % higher; *P*<0.001) for litters of females fed with diet C. When we considered all females housed at the production farm, the cumulative number of liveborn and weaned kittens was higher for females fed on diet F (on average +6.1 liveborn and +4.8 weaned kittens; *P*<0.001) compared to those fed with diet C.

Regardless of the rearing diet (Table 4), the covariate birth weight only affect the number of weaned kittens of females reaching the reproductive life. Each unit of increment of birth weight value increased the number of weaned kittens by 0.5. In the case of the covariate PFT, it negatively affected the number of liveborn and weaned kittens (*P*<0.001). Each unit of increment in the PFT value reduced the cumulative number of liveborn and weaned by 3.2 and 3.0 kittens, respectively.

From the 673 females entering the production farm, 34 females reaching the insemination age never conceived (diet C = 16 and diet F = 18). For those females fed with diet C, the average number of artificial insemination attempts was 1.5 ± 0.3. The value for females fed with diet F was similar (1.7 ± 0.3). Among females having at least one litter, the number of parities over the number of artificial inseminations was not influenced by the diet (diet C = 85.8 ± 1.2 % *vs.* diet F = 83.5 ± 1.1 %; Table 2). No effect of the covariates birth weight or PFT was observed.

Productive lifespan

The proportion of females fed with diet C or F according to their maximum parity order is in Figure 3. From the 673 females transferred to the production farm (diet C = 335 and diet F = 338), 31 % never conceived (diet C = 125 and diet F = 87). From the 461 females having at least one litter (diet C = 210 and F = 251), 274 females (41 %) had between one and five litters, from which 129 (47 %) were fed with diet C and 145 (53 %) were fed with diet F. Females having between six and ten litters represented 23 % of the data, from which 41 % received diet C and 59 % the diet F. Only 31 females had 11 litters or more (55 % on diet C and 45 % on diet F).

The overall relative hazard risk of culling or death for females fed with diet F was 13 % lower than females fed with diet C ($P<0.05$). From Figure 4 (left panel), which represents the percentage of live females throughout the experimental period according to the rearing diets, two drops in the percentage of live females can be distinguished, one around day 91 and another around day 120. Higher losses were observed for females on diet C than females on diet F.

Independently of the diet, survival analysis showed that per unit of augmentation in the PFT, the overall relative hazard risk of culling or death incremented by 13 % ($P<0.05$). However, no difference in the relative hazard risk was observed between females reared with diet C or F (13 % *vs.* 14 %) during their reproductive life. Figure 4 (right panel) exemplifies the effect of PFT on the proportion of females alive throughout the experimental period for three hypothetical PFT levels: 4.5, 6.0, and

7.5 mm. No diet differences were observed within these proposed PFT levels, the females with low PFT levels at insemination being the ones at lower risk of death or culling.

Discussion

Survival during lactation

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

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

Development during rearing

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

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

Reproductive performance

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

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

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

Productive lifespan

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

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

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

Body fatness at first mating. Starting reproductive life with ‘optimal’ development and an adequate level of reserves is expected to improve fertility and lifespan. In contrast, an inadequate development or inappropriate levels of body reserves increase the risk of death and culling (Theilgaard *et al.*, 2006) and impair fertility (Savietto *et al.*, 2016). Among the many factors associated with the high risk of death observed around parturition (Rosell and de la Fuente, 2009), an excess of fat at first effective mating followed by a high pre-partum mobilization of reserves, lower DE intake, high non-esterified fatty acids and low glucose levels are all related to pregnancy toxaemia (Martínez-Paredes *et al.*, 2012). In this context, our results support the hypothesis that when an adequate level of body reserves is attained, both reproduction and lifespan are reinforced. Although we could not specify whether the extended lifespan is a result of reducing the risks related to the mobilization patterns of PFT (Savietto *et al.*, 2016), our results indicate that PFT at first mating are a good predictor of the future lifetime reproductive performance of rabbit females. Although we should be cautious before recommending a target level of PFT over which females would be at higher risk of being culled, for this particular population we observed that PFT values at first mating above 6.0 mm should be avoided (Figure 4B). So, it is advisable to control the body condition of young rabbit does to avoid excessive fatness at first mating. In gilts, for example, having an adequate body condition at first mating appears to improve fertility (O’Downd *et al.*, 1997) and lengthen their lives (Tarrés *et al.*, 2006). In adult captive rabbits deviation from the adequate levels reduces lifespan (Theilgaard *et al.*, 2006).

Conclusions

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

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References

Argente M, Santacreu MA, Climent A and Blasco A 1999. Phenotypic and genetic parameters of birth weight and weaning weight of rabbits born from unilaterally ovariectomized and intact does. Livestock Production Science 57, 159-167.

Arias-Álvarez M, García-García RM, Rebollar PG, Nicodemus N, Revuelta L, Millán P and Lorenzo PL 2009. Effects of a lignin-rich fibre diet on productive, reproductive and endocrine parameters in nulliparous rabbit does. Livestock Science 123, 107-115.

Bautista A, Zepeda JA, Reyes-Meza V, Martínez-Gómez M, Rödel HG and Hudson R 2015. Contribution of within-litter interactions to individual differences in early postnatal growth in the domestic rabbit. Animal Behaviour 108, 145-153.

Coureaud G, Schaal B, Coudert P, Rideaud P, Fortun-Lamothe L, Hudson R and Orgeur P 2000. Immediate postnatal sucking in the rabbit: Its influence on pup survival and growth. Reproduction Nutrition and Development 40, 19-32.

Coureaud G, Fortun-Lamothe L, Langlois D and Schaal B 2007. The reactivity of neonatal rabbits to the mammaty pheromone as a probe for viability. Animal 1, 1026-1032.

De Blas C and Mateos GG 2010. Feed formulation. In Nutrition of the rabbit (ed. C De Blas and J Wiseman), pp. 222-232. CABI Publishing, Wallingford, UK.

Drummond H, Vázquez E and Sánchez-Colón S 2000. Competition for milk in the domestic rabbits: Survivors benefit from littermate deaths. Ethology 106, 511-526.

Fontana L, Partridge L and Longo VD 2010. Extending healthy life span: from yeast to humans. Science 328 (5976), 321-326.

Fortun-Lamothe L, Prunier A, Bolet G and Lebas F 1999. Physiological mechanisms involved in the effects of concurrent pregnancy and lactation on foetal growth and mortality in the rabbit. Livestock Production Science 60, 229-241.

Fox J 2002. Cox proportional hazard regression for survival data. Appendix to an R and S-Plus companion to applied regression. In An R and S-plus companion to applied regression (ed. J Fox), pp. 1-18. Sage publications, Inc. London, UK.

García-Torres E, Hudson R, Castelán F, Martínez-Gómez M and Bautista A 2015. Differential metabolism of brown adipose tissue in newborn rabbits in relation to position in the litter huddle. Journal of Thermal Biology 51, 33-41.

Garreau H, Bolet G, Larzul C, Robert-Granie C, Saleil G, SanCristobal M and Bodin L 2008. Results of four generations of a canalising selection for rabbit birth weight. Livestock Science 119, 55-62.

Gidenne T 2015. Dietary fibres in the nutrition of the growing rabbit and recommendations to preserve digestive health: a review. Animal 9 (2), 227-242.

Jilge B, Kuhnt B, Landerer W and Rest S 2001. Circadian temperature rhythms in rabbit pups and in their does. Laboratory animals, 35, 364-373.

Martínez-Paredes E, Ródenas L, Martínez-Vallespín B, Cervera C, Blas E, Brecchia G, Boiti C and Pascual JJ 2012. Effects of feeding programme on the performance and energy balance of nulliparous rabbit does. Animal 6, 1086-1095.

O'Downd S, Hoste S, Mercer JT, Fowler VR and Edwards SA 1997. Nutritional modification of body composition and the consequences for reproductive performance and longevity in genetically lean sows. Livestock Production Science 52, 155-165.

Pascual JJ, Cervera C and Fernández-Carmona J 2002a. A feeding programme for young rabbit does based on lucerne. World Rabbit Science 10, 7-13.

Pascual JJ, Motta W, Cervera C, Quevedo F, Blas E and Fernández-Carmona J 2002b. Effect of dietary energy source on the performance and perirenal fat thickness evolution of primiparous rabbit does. Animal Science 75, 267-279.

Pascual JJ, Blanco J, Piquer O, Quevedo F and Cervera C 2004. Ultrasound measurements of perirenal fat thickness to estimate the body condition of reproducing rabbit does in different physiological states. World Rabbit Science 12, 7-21.

Pascual JJ, Savietto D, Cervera C and Baselga M 2013. Resources allocation in reproductive rabbit does: a review of feeding and genetic strategies for suitable performances. World Rabbit Science 21, 123-144.

Poigner J, Szendrö Z, Lévai A, Radnai I and Biró-Németh E 2000. Effect of birth weight and litter size at suckling age on reproductive performance in does as adults. World Rabbit Science 8, 103-109.

Quevedo F, Cervera C, Blas E, Baselga M, Costa C and Pascual JJ 2005. Effect of selection for litter size and feeding programme on the performance of young rabbit females during rearing and first pregnancy. Animal Science 80, 161-168.

R Core Team 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Retrieved on 10 December 2016, from http://www.R-project.org.

Rommers JM, Meijerhof R, Noordhuizen JPTM and Kemp B 2004a. Effect of feeding program during rearing and age at first insemination on performances during subsequent reproduction in young rabbit does. Reproduction Nutrition and Development 44, 321-332.

Rommers JM, Meijerhof R, Noordhuizen JPTM and Kemp B 2004b. The effect of level of feeding in early gestation on reproductive success in young rabbit does. Animal Reproduction Science 81, 151-158.

Rosell JM and de la Fuente LF 2009. Culling and mortality in breeding rabbits. Preventive Veterinary Medicine 88, 120-127.

Ross MH 1972. Length of life and caloric restriction. The American Journal of Clinical Nutrition 25, 834-834.

Savietto D, Marono S, Martínez I, Martínez-Paredes E, Ródenas L, Cervera C and Pascual JJ 2016. Patterns of body condition use and its impact on fertility. World Rabbit Science. 24 (1), 39-45.

Sonnenburg ED, Smits SA, Tikhonov M, Higginbottom SK, Wingreen NS and Sonnenburg JL 2016. Diet-induced extinctions in the gut microbiota compound over generations. Nature 529 (7585), 212-215.

Szendrö Zs, Gyovai M, Maertens L, Biró-Németh E, Radnai I, Matics Zs, Princz Z, Gerencser Zs and Horn P 2006. Influence of birth weight and nutrient supply before and after weaning on the performance of rabbit does to age of the first mating. Livestock Science 103, 54-64.

Tamburini S, Shen N, Wu HC and Clemente JC 2016. The microbiome in early life: implications for health outcomes. Nature Medicine 22, 713-722.

Tarrés J, Tibau J, Piedrafita J, Fábrega E and Reixach J 2006. Factors affecting longevity in maternal Duroc swine lines. Livestock Science 100, 121-131.

Theilgaard P, Sánchez JP, Pascual JJ, Friggens NC and Baselga M 2006. Effect of body fatness and selection for prolificacy on survival of rabbit does assessed using a cryopreserved control population. Livestock Science 103, 65-73.

Vicente JS, Llobat L, Viudes-de-Castro MP, Lavara R, Baselga M and Marco-Jiménez F 2012. Gestational losses in a rabbit line selected for growth rate. Theriogenology 77(1), 81-88.

Villamide MJ, Carabaño R, Maertens L, Pascual JJ, Gidenne T, Falcao-e-Cunha L, Xiccato G 2009. Prediction of the nutritional value of European compound feeds for rabbits by chemical components and in vitro analysis. Animal Feed Science and Technology 150 (3/4), 283-294.

Wells K, O'Hara RB, Cooke BD, Mutze GJ, Prowse TA, Fordham DA 2016. Environmental effects and individual body condition drive seasonal fecundity of rabbits: identifying acute and lagged processes. Oecologia 181 (3), 853-864.

Xiccato G and Trocino A 2010. Energy and protein metabolism and requirements. In Nutrition of the Rabbit (ed. C De Blas and J Wiseman), pp. 83-118. CABI Publishing, Wallingford, UK.

Xiccato G, Parigi-Bini R, Dalle Zotte A, Carazzolo A, Cossu ME 1995. Effect of dietary energy level, addition of fat and physiological state on performance and energy balance of lactating and pregnant rabbit does. Animal Science 61, 387-398.

Xiccato G, Bernardini M, Castellini C, Dalle Zotte A, Queaque PI and Trocino A 1999. Effect of postweaning feeding on the performance and energy balance of female rabbits at different physiological states. Journal of Animal Science 77, 416-426.

Table 1 *Ingredients and chemical composition of the experimental rearing diets for young rabbit females.*

Ingredients & chemical composition (g per kg of DM)	Control diet (C)	Fibrous diet (F)
Alfalfa meal	290	412
Wheat bran	300	181
Beet pulp	87.4	156
Cereal straw	-	151
Sunflower meal	160	50.0
Soybean meal	27.7	-
Barley	71.8	20.0
Soybean oil	16.9	10.0
Sugarcane molasses	20.0	-
L - Lysine HCL	1.5	-
Methionine OH	1.0	-
L - Threonine	-	1.7
Calcium carbonate	12.7	-
Dicalcium phosphate	-	6.3
Sodium chloride	4.0	5.0
Vitamin and mineral premix[1]	2.0	2.0
Cycostat 66G®[2]	5.0	5.0
Dry matter (DM; g per kg)	905	910

Ash	80	114
Crude protein	184	134
Ether extract	50	26
Neutral detergent fibre	381	436
Acid detergent fibre	195	234
Acid detergent lignin	24	41
Digestible energy (MJ per kg of DM)[3]	11.8	10.0

[1] Vitamin and mineral premix (g per kg): thiamine, 0.25; riboflavin, 1.5; calcium pantothenate, 5.0; pyridoxine, 0.1; nicotinic acid, 12.5; retinol, 2.0; cholecalciferol, 0.1; α-tocopherol, 15.0; phytylmenaquinone, 0.5; cyanobalamin, 0.0006; choline chloride, 100.0; $MgSO_4$ H_2O, 7.5; ZnO, 30; $FeSO_4$ $7H_2O$, 20.0; $CuSO_4$, 5 H_2O, 3.0; KI, 0.5; $CoCl_2$ 6 H_2O, 0.2; Na_2SeO_3, 0.03. [2] Cycostat 66G® Alpharma (Antwerp, Belgium): 66 g of robenidine HCL per kg of product. [3] Digestible Energy (MJ per Kg DM): Estimated using the equation proposed by Villamide *et al.* (2009): $DE = 16.43 - 0.0191 \cdot Acid\ Detergent\ Fibre - 0.0208 \cdot Ash + 0.0148 \cdot Ether\ extract$

Table 2 *Least square means and SE for insemination weight, perirenal fat thickness and fertility rate of rabbit females fed with control (C) or fibrous (F) diet during their rearing period.*

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

Contrast of means followed by a star (*) are significant different from zero at P <0.05 (*t*-test).

[1] Fertility rate was defined as the number of parities per insemination through a female reproductive life. No difference was observed in the number of inseminations spend for females that never conceived (Diet C: n = 16; 1.5 ± 0.3 inseminations. Diet F: n = 18; 1.7 ± 0.3 inseminations).

Table 3 *Cumulative number of liveborn, stillborn, weaned, death kittens during lactation and their SEM according to the diet females received during their rearing period [control (C) or fibrous (F) diet].*

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

[1] Success Reproduction: females having at least one partum; Whole Population: females house at the production farm.

[2] Offspring that died during lactation.

Table 4 *Estimated regression coefficients for model 2.1 and 2.2 used to evaluate the effect of birth weight, perirenal fat thickness (PFT) and the fibrous diet (F) rabbit females received during rearing on reproductive traits.*

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

[1] Success Reproduction: females having at least one partum; Whole Population: females housed at the production farm.

[2] Regression coefficients followed by two stars (**) significantly differed from zero at P<0.001 and those followed by a one star (*) differed from zero at P<0.05.

[3] Diet effect was coded as 0 for diet C and 1 for diet F. Birth weight and PFT covariates were standardized to perform the multiple regression.

Figure captions

Figure 1 Predicted probability (%) of newborn rabbit female survive to weaning age (38 days) depending on its birth weight and whether it had suckled milk or not just after birth. Shaded areas represents the 95% confidence interval.

Figure 2 Weibull growth curves for young rabbit females fed with control or fibrous diet from 63 to 188 days. Points represent the average live weight and the vertical bars around means represent the 95 % confidence interval (no overlapping bars are significantly different). Left panel shows growth curves from 0 to 188 days. Right panel shows growth curves from 63 to 188 days old.

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

Figure 4 Left panel: Estimated proportion of live rabbit females fed with the control (C) or fibrous (F) diet during rearing period. Right panel: Estimated proportion of live females considering the diet and three hypothetical levels of perirenal fat thickness: 4.5, 6.0 and 7.5 mm.

Figure 1. Survival to weaning age

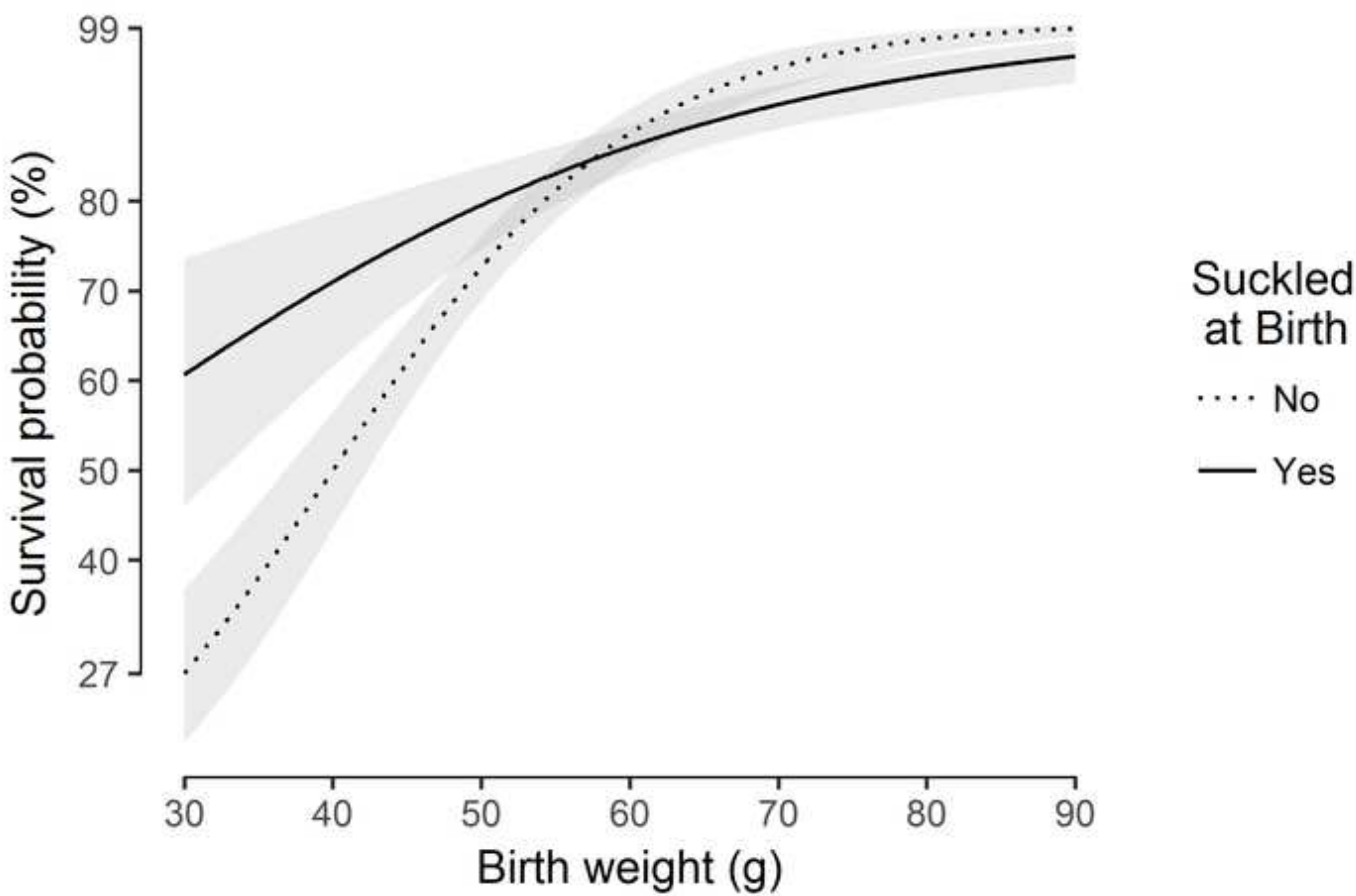


Figure 3. Proportion of alive females

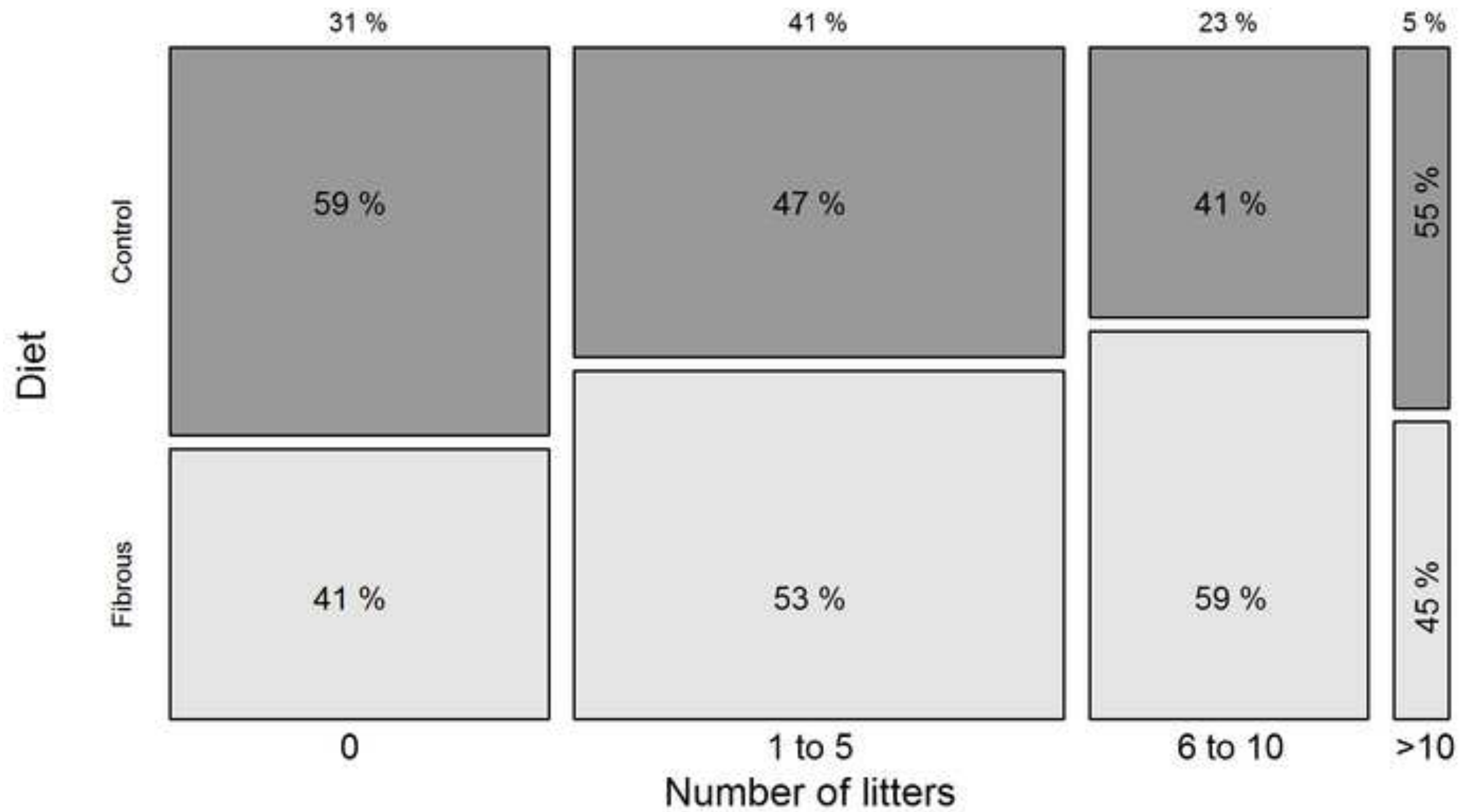
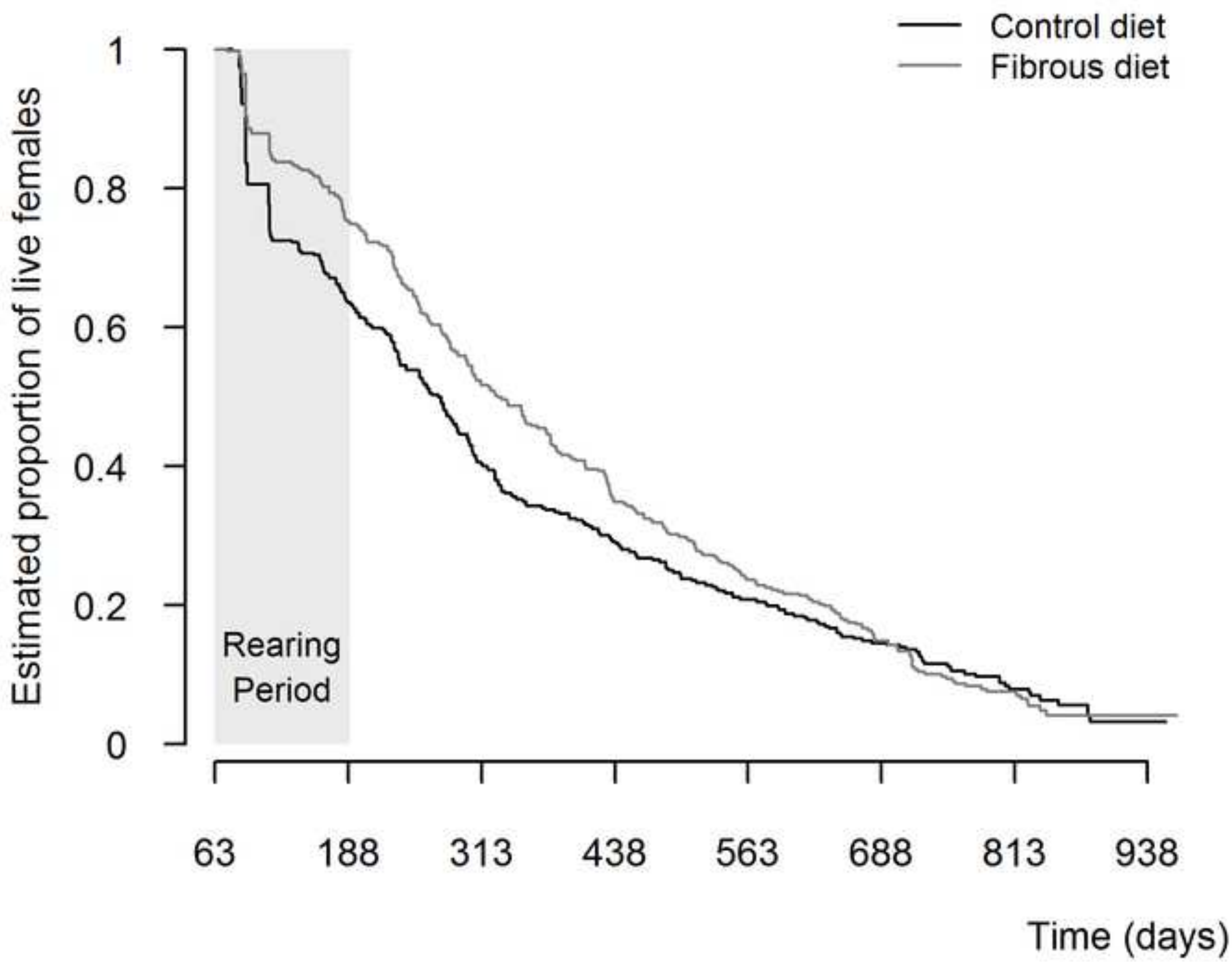
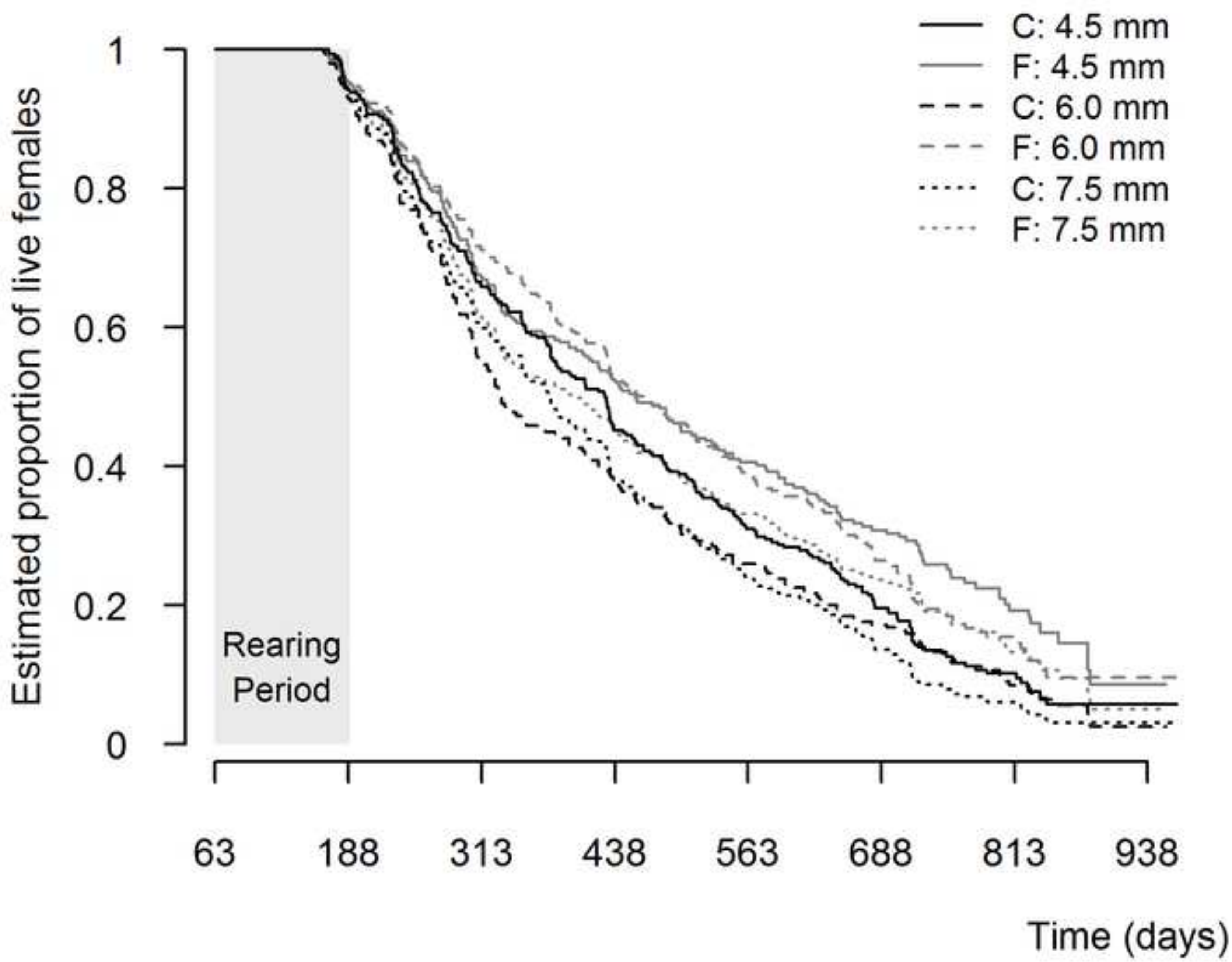


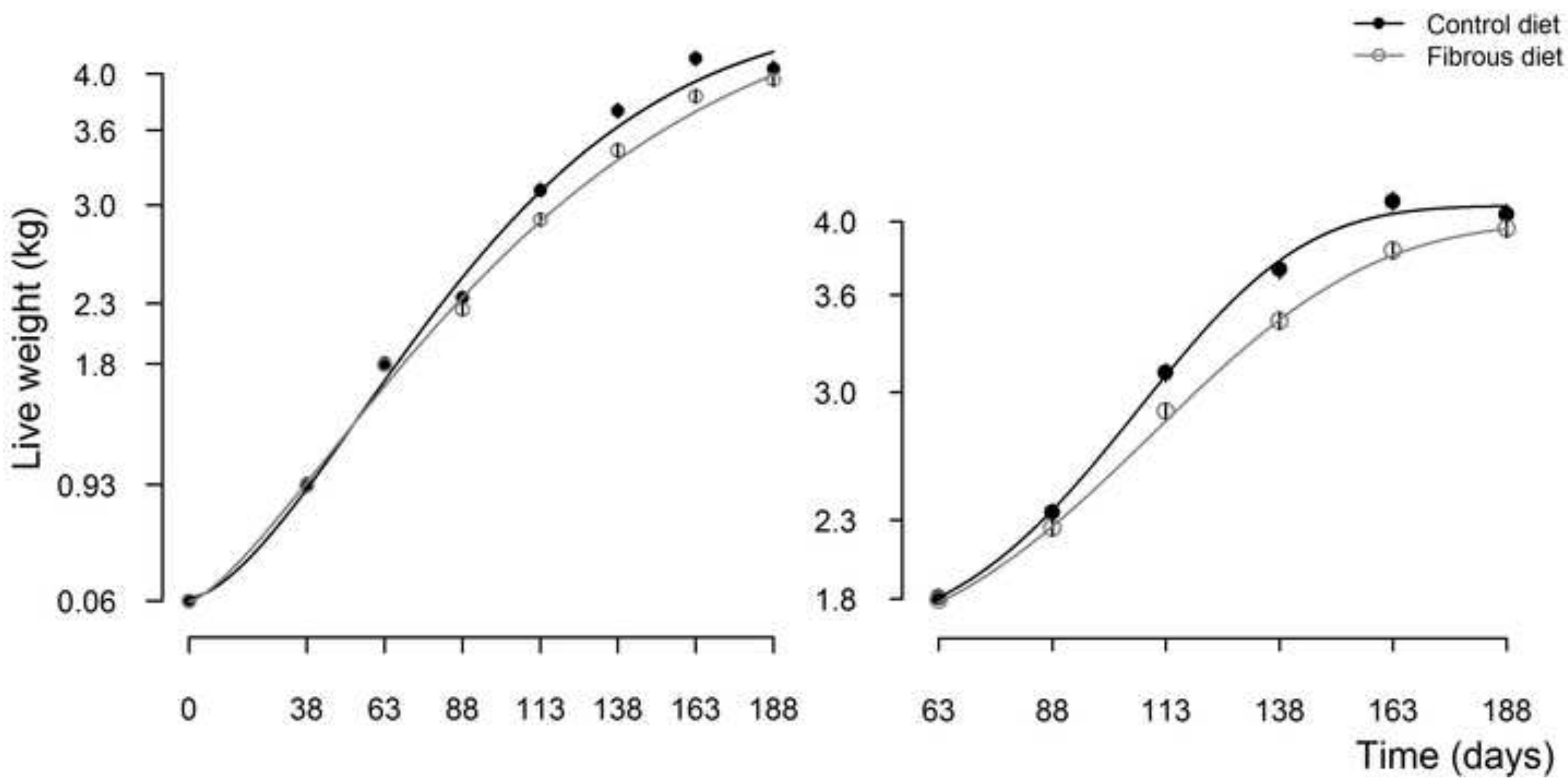
Figure 4 Left pannel. Diet effect on survival





Estimated proportion of live females
1
0.8
0.6
0.4
0.2
0
Rearing
Period
63
188
313
438
563
688
813
938
Time (days)
C: 4.5 mm
F: 4.5 mm
C: 6.0 mm
F: 6.0 mm
C: 7.5 mm
F: 7.5 mm

Figure 2. Growth rate



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

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Supplementary Table S1 *Parameters of the Weibull growth curves of rabbit females fed with control (C) or fibrous (F) diet during their rearing period. SE is the standard error of each estimated parameter.*

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

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

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