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Additional Information

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Abstract

This study examined the effect of long-term selection of a maternal rabbit line, solely for a reproductive criterion, on the ability of female rabbits to deal with constrained environmental conditions. Female rabbits from generations 16 and 36 ($n = 72$ and 79 , respectively) of a line founded and selected to increase litter size at weaning were compared simultaneously. Female rabbits were subjected to normal (NC), nutritional (NF) or heat (HC) challenging conditions from first to third parturition. Animals in NC and NF were housed at normal room temperatures (18 to 24°C) and respectively fed with control (11.6 MJ DE/kg DM, 126 g DP/kg DM, and 168 g of ADF/kg DM) or low-energy fibrous diets (9.1 MJ DE/kg DM, 104 g DP/kg DM and 266 g ADF/kg DM), whereas those housed in HC were subjected to high room temperatures (25 to 35°C) and the control diet. The litter size was lower for female rabbits housed in both NF and HC environments, but the extent and timing where this reduction took place differed between generations. In challenging conditions (NF and HC), the average reduction in the reproductive performance of female rabbits from generation 16, compared with NC, was -2.26 ($P < 0.05$) and -0.51 kits born alive at second and third parturition, respectively. However, under these challenging conditions, the reproductive performance of female rabbits from generation 36 was less affected at second parturition (-1.25 kits born alive), but showed a greater reduction at the third parturition (-3.53 kits born alive; $P < 0.05$) compared with NC. The results also showed differences between generations in digestible energy intake, milk

yield and accretion, and use of body reserves throughout lactation in NC, HC and NF, which together indicate that there were different resource allocation strategies in the animals from the different generations. Selection to increase litter size at weaning led to increased reproductive robustness at the onset of an environmental constraint, but failure to sustain the reproductive liability when the challenge was maintained in the long term. This response could be directly related to the short-term environmental fluctuations (less severe) that frequently occur in the environment where this line has been selected.

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

Implications

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

Introduction

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

Some authors (Sánchez et al., 2006; Theilgaard et al., 2006; Piles et al., 2006b) found no evidence of antagonism between selection to increase litter size at weaning and lifespan, in relatively abundant conditions. However, it has been suggested that selection for litter size could increase the reproductive sensitivity when environmental conditions limit the availability of resources (Theilgaard et al., 2007). In fact, reproduction is enhanced during spring and early summer under natural conditions, which means that the rabbits reproduce with high grassland quality and comfortable temperatures, but

when the conditions are not suitable reproduction declines. Domestic breeds are also predisposed to express their genetic potential under adequate environmental conditions, reducing their reproductive performance when exposed to high temperature or low-energy diets (Fernández-Carmona et al., 1995 and 2003), which could lead to higher culling rates.

However, it may be that different physiological responses are induced, depending on the nature of the environmental constraint. For example, low-energy diets usually lead to a restriction in nutrient supply, whereas high temperatures would mainly bring about a constriction of metabolic rate (and thereby a negative feedback on intake) (Cervera and Fernández-Carmona, 2010). In addition, the challenge length may also be considered, as during the normal selection process these animals have been faced with short-term challenges (such as the seasonal hot period; Baselga, 2004). There is evidence that when selection to increase a trait is carried out under adequate environmental conditions, the environmental sensitivity could also increase (Falconer, 1990).

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

Material and methods

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

Animals

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

information concerning the cryopreservation and embryo transfer techniques used in this study are available in Vicente et al. (1999) and Besenfelder and Brem (1993), respectively.

Environments

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

Experimental procedures

Young female rabbits were reared following the management scheme proposed by Ragab and Baselga (2011) from birth to 63 days of age, and then transferred to the experimental farm. From 63 days to first parturition, animals were fed with a commercial diet (CP = 15.3 g/kg DM, ether extract = 2.5 g/kg DM, and crude fibre = 23.1 g/kg DM) supplied *ad libitum* and daily exposed to 16 h of light. Female rabbits were first artificially inseminated at 125 days of age, reaching the first parturition with an average live weight (LW) of 3583 ± 240 g (mean ± s.d.). At first parturition, female rabbits from both generations (V16 and V36) were randomly assigned to one of the three environments (HC, NC, NF) in a 2 × 3 factorial design. The number of animals differed owing to the availability of young female rabbits from the selection nucleus (V16HC = 31, V16NC = 22, V16NF = 19, V36HC = 29, V36NC = 25 and V36NF = 25). During the experimental period, which lasted until third parturition, female rabbits followed a programmed reproductive interval of 42 days, with insemination at 11 days post-parturition (**dpp**). Non-pregnant

female rabbits were re-inseminated 21 days later and so on, up to a maximum of three consecutive failures, whereupon they were culled because of infertility. The total number of kits born (**TB**) and kits born alive (**BA**) was recorded at each parturition. Litter size was standardised at birth to nine kits in the first lactation and 10 in the second. Subsequently, dead kits were not replaced. The number of weaned kits was recorded at 28 dpp.

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

Blood plasma parameters

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

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

Statistical analysis

A mixed model (SAS Institute Inc., Cary, NC, USA), with a repeated measure design, was used to analyse performance, hormonal and metabolic data of rabbit does until third parturition. The model considered the variation between animals and the co-variation within them. The covariance structure was estimated using the spatial power function, after objectively comparing among other covariance structures as suggested by Littell et al. (1998). The spatial power function is a direct generalisation of first-order autoregressive covariance function for equally time-spaced data, with the advantage of accounting for different lag times between two measurements. The model used to analyse reproductive performance (Table 4.1) included the generation (V16 and V36), environment (NC, HC and NF), parturition (first, second and third) and their interactions. The model used to analyse other performance traits (Table 4.2) and blood plasma parameters (Table 4.3) included the generation, environment, reproductive cycle (two levels: first (all measures between first and second parturition; only second parturition included) and second (all measures between second and third parturition; only third parturition included) and their interactions. This model also included measurement day (different depending on the variable studied; see experimental procedure) and its interactions with generation and environment as fixed effects. Finally, differences in the evolution of DE intake, milk yield and PFT with time within lactation (Figures 4.1 to 4.3) were analysed considering the generation, environment, lactation week and their interactions. All models included the random effect of animal [$p \sim N(0, \sigma_p^2)$]. The models for intake and milk yield included the average litter size during lactation as a covariate. Plasma concentrations of total T₃, leptin, NEFA, BHB and lactate did not follow a normal distribution; hence, log₁₀ transformation was applied before analysis. Variables were presented as least square means followed by their standard errors, and different contrasts were computed to test the effect of the environmental challenge and selection for litter size at weaning in each reproductive cycle as follow:

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

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

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

Results

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

Performance traits

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

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

Table 1 The effect of environment and generation of selection for litter size at weaning on reproductive performance of females rabbit at first, second and third parturition

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

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

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

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

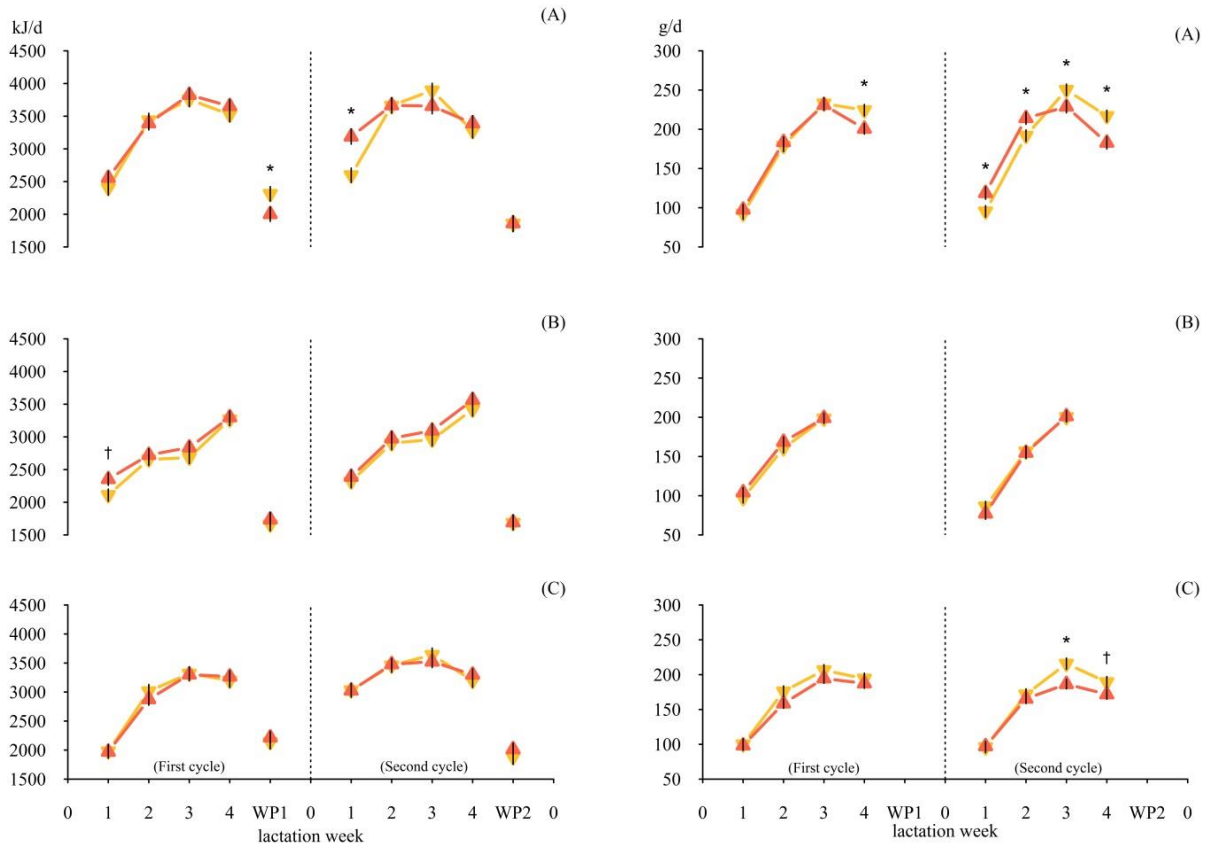


Figure 1 Digestible energy intake (kJ/d) of V16 (gold) and V36 (red) female rabbits housed in: (A) normal [normal room temperature (18 to 24°C) and fed with diet C (11.6 MJ DE/kg DM)], (B) heat [high room temperature (25 to 35°C) and diet C] or (C) nutritional [normal room temperature and fed with diet F (9.1 MJ DE/kg DM)] challenging conditions. Vertical bars represent the standard errors of means. WP represents the weaning to partum interval. *P<0.05 and †P<0.10.

Figure 2 Milk yield (g/d) of V16 (gold) and V36 (red) female rabbits housed in: (A) normal, (B) heat or (C) nutritional challenging conditions. Vertical bars represent the standard errors of means. WP represents the weaning to partum interval. *P<0.05 and †P<0.10.

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

Table 2 The effect of environment and generation of selection for litter size at weaning on average dry matter (DM) and digestible energy (DE) intake, milk yield, live weight, perirenal fat thickness (PFT), and weaning to parturition interval of rabbit does at first and second reproductive cycles. Survival rate of female rabbits is also presented

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

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

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

Blood plasma parameters

At first parturition, before random allocation of female rabbits to treatments, plasma concentrations of leptin (1.74 ng/ml), NEFA (506.4 μ ekv/l), BHB (0.268 mM), glucose (6.76 mM) and lactate (4.70 mM) were similar in both generations, whereas total T₃ was slightly lower for V16 than V36 (1.67 and 1.97 nM, respectively; $P < 0.10$). Blood plasma parameters in the first and second reproductive cycle are in Table 4.3. In NC, V16 had a higher plasma concentration of

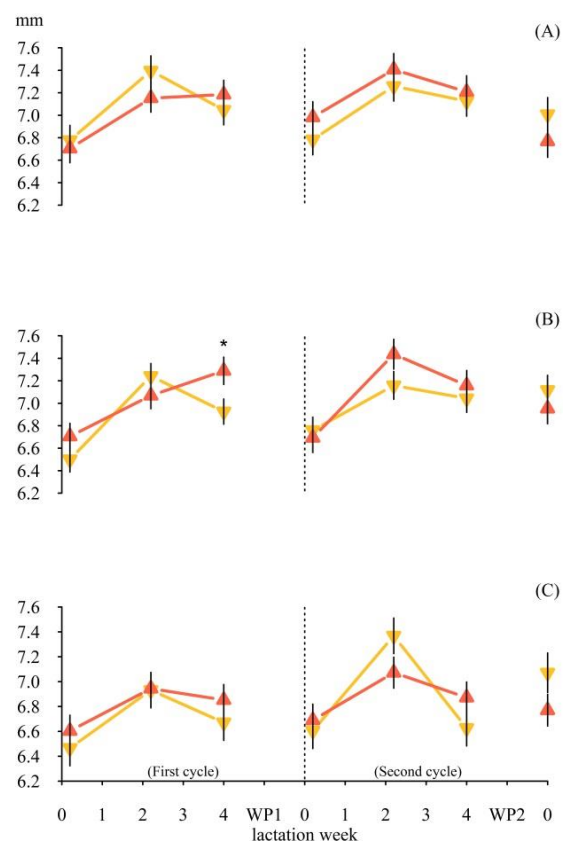


Figure 3 Perirenal fat thickness (mm) of V16 (gold) and V36 (red) female rabbits housed in: (A) normal, (B) heat, or (C) nutritional challenging conditions. Vertical bars represent the standard errors of means. WP represents the weaning to partum interval. * $P < 0.05$.

glucose (+11.0%; $P < 0.05$) during the first reproductive cycle and higher BHB (+13.0%; $P < 0.05$) during the second cycle than V36. However, when housed in NF, V36 had a higher total T_3 level (+42.0%; $P < 0.05$) during the first reproductive cycle than V16. During the first reproductive cycle, and always compared with NC, V16 female rabbits showed significantly lower total T_3 (-33.0%) and glucose levels (-9.0%) in HC, and lower total T_3 (-36.0%), leptin (-76.0%), NEFA (-4.0%) and higher BHB (+14.0%) levels in NF. During the second reproductive cycle, V16 female rabbits in NF also had significantly lower leptin (-61.0%) and NEFA (-3.0%) levels, but higher BHB (+16.0%) and lactate (+22.0%), compared with NC. Housing V36 female rabbits in HC resulted in significantly higher NEFA (+4.0%) and BHB (+18.0%) levels during the second reproductive cycle compared with NC, whereas V36 in NF had a reduced lactate (-17.0%) in the first cycle and higher BHB (+27.0%) level in both reproductive cycles.

Discussion

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

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

In general, the results suggest that selection to increase litter size at weaning had changed the way in which the female rabbits interact with environment. To aid interpretation, it should be noted that litter traits at first parturition represent differences purely because of genetic selection, as animals were allocated to different environments just after the first parturition, whereas differences in the second and third parturition reflect the effect of genotype-environment interaction. Observed performance differences after the first parturition were the result of differences in adaptive capacity of V16 and V36 female rabbits to the

different environmental constraints, with results in the first lactation indicating the effect of the constraint being applied in lactation only, whereas during the second reproductive cycle the effects were the result of the constraint being applied both in that lactation and the preceding gestation. As no differences between V16 and V36 within environments were found in the percentage of female rabbits completing the first and second cycle nor in the LW at the end of first lactation, results concerning the second lactation and the third parturition can be interpreted as the response of selection to a long-term environmental constraint.

Effect of selection for reproduction (NC)

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

Table 3 The effect of environment and generation of selection for litter size at weaning on plasma concentrations of total T₃, leptin, non-esterified fatty acids (NEFA), β-OH-butyrate (BHB), lactate and glucose of rabbit does during first and second reproductive cycle

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

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

Response to heat stress restriction (HC)

The drop in feed intake at high temperatures (i.e. sows: Quiniou et al., 2000; ruminants: Morand-Fehr and Doreau, 2001; rabbits: Fernández-Carmona et al., 2003) is a well-known physiological adaptation to prevent an increment in body core temperature above the normal state, and can be considered a metabolic restriction. Female rabbits of generation 16 had a quicker response to heat challenge than V36. They reduced both the DE intake and the plasma concentrations of glucose and total T₃ (both positively correlated with energy intake; Dauncey, 1990; Rommers et al., 2004; Brecchia et al., 2006), in the first reproductive cycle, with respect to NC female rabbits. In contrast, in the first reproductive cycle, V36 female rabbits did not reduce glucose and total T₃ concentrations, and maintained a higher PFT at weaning. This different adaptation between V16 and V36 female rabbits to the HC environment could be associated with their different reproductive performances at second parturition, where V16 female rabbits had higher reduction in TB and BA than V36 rabbits. These results suggest that female rabbits coming from more recent generations of selection for reproduction conserved, in the short term, their reproductive effort after being exposed to high temperatures. The negative effect of HC environment on V16 female rabbits in the second lactation was similar to that observed in the previous one. However, symptoms of exhaustion from prolonged heat exposure appeared in the V36 female rabbits; the reduction in DE intake and milk yield in early lactation and the high levels of plasma NEFA and BHB of V36 female rabbits owing to HC were greater in the second reproductive cycle. The low plasma NEFAs (positively correlated to mobilisation of body reserves Brecchia et al., 2006) reflected the absence of body reserve accretion – fuel for reproduction – by V36 female rabbits from second insemination to third parturition. This clearly affected their reproductive performance at third parturition.

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

Response to dietary energy restriction (NF)

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

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

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

Conclusions

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

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References

- Baselga, M.** (2004). Genetic improvement of meat rabbits. Programmes and diffusion. In *Proceedings of 8th World Rabbit Science Congress*. Puebla, Mexico. Available online at: <http://world-rabbit-science.com/WRSA-Proceedings/Congress-2004-Puebla/Papers/Genetics/G0-Baselga.pdf>
- BOE** (2005). Real decreto 1201/2005, de 10 de octubre, sobre protección de los animales utilizados para experimentación y otros fines científicos. *Boletín Oficial del Estado* **252**, 34367-34391.
- Brecchia, G., Bonanno, A., Galeati, G., Federici, C., Maranesi, M., Gobetti, A., Zerani, M. and Boiti, C.** (2006). Hormonal and metabolic adaptation to fasting: effects on the hypothalamic-pituitary-ovarian axis and reproductive performance of rabbit does. *Domest Anim. Endocrin.* **31**, 105-122.
- Bresenfelder, U. and Brem, G.** (1993). Laparoscopic embryo transfer in rabbits. *J. Reprod. Fertil.* **99**, 53-56.
- Cervera, C., and Fernández-Carmona, J.** (2010). Nutrition and the climatic environment. In *The Nutrition of the Rabbit* (ed. C. de Blas and J. Wiseman), pp. 267-284. Wallingford: CAB International.
- Coureaud, G., Fortun-Lamothe, L., Langlois, D. and Schaal, B.** (2007). The reactivity of neonatal rabbits to the mammary pheromone as a probe of viability. *Animal* **1**, 1026-1032.
- Dauncey, M. J.** (1990). Thyroid hormones and thermogenesis. *P. Nutr. Soc.* **49**, 203-215.

- Estany, J., Baselga, M., Blasco, A. and Camacho, J.** (1989). Mixed model methodology for the estimation of genetic response to selection in litter size of rabbits. *Livest. Prod. Sci.* **21**, 67-75.
- Falconer, D. S.** (1990). Selection in different environments: effects on environmental sensitivity (reaction norm) and on mean performance. *Genet Res.* **56**, 57-70.
- Fernández-Carmona, J., Cervera, C., Sabater, C., and Blas, E.** (1995). Effect of diet composition on the production of rabbit breeding does housed in a traditional building and at 30°C. *Anim. Feed Sci. Tech.* **52**, 289-297.
- Fernández-Carmona, J., Alqedra, I., Cervera, C., Moya, J. and Pascual, J. J.** (2003). Effect of lucerne-based diets on performance of reproductive rabbit does at two temperatures. *Animal Sci.* **76**, 283-295.
- Friggens, N. C.** (2003). Body lipid reserves and the reproductive cycle: towards a better understanding. *Livest. Prod. Sci.* **83**, 219-236.
- García, M. L. and Baselga, M.** (2002). Estimation of genetic response to selection in litter size of rabbits using a cryopreserved population. *Livest. Prod. Sci.* **74**, 45-53.
- García-Diego, F. J., Pascual, J. J. and Marco-Jiménez, F.** (2011). Technical note: design of a large variable temperature chamber for heat stress studies in rabbits. *World Rabbit Sci.* **19**, 225-231.
- Garreau, H., Larzul, C. and Ducrocq, V.** (2001). Analyse de longévité de la souche de lapins INRA 1077. In *9èmes Journées de la Recherche Cunicole* (ed. B Gérard), pp. 217-220., Paris: ITAVI.
- Harano, Y., Ohtsuki, M., Ida, M., Kojima, H., Harada, M., Okanishi, T., Kashiwagi, A., Ochi, Y., Uno, S. and Shigeta, Y.** (1985). Direct automated assay method for serum or urine levels of ketone bodies. *Clin. Chim. Acta* **151**, 177-183.
- Littell, R. C., Henry, P. R. and Ammerman, C. B.** (1998). Statistical analysis repeated measured data using SAS procedures. *J. Anim. Sci.* **76**, 1216-1231.
- Martínez-Paredes, E., Ródenas, L., Martínez-Vallespín, B., Cervera, C., Blas, E., Brecchia, G., Boiti, C. and Pascual J. J.** (2012). Effects of feeding programme on the performance and energy balance of nulliparous rabbit does. *Animal* **6**, 1086-1095.
- Morand-Fehr, P. and Doreau, M.** (2001). Ingestion et digestion chez les ruminants soumis à un stress de chaleur. *INRA Prod. Anim.* **14**, 15-27.
- Nicodemus, N., Redondo, R., Pérez-Alba, L., Carabaño, R., De Blas, J. C. and García J.** (2010). Effect of level of fibre and type of grinding on the performance of rabbit does and their litters during the first three lactations. *Livest. Sci.* **129**, 186-193.

- Pascual, J. J., 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 Sci.* **12**, 7-21.
- Piles, M., García, M. L., Rafel, O., Ramon, J. and Baselga, M.** (2006a). Genetics of litter size in three maternal lines of rabbits: repeatability versus multiple-trait models. *J. Anim. Sci.* **84**, 2309-2315.
- Piles, M., Garreau, H., Rafel, O., Larzul, C., Ramon, J. and Ducrocq, V.** (2006b). Survival analysis in two lines of rabbit selected for reproductive traits. *J. of Anim. Sci.* **84**, 1658-1665.
- Quevedo, F., Cervera, C., Blas, E., Baselga, M. and Pascual, J. J.** (2006). Long-term effect of selection for litter size and feeding programme on the performance of reproductive rabbit does. 2. Lactation and growing period. *Animal Sci.* **82**, 751-762.
- Quiniou, N., Renaudeau, D., Dubois, S. and Noblet, J.** (2000). Influence of high ambient temperatures on food intake and feeding behaviour of multiparous lactating sows. *Animal Sci.* **70**, 471-479.
- Ragab, M. and Baselga, M.** (2011). A comparison of reproductive traits of four maternal lines of rabbits selected for litter size at weaning and founded on different criteria. *Livest. Sci.* **136**, 201-206.
- Ramón, J., Rafel, O. and Piles, M.** (2004). Resultados de gestión en España. GTE 2002: Algo falla. *Boletín de Cunicultura* **133**, 24-28.
- Rommers, J. M., Boiti, C., Brecchia, G., Meijerhof, R., Noordhuizen, J. P. T. M., Decuyper, E. and Kemp, B.** (2004). Metabolic adaptation and hormonal regulation in young rabbit does during long-term caloric restriction and subsequent compensatory growth. *Animal Sci.* **79**, 255-264.
- Sánchez, J. P., Baselga, M. and Ducrocq, V.** (2006). Genetic and environmental correlations between longevity and litter size in rabbits. *J. Anim. Breed. Genet.* **123**, 180-185.
- Savietto, D., Blas, E., Cervera, C., Baselga, M., Friggens, N. C., Larsen, T. and Pascual J. J.** (2012). Digestive efficiency in rabbit does according to environment and genetic type. *World Rabbit Sci.* **20**, 131-140.
- Theilgaard, P., Sánchez, J. P., Pascual, J. J., Friggens, N. C. and Baselga, M.** (2006). Effect of body fatness and selection for prolificacy on survival of rabbit does assessed using a cryopreserved control population. *Livest. Sci.* **103**, 65-73.
- Theilgaard, P., Sánchez, J. P., Pascual, J. J., Berg, P., Friggens, N. C. and Baselga, M.** (2007). Late reproductive senescence in a rabbit line hyper selected for reproductive longevity, and its association with body reserves. *Genet. Sel. Evol.* **39**, 207-223.

Vicente, J. S., Viudes-de-Castro, M. P. and García, M. L. (1999). In vivo survival rate of rabbit morulae after vitrification in a medium without serum protein. *Reprod. Nutr. Dev.* **39**, 657-662.