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Comparison of immune response to lipopolysaccharide of rabbit does selected for litter size at weaning or founded for reproductive longevity.

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ABSTRACT

To evaluate differences in maternal lines to the immune response of reproductive rabbit does, a total of 64 animals of two different lines: (1) founded for hyper-longevity and litter size criteria (LP) and (2) selected for litter size at weaning (V) were used. Females were subjected to three different reproductive efforts: post-partum (PP) mating at first lactation and 9 kits during the second; post-weaning (PW) mating at first lactation and 9 kits during the second; and PW mating at first lactation and 5 kits during the second. At second weaning (30 days PP), an acute response was induced by intravenous infusion of lipopolysaccharide (LPS). LP females seemed to be lower affected during the hyper-acute phase than V females, showing lower plasma glucose content at 1.5 h post infusion (pi) and rectal temperature at 6 h pi; and showed higher ulterior immune response, with higher levels of c-reactive protein at 48 h pi and haptoglobin in plasma from 24 h pi. Survival test conferred a higher risk of culling for V than for LP females during the first hours after challenge. These results may suggest that, regarding immune response to LPS challenge, foundation by hyper-longevity productive criteria lead to obtain a more robust population of rabbit does, characterized by improved response ability.

Keywords: Rabbit doe; Immunological challenge; Genetic selection; Litter size; Longevity.

1. Introduction

Although improvements in production have been achieved by genetic selection programs, in some species selection for productive criteria has been associated with undesirable physiological and/or immunological traits (Burkhart et al., 1983; Schinkel et al., 1999; Ravagnolo and Miztal, 2000; Rauw et al., 2002). Rabbit production has become more intensive due to improved genetic programmes, reproductive management and feeding systems. However, unfavourable changes in body condition, lifespan of females, and general health have been also associated with this trend (Rosell and de la Fuente, 2009; Pascual, 2010). Rabbit health may be considered one of the main handicaps to current rabbit production under commercial conditions (Pascual , 2010).

In rabbit does, the genetic selection programmes for reproductive traits have mainly focused on improving litter size, either at partum or weaning (Pascual et al., 2010). This may have affected the capacity of rabbits to respond to immune challenges (Ferrian et al., 2012). In other species there is evidence that immunological capability may differ depending on the genetic origin of the animals (Rauw et al., 1998; Siegel and Honaker, 2009). Recently, a rabbit line founded by selecting commercial females based on their reproductive longevity (LP, Sánchez, 2006), showed they were more robust and able to withstand environmental and productive challenges by the greater plasticity to use their greater soma to overcome these demanding situations (Theilgaard et al., 2007, 2009) than other line highly selected for litter size at weaning (V), which could explain their greater life expectancy on the farm of LP females (Sánchez et al., 2008). However, life expectancy is not only defined by the success of females to confront reproductive and environmental challenges, but also immunological ones. Thus it might be hypothesized that "more robust" animals might also be characterized for better immune systems, and perhaps the introduction of this type of animals could contribute to improve the overall

sanitary status of the farm.

However, it has also been observed that the sustained reproductive effort required of rabbits might affect their health status. Thus, Martínez-Vallespín et al. (2011) showed greater physiological wear, and culling rates, for rabbit females subjected to more demanding conditions (poorer feed and delayed weaning age). Guerrero et al. (2011) described how prolonged lactation led to lymphopaenia and lesser modulation of lymphocyte populations during the pregnancy-lactation cycle of rabbit does.

Our aim was to evaluate the effect of selection of rabbit does for either longevity and litter size or litter size at weaning on their immune responses to lipopolysaccharide.

2. Materials and methods

2.1. Sources of animals

Sixty-four rabbit females from two genetic lines (31 and 33 from V and LP, respectively) at second weaning were used in the present trial, coming from an initial group of 132 females. The V line was selected for litter size at weaning for 31 generations, using as selection criterion the best linear unbiased prediction (BLUP) under a single-trait repeatability animal model (Estany et al., 1989; García and Baselga, 2002). The LP line was founded by selecting females from commercial farms showing an extreme longevity and an average life-time prolificacy per partum close to the average of the Spanish commercial population: i.e. at least 25 litters, with a minimum average litter size per partum of 7.5 kits born alive, as described by Sánchez et al. (2008). After the foundation this line has been selected for litter size at weaning for six generations.

The Committee of Ethics and Animal Welfare of the Universitat Politècnica de Valencia approved this study. All the animals were handled according to the principles of animal

97 care published by Spanish Royal Decree 1201/2005 (BOE, 2005).

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99 *2.2. Experimental procedure*

100 Throughout the experiment, females were housed in a conventional housing (with light
101 alternating cycle of 16 light hours and eight dark hours, and under controlled
102 environmental conditions: average daily minimum and maximum temperature of 17.5
103 and 25.5ºC, respectively), using individual cages (700×500×320 mm) provided with a
104 nest for the litter from 28th day of gestation. Animals were fed *ad libitum* with a
105 commercial diet for reproductive rabbit does (218 g acid detergent fibre and 174 g crude
106 protein per kg of dry matter; Cunilactal, Nutreco) throughout the whole experiment.
107 After first parturition litters of 132 females (60 from the V line and 72 from the LP line)
108 were standardised to nine kits. A total of 43 females from both lines were successfully
109 artificially inseminated (AI) at day 4 post first partum (PP), while the other 89 females
110 were AI after first weaning (PW; day 30 post first partum). At second parturition, litter
111 size was standardised to 9 kits in all PP females (PP9), and to 5 or 9 kits for PW females
112 (PW5 and PW9, respectively). Therefore within each line, three experimental groups
113 with different levels of productive effort until second weaning were obtained: high
114 (PP9), short recovery time after first post-weaning and high litter size at second
115 lactation (9 and 11 does for lines V and LP, respectively); medium (PW9), long
116 recovery time after first post-weaning and high litter size at second lactation (11 does
117 from each line); and low (PW5), long recovery time after first post-weaning and low
118 litter size at second lactation (11 does from each line). Females were not mated during
119 the second lactation to avoid heterogeneity.

120

121 *2.3. Performance traits*

To evaluate the possible correlation between the previous energy balance and the immunological response of females at second weaning, body weight (BW), perirenal fat thickness (PFT) and estimated body energy (EBE) at day 0, 10 and 30 post second parturition (pp) were recorded. The PFT of does was measured by ultrasound to evaluate body condition, as described by Pascual et al. (2000, 2004). The average of the left- and right-side PFT was used for further calculations. The estimated body energy (EBE) content of does was determined from BW and PFT of does, using the equations proposed by Pascual et al. (2004) for body energy estimation at different physiological stages.

2.4. LPS challenge

An acute phase response was induced according to Saitoh et al. (2000), by lipopolysaccharide (LPS) challenge at day 30 post second parturition. LPS from *Escherichia coli* (serotype 0111:B4, L2630, Sigma Chemical Company, St. Louis, MO, USA) was dissolved in saline (0.25 mg/mL) and injected via marginal ear vein (50 µg/kg). Rectal body temperatures were measured (digital thermometer Citizen CT561C) and blood samples collected from the central ear artery at 0, 1.5, 3, 6, 24 and 48 h after the LPS inoculation (11:00 a.m.) using vacuum tubes with EDTA. Plasma was obtained by centrifugation (3,000g, 10 min) at 4 ºC and stored at -80 ºC until analysis for glucose, non-esterified fatty acids (NEFA), haptoglobin and C-reactive protein.

2.5. Plasma analyse

Blood plasma glucose was determined according to standard procedures (Siemens Diagnostics® Clinical Methods for ADVIA 1650). NEFA were determined using the

147 Wako, NEFA C ACS-ACOD assay method. Haptoglobin was determined chemically
148 due to its ability to bind to haemoglobin, Phase ™, Tridelta Developments, Wicklow,
149 Ireland. All analyses were performed using an autoanalyzer, ADVIA 1650® Chemistry
150 System (Siemens Medical Solutions, Tarrytown, NY 10591, USA). The intra assay
151 variabilities were in all instances below 2 % (CV); inter assay variations were in all
152 instances below 4.5 % (CV).
153 Rabbit C-reactive protein was analysed by a commercial ELISA assay (Life
154 Diagnostics, Inc., West Chester, PA 19380, USA). Manufacturer's instructions were
155 followed. Intra- and inter assay variations were below 8%.
156
157 *2.6. Statistical analysis*
158 *Rectal temperature and plasma traits data*
159 To analyse the evolution of corporal temperature and blood plasma traits with time after
160 LPS infusion, a mixed model (PROC MIXED; Statistical Analysis System, 2002) was
161 fitted, accounting for the repeated measures design in the data that takes into account
162 the variation between animals and covariation within them. Covariance structures were
163 objectively compared using the most severe criteria (Schwarz Bayesian criterion), as
164 suggested by Littell et al. (1998). The model included the times (0, 1.5, 3, 6, 24 and 48
165 h), and their interactions with the genetic type (LP, and V) and the group (PP9, PW5 and
166 PW9) to gather differences in the evolution of the traits in function of these main
167 effects. Randoms terms in the model included a permanent effect of each animal (p) and
168 the error term (e). Contrast tests were also performed at each time to define punctual
169 differences between genetic types and groups. To test the relationship between the
170 previous performance traits during second lactation and both the rectal temperature and
171 plasma traits at maximum response time after LPS challenge, Pearson's correlation

coefficients (ρ) were obtained using PROC CORR of the Statistical Analysis System (2002).

Survival after the immunological challenge

Cumulative mortality of rabbit does after a LPS-induced challenge at second weaning was analysed by a x^2 test using the PROC GENMOD of the Statistical Analysis System (2002) Survival analyse techniques were also used, to evaluate the effect of plasma traits on female survival. Females having a last control alive equal to 48 h were assumed to have a censored record for time until death. The following proportional hazard model was fitted

$$h_{ik}\left(t \mid \mathbf{x}_i'(t)\right) = h_0(t)_k \times \exp\left\{\mathbf{x}_i'(t)\mathbf{b}\right\}$$

where $h_{ik}\left(t \mid \mathbf{x}_i'(t)\right)$ is the hazard associated to the animal i at time t, $h_0(t)_k$ is a baseline hazard function of time t,; $\mathbf{x}_i'(t)$ is an incident vector for animal i relating covariates to the observations; **b** is a vector with the effect of covariates (C-reactive protein, glucose, NEFA haptoglobin and temperature). It was decided to use a stratified model in order to avoid the constraint of proportionality between hazards across levels of the combination genetic type by group. The presented proportional hazard model was fitted assuming the Cox likelihood and implemented using the Survival Kit software.

The survival function for six animals, one in each level of the interaction genetic type×group was predicted, for these animals the covariates in $\mathbf{x}_i'(t)$ were set to their mean value. An estimate of the log-hazard between two levels genetic type×group in a given time could be obtained from these predictions. At a given time (t) the survival function for an animal in a given combination genetic type by group L1 (for example, V in PW9) could be named $\hat{S}_{L_1}(t_t)$; for another animal in other combination L2 (for

example LP in PW9) it will be $\hat{S}_{L_2}(t_t)$. At this time the proportionally between hazards holds, thus

$$\hat{h}_{L_1}(t_t) = \hat{h}_{L_2}(t_t) \times \gamma_{L1-L2,t}$$

where, $\gamma_{L1-L2,t}$ is the hazard ratio between L1 and L2 at time t (times more likely to die a L1 than a L2 female at time t); being $\theta_{L1-L2,t}$ the log-hazard ratio ($\log(\gamma_{L1-L2,t})$). Given the relationships between hazard and survival functions $\hat{S}_{0,L_1}(t_t) = \hat{S}_{0,L_2}(t_t)^{\gamma_{L1-L2,t}}$. In order to obtain a measurement on the uncertainty for the estimated differences between genetic types in each group, 10,000 bootstrap samples of the data set were obtained and for each model fitted, determining log-hazard ratios for each genetic type×group combination. Each bootstrap sample consisted of a random extraction with reposition from the original data set of as many records as the original data set had (64), in this way for each replication one particular record could be present several times and perhaps other records will not appear, simulating random repetitions of a given experiment. The estimated effect for each level of the combination genetic type×group was the average across replicates, while its error was the standard deviation across replicates.

3. Results

3.1. Rectal temperature and plasma traits

As a consequence of the different genetic types and previous reproductive efforts used, females arrived at the second weaning with relative differences in body condition (coefficient of variation (CV) from 8 to 11%), ranging in BW from 3.3 to 5.2 kg and PFT from 4.8 to 10.0 mm (Table 1). During the induced challenge, the concentrations of all the plasma metabolites had high variability (CV from 34 to 152%).

Time from challenge significantly affected all the variables analysed (P<0.001). As can be seen in Figure 1, the evolution of rectal temperature of the females after infusion was very similar for both genetic types, with minor exceptions at one time-point (P=0.09), showing a high increase just after infusion (+1.45ºC at 3 h; P<0.001) and then a return to normal ranges at 48 h post infusion (pi). LP females showed a lower rectal temperature than V females (–0.3ºC; P<0.05) just at 6 h pi.

The evolution of plasma NEFA concentration was highly affected by the group (P<0.001; Figure 2). PP9 females showed similar plasma NEFA concentrations for all the time-points after the challenge, but NEFA was highly increased in PW9 and PW5 groups at 24 (on av. +580 μeKv/L respect to PP9; P<0.05) and 48 h pi (on av. +1425 μeKv/L respect to PP9; P<0.001). Genetic type had no effect on the evolution of plasma NEFA levels after the challenge (P=0.709; Figure 3a). Glucose concentrations in plasma evolved in a similar way for both genetic types (P=0.077), reaching a peak after infusion (+2.5 mM at 1.5 h; P<0.001) and decreasing to a low at 24 h (–3.1 mM; P<0.001) before recovering to normal ranges at 48 h pi (Figure 3b). V females showed a higher plasma glucose content than LP females (+1.2mM; P<0.01) only at 1.5 h pi.

Both acute phase proteins measured showed a similar trend during the challenge (Figures 3c and 3d). They were maintained at basal level until 6 h pi (on av. 38 mg/L and 0.47 mg/mL of C-reactive protein and haptoglobin, respectively), but showed important progressive increases from this time up to 48 h pi (on av. + 685 mg/L and +2.89 mg/mL respect to the basal level, respectively). Differences between genetic types were found at 48h pi for the level of C-reactive protein (P=0.08) and haptoglobin in plasma (P<0.001). LP females had higher concentrations of haptoglobin at 24 h pi (+0.46 mg/mL; P<0.01), and of haptoglobin (+0.80 mg/mL; P<0.001) and C-reactive protein (+67 mg/L; P<0.10) at 48 h pi, than V females.

Table 2 shows the linear relationships found between body condition traits of females before the induced challenge with the peak of the rectal temperature and plasma traits registered during the challenge. The greater the mobilization of reserves between 10 and 30 d pp (r= –0.34; P<0.05) the greater the rectal temperature measured at 3 h pi. The smaller the amount of PFT and EBE at 10 d pp the higher the plasma concentration of glucose at 1.5 h pi (r= –0.26 and -0.28, respectively; P<0.05). Finally, the lower the PFT at 0, 10 and 30 d pp the greater the plasma concentration of NEFAs recorded at 48 h pi (r= –0.35, –0.36 and –0.36; P<0.05).

3.2. Survival after immunological challenge

As a consequence of the induced challenge, V females had a higher cumulative mortality up to 6 h pi than LP females (+20% at 3 and 6 h pi; P<0.05) and, although differences remained after that, they were not significant (Figure 4). Females from PP9 group presented a significantly (P<0.05) lower mortality rate at 6 h (5 vs. 20%) and 48 h pi (20 vs. 41%) with respect to the PW5 and PW9 groups.

The ratios between the probabilities of culling associated with genetic type for the different treatment groups and across time are presented in the Table 3. For females mated at weaning after the first cycle, a V female was 166,791 times more likely to die than an LP female until 1.5 h pi when the females were under treatment PW9. If they were under treatment PW5, the relative risk was also very high, 232,838, and it extended until 6 h pi. However, when females were mated at parturition after the first cycle (PP9), the risk was similar for both genetic types until 3 h pi. From 3-6 h pi, hazard differences in favour of LP females disappeared or even tended to reverse, there being a lower risk of death for the V line. Having observe extreme values for the relative risks between lines at given times it is a consequence of a lack of animals dying

at these times. The solution to avoid this issue would be to have a larger data set in order to give the chance of observing a die in those combinations of genetic type and group for which so far none death have been observed at that certain control times. In spite of this, the lack of dying animals from a specific genetic time in a given time could be an evidence of the lower risk of culling for this genetic type with respect to the other at that time.

To evaluate the role of the controlled blood plasma traits as mediators of the different survival rates associated with the different levels of the combinations between genetic type and treatment group, different stratified analyses were conducted (Table 4), where the base hazard function was assumed to be defined by the different component of the combination genetic type by group. When a unique baseline hazard function was considered, i.e. the effect of the genetic type and group were not considered, a significant association between NEFA plasma concentration and the hazard was found, being the estimate in this case 0.46×10^{-3}. Nearly the same estimate was obtained when the baseline hazard function was defined exclusively either by the genetic type or the treatment group, indicating that the effect of these factors it was not mediated throughout differences in NEFA levels. However, when both factors were used for defining the baseline hazard function the NEFA estimate of risk dropped to 0.39×10^{-3} and it became non-significant.

4. Discussion

Bacterial infections are accompanied by potent host responses that are often followed by opposing anti-inflammatory effects (Lewkowicz et al., 2006). LPS, the primary toxic component of endotoxin, located in the cell wall of Gram-negative bacteria, elicits a complex acute phase response (Krueger and Majde, 1990), which can be followed by

fever and changes in the the blood concentrations of some physiological metabolites and acute phase proteins. Although different studies have evaluated the response in rabbits after moderate (<5 mg/kg BW; Kimura et al., 1994; Amador et al., 2007; Huang et al., 2008; Marca et al., 2009) or severe LPS challenges (50 to 85 mg/kg BW; Mathison and Ulevitch, 1979; Saitoh et al., 1999, 2000) at hormonal, cellular and tissue levels, knowledge related to other factors affecting the immunological response is scarce.

The fever induced after challenge (between 0 and 3 h pi) is a normal adaptation in response to the LPS pyrogenic stimulus, that leads to a proportional rise of plasma glucose content (Kivirante et al., 1995) but without associated changes in NEFA level. Increased glucose utilization to support immune system functions and reduced liver fatty acid oxidation just after infection has been widely described (Blackburn, 1977; Grungfeld and Feingold, 1992). This has also been observed in rabbits, where the maximum temperature is reached between 1.5 to 3 h pi both in mild (Huang et al., 2008; Shibata et al., 2000; Kimura et al., 1994) and severe inductions (Saito et al., 2000), with recovery to the normal ranges from 24-48 h pi. The rectal temperature peak is reached in cows and sheep about 4 h after LPS inoculation (Waggoner et al., 2009; Yates et al., 2011), with an increase of plasma glucose content at 2 h pi as a result (Waggoner et al., 2009; Stengel et al., 2010; Bernhard et al., 2012).

However, a subsequent decrease of the plasma glucose level was observed from 6 to 24 h pi just as NEFA levels began to rise. LPS induction usually drives the liver to a total depletion of hepatocyte glycogen. This, together with the concomitant lack of feed consumption, might lead to the pronounced hypoglycemia (Ferrante et al., 1984; Fukuzumi et al., 1996; Leininger et al., 2000) and NEFA mobilization (Webel et al., 1997; Leininger et al., 2000; Kushibiki et al., 2009), commonly observed after LPS

challenges in other species.

Another systemic response to disease is an increase in liver production of acute-phase proteins (Jain et al., 2011), as was observed in the present study from 6 h pi, and in previous works with rabbits (Murray and Connel, 1960; Mackiewickz et al., 1988; Baker and Long, 1990; Peterson et al., 2004; Georgieva et al., 2009). Haptoglobin plays both an antioxidative (Carter and Worwood, 2007) as well as a bacteriostatic role by restricting the free iron needed for bacterial growth (Eaton et al., 1982), while C-reactive protein has the ability to increase opsonization and activation of the complement system (Petersen et al., 2004). Therefore, the greater the increase of these proteins in the blood, the greater was the response against the infectious agent.

Although both lines have shown a very similar response pattern for most of the parameters measured after LPS challenge, punctual differences between them should be highlighted. LP females showed an earlier drop of maximum temperature and lower glucose release during the initial acute phase, as well as higher amount of plasma C-reactive protein and especially haptoglobin after 24 h pi, with respect to V females (Figures 1 and 3). The lower early acute reaction of LP females (first 6 h pi) coincided with the period where the gap in survival between the genetic lines occurred (+20% at 6h pi; $P<0.05$). This gap was subsequently maintained. In rabbits (Kluger and Vaughn, 1978), as in other species (Bernheim and Kluger, 1976; Covert and Reynolds, 1977), an initial positive correlation between the fever magnitude and survival has been reported, although the correlation was reversed when the fever was too high during acute challenges, with high and maintained temperatures increasing the risk of death. Our results showing genetic differences during the early severe phase of the challenge, leading to longer survival for those animals showing a lower acute response at this critical stage followed by a higher later response to the inoculum via increased acute

phase proteins, indicating clear differences in the ability to mount an immune response to the LPS challenge.

Why should the criteria used during the foundation or selection have affected the ability of the females to confront immunological challenges? The response to this question seems to lie in the differences between the studied lines in resource allocation. From the correlations reported in Table 2, it might be deduced that the better the body condition of females before challenge, the lower the acute impact of LPS (lower rise of the rectal temperature and glucose release to plasma), the lower the mobilization of reserves via NEFA, and the higher the response after inoculation of LPS via acute phase proteins. Further, the only blood trait correlated with survival was NEFA (Table 4). In that respect, previous works have indicated that the greater was the body condition of rabbit females the greater their lymphocyte count (Guerrero et al., 2011), and that rabbit does in a good body condition showed a lower risk of culling or death (Theilgaard et al., 2006). These results may lead to hypothesize that body reserves might actively participate in the modulation of the immune system response.

Rabbit females from the LP line have been characterized by a longer reproductive life than females only selected for reproductive traits (Sánchez et al., 2008). This difference is associated with their greater soma (body weight and body condition) at the beginning of their reproductive life and their greater robustness in productive and environmental challenges (Theilgaard et al., 2007, 2009; Savietto et al., 2012). Pascual et al. (2012) proposed that the nutrient partitioning capacity of these robust females enabled them to better cope with the possible reproductive, environmental, and probably immunological challenges, that they might meet in the course of their productive life. This might explain their higher life expectancy on the farm. In fact, Ferrian et al. (2012), comparing the lymphocyte population in blood of rabbit does from the 36th generation of line V

with that of does from line LP, described higher lymphocyte counts and better responses under heat stress conditions for LP females. It must be considered that the robustness definition refers mainly to health, and as proposed by Ellen et al. (2009), robust animals must be less sensitive to disease and their immunological response must allow a quicker recovery than less robust animals. This was the case in the present study.

Finally, our results showed that the advantage in terms of survival rate of the LP over the V line came exclusively from females mated after first weaning (PW; Table 4), which were also the main group responsible for the increase of NEFA in blood after 6 h pi (Figure 2). Females mated just after first parturition (PP9) did not show genetic differences on survival rate or increased NEFA blood level during the challenge. This difference should be interpreted carefully since the PP9 group was set up from females successfully pregnant just after first parturition, whereas the PW groups were constituted from those does that failed to become pregnant just after first parturition. It can be hypothesized that this mating procedure could have performed a pre-selection for especially robust rabbit does. In fact PP9 does were characterized by lower mortality rate during the challenge, independently of their genetic type.

In conclusion, a selection line (LP) founded using rabbit does with exceptional productive hyper longevity was found to be more robust with respect to health than a line selected only for reproductive intensity. The LP does showed an improved response to immunological challenge with LPS. In addition, the results of the present work suggest that the utilization of animals characterized by greater health robustness might contribute to maintaining an adequate productive level and health in commercial rabbit farms.

5. Conflict of interest statement

None declared.

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587

1

Table 1. Means, standard deviations and coefficients of variation for the body condition traits and plasma metabolite concentrations measured.

Variable	No.	Mean	SD	Minimum	Maximum	CV
Body weight at weaning (g)	64	4076	341	3365	5155	8.35
Perirenal fat thickness at weaning (mm)	64	7.83	1.28	4.80	10.0	10.14
Estimated body energy at weaning (MJ/kg BW)	64	9.32	1.04	7.41	12.42	11.19
Rectal temperature (ºC)	339	40.5	0.8	38.4	42.0	1.97
Non esterified fatty acids (µeqv/L)	310	947	732	73	5061	77.35
Glucose (mM)	307	7.22	2.48	0.86	16.89	34.37
c-reactive protein (mg/L)	305	179	272	1.1	1057	152.4
Haptoglobin (mg/mL)	306	1.09	1.22	0.02	5.99	112.1

No.: number of observations
SD: standard deviation
CV: coefficient of variation (%)

2

Table 2

Table 2. Correlation coefficients of some body condition traits with the rectal temperature (T) and plasma concentration on glucose, non-esterified fatty acids (NEFAs) and haptoglobin at maximum response time after LPS challenge.

	No.	PFT_{0d}	PFT_{10d}	PFT_{30d}	EBE_{10d}	$\Delta EBE_{10\text{-}30d}$
Rectal T_{3h}	56	+0.0456	+0.2573	–0.1211	+0.2069	–0.3384*
$Glucose_{1.5h}$	59	–0.0897	–0.2574*	–0.1544	–0.2778*	+0.0987
$NEFA_{48h}$	32	–0.3508*	–0.3612*	–0.3611*	–0.2023	–0.0472
$Haptoglobin_{48h}$	32	+0.2950+	+0.0727	+0.0616	+0.0346	+0.0960

PFT: perirenal fat thickness
EBE: estimated body energy
ΔEBE: change of estimated body energy between 10 and 30 days

1
2

Table 3. Hazard ratio of death between different genetic types for each group (PP9, previous AI post-partum and 9 kits; PW9, previous AI post-weaning and 9 kits; and PW5, previous AI post-weaning and 5 kits) across time.

			Hazard ratio[1]		
Genetic type contrast	Group	Time (h)	Log_e(hazard)	SD Log_e(hazard)	Hazard ratio ($e^{Loge(hazard)}$)
V-LP	PW9	0.5	12.02	5.64	166791.53
		1.5	12.02	5.63	166791.53
		3	1.57	8.13	4.79
		6	1.91	7.94	6.79
		24	1.91	7.94	6.79
		48	1.68	5.59	5.39
V-LP	PW5	0.5	12.36	3.33	232838.42
		1.5	12.36	3.54	232838.42
		3	12.36	3.54	232838.42
		6	12.36	3.54	232838.42
		24	1.48	8.01	4.41
		48	0.17	3.00	1.18
V-LP	PP9	0.5	0.00	0.00	1.00
		1.5	0.00	0.00	1.00
		3	0.00	0.00	1.00
		6	-11.40	7.81	0.00
		24	-1.09	8.75	0.34
		48	-1.09	8.75	0.34

[1] Hazard ratio (ratio of the probabilities of death or culling) between genetic types at each group.
SD: standard deviation.

Table 4

Table 4. Stratified analyses for the regression coefficients (b) of time dependent traits on the $\log_e$(hazard) of death or culling.

Model design	complete		without genetic type		without group		without genetic type nor group	
	b[1] ± SE	P-value	b ± SE	P-value	b ± SE	P-value	b ± SE	P-value
Rectal temperature (ºC)	0.227 ± 0.408	0.5785	0.305 ± 0.393	0.4380	0.518 ± 0.383	0.1760	0.508 ± 0.375	0.1757
NEFA[2] (µeqv/L)	$0.391\times10^{-3} \pm 0.231\times10^{-3}$	0.0897	$0.490\times10^{-3} \pm 0.231\times10^{-3}$	0.0309	$0.456\times10^{-3} \pm 0.194\times10^{-3}$	0.0188	$0.465\times10^{-3} \pm 0.192\times10^{-3}$	0.0156
Glucose (mM)	0.206 ± 0.119	0.0822	0.156 ± 0.110	0.1558	0.127 ± 0.109	0.2438	0.138 ± 0.109	0.2042
c-reactive protein (mg/L)	$0.104\times10^{-3} \pm 0.142\times10^{-2}$	0.4616	$0.558\times10^{-3} \pm 0.136\times10^{-2}$	0.6819	$0.763\times10^{-3} \pm 0.135\times10^{-2}$	0.5709	$0.629\times10^{-3} \pm 0.133\times10^{-2}$	0.6291
Haptoglobin (mg/mL)	–0.237 ± 0.326	0.4663	–0.116 ± 0.300	0.6988	–0.170 ± 0.315	0.5900	–0.107 ± 0.293	0.7151

[1] e^b: increase of the probability of death or culling of the animal per unit of trait increased. [2] NEFA: Non esterified fatty acids

Figure 1

Figure 1. Evolution of rectal temperature in LP (solid line) and V (broken line) rabbit does after a LPS induced challenge at second weaning. *Significant differences between means at P<0.05.

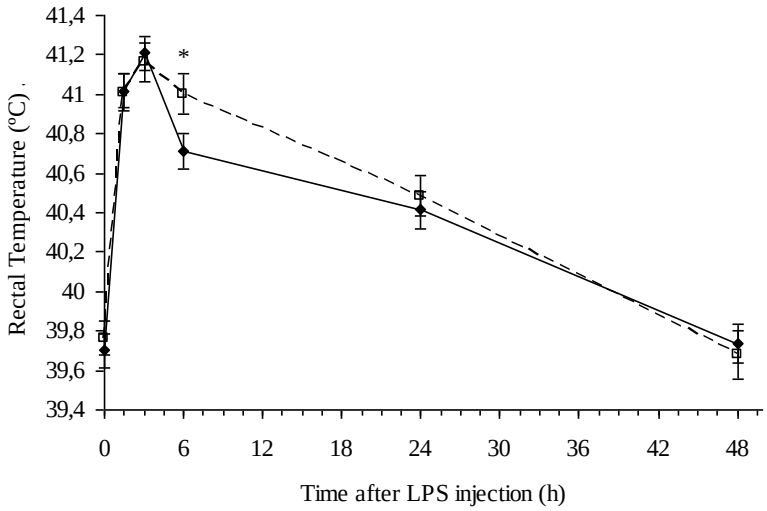


Figure 2

Figure 2. Evolution of plasma non-esterified fatty acids after a LPS induced challenge at second weaning, according to the previous reproductive effort (PP9, previous AI post-partum and 9 kits; PW9, previous AI post-weaning and 9 kits; and PW5, previous AI post-weaning and 5 kits). Significant differences between PW5 and PW9 with PP9 means at each time are presented as *P<0.05 or ***P<0.001.

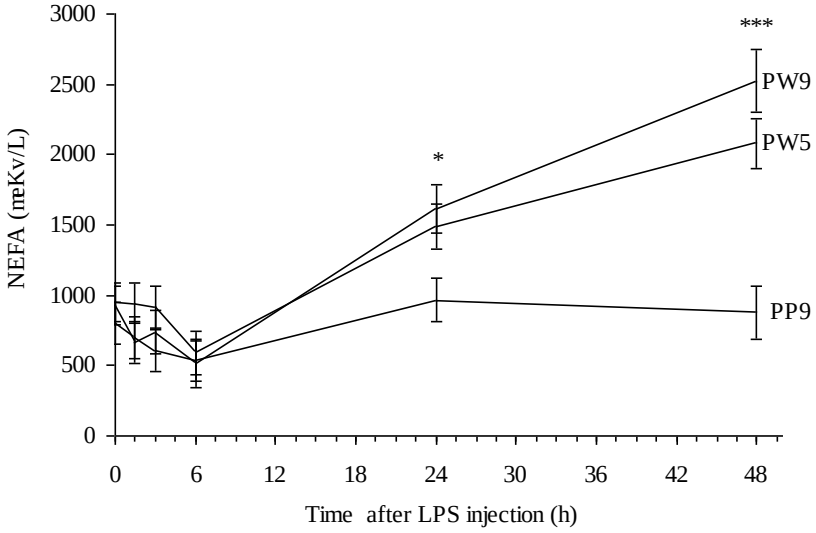
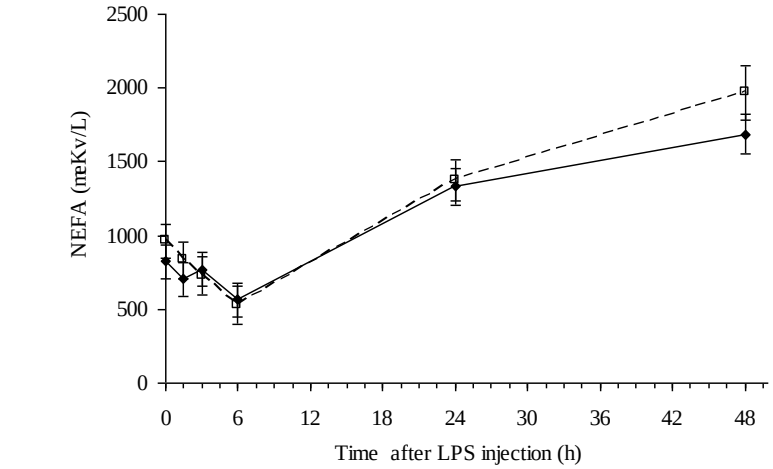
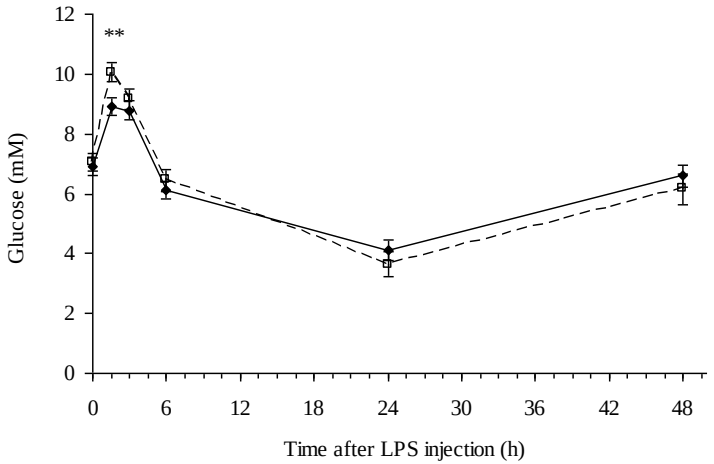


Figure 3

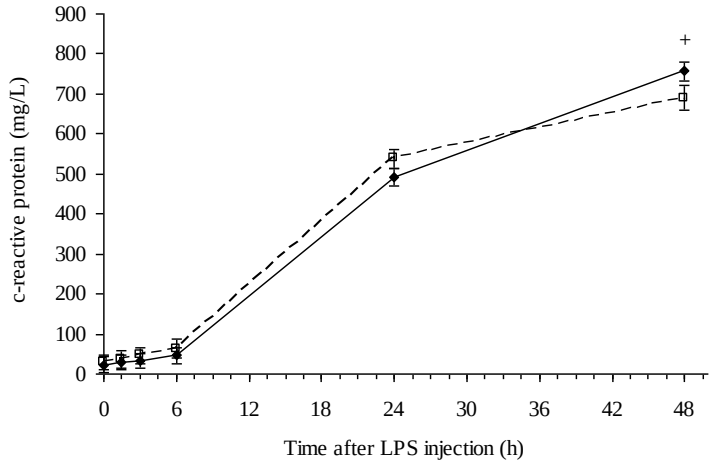
Figure 3. Evolution of plasma concentrations of: (a) non-esterified fatty acids (NEFA), (b) glucose, (c) C-reactive protein and (d) haptoglobin in LP (solid line) and V (broken line) rabbit does after a LPS induced challenge at second weaning. Significant differences between means at $^{+}$P<0.10, *P<0.05, **P<0.01, ***P<0.001.



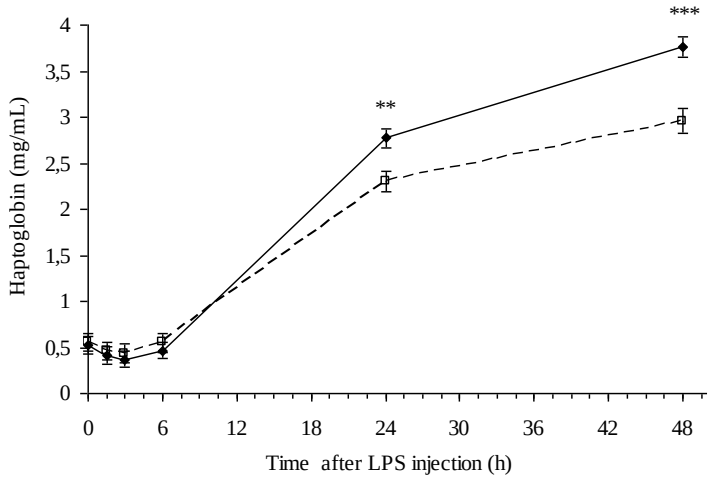
(a)



(b)



(c)



(d)

Figure 4

Figure 4. Cumulative mortality of LP (dark bars) and V (white bars) rabbit does after a LPS induced challenge at second weaning. Means in a same time not sharing superscripts are different at $P<0.05$ (x^2 test).