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

S. Ferrian [a], E. Blas [b], T. Larsen [c], J.P. Sánchez [d], N.C. Friggens [e], J.M. Corpa [a,*], M. Baselga [b], J.J. Pascual [b]

[a] Instituto de Ciencias Biomédicas, Universidad CEU Cardenal Herrera, Avda. Seminario s/n, 46113 Moncada, Valencia, Spain
[b] Institute for Animal Science and Technology, Universitat Politècnica de Valencia, Camino de Vera 14, 46071 Valencia, Spain
[c] Department of Animal Science – Integrative Physiology, Aarhus University, Blichers Allé 20, 8830 Tjele, Denmark
[d] Genetica i Millora Animal, Institut de Recerca i Tecnologia Agroalimentàries, Alcalde Rovira Roure 191, 25198 Lleida, Spain
[e] INRA, UMR 791 Modélisation Systémique Appliquée aux Ruminants (MoSAR), AgroParisTech, 16 rue Claude Bernard, 75231 Paris, France

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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.

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, 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 (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 another 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.

* Corresponding author. Tel.: +34 96 1369000x1374; fax: +34 96 1395272.
E-mail address: jmcorpa@uch.ceu.es (J.M. Corpa).

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

2.2. Experimental procedure

Throughout the experiment, females were housed in a conventional housing (with light alternating cycle of 16 light hours and eight dark hours, and under controlled environmental conditions: average daily minimum and maximum temperature of 17.5 and 25.5 °C, respectively), using individual cages (700 × 500 × 320 mm) provided with a nest for the litter from 28th day of gestation. Animals were fed *ad libitum* with a commercial diet for reproductive rabbit does (218 g acid detergent fibre and 174 g crude protein per kg of dry matter; Cunilactal, Nutreco) throughout the whole experiment.

After first parturition litters of 132 females (60 from the V line and 72 from the LP line) were standardised to 9 kits. A total of 43 females from both lines were successfully artificially inseminated (AI) at day 4 post first partum (PP), while the other 89 females were AI after first weaning (PW; day 30 post first partum). At second parturition, litter size was standardised to 9 kits in all PP females (PP9), and to 5 or 9 kits for PW females (PW5 and PW9, respectively). Therefore within each line, three experimental groups with different levels of productive effort until second weaning were obtained: high (PP9), short recovery time after first post-weaning and high litter size at second lactation (9 and 11 does for lines V and LP, respectively); medium (PW9), long recovery time after first post-weaning and high litter size at second lactation (11 does from each line); and low (PW5), long recovery time after first post-weaning and low litter size at second lactation (11 does from each line). Females were not mated during the second lactation to avoid heterogeneity.

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 (3,000*g*, 10 min) at 4 °C and stored at −80 °C 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 Wako, NEFA C ACS-ACOD assay method. Haptoglobin was determined chemically due to its ability to bind to haemoglobin, Phase™, Tridelta Developments, Wicklow, Ireland. All analyses were performed using an autoanalyzer, ADVIA 1650® Chemistry System (Siemens Medical Solutions, Tarrytown, NY 10591, USA). The intra assay variabilities were in all instances below 2% (CV); inter assay variations were in all instances below 4.5% (CV).

Rabbit C-reactive protein was analysed by a commercial ELISA assay (Life Diagnostics, Inc., West Chester, PA 19380, USA). Manufacturer's instructions were followed. Intra- and inter assay variations were below 8%.

2.6. Statistical analysis

2.6.1. Rectal temperature and plasma traits data

To analyse the evolution of corporal temperature and blood plasma traits with time after LPS infusion, a mixed model (PROC MIXED; Statistical Analysis System, 2002) was fitted, accounting for the repeated measures design in the data that takes into account the variation between animals and covariation within them. Covariance structures were objectively compared using the most severe criteria (Schwarz Bayesian criterion), as suggested by Littell et al. (1998). The model included the times (0, 1.5, 3, 6, 24 and 48 h), and their interactions with the genetic type (LP, and V) and the group (PP9, PW5 and PW9) to gather differences in the evolution of the traits in function of these main effects. Randoms terms in the model included a permanent effect of each animal (p) and the error term (e). Contrast tests were also performed at each time to define punctual differences between genetic types and groups. To test the relationship between the previous performance traits during second lactation and both the rectal temperature and 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).

2.6.2. 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