Selection for Genetic Environmental Sensitivity of Litter Size Changes Resilience in Rabbits

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Summary

Resilience is the ability of an animal to recover from the challenge of new environmental conditions. Resilience is genetically related to environmental sensitivity. In prolific species, females with low variability in litter size may have higher resilience. We have divergently selected two lines for litter size environmental sensitivity; i.e., one for high litter size residual variance and the other one for low litter size residual variance. We have proved that the homogeneous line is less sensitive to stress and less sensitive to diseases than the line selected for litter size heterogeneity. This means that selection for more resilient animals can be carried out by selecting for uniformity of litter size. We have also shown that selection for litter size uniformity is not damaging the reproductive aptitude of the female, which fitness is even better than the line selected for increasing litter size variability.

Keywords: disease resistance, resilience, environmental variance, stress, rabbits, divergent selection

Introduction

Resilience is a term widely used in material science, ecology and a large range of topics. In livestock science, resilience is the ability of adaptation of a farm animal to a disturbing agent or adverse condition or situation. Resilience is directly related to welfare, defined by Broom (2008) as the capacity of animals to cope with their environment. Albers *et al*. (1987) distinguish between "resistance"-the ability to suppress establishment and/or subsequent development of infection, and "resilience'‘-the ability to maintain a relatively undepressed production level when infected. This definition relates resilience with robustness. Resilience can have a genetic basis, and Mulder & Rashidi (2017) have recently examined by computer simulation the possibility of selecting for resilience for litter size in pigs.

In the case of litter size, a trait directly related to fitness, environmental variability could be related to resilience. Females with more adaptable genotypes to changes in environmental conditions would show less sensitivity to diseases and to stress and less variability in litter size. This can be tested using some biomarkers. Acute phase proteins are an adequate biomarker for disease sensitivity; they are plasma proteins that change their concentration in response to an inflammatory or infectious process, regardless of the causative agent. Another biomarker could be the concentration of lymphocytes and

neutrophils in blood, a hemogram can show the number and type of leucocytes. A biomarker for stress in animals is serum cortisol concentration.

Recently, Blasco *et al.* (2017) divergently selected two rabbit lines for environmental sensitivity of litter size during 10 generations. The experiment was successful and the lines diverged around 5% of the original mean per generation. The response was slightly asymmetric, being more efficient the line selected for higher heterogeneity. In the same experiment, we measured resilience indicators in the dams at the eight generation of selection. The objective of this paper is to analyse these resilient indicators in order to find whether selection for litter size homogeneity also lead to animals genetically more resilient.

Material and methods

Animals

Rabbits used in this study come from a maternal synthetic line created from commercial crossbred does. Reproduction was organized in discrete generations. Does were first mated at 18 weeks of age, thereafter 10 d after parturition. They were under a constant photoperiod of 16:8 h and controlled ventilation. Animals were fed with a standard commercial diet. A divergent selection experiment on environmental sensitivity of litter size was carried out during ten generations. Environmental sensitivity was measured as intra-doe variance of litter size after pre-correcting litter size for year-season and lactation status. As the genetic determination of all parities of a rabbit doe is approximately the same and the permanent effects are the same along parities (Piles *et al.,* 2006), the intra-doe phenotypic variance records the environmental variability if no other systematic environmental effects are acting. The experiment is described in Blasco *et al*. (2017).

Resilience indicators

Acute phase protein: Plasma concentration of C-reactive protein (CRP) was measured in 69 does of the High line and 80 does of the Low line of the eight generation of selection. One blood sample was taken at 18 wk of age. After that, half of the females were vaccinated subcutaneously against viral haemorrhagic disease (CUNIPRAVAC® RHD), and the other half were vaccinated subcutaneously against myxomatosis (MIXOHIPRA® H). Three days after the vaccination, another blood sample was collected. C-reactive protein concentration was measured in both samples using a commercially available enzyme-linked immunoassay (ELISA) kit for rabbits (Life Diagnostics, Inc, PA, USA catalogue number 2210-5).

Hemogram: Lymphocytes and Neutrophils were measured in a subset of 20 females of the High line and 20 females of the Low line of the former animals by ADVIA 120 Hematology Analyzer.

Cortisol: Plasma concentration of cortisol was determined in 29 does of the High line and 25 does of the Low line of the eight generation of selection. One blood sample was taken at 18 wk of age. After that, 30 µg/kg of ACTH i.m. were injected in all does. Another blood sample was extracted 4 h after the injection, following the procedure described by Guelphi *et al.* (2011). Plasma cortisol levels were measured using an ELISA kit (Endocrine Technologies, Inc., CA, USA, catalogue number ERK R7003).

All blood samples were obtained from the central ear artery using vacuum tubes with EDTA. All samples were immediately centrifuged 15 min at 4000 rpm, and plasma was frozen at -80ºC for further analyses.

Statistical analysis

Response to selection was estimated as the difference between lines in the 8th generation, and as the average of the genetic values in the eight generation, estimated using a mixed model with generation as fixed effect and genetic value as random effect. Correlated response in resilience indicators was estimated as the differences between lines in the 8th generation, correcting by type of vaccine, season and dam weight.

Bayesian analyses were performed, with bounded flat priors for all unknowns. Features of the marginal posterior distributions were estimated using Gibbs sampling. For the differences between lines, we used a chain of 60,000 samples, with a burn-in period of 10,000. For the genetic analyses, we used a chain of 1,000,000 samples, and burn-in of 500,000. In all Bayesian analysis, Monte Carlo standard errors were small and lack of convergence was not detected by the Geweke test. Details of the procedure can be found in Blasco (2017). Software was developed for performing the analyses of differences between lines, and TM program was used for the genetic one.

Results and Discussion

Response to selection estimated as the phenotypic difference between lines was 1.16 kits^2 in the 8th generation with a confidence interval [0.24, 2.05] with 95% probability. The difference between genetic values was 1.06 kits^2, which agrees with the phenotypic difference validating the genetic model used in the analysis. Response to selection was slightly asymmetric. Table 1 shows the correlated responses in resilience indicators.

Acute phase protein: Before vaccination, the High line had a higher concentration of C-reactive protein than the Low line, showing a higher base level (subclinic) of immune response, which is related to a higher sensitivity to diseases or to a lesser tolerance to usual microorganisms in the farm microenvironment (Rauw, 2012). After vaccination, the Low line showed a higher level of C-reactive protein; line Low had a quicker and higher response to invader agents, showing a higher resilience. No difference in the type of vaccine was detected.

Hemogram: The High line showed a higher base level of Lymphocytes before vaccination, and a lower response to pathogen agents, the same patter as the CRP, and the interpretation could be the same, a lower tolerance to common microorganisms in the farm. A higher count of neutrophils found in the Low line could help a rapid elimination of pathogens, inactivating the infection. Neutrophils provide the first-line defence against infection in innate immune response; if infection persists, lymphocytes are involved in the activation of adaptive immune response (Janeway et al., 2001).

Cortisol: ACTH is naturally produced in an organism as response to stress, and leads to an increment in the levels of glucocorticoids (cortisol, corticosterone) in the bloodstream (Herman *et al.,* 2003). Injecting ACTH produces the same response, and the organism increases its cortisol level. Before injecting ACTH, the High line had a higher cortisol base level, showing a higher base level stress than the Low line. The difference between lines substantially increased after the injection of ACTH, showing that line Low coped better with stress than the High line, and consequently showed a higher resilience as well.

The line selected for low litter size environmental variance showed higher litter size in all generations than the High line (Blasco *et al.,* 2017). Survival at birth and weaning (4 wk of age) were in both cases 3% higher in the Low line (P=0.92 and 0.82 respectively), which is

coherent with the results we obtained in doe's resilience. No experiment of this kind has been performed hitherto, thus we cannot compare our results with others, but the general picture is coherent. We can conclude that resilience of does, their ability to face an aggression, is genetically related to environmental sensitivity. Selection for reducing litter size residual variance leads to does with more ability to face external pathogens and more resistance to stress than does selected for higher environmental variance of litter size.

Table 1. Correlated response in resilience indicators.

		Mean	H−L	HPD95%	P
C-Reactive Protein, µg/ml	Before vacc.	25.4	4.81	-1.25 , 11.15	0.96
	After vacc.	45.2	-15.1	-26.4 , -4.44	1.00
Cortisol, ng/ml	Before ACTH	0.73	0.16	0.06 , 0.25	1.00
	After ACTH	1.37	0.64	0.26 , 1.05	1.00
Lymphocytes, %	Before vacc.	64.6	4.0	-1.36, 9.53	0.93
	After vacc.	68.1	-4.4	-10.6, 1.9	0.93
Neutrophils, %	Before vacc.	25.7	-4.2	-9.3, 1.1	0.95
	After vacc.	23.1	2.7	-3.2, 8.0	0.83

H−L, differences between High (H) and Low (L) lines at generation 8th; P, Probability of the difference between lines being positive when the difference is positive, or being negative when the difference is negative.

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