

EFFECT OF DIVERGENT SELECTION FOR RESIDUAL VARIANCE OF LITTER SIZE ON HEALTH STATUS AND WELFARE

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ABSTRACT

The objective of this study was to relate does health status and welfare with litter size variability. To assess welfare status, a measure of plasmatic levels of cortisol was taken, since levels of cortisol are related to stress. To assess health status, reactive protein (CRP), haptoglobin (HP) and amyloid A (SAA) were taken. Does come from a divergent selection experiment for litter size variability were used in the experiment. In order to compare extreme groups of does, dams with the highest litter size variability of the high line and dams with the lowest litter size variability of the low line were used in the experiment. No difference between groups was found for cortisol concentration. However, concentration for CRP and SAA were different with probability of the difference being higher than zero always larger than 95 %. No clear conclusions can be drawn for HP. A higher concentration of CRP and SAA suggests that the females with higher litter size variability are more susceptible to pathogens. This susceptibility associated to higher variability of litter size may be under genetic control. If so, selection for reducing litter size variability may lead to does with a higher health status and disease resistance. Serum concentration of CRP and SAA seems to be better markers of health status than HP.

Key words: Litter size, homogeneity, cortisol, C reactive protein (CRP), serum amyloid A (SAA), haptoglobin (Hp), animal welfare.

INTRODUCTION

The acute phase proteins (APPs) are plasma proteins that change their concentration in response to an inflammatory or infectious process, regardless of the causative agent (Kushner and Mackiewicz, 1987). Recent studies have shown that changes in the handling and transportation of animals can also cause an increase of the APPs in blood (Murata *et al.*, 2004; Piñeiro *et al.*, 2007). Because of this, the APPs have been proposed as useful biomarkers that can help measure the health and welfare of the animal (review by Rodríguez-Gómez *et al.*, 2009). These biomarkers include C-reactive protein (CRP), haptoglobin (HP) and serum amyloid A (SAA) (review by Eckersall and Bell, 2010).

Several studies have reported that stress affects negatively the immune system, and therefore the immune response to pathogenic agents (see review by Webster and Glaser, 2008). Stress is also highly related to animal welfare. Serum cortisol concentration has been usually used as a physiological marker of stress in animals (Cohen *et al.*, 1997), and can be used as an indicator of welfare.

In a divergent selection experiment for residual variance of litter size in rabbits, the females of the low line (L) showed a more homogeneous litter size than those of the high line (H) (Argente *et al.*, 2012). This difference can be due to differences between lines in stress or disease resistance. To evaluate welfare and health status, it is important to find metabolic indicators of these traits as cortisol for stress or APPs for health status. Before evaluating differences between lines, these indicators can be used to

examine whether differences in litter size variability are associated to differences in stress or health status.

The objective of this study was to relate litter size variability with does' health status and welfare, by measuring plasma levels of C reactive protein, serum amyloid A, haptoglobin and cortisol in groups of does differing in their litter size variability.

MATERIALS AND METHODS

Animals

All experimental procedures involving animals were approved by the University Miguel Hernández of Elche Research Ethics Committee. Does come from a divergent selection experiment for litter size variance (see details in Argente et al., 2012). Two groups were compared, the group of the 27 does with the highest litter size variability of the high line and the group with the 27 does with the lowest litter size variability of the low line. All Animals were bred at the farm of the University Miguel Hernández of Elche. This farm has two large rooms which are accessed through a common warehouse. The photoperiod used was 16-h light: 8-h dark in both premises. The animals stayed in the first room until 18 wk of age. At the 18th wk of age, the animals were relocated into individual cages of the second room. Three days later, the females were mated thereafter 10 d after parturition. Food and water were provided ad libitum. Blood samples were collected by venepuncture of marginal vena ear immediately after the first and second mating, for each blood sample was used one tube containing EDTA. All blood samples obtained were maintained for 1 h at room temperature in laboratory. The serum was separated by centrifugation (5000 g for 15 min) and then frozen at -20°C until testing.

Determination of acute phase proteins

Serum concentration of CRP, HP, SAA and cortisol were determined by a commercially available enzyme-linked immunoassay (ELISA) kit using the Phase Rabbit CRP (Helica Biosystems, Inc. USA), the Phase Rabbit Hp (Immunology Consultants Laboratory, Inc, USA), the Phase Rabbit SAA (Tridelta Development Ltd., Ireland) and the Rodent Cortisol (Endocrine Technologies, Inc, CA, USA), respectively. Two replicates were measured for each experimental point.

Traits

The following traits were analyzed: serum concentration of CRP, HP and SAA and cortisol at first and second mating, which we name CRP₁, HP₁, SAA₁, Cortisol₁ and CRP₂, HP₂, SAA₂, Cortisol₂ respectively.

Statistics analysis

All statistical analyses were performed using Bayesian methodology. The traits CRP₁, HP₁, SAA₁ and Cortisol₁ were analyzed using a model with the effects of group (with two levels: high litter size variability and low litter size variability) and station (with three levels: autumn, winter and spring). The model used to analyze CRP₂, HP₂, SAA₂ and Cortisol₂ also included the effect of lactation status (with two levels: lactating or nonlactating females). Bounded uniform priors were used for all unknowns. The priors for the variances were also bounded uniform. Features of the marginal posterior distribution of differences between groups were estimated using Gibbs sampling. After some exploratory analyses, we used a chain of 200,000 samples each, with a burn-in period of 20,000. Only one of every 50 samples was saved for inferences. Convergence was tested using the Z criterion of Geweke (Sorensen and Gianola, 2002) and Monte Carlo sampling errors were computed using time-series procedures described in Geyer (1992).

RESULTS AND DISCUSSION

Table 1 displays the means in the high line for all traits, and the parameters of the marginal posterior distributions of the differences (D) between the two groups of high and low litter size variability respectively. All Monte Carlo standard errors were very small and lack of convergence was not detected by the Geweke test. The values are within the wide range of those found by other authors in rabbits, e.g. Sun *et al.* (2005) for CRP, Bauer *et al.* (2009) for HP, Van Lenten *et al.* (2007) for SAA, and Szeto *et al.* (2004) for cortisol.

No difference was found between groups for cortisol concentration at first and second mating. Conversely, the group with higher litter size variability showed a higher concentration of CRP and SAA at first and second mating, since $P(D>0)$ was always larger than 95 %. No clear conclusions can be drawn for HP. A higher concentration of CRP and SAA suggest that females of the group with higher litter size variability are more susceptible to pathogens.

The differences in CRP and SAA found between groups of high and low litter size variability do not prove that selection for lower variability will lead to animals with higher disease resistance or better health status. We have only observed that animals with high variability in litter size show a poorer health status than animals with lower litter size variability, but this can be due only to environmental causes, and genetic correlation between litter size variability and concentration of CRP and SAA can be null. As the groups come from an experiment of divergent selection for litter size variability, both lines could have been compared. However the difference between lines is not high enough (see Argente *et al.*, 2012) to find differences in APPs concentration, thus two extreme groups of litter size variability were formed and phenotypic differences were found. Cheverud (1988) has noted that genetic correlations are quite often similar to phenotypic correlations, thus observing a phenotypic association between extreme groups of litter size variability with APPs concentration suggest that a genetic relationship may exist. When the difference between divergent lines will be great enough, it will be a worth to measure APPs concentration in order to find whether a genetic correlated response in health status was obtained

Table 1. Features of the estimated marginal posterior distributions of the differences (D) between groups of high and low litter size variability for serum concentration of cortisol, serum concentration of C reactive protein (CRP), serum concentration of haptoglobin (HP) and serum concentration of serum amyloid A (SAA) at first and second mating (subindex 1 and 2 respectively).

	Mean H	SD	D	HPD _{95%}	P (%)
Cortisol ₁ , ng/ml	6.40	5.26	-0.92	-3.91, 1.50	76
Cortisol ₂ , ng/ml	9.21	6.72	-2.12	-5.94, 0.98	88
CRP ₁ , µg/ml	18.35	9.29	7.44	1.73, 12.04	100
CRP ₂ , µg/ml	13.99	13.04	4.87	1.43, 9.98	95
HP ₁ , µg/ml	69.10	56.79	12.90	-19.62, 39.13	79
HP ₂ , µg/ml	133.9	134.1	26.3	-37.7, 78.2	79
SAA ₁ , µg/ml	78.75	40.63	23.36	-1.41, 43.35	98
SAA ₂ , µg/ml	79.67	39.92	20.03	-2.60, 38.88	97

Mean H: mean in the group with high litter size variability. SD: standard deviation. D: posterior median of the difference between groups. HPD_{95%}: highest posterior density region at 95%. P: $P(D>0)$ when $D>0$ and $P(D<0)$ when $D<0$.

CONCLUSION

An association between two acute phase proteins serum concentration (CRP and SAA) and high litter size variability has been found, showing that does with higher litter size variability have poorer health status than does with lower litter size variability.

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REFERENCES

- Argente M.J., Garcia M.L., Muelas R., Blasco A. 2012. Divergent selection for residual variance of litter size. *In Proc. 10th World Rabbit Congress, 2012, September, Sharm El-Sheikh, Egypt. 97-101*
- Bauer N.B., Brinke N., Heiss C., Skorupa A.B., Peters F., Kraus R., Schnettler R., Moritz A. 2009. Biodegradable beta-Tri-Calciumphosphate/hydroxyethyl methacrylate enhanced three component bone adhesive demonstrates biocompatibility without evidence of systemic toxicity in a rabbit model. *J Biomed Mater Res B Appl Biomater, 90(2), 767-77.*
- Cheverud, J.M. 1988. A comparison of genetic and phenotypic correlations. *Evolution, 42, 958-968.*
- Cohen S., Kessler R.C., Gordon L.U. 1997. Measuring stress: a guide for health and social scientists. *Oxford University Press, New York.*
- Eckersall P.D., Bell, R. 2010. Acute phase proteins: biomarkers of infection and inflammation in veterinary medicine. *The Veterinary Journal, 185 (1), 23-27.*
- Geyer, C. M., 1992. Practical Markov chain Monte Carlo (with discussion). *Stat. Sci., 7, 467-511.*
- Kushner I., Mackiewicz A., 1987. Acute phase proteins as disease markers. *Dis. Markers, 5, 1-11.*
- Murata H., Shimada N., Yoshioka M. 2004. Current research on acute phase proteins in Veterinary diagnosis: an overview. *The Veterinary Journal, 168, 28-40.*
- Piñeiro M., Piñeiro C., Carpintero R., Morales J., Campbell F.M., Eckersall P.D., Toussaint M.J.M., Lampreave F. 2007. Characterisation of the pig acute phase protein response to road transport. *The Veterinary Journal, 173, 669-674.*
- Rodríguez-Gómez I.M., Barranco I., Pallarés F.J., Rodríguez-Estévez V., Gómez-Laguna J., Carrasco L. 2009. Las proteínas de fase aguda como biomarcadores de bienestar y nivel sanitario de las explotaciones de porcino. *Real Academia De Ciencias Veterinarias De Andalucía Oriental, Vol 22(1).*
- Sorensen D., Gianola D. 2002. Likelihood, Bayesian, and MCMC Methods in Quantitative Genetics. *First Edition. Springer-Verlag, New York.*
- Sun H., Koike T., Ichikawa T., Hatakeyama K., Shiomi M., Zhang B., Kitajima S., Morimoto M., Watanabe T., Asada Y., Chen YE., Fan J. 2005. C-reactive protein in atherosclerotic lesions: its origin and pathophysiological significance. *Am. J. Pathol., 167, 1139-1148*
- Szeto Y.T., Kwok T.C.Y., Benzie F.F. 2004. Effects of a long-term vegetarian diet on biomarkers of antioxidant status and cardiovascular disease risk. *Nutrition, 20, 863-866.*
- Van Lenten, B. J., Wagner A. C., Navad N., Anantharamaiah G. M., Hama S., Reddy S. T., Fogelman A. M. 2007. Lipoprotein inflammatory properties and serum amyloid A levels but not cholesterol levels predict lesion area in cholesterol-fed rabbits. *J. Lipid Res., 48, 2344-2353.*
- Webster Marketon J.I., Glaser R. 2008. Stress hormones and immune function. *Cellular Immunology, 252 (1-2), 16-26.*