Genetic Parameters For ACTH Response In Pig

C. Larzul[*], A. Foury[†], E. Terenina[†], Y. Billon[‡] and P. Mormède[†]

Introduction

Adrenal hormones, essential for survival, play important roles in stress responses, metabolism regulation, immunity, reproduction, water and salt balance and various brain functions. Hyperactive HPA axis has an unfavorable effect on production traits such as growth rate and feed efficiency (Hennessy and Jackson (1987), Knott et al. (2008)) or body composition with an increase in lipids/proteins ratio (Foury et al. (2005), Foury et al. (2007)). Few studies established a positive relationship between HPA axis activity and robustness traits such as newborn survival (Leenhouwers et al. (2002)), heat tolerance (Michel et al. (2007)) and resistance to diseases (Gross (1976)). HPA axis activity could be considered as a valuable criterion selection to improve stress resistance, newborn survival and disease resistance, without impairing (re)production traits. Selection for higher cortisol levels would benefit to animal welfare through better health, lower sensitivity to stress and improved longevity. Divergent selection experiments involving HPA activity were carried out in several species through social stress in layers (Gross and Siegel (1985)), tonic immobility in Japanese quail (Satterlee and Johnson (1988)), cold stress in turkey (Brown and Nestor (1973)), or restraint stress in mice (Touma et al. (2008)). In all those experiments, it was shown that the main selected physiological process was the production of corticosteroid hormones linked to adrenal cortex sensitivity to ACTH (Pottinger and Carrick (2001)). A direction selection on the response to ACTH was also carried out in chicken (Edens and Siegel (1975)). In pig, differences in cortisol secretion were shown in response to ACTH among individuals but stable through time (Hennessy et al. (1988)). Metabolic clearance of cortisol is not related to the cortisol response to ACTH (Zhang et al. (1993)). It is assumed that variability between individuals is mainly due to the sensitivity of the adrenal gland to ACTH. Additionally, genetic determinism of cortisol response has been studied in pigs and has shown a moderate heritability value (h^2= 0.26, D.P. Hennessy, personal communication).

The aim of this study was to estimate the genetic variability of the cortisol secretion in response to ACTH stimulation in pigs.

Material and methods

Animal. Thirty Large White sows bred in an INRA experimental farm were inseminated each once with semen from 30 Large White boars. A total of 298 male and female piglets

[*] INRA, UMR1313, 78350 Jouy-en-Josas

[†] INRA, UMR1286, 33076 Bordeaux

[‡] INRA, GEPA, Le Magneraud, 17700 Surgères

were studied 2 weeks after weaning at the age of 6 weeks. All piglets were weighed at birth and at weaning.

Measures. A first blood sample was collected in heparined tubes by direct puncture from the jugular vein. Piglets were then injected in the neck muscles with mammalian 1–24 ACTH (Immediate Synacten, Novartis France) at the dose of 250 µg per animal and put back in their pen. A second blood sample was collected one hour after ACTH injection. The blood samples were centrifuged and plasma frozen at -80°C until measurement of glucose and cortisol. Plasma total cortisol was measured using a specific radio immunoassay (RIA DiaSorin). Glucose was measured by spectophotometry with the glucose oxidase technique.
The corticosteroid-binding globulin (CBG) capacity to bind cortisol (Bmax) was measured in the basal blood sample by radiocompetition.

Statistical analyses. Glucose, cortisol and CBG levels were transformed to base 10 logarithmic scores. ANOVA was used to study the fixed effects of birth and weaning weights, batch and sex. Birth and weaning weights were also considered as covariates.
Genetic parameters were estimated using restricted maximum likelihood methodology applied to a multiple trait animal model, with the VCE6 software. Random effect of litter was estimated but removed from the final analysis except for cortisol measured after ACTH injection.

Results and discussion

Means of the traits are shown in table 1. As expected, the cortisol level was increased after ACTH injection. Sex effect was significant ($P<0.05$) for all trait but cortisol measured after injection. Males had a higher level for glucose and cortisol and a lower CBG binding capacity. Effect of birth weight was found significant only for glucose levels after injection. Weaning weight was not significant.

Table 1: Means, heritability and litter effect estimates[α]

Trait	CBG	Cortisol_b	Cortisol_a	Glucose_b	Glucose_a
Mean	1.02	1.97	2.41	0.05	0.02
Standard deviation	1.54	0.22	0.11	0.08	0.07
h^2	0.161	0.175	0.512	0.163	0.226
c^2	-	-	0.089	-	-

[α] _b: before ACTH injection, _a: after ACTH injection; heritabilities h^2 and litter effect c^2. All variables are log-transformed (CBG and cortisol in nMol, glucose in g/L).

Estimates of genetic parameters are shown in table 1 and 2. The only significant litter effect was found for cortisol response to ACTH. For all the other traits, litter effect was estimated with a very low value and was removed from the model for genetic parameter estimations. The highest heritability value was estimated for cortisol response to ACTH ($h^2=0.5$), higher than the previous estimate communicated by Hennessy ($h^2=0.26$). All other heritability values were moderate, estimated around 0.2. In duck, it has also been shown that heritability

value was higher (h^2=0.37 vs 0.15) for corticosterone level after ACTH injection than before (Brun et al. (2008)).
Cortisol (respectively, glucose) before and after ACTH injection were moderately phenotypically correlated. CBG binding capacity was not significantly correlated to the other traits. Cortisol before injection was positively correlated to glucose before injection whereas the correlation for the same traits after injection was not significant.

Table 2: Correlation estimates[α]

Traits	CBG	Cortisol_b	Cortisol_a	Glucose_b	Glucose_a
CBG		0.19	0.02	0.12	0.09
Cortisol_b	-0.27		0.55	0.23	0.15
Cortisol_a	0.02	0.96		0.09	0.00
Glucose_b	-0.81	0.04	0.22		0.49
Glucose_a	-0.54	-0.19	-0.04	0.44	

[α] _b: before ACTH injection, _a: after ACTH injection; Genetic correlations below the diagonal, and phenotypic correlations above the diagonal. Standard errors of h^2 and c^2 estimates are 0.1 and standard errors of genetic correlations are about 0.4.

The highest genetic correlation was estimated between cortisol before and after injection. It has been previously shown that the cortisol response to ACTH or to stress was proportional to basal cortisol level (Foury et al. (2007); Solberg et al. (2006)), which is consistent with a high genetic correlation. As a selection criterion, cortisol level after ACTH injection would be more efficiently selected than basal cortisol level due to a higher heritability value, but cortisol level would also be increased.
CBG is an important source of genetic variation of HPA axis function and could influence circulating levels (Moisan (2010)). It was expected that the levels of cortisol measured after ACTH should have been corrected for CBG levels to measure the adrenal response to ACTH. Unexpectedly, post-ACTH cortisol levels were independent from CBG levels, both phenotypically and genetically. Glucose levels were measured as a simple index of cortisol function. It was expected that glucose levels would increase after ACTH, which was not the case, and glucose and cortisol levels were lowly correlated.

Conclusion

These results confirm the strong influence of genetic factors on the response of the adrenal gland to ACTH, a major source of variation in HPA axis activity and function. Intense selection for lean tissue during the last decades has reduced concomitantly the activity of the corticotropic axis, with negative consequences of selection on piglet survival for instance. One strategy to improve robustness is to select animals with higher HPA axis activity by increasing cortisol level after ACTH injection. Considering the high heritability value associated with this trait, a selection experiment will be carried out and consequences on robustness, health and behavior will be evaluated. In addition, the analysis of the molecular polymorphisms of genes involved in adrenal sensitivity to ACTH, previously identified by Hazard et al. (2008) will also deliver powerful tools for selection of animals with better robustness towards environmental stressors.

References

Brown, K.I., and Nestor K.E. (1973). *Poult. Sci.,* 52:1948-1954.

Brun, J.M., Basso B., Arnaud I. *et al.* (2008). In *Proc 8ème JRPFG*.

Edens, F.W., and Siegel H.S. (1975). *General and Comparative Endocrinology*, 25:64-73.

Foury, A., Devillers, T., Sanchez, M.P. *et al.* (2005). *Meat Science*, 69:703-707.

Foury, A., Geverink, N.A., Gil, M. *et al.* (2007). *Animal*, 1:973-982.

Gross, W. B. (1976). *Poult. Sci.*, 55:1508-1512.

Gross, W. B., and Siegel P.B. (1985). *Poult. Sci.*, 64: 2230-2233.

Hazard, D., Liaubet, L., SanCristobal, M. and Mormede, P. (2008). *BMC Genomics*, 9:101

Hennessy, D.P., and Jackson P.N. (1987). *A. P. S. A. Committee*: 23.

Hennessy, D. P., Stelmasiak, T., Johnston, N.E. *et al.* (1988). *American Journal of Veterinary Research*, 49:1276-1283.

Knott, S. A., Cummins, L.J., Dunshea, F.R. *et al.* (2008). *Domest. Anim. Endocrinol*., 34:261-268.

Leenhouwers, J. I., Knol, E.F., de Groot, P.N. *et al.* (2002). *J. Anim. Sci*., 80:1759-1770.

McEwen, B.S. (2007). *Physiol. Rev.*, 87:873-904.

Michel, V., Peinnequin, A. Alonso, A. *et al.* (2007). *Neuroscience*, 147:522-31.

Moisan, M.P. (2010). *Molecular and Cellular Endocrinology,* 316:35-41.

Pottinger, T.G., and Carrick, T.R. (2001). *Comp. Biochem. Physiol A Mol.Integr.Physiol.,* 129:399-404.

Satterlee, D.G., and Johnson W.A. (1988). *Poult. Sci.*, 67:25-32.

Solberg, L.C., Baum, A.E., Ahmadiyeh, N. *et al.* (2006). *Physiological Genomics* 27:362-369

Touma, C., Bunck, M., Glasl, L. *et al.* (2008). *Psychoneuroendocrinology*, 33:839-862.

Zhang, S. H., Hennessy, D.P., McCauley, I. *et al.* (1993). *Comp. Biochemistry and Physiology a-Physiology*, 104:43-49.