Molecular Genetics Of Stress Responses To Increase Robustness In The Context Of Sustainable Production

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Introduction

The concept of robustness in farm animals was defined by Knap (2005) as 'the ability to combine a high production potential with resilience to stressors, allowing for unproblematic expression of a high production potential in a wide variety of environmental conditions'. Indeed, genetic progress in production traits realized at the nucleus level may become constrained in commercial practice if the resulting animals (end products of the breeding system) are raised in conditions that do not support full expression of their genetic potential. Robustness may then be seen as a global measure – as evaluated for instance by the realized level or functional longevity – of the sensitivity of the animal to the climatic, physical, nutritious, infectious, and social environment, and to the metabolic load of its genetic potential for production traits. This concept also includes traits that are specifically sensitive to inadequate environmental conditions, such as skeletal and cardiovascular integrity, disease resistance, and mortality in various stages, altogether known as 'functional traits'. Such traits are important not only in terms of performance levels but also for animal health and welfare (Knap 2009).

Robustness as a breeding goal

In its 'Sustainable Farm Animal Breeding and Reproduction, a vision for 2025', the FABRE Technology Platform (2006; http://www.fabretp.org/images/vision.fabretp.def1.pdf) described the farm animal of the future as 'robust, adapted and healthy'. The importance of robustness-related traits in breeding objectives is progressively increasing towards the production of animals with a high production level in a wide range of climatic conditions and production systems, together with a high level of animal welfare. As stated by Knap (2009), 'Sustainable breeding goals combine robustness traits with production traits to such an extent that selection balances genetic change in production potential with genetic change in environmental sensitivity'. Indeed, when selection focuses on production traits only, the abovementioned functional traits are likely to become compromised (Rauw et al. 1998; Star et al. 2008; Knap and Rauw 2009; Siegel et al. 2009; Veerkamp et al. 2009). The current evolution of animal production systems (increase of economic pressure, diversification of production environments, reduction of individual animal management, increase of parasitic load with outdoor production), combined with global warming, increases the importance of adaptation and robustness traits in sustainable breeding goals.

Several strategies have been described to reach this goal. Global sensitivity to the environment is measured by such techniques as the reaction norm analysis by comparing

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animals with identical genotypes in different environments (Knap and Su 2008). This is a difficult endeavour and heritability of the character is low. The sensitivity to the environment may also contribute to the environmental variance of a trait, which has been shown to be under genetic control (SanCristobal-Gaudy et al. 2001). The reduction of trait variance by genetic selection is also known as canalising selection or canalisation (e.g. SanCristobal-Gaudy et al. 1998, Bolet et al. 2007). Another strategy is direct selection for robustness-related traits. Genetic improvement in functional traits such as leg soundness, mortality rates in various stages of the animal's life, and functional longevity is possible when these traits are properly included into breeding goals and selection criteria, and is being realized in existing breeding programs (Knap 2009). Disease resistance traits are more difficult to select for, except in specific cases such as the somatic cell count in milk that is a good indicator of sensitivity to mammary infections in dairy cattle (Colleau and Regaldo 2001). Current efforts towards the discovery of molecular bases for genetic variation of these complex traits will eventually deliver DNA polymorphisms to be used for genomic selection. The third strategy that will be developed in this paper focuses on the molecular genetics of neuroendocrine stress responses, more specifically the hypothalamic-pituitary-adrenocortical (HPA) axis.

The hypothalamic-pituitary-adrenocortical (HPA) axis

The HPA axis is the cornerstone of biological stress responses, together with the autonomic nervous system, in concert with behavioural adaptive processes. The main output elements of the axis are the glucocorticoid hormones cortisol (mammals, fish) or corticosterone (birds) synthesized in and released by the adrenal cortex in response to the hormone ACTH released by the anterior pituitary gland under the control of hypothalamic neurohormones corticotrophin-releasing hormone (CRH) and vasopressin. Glucocorticoid hormones act on a wide range of cells and tissues via the glucocorticoid (GR) and mineralocorticoid (MR) nuclear receptors. They influence numerous metabolic pathways, the immune system, inflammatory processes and brain functions, just to mention the most important. They also exert a strong feedback on the HPA axis (Chrousos 1998).

Cortisol, production and robustness

Cortisol and production traits. Cortisol has complex, and mostly negative, effects on production traits. Hennessy et al. (1988) showed that the adrenal response to ACTH in pigs is an individual trait and that growth rate and feed efficiency are negatively related to the intensity of this response (Hennessy and Jackson 1987). Similar results have been obtained in sheep, with residual feed intake being directly proportional to the release of cortisol after injection of ACTH (Knott et al. 2008). An extensive study of the effects of corticosterone in chickens chronically infused with ACTH describes the effects of adrenal stimulation on production (e.g. reduced feed intake, body and carcass weight), and physiological (e.g. increased liver weight and lipid content, increased adrenal glands weight and plasma concentrations of glucose and lipids) traits (Thaxton and Puvadolpirod 2000). In pigs, several examples show that leanness is influenced by the cortisol production rate as measured for instance by the excretion level in urine (Foury et al. 2005; Foury et al. 2007). All these changes result mainly from the physiological effects of glucocorticoid hormones on metabolism, with an increase of energy storage (fat and glycogen) at the expense of tissue proteins (Devenport et al. 1989).

Cortisol and robustness. By contrast, several lines of evidence show that cortisol has positive effects on robustness traits although experimental data are still fragmentary, especially in farm animal species. Glucocorticoid hormones strengthen adaptation processes. In rats for example, animals with the strongest HPA axis response to heat stress, as measured by circulating corticosterone levels, displayed a more efficient physiological adaptation to the heat stimulus, with a lower increase of core temperature and hemoconcentration, and a reduced inflammatory response in the brain (Michel et al. 2007). Another example of the positive influence of stress hormones on robustness traits can be found in the work on newborn piglet survival of (Leenhouwers et al. 2002), who showed that piglet viability is a heritable and piglet-intrinsic trait. The only biological characteristics correlated (positively) with the estimated breeding value for piglet survival were the size of the adrenal glands and the concentration of cortisol in cord blood collected at birth. Finally, experimental evidence in poultry shows that genetic selection for the intensity of the HPA axis stress response has a complex influence on immune responses and resistance to diseases (Gross 1976; Minozzi et al. 2008). Altogether, these data suggest that glucocorticoids have a positive influence on several robustness-related traits. Considering the abovementioned general development towards less supportive production conditions, this positive influence is worth being explored in more detail.

Cortisol: trade-off factor between production and robustness. In the French Large White pig breed, a comparison of progeny from sires born in 1977 (frozen semen) versus 1998-2000 (Foury et al. 2009) shows a decrease of the production of cortisol (urinary cortisol at slaughter), together with an improvement of production traits (growth rate, feed efficiency, leanness). This illustrates the abovementioned negative effect of cortisol on production traits. This decrease in HPA axis activity may explain part of the compromised robustness that coincides with single-minded genetic improvement of production traits in farm animals (see above). This trade-off between productivity and robustness is predicted by resource allocation theory (Beilharz 1998, Glazier 2009): the energetic resources of an individual are limited and their allocation across metabolic functions is optimized towards the best adaptation of the individual to its environment (= fitness). Genetic selection for production traits logically redirects resources towards those production traits, at the expense of other traits (such as robustness traits). When resources are not sufficient to support full expression of the production potential this becomes problematic, and leads to genotype x environment interaction.

Therefore the HPA axis appears as a putative physiological element of the trade-off between production traits and robustness traits that are influenced negatively and positively respectively by cortisol. So, an additional strategy to those listed above to increase farm animal robustness would be strengthen the HPA axis activity, but without the possible side effect of increased cortisol production to compromise productivity. This objective does not appear to be out of reach. Indeed, the functional variability of the HPA axis is usually very large, even in genetically homogeneous populations. Foury et al. (2007) found a 30-fold range of urine cortisol concentrations in each of five pure pig lines, much more than the variation of production traits. In the abovementioned study of genetic trends of stress-responsive systems in the French Large White, (Foury et al. 2009) found a -0.27 correlation

coefficient between cortisol levels (in urine collected from the bladder after slaughter) and carcass lean content, so that only $0.27 \times 0.27 = 7.3$ % of the variance of leanness is related to differences in cortisol production. It is therefore possible to envisage the selection for a more active/reactive HPA axis to improve robustness without compromising production traits. To this end, several strategies are possible, based on our knowledge of genetic influences on cortisol production, bioavailability and efficiency.

Genetics and the HPA axis

Genetics of stress responses. Basal activity of the HPA axis and the response to stress are strongly influenced by genetic factors (see Mormede et al. 2002; Redei 2008; DeRijk 2009 for reviews). Divergent genetic selection for the HPA axis response to various stimuli has been successful in a wide range of farmed species and mice (Touma et al. 2008). The response to selection is usually very strong, with realized heritabilities between 0.2 and 0.4.

Adrenal cortex sensitivity to ACTH. Genetic variation is present in every component of the system, at the level of hormone production, bioavailability and action. The production rate of cortisol is primarily regulated by the sensitivity of the adrenal cortex to ACTH. Hennessy et al. (1988) demonstrated in pigs that the adrenal response to ACTH is variable among individuals but stable across time for a given animal. Similar differences in cortisol secretion were shown in response to CRH (Zhang et al. 1990), physical exercise or insulin-induced hypoglycemia (Zhang et al. 1992), although the ACTH response was not different among individuals, so that the effect must have been due to adrenal sensitivity to ACTH. Metabolic clearance of cortisol bears no relationship with the response to ACTH (Zhang et al. 1993). Altogether, these data demonstrate that adrenal sensitivity to ACTH is a key index of individual differences in HPA function. As noted previously, the magnitude of the adrenal response to ACTH is negatively correlated with body weight and growth rate (Hennessy and Jackson 1987) but did not show, in this study, any relationship to body fat content or muscle pH. The adrenal response to ACTH is highly heritable ($h2 = 0.51$; Larzul et al. this meeting, data obtained in a Large White pig population). Indeed, as mentioned in the previous paragraph, Brown and Nestor (1973) could select divergent lines of turkey based on their response to ACTH injection, with a realized heritability of 0.28. The same kind of variability in adrenal response to ACTH has been shown in humans (Bertagna et al. 1994; Coste et al. 1994). Differential gene expression studies in pigs (Hazard et al. 2008; Li et al. 2008a&b; Jouffe et al. 2009) and chickens (Bureau et al. 2009) have produced candidate genes for differences in sensitivity to ACTH.

Bioavailability of glucocorticoid hormones. Bioavailability of glucorticoid hormones is regulated by metabolic enzymes and carrier proteins. The enzymes 11β-hydroxysteroid dehydrogenase 1 and 2 convert cortisol and corticosterone into their inactive 11-oxo derivatives (type 2) and back (type 1). This mechanism is an important regulator of glucocorticoid hormone activity (Remer et al. 2008). Although research in this field is very active towards the development of drugs for the control of obesity and metabolic diseases in humans (Hale and Wang 2008), very little information is available in farm animals.

In plasma, glucocorticoid hormones bind with high affinity to a specific glycoprotein, corticosteroid-binding globulin (CBG) and with a lower affinity to albumin, so that the free, active fraction of the hormones is small and highly regulated by CBG levels. A genetic mapping experiment in a F2 intercross between Meishan and Large White pig breeds showed an association between a locus on pig chromosome 7q26 and cortisol levels, especially after the pigs were exposed to the stress of a novel environment (Desautes et al. 2002). By comparative genomics the gene encoding CBG (SERPINA6) appeared to be a good candidate, and further research strongly supported the implication of mutations in the SERPINA6 gene in cortisolemia, carcass composition and meat quality (Ousova et al. 2004; Geverink et al. 2006; Guyonnet-Duperat et al. 2006). Several recent animal and human studies confirm the role of CBG or its genomic locus in traits related to neuro-psychiatry, obesity and diabetes, immunity and inflammation, as well as growth (see Moisan (2010), for a review).

Receptors and transduction mechanisms. Large genetic variations in the efficiency of corticosteroid hormones have been described (e.g. Harizi et al. (2007) in mice). In humans and laboratory animals, numerous molecular variations have been described in the sequence of receptors, with functional consequences for health and disease (van Rossum and Lamberts 2004, DeRijk 2009), but little information is available in farm animal species (Perreau et al. 1999).

This review of the literature shows that glucocorticoid secretion and function is highly variable, due to numerous genetic differences in all the components of the HPA axis. Several molecular variations in gene structure have functional consequences on various traits related to stress responses, production and robustness. Data in farm animals are still incomplete but research is active in this field. We also need more information about the system genetics of the HPA axis for a more integrated understanding of the whole system. Indeed, several sources of genetic variability are usually found in the same model (Marissal-Arvy et al. 2004), but very little is known about the interactions among various sources of variability within the axis, and how they eventually compensate for or potentiate each other. Modelling the various sources of genetic variability and their functional consequences should provide insight in the best use of DNA markers to influence the function of the HPA axis towards the breeding goals of improved robustness without negative effects on production traits.

Conclusion and perspectives

The HPA axis is a neuroendocrine system of critical importance in the regulation of energy metabolism and stress responses. Its level of activity influences production traits negatively and several robustness traits positively. The recent history of genetic selection for production traits such as growth rate, feed efficiency, and leanness (all negatively influenced by glucocorticoid hormones), has probably contributed to the reduction of HPA axis activity and consequently to a decrease of the robustness of modern, high-productivity animals. In the context of sustainable breeding, the genetic selection objective aims at a better balance between production and robustness traits. HPA axis activity should then be increased to improve robustness, but at the same time, genetic selection should not compromise the high production level of modern genotypes. A high genetic variability is present in the various

components of the HPA axis, and molecular genetics research is producing fundamental knowledge towards marker-assisted selection for an optimized balance between production and robustness. This selection strategy based on genetic variation of neuroendocrine stress systems is complementary to already implemented approaches such as the integration of robustness phenotypes or environmental sensitivity in selection programmes.

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References

Beilharz, R.G. (1998). *J. Anim. Breeding Genet.,* 115:433-437.

Bertagna, X., Coste, J., Raux-Demay, M.C. *et al.* (1994). *J. Clin. Endocrinol. Metab.,* 79:390-394.

Bolet, G., Gaffeau, H., Joly, T. *et al.* (2007). *Livest. Sci,.* 111:28-32.

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

Bureau, C., Hennequet-Antier, C., Couty, M. *et al.* (2009). *BMC Genomics,* 10:430.

Chrousos, G.P. (1998). In *Stress of Life - from Molecules to Man* (ed. P Csermely), pp. 311-335.

Colleau, J.J. and Regaldo, D. (2001). In *8th Conference on Ruminant Research*, Paris, France, pp. 329-332.

Coste, J., Strauch, G., Letrait, M. *et al.* (1994). *Brit. J. Pharmacol*., 38:474-479.

DeRijk, R.H. (2009). *Neuroimmunomodulation*, 16:340-352.

Desautes, C., Bidanel, J.P., Milan, D. *et al.* (2002). *J. Anim Sci.*, 80:2276-2285.

Devenport, L., Knehans, A., Sundstrom, A. *et al.* (1989). *Life Sci.,* 45:1389-1396.

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

Foury, A., Tribout, T., Bazin, C. *et al*. (2009). *Animal*, 3:1681-1687.

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

Geverink, N., Foury, A., Plastow, G.S. *et al.* (2006). *J Anim Sci*., 84:804-811.

Glazier, D.D. (2009). In *Resource Allocation Theory Applied to farm Animal Production* (ed. WM Rauw), pp. 44-60, CABI, Wallingford.

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

Guyonnet-Duperat, V., Geverink, N., Plastow, G.S. *et al.* (2006). *Genetics* 173:2143-2149.

Hale, C. and Wang, M. (2008). *Mini-Reviews Med. Chem*., 8:702-710.

Harizi, H., Homo-Delarche, F., Amrani, A. *et al.* (2007). *J. Neuroimmunol*., 189:59-68.

Hazard, D., Liaubet, L., Sancristobal, M. *et al.* (2008). *BMC Genomics*, 9:101.

Hennessy, D.P. and Jackson, P.N. (1987). In *Manipulating Pig Production* (Ed. APSA Committee), p. 23.

Hennessy, D.P., Stelmasiak, T., Johnston, N.E. *et al*. (1988). *Amer. J. Vet. Res*., 49, 1276-1283.

Jouffe, V., Rowe, S.J., Liaubet, L. *et al.* (2009). *BMC Proceedings,* 3(S4):S14.

Knap, P.W. (2009). In *Resource Allocation Theory Applied to Farm Animal Production* (ed. WM Rauw), pp. 288-301, CABI, Wallingford.

Knap, P.W. and Su, G. (2008). *Animal,* 2:1742-1747.

Knap, P.W. and Rauw, W.M. (2009). In *Resource allocation theory applied to farm animal production* (ed. WM Rauw), pp. 210-229, CABI, Wallingford, UK.

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.

Li, L.A., Xia, D., Wei, S. *et al.* (2008a). *Steroids*, 73:806-814.

Li, L.A., Xia, D., Wei, S. *et al*. (2008b). *Domest. Anim. Endocrinol.*, 35:1-7.

Marissal-Arvy, N., Lombes, M., Petterson, J. *et al*. (2004). *J. Biol. Chem*., 279:39232-39239.

Michel, V., Peinnequin, A., Alonso, A. et al. (2007). *Neuroscience,* 147:522-531.

Minozzi, G., Bidanel, J.P., Minvielle, F. et al. (2008). *Livest. Sci*., 119:221-228.

Moisan, M.P. (2010). *Mol. Cell. Endocrinol*., 316:35-41.

Mormede, P., Courvoisier, H., Ramos, A. *et al.* (2002). *Psychoneuroendocrinology*, 27:563-583.

Ousova, O., Guyonnet-Duperat, V., Iannuccelli, N. *et al*. (2004). *Mol. Endocrinol*., 18:1687-1696.

Perreau, V., Sarrieau, A. and Mormede, P. (1999). *Life Sci*., 64:1501-1515.

Rauw, W.M., Kanis, E., Noordhuizen-Stassen, E.N. et al. (1998). *Livest. Prod. Sci*., 56:15-33.

Redei, E.E. (2008). *Ann. Med*., 40:139-148.

Remer, T., Maser-Gluth, C. and Wudy, S.A. (2008). *Mini-Rev. Med. Chem*., 8:153-170.

SanCristobal-Gaudy, M., Elsen, J.M., Bodin, L. *et al.* (1998). *Genet. Sel. Evol*., 30:423-451.

SanCristobal-Gaudy, M., Bodin, L., Elsen, J.M. *et al*. (2001). *Genet. Sel. Evol*., 33:249-271.

Siegel, P.B., Honaker, C.F. and Rauw, W.M. (2009). In *Resource allocation theory applied to farm animal production* (ed. WM Rauw), pp. 230-242, CABI, Wallingford, UK.

Star, L., Ellen, E.D., Uitdehaag, K. *et al.* (2008). *J. Agric. Environ. Ethics,* 21:109-125.

Thaxton, J.P. and Puvadolpirod, S. (2000). *Poult. Sci*., 79:391-395.

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

van Rossum, E.F.C. and Lamberts, S.W.J. (2004). In *Recent Progress in Hormone Research*, Vol 59, pp. 333-357.

Veerkamp, R.F., Windig, J.J., Calus, M.P.L. et al. (2009). In *Resource allocation theory applied to farm animal production* (ed. WM Rauw), pp. 243-260, CABI, Wallingford, UK.

Zhang, S.H., Hennessy, D.P. and Cranwell, P.D. (1990). *Amer. J. Vet. Res.,* 51:1021-1025.

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

Zhang, S.H., Hennessy, D.P., Cranwell, P.D. *et al.* (1992). *Comp. Biochem. Physiol. a-Comp. Physiol.,* 103:695-703.