Molecular genetics of the adrenocortical axis and breeding for robustness

P. Mormede*, E. Terenina
INRA, UMR 444 Génétique Cellulaire, F-31326 Castanet-Tolosan, France
Received 3 February 2012; received in revised form 20 April 2012; accepted 14 May 2012

Abstract

The concept of robustness refers to the combination of a high production potential and a low sensitivity to environmental perturbations. The importance of robustness-related traits in breeding objectives is progressively increasing toward 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. Current strategies to increase robustness include selection for “functional traits,” such as skeletal and cardiovascular integrity, disease resistance, and mortality at various stages. It is also possible to use global evaluation of sensitivity to the environment (e.g., reaction norm analysis or canalization), but these techniques are difficult to implement in practice. The glucocorticoid hormones released by the adrenal cortex exert a wide range of effects on metabolism, the cardiovascular system, inflammatory processes, and brain function, for example. Protein catabolism toward energy production and storage (lipids and glycogen) supports their pivotal role in stress responses aiming at the adaptation and survival of individuals under strong environmental pressure. Large individual variations have been described in adrenocortical axis activity, with important physiological consequences. In terms of animal production, higher cortisol levels have negative effects on growth rate and feed efficiency and increase the fat: lean ratio of carcasses. On the contrary, cortisol has positive effects on functional traits and adaptation. Intense selection for lean tissue growth and more generally high protein output during the past decades has concomitantly reduced cortisol production, which may be responsible for the negative effects of selection on functional traits. In this paper, we review experimental evidence suggesting that the balance between production and functional traits was modified in favor of improved robustness by selecting animals with higher adrenocortical axis activity, as well as the molecular genetic tools that can be used to fine-tune this objective.

Keywords: Stress; Adaptation; Welfare; Cortisol; Functional traits; Genomic selection

1. Introduction: The new challenges to breeding food-producing animals

In its “Sustainable Farm Animal Breeding and Reproduction, a vision for 2025,” the European FABRE Technology Platform described the farm animal of the future as “robust, adapted and healthy” and “producing a safe and healthy food” (2006; http://www.euroqualityfiles.net/vision_pdf/vision_fabre.pdf). Intense genetic selection of farm animals during recent decades has considerably increased the production (and productivity) of modern food-producing animals at the expense of their robustness, as shown by the degradation of numerous indicators (known as functional traits) such as the efficiency of reproduction, sensitivity to disease, vulnerability to environmental pressure (such as exposure to heat), and...
duration of productive life. At the same time, an increasing awareness toward the negative impact of animal breeding on the environment (such as the production of nitrates, phosphates, and greenhouse gases), an increasing concern of consumers about the deleterious or positive effects of feedstuffs on human health and of citizens about the welfare of farm animals, and the changes in rural lifestyle that result in a reduced workforce have raised new challenges for farm animal production. The necessary increase of production to match the demand of food of animal origin is therefore constrained by new challenges for commercial enterprises involved in the genetic selection of food-producing animals that must be answered to keep and improve their position in an open, highly competitive market. Innovative strategies must be developed urgently to analyze and take into consideration these new characteristics, to be combined with the necessary high level of production and an increasing quality of commercial products, which are prerequisite to the sustainability of animal production.

2. Robustness is the central concept integrating farm animal breeding objectives

The concept of robustness integrates these different breeding goals, as it refers to the combination of a high production potential and a low sensitivity to environmental perturbations [1,2]. For the sustainability of animal breeding, the high production potential must be understood not only in terms of economic profitability but also in terms of minimal environmental impact. In designing the animal of tomorrow, three features should be considered as constituting the core of trait development focus: feed efficiency, adaptability, and product quality.

Increasing feed efficiency is a main avenue to reducing the cost of production (animal feed represents more than 50% to 60% of the total production cost for pig, chickens, milk, and beef) and the environmental burdens caused by animal production systems (reduction of effluents, nitrogen and phosphorus excretion, methane emission). This goal can also be reached using alternative sources of food, such as by-products of agro food and biofuel industries, which would also reduce the competition between animals and humans for noble feedstuff.

Adaptability is central to animal efficiency. Genetic progress may become constrained in commercial practice if animals are raised under conditions that do not support full expression of their genetic potential because of suboptimal environmental conditions in terms of nutrition, stocking density, temperature, humidity, and pathogen exposure. For instance, pigs raised in commercial environments do not express more than 80% of their genetic potential [3]. Adaptability is a global measure of the sensitivity of the animal to the environment and to the metabolic load of its genetic potential for production traits. Adaptability also includes traits that are sensitive to inadequate environmental conditions, such as disease resistance and mortality in various stages (eg neonatal), altogether known as “functional traits.” Such traits are important not only for performance levels but also for animal health and welfare [4]. Their improvement also has a positive impact on the environment by reducing production losses as well as the need for the preventive and therapeutic use of drugs (such as antibiotics). Finally, changes in rural lifestyle and economic constraints reduce the workforce available for the care of farm animals, which must therefore become more independent.

The quality of animal products is the third category of traits to take into consideration in designing the animals of tomorrow. Food safety is of primary importance for products of animal origin, together with their sensory and technological qualities. However, there is a growing demand for food products that improve consumer health status (eg specific fatty acid composition).

The solution to this multidimensional equation is a major challenge to animal breeding. Numerous avenues are explored to reach these goals and genetics is a major lever in designing the animal of tomorrow. Indeed, all traits cited above and contributing to the sustainability of farm animal production are more or less heritable. However, the challenge is to combine the number and diversity of traits in the selection goals. Genomic selection is a unique opportunity to combine a complex set of traits as long as these traits can be measured accurately in the reference population. Many traits contributing to sustainability are difficult to measure and a considerable effort of detailed and extensive phenotyping will be necessary before it is possible to use this information in genomic selection. In this paper, we present our view that stress neuroendocrine systems, and more specifically the hypothalamic-pituitary-adrenocortical (in short, adrenocortical) axis, are primary contributors to the major phenotypes described above, including stress and adaptation, robustness, zootechnical performances including feed efficiency, and product quality. Furthermore, a considerable genetic variation has been described in adrenocortical axis function that may be used for genetic selection toward the objective of sustainability of farm animal production.
3. Physiology of the adrenocortical axis

The adrenocortical axis [5] and sympathetic nervous system [6] are the primary mediators of the neuroendocrine stress response and, together with behavioral adjustments, contribute to physiological adaptation [7] (Fig. 1). The main output elements of the axis are the glucocorticoid (GC) hormones; cortisol in bovine, pigs, and fish; or corticosterone in birds, synthesized in and released by the adrenal cortex (or suprarenal glands in fish) in response to the adrenocorticotropic hormone (ACTH) released by the anterior pituitary gland under the control of hypotalamic neurohormones corticotropin-releasing hormone and vasopressin [8,9]. Glucocorticoid hormones are not soluble in water and therefore circulate in blood bound to plasma proteins, such as albumin and the specific carrier corticosteroid-binding globulin (transcortin or CBG). Their liposolubility allows GC hormones to diffuse largely in all tissues and cells, where they act via the glucocorticoid (GR) and mineralocorticoid (MR) nuclear receptors that act as transcription factors, influencing the expression of several hundreds of genes via a wide range of transduction mechanisms.

Fig. 1. Organization and regulation of the adrenocortical axis. The adrenocortical axis proper is constituted by the hypothalamic (parvicellular part of the paraventricular nucleus)-anterior pituitary (corticotroph cells)-adrenal cortex functional chain releasing glucocorticoid hormones in the general circulation. The hormones act through their receptors on a wide range of tissues. The axis is controlled by central mechanisms and self-regulated by hormonal feedback. The main sources of genetic variation are shown on a gray background: sensitivity of the adrenal gland to ACTH, which is also under sympathetic control by the splanchnic nerve, hormone bioavailability (including transport mechanisms and catabolic processes), and functional efficiency of receptor transduction mechanisms (see text for details).
mechanisms [10–13]. They influence numerous metabolic pathways, the immune system, inflammatory processes, and brain functions, to mention the most important. They also exert strong feedback on the axis [14]. Glucocorticoid hormones are metabolized in the liver by oxdoreduction reactions and then conjugated (sulfo- and glucuronon conjugates that are eliminated in urine as 17 hydroxysteroids and a small fraction of untransformed steroids that can be measured as an index of their production). The 11β-hydroxysteroid dehydrogenase (11β-HSD) shuttle plays an important role in regulating glucocorticoid hormone availability. The type 1 enzyme (11β-HSD1) allows the regeneration of cortisol (or corticosterone) from inert cortisone (or 11-dehydrocorticosterone) and is present in various tissues of the body—including the liver, adipocytes, bones, and brain—acting as an intracellular glucocorticoid amplifier. It is also involved in the regulation of the axis. The type 2 enzyme (11β-HSD2) has a localization restricted to mineralocorticoid responsive tissues and the fetoplacental unit; it allows the reverse reaction, metabolizing glucocorticoids to inert compounds, and therefore protects access to the MR by glucocorticoid hormones [15].

The activity of the adrenocortical axis is highly variable because of the combination of numerous influences on secretory activity and pharmacokinetic properties. The secretion of ACTH and cortisol is pulsatile in most species, with a pulse frequency of about 90 min, follows a diurnal cycle and is influenced by meals, physical activity, and environmental conditions (see [16] for a review and species-specific features). Furthermore, short half-lives of distribution and elimination of cortisol (for example < 2 min and 20 min, respectively, in sheep [17] and 3.5 min and 36.5 min, respectively, in pigs [Mormede and Galtier, unpublished data]) allow a rapid adaptation of circulating levels to physiological needs. However, accurate measurement of the activity of the axis is challenging [16].

The effects of GC hormones on energy metabolism, in concert with insulin, are central to their role in adaptation [18–20]. They induce protein and lipid catabolism in peripheral tissues (eg muscle, skin, and thymus), releasing amino acids, glycerol, and free fatty acids that are used in the liver for anabolism, including new protein synthesis and gluconeogenesis [21]. Glucocorticoid hormones have also an anti-insulin effect. Altogether these processes increase the availability of energetic metabolites for behavioral adjustments (the “fight-or-flight response”) and metabolic adaptation (eg thermoregulation). However, when the mobilized energy is not consumed and in the presence of insulin, metabolites are taken up by adipose tissue, promoting the synthesis of lipids. The result is an increase of energy storage (fat and glycogen) at the expense of tissue proteins. It has been shown in rats that the lipid:protein ratio of the carcass, measured 2 wk after adrenalectomy and subcutaneous implantation of a corticosterone pellet, is directly proportional to the dose of corticosterone [22]. In the same experiment, Devenport and colleagues showed that the final body weight and feed efficiency followed a bell-shape curve with increasing dosages of corticosterone. Several mechanisms are involved in these effects. At the lowest doses, feed intake is reduced because corticosterone plays an important role in the regulation of feeding behavior [23,24]. Under normal physiological conditions, the MR is tonically activated by low basal levels of circulating corticosterone. The activation of MR is required for the maintenance of fat ingestion and fat deposition that occurs during most meals of the feeding cycle. In contrast, the GR is phasically activated by moderate levels of corticosterone normally reached during the circadian peak or during periods of increased energy requirements, such as after exercise and food restriction or during stress, when corticosterone levels increase further. Activation of this receptor is required for the natural surge in carbohydrate ingestion and metabolism that is essential at the onset of the active feeding cycle when the body’s glycogen stores are at their nadir, and gluconeogenesis is needed to maintain blood glucose levels or to provide additional substrates for glucose when necessary [23]. Reduced feed efficiency at the lowest corticosterone levels may also result from an excessive sympathetic tone, with catabolic effects, that is negatively regulated by GC [25,26]. At higher dosages of corticosterone, the direct effects of GC via GR described above (peripheral catabolism and promotion of energy storage) are responsible for the lower than optimal feed efficiency and body weight gain. These metabolic effects of GC are not favorable to animal production when growth rate, feed efficiency, and lean carcasses are the main selection objectives; however, they may positively influence adaptation and functional traits.

Glucocorticoid hormones have an important effect on the immune system. Indeed, the decrease in thymus size is a historical symptom of the stress syndrome as initially described by Hans Selye [5] and is a perfect bioassay of glucocorticoid levels [27,28]. Glucocorticoid hormones and their synthetic analogs are still the most potent anti-inflammatory drugs for clinical use.
Cortisol and stress also influence the specific immune response in a complex manner [29,30]. Conversely, acute inflammation is a powerful trigger of the adrenocortical axis, as shown, for instance, under experimental conditions by the response to bacterial lipopolysaccharide [31,32]. In chronic inflammation however, the cortisol-inflammation relationships become more complicated. Glucocorticoid resistance develops and activation of the adrenocortical axis, which may appear as an adaptive program positively selected for short-lived inflammatory responses (energy appeal reaction), becomes a disease-inherent pathogenetic factor, if it continues too long, that can drive systemic disease sequelae of chronic inflammatory diseases such as metabolic syndrome [33].

4. Genetics of adrenocortical axis activity and function

Individual variations of adrenocortical axis activity are well documented, and the reproducibility of the tests for adrenocortical axis function discloses stable individual characteristics [34–38].

The influence of genetic factors has been suggested by twin and family studies in humans [39–49] and pigs [50]. Large variations have been described between inbred strains of mice [51–53] and rats [54–61] and between farm animal breeds [62–72]. A few contrasted lines or breeds have played an important role in the study of the molecular bases of genetic variation in stress responses. In rats, most research has been done with Lewis, Fischer 344, Brown Norway, and Wistar Kyoto inbred strains and their intercross [73–79]. In mice the C57BL/6 and DBA/2 inbred strains are contrasted for almost all characteristics of the adrenocortical axis [53,80–81] and the BXD panel of recombinant inbred strains derived from these 2 parental strains is a powerful tool for an integrated study of the molecular bases of these differences (eg [82] and http://www.genenetwork.org). In pigs, the Meishan (MS) Chinese breed has been frequently compared with and bred with European White-type breeds. Meishan pigs give birth to larger and more lively litters [83–86] and have a lower growth rate and feed efficiency, as well as fattier carcasses with lower muscle content and a better meat quality index [87,88]. As with adrenocortical axis activity, circulating levels of cortisol is much higher, close to the values measured in wild boars [66,89–91]. These high levels result not only from a higher production of hormones, as can be measured in urine [68,92], but also from higher circulating levels of CBG [93–95].

The higher production of cortisol is at least partly the result of a higher susceptibility of the adrenal cortex to ACTH [67,96]. Exploration of corticosteroid receptor properties showed higher densities of hippocampal MR in MS pigs and of pituitary GR in Large White (LW) pigs [97]. The difference in the MR/GR balance in the hippocampus and pituitary could be involved in the different adrenocortical axis activity between the 2 breeds [98–100]. These breeds have been largely used to search for loci involved in genetic variation of production traits, as well as behavioral and neuroendocrine stress responses [101].

Finally, it has been shown in a wide range of animal species that adrenocortical axis activity is responsive to genetic selection: confinement stress in trout [72,102,103], adrenal response to ACTH [104] and social stress [105] in chickens, cold stress in turkeys [106], immobilisation stress in Japanese quail [107] and mice [108], and suspension test in ducks [109]. The response to selection is usually strong, with realized heritability between 0.4 and 0.5, showing that genetics is a powerful tool to tune adrenocortical axis activity in the desired direction.

5. Sources of genetic variation in adrenocortical axis activity and functional consequences

5.1. Sensitivity of the adrenal gland to ACTH

All components of the adrenocortical axis and its regulatory inputs may contribute to genetic variation (Fig. 1). The best documented process is the sensitivity of the adrenal gland to ACTH, which tunes the production of GC hormones. It has been shown in humans [35,36] and in pigs [37] that this response is an individual trait (“the corticotroph phenotype” [35]) and we have calculated in a family study with LW pigs a heritability of 0.51 [50]. Divergent lines of turkey could be selected on the basis of their response to ACTH injection, with a realized heritability of 0.28 [106]. In both trout [110] and duck [109], the adrenal response to ACTH appears to be a major component of the differences between lines divergently selected for the adrenocortical axis response to confinement and suspension stress, respectively. Differential gene expression studies in pigs [96,111–113] and chickens [114] have produced a list of candidate genes related to differences in sensitivity to ACTH. The relationship between adrenal sensitivity to ACTH and production traits is well documented. In a large population study in pigs, Hennessy and Jackson showed that animals with higher sensitivity to ACTH (as measured at 3 wk) were lighter, with a slower growth curve and a lower feed efficiency.
In rams, residual feed intake was shown to be directly proportional to the release of cortisol after injection of ACTH [116]. The same tendency was found in Brahman steers, with a significant correlation with the marbling score of meat [117]. However, we did not find any difference in adrenocortical axis activity between 2 lines of LW pigs genetically selected for divergent feed efficiency ([118] and unpublished results), showing that these 2 functions may be independently influenced by genetic factors.

5.2. Hormone bioavailability

Hormone bioavailability is another important mechanism of genetic variation. Little information is available in farm animals on the metabolic disposal of GC hormones and its genetic variation, despite their importance in the regulation of GC hormone activity [119]. In contrast, the influence of CBG has been raised by the discovery of the linkage between circulating cortisol levels (basal and poststress) and a genetic locus including the CBG gene (SERPINA6) in an F2 intercross between MS and LW pigs, explaining 20% of the variance of the F2 population [101]. The same region was also found to be linked, although more weakly, to several parameters of body composition, findings consistent with an increased adrenocortical axis function. The association with CBG was confirmed using CBG levels instead of cortisol, and in this study we confirmed in some F2 families that the CBG gene could be a regulator of fat accumulation and muscle content in the carcass, suggesting that specific haplotypes are involved in this association [95]. Indeed, numerous polymorphisms (SNP for single nucleotide polymorphism) were detected, both between and within breeds, 4 of which lead to amino acid substitution. The in vitro and in vivo analysis revealed that at least 1 of these polymorphisms could influence CBG binding of cortisol or CBG mRNA expression [94]. We have also explored the relationships between CBG levels and various measures (carcass composition and meat quality) in 5 different breeds with inconsistent results [120]. Only 2 genetic stocks (pure LW and an LW by MS advanced intercross line) showed correlations between CBG levels and circulating cortisol levels and production traits. Furthermore, the nature and the direction of correlations differed in the 2 lines. Indeed, in humans as well, complex relationships were found among CBG genotypes, adrenocortical axis phenotypes, and obesity [121,122]. It is interesting to note here that even when plasma cortisol levels are correlated with plasma CBG, urinary levels of cortisol are always independent of CBG levels and therefore give a more accurate evaluation of cortisol production than circulating levels, as shown in humans [123,124]. The same locus was found to influence the adrenocortical axis response to stress in rats [125] and several phenotypes that may be influenced by adrenocortical axis activity (see review in [126]). The role of CBG in the regulation of adrenocortical axis activity and related traits was studied in knockout mouse models, showing that this carrier protein may play an important role in GC hormone activity, particularly in response to stress [127,128]. At this stage, we need more information about the physiological role of CBG that has always been balanced between the “free hormone hypothesis,” stating that only the unbound hormone is available for transfer into the target cells, and the “reservoir hypothesis,” stating that CBG sequestrates glucocorticoids in plasma and is therefore able to release large amounts of hormone in the tissues [129,130]. The influence of genetic polymorphisms on these processes and the final physiological effects of GC hormones should be explored further.

5.3. Receptor function and transduction mechanisms

Genetic variability at the level of corticosteroid receptors is well documented in humans, as is its contribution to a large range of pathologic conditions [13,131–133]. We showed in rats that the adipogenic effects of GC hormones vary considerably across strains as a consequence of different functional activity of their receptors [134,135]. In contrast, little information is available in farm animal species. Binding properties of MR and GR have been studied in LW and MS pigs [97], and an SNP of the NR3C1 gene was shown to influence circulating cortisol levels and adrenal gland weight [136], but little is known about the efficiency of the different transduction pathways involved in corticosteroid hormone function. It may be anticipated that genetic polymorphisms in specific pathways would be a powerful lever to potentate the favorable effects of these hormones and downregulate their undesirable consequences. This should be an important line of research for the future.

5.4. Higher control mechanisms

Finally, higher levels of control of the adrenocortical axis (pituitary, hypothalamic, and suprahypothalamic) are difficult to explore directly in farm animals. A promising approach consists of the search of molecular polymorphisms in the genes encoding constitutive or regulating proteins of the adrenocortical axis and its regulatory nervous pathways [8]. Murani and colleagues found multiple...
and consistent associations with SNP in NR3C1 and AVPR1B (a receptor for vasopressin that interacts with corticotrophin-releasing hormone to release ACTH from the pituitary), providing convincing evidence for genuine effects of their DNA sequence variation on stress responsiveness and aggressive behavior [136]. Terenina and colleagues (unpublished observations) extended these findings to the monoaminergic neural pathways regulating adrenocortical axis activity.

5.5. System genetics

A major point to consider here is how these different sources of genetic variation in the adrenocortical axis, its receptor and postreceptor transduction mechanisms, and its regulatory pathways contribute to the final function(s) of corticosteroid hormones. The adrenocortical axis is strongly regulated by various feedback mechanisms involving the effects of corticosteroid hormones on different components of the axis and higher regulating pathways such as the hippocampus, via the classical MR and GR, as well as membrane receptors [98,137,138]. Consequently, it is expected that any mutation at one level of the system will reorganize the whole axis so that the functional consequence of the initial change may be dampened or amplified, depending on the plasticity of the other components. Indeed, several sources of genetic variability are usually found in the same model [60,61,74], but little is known about the interactions among various sources of variability within the axis and how they eventually compensate for or potentiate each other. In this context, it is important to question the functional significance of various parameters classically used to evaluate adrenocortical axis activity. For instance, when comparing the response of 3 mouse strains to various stressors, the strain with the lowest biological response as measured by the increase of glucose or decrease of interleukin 6 plasma levels [80,81]. These results clearly show that plasma concentration of GC hormones, the gold standard to measure the intensity of adrenocortical axis activity and response to stress, is not necessarily a reliable index of the adrenocortical axis functional tone. For a complete characterization of the genetic variation in adrenocortical axis activity and its consequences on robustness traits, we need a more exhaustive evaluation of the different components of the axis, such as the urinary concentration of GC hormones and metabolites that reflect the production rate and metabolism, the salivary concentration proportional to the free circulating hormone, CBG levels, and, perhaps more importantly, robust measures of functional activity. Indeed, GC hormone excretion in urine is suited to studying individual differences in adrenocortical axis activity because it is not influenced by CBG concentration, unlike plasma concentration [93], nor by rapid (frequently pulsatile) changes in hormone secretion (urine accumulates over several hours), nor by sample handling (urine collection is a noninvasive method). Finally, urine collection allows the measurement of catecholamine excretion as an index of the sympathetic nervous system activity [16,139]. Several studies have shown a relationship between GC hormone levels in urine and carcass composition, as well as catecholamine levels and meat quality [92,140–143]. Little information is available with regard to the functional output of the system. The use of functional genomics has been recently promoted by Cole [144] to study stress and coping in humans. This approach is based on a genome-wide transcriptional profiling of circulating blood cells associated with bioinformatic analysis of the balance between GR- and nuclear factor kB-regulated genes in the differentially expressed transcripts, with these 2 systems—cortisol transduction and inflammatory responses—opposing each other. The activation of the sympathetic nervous system can also be analyzed through the expression of genes regulated by CREB/ATF transcription factors [144,145]. On one hand, the autonomic nervous system plays a specific role in metabolic regulation and stress responses [6]. It has also been shown to have specific genetic variation (see [146] for review). On the other hand, the adrenocortical axis and sympathetic nervous system have strong relationships. We have shown, for instance, that cortisol and adrenaline levels measure in urine collected after slaughter in a genetically mixed population of pigs were highly correlated with each other, much more so than cortisol and noradrenaline levels [140,143]. One mechanism to explain this relationship is the regulation by cortisol of the enzyme PNMT, which catalyzes the methylation of noradrenaline into adrenaline [147]. It is also possible that the adrenal cortex (cortisol) and medulla (adrenaline) are somehow coactivated, but that the adrenocortical axis (cortisol) and the sympathetic nervous system (noradrenaline) are largely independent. Further experiments should sort out the respective influence of these mechanisms.

6. Adrenocortical axis contribution to adaptation-related traits

As mentioned previously, adaptability is a global measure of the sensitivity of the animal to the environ-
ment and to the metabolic load of its genetic potential for production traits. Adaptability also includes traits that are sensitive to inadequate environmental conditions, such as disease resistance and mortality in various stages (e.g., neonatal) or leg soundness, altogether known as “functional traits.” Such traits are important not only for performance levels but also for animal health and welfare.

A few examples are available to demonstrate the positive influence of the adrenocortical axis on several functional traits. The adrenocortical axis is essential for regulating intrauterine fetal homeostasis, controlling the normal timing of parturition, and ensuring timely differentiation and maturation of vital organ systems for postpartum survival. These effects are mediated by a surge of cortisol production and CBG synthesis in late gestation (e.g., [148–155]). In perinatal piglets, for instance, carbohydrate metabolism is described as being closely related to piglet survival. Prenatal glycogen deposition, glucose homeostasis, and thermoregulation are important factors for maturity [156], and cortisol is a key hormonal factor in increasing glycogen synthesis [157]. The low level of cortisol in neonatal calves may also be responsible for the lower endogenous production of glucose in preterm calves [158]. Heart glycogen is important for resistance against anoxia during delivery. Liver glycogen maintains glucose homeostasis during parturition and the immediate postpartum period. Muscle glycogen stores have a function first in postnatal thermogenesis, especially before colostrum intake, and later if energy intake is inadequate. Availability of glycogen stores is critical in the absence of brown adipose tissue for temperature regulation in neonatal animals [159,160]. In their search for biological traits in piglets related to their own genetic merit for survival, Leenhouders and colleagues [161,162] found that 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. These endocrine measurements were also correlated positively with the relative weight of the small intestine and higher concentrations of glycogen in the liver and muscle that reflect the gluconeogenic properties of cortisol [155,163] and its influence on intestinal maturation [164,165]. The high level of adrenocortical axis activity in MS pigs may at least partly explain the exceptional viability of their piglets [84], although data are still incomplete on the evaluation of adrenocortical axis activity in fetuses from different breeds [166].

Experimental data also show that adrenocortical axis activity may contribute to heat resistance. Michel and collaborators studied individual variations in responses to heat stress in rats [167]. The animals with the strongest adrenocortical axis response, as measured by the circulating corticosterone levels, displayed a more efficient physiological adaptation to the heat stimulus, with a lower increase of core temperature and hemocoagulation and a reduced inflammatory response in the brain. These differences reflect the physiological effects of glucocorticoid hormones [168] and show that the animals that mounted a strong stress response adapt better to the stressor. Similar results were obtained in chickens. Animals from the Red Jungle fowl or the indigenous village fowl genotypes, with high or medium basal levels of plasma corticosterone, respectively, did not show any response to acute heat exposure (36°C, 3 h, as measured by deep body temperature, expression of heat shock protein 70 in the brain, plasma corticosterone concentration, and blood heterophil:lymphocyte ratio). On the contrary, chickens from a selected line (Cobb 500) with low basal concentrations of corticosterone showed a marked response [169].

Finally, experimental evidence in poultry shows that genetic selection for the intensity of the adrenocortical axis stress response has a complex influence on immune responses and resistance to diseases. For instance, chickens from a line selected for high levels of plasma corticosterone when housed in an environment facilitating considerable social interaction were more resistant to parasitic infestation by *Eimeria necatrix* than those from a line selected for low levels of plasma corticosterone housed in an environment that minimized social interaction [170]. Recently, Minozzi and colleagues showed that genetic selection of Leghorn chickens for different immune traits did not modify corticosterone response to stress or to ACTH, but within lines, several endocrine traits correlated with the level of immune parameters. It is worth noting, for instance, that in the line selected for high antibody response to Newcastle disease virus, vaccine basal corticosterone concentrations were negatively correlated to phagocytic activity measured by carbon clearance, but stress corticosterone response was positively correlated with the antibody response [171]. These differences reflect the complex effects of corticosteroid hormones on the immune system and inflammatory processes [29,30].

7. Adrenocortical axis and selection for robustness

As explained earlier, the concept of robustness integrates diverse components that frequently appear to be
contradictory. Indeed, local breeds of minimally selected animals are usually well adapted to their (eventually harsh) environment, but their absolute production level is frequently low compared with selected genotypes. Conversely, genetically selected, highly productive stocks frequently show signs of reduced robustness [4,172–175]. Reduced robustness may be associated with increased pain and reduced animal welfare, caused, for example, by increased lameness and susceptibility to other diseases, reduced survival of newborns, and lower functional longevity. The trade-off between productivity and robustness is predicted by the resource allocation theory [176,177]: the energetic resources of an individual are limited and their allocation across metabolic functions is optimized toward the best adaptation of the individual to its environment (ie fitness). Genetic selection for production traits logically redirects resources toward these production traits, at the expense of other traits (such as functional traits). When resources are not sufficient to support full expression of the production potential, the interaction between the selected genotype and a restrictive environment may reduce the resilience of the animal. Genetic selection for robust animals must balance these different components. It must be noted here that the genetic gain from intense selection on production traits is only partly maintained at the level of commercial production because of the suboptimal level of environmental conditions [3], leaving some space for a small reduction in absolute production potential as long as a significant gain is obtained in resilience to environmental negative factors.

Several breeding strategies can be implemented to increase robustness [2]. First, genetic improvement in functional traits, such as leg soundness, mortality rates at various stages of the animal’s life, and functional longevity, is possible when these traits are properly included in breeding goals and selection criteria. This goal is already implemented in existing breeding programs (eg [178]). Although valuable, this approach requires extensive and time-consuming phenotyping of the animals for multiple and frequently difficult-to-measure traits. Current efforts toward the discovery of molecular bases for genetic variation of these complex traits will likely nominate DNA polymorphisms to be used for genomic selection. Second, the global sensitivity to the environment can also be measured by techniques such as the reaction norm analysis that compares animals with identical genotypes across different environments. This is a difficult endeavor and heritability of the character is often low [179]. Sensitivity to the environment may also contribute to the environmental variance of a trait, which has been shown to be under genetic control. The reduction of trait variance by genetic selection is also known as canalizing selection, or canalization [180]. To date, this approach has not yielded practical solutions for genetic selection. The third strategy, which is the topic of the present review, focuses on the molecular genetics of neuroendocrine stress responses, more specifically the adrenocortical axis.

The adrenocortical axis has a central role in the regulation of the genetic trade-off mechanisms between production and adaptation. Genetic selection for domesticated phenotypes usually reduces the activity of the adrenocortical axis, as shown, for instance, in pigs [90], chickens [169], sheep [181–184], guinea pigs [185], silver foxes [186], or rats [187]. This downregulation of stress responses may be considered an adaptation to living in a biologically safe, predator-free environment that is favorable to improve production traits mostly based on protein anabolism (the Darwinian concept of stress [188]). However, intense selection for production traits has further driven down the activity of the adrenocortical axis, as we showed in the LW breed of pigs by comparing progeny from sires born in 1977 (frozen semen) vs 1998–2000 [189]. This trend results from the above-mentioned negative effect of cortisol on production traits and carcass composition, so that adrenocortical axis activity was counterselected in the selection process. As a consequence, this decrease in adrenocortical axis activity may explain part of the compromised robustness that coincides with overfocused genetic improvement of production traits in farm animals (Fig. 2).

We have shown that genetic variation can be found at every step of the adrenocortical axis function [133]. Genetic polymorphisms could therefore be used in marker-assisted or genomic selection to increase robustness by improving functional traits without compromising the high level of production. This objective does not appear to be out of reach. Indeed, the functional variability of the adrenocortical axis is usually large, even in genetically homogeneous populations. A 30-fold range of urine cortisol concentrations was found in each of 5 pig lines, much more than the variation of production traits (Fig. 3) [92]. In the above-mentioned study of genetic trends of stress-responsive systems in the French LW, we found a −0.27 correlation between cortisol levels (in urine collected from the bladder after slaughter) and carcass lean content, so that only $(0.27)^2 = 7.3\%$ of the variance of leanness is related to differences in cortisol production [189]. It is
therefore possible to envisage the selection for a stronger adrenocortical axis to improve robustness without compromising the genetic gain on production traits [178]. On the other hand, even if the maximal genetic potential of the animals is slightly reduced, the gain can be obtained via a better realization of this potential under commercial conditions (Fig. 2).

Furthermore, we should be able to finely tune this genetic approach by sorting the polymorphisms according to their functional outcome. Considering the complexity of the physiological effects of glucocorticoid hormones that up- or downregulate hundreds of genes, it would not be surprising that specific gene polymorphisms would more specifically improve functional traits (and should therefore be positively selected), whereas others would be more deleterious on production traits (and should therefore be negatively selected).

However, we have no overall view integrating these diverse sources of genetic variability within the system and their functional consequences on the different components of robustness. System genetics and modeling approach should deliver the necessary knowledge to solve this challenge of selecting more robust animals in the context of strong economic pressure.

8. Conclusion

The adrenocortical axis plays a central role in the physiology of food-producing animals as a trade-off mechanism between production and functional traits that are directly related to adaptation of the animal to environmental constraints. These functional traits have
suffered from several decades of intense selection focused on production traits and at the same time have gained importance in breeding objectives because of new challenges such as the environmental impact of farm animal production, animal welfare, and the changes in rural lifestyle resulting in a reduced workforce. Increasing animal robustness will be obtained by improving the functional traits and at the same time maintaining as much of the high production potential as possible. Strong genetic factors influence adrenocortical axis activity and function, so it is conceivable to increase robustness by selecting animals with higher adrenocortical axis activity. A more detailed analysis of the molecular bases of genetic variability should allow the development of a fine-tuned strategy for genomic selection of the alleles maximizing the gain on functional traits while minimizing the impact on production.

**References**


[175] Veerkamp RF, Windig JJ, Calus MPL, Ouweltjes W, de Haas Y, Beerd A. Selection for high production in dairy cattle, In:


