# Robustness and adaptability in relation to corticotrope axis functionality in poultry species

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Robustness has been defined as the ability of the animals to maintain homeostasis allowing them to combine a high production potential and function well in the wide range of environments and production systems they are submitted to worldwide, or so called adaptability (Knap 2005). An opposite relationship between productivity and robustness is predicted by the 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, so called fitness, of the individual to its environment. Genetic selection for production traits logically redirects resources towards those production traits, at the expense of other traits. When resources are not sufficient to support full expression of the production potential this becomes problematic, and leads to genotype x environment interaction. Poultry species are subjected to relatively intensive genetic selection for various production traits and this selection is thought to affect their ability to cope with common production stressors related to production systems. In the meantime, animal welfare has been an issue of increasing concern in recent decades and resulted in the adoption of new regulation in Europe. As a consequence, the diversity and complexity of rearing systems have increased greatly and result in a wider range of potentially stressful situations.

Stress can cause disruption of homeostasis and induce a series of adaptative responses by several systems among which are the autonomic nervous system (ANS) and the hypothalamic-pituitary-adrenal (HPA) axis (Mormede et al., 2007). These neuroendocrine systems are primarily involved in homeostatic metabolic processes and, particularly, in the regulation of energy fluxes. The energy supply is used by the defence mechanisms to cope with the stressor. Consequently, any change in the HPA axis, beside a competition for energetic resources, can impact performance, disease resistance or carcass quality of the animal and ultimately the profitability of farmers. As a result, corticotropic axis functionality plays a key role when it refers to robustness and adaptability.

The HPA axis mainly acts on the long term stress response of the animal, allowing the organism to deal with stress without exhausting internal resources (Mormede et al., 2007). The hypothalamus synthesizes neuropeptides, mainly vasopressin (AVP) and corticotrophin releasing hormone (CRH), which trigger the release of ACTH from the pituitary gland (Hazard et al., 2007). ACTH acts on the adrenal glands stimulating the synthesis in and the release out into the blood circulation of glucocorticoid hormones, mainly corticosterone in birds (Mormède et al., 2007). These hormones act on a wide range of cells and tissues via the glucocorticoid and mineralocorticoid nuclear receptors. Corticosterone has complex effects on production traits. It negatively affects numerous metabolic pathways, the immune system, inflammatory processes, growth rate and brain functions, to mention the most important. By contrast, corticosterone has positive effects on robustness traits, at least indirectly, by contributing to homeostasis maintenance.

**Table 1.** Range in corticosterone concentrations (ng/ml plasma) for basal levels and following a physical (constraint in a crush cage, box or placement in a net for 10 to 15min) or a pharmacological challenge with an ACTH agonist (1-24 ACTH, Immediat Synacthen, 5 to 10 µg - kg B.W. i.m.), in different species of domestic birds assessed by specific radioimmunoassay (Etches, 1976).

| Species and strain | Sexe and physiological stage | Corticosterone concentration range (ng/ ml plasma) | | |
|---|---|---|---|---|
| | | Basal or initial levels | 10-15min after restraint in a crush cage, a box or a net | 10-15 min after i.m. injection of 1-24 ACTH (5-10µg /kg B.W.) |
| Turkey | Immature male | 1 - 5 | 20 - 30 (cage) | 80 - 100 |
| | Immature female | 5 - 10 | 50 - 80 (cage) | 100 - 150 |
| Pekin duck | Immature male | 10 - 20 | 50 - 80 (net) | 150 - 200 |
| Muscovy duck | Immature male | 10 - 20 | 20 - 30 (net) | 80 - 120 |
| Mule duck | Immature male | 10 - 20 | 50 - 80 (net) | 80 - 150 |
| Geese | Immature male | 10 - 20 | 100 - 250 (net) | 150 - 350 |
| Quails | Short tonic immobility | 5 - 10 | 30 - 50 (box) | 40 - 80 |
| | Long tonic immobility | 2 - 7 | 15 - 30 (box) | 30 - 50 |
| Gallus: low growth rate broiler strain | Immature female | 2 - 10 | 2 - 30 (cage) | 20 - 50 |
| | Mature female | 1 - 10 | 5 - 30 (cage) | 50 - 200 |
| | Immature male | 2 - 10 | 10 - 20 (cage) | 20 - 50 |
| | Mature male | 1 - 15 | 10 - 50 (cage) | 50 - 200 |
| Gallus: laying strain | Mature female | 1 - 5 | 5 - 15 (cage) | 20 - 30 |

There exists a huge variability – across species, breeds, and individuals – in the basic functioning of adaptation mechanisms and in their corticosterone responses to environmental or pharmacological challenges. This variability can have multiple origins, such as genetic, developmental, and experiential ones. Birds have a relatively low sensitivity to social stress in terms of adrenal responses (Hazard *et al*., 2008a,b). On the other hand, various physical stressors will generally elicit adrenal responses. However, a large variability not only between species but also within species in term of adrenal responsiveness to comparable physical and pharmacological challenges, as well as in the maximal adrenal responsiveness, is observed (Table 1, based published and unpublished data from our lab). It is of interest to notice that the response is elicited in a few minutes and that the peak is occurring after 2 to 20 min depending upon the intensity of the physical stress or pharmacological challenge. Prolonged

physical stress or high doses of ACTH only marginally increase peak levels but increase the duration of the corticosterone response.

Concerns have been expressed, in recent years, regarding the consequence of selective programs for higher body weights or other production traits in poultry species in the overall capacity to cope with stressful environments. In order to investigate this claim, we have extensively assessed adrenocorticotrope axis responsiveness in domestic poultry by comparing different genetic breeds of various species (Gallus, turkey, quails, Pekin and Muscovy ducks).

A first study consisted in comparing male turkeys from two commercial genotypes and their intercross differing in their growth rate and final body weight (Guémené et al., 2001). It was concluded that, under our experimental set up, we had no indication that current selective programs for body weight did affect HPA axis responsiveness and their overall capacity to cope with stressful environments. HPA axis functionality was assessed in different lines of chickens derived from five different commercial and experimental genetic selection programs. The lines examined originated from two divergent and a unilateral experimental selection programs aiming at modifying feed efficiency (increasing or lowering the residual feed intake - R+ and R- lines (Bordas *et al*., 1992)), modifying abdominal fat content (lean and fat lines (Leclercq, 1988, Leclercq *et al*., 1989)) and improving meat quality and growth rate and reducing abdominal fat content (Lines Y33 and Y11 (unselected control) (Ricard and Touraille, 1988, Le Bihan-Duval *et al*., 1998). Lastly, two lines selected for fast or slow (Lines F and S) growth rates, commercially referenced as standard and "Label" broilers for marketing, respectively, were included in the resarch (Debut et al., 2005). Results from this survey indicated that birds originating from different genetic selection programs differed in their adrenal responsiveness in a manner coherent with the broadly expected physiological effect of corticosteroids toward the different selected parameters. These different genetic lines thus appeared to be valuable biological models for further investigations aimed at identifying genes responsible for variation in stress responsiveness and at establishing relationships between these genes and product quality and animal welfare issues.

The effect of genetic selection on immune responses was assessed by measuring corticosterone concentrations before and after physical stress as well as ACTH injection in three White Leghorn chickens lines previously selected for three different *in-vivo* immune responses: high antibody response to Newcastle disease virus vaccine 3 wk after vaccination (ND3), high cell-mediated immune response (response to phytohemagglutinin, PHA) and high phagocytic activity measured as carbon clearance (CC), and in a random bred control line (Pinard van der Laan, 2002). Corticosterone responses did not differ between lines, indicating that selection for immune responses of different nature had little effect on response to stress. However, it is worth noting that within line, significant negative correlations were found between cell mediated immunity and corticosterone plasma concentrations in three of the four lines, and that corticosterone levels were positively correlated to ND3 and negatively correlated to CC in line ND3-L selected for high antibody titers (Minozzi *et al*., 2008).

Two independent studies were undertaken to further investigate the possible functional relationship between feather pecking and adrenocorticotropic axis reactivity in slow growth broilers (Guémené *et al*., 2002) and egg laying hens (Kjaer *et al*., 2009). Selection was performed using an indirect electronic recording method, the "peckometer", and a direct method by counting feather pecking bouts. Interestingly, in both studies, the highest adrenal reactivity was associated with the highest levels of feather pecking.

Further investigation regarding HPA axis functionality was performed using experimental lines of quail (Mills and Faure, 1991) and Pekin ducks (Arnaud *et al*, 2008) respectively divergently selected on tonic immobility duration and corticosterone responses to physical stress. Surprisingly, quail from the short tonic immobility line (STI), considered to be less fearful, exhibited higher corticosterone responses to restraint than the long tonic immobility line (LTI) (Hazard et al., 2008a,b). Further investigations of their respective HPA axis functionality indicated that this large difference in corticosterone response was unlikely to be due to difference in adrenal and pituitary functions. Interestingly enough, behavioural observations indicated that STI quail exhibited higher levels of activity corresponding to the fight-flight strategy during the restraint test than LTI ones. It is likely that this difference would be at least partially the cause of the observed differences in corticosterone response to severe stressful treatment. Similarly, in the divergent selection on corticosterone responses to shackling conducted on Pekin ducks; birds from the HCort line (High corticosterone response to restraint) expressed higher levels of physical activity in various stressful situations and behavioural tests, including the selection test (Arnaud et al., 2008). As in other birds species such as (drenal response to ACTH in chickens (Edens and Siegel, 1975); social stress: (Gross and Siegel, 1985); cold stress in turkeys (Brown and Nestor, 1973); immobilization stress in Japanese quail (Satterlee and Johnson, 1988), the divergent genetic selection for HPA axis response has been successful with a very important response to selection, with realized heritability of around 0.4. Interestingly, similar differences in corticosterone responses were observed in the mule ducks resulting from the intercross with a Muscovy duck. Interestingly enough, further studies indicated that this selection program induced positive effects in favour of the low responders on reproductive function in ducks (Basso et al., 2010). Unexpectedly, positive effects in favour of STI quail line, being the highest responders, were observed on meat quality parameters (pHu, drip losses) (Rémignon *et al*., 1998).

Current efforts towards the discovery of molecular bases for genetic variation of these complex traits will possibly deliver DNA polymorphisms to be used for genomic selection. In the aim to identify genes involved in the regulation of adrenal activity following ACTH stimulation we examine adrenal genes differentially expressed in individuals with high and low plasma corticosterone response following ACTH treatment (Bureau *et al*., 2009). ACTH treatment affected the expression of 134 genes among which several transcripts assigned to genes involved in the adrenal ACTH signalling pathway and steroidogenic enzymes were identified as differentially expressed. Some genes (MC2R, CREM, Cry, Bmal1, Sqle, Prax1 and StAR) were differentially expressed between higher and lower adrenal responders to ACTH treatment. The major mechanism explaining differences between animals with high or low response to ACTH treatments may be in the transport of cholesterol into the mitochondria for steroidogenesis with PRAX-1 being putatively a good candidate to target the sensitivity of animals to stress.

In conclusion, available results illustrate that paying more attention to HPA axis functionality in commercial breeding programs might be one perspective to improve general robustness and resilience to environmental diversity. However, regulatory mechanisms may differ between species and it is worth remembering that major differences in HPA functionality appeared to involve mainly central mechanisms in poultry species, while it mainly involves differences at the adrenal level in other domestic species (Mormede *et al*., 2007). In any case, genomics technologies can be used to identify genetic markers for specific traits and should become increasingly powerful as the genomes of poultry species become more fully understood in terms of both structure and function.

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