Innovative phenotyping strategies exploiting immune response to heat stress conditions

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Abstract

Evaluating the heat stress status of an animal is a challenge. Indeed, currently, the heat stress status is determined on the basis of a significative change of a response variable known to be correlated with a temperature-humidity related variable. These response variables are, most often, milk yield or similar traits. However, it is known that heat stress has a negative effect on the immune system, but this information is still very little used in the context of heat stress phenotyping. Indeed, only a few studies highlight immune genes involved in heat stress. In addition, these studies provide very little information on the molecular mechanisms that link these genes to the observed phenotype. Immune response based deep-phenotyping strategy would provide a more comprehensive understanding of heat stress and could also serve as a basis for identifying heat stress indicators through genomics.

Introduction

Currently, phenotypes used for genetic improvement remain often defined in a very straightforward fashion, e.g., to improve production traits, these traits are recorded. Recently, interest for breeding of resilient and healthy livestock has strongly increased. Indeed, the idea has emerged that by selecting animals based on immune traits, it would be possible to reduce the occurrence of diseases and thus also increase profitability of the farm (Mallard *et al.*, 2015; Thompson-Crispi *et al.*, 2014). In addition, it is shown that, generally, healthier animals show better growth and better reproductive abilities (Mallard *et al.*, 2015). However, in this condition direct phenotyping quickly reaches its limits as disease traits are manifestations of the reaction of the animal to underlying mostly unknown solicitations. Moreover, direct recording of this data is notoriously unreliable and biased as only recognized positive cases are recorded and the quantity of false-negative cases is expected to be high.

Recently, some studies have developed technologies allowing to predict cell mediated response and humoral immune response of an animal. Even if this type of studies is still rather rare, the heritability for the immune response was estimated to be between 0.25 and 0.35 which was clearly higher than traditional disease traits and at a level where important responses to selection can be expected (Mallard *et al.*, 2015; Thompson-Crispi *et al.*, 2012). However, these technologies can hardly be used to determine the immune status of an animal in real time.

Heat stress is known to have a negative effect on the immune system (Bagath *et al.*, 2019; Dahl *et al.*, 2020). However, this knowledge is not currently used as an indicator of heat stress. The most used approach is to relate a response variable, often milk yield or similar traits, to a temperature-humidity related variable. The status of heat stress is then associated with a significant change in this response variable, i.e., decrease for production related traits, or an increase for traits like somatic cell count. The temperature at which this decrease, or increase, begins, and its slope vary from animal to animal. In current implementations, reaction norm models are used with, in general, a heat stress threshold set and only the slope being assessed for each animal. The already implemented genomic breeding value estimation for thermotolerance is based on this type of phenotypes (Nguyen *et al.,* 2017).

Only a few studies highlight the genes and pathways that could be involved in thermotolerance (Garner *et al*., 2020; Halli *et al*., 2021; Otto *et al*., 2019; Sigdel *et al*., 2019; Srikanth *et al*.,

2017). And, even if these studies provide a lot of information, few of those are looking at the molecular mechanisms which lead to the observed phenotype, and therefore could serve as basis for the definition of indicators (i.e., biomarkers) of heat stress. For this reason, the objective of the present paper was to extend the work of Otto *et al.* (2019) toward innovative potential deep-phenotyping strategies exploiting immune response in the context of heat stress.

Innovative deep phenotyping strategies for biomarkers of heat stress

From heat stress to cortisol secretion. In the event of heat stress, the paraventricular nucleus of the hypothalamus will produce the corticotropin-releasing hormone (CRH) (Mishra, 2021). This hormone will bind to the CRH receptors expressed on the surface of the anterior pituitary cells, which triggers exocytosis of the adrenocorticotrophic hormone (ACTH) stored in secretory vesicles (Rehfeld and Bundgaard, 2010). ACTH enters the adrenal gland through the bloodstream and stimulates cortisol synthesis (Feher, 2012). Once released into the bloodstream, cortisol is able to passively cross the plasma membrane of target cells. Its receptor, the glucocorticoid receptor (GR) is located in the cytoplasm where it is sequestered by several proteins including HSP90. The binding of cortisol to the GR causes its dissociation from this protein complex and its translocation to the nucleus. The GR can then bind to the glucocorticoid response element (GRE) regions of the promoter of the target genes. The GR-cortisol complex is also able to modulate the function of other transcription factors by interacting directly with them (Nicolaides *et al.*, 2020).

From cortisol secretion to greater occurrence of diseases. Among the target cells of cortisol are immune cells including macrophages. These phagocytic cells exhibit a wide variety of phenotypes *in vivo*. This panel of phenotypes has two extremes, M1 and M2. The former is associated with the initiation of inflammation and the eradication of pathogens and the latter with the inhibition of inflammation and the prevention of damages (Maciuszek *et al.*, 2020). The polarization of macrophages in M1 is initiated, among other, by lipopolysaccharide (LPS) of bacterial origin. This is because LPS binds to the CD14 receptor expressed on the surface of macrophages. The complex formed then interacts with Toll like receptor 4 (TLR4) to trigger a signaling cascade dependent on p50/p65 nuclear factor kappa B (NFκB). This leads to the activation of gene transcription encoding proteins involved in the antimicrobial activity of macrophages such as the inducible nitric oxide synthase (iNOS) as well as pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α) (Tugal *et al.*, 2013).

However, after binding to its receptor, cortisol is capable of sequestering the p65 subunit of NFκB and inducing the expression of IκB, an inactivator of NFκB (Feher, 2012; Nicolaides *et al.*, 2020). Moreover, the glucocorticoid-induced leucine zipper (GILZ) whose expression is controlled by the glucocorticoid receptor, is also capable of inhibiting NFκB (Cannarile *et al*., 2019). In this way, heat stress appears to prevent M1 polarization and thus promote M2 polarization which is associated with reduced antimicrobial activity.

In addition, NFκB was also shown to be involved in the activation of T and B lymphocytes (Haselager *et al.,* 2020; Krawczyk and Penninger, 2014). The type of cytokines produced by dendritic cells are also modulated by the activation of NFκB (Walsh and Mills, 2013). All these data suggest that heat stress through, among others, the production of cortisol and inactivation of NFκB, may lead to a weaker immune response against pathogens by reducing the ability of immune cells to react to infection.

From heat stress to pro-inflammatory response. However, cortisol levels vary greatly with the time of exposure to heat stress (Mishra, 2021). Chronic heat stress is even associated with increased inflammation, possibly as a result of decreased cortisol levels in affected animals (Most and Yates, 2021). The increased inflammation in the absence of cortisol could be due to

cell death induced by increased body temperature as well as the overexpression of heat shock proteins (HSPs). Indeed, in cells such as macrophages which express TLR4 and CD14, HSPs have been shown to be able to generate a pro-inflammatory response like other molecules released during cell damage. In this way, this pro-inflammatory response is possible via the activation of NFκB (Asea and Kaur, 2017).

From pro-inflammatory response to greater occurrence of diseases. It has been shown that with greater inflammation the symptoms of a disease can also be greater (Hunter, 2012). In this way, if the heat stress induces an increase in inflammation, it would not lead to an increase in the occurrences of infectious disease but could cause more severe symptoms which would cause more animals to pass from subclinical to clinical level.

From deep phenotyping to identification of heat stress markers. On this basis, it is expected that NFκB activity and therefore the expression of its target genes will be different between stressed and unstressed animals. This difference could then be exploited as a marker of heat stress.

From biomarkers identification to genetic progress for heat stress resilience. Translating heat stress markers into genetic progress requires several conditions. First, deep phenotypes must be acquired in sufficient quantities and on relevant animals to serve as a basis for heat stress indicators. Indeed, it is necessary to confirm the heat stress status of the animals to find reliable markers of heat stress based on these animals. Based on Otto *et al.* (2019), these markers could be target genes of NFκB. Of course, as already mentioned, significant differences must be obtained between the groups formed for the markers tested. Ideally, these markers should also be validated to determine whether they are capable, on their own, of discriminating between stressed and non-stressed individuals and be considered as biomarkers (Ou *et al*., 2021). In the case of selection purpose, once biomarkers obtained, it is still required to link variations of these indicators to genetic variation. Moreover, this genetic variation has to be additive, therefore heritable and can be improved by selection. Many strategies are possible to implement biomarkers for selection. These can be very traditional approaches where immune response-based biomarkers are added in multi-trait settings. Indeed, these precise biomarkers not available in large quantities could be added with less precise traits but available in larger quantities. Moreover, very innovative approaches using novel methods as machine learning and these biomarkers as input could be used to generate predictions of heat stress resilience.

Conclusion

We followed the direction shown by Otto *et al.* (2019), who have identified among others, that NFκB as a transcription factor is associated with heat stress. Extending on this study, we showed how it is possible to go further and directly link NFκB to heat stress and to the higher occurrence of infectious diseases which results from it. This is far from the only opportunity to perform deep innovative phenotyping exploiting immune response to heat stress conditions. Indeed, the information given above is, of course, far from exhaustive, but it does allow to link genomic studies to molecular mechanisms, and therefore also to potential molecular phenotypes (i.e., biomarkers) associated with heat stress. Linking studies in this systematic way should allow a better understanding of the mechanisms involved at the systemic level but also facilitate the identification of biomarkers. Heritable variation of these identified biomarkers could subsequently be used in animal breeding to generate genetic progress for heat stress resilience.

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