Advances in Animal Biosciences, (2016), **7:2**, pp 218–222 © The Animal Consortium 2016
doi:10.1017/S2040470016000169

advances in animal biosciences

The challenge of genetic selection for heat tolerance: the dairy cattle example

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Exposure of livestock to high heat loads negatively affects animal's productivity. Genetic selection for heat tolerance using quantitative approaches has been developed by making use of the milk recording information merged with the meteorological information in the test date. The main conclusions of the studies following this approach have been that there is genetic variability in the response to heat stress (HS), that a genotype by environment interaction between thermal comfort and HS conditions exists and that there is a genetic antagonism between HS tolerance and high milk production. This approach has the advantage of adding no extra costs to the existing milk recording schemes, but it has some disadvantages. Current milk recording information does not seem to fully capture the productive response to high heat loads. Moreover, the antagonism between heat tolerance and high milk production may offset the benefits of selection for this trait if heat-tolerant animals are less productive. The use of new tools from phenomics, genomics and transcriptomics can help in achieving an accurate selection of heat-tolerant animals without damaging progress in milk production. New phenotypes for this selection are measure of body temperature and respiration rates together with measures of indicators of heat tolerance in milk, through the use of mid IR spectroscopy. The use of commercial DNA chips to perform genome wide association studies or comparison of whole genome DNA sequence of animals of heat adapted and temperate climate breeds could also provide useful genomic information. Finally, RNA sequencing together with the recent discovery of the possibility to use the milk transcriptome instead of tissue biopsies could help to discover differentially expressed genes under HS and thermoneutrality. The existence of powerful tools to achieve better phenotypes to identify tolerant v. *susceptible animals and to gain insights about the genetic mechanisms underlying HS response to be able to disentangle the genetic puzzle of heat tolerance may make possible the selection of heat-tolerant and still productive animals.*

Keywords: heat tolerance, genetic improvement, dairy cattle

Introduction

The physiological stress produced by exposure to high thermal loads has negative effects on productive and reproductive performance of livestock, as well as on morbidity and mortality rates (see, e.g. West, 2003; Hansen, 2009). Animals selected for dairy purposes suffer those negative effects to a larger extent than animals selected for growth rates because of the high metabolic heat production associated with milk production (Baumgard and Rhoads, 2012). Moreover, the expected consequences of climate change are likely to aggravate the heat stress (HS) problem in dairy farms in the future.

The negative impact of HS can be mitigated in several ways, through improvement in the barn conditioning, selection of heat-tolerant animals or crossing with adapted breeds and by changes in management and nutrition. However, none of those three ways alone or in combination will eliminate completely the negative effects of high heat loads. St-Pierre *et al*. (2003) find yearly estimated economic losses associated with HS ranging between 100 and 167 $/cow under optimal barn conditions or with natural ventilation alone.

Genetic selection of heat-tolerant animals provides a cumulative and permanent solution at a relatively low cost. West (2003) reviews the effects of HS on dairy cattle and concludes that selection of heat-tolerant animals is possible and it should be considered to prevent the increasing susceptibility to high heat loads associated with the intensive selection for production. However, selection of heat-tolerant animals is challenging because of the complexity of the mechanisms that determine the HS response and the antagonistic relationship with high milk production.

In this study, previous results about the physiological response to HS and associated genes are reviewed and possible approaches to select heat-tolerant animals without damaging productive levels by making use of available phenomic, genomic and transcriptomic tools are discussed.

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https://doi.org/10.1017/S2040470016000169 Published online by Cambridge University Press

Measurement of thermal load

An important measure in the analysis of HS effects is the amount of thermal load to which the animal is exposed. Thermal load is determined by several meteorological variables such as temperature, air humidity, wind speed and direction, or solar radiation. Various indices combining ambient temperature with other meteorological variables have been developed (see Hahn *et al*., 2009 for a review). An index value represents the effect produced by the heat exchange process, which can alter the biological response that might be associated with changes in temperature alone (Hahn *et al*., 2009). The index that has become the standard to characterise the thermal load supported by the animals in many livestock species is the so called THI, a temperature and humidity index adapted for livestock from an index derived initially for humans (Thom, 1959):

$$\mathrm{THI} = (1.8 \times T + 32) - (0.55 - 0.0055 \times \mathrm{RH}) \times (1.8 \times T - 26) \quad (1)$$

where T is the dry bulb temperature in ºC and RH the relative humidity.

Although other indices including wind speed and solar radiation have been developed more recently (black globe-humidity index (BGTHI) by Buffington *et al*., 1981; equivalent temperature index (ETI) by Baeta *et al*., 1987; Environmental Stress Index (ESI) by Moran *et al*., 2001, 2003; heat load index (HLI) by Gaughan *et al*., 2008), the THI index described before continues to be the most used index in cattle. However, the THI index may not be optimal for all breeds, climates and production systems (see, e.g. Bohmanova *et al*., 2007; Hammami *et al*., 2013).

Heat stress effects

High thermal loads affect physiological and metabolic processes resulting in decreased feed intake, changes in the metabolic source of energy (glucose is used as main energy source as opposed to fat, which is the main source of energy in pair fed animals under thermoneutrality (see, e.g. Baumgard and Rhoads, 2012)), in the endocrine response and in an activation of the mechanisms of heat dissipation mainly through sweating and panting. Negative effects on milk, fertility and immune system (West, 2003; Hansen, 2009) have also been found as a consequence of all those changes.

The effect of HS on milk production in specialised dairy breeds has been profusely studied. A large part of the studies aim at determining the thermo-tolerance threshold and the subsequent slope of decay in production. Although a value of 72 for the THI value has been widely accepted as the HS threshold for dairy cattle (see, e.g. review by Hahn *et al*., 2009), estimates of the thermo-tolerance threshold range from values around 60 in Holstein populations in Central Europe (Hammami *et al*., 2013; Lambertz *et al*., 2014) and values above 70 for Holsteins in warm areas of the USA (Ravagnolo *et al*., 2000) and the Mediterranean basin (Barash *et al*. 2001; Bernabucci *et al*., 2014; Carabaño *et al*., 2014). These differences could be explained by the climatic differences between regions. In the more temperate areas of Central Europe with sporadic heat waves, an acute HS response is expected, as opposed to regions with steadily hot temperatures along the summer where animals are expected to develop acclimatisation mechanisms to deal with HS. Apart from climatic characteristics, differences in thermo-tolerance thresholds have been observed for milk yield *v*. milk quality (fat and protein content), with lower thresholds found for milk components (Bernabucci *et al*., 2014; Carabaño *et al*., 2014).

Estimates of the slopes of production decay also vary widely across studies, although no clear pattern depending on regional or production system characteristics has been observed. The range of estimated slopes of decay extends from values close to 1 kg milk/day and THI degree above the threshold in Bernabucci *et al*. (2014) for the Italian Holstein to values around 100 g/day and THI degree in the Holstein populations of Luxembourg (Hammami *et al*., 2013), Germany (Lambertz *et al*., 2014) and Spain (Carabaño *et al*., 2014). Decays for fat and protein production range from 30 to 40 g/day and THI degree in Bernabucci *et al*. (2014) and 3 to 10 g/day and THI degree in the other studies mentioned before. Differences in statistical modelling and data editing, apart from climate and production systems could explain the differences in estimated loss of production associated with HS. All those studies have used the official milk recording information, which is normally done monthly in commercial farms. However, Freitas *et al*. (2006) found that the use of monthly information only captures a part of the production loss associated with HS.

Evidence of genetic control of heat stress effects in livestock

The complexity of the response to HS is associated with a variety of mechanisms that are genetically regulated (see reviews by Sonna *et al*., 2002; Collier *et al*., 2008). Collier *et al*. (2008) group the genes involved in the bovine HS response in three categories, genes associated with hair and coat characteristics, genes involved in the cellular response and genes acting at the systemic level. In the first category, Olson *et al*. (2003) found a major gene with a dominant type of inheritance determining a type of here, the 'slick hair', which is associated with tolerance to HS (by maintaining lower rectal temperatures). An extensive number of genes have also been found to be associated with HS effects in genomic (Gautier *et al*., 2009; Hayes *et al*., 2009; Chan *et al*. 2010) and transcriptomic (Sørensen *et al*., 2005; Collier *et al*., 2006; Salama *et al*., 2012) analyses in different species. The main group of genes related with response to HS is the group of heat shock transcription factors (HSF) and the associated heat shock protein genes (HSPs). Sonna *et al*. (2002) found at least 50 genes not belonging to the HSF or HSP families showing differential expression under HS conditions. Collier *et al*. (2008) summarising the groups of genes involved in the bovine HS response through changes in expression also mentions genes

https://doi.org/10.1017/S2040470016000169 Published online by Cambridge University Press

involved in increased glucose and amino acid oxidation and reduced fatty acid metabolism, endocrine system activation of the stress response and immune system activation via extracellular secretion of HSP. Apart from the evidences found in transcriptomic analyses, genomic analyses have also pointed at the existence of genes related with hair and skin properties, immune response, nervous system and tick resistance (Gautier *et al*., 2009; Chan *et al*., 2010).

There is therefore a wide evidence of the genetic control of the response to HS, which supports the possibility of effective genetic selection, but also provides evidence of the complexity of the selection strategy. Modification of the mechanisms of tolerance to heat may involve other aspects of the animal productivity, with unwanted consequences for the overall economic value of the animals.

Selection strategies

The use of milk recording plus meteorological information

After the seminal work of Misztal (1999) and Ravagnolo *et al*. (2000), where the use of milk recording information together with data from meteorological stations to obtain individual measures of heat tolerance is shown, there has been a series of studies oriented towards the use of this approach in selection of dairy breeds from a quantitative perspective (Bohmanova *et al*., 2005; Finocchiaro *et al*., 2005; Brügemann *et al*., 2011; Hammami *et al*., 2013 and 2014; Bernabucci *et al*., 2014; Carabaño *et al*., 2014). The main conclusions of these studies can be summarised as (1) there is genetic variability in the response in production to HS; (2) a small to moderate G × E interaction has been found between productive merit under thermal comfort and HS conditions; (3) the G × E interaction found across countries (e.g. Interbull across country evaluations) can be aggravated under HS; (4) there is a genetic antagonism between milk production level and heat tolerance. In summary, because of the existing genetic variability, genetic improvement for heat tolerance could be accomplished under this approach at a low cost, but some drawbacks exist. On one hand, the monthly information obtained from milk recording systems do not seem to capture all the productive response to heat load increase (Misztal *et al*., 2006). On the other hand, the antagonism between heat tolerance merit and milk production merit could (at least partially) cancel the gain from having animals that can stand hot conditions but with a lower merit for production.

The use of adapted breeds

Bos indicus breeds or *Bos taurus* breeds raised during many generations in hot climates show a better body temperature control than *B. taurus* breeds in temperate climates (Hansen, 2004). Hair and coat characteristics that facilitate heat dissipation, lower metabolic rates and a larger cytoprotective capacity observed, for example, in lymphocyte cells, oocytes and first embryo stages in hot climate breeds are mechanisms that favour their heat tolerance (Hansen, 2004). Berman (2011) indicates that the ancestral adaptation of those breeds to survive in environments poor in feed resources during some periods of the year as a consequence of the high temperatures has favoured selection of animals with lower metabolic rates. This would be a positive quality for heat tolerance but, on the other hand, could prevent an increase of the productive levels when food resources improve. This would be a disadvantage of these breeds when an improvement in heat mitigation facilities and an adequate feeding management could be provided.

New tools to select heat-tolerant animals

Despite of the large improvement in the knowledge of physiological, metabolic and cell mechanisms that characterise HS response, there are many unknowns to solve in order to be able to select heat-tolerant animals that are able to maintain productive levels that guarantee the economic sustainability of milk production in warm/hot areas. The availability of *phenomic*, genomic and transcriptomic tools can provide means to move forward in disentangling the genetic mechanisms that determine heat tolerance.

The use of phenotypes that allow measures of tolerance more precisely than decays in production from milk recording is one of the paths to explore. One of those measures could be body temperature, which might be considered as the gold standard indicator of heat tolerance. The use of this trait in selection, despite of showing a reasonable heritability (0.17 in Dikmen *et al*., 2012), has been limited because it is labour intensive and thus expensive to obtain. However, the growing availability of electronic devices to obtain individual information in dairy farming could provide means to obtain cheaper and, hence, large-scale recording of body temperature. Another path to explore is the use of milk medium infrared spectra (MIRs), which can be obtained at a low cost as they are routinely used to determine fat, protein and lactose contents in milk. MIRs together with the measurement of metabolites or compounds in milk that can be HS indicators would be used to calibrate prediction equations which would be subsequently used to obtain measures of HS tolerance at a low cost. The use of MIR as a way of obtaining fine phenotypes in dairy cattle is extending rapidly (Gengler *et al*., 2013) and has been recently explored by Hammami *et al*. (2014) in relation to HS tolerance.

Another powerful tool for the refinement of selection of heat tolerance while maintaining productivity is the use of genome wide SNP markers and DNA sequences. Previous genome wide association studies (GWAS) using available commercial SNP platforms and phenotypes for tolerance obtained from milk production decays under high temperatures have detected genomic signals pointing at only a few number of genes (Hayes *et al*., 2009; Biffani *et al*., 2015). This scarcity might be associated with the use of relatively inaccurate phenotypes to measure heat tolerance. The use of new phenotypes in GWAS studies together with genome-wise comparison of DNA sequences from hot *v.* temperate climate breeds might provide valuable and complementary information from the DNA analyses.

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Transcriptomic analyses have already provided a wide range of genes related with HS response, as previously mentioned. Nearly all those studies have been accomplished making use of expression microarrays or through Real Time PCR analyses for a small number of candidate genes. A more powerful tool in this field is RNA sequencing (RNA-Seq), which is increasingly used in different animal species. Wickramasinghe *et al.* (2014) highlight the advantages of RNA-Seq over microarray technologies as a way of improving the quantification of transcripts, of providing a comprehensive coverage of all expressed sequences in a given tissue and as a way of SNP discovery among other important features. The recent discovery of the possibility of using the milk transcriptome in substitution of the transcriptome from mammary gland tissue (Cánovas *et al.*, 2014) is another way to improve the power of transcriptomics in the discovering of genes and SNPs associated with HS response and HS tolerance.

An important issue related with the implementation of new tools for selection is the need for cross-validating the results obtained under certain conditions (thermal conditions and production systems, breeds) in a different scenario. Therefore, a prediction equation of HS using, for example, MIR has to be tested on independent data to match it in a different environment. Also, the genes detected by GWAS or RNA analyses for breeds raised in temperate areas may not be the same as the ones acting in warm climates even for the same breeds because acute events *v.* chronic high heat load reactions may not be regulated in the same manner.

Conclusions

Evidence of the genetic background underlying heat tolerance and of existing genetic variability permits to foresee an efficient selection for this trait. However, the complexity of the mechanisms underlying the HS response and the genetic antagonism with production make the selection strategy challenging. The existence of powerful tools to achieve better phenotypes to identify tolerant *v.* susceptible animals and to gain insights about the genetic mechanisms underlying HS response to be able to disentangle the genetic puzzle of heat tolerance may make possible the selection of heat-tolerant and still productive animals.

Acknowledgements

The author acknowledges the invitation of ALPA to present the invited contribution and attend the XXIV Congreso de la Asociación latinoamericana de producción animal and XL Congreso de la sociedad Chilena de producción animal and EAAP financing the travel expenses.

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