

A multi-omics approach to selective breeding for heat tolerance in pigs

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Introduction

Global warming is an urgent crisis of the 21st century as the temperature continues to increase across the world. In Europe, the temperature rise is faster compared to the global average, an additional increase of up to 6.2°C is expected over the next century [1]. The crisis associated with the rise in temperature (e.g., summer heatwave) has detrimental effects on livestock production and welfare, whereas livestock production is an important sector of the global economy and is directly important for the livelihood of a vast number of people. The pig sector alone contributes up to 9% of total EU agriculture revenue and 35% of all revenue from meat production in 2018 (source: <https://ec.europa.eu/eurostat/fr/>). With the detrimental effects of climate change, the productivity and welfare of pigs are under threat.

The effects of the climate crisis in monogastric conditions are subtle and have been largely ignored compared to the ruminants. Partly because ruminants are thought to be more vulnerable as they are directly impacted by outdoor climatic conditions whereas pigs' housing is equipped with mechanical ventilation systems. But high ventilation rate does not provide enough to eliminate heat from the animals in the summer months when they might be under intense heat stress [2]. Pigs are particularly sensitive to temperature crises as they cannot adequately dissipate heat from the body above the upper limit of the thermoneutral zone. The physiological effects of heat stress in pigs are numerous and vary depending on the physiological state, such as in lactating sows or finishing pigs. Severe heat stress could lead to the death of pigs [1]. The increased frequency and severity of heatwaves in the past 20 years have resulted in many livestock deaths across the world. For example, there were thousands of temperature-related deaths in pigs across Europe in 2003, resulting from summer heatwaves during which the average temperature was 3.5°C above normal [2]. Lactating sows and their litters are more at risk during severe heat stress. Temperature above 22°C at parturition increases the risk of stillbirth and hypoxia for piglets due to the prolonged duration of farrowing.

Animal characteristics, including their genotype, determine the threshold by which they experience a decrease in their performance. The level of metabolic and physiological adjustments required to achieve acclimatisation is also dependent on the genetic potential of the animals [3]. Evidence has shown variation in heat tolerance levels among pigs, but the current selection tools do not include heat tolerance and adaptability in swine genetic evaluation [3]. Heat stress in pigs results in economic loss as a consequence of poor productivity and impairs the welfare of pigs. Pigs' adaptability during heat stress should get more attention than the current state. Heat tolerance is a heritable trait and thus can be improved, but the genetic mechanism underlying heat tolerance is elusive to researchers.

New genomic technology offers us the chance to elucidate the complex biological mechanism that can reveal novel information for improving livestock that is adaptable to current environmental challenges. A notable approach is to integrate different 'omics' tools to reveal new biomarkers for

selecting heat tolerance in pigs. High throughput and rapid data generation for genomes, transcriptomes, epigenomes, and metabolomes have been made possible with the advent of next-generation sequencing (NGS) technologies. With these high-throughput molecular technologies, the entirety of molecules and their interactions on all levels of biological configuration can be studied. They start from the DNA level (genomics) to its modification prior to transcription (epigenomics), the abundance of expressed transcripts and resulting proteins (transcriptomics and proteomics) and metabolites (metabolomics). The integration of these omics could elucidate gene functions and networks under conditions of physiological and environmental stress [4].

The societal expectation for animal welfare is high, productivity is no longer the only concern of stakeholders and consumers. Heat stress impairs both productivity and welfare, but we could now harness new genomic tools to satisfy animal welfare standards while also maintaining productivity.

Our Solution

We aim to develop a new model for improving animal welfare by integrating multi-omics approaches (genomics, epigenomics, transcriptomics and metabolomics) to find novel quantitative trait loci (QTL) and to enrich selection models with both genetic and non-genetic inheritance factors that contribute to heat tolerance in pigs. Our method would not only provide new knowledge to stakeholders to enhance pig production but also contribute to minimizing the effect of climate change on pigs and hence leads to more ethical and sustainable production.

Our approach

Our approach is to run an extensive molecular comparison between different genetic groups of pigs that are exposed to heat stress and a control group raised in normal temperature. Application of the omics technologies would reveal specific genes and biological pathways that would be included in the prediction model for heat tolerance.

Animal Data

Following ethical approval from the appropriate authorities, experimental animals will be obtained from farm units with a population size of at least 1000. Whole selected Landrace-Yorkshire swine population will be assigned to the following groups with an approximately equal number of males and females: a control group without heat stress (~500); a group exposed to constant heat stress (~500). For maintaining the necessary conditions, the exposed group will be allocated to the environmental control cabins with 30°C temperature 24h daily for 90 days. The control group will be kept in a normal environment in a well-ventilated barn (no artificial temperature and humidity) under natural light/dark cycle. All pigs will be ear-tagged and grown in pens with a feeding station measuring feed intake and weight of the pig.

Sample collection

Tissue biopsy from Longissimus dorsi muscles and plasma samples will be used for all omics investigations. Profiling before and after exposure to stress in the treatment group will also be considered to differentiate the causal relationships or associations. Profiling of the control group will also be done at the same time points (ages) to differentiate the effect of age from the effect of heat stress. Samples will be collected from 20 animals with maximum values for a production trait and 20 animals with minimum values both from control and treatment groups.

Environmental Data

Mean daily temperature (°C) and humidity (%) will be collected from appropriate meteorological stations from which the temperature-humidity index will be calculated for each group using [6].:

$$\text{THI (C)} = (1.8 * T + 32) - [(0.55 - 0.0055 * H) * (1.8 * T - 26)]$$

According to Brown-Brandl et al., the index values can be categorized by normal, alert, danger, and emergency types [7]. The experimental group will be with the assigned parameters in the environmental control cabins.

Pipeline for data analysis

Genetic characteristics, gene expression, DNA methylation, and metabolomic biomarkers will be found using genotyping-by-sequencing (GBS), differential expression analysis, methylated-DNA-immuno-precipitation (MEDip) and metabolomic analysis respectively. After that, the pipeline would be used to evaluate the combinatorial effect of selected biological markers by measuring the prediction accuracy of a model with all omics data included (Fig. 1).

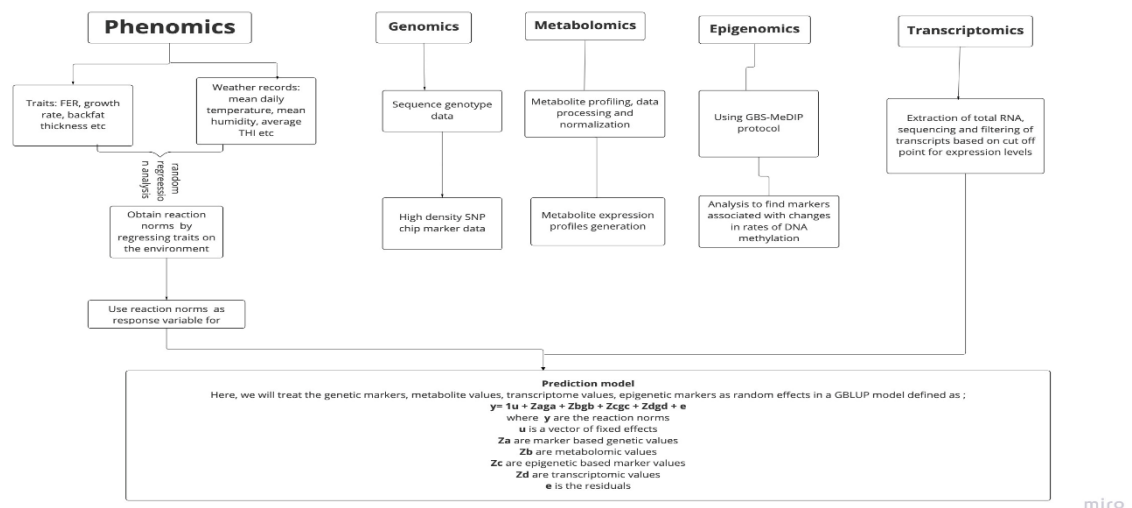


Fig. 1: Pipeline of our study. Genomics by GBS, Metabolomics by GC-MS/NMR. Epigenomics by MEDip, Transcriptomics by RNAseq.

Conclusion

Here, we propose an innovative selection model integrated with multi-omics (genomics, epigenomics, transcriptomics, and metabolomics) data to breed heat-tolerant pigs. With global temperature predicted to increase over the coming years, our goal is to optimize stress resilience in pigs associated with increased temperature while also helping farmers to keep productivity. The tools proposed can be easily implemented as they meet ethical standards and are therefore expected to have wider social acceptability.

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