(antibody titer, cytokine profile), pathogen challenge experiments, and transcriptomic analyses can be combined to comprehensively assess infection rate, recovery time, and survival rate (König and May, 2019).

In animal welfare and adaptability, behavioral performance, stress physiological indicators (such as cortisol level), heat stress scores, feeding patterns, and activity rhythms are monitored to prevent excessive pursuit of productivity at the expense of animal welfare, while meeting the ethical concerns of regulators and the public (König and May, 2019).

4.3 Environmental influences on phenotypic expression and their control

The environment is a key external variable determining the reproducibility and generalizability of phenotypic traits. Genotype × Environment interactions (G×E) can cause the same genetic modification to exhibit heterogeneous performance under different ecological and management systems (Figure 1).

Common influencing pathways include: heat and humidity stress altering energy allocation and feed intake; diet composition regulating lipid metabolism and immunity; and density and light exposure affecting reproductive hormones and behavior. To ensure scientific validity and comparability, randomized block or stratified designs should be adopted during the design stage, and continuous records of temperature, humidity, feed composition, pathogen exposure, and stress levels should be maintained. During statistical analysis, covariate correction and stratified analysis should be introduced to ensure scientific rigor and comparability.

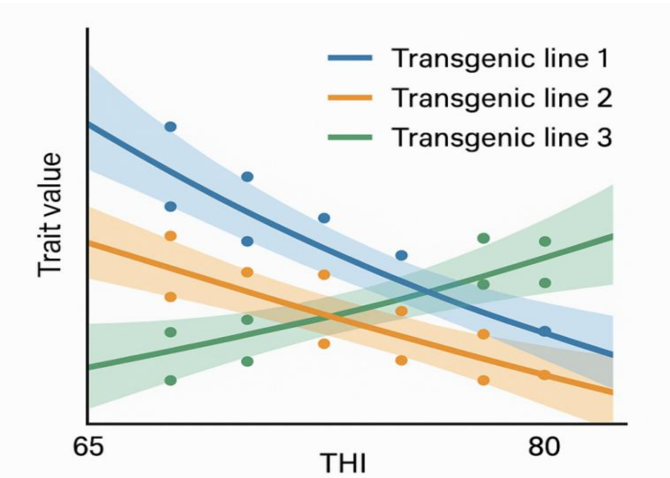

Figure 1 Reaction norms of key traits across THI gradients with random regression fits

5 Phenotypic Evaluation Methods for Transgenic Livestock

5.1 Principles of experimental design: control group setup and sample size

For the setup of control groups, non-transgenic individuals of the same breed, age, and management conditions should be selected as negative controls to minimize interference from genetic background and environmental factors. When studies involve different transgenic constructs, multiple treatment groups should be established, including a vector control, to identify potential nonspecific effects caused by regulatory elements. To further reduce environmental variation, it is recommended to use randomized grouping and block design, maintaining consistency in feeding management, housing density, lighting conditions, and disease prevention measures.

In determining sample size, a balance should be achieved between statistical power and experimental feasibility. Too small a sample size may lead to insufficient statistical power and high variability in results, while excessively large sample sizes may waste resources. For transgenic livestock, especially in large animal experiments, power analysis should be conducted based on the expected effect size, significance level (α), and statistical power ($1-\beta$) to determine a reasonable sample size. For high-dimensional studies such as metabolomics, simulation-based sample optimization methods can be used, combined with hybrid sampling strategies that integrate random sampling and extreme phenotype sampling, thereby improving parameter estimation and model prediction accuracy.