

Research Report

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Progress in Genetic Research on Behavioral Traits in Livestock: Application of Whole Genome Association Analysis

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Abstract This study reviewed the application of genome-wide association analysis (Genome-wide association study, GWAS) and its progress in genetic studies of behavioral traits in domestic animals. By combing the cases of GWAS applications for behavioral traits such as stress response, social behavior, and maternal behavior in different livestock species (such as cows, pigs, sheep, poultry, etc.), this study demonstrates the contribution of GWAS in revealing the genetic basis of these complex traits, and discusses the challenges faced by GWAS research, such as the genetic heterogeneity of the complex traits, the effects of population structure and environmental factors, and the difficulty of detecting rare variants. This study also emphasizes the importance of integrating genetics, epigenetics, and environmental factors in the study of behavioral traits in livestock, and explores the potential of new technologies, such as gene editing and integrated multi-omics analyses, to be applied in future studies. The study concludes with a summary of the practical applications of GWAS for livestock genetic improvement and breed selection, as well as possible improvements in livestock productivity and animal welfare. The analysis of existing studies aims to provide a reference for genetic studies of behavioral traits in livestock and to provide suggestions for future research directions and applications.

Keywords Genome-wide association analysis; Livestock behavioral traits; Genetic improvement; Animal welfare; Integrated multi-omics analysis

In modern animal husbandry, the behavioral traits of livestock are of great importance for improving production efficiency and ensuring animal welfare. These behavioral traits, including stress response, social interaction, feeding behavior, reproductive behavior, etc., not only affect the growth, development and health status of animals, but also directly relate to the economic efficiency and social sustainability of animal husbandry. Exploring the genetic basis of behavioral traits in livestock and how to optimize these traits by means of genetic improvement is of great theoretical significance for improving breeds, increasing productivity, and ensuring animal welfare (Guo et al., 2021).

Genetic studies of behavioral traits are not only important for scientific understanding, but also directly related to the application value in actual production. Genetic improvement can select and breed livestock breeds with stronger adaptability and better production performance, thus improving the productivity and economic efficiency of animal husbandry. At the same time, to improve the welfare state of animals and improve their quality of life is also an important requirement of modern society to the livestock industry, in-depth investigation of the genetic mechanism of livestock behavioral traits is of great practical significance for the realization of the sustainable development of the animal husbandry industry.

Genome-wide association study (GWAS) technology has been widely used as a powerful tool in livestock genetics research in recent years, and GWAS is able to identify genetic variants associated with specific traits on a genome-wide scale, thus revealing the genetic basis of these traits. Compared to traditional genetic research methods, GWAS has higher resolution and stronger discovery capabilities, enabling researchers to effectively uncover key genes and genetic markers associated with complex traits without a priori genetic information. This high-throughput and unbiased analysis has greatly contributed to the advancement of livestock genetics research (Tang et al., 2020). With the help of genome-wide association analysis, researchers are able to more precisely

locate the key gene regions affecting livestock behavioral traits, which is of great significance in resolving the genetic regulatory mechanisms of traits and guiding livestock genetic improvement and management.

This study will explore the current status and prospects of the application of GWAS technology in the genetic study of behavioral traits in livestock by reviewing and analyzing the current research results, aiming to provide valuable information and insights for researchers and production practitioners in the field of animal husbandry. By summarizing the cases of GWAS application in different livestock species, we will evaluate its role and potential in revealing the genetic mechanisms of behavioral traits, promoting livestock genetic improvement and enhancing animal welfare. This study will also discuss the challenges encountered in the application of GWAS for livestock behavioral trait research and the possible directions for future development, which will serve as a reference for subsequent research and practice.

1 Genetic Basis of Behavioral Traits in Domestic Animals

1.1 Definition and classification of behavioral traits

Livestock behavioral traits usually refer to the behavioral patterns exhibited by livestock under specific environmental and management conditions, and these traits are largely influenced by genetic factors. Based on the function and expression of behavior, livestock behavioral traits can be classified into several major categories, including stress response, social behavior, reproductive behavior, feeding behavior, and exploratory behavior.

Stress response: It reflects the physiological and behavioral responses of animals to environmental stress, and has a direct impact on their growth, production performance, and health status.

Social behavior, including social habits, leader follower behavior, competition, and cooperation, affects the harmony and production efficiency within a group.

Reproductive behavior: It involves animal mating, pregnancy, and maternal behavior, and has a significant impact on reproductive efficiency and offspring survival rate.

Feeding behavior: reflects the animal's food selection, feeding amount, and feeding rhythm, which are directly related to growth efficiency and health status.

Exploration behavior: including environmental exploration and curiosity, reflecting the adaptability and welfare status of animals.

1.2 Evidence and research methods for the heritability of behavioral traits

In recent years, an increasing number of studies have revealed through genetic methods that behavioral traits in livestock have a clear genetic basis. For example, through paired reproduction experiments, family analysis, and genetic correlation studies, scientists have found significant genetic variation in behavioral traits among different livestock breeds, and the heritability of these traits (i.e. the proportion of genetic factors in trait variation) fluctuates within a certain range, indicating that genetic factors play an important role in the formation of behavioral phenotypes.

With the development of molecular biology technology, researchers can directly explore the genetic basis of behavioral traits at the DNA level. Genome-Wide Association Analysis (GWAS) and candidate gene method are two commonly used molecular genetics research methods. GWAS allows scientists to screen for genetic markers related to behavioral traits across the entire genome, while candidate gene methods focus on specific genes known or predicted to be associated with specific behavioral traits.

Scientists have identified genetic factors related to behavioral traits in multiple livestock species, providing valuable information for understanding the genetic mechanisms underlying these complex traits. These studies not only enhance the understanding of animal behavioral genetics, but also provide scientific basis for improving livestock breeds, improving production efficiency, and animal welfare. With the continuous progress of molecular genetic technology and the arrival of the big data era, the research on the inheritance of behavioral traits will enter a new stage of development, contributing to the sustainable development of animal husbandry.

1.3 Case study on the genetic mechanism of behavioral traits in livestock

Through genome-wide association analysis (GWAS) and other molecular genetic methods, researchers have identified genes and genetic markers associated with important behavioral traits in multiple livestock species. Here are several representative cases:

Stress response: In pigs, multiple genes related to stress response have been discovered, such as the NR3C1 gene, which is involved in regulating the response to cortisol and affecting the stress sensitivity of pigs (Niu et al., 2023).

Social behavior: In poultry, research has identified genetic markers related to social ranking, which are closely related to animal social interaction and population structure formation.

Reproductive behavior: In cattle, multiple genetic loci related to sexual behavior and reproductive efficiency were identified through GWAS analysis, providing molecular genetic evidence for improving cattle reproductive performance.

These case studies not only reveal the genetic basis of behavioral traits in livestock, but also provide possible pathways for optimizing these traits through genetic selection and improvement. Understanding and utilizing the genetic mechanisms of behavioral traits can help livestock practitioners develop more scientific management and breeding strategies, thereby improving production efficiency and animal welfare.

2 Principles and Methods of Genome-Wide Association Analysis (GWAS)

2.1 Basic principles and technical processes of GWAS

Genome-Wide Association Analysis (GWAS) is a research method used to identify genetic variations associated with specific traits across the entire genome. Since its introduction, GWAS has become an extremely powerful tool in genetic research, especially in revealing the genetic basis of complex traits, including behavioral traits in livestock.

The core principle of GWAS is based on genotyping and phenotype observation of a large number of individuals to discover statistical correlations between genotypes and phenotypes. This process involves the following key steps:

Sample collection and phenotype data recording: Select a sufficient number of research subjects and record their phenotype data in detail, such as the specific manifestations of behavioral traits.

DNA sample extraction and genotyping: DNA is extracted from each individual and high-throughput genotyping technology is used to measure single nucleotide polymorphisms (SNPs) across the entire genome.

Association analysis: Using statistical methods to analyze the correlation between genotype data and phenotype data, in order to identify genetic markers significantly associated with the target trait.

2.2 Sample preparation, genotype analysis, and statistical methods

Sample preparation: Choosing the appropriate sample set is crucial for the success of GWAS. This usually requires a sufficiently large sample size to ensure sufficient statistical ability to detect even small effects of genetic variation. The sample should represent the research target population as much as possible to avoid selection bias (Deng et al., 2022).

Genotype analysis: Currently, high-density SNP chips and next-generation sequencing technology are the main tools for genotype analysis in GWAS. These technologies can efficiently identify millions of SNPs across the entire genome.

Statistical methods: GWAS typically uses multiple statistical models to analyze the relationship between genotype and phenotype, including linear regression, logistic regression, etc. The key is to adjust potential confounding variables such as age, gender, and group structure to ensure the accuracy of the analysis results.

2.3 Challenges and solutions faced in GWAS research

Although GWAS has made significant achievements in genetic research, it also faces some challenges in practical applications, mainly including:

Group structure: Animal populations often have complex group structures, which may lead to false positive findings. To address this issue, researchers can use various methods such as Principal Component Analysis (PCA) or Mixed Linear Model (MLM) to correct for the influence of population structure.

Multiple testing: In GWAS, thousands of SNPs are typically tested for associations with specific traits, which increases the risk of accidentally discovering significant associations. To control the false positive rate, strict statistical correction methods such as Bonferroni correction or false discovery rate (FDR) control can be used.

Effect size and genetic heterogeneity: Many genetic variations related to traits have smaller effects, and the genetic basis of traits may involve the interaction of multiple genes and environmental factors. Increasing sample size, adopting more detailed phenotype classification, and integrating multi omics data are potential strategies to address this issue (Zhang et al., 2023).

Overall, GWAS provides a powerful tool for revealing the genetic basis of livestock behavioral traits, but it also requires researchers to adopt appropriate strategies to overcome the challenges in analysis. By continuously optimizing methods and technologies, GWAS is expected to play a greater role in the field of animal genetics research.

3 The Application of GWAS in Genetic Research of Behavioral Traits in Livestock

3.1 Important cases of GWAS technology applied in genetic research of behavioral traits in livestock

Vallee's research team conducted a GWAS study in 2016 to examine the behavioral, typological, and muscle development characteristics of Charlotte cattle. This study found a significant association between aggression, maternal care, and various types of characteristics during pregnancy, indicating that these characteristics have a complex genetic basis. The creatinine genes known to affect muscle properties have been identified to be significantly associated with muscle development (Vallee et al., 2016).

Schmid and Bennewitz provided a selective review of the statistical model and experimental design of GWAS for quantifying features in livestock in 2017. They emphasized the importance of considering non additive genetics and genotype and environmental effects in GWAS data analysis (Schmid and Bennewitz, 2017).

Fonseca et al conducted a systematic review of GWAS results on sperm and testicular characteristics in livestock in 2018. They used systems biology methods to identify key functional candidate genes for reproductive characteristics, highlighting the genetic mechanisms underlying these features (Fonseca et al., 2018).

Freeborn et al. (2019) used GWAS, fine mapping, and multi tissue transcriptome data analysis to investigate the genetic basis of seven health characteristics in cows. This study detected significant associations and identified 20 candidate genes related to cow health, providing insights into the genetic structure of complex features and diseases (Freeborn et al., 2019).

3.2 Key genes and genetic variations discovered and their biological significance

GWAS research can not only identify genetic markers related to livestock behavioral traits, but also further reveal the biological mechanisms behind these markers. For example, the NR3C1 gene associated with stress response in pigs is a gene encoding glucocorticoid receptors, which play a core role in regulating stress response. Similarly, the discovery of genetic markers related to bovine social behavior suggests that social interaction behavior may be associated with specific signaling pathways and neurotransmitter systems (Figure 1). These findings provide a new perspective on the genetic basis of behavioral traits in livestock and specific molecular targets for genetic improvement.

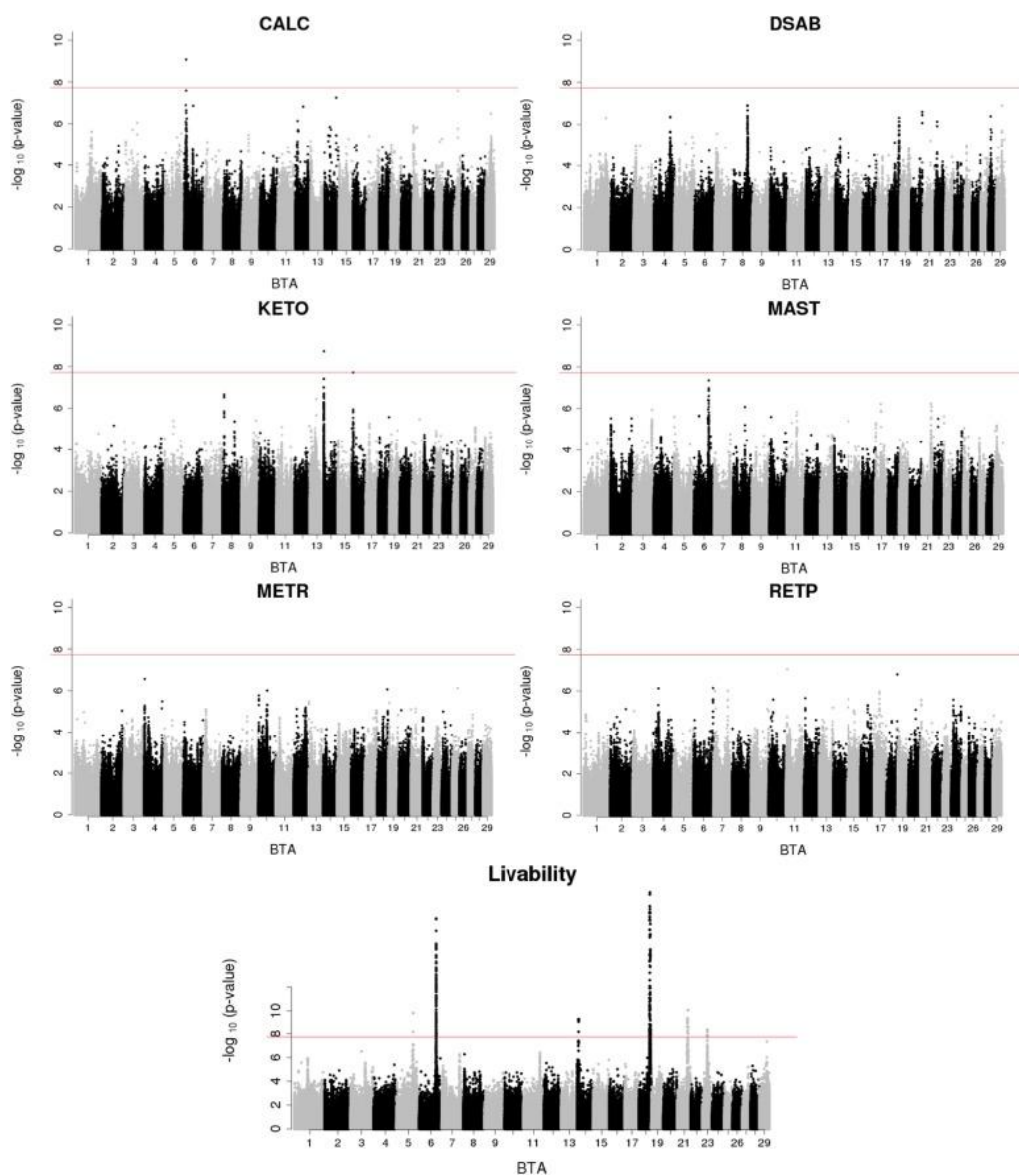


Figure 1 Manhattan plot of the survival rate of cows under complex diseases (Freebern et al., 2019)

Note: Manhattan plots for hypocalcemia (CALC), displaced abomasum (DSAB), ketosis (KETO), mastitis (MAST), metritis (METR), retained placenta (RETP) and cow livability. The genome-wide threshold (red line) corresponds to the Bonferroni correction

In the genetic research of behavioral traits in livestock, the application of whole genome association analysis (GWAS) has successfully identified multiple key genes and genetic variations related to important behavioral traits. These findings are of great value for a deeper understanding of the genetic basis and biological significance of behavioral traits in livestock.

Genome-Wide Association Analysis (GWAS) has made remarkable progress in unraveling the genetic basis of behavioral traits in livestock, and the key genes and genetic variants identified not only increase the understanding of the mechanisms of genetic regulation of these traits, but also provide a scientific basis for future genetic improvement and welfare enhancement. These findings demonstrate the potential of modern genetics in analyzing the molecular mechanisms behind complex traits, and open up new avenues for sustained improvements in livestock productivity and animal welfare.

3.3 Methods for validation and functional analysis of GWAS results

Although GWAS can effectively identify genetic markers associated with traits, the results require further validation and functional analysis to determine the mechanism of action of specific genes. Validation methods usually include:

Bioinformatics analysis: Bioinformatics annotation of gene loci identified by GWAS to determine whether they are located near known functional genes or regulatory elements, or whether they are associated with known biological pathways.

Gene expression analysis: analyze the expression patterns of candidate genes in different tissues or under different physiological states by techniques such as quantitative PCR or RNA sequencing to verify their relevance to specific behavioral traits (Figure 2).

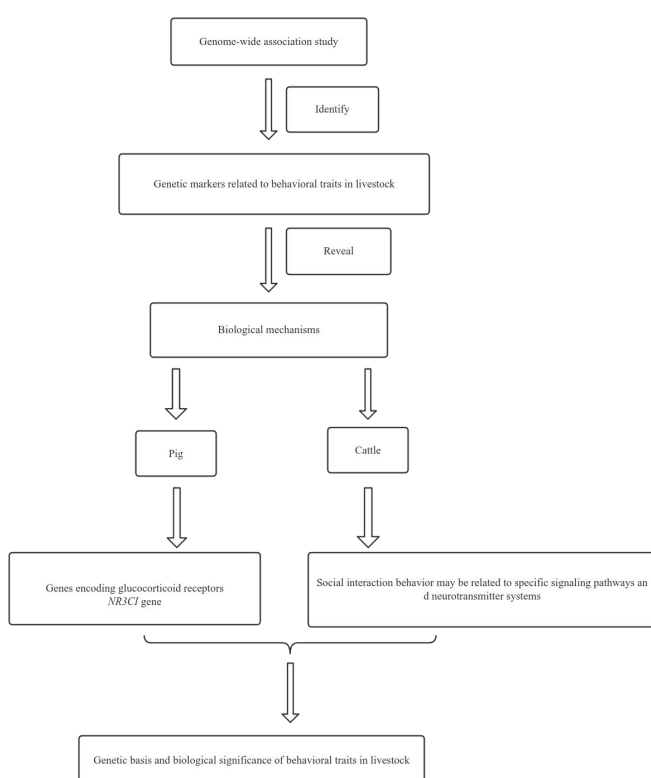


Figure 2 GWAS study identification of genetic markers and their biological significance

Functional validation experiments: using gene editing techniques, such as CRISPR-Cas9, to directly manipulate candidate genes and observe changes in behavioral traits in order to confirm the role of these genes in trait expression.

Through the application of these methods, researchers are able to not only confirm the accuracy of GWAS results, but also explore the function and mechanism of action of the relevant genes in depth, providing a more scientific and precise basis for the genetic improvement and management of livestock. In conclusion, the application of GWAS technology in the genetic study of livestock behavioral traits provides valuable tools and insights to reveal the genetic complexity and biological nature of traits. With the continuous progress of molecular genetics technology and the continuous optimization of data analysis methods, GWAS will play a greater role in livestock genetics research in the future.

4 Research Progress and Case Studies

4.1 Summary of progress in GWAS studies of behavioral traits in different livestock species

With the wide application of genome-wide association analysis (GWAS) technology, genetic studies on behavioral

traits in livestock have made remarkable progress. These studies have not only revealed the genetic basis of behavioral traits in a variety of livestock species, but also provided new strategies for livestock genetic improvement and breed selection.

Cattle: In dairy cattle, GWAS research has focused on social behavior, stress responses, and behavioral traits related to production performance. For example, certain genetic variants have been found to be strongly associated with social hierarchies and group interaction behaviors in cattle, and these findings can help understand herd structure and optimize herd management.

Swine: GWAS studies of behavioral traits in pigs have focused on stress response, aggression, and maternal behavior. The discovery of specific genes, such as variants in the *NR3C1* gene associated with stress, offers possible avenues for genetic improvement to reduce stress response and improve reproductive efficiency in pigs.

Sheep: In sheep, GWAS studies have looked at exploring genetic variants associated with social behavior, stress response, and maternal behavior, among others. These studies provide a genetic basis for improving sheep welfare and enhancing production performance.

Poultry: GWAS studies of behavioral traits in poultry have focused on maternal behavior, stress responses, and social interaction behaviors. Studies have revealed multiple genes and genetic markers associated with these traits, contributing to improved hatching efficiency and improved breeding environments.

4.2 Important findings on the genetic basis of specific traits

Stress tolerance: In several livestock species, GWAS studies have identified multiple genes and genetic variants associated with stress tolerance. For example, in pigs, variants in the *NR3C1* gene associated with the glucocorticoid receptor have been found to be associated with the intensity of the stress response, a finding that has important implications for understanding how animals adapt to environmental stress.

Maternal behavior: Maternal behavior in poultry and pigs is another focus of GWAS research. In poultry, specific genes have been found to be strongly associated with maternal behavior, which has a direct impact on improving hatchability and chick survival. In pigs, certain genetic variants are also associated with maternal behavior, which is expected to be genetically improved to enhance the reproductive efficiency of pigs.

4.3 Practical applications of GWAS research for livestock genetic improvement and breed selection

The results of GWAS research have begun to play a role in livestock genetic improvement and breed selection. By identifying genetic variation associated with important behavioral traits, breeders can make more precise genetic selections to optimize the behavioral performance of livestock and improve productivity and animal welfare. For example, by selecting for genetic markers for better stress tolerance or maternal behavior, pig and poultry breeds can be improved to adapt to the breeding environment and increase reproductive efficiency (Nguyen et al., 2021).

The application of GWAS results also includes the development of molecular marker-assisted selection (MAS) strategy, which can improve selection efficiency and accuracy. With the output of more and more GWAS research results, its application in animal breeding will be more extensive, which will help to realize the sustainable development of the livestock industry.

In conclusion, GWAS has made remarkable progress in genetic studies of livestock behavioral traits, providing valuable resources for understanding the genetic basis of livestock behavior and promoting livestock genetic improvement. These studies have not only improved the understanding of the genetic regulatory mechanisms of livestock behavior, but also provided new strategies and tools for future genetic improvement and breed selection. With further research and technological advances, GWAS will continue to play a key role in revealing the genetic secrets of livestock behavioral traits and promoting the development of the livestock industry.

5 Challenges and Future Directions

5.1 Limitations and challenges of the current GWAS approach in livestock behavioral traits research

Although genome-wide association analysis (GWAS) has made significant progress in revealing the genetic basis of behavioral traits in livestock, this research method still faces many challenges and limitations. At the same time, the importance of integrating genetics, epigenetics, and environmental factors has become increasingly important, and the development of emerging technologies has opened up new possibilities for future research (Guo et al., 2023).

Genetic heterogeneity of complex traits: livestock behavioral traits are often the result of multiple genes acting together in complex ways and are significantly influenced by environmental factors. This makes it more difficult to identify specific genetic variants associated with traits through GWAS.

Influence of population structure and genetic background: differences in genetic background within livestock populations can lead to biased GWAS results, and the correct interpretation of these results needs to take into account the complexity of population structure and genetic background.

Difficulty in detecting rare variants: GWAS mainly recognizes genetic variants with high frequency and has limited ability to detect rare variants that affect traits.

5.2 Importance of integrating genetics, epigenetics and environmental factors in the study of behavioral traits in livestock

Intersection of genetics and epigenetics: the expression of behavioral traits in livestock is not only influenced by genetic factors, but also regulated by epigenetic modifications. Epigenetic studies have revealed the role of DNA methylation, histone modification, and other mechanisms in the regulation of gene expression, which play important roles in animal behavioral adaptation and variability.

Role of environmental factors: the influence of environmental factors on behavioral traits in livestock should not be ignored as well. The interaction between genetics and the environment has a decisive influence on the expression of traits, and future research needs to pay more attention to this interaction.

5.3 Potential application of new technologies in future research

Gene editing technology: gene editing technologies such as CRISPR-Cas9 offer the possibility of precisely modifying specific genes, which is of great significance for verifying the functional loci discovered by GWAS and exploring the molecular mechanisms of behavioral traits. Through gene editing, it is possible to directly observe the effects of specific gene variants on behavioral traits, thus providing an in-depth understanding of the relationship between genetics and behavior.

Multi-omics integrated analysis: Using the integrated analysis of transcriptomics, proteomics, metabolomics and other multi-omics data, the molecular mechanisms related to behavioral traits of livestock can be comprehensively revealed. The integrated multi-omics analysis can help to construct a direct link between genes, expression products and traits, providing more detailed and comprehensive biological information.

Future research on livestock behavioral traits will face the challenge of integrating genetics, epigenetics, and environmental factors, and the application of emerging technologies will bring new opportunities for the development of this field (Su et al., 2023). Technologies such as gene editing and integrated multi-omics analysis will enable researchers to probe more deeply into the genetic mechanisms and biological basis of behavioral traits. With the continuous progress and application of these technologies, it is expected that more significant results will be achieved in the genetic improvement and welfare enhancement of livestock behavioral traits in the future.

6 Conclusion

Genome-wide association analysis (GWAS), as a powerful genetics research tool, has made remarkable achievements in genetic studies of behavioral traits in livestock. Through high-throughput genotyping and sophisticated statistical methods, GWAS has successfully revealed the genetic basis behind a wide range of

livestock behavioral traits, providing valuable scientific evidence for understanding the genetic mechanisms of behavioral traits, guiding livestock genetic improvement and enhancing animal welfare.

The application of GWAS has enabled researchers to identify key genes and genetic variants associated with behavioral traits in livestock on a genome-wide scale. These findings have not only improved the understanding of the mechanisms of genetic regulation of animal behavior, but also provided new selection criteria for breeding programs that can help to optimize the production performance and behavioral welfare of domestic animals. Through GWAS, it has been possible to identify multiple genetic markers associated with key traits such as stress response, social behavior, and maternal behavior in different livestock species, and the discovery of these markers has provided new perspectives and approaches for livestock genetic improvement and management.

Although GWAS have provided insights into the genetics of behavioral traits in livestock, the mechanisms underlying the inheritance of behavioral traits are extremely complex and are influenced by a combination of polygenic and environmental factors. Ongoing research efforts are necessary to reveal more key genetic variants, understand genetic-environmental interactions, and explore the role of epigenetics in the formation of behavioral traits. With the development of new technologies, such as gene editing and integrated multi-omics analyses, future studies are expected to provide more detailed and comprehensive biological information, further advancing the field of livestock behavioral genetics (Wang et al., 2022).

GWAS research is not only important for scientific understanding, but the application of its results can also directly contribute to improved livestock productivity and animal welfare. Through genetic selection, it is possible to breed livestock breeds that are better adapted to the production environment and have better behavioral traits. For example, by selecting for individuals with lower stress responses and better social interactions, behavioral problems during breeding can be reduced, improving the overall welfare and productivity of the population. A better understanding of the genetic basis of behavioral traits in livestock can also help to design more humane management and breeding strategies, thereby improving the quality of life of the animals.

In summary, GWAS has made significant progress in the genetic study of behavioral traits in livestock, providing a new scientific basis and methodology for genetic improvement, breed selection and welfare enhancement. Despite the many challenges, continued research and the application of new technologies will continue to advance the field and are expected to make greater contributions to the sustainable development of the livestock industry and the improvement of animal welfare. As research progresses, the livestock of the future will not only be more efficient in production, but also healthier and happier in behavior.

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