



Research Article Open Access

Whole-Genome Association Analysis in Revealing the Application of Genetic Factors Affecting Livestock Production Traits

Siping Zhang **Z**

Tropical Animal Medicine Center, Hainan Institute of Tropical Agricultural Resources, Sanya, 572024, Hainan, China

Corresponding author email: 2495757304@qq.com

Animal Molecular Breeding, 2024, Vol.14, No.1 doi: 10.5376/amb.2024.14.0006

Received: 20 Nov., 2023 Accepted: 02 Jan., 2024 Published: 26 Jan., 2024

Copyright © 2024 Zhang and Lin, This is an open access article published under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Preferred citation for this article:

Zhang S.P., and Lin X.F., 2024, Whole-genome association analysis in revealing the application of genetic factors affecting livestock production traits, Animal Molecular Breeding, 14(1): 45-53 (doi: 10.5376/amb.2024.14.0006)

Abstract This study reviews the application of whole-genome association analysis in revealing genetic factors affecting livestock production traits. With the continuous development of biotechnology and genomics, whole-genome association analysis has gradually received attention as a powerful genetic research tool. This method detects associations between a large number of genetic markers and traits of interest, revealing the genetic basis of livestock production traits and providing a scientific basis for breeding and genetic improvement. This study first introduces the definition and classification of livestock production traits, as well as their genetic background and influencing factors. Next, an overview of the principles and methods of whole-genome association analysis is provided, along with a comparison of the advantages and disadvantages of traditional genetic research methods and whole-genome association analysis. Subsequently, through typical case analysis, the application and technological progress of whole-genome association analysis in the study of livestock production traits are elaborated in detail. Further discussion includes key genes and genetic markers identified through this method, as well as their practical applications in livestock genetic improvement. Finally, the importance of whole-genome association analysis in deciphering the genetic basis of livestock production traits is summarized, and the potential application value of it in the sustainable development of animal husbandry is discussed.

Keywords Whole-genome association analysis; Livestock production traits; Genetic factors; Breeding; Genetic improvement

Production traits in livestock have significant implications for the economic efficiency and sustainable development of the agriculture industry (Ridha, 2023). Traits such as the fertility of female animals, meat quality and growth rate of beef cattle, and the milk yield and quality of dairy cows are focal points in livestock production. However, the expression of these traits is influenced by various factors, including environmental and genetic factors (Berghof et al., 2019). The study of genetic factors is particularly crucial because genetics is one of the primary sources of trait variation. Understanding genetic factors can help researchers better comprehend the mechanisms behind trait formation and provide more accurate guidance for breeding selection.

Optimizing and improving livestock production traits has always been a significant issue in the agriculture industry. Over the past few decades, with the rapid development of biotechnology and genomics, genome-wide association studies (GWAS) have gradually attracted widespread attention as a powerful tool for genetic research (Xu et al., 2020). GWAS, by detecting associations between a large number of genetic markers and traits of interest, can help reveal the genetic basis of livestock production traits and provide a scientific basis for breeding and genetic improvement.

The research objective of this study is to explore the application of GWAS in revealing the genetic factors affecting livestock production traits. By reviewing the latest progress and application cases of GWAS in the study of livestock production traits, this study aims to deepen the understanding of the role of GWAS in analyzing the genetic basis of livestock production traits and discuss its potential application value in breeding and genetic improvement. This study hopes to provide theoretical support and scientific guidance for the sustainable development of the agriculture industry and offer references for further development in related research fields.



1 Genetic Background of Livestock Production Traits

The study of livestock production traits is a critical aspect of the agriculture industry, directly related to the development and improvement of animal production. Understanding the genetic background of livestock production traits is crucial for achieving genetic improvement and enhancing production performance.

1.1 Definition and classification of livestock production traits

Livestock production traits refer to characteristics that affect the growth, development, reproduction, and product quality of farm animals. These traits can be categorized into growth traits, reproductive traits, and product traits, among others.

Growth Traits include body weight, height, and length, which are important indicators for evaluating the growth rate and body size of livestock. Reproductive Traits, such as egg production rate, fertility, and mating rate, directly influence livestock's breeding efficiency. Product Traits, such as meat quality, milk yield, and egg quality, are key factors in assessing the quality of livestock products.

1.2 Genetic background and influencing factors

The expression of livestock production traits is influenced by various factors, among which genetic factors play a significant role (Li et al., 2019). The genetic background determines the variation in trait expression among individual animals, and its impact can even surpass environmental factors.

The genetic background of livestock production traits mainly includes genotype and gene frequency. The genotype determines an individual's genetic characteristics, while gene frequency reflects the distribution of different alleles within a population. Additionally, there is an interaction between genotype and environment, where environmental factors can modulate the expression level of genotypes in trait manifestation.

1.3 Overview of GWAS

GWAS (Genome-wide association studies) is a method to identify genetic variations that affect traits by comparing the association between genotypes and phenotypes within a population (Figure 1). Its principle is based on the correlation between genetic polymorphisms and traits differences among individuals. By using statistical methods, GWAS aims to determine genetic markers or genes associated with target traits.

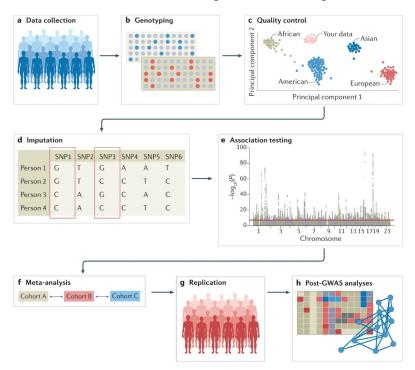


Figure 1 Overview of steps for conducting GWAS (Uffelmann et al., 2021)

M)

Animal Molecular Breeding 2024, Vol.14, No.1, 45-53

http://animalscipublisher.com/index.php/amb

The methods used in GWAS involve various statistical techniques, such as linear regression, analysis of variance (ANOVA), and chi-square tests. By analyzing large-scale genotype and phenotype data, GWAS can identify genetic markers associated with target traits, providing critical evidence for the genetic improvement of livestock production traits.

The advancement of GWAS has opened new avenues for unraveling the genetic basis of complex traits, especially offering advantages in areas such as polygenic effects and low-frequency genetic variations. With the continuous progress of technology and the accumulation of data, the application prospects of GWAS in the field of animal genetics and breeding are set to become even more extensive.

2 Progress in the Genetic Research of Livestock Production Traits

Genetic research on livestock production traits has always been an important research direction in the field of animal husbandry. In the past, researchers primarily relied on traditional genetic methods to explore the genetic basis of livestock production traits. With the rapid development of genomics and biotechnology, new generations of genetic research methods have emerged, with GWAS being the most representative. This section will introduce the traditional methods of genetic research on livestock production traits as well as the progress and advantages of new technologies.

2.1 Traditional genetic research methods

Traditional genetic research methods were the main means used to study livestock production traits in the early days, including genetic linkage analysis and candidate gene approaches.

Genetic linkage analysis is a method to identify genes affecting trait phenotypes by studying the linkage relationships between genetic markers and traits of interest (Teare and Barrett, 2005). Researchers use the genetic information of livestock pedigrees to determine the approximate location of trait genes by constructing pedigrees and genetic maps. However, due to long breeding cycles and high generation numbers in livestock, genetic linkage analysis requires a large amount of pedigree data and time, and its precision is limited.

The candidate gene approach is a method of screening and studying genes based on known functions or locations related to traits (Zhu and Zhao, 2007). Researchers select potential candidate genes related to the target trait based on existing biological knowledge and previous research results. This method has higher efficiency and precision but is limited by prior knowledge and assumptions, possibly overlooking important genes.

2.2 Technological advances and advantages

With continuous advances in biotechnology, GWAS have become a new tool for studying the genetics of livestock production traits. The advent of this technology has significantly advanced livestock genetic research, bringing many advantages.

GWAS is a method that identifies genes affecting traits by detecting associations between single nucleotide polymorphisms (SNPs) or other genetic markers and traits across the entire genome. Compared to traditional methods, GWAS does not require prior knowledge of the gene's function or location and can comprehensively and efficiently screen genes related to traits. Moreover, with the continuous development of high-throughput sequencing technologies and bioinformatics methods, the resolution and efficiency of GWAS have been steadily increasing.

GWAS offers several advantages. It is not limited by prior knowledge, allowing for a comprehensive and unbiased screening of genes. It can also be conducted in a wider population and sample size, improving the universality and reliability of research results. Additionally, this method can identify the polygenic effects of complex traits, helping to better understand the genetic mechanisms of livestock traits.

Both traditional genetic research methods and GWAS have their advantages and disadvantages, but the latter has clear benefits in terms of efficiency and precision, making it the mainstream method for current research on the genetics of livestock production traits. With ongoing technological progress and methodological improvements, GWAS is expected to play an increasingly important role in the future of livestock genetic improvement.



Animal Molecular Breeding 2024, Vol.14, No.1, 45-53

http://animalscipublisher.com/index.php/amb

3 Key Genes and Genetic Markers in Livestock Production Traits

The genetic background of livestock production traits is one of the crucial factors affecting their production characteristics. In recent years, with the development of GWAS technology, a series of key genes and genetic markers have been revealed, playing a significant role in deciphering the genetic basis of livestock production traits.

3.1 Key genes identified through GWAS

GWAS is a systematic approach that identifies genes associated with target traits by analyzing large-scale genotype and phenotype data. This technology enables researchers to identify key genes related to livestock production traits at the whole-genome level.

In revealing key genes for livestock production traits, it's essential first to understand the function and mechanism of these genes. For example, in dairy cows, genes encoding crucial proteins like milk fat synthase and milk proteins have been identified, which significantly impact the production and quality of dairy products (Zhao et al., 2024). Moreover, key genes regulating growth, reproduction, and other production traits have been discovered, participating in complex biochemical processes within the organism and affecting livestock production performance.

The key genes identified through GWAS are closely associated with livestock production traits. Through the analysis of large-scale genotype and phenotype data, researchers can establish the relationship between specific genotypes and traits. This provides important evidence for selecting breeding targets. For instance, in pig growth traits, key genes related to growth rate and body weight gain have been found, where gene variations directly affect pigs' growth performance, thus offering crucial clues for breeding selection.

3.2 Application and interpretation of genetic markers

Genetic markers are specific loci on the genome used to mark genes related to target traits. Among them, SNP (Single nucleotide polymorphism) and QTL (Quantitative trait loci) are two common types of genetic markers that play an essential role in revealing the genetic basis of livestock production traits.

SNPs are common types of genetic variations in the genome. In GWAS, SNPs are widely used to identify genetic markers related to livestock production traits. By analyzing the SNP of livestock populations, researchers can pinpoint SNPs closely associated with target traits and further explore these loci's functions and mechanisms. This provides important molecular markers for genetic improvement, helping to accelerate the breeding process.

QTLs are genes or genomic regions that affect quantitative traits. In the research of livestock production traits, QTLs are extensively used to locate genetic areas related to target traits. Through QTL mapping, researchers can determine genomic regions affecting specific production traits, thereby identifying key genes and functional elements (Figure 2). This offers vital clues for understanding the genetic mechanisms behind livestock production traits, providing more precise targets for genetic improvement.

The key genes and genetic markers identified through GWAS provide critical evidence for understanding the genetic basis of livestock production traits. These research findings offer significant theoretical support and practical guidance for livestock genetic improvement, contributing to enhanced livestock production performance and economic benefits.

4 Application of GWAS in Improving Livestock Production Traits

In improving livestock production traits, genome-wide association studies (GWAS) are being extensively applied as a powerful genetic tool. By analyzing large-scale genomic data, GWAS can reveal key genes and genetic markers that influence livestock production traits, providing new approaches and strategies for genetic improvement in livestock.

http://animalscipublisher.com/index.php/amb

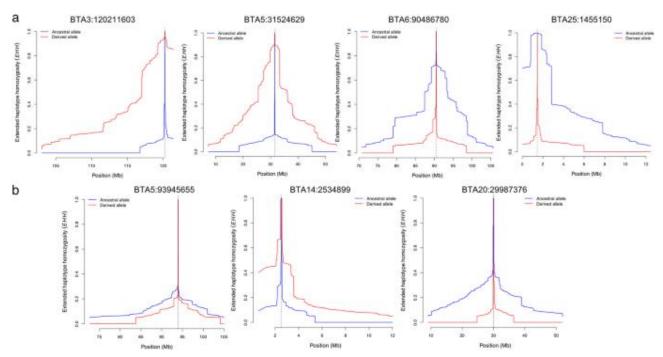


Figure 2 Extended haplotype homozygosity (EHH) decay surrounding the lead SNPs at QTL affecting body conformation and milk production (Fang and Pausch, 2019)

Note: a: the lead SNPs of the QTL associated with body conformation; b: the lead SNPs of the QTL associated with milk production, where the ancestral alleles could be determined. The dashed line marks the location of the lead SNPs relative to which EHH scores were calculated. Blue and red lines indicate EHH decay of the ancestral and derived alleles, respectively

4.1 Livestock genetic improvement strategies

Genetic improvement has always been a key component in the development of the livestock industry. Traditionally, genetic improvement in livestock relied on breeding selection and artificial insemination. However, these methods have certain limitations in efficiency and precision (Hagan et al., 2020). With the rapid advancement of genomics and biotechnology, GWAS has become one of the essential tools for livestock genetic improvement.

GWAS allows for the screening of candidate genes closely associated with target traits. These genes can serve as breeding targets, helping to accelerate the breeding process. GWAS identifies key genes and genetic markers that affect target traits, thereby optimizing the genetic background of livestock. By breeding individuals with favorable genetic foundations, the genetic quality of the entire population can be gradually improved. Guided by GWAS, more precise selection targets can be established, leading to more efficient selective breeding. Using individuals with favorable genotypes as breeding subjects can effectively enhance the genetic level of the next generation, speeding up the selection of superior breeds.

4.2 Optimization of genetic background and selection targets

In livestock genetic improvement, determining and optimizing selection targets is crucial. GWAS provides researchers with more accurate genetic background information and the identification of trait-related genes, thereby helping researchers better select and optimize breeding targets. Analyzing large-scale genomic data enables the identification of genes and genetic markers closely related to production traits, providing a scientific basis for selecting breeding targets. This allows for more effective targeted breeding, accelerating the improvement process of livestock production traits.

GWAS facilitates comprehensive screening and assessment of the entire genome, discovering genetic markers and genes closely associated with target traits. During the breeding process, individuals with favorable genotypes can be selectively bred based on this information, thereby optimizing the population's genetic background. GWAS helps determine the specific scope and priority of selection targets. Establishing corresponding selection targets



based on the genetic contribution and importance of different traits helps achieve more precise and effective selective breeding. GWAS allows for a comprehensive assessment and analysis of the genetic background of livestock populations. Keeping up with the population's genetic characteristics and issues helps adjust breeding strategies and better guide genetic improvement efforts.

4.3 Practical cases of genetic marker-assisted selection

In practice, many livestock genetic improvement projects have started to adopt GWAS-assisted selection strategies and achieved certain successes. For example, in dairy cow production trait improvement, GWAS analysis has successfully identified a series of genes and genetic markers related to milk yield and protein content. Based on this information, breeders can more precisely select individuals with excellent production traits as parental groups, thereby enhancing the genetic level of offspring. Similarly, genetic improvement projects for pigs, chickens, and other poultry are actively adopting GWAS-assisted selection strategies and achieving certain results.

In beef cattle breeding, GWAS technology has identified genetic markers and genes related to meat quality and growth rate (Raza et al., 2020). Through genetic marker-assisted selection, beef cattle breeds with better meat quality and faster growth were successfully bred, greatly improving the efficiency and quality of beef cattle breeding. In dairy cattle breeding, GWAS is widely used to discover genetic markers and genes related to key traits such as milk yield and fat percentage (Figure 3). Based on this information and through genetic marker-assisted selection, dairy cattle strains with higher milk yield and better fat percentages were successfully bred, providing high-quality raw materials for dairy production. In poultry breeding, GWAS has also achieved significant results. Using this technology, poultry strains with faster growth rates and stronger disease resistance were successfully bred, injecting new vitality into the poultry farming industry (Tang et al., 2024).

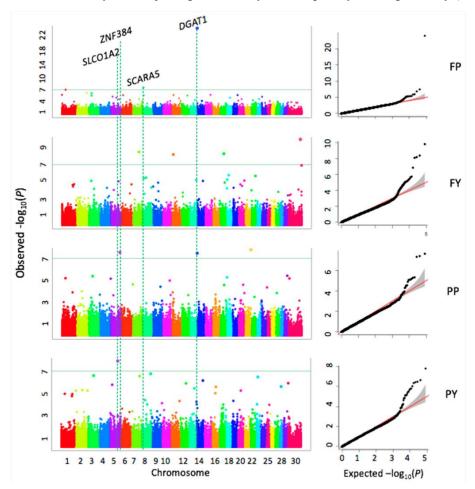


Figure 3 Associations between 124,743 SNPs and milk traits (Liu et al., 2020) Note: FY: fat yield; PY: protein yield; FP: fat percentage; PP: protein percentage

M)

Animal Molecular Breeding 2024, Vol.14, No.1, 45-53

http://animalscipublisher.com/index.php/amb

The application of GWAS in improving livestock production traits holds significant importance. Through GWAS, researchers can more accurately identify the key genes and genetic markers affecting production traits, providing a scientific basis for the selection and optimization of breeding targets. This accelerates the process of livestock genetic improvement, enhancing the productivity and economic benefits of the livestock industry.

5 Frontiers and Challenges in Livestock Production Trait Research

5.1 The rise of emerging technologies

With technological advancements, emerging technologies continue to rise in the study of livestock production traits. Among these, gene editing technology is a field that has garnered significant attention (Wang and Doudna, 2023). Through techniques such as CRISPR/Cas9, researchers can precisely edit the animal genome, directly regulating target genes to achieve rapid genetic improvement. The advent of this technology has provided a new approach and methodology for livestock production trait research, allowing scientists to more accurately explore the genetic factors affecting livestock traits.

Despite the vast potential of gene editing technology, its application still faces many challenges. The use of gene editing in livestock is still in its early stages, and further in-depth research is required. Gene editing may induce unpredictable side effects, such as unintended mutations, which could adversely affect livestock health and production traits. Scientists need to proceed cautiously with gene editing research, respecting animal welfare and genetic stability, while continuously refining the technology and safety assessment methods.

5.2 The role of GWAS in livestock genetic research

Genome-wide association studies (GWAS) as a powerful genetic analysis tool, have been widely applied in livestock genetic research. GWAS enables researchers to search the entire genome for genes and genetic markers associated with specific traits, thus revealing the genetic factors influencing livestock production traits.

The advantage of GWAS lies in its high-throughput and comprehensive nature, allowing for the simultaneous analysis of a large number of genes and sample data, effectively identifying key genes and genetic markers related to traits. Compared to traditional candidate gene methods, GWAS is not limited by prior hypotheses, capable of discovering new candidate genes and providing a broader selection space for livestock genetic improvement.

However, GWAS also has some limitations. GWAS typically requires a large sample size to obtain reliable results and necessitates strict data correction and validation to eliminate the impact of false-positive results. GWAS may be affected by genetic heterogeneity and environmental factors when detecting complex traits, leading to uncertainty in the results. Researchers need to consider various factors to improve the accuracy and reliability of GWAS analysis.

The rise of emerging technologies and the application of GWAS present new opportunities and challenges for research on livestock production traits. By fully utilizing these technologies and methods, scientists can better understand the genetic basis of livestock traits, providing more effective strategies and approaches for livestock genetic improvement and precision breeding.

6 Summary and Outlook

This study delves into the application of GWAS in revealing the genetic factors influencing livestock production traits. It provides an overview of the genetic background of livestock production traits, including definitions, classifications, genetic backgrounds, and their influencing factors. It details the principles and methods of GWAS, highlighting its advantages and application prospects in the study of livestock production traits. The study reviews traditional genetic research methods, such as genetic linkage analysis and candidate gene approaches, and focuses on discussing typical cases and technological advancements of GWAS in livestock production trait research. Furthermore, it presents key genes and genetic markers identified through GWAS and their practical applications in livestock genetic improvement. The main content of the paper is summarized, reviewing the study's focal points and findings, and suggestions for future research and practice are proposed.

Animal Molecular Breeding 2024, Vol.14, No.1, 45-53



http://animalscipublisher.com/index.php/amb

Despite the significant role of GWAS in revealing the genetic factors of livestock production traits, there remain challenges and room for improvement (Li and Ritchie, 2021). Sample size and quality are crucial in conducting GWAS. Future research should aim to increase sample sizes and adopt effective methods to control data quality, enhancing the reliability and accuracy of research findings. In identifying key genes and genetic markers, further exploration into their functions and regulatory mechanisms is needed. This will help better understand the molecular mechanisms behind the formation of livestock production traits and provide more theoretical support for future genetic improvement strategies. GWAS involves knowledge from multiple disciplines, such as bioinformatics and genetics. Future research needs to strengthen interdisciplinary collaboration and communication to address technical and methodological challenges. Active promotion of data sharing and openness is also essential to provide researchers with more resources and support.

Livestock production traits are influenced by multiple genes, so a single genetic improvement strategy may not meet the needs of different livestock types and production environments. Future research should develop diverse genetic improvement strategies, optimizing livestock's genetic background based on GWAS results for better production trait improvement outcomes (Yu et al., 2024). GWAS is an evolving field, and future studies should enhance technological innovation and method optimization, continuously improving analysis efficiency and accuracy. Especially in data processing, statistical analysis, and functional prediction, related techniques and methods need ongoing refinement.

Future research should continue to explore the potential of GWAS in livestock production trait improvement, strengthen interdisciplinary cooperation and data sharing, develop diverse genetic improvement strategies, and keep pushing for technological innovation and method optimization. This will contribute more significantly to the continuous improvement of livestock production traits and the enhancement of agricultural productivity.

References

Berghof T.V., Poppe M., and Mulder H.A., 2019, Opportunities to improve resilience in animal breeding programs, Frontiers in genetics, 9: 410180.

https://doi.org/10.3389/fgene.2018.00692

PMid:30693014 PMCid:PMC6339870

Fang Z.H., and Pausch H., 2019, Multi-trait meta-analyses reveal 25 quantitative trait loci for economically important traits in Brown Swiss cattle, BMC genomics, 20: 1-15.

https://doi.org/10.1186/s12864-019-6066-6

PMid:31481029 PMCid:PMC6724290

Hagan B.A., Moro-Mendez J., and Cue R.I., 2020, Realized genetic selection differentials in Canadian Holstein dairy herds, Journal of dairy science, 103(2): 1651-1666.

https://doi.org/10.3168/jds.2019-16890

PMid:31759593

Li B.L., and Ritchie M.D., 2021, From GWAS to gene: transcriptome-wide association studies and other methods to functionally understand GWAS discoveries, Frontiers in Genetics, 12: 713230.

https://doi.org/10.3389/fgene.2021.713230

PMid:34659337 PMCid:PMC8515949

Li F.Y., Li C.X., Chen Y.H., Liu J.H., Zhang C.Y., Irving B., Fitzsimmons C., Plastow G., and Guan L.L., 2019, Host genetics influence the rumen microbiota and heritable rumen microbial features associate with feed efficiency in cattle, Microbiome, 7: 1-17.

https://doi.org/10.1186/s40168-019-0699-1

PMid:31196178 PMCid:PMC6567441

Liu L.Y., Zhou J.H., Chen C.J., Zhang J., Wen W., Tian J., Zhang Z.W., and Gu Y.L., 2020, GWAS-based identification of new loci for milk yield, fat, and protein in Holstein cattle, Animals, 10(11): 2048.

https://doi.org/10.3390/ani10112048

PMid:33167458 PMCid:PMC7694478

Raza S.H.A., Khan S., Amjadi M., Abdelnour S.A., Ohran H., Alanazi K.M., El-Hack M.E.A., Taha A.E., Khan R., Gong C., Schreurs N.M., Zhao C.P., Wei D.W., and Zan L.S., 2020, Genome-wide association studies reveal novel loci associated with carcass and body measures in beef cattle, Archives of Biochemistry and Biophysics, 694: 108543.

https://doi.org/10.1016/j.abb.2020.108543

PMid:32798459



Animal Molecular Breeding 2024, Vol.14, No.1, 45-53

http://animalscipublisher.com/index.php/amb

Ridha S.N., 2023, Investigating the genetic basis of disease resistance in animal populations, World Journal of Advanced Research and Reviews, 18(1): 073-079.

https://doi.org/10.30574/wjarr.2023.18.1.0443

Tang X.X., Zheng J.M., Luo N., Ying F., Zhu D., Li S., Liu D.W., An B.X., Wen J., Zhao G.P., and Li H.G., 2024, Genetic mechanism of broiler leg disease based on genome-wide association analysis, ACTA VETERINARIA ET ZOOTECHNICA SINICA, 55(1): 99-109.

Teare M.D., and Barrett J.H., 2005, Genetic linkage studies, The Lancet, 366(9490): 1036-1044.

https://doi.org/10.1016/S0140-6736(05)67382-5

PMid:16168786

Uffelmann E., Huang Q.Q., Munung N.S., De Vries J., Okada Y., Martin A.R., Martin H.C., Lappalainen T., and Posthuma D., 2021, Genome-wide association studies, Nature Reviews Methods Primers, 1(1): 59.

https://doi.org/10.1038/s43586-021-00056-9

Wang J.Y., and Doudna J.A., 2023, CRISPR technology: A decade of genome editing is only the beginning, Science, 379(6629): eadd8643.

https://doi.org/10.1126/science.add8643

PMid:36656942

Xu Y.B., Liu X.G., Fu J.J., Wang H.W., Wang J.K., Huang C.L., Prasanna B.M., Olsen M.S., Wang G.Y., and Zhang A.M., 2020, Enhancing genetic gain through genomic selection: from livestock to plants, Plant Communications, 1(1): 100005.

 $\underline{https://doi.org/10.1016/j.xplc.2019.100005}$

PMid:33404534 PMCid:PMC7747995

Yu H., Li R., Yi X.D., and Pang W.J., 2024, Research progress on application of genome sequencing in the meat quality improvement in domestic animals, Journal of Agricultural Biotechnology, 32(2): 471-483.

Zhu M., and Zhao S.H., 2007, Candidate gene identification approach: progress and challenges, International journal of biological sciences, 3(7): 420.

https://doi.org/10.7150/ijbs.3.420

PMid:17998950 PMCid:PMC2043166

Zhao T.D., Wang R.W., Wei X.F., Yang H.L., Fu H.X., and Wang C.M., 2024, Effects of miR-223 on lipopolysaccharide-induced inflammatory response of dairy mammary epithelial cells, China Dairy Industry, 52(1): 28-32.