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Molecular genetic approaches on cattle and chicken breeding: A Review

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ABSTRACT

In recent years, the application of molecular genetic methods and techniques has made a significant contribution in various fields, including animal husbandry. Traditional approaches to livestock breeding have gained new insights from molecular genetics, resulting in increased efficiency and optimization of breeding programs. Genome selection has emerged as a revolutionary technique, enabling comprehensive selection at the early stages of breeding. Moreover, the manipulation of economically important candidate genes at the cellular level contributes to future advances in livestock breeding. In cattle, the integration of gene editing into breeding programs is considered a tool for genetic modification, with particular emphasis on its potential implications across sectors and regions. Numerous studies on the use of genes as the basis for breeding chickens have been conducted extensively. These studies can be categorized into several sections, such as disease resistance, physical performance, and physiological aspects. The significant contribution of molecular genetics to livestock breeding and genetic improvement still requires further research to capitalize on technological advances in this field.

Keywords: Breeding, cattle, chicken, gene selection, molecular genetic

ABSTRAK

PENDEKATAN GENETIK MOLEKULER TERHADAP PEMULIAAN TERNAK AYAM DAN SAPI. Dalam beberapa tahun terakhir, penerapan metode dan teknik genetika molekuler telah memberikan kontribusi yang signifikan dalam berbagai bidang, termasuk bidang peternakan. Pendekatan tradisional pada pemuliaan ternak telah mendapat wawasan baru dari genetika molekuler, menghasilkan peningkatan efisiensi dan optimalisasi program pemuliaan. Seleksi genom telah muncul sebagai teknik revolusioner, memungkinkan seleksi komprehensif pada tahap awal pemuliaan. Selain itu, manipulasi gen kandidat penting secara ekonomi pada tingkat sel berkontribusi pada kemajuan pemuliaan ternak di masa mendatang. Pada ternak sapi, integrasi penyuntingan gen ke dalam program pemuliaan dianggap sebagai alat untuk modifikasi genetik, terutama penekanan pada integrasi luas dengan mempertimbangkan potensi implikasinya di berbagai sektor dan wilayah. Beberapa penelitian mengenai penggunaan gen sebagai dasar pemuliaan ternak ayam telah dilakukan secara intensif. Pada dasarnya kajian-kajian tersebut dapat dikategorikan dalam beberapa bagian seperti resistensi penyakit, performa fisik, dan aspek fisiologis. Kontribusi yang signifikan dari genetika molekuler untuk pemuliaan ternak dan perbaikan genetik tetap perlu untuk ditingkatkan melalui penelitian lanjutan sesuai dengan kemajuan teknologi di bidang ini.

Kata Kunci: Pemuliaan ternak, sapi, ayam, seleksi gen, genetik molekuler

INTRODUCTION

In recent years, molecular genetic methods and techniques have witnessed extensive utilization across diverse applications. The profound impact of molecular genetics has been extensively deliberated, encompassing solutions to both elementary and intricate problems. This remarkable efficacy of molecular genetics can be attributed to its aptitude in discerning pathogenic genes, elucidating mechanisms governing gene expression, and unravelling the interplay between genes as well as their interaction with external factors.

In the domain of livestock breeding, conventional methods have traditionally sought to enhance the complex traits exhibited by livestock in alignment with breeding objectives. This pursuit has been accomplished through a combination of performance testing, estimation of breeding values, and selection (Simianer, 2019). Classic livestock breeding programs have been developed, incorporating one or multiple of these approaches, with valuable insights gained from the field of molecular genetics. Such integration has ultimately contributed to the optimization of efficiency within livestock breeding programs. Notably, genomic selection has emerged as a paradigm-shifting technique, enabling the comprehensive selection of livestock breeding programs from their inception. Furthermore, the potential to engineer cellular-level processes to manipulate economically valuable candidate genes represents a plausible avenue for future advancements in livestock breeding. These endeavours have demonstrated the ability to further augment the efficiency of livestock breeding programs. Advancements in molecular genetic techniques and methods are being continuously pursued in response to technological advancements, aiming to render the obtained results more quantifiable and controllable.

Molecular genetic approaches in support of livestock breeding programs have benefitted greatly from the decreased cost of DNA sequencing, which has expedited the assembly of genomes for various organisms, including livestock. The availability of recent and reliable livestock genome references facilitates the design of breeding programs for livestock breeders. Notably, the National Center for Biotechnology Information (NCBI) serves as a comprehensive database for livestock genetic information. The NCBI page provides access to the latest data on the complete genome of cows (genome assembly ARS-UCD1.3), with relevant references including RefSeq GCF_002263795.2 and submitted sequence GenBank GCA_002263795.3, submitted by USDA, ARS (U.S. Department of Agriculture, Agricultural Research Service) on April 14, 2018 (NCBI, 2023a). Additionally, several studies have contributed to the analysis of the bovine genome, such as Estrada *et al*. (2022), who successfully mapped the Peruvian *Bos taurus* genome, with the dataset documented at GenBank NCBI under the accession number JANIWY000000000. The associated projects include Bioproject, Biosample, and Sequence Read Archive (SRA) with the respective numbers PRJNA849594, SAMN29095626, and SRS13407845. Daetwyler et al. (2014) also performed genome mapping using a sample of 234 cattle, including data from 129 Holstein-Friesian individuals, 43 individuals from the Fleckvieh breed, and 15 individuals from the Jersey breed. On the other hand, the latest documented chicken genome is bGalGal1 maternal broiler GRCg7b (GCF_016699485.2; submitted sequence GenBank GCA_016699485.1) (NCBI, 2023b). The most recent genomes of other farm animals can also be accessed on the latest NCBI page.

The dataset provided by NCBI presents a valuable resource for livestock breeders to estimate the economic value of livestock breeding. The accomplishments of geneticists in sequencing entire DNA sequences enable a broader observation of loci that hold economic significance in livestock, including those integrated within quantitative trait loci (QTL). Consequently, research efforts focused on QTL analysis for commonly reared livestock species such as cows, chickens, pigs, and others will intensify. The continuous advancements in molecular genetic techniques and methods have expanded opportunities for livestock breeders to design livestock breeding programs with greater ease both presently and in the future.

Classic vs modern selection

Traditional selection methods in livestock breeding rely on the assessment of observable traits, either through visual evaluation or measurements such as weight, height, or milk yield. This approach has long been the primary method employed in livestock breeding and has achieved considerable success in enhancing the performance of various livestock species. Classic selection offers the advantage of being relatively straightforward and practical to implement. It allows breeders to make decisions based on easily identifiable characteristics, facilitating the selection process

However, classic selection does have certain limitations. One major drawback is the lack of precision in selecting for specific traits. Since classic selection is based on observable traits, it may not capture the underlying genetic factors that contribute to those traits. This can lead to slower progress in improving traits that are more challenging to assess visually or that have a complex genetic basis. Additionally, classic selection may overlook valuable genetic variations that are not readily visible but could be beneficial in terms of productivity, disease resistance, or other important traits

Another limitation of classic selection is the potential influence of environmental factors on trait expression. Observable traits can be influenced by various environmental conditions, such as nutrition, housing, or management practices. This environmental influence can mask the true genetic potential of animals and introduce variability in trait performance. As a result, classic selection may not accurately reflect an animal's genetic merit, limiting the effectiveness of breeding programs

To overcome these limitations, modern livestock breeding programs increasingly incorporate molecular genetic techniques and gene-based selection methods. These approaches provide a more precise understanding of an animal's genetic composition and allow breeders to directly select for specific genes or genetic markers associated with desirable traits. By incorporating genetic information, breeders can enhance the accuracy and efficiency of trait selection, leading to more rapid genetic improvement.

Gene selection, a more contemporary approach to livestock breeding, revolves around the selection of animals based on their genetic composition. This is achieved by identifying specific genes or genetic markers associated with desirable traits such as meat quality, milk yield, or disease resistance. By focusing on animals with favourable genotypes for these markers, breeders can enhance the performance and characteristics of their herds. Gene selection offers several advantages over classic selection methods

Firstly, gene selection is a more precise approach as it enables breeders to directly target specific genes or markers associated with desired traits. This allows for a more accurate selection process, resulting in the desired traits being passed on to future generations. By pinpointing and selecting animals with the desired genetic variations, breeders can expedite the genetic improvement of their herds

Secondly, gene selection has the potential to accelerate the rate of genetic improvement in livestock. Compared to classic selection methods, gene selection allows for a faster identification of favorable genes or markers associated with desirable traits. This can lead to more rapid genetic progress and the development of improved livestock populations.

However, it is important to note that gene selection also has its challenges and limitations. Successful implementation of gene selection requires access to genomic data and advanced computational tools for genotyping and genetic analysis. These resources can be costly, making it challenging for all breeders to adopt this approach, particularly in resource-limited settings. Additionally, the interpretation and validation of genomic data require specialized knowledge and expertise, adding to the complexity of gene selection.

Recent advancements in DNA technologies have led to the emergence of a multitude of genetic variations at the DNA sequence level, referred to as DNA-based markers, over the past few decades. These markers have revolutionized various aspects of livestock research and breeding. They have facilitated the development of marker-based gene tags, enabled the mapping and cloning of crucial genes in livestock, supported studies on genetic variability and phylogenetic analysis, aided in synteny mapping, and enhanced marker-assisted selection for desirable genotypes. DNA-based markers offer several advantages over traditional phenotypic and biochemical markers, primarily because they provide objective and analysable data. This article provides a comprehensive review of the primary applications of molecular markers in modern livestock breeding strategies, including parentage determination, estimation of genetic distances, assessment of genetic diversity, gene mapping, and marker-assisted selection (Teneva and Petrovic, 2010).

Genetic approaches on cattle breeding

In the past few years, there has been a renewed focus on adapting cattle to challenging environments, driven primarily by advancements in genomic techniques. The current emphasis on tick resistance is justified due to the significant impact tick infestations have on production and animal well-being in tropical and subtropical regions, where approximately 70% of beef cattle are found. Studies have demonstrated that the heritability of tick burden in cattle ranges around 0.30, indicating that selective breeding programs for tick resistance can be successful. Research on gene expression has strongly suggested that both immune and non-immune mechanisms play a role in tick resistance among cattle. Recent investigations using quantitative-trait mapping have identified specific chromosome segments and single nucleotide polymorphisms associated with tick burden, but a definitive causal variant has not yet been pinpointed. The majority of genetic markers linked to tick burden explain only a small portion of the overall variance, which is typical for markers associated with quantitative traits. Consequently, it is likely that panels of multiple markers for tick resistance, potentially tailored to zebu or taurine breeds, will be developed for use in future cattle breeding and selection programs (Laercio *et al*., 2011).

The importance of genetic improvement in cattle is important for sustainable animal agriculture. The use of reproductive and molecular biotechnologies in this process and emphasizes the growing popularity of gene editing as a tool for precise genetic modification in cattle. To ensure successful genetic improvement, gene editing should be seamlessly integrated into existing breeding programs. The strategies for incorporating gene editing effectively, disseminating improved traits, and considers how these approaches may vary across the dairy and beef sectors and different regions of the world are very useful in order to increase performance of cattle (Mueller and Eenennaam, 2022).

Shamshidin *et al.* (2019) recorded that the utilization of modern cattle breeding methods includes the active implementation of new breeding programs. The integration of molecular genetic research paves the way for the application of DNA microsatellites (STR-loci), which are widely utilized in analyzing the allele pool of livestock. Given these circumstances, the objective of this study was to examine the genetic structure and assess the level of genetic differentiation among cattle breeds in the Republic of Kazakhstan using molecular genetic information.

Semen from bulls was employed as the biological material for the research. The study encompasses DNA profiles of 11 microsatellite loci, which include dairy breeds (Holstein, Black-and-white, Aulie-Ata), combined breeds (Alatau), and meat breeds (Auliekol, Kazakh whiteheaded). The microsatellite profile of the animals was characterized by the following loci: BM1824, ETH225, INRA23, BM2113, SPS115, ETH10, TGLA122, TGLA126, TGLA227, ETH3, TGLA53, which are part of the recommended ICAR and ISAG panel. The genetic structure of the populations was assessed through F-statistics, while genetic identity indicators were determined using Nei's methodology. Populations' heterozygosity was evaluated based on Wright's fixation index. Furthermore, variations of microsatellite loci were analysed to explore differences among breeds in terms of productivity (Shamshidin *et al.*, 2019).

In Cai *et al.* (2019) study, 15,551,021 SNPs were associated with the female fertility index in 5038 Nordic Holstein cattle. Seven quantitative trait loci (QTL) on six chromosomes in cattle were identified. A list of potential candidate genes affecting fertility in cattle was generated by utilizing gene-based analysis and spread of linkage disequilibrium (LD) information, along with considering the nearest genes to GWAS hits. Subsequently, the list of candidate genes for fertility was refined using prior knowledge on genes related to fertility from Gene Ontology terms, Kyoto Encyclopedia of Genes and Genomes pathway analysis, the mammalian phenotype database, and publicly available RNA-seq data. Candidate mutations within the prioritized candidate genes were investigated using variant annotations. Through the integration of multiple sources of information, candidate genes with biological relevance underlying each of these seven QTL were proposed. On chromosome 1, ten candidate genes for two QTL were identified. For the rest of the chromosomes, one candidate gene was proposed for each QTL. FRAS1, ITGB5, ADCY5, and SEMA5B were supported as candidate genes for cow fertility based on differential expression in different studies, as indicated in the candidate genes list.

Estrada *et al.* (2022) analyzed the Peruvian creole cattle (PCC) that is a vital but overlooked breed in the Andean region of Peru. The entire PCC genome using the Illumina HiSeq 2500 platform was tested. This yielded 320 GB of data, which is enhanced using a reference scaffolding technique. The resulting PCC genome size was 2.81 Gb, comparable to *Bos taurus* and *B. indicus* genomes. It is revealed that 40.22% of the genome consists of repetitive DNA, with retroelements accounting for 32.39%. As much as 19,803 protein-coding genes and obtained similar SSR statistics compared to other breeds had been identified. The availability of the PCC genome will enhance understanding of its genetics and adaptation to the challenging Andean ecosystem.

The 1000 bull genomes project that aims to accelerate genetic improvement in domestic cattle while considering animal health and welfare by providing annotated sequence variants and genotypes of key ancestor bulls was done by Daetwyler *et al*. (2014). In the initial phase, the genomes of 234 cattle, including individuals from Holstein-Friesian, Fleckvieh, and Jersey breeds was sequenced. Result of this experiment identified 28.3 million variants, with an average of 1.44 heterozygous sites per kilobase. This research successfully identified recessive and dominant mutations related to embryonic death and lethal chondrodysplasia, respectively. Additionally, genome-wide association studies for milk production and curly coat traits has been performed, identifying specific variants associated with these traits in cattle.

Genetic approaches on chicken breeding

Several studies concerning the use of genes as the basis for breeding chickens have been carried out intensively. Basically, these studies can be divided into several subjects such as disease resistance, physical performance, and physiological aspects.

Disease resistance

Disease resistance plays a crucial role in the poultry industry as it directly impacts production capacity and has implications for food security. Võ *et al*. (2021) conducted a study with the aim of developing vaccines by examining the microneme protein 2 (MIC2) and surface antigen 1 (SAG1) of *Eimeria tenella*, the genus responsible for avian coccidiosis outbreaks. This group of researchers analysed the genetic diversity and natural selection of etmic2 and etsag1 in Korean *E. tenella* isolates, which provides valuable information for the design of effective coccidia vaccines.

Another study focused on the induction of host defence peptides (HDPs) in two lines of chickens: one with disease resistance and the other susceptible to disease. Jang *et al*. (2020), by examining the mRNA expression patterns of HDPs, found that the disease-resistant *Fayoumi* line exhibited higher expression compared to the disease-susceptible *Leghorn* line, indicating the involvement of HDPs in chicken disease resistance.

Manjula *et al*. (2021) investigated the chicken major histocompatibility complex (MHC)-B region using 11 MHC-linked microsatellite markers from four different countries (Sri Lanka, Bangladesh, South Korea, and Nigeria). Through Set 1 and Set 2 analysis, it was identified as much as 409 haplotypes, which can be further utilized to study MHC polymorphisms and their impact on differential immune responses

Physical performance

The development of muscle in broilers is a significant economic characteristic. Zhang *et al*. (2021) conducted a study investigating the impact of miRNA, specifically miR-21-5p, on the proliferation and differentiation of skeletal muscle satellite cells (SMSCs). The study findings confirmed that miR-21-5p targets the gene KLF3. Thus, this research demonstrates the role of miR-21-5p in influencing the growth and development of skeletal muscle in chickens.

Following the publication of the chicken genome in 2004, there has been a surge in large-scale genome research. As a result, a study was conducted as part of a Genome-Wide Association Study (GWAS) using a 60K single nucleotide polymorphism (SNP) chip, which identified 12 significant SNPs associated with body weight at eight weeks of age in Korean native chickens (KNCs). These SNPs can serve as useful markers for selecting chickens based on their body weight (Cha *et al*., 2021).

Another study has been done that focused on investigating selection signatures between Korean native chicken breeds and commercial chicken breeds, utilizing data from a 600K SNP chip (Cho *et al*., 2021). This analysis led to the identification of several regions exhibiting selection signatures, indicating that these genomic locations could be utilized to identify the specific genes and mutations responsible for enhancing meat and egg-related traits in native chicken breeds.

Physiological aspects

Serotonin, also known as 5-hydroxytryptamine (5-HT), plays a role in various physiological functions in vertebrates. However, there is limited information available regarding its involvement in avian species. To address this gap, Sun *et al*. (2021) conducted a study focusing on the full-length cDNA and expression analysis of three serotonin receptor genes (HTR1B, HTR1E, and HTR1F) in the pituitaries of chickens. By investigating these receptor genes, the study aimed to enhance understanding of serotonin's function in chickens. The findings from this research will contribute to expanding our knowledge of serotonin's role in chicken physiology through the examination of its receptor genes.

Mukae *et al*. (2021) have conducted research on transgenic chickens with the objective of generating monoclonal antibodies (mAbs) in egg whites. The study focused on incorporating genes for the heavy and light chains of humanized anti-HER2 mAb into the chicken ovalbumin gene locus using CRISPR/Cas9 techniques. The outcomes of the study demonstrated that chickens can be effectively utilized for mAb production, including the potential for commercial-scale manufacturing of mAbs.

Bone health in poultry is gaining importance. Studies by Adhikari *et al*. (2020) and Jansen *et al*. (2021) investigate factors influencing bone health in broilers and laying hens, respectively. Adhikari *et al*. (2020) explore the effect of 20(S)-hydroxycholesterol on mesenchymal stem cells from broiler chicken bones, while Jansen *et al*. (2021) identify candidate genes and SNPs associated with bone breaking strength and mineral density in laying hens, potentially aiding in reducing bone disorders.

No chicken poultry species

Li *et al*. (2021) investigate G protein-coupled receptors (GPR3, GPR6, GPR12) and a novel GPR12-like receptor (GPR12L) in chickens and ducks, providing valuable insights into their structure, function, and expression. They find that progesterone upregulates the expression of GPR12, contributing to understanding of these receptors in avian species from an endocrinology perspective.

In one of the studies, Melak *et al*. (2021) investigated single nucleotide polymorphism (SNP) markers associated with marketing weight in male Yangzhou geese. The researchers employed the specific locus amplified fragment sequencing (SLAF-seq) method combined with bulked segregant analysis (BSA) to identify the SNP markers. From a sequence depth of 44.97, a total of 12,917 SNPs were discovered, and among them, 10 novel SNPs were identified as the most significant markers related to the target trait of marketing weight in geese. The researchers also conducted expression analysis using quantitative PCR, which revealed significant differences in seven genes. These identified genes and SNPs hold potential as valuable markers for enhancing marketing weight in geese.

Another study focused on geese involved the identification of SNPs and a phylogenetic analysis using genotyping-by-sequencing (GBS) in 12 different geese breeds (Grzegorczyk *et al*., 2021). Through GBS, a total of 3,833 SNPs were identified, and 791 SNPs were selected for population analysis. These studies contribute to the understanding of geese genetics and provide valuable insights into improving traits such as marketing weight and genetic diversity within geese populations.

One of the identified variants in geese was found to be associated with the EDAR gene, which plays a role in plumage development and can be utilized for selection purposes. To gain a better understanding of the enzyme involved in goose follicular development, Yuan *et al*. (2020) conducted a study focusing on the expression of the SCD gene. They employed quantitative reverse-transcription PCR (qRT-PCR) to assess gene expression levels. Additionally, they used liquid chromatography-tandem mass spectrometry (LC-MS/MS) to investigate the function of SCD in granulosa cells (GCs) of geese. Through qRT-PCR and LC-MS/MS analysis, the study identified cholesterol and pantothenol (or pantothenate) as potential metabolite biomarkers associated with SCD-related lipid metabolism in goose GCs. These findings shed light on the role of SCD and its impact on lipid metabolism in geese, providing insights into the molecular mechanisms underlying follicular development.

Bernini *et al*. (2021) analyse genomic diversity in heritage turkey breeds, identifying regions associated with reproduction efficiency. In the Japanese quail study by Shumaker et al., SNPs related to immune responses, DNA repair, and neurological signalling are identified, potentially influencing poultry production. Finally, a study on pigeons by Ge *et al*. (2021) investigates microRNA-associated-ceRNA networks involved in regulating crop milk production, identifying key microRNAs and providing insights into lactation regulation.

Both studies contribute to our understanding of the genetic and molecular mechanisms underlying important traits in Japanese quail and pigeons, respectively, offering potential avenues for improving poultry production and reproductive performance in these species.

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