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A Review on Bioinformatics in Animal Breeding and Research on Disease Resistance

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ABSTRACT

Integrating bioinformatics in animal breeding has transformed how genetic information is utilised to improve disease resistance and overall productivity in livestock. This article examines the roles of bioinformatics in animal breeding, with a particular focus on research related to disease resistance. It explores the latest advances in genome sequencing, genomic selection, and the identifi cation of Quantitative Trait Loci (QTLs) that contribute to disease resistance. By leveraging recent literature, it highlights the potential of bioinformatics tools and techniques to accelerate breeding programmes, improve animal health, and enhance the effi ciency of livestock production. It also discusses the challenges associated with bioinformatics in animal breeding,

such as data management and the need for interdisciplinary collaboration.

Keywords: *Bioinformatics, Animal Breeding, Disease Resistance, Genomic Selection, Quantitative Trait Loci (QTLs)*

Introduction

Animal breeding has long relied on traditional methods of selection based on phenotypic traits to improve the productivity and health of livestock. However, molecular genetics and bioinformatics have revolutionised this fi eld by providing tools to analyse genetic information at a much deeper level. Applying computational and statistical techniques to manage and analyse biological data, bioinformatics is crucial in modern animal breeding programmes.

Animal breeding has traditionally relied on selective breeding practices, where animals with desirable traits are chosen to reproduce, gradually improving the genetic quality of the herd over generations. However, this approach is often slow and limited by the animals' observable traits. The advent of genomic technologies has revolutionised this process by allowing breeders to assess the genetic potential of animals at a much earlier stage and to select for traits that are diffi cult to measure, such as disease resistance.

Disease resistance is a critical trait in animal breeding, because it directly impacts livestock health, productivity, and welfare. Infectious diseases can cause significant economic losses in the livestock industry, biotechnology, revolutionising fields like plant and animal breeding, forensic science, medicine, and conservation biology. These markers can help in distinguishing between individuals or populations based on genetic differences and are widely used to track the inheritance of traits across generations.

Types of DNA Markers

Several types of DNA markers are commonly used in research, each with its unique properties, advantages, and applications. They can be broadly classified into three categories: morphological markers, biochemical markers, and molecular markers. The latter has gained prominence with advances in molecular genetics due to its precision and reliability.

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and the emergence of antibiotic-resistant pathogens has heightened the need for alternative approaches to disease management. Bioinformatics provides the tools to identify the genetic variants that confer resistance to diseases, enabling the development of breeding programmes that can produce more resilient animals.

This article aims to explore the role of bioinformatics in animal breeding, with a particular focus on research related to disease resistance. By examining recent advances in genomic technologies, computational methods, and their applications in breeding programmes, it highlights bioinformatics's potential to transform the future of livestock production and disease management.

DNA Markers

DNA markers are specific sequences of nucleotides within the genome that serve as landmarks to identify particular traits, genes, or regions of DNA. They have become essential in genetics, molecular biology, and

Restriction Fragment Length Polymorphisms (RFLPs)

RFLPs are one of the earliest forms of DNA markers. They are based on variations in the DNA sequence that cause differences in the lengths of restriction enzymedigested DNA fragments. These markers are highly informative and co-dominant, meaning they can distinguish between heterozygous and homozygous individuals. However, their use has declined due to the labour-intensive and time-consuming processes.

Microsatellites or Simple Sequence Repeats (SSRs)

Microsatellites consist of short, repetitive DNA sequences (usually 1-6 base pairs long) scattered throughout the genome. These highly polymorphic markers are ideal for genetic diversity, parentage analysis, and population genetics studies. Their codominant inheritance allows for the detection of both alleles in heterozygous individuals. SSRs are widely used in breeding programmes to identify genetic

diversity and to link specific traits with particular genes.

Single Nucleotide Polymorphisms (SNPs)

SNPs are the most abundant and widely used DNA markers in current genetic research. They represent single base-pair differences in the DNA sequence between individuals. SNP markers are favoured for high-throughput genotyping due to their ubiquity across the genome and ease of detection. They are commonly used in Genome-Wide Association Studies (GWAS) to link genetic variants with traits or diseases in both plants and animals. SNP arrays, which allow the simultaneous analysis of thousands of SNPs, have become a powerful tool in genomics, enabling genomic selection and personalised medicine.

Random Amplified Polymorphic DNA (RAPD)

RAPD markers are generated by amplifying random genome segments using short primers under lowstringency PCR conditions. While they are easy to produce and do not require prior knowledge of the genome, they are less reproducible than other markers and have been largely replaced by more reliable methods.

Amplified Fragment Length Polymorphisms (AFLPs)

AFLP markers combine the principles of RFLP and PCR amplification. They involve digesting genomic DNA with restriction enzymes and amplifying specific fragments. AFLPs are highly polymorphic and can detect many markers in a single reaction. However, like RFLPs, they are labour-intensive and have been largely supplanted by SNP and SSR markers in modern applications.

Advances in Genome Sequencing

The rapid development of Next-Generation Sequencing (NGS) technologies has significantly reduced the cost and time required for whole-genome sequencing (WGS). In animal breeding, NGS has enabled the identification of genetic variants associated with disease resistance, facilitating the discovery of new Quantitative Trait Loci (QTLs) and the development of Genomic Selection (GS) strategies.

Whole-Genome Sequencing

Whole-Genome Sequencing (WGS) provides a comprehensive view of an organism's genetic makeup, allowing researchers to identify Single Nucleotide Polymorphisms (SNPs), insertions, deletions, and other genetic variations that may contribute to disease resistance. Studies have shown that WGS can effectively identify SNPs associated with resistance to diseases such as bovine tuberculosis and mastitis in cattle (Banos *et al.*, 2020). The availability of highquality reference genomes for various livestock species has further facilitated the application of WGS in animal breeding.

Genome-Wide Association Studies (GWAS) Genome-Wide Association Studies (GWAS) have become a powerful tool for identifying genetic variants associated with complex traits, including disease resistance. GWAS involves scanning the genomes of many individuals to identify SNPs that are significantly associated with a trait of interest. In livestock, GWAS has been used to identify genetic markers associated with resistance to diseases such as foot-and-mouth disease in pigs (Bishop & Woolliams, 2014). The integration of GWAS with bioinformatics tools allows for the efficient analysis of large datasets, identifying novel genetic markers and pathways involved in disease resistance.

Genomic Selection in Animal Breeding

Genomic Selection (GS) is a modern breeding approach that uses genomic information to predict the breeding value of individuals. By incorporating genomic data into selection models, GS enables a more accurate and efficient selection of animals with desirable traits, such as disease resistance.

Genomic Prediction Models

Genomic prediction models use genetic markers like SNPs to estimate individuals' genomic breeding values (GEBVs). These models have been successfully applied in livestock breeding to improve disease resistance, growth rate, and reproductive performance. For example, Meuwissen *et al.* (2016) demonstrated the effectiveness of GS in improving cattle's resistance to bovine respiratory disease. Bioinformatics tools are essential for developing and validating these models, because they allow for the efficient analysis of large genomic datasets.

Integration of Omics Data

Integrating various omics data, such as genomics, transcriptomics, and proteomics, into genomic selection models can further enhance the accuracy of predictions. Researchers can better understand the genetic architecture underlying disease resistance by incorporating information on gene expression and protein function. For instance, Li *et al.* (2019) integrated transcriptomic data into a genomic selection model to improve resistance to Salmonella in poultry, demonstrating the potential of this approach.

Identification of Quantitative Trait Loci (QTLs) Quantitative Trait Loci (QTLs) are genome regions associated with variation in quantitative traits, such as disease resistance. Identifying QTLs is critical in understanding the genetic basis of complex traits and

developing strategies for marker-assisted selection (MAS).

QTL Mapping

QTL mapping involves the identification of regions of the genome associated with a particular trait. This process typically involves using statistical methods to analyse the relationship between genetic markers and phenotypic traits in a population. QTL mapping has been widely used in livestock breeding to identify genome regions associated with resistance to diseases such as avian influenza in chickens (Sun *et al.*, 2021). Bioinformatics tools are essential for analysing QTL data, because they allow for the efficient management and interpretation of large datasets.

Fine Mapping and Candidate Gene Identification Once QTLs have been identified, fine-mapping techniques can narrow down the regions of interest and identify candidate genes responsible for the trait. Fine mapping involves using high-density SNP arrays or sequencing data to refine the location of QTLs and identify the causal variants. Bioinformatics plays a crucial role in this process by enabling the integration of multiple data types, such as GWAS results, expression quantitative trait loci (eQTL) data, and functional annotation data, to identify candidate genes. For example, Zhang *et al.* (2018) used fine mapping to identify candidate genes associated with resistance to Marek's disease in chickens, providing valuable insights into the genetic basis of this trait.

Understanding Immunogenetics

The study of the genetic basis of the immune response is known as immunogenetics. The term was introduced with the discovery of ABO blood groups and was first demonstrated through the existence of "natural" antibodies, that is iso-antibodies (Landsteiner, 1901).

The broad field of immunogenetics includes the study of normal immunological pathways and identifying genetic variations that result in immune defects, which may facilitate the detection of new therapeutic targets for immune diseases. Hence, understanding and subsequent manipulation of host immune response (immunomodulation) is the most precise and effective tool to reduce disease incidences and nullify the limitations associated with antibiotic treatment or vaccination. Therefore, breeding for disease resistance has gained considerable attention from researchers in the recent past.

Immune Response Genes

Often, it was observed that individuals respond differently to the same infectious agent. A possible explanation may be the genetic variability between them. Indeed, many studies have looked for associations between genes involved in immunity and disease outcome (Buniello *et al.* 2019), and it has been found that specific Immune response (Ir) genes play a crucial role. This concept was discovered in the mid-1960s (McDevitt and Benacerraf, 1969). It introduced a new level of antigen recognition, whose diversity and specificity had to be explained in addition to those of familiar immunoglobulins. Hence, immune response (Ir) genes were defined as antigen-specific genes that control the ability of an animal to raise an immune response, either humoral or cellular, to a particular antigen (Berzofsky, 1980). This includes Major Histocompatibility Complex (MHC I, II and III), Interleukins (IL–6, IL-β), Tumor Necrosis Factor (TNF-α), Cluster of Differentiation (CD-14) and Tolllike Receptor (TLR-4), which are responsible for conferring innate immunity. The code for a set of cytokine or anti-inflammatory response complement proteins (C1-C4) that adhere to pathogens and

cytokines (interferons and chemokines) that attract immune cells to the site of infection.

The MHC gene complex is central to all immune functions and disease resistance. All the higher animals possess an MHC gene complex that codes for the predominant cell surface proteins on the cells and tissues of each individual of the species (Snell *et al.* 1976). The MHC encodes three classes of protein molecules: Class I, II, and III (Matzinger and Zamoyska. 1982). The first class of molecules consists of a membrane-bound glycoprotein heavy chain with a molecular weight of 40,000 to 50,000 and a nonmembrane-bound light chain, 32-microglobulin, with a molecular weight of 12,000. The class II molecules are membrane-bound glycoproteins consisting of two non-covalently associated chains, α and β, each with a molecular weight of about 30,000. Class III molecules are components of serum complement. A study by Kannaki *et al.* (2017) highlighted that LEI0258 microsatellite-based MHC typing would be a helpful tool in sorting cross-bred and indigenous chicken populations and selecting birds for breeding programmes. In another study, Kannaki *et al.* (2018) attempted to explore the TLR gene family and TLR gene expressions in day-old duckling tissues using real-time PCR. Also, they investigated the cytokine expression in peripheral blood mononuclear cells (PBMCs) upon TLR agonist's stimulation in an in vitro assay. It was found that TLR gene expression in young ducklings and cytokine response upon LPS stimulation demonstrated the innate preparedness of younger birds to encounter pathogens and their functional ability to respond to their ligands. The relative expression of interleukins (IL)-1β, IL2, IL-6, IL-17 and interferon (IFN)-Y genes were explored in response to coccidial challenge in Kadaknath, Cari-Vishal and Cobb broiler chicken using quantitative PCR (Thakur *et al.,* 2020). It was concluded that the

differential expression of cytokine genes in the three genetic groups showed different degrees of mucosal immune response to Eimeria infection. It depends upon the birds' genetic background or genotype, coccidial dosage, and age of infection. Some of the Ir genes and their association with disease resistance in livestock and poultry are given in Table 1.

Broad Strategies of Breeding for Disease Resistance Selection of healthy animals based on natural infection

This method will randomly choose only healthy animals without any illness-related signs or symptoms. Nevertheless, if animals are not randomly subjected to or exposed to pathogens, the accuracy of selection declines. The main benefits of this approach are that it is less expensive, easier to use, and ethically unquestionable. This has been used to select Red Maasai sheep in Kenya, which are more resistant to Haemonchus infection than the Dorper breed of sheep from South Africa (Mugambi *et al.,* 1996).

Selection of Animals after Artificial Infection In this case, uniformly confronting research animals with infections of identical dosages of the infectious agent is an attempt to increase the selection accuracy. The random distribution of the disease among the research animals makes this methodology more exact. The primary limitation is that the procedure raises ethical questions and can be expensive depending on the pathogen's virulence and clinical manifestation of the illness. The population may need to be isolated to stop the spread to other stocks. For instance, challenge studies have been employed to select disease resistance in sheep against Strongyle infection (Terefe *et al.,* 2007). In research using vulnerable INRA 401 (INRA) breeds of lambs and resistant Barbados Black Belly (BBB) breeds, the immunological responses to Haemonchus contortus infection were compared. During initial and secondary artificial challenges, BBB lambs showed signs of blood eosinophilia and a more sustained and increased Th2 cytokine mRNA transcription.

Bioinformatics Tools and Techniques in Animal Breeding

Bioinformatics's application in animal breeding relies on various tools and techniques for analysing genetic data. These tools are essential for managing large datasets, identifying genetic variants, and predicting the effects of genetic changes on phenotypic traits.

Bioinformatics Software for Sequence Analysis Several bioinformatics software packages are available for analysing genomic data in animal breeding. These tools are used for sequence alignment, variant calling, and annotation tasks. For example, BWA (BurrowsWheeler Aligner) and GATK (Genome Analysis Toolkit) are commonly used for sequence alignment and variant calling. In contrast, ANNOVAR is used to annotate genetic variants (Li & Durbin, 2009). These tools are essential for identifying genetic variants associated with disease resistance in livestock.

Burrows-Wheeler Aligner (BWA)

BWA is a software package for aligning short DNA sequences to a reference genome. In animal breeding, BWA helps map the DNA sequences from livestock to a reference genome, essential for identifying genetic variations, such as single nucleotide polymorphisms (SNPs) and insertions/deletions (indels). This alignment is a crucial first step in understanding the genetic makeup of animals and identifying traits related to disease resistance, productivity, or other desirable characteristics.

Genome Analysis Toolkit (GATK) GATK is a software suite designed for variant discovery in highthroughput sequencing data. In animal breeding, after the DNA sequences are aligned to the reference

genome using BWA, GATK detects and analyses genetic variants such as SNPs and indels. GATK also provides tools for variant filtering and quality control, ensuring that only the most reliable genetic markers are used in breeding programmes. This information is critical for marker-assisted selection, where animals with desirable genetic traits are selected for breeding.

ANNOVAR

ANNOVAR is a tool for annotating genetic variants. It takes the variants identified by GATK and provides functional annotations, such as predicting the impact of a variant on gene function or identifying whether a variant is associated with a known disease or trait. In animal breeding, ANNOVAR helps breeders understand the biological significance of the genetic variants present in their livestock, enabling them to make informed decisions about which animals to select for breeding based on the genetic potential for improved traits.

Data Management and Integration

Managing and integrating large genomic datasets is a significant challenge in bioinformatics. Tools such as Galaxy and KBase provide platforms for analysing and integrating diverse datasets, allowing researchers to perform complex analyses without requiring extensive computational expertise (Afgan *et al.*, 2018). These platforms also facilitate collaboration and data sharing, which is essential for advancing research in animal breeding.

Functional Annotation and Pathway Analysis Functional annotation involves assigning biological functions to genetic variants, while pathway analysis identifies biological pathways affected by these variants. DAVID and Enrichr are commonly used for functional annotation and pathway analysis in animal breeding (Huang *et al.*, 2009). These tools are essential for understanding the biological mechanisms underlying disease resistance and identifying potential targets for genetic improvement.

Roles of Bioinformatics in Vaccine Development, Disease Diagnosis and Drug Discovery

Emerging infections seriously threaten livestock productivity, necessitating the identification of potential vaccine candidates to guarantee long-term animal protection (Kaikabo and Kalshingi, 2007; Ganguly *et al.*, 2013). New vaccine development approaches must offer broad-spectrum and long-term protection against viral and bacterial illnesses (Pathak *et al.*, 2020; Gebre *et al.*, 2021). Bioinformatics has made it easier to identify vaccine targets from sequenced biological data of organisms; research and the production of future veterinary vaccines have advanced. The time and expense associated with Vaccinology have been significantly reduced by using bioinformatics in discovering vaccine targets (Kaikabo and Kalshingi, 2007). Researchers can now use cutting-edge vet informatics techniques to create vaccines that offer defence against animal diseases (Kaikabo and Kalshingi, 2007; Mugunthan and Mani Chandra, 2021; Ganguly *et al.*, 2013). Thus, naked DNA vaccines were developed by identifying target antigenic peptides in a genomic sequence of pathogens (Kaikabo and Kalshingi, 2007).

Metabolomics can be used to determine predictors of inter-individual variability in drug response, give clinicians information to aid in drug selection decisions, and provide information on the pharmacokinetics and pharmacodynamics of a particular medication (RiveraVelez *et al.*, 2021). By

examining transcription start sites and promoter regions of bovine olfactory receptors using in silico analysis, five candidate motifs (MOR1, MOR2, MOR3, MOR4, and MOR5) that are crucial for gene regulation were discovered, according to Samuel and Dinka, (2020).

Both human and animal viruses have given rise to many bioinformatics tools that can help analyse genetic material related to the virus and design medically beneficial success plans that can avert disasters before they start. An Open Reading Frame (ORF) is a hereditary information unit translated into a protein. Viral genome analysis is the method by which ORF is found. It serves as the foundation for additional study, including searching for similar structures or positions, describing potential future events in proteins, functional analysis, therapeutic strategies to prevent viral diseases, and identifying virus-killing targets (Latchman, 2005). Bioinformatics may be utilised to pinpoint diagnostic issues and develop creative answers for the ongoing development and enhancement of molecular diagnostics (Kaikabo and Kalshingi, 2007). This is similar to Kumar (2003), who employed bioinformatics to find antigenic epitopes from the Rabies virus glycoprotein G that might be exploited to create an anti-rabies subunit vaccine.

Role of Bioinformatics in Animal Genetics

The field of bioinformatics creates and offers techniques and resources to assist biologists in identifying genuine signals, which are biological activity expressions mixed in a large data set. Veterinary science utilises tools and techniques from bioinformatics to enhance cattle breeds by utilising massive biological datasets to comprehend the system's genetics of complex traits (Kadarmideen,

2014). To identify functionally linked features that may contribute to genetic diversity, it is also helpful to detect different sequence variants, such as single nucleotide polymorphisms, copy number variations, insertions, and deletions (Daetwyler *et al.*, 2013). A growing need for food in the livestock industry necessitates improved production. Animal heredity is studied by animal genetics. People questioned how some diseases are passed down through the centuries, why children look like their parents, and what keeps them unique. Mendel initially recognised the idea that genes are passed down regularly through generations. Science knows that DNA molecules carrying sequences of four nucleotides (A, T, G, and C) are passed down from generation to generation. The genome, the blueprint for all biological structures and functions, contains the whole sequence information of an organism. The study of the genome is known as genomics (Cooper, 1994). It looks at the molecular data to comprehend disorders and natural variation. Large data sets, spanning terabytes, are produced by DNA sequencing and alignment, gene expression measurement techniques, and more intricate modelling studies like protein folding patterns. These sets would only be able to analyse and interpret with contemporary computational and machine-learning capabilities. One must possess mathematical and statistical expertise to properly apply such analytical techniques to studying biological systems. To sequence the genome of a domestic cow (Bos taurus), Zimin *et al.* (2009), combined whole-genome shotgun sequencing and hierarchical sequencing methods (Zimin *et al.*, 2009). Using sequence alignment and analysis, Ajayi *et al.* (2018) discovered sixty-seven genes in the bovine genome members of heat shock protein families. In pigs affected by mycoplasma

pneumonia of swine, Uemoto *et al.* (2021), used GWAS (Genome-Wide Association Studies) to identify six significant quantitative trait loci for immunerelated features, providing new insights into the genetic factors affecting pig production, respiratory illness, and immune-related qualities (Uemoto *et al.*, 2021). Another GWAS-based analysis found candidate genes for individual birth weight features in Korean Yorkshire piglets and milk production variables in Korean Holstein cattle (Kim *et al.*, 2021; Lee *et al.*, 2020). Therefore, GWAS-based methods can potentially unravel crucial and intricate features connected to cattle productivity (Pathak and Kim., 2022).

Applications of Bioinformatics in Research on Disease Resistance

The application of bioinformatics in research on disease resistance has led to significant advances in understanding the genetic basis of disease resistance and developing more resilient breeds.

Bovine Tuberculosis (BTB) is a significant disease affecting cattle worldwide, with implications for animal and public health. Recent studies have used bioinformatics tools to identify genetic variants associated with resistance to BTB. For example, GWAS and whole-genome sequencing have been used to identify SNPs associated with BTB resistance. At the same time, functional annotation and pathway analysis have provided insights into the biological mechanisms underlying this trait (Banos *et al.*, 2020). These findings could inform breeding programmes to improve resistance to BTB in cattle.

Marek's Disease (MD) is a viral disease that affects chickens, leading to significant economic losses in the poultry industry. Research on disease resistance

to MD has benefited from applying bioinformatics tools, such as QTL mapping and fine mapping, to identify genetic variants associated with resistance to MD. For example, Zhang *et al.* (2018) identified several candidate genes associated with resistance to MD using fine mapping and functional annotation. These findings could inform the development of more resilient chicken breeds.

Salmonella is a major pathogen that affects pigs, leading to significant economic losses and public health concerns. Recent research has used bioinformatics tools to identify genetic variants associated with resistance to Salmonella in pigs. For example, GWAS and genomic selection have been used to identify SNPs associated with resistance to Salmonella. At the same time, transcriptomic and proteomic data have been integrated into genomic prediction models to improve selection accuracy (Li *et al.*, 2019). These findings inform breeding programmes aimed at improving resistance to Salmonella in pigs.

Conclusion

Bioinformatics has transformed the field of animal breeding by providing tools to analyse genetic information in unprecedented detail. The application of bioinformatics in research on disease resistance has led to significant advances in understanding the genetic basis of disease resistance and developing more resilient breeds. By leveraging recent advances in genome sequencing, genomic selection, and QTL mapping, bioinformatics has the potential to accelerate breeding programmes, improve animal health, and enhance the efficiency of livestock production. However, challenges such as data management, interdisciplinary collaboration, and ethical

considerations must be addressed to realise bioinformatics's potential in animal breeding fully.

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