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PREFACE

It is with great enthusiasm that I present Advances in Veterinary and Agricultural Sciences, a comprehensive volume that encapsulates the latest breakthroughs and innovative perspectives in our shared pursuit of sustainable agricultural and veterinary practices. In an era marked by global challenges such as climate variability, food security, and zoonotic disease management, this book serves as a vital resource for researchers, practitioners, policymakers, and students alike. Drawing on diverse expertise from across the globe, it underscores the interdisciplinary nature of modern science, where veterinary medicine and agricultural innovation intersect to foster resilience and progress.

This collection comprises 24 meticulously curated chapters, each authored by leading scholars and experts who explore cutting-edge topics ranging from genomic advancements in livestock breeding to precision agriculture techniques and emerging therapeutic strategies in animal health. These contributions not only reflect the breadth of current research but also illuminate pathways for future discoveries, emphasizing practical applications that can transform farming systems and enhance animal welfare worldwide.

We are delighted to announce that Advances in Veterinary and Agricultural Sciences will be published under the auspices of ISRES in Turkey, a hub of scholarly excellence and international collaboration. This partnership amplifies our commitment to disseminating knowledge that bridges continents and cultures, ensuring that insights from this work reach a truly global audience.

In compiling this volume, I extend my deepest gratitude to all the chapter authors—the multiple voices behind these 25 insightful works—for their unwavering dedication and scholarly rigor. Their analyses and forward-thinking ideas form the cornerstone of this endeavor. I also sincerely thank the National Centre for Livestock Breeding, Genetics & Genomics (NCLBG&G), Pakistan, for their valuable contributions and support. Their work has greatly advanced livestock research and innovation, providing foundational expertise that enriches the discussions herein.

As editor, I hope this book inspires continued dialogue and collaboration, propelling the fields of veterinary and agricultural sciences toward a more equitable and sustainable future. Thank you for joining us on this journey.

November 2025

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Sustainable Livestock Breeding

Muhammad SAFDAR

Muhammad TANVEER

Mehmet OZASLAN

1. Introduction

Farmers are facing the impact of climate change that makes livestock farming more impulsive than ever (Beilin et al., 2012). For example, heatwaves, droughts and erratic weather patterns are affecting the availability of grazing land, water and feed that set immense pressure on farmers to adapt (Giridhar & Samireddypalle, 2015). Moreover, livestock production itself contributes to climate change that significant through methane emissions from cattle. This double challenge has led to a global push for sustainable livestock breeding and climate adaptation strategies to ensure that animals survive and thrive to reduce their environmental footprint (Nardone et al., 2010).

Therefore, farmers have bred animals for traits e.g. high milk yield, quality meat and fast growth (Hammond, 1947). Moreover, breeders now focus on heat tolerance, disease resistance and efficient feed use. In Africa and South Asia, hardy local breeds N'Dama cattle and Tharparkar cows thrive in extreme conditions with negligible resources (Ahlawat et al., 2015). Scientists are now integrating these traits into modern breeding programs that create resilient livestock to rise temperatures.

Genetics is playing a crucial role in sustainable livestock breeding. Researchers have identified genes that make cattle more heat-resistant (Osei-Amponsah et al., 2019). This allows them to maintain normal body temperatures even in extreme heat. Furthermore, some farmers in Australia and the U.S. are already crossbreeding local heat-tolerant breeds the Brahman cattle with high-yield dairy breeds to create animals that produce milk efficiently without suffer in heat stress (Usala, 2020). Therefore, genetic selection is also being used to reduce methane emissions. A recent discovery in Scotland led to the breeding of calves like Hilda that were significantly selected for their ability to produce less methane (Hellwing et al., 2019).

For instance, farmers are turning innovative farming systems to make livestock production more sustainable (Elzen et al., 2012). Silvopastoral farming that combines trees, shrubs and pasture to create a better environment for livestock (Jose & Dollinger, 2019). In Colombia, cattle farmers are using silvopastoral systems to provide natural shade and cooling for their animals that lead to higher milk yields and better meat quality (Huertas et al., 2021). These systems also improve soil

health, increase biodiversity and help capture carbon from the atmosphere that makes a solution for both farmers and the planet (Lal, 2016).

Feed efficiency is another key focus area. Moreover, traditional livestock feed production that significantly soy and corn farming has been linked to deforestation and land degradation (Thornton & Herrero, 2010). For instance, farmers are exploring alternative protein sustainable and affordable sources. In Zimbabwe, some farmers are pioneering maggot farming (Havlíček). Therefore, black soldier fly larvae transform organic waste into high-protein animal feed. Moreover, this offers a cost-effective sustainable feed for poultry and fish that efficiently managing waste and lowering livestock farming's environmental impact (Siddiqui et al., 2024).

In the dairy industry, researchers and farmers test with methane-reducing diets. Moreover, these studies show that seaweed supplements in cow feed cut methane emissions by up to 80% (Reyes et al., 2023). Therefore, some farms already use these supplements that show cows maintain productivity and emit significantly less methane. For example, biogas digesters are being used to capture methane and convert it into renewable energy that farmers use for electricity or heating (Kabeyi & Olanrewaju, 2022).

Sustainability, in livestock farming is also being driven in financial incentives and recognition programs. In Australia, the 2024 Farmer of the Year Awards emphasized beef and dairy farmers successfully applied carbon-neutral farming techniques (Küfeoğlu, 2024). These farmers have adopted precision feeding, rotational grazing and regenerative farming to reduce their carbon footprint to increase productivity. Therefore, their success stories are inspiring other farmers to follow suit that prove sustainable practices profitable and environmentally friendly (Fisk, 2010).

The transition to sustainable livestock farming requires strong support from governments, research institutions and consumers. Furthermore, farmers need access to financial resources, training and climate-resilient breeds to successfully implement these strategies (Singh et al., 2023). Governments play a role to support sustainable practices and invest in research to develop a better breeding and feeding techniques. Therefore, consumers drive sustainability in choose products from green farms that encourage businesses to adopt climate livestock practices (Bist et al., 2024).

Animal welfare is another critical aspect of climate adaptation (Narayan et al., 2021). For example, heat stress, disease outbreaks and water shortages decrease productivity of animal welfare. Moreover, farmers enhance housing, cooling and veterinary care to protect livestock health amid climate change. Therefore, some dairy farms in India are now using to keep cows comfortable during extreme summer heat that led to higher milk production and healthier animals (Das, 2018).

In addition to, climate change increases the future of livestock farming will depend on innovation, adaptation and collaboration. Moreover, sustainable breeding, better feed,

regenerative farming and strong policies are key to build a strong climate-smart in livestock industry. The challenge is significant that progress proves a sustainable, efficient and responsible livestock sector (Steenwerth et al., 2014).

2. Genetic Adaptation to Climate Change

Genetic adaptation dynamic for species to survive and adjust in climate change. Recent research climaxes in organisms genetically adapt to environmental changes that offer perceptions into their survival and biodiversity (Petrokas et al., 2020). Moreover, genetic accommodation permits phenotypic plasticity to evolve that allows organisms to adapt environmental changes. Levis and Pfennig (2019) explore evolved plasticity to use two methods: space-for-time swaps and experimental evolution. These approach help elucidate populations adjust to new climates in selecting for traits that enhance survival transformed conditions. The study emphasizes the significant role of divergence in environmentally responsive traits that facilitate limited adaptation along climatic gradients (Kremer et al., 2014).

Epigenetic changes switch genes on or off lack in altering DNA that help organisms adapt to climate change. Gugger et al. (2020) show that epigenetic changes help species cope with environmental stress (McCaw et al., 2020). These modifications enable rapid phenotypic adjustments that provide a flexible mechanism for cope up with change of climates. The study suggests to integrate epigenetic data with traditional genetic studies that offer a more comprehensive empathetic of adaptive processes (LaFreniere & MacDonald, 2013).

Genomics reveals species' climate adaptation. Bálint et al. (2020) shows genomics reveals animal adaptation and range shifts in warm climates. These studies emphasize the importance of genetic diversity and the potential for certain alleles to confer advantages new environmental pressures (Pius et al., 2024).

A systematic literature review conducted by Bay et al. (2024) identified 40 empirical examples of wild populations suffer the genetic adaptation in response to climate change (Bay et al., 2017). This comprehensive analysis emphasizes the prevalence of genetic changes a direct response to environmental pressures that importance of evolutionary processes in real-time. The study provides concrete instances genetic changes that have been documented to offer valuable case studies for empathetic adaptive mechanisms (Slavin & Kriegman, 1992).

Exposito-Alonso et al. (2024) explored changes in climate influence gene variations within a species that look link between allele frequencies and climate factors. The research aimed to test for genetic repeatability in resident adaptation to climate that provide visions into similar environmental pressures lead to convergent genetic solutions (Table 1). The study's finds help to predict species evolve the climate continues to change (Parmesan, 2006).

Table 1. Changes in Climate Variables of Relevance to Livestock Production

Sr.	Climate related variable	Projected change	Confidence in projection
1	No. of hot days and nights	Increase	Virtually certain
2	No. of cold Nights	Decrease	Virtually certain
3	No. of warm spells and heat waves	Increase	Very likely
4	No. of heavy precipitation events	Increase	Very likely
5	Extent of drought affected areas	Increase	Likely
6	Fire weather risk	Increase	Likely
7	Soil moisture	Decrease	Likely (Southern Australia

Shaw and Etterson (2022) explain it's tough to predict species will adapt to climate change, importance the roles of gene flow, genetic diversity and the speed of environmental changes. The authors climax that gene flow introduce beneficial alleles (Nielsen et al., 2021). Moreover, it also impedes resident adaptation to dilute specialized gene pools. This duality emphasizes the need for nuanced conservation strategies that consider species-specific genetic architectures and ecological contexts.

Genetic diversity in populations is a cornerstone of adaptive potential. A recent global analysis conveyed a significant decline in genetic diversity among more than 600 species over the past 30 years that mainly due to habitat destruction, climate change, invasive species and diseases. Genetic diversity helps species to adapt and survive that says researcher Catherine Grueber. The study also emphasized effective conservation efforts that genetic diversity was restored to determine the feasibility and importance of targeted actions (Weeks et al., 2011).

Chimpanzees have genes that help them flourish in different African habitats to

support their survival in diverse environments. A study of genetic data analyzed from 388 chimpanzees found that forest-dwelling populations exhibited changes in genes associated with disease resistance that mainly against pathogens. This parallel evolution in response to similar environmental pressures climaxes the elaborate relationship between habitat-specific challenges and genetic adaptation (Wiens, 1976).

Moreover, the erosion of genetic diversity poses significant challenges for conservation. Seed banks help and save the genetic material needed for plants to adapt in the future. For instance,

Kenya's National Gene Bank houses over 50,000 seed varieties that aimed to respond the loss of resilient traditional seeds (Ayieko et al., 2021). These sources serve the lakes of genetic diversity that offer resources to develop crop varieties capable of bear changed the climatic conditions.

Scientists discovered that species rapidly change their genes to adapt and survive in a changing climate. Researchers organized genetic, epigenetic and ecological data to better recognize species adapt to changing environments (Franks & Hoffmann, 2012). Therefore, these interdisciplinary approaches help us understand species will adapt to change environments and guide better conservation efforts.

3. Sustainable Breeding Strategies

Sustainable breeding strategies aim to develop livestock adapt to environmental changes that maintain productivity and minimum environmental impact (Hoffmann, 2010). These strategies focus on long-term genetic improvements to enhance resilience, efficiency and animal welfare. Breeders help livestock flourish in changing climates in selection of animals that handle heat better, resist diseases and make the maximum of their feed. Sustainable breeding boosts growth and protects nature of traditional farming. They maintain genetic diversity that reduce the risk of disease epidemics and enhanced adaptability (Qaim, 2020). These strategies also consider ethical aspects that ensure animal welfare and social acceptance. Farmers, researchers, and policymakers essential work organized for successful breeding programs. Moreover, sustainable breeding contributes to food security and environmental conservation. Therefore, it is a vital component of climate adaptation in livestock farming (Hoffmann, 2010).

Genetic selection is a powerful tool used to develop climate-resilient livestock. Furthermore, it's about breeding animals with strong traits that resist heat and disease to make them even better (Renaudeau et al., 2012). Genomic tools GWAS help pinpoint genes tied to climate resilience. For instance, certain cattle breeds possess genetic markers for effective heat dissipation and parasite resistance. These markers help in breed livestock to handle climate stress. Moreover, this approach reduces the need for external interventions that used in cooling systems or chemical treatments. They also enhance productivity and profitability for farmers. Therefore, genetic selection accelerates adaptation to change the environmental conditions. They ensured that livestock populations remain healthy and productive. This strategy is crucial for sustainable livestock breeding (Naskar et al., 2015).

Cross-breeding combines genetic traits from different breeds to create hybrids with enhanced performance and resilience. Moreover, this strategy leverages heterosis, or hybrid vigor that result in better growth rates, fertility and disease resistance (Dutta et al., 2024). For example, cross-breeding original breeds with commercial ones produces livestock that are both high-yielding and well-adapted to resident climates. Moreover, this approach maximizes productivity to maintain genetic diversity. Therefore, it enhanced adaptability to environmental stressors that temperature fluctuations and disease epidemics. They also optimize feed utilization to reduce resource requirements (Naskar et al., 2012). Therefore, this strategy supports sustainable

livestock systems to balance productivity with environmental conservation. They minimize dependency on chemical contributions of antibiotics and pesticides. Moreover, crossbreeding is a practical solution to enhance climate resilience in livestock. They contribute to food security and economic sustainability.

Livestock breeds are naturally adapted to resident environments that make them valuable genetic resources for climate resilience (Hoffmann, 2010). These breeds possess unique traits e.g. heat tolerance, disease resistance and efficient resource utilization. For example, African N'Dama cattle resist trypanosomiasis that a common tropical disease. These breeds maintain and conserve genetic diversity and enhances adaptability. They help them adapt a better climate challenge that add original genetics to modern breeding makes livestock stronger (Rauw & Gomez-Raya, 2015). They reduce reliance on external inputs and enhance sustainability. Community-based conservation programs allow limited farmers to preserve traditional breeds. Moreover, this approach maintains cultural heritage and promotes sustainable livelihoods. They also ensure that livestock systems remain resilient to climate change. Therefore, conservation of original breeds is essential for sustainable livestock breeding and biodiversity preservation (Hoffmann, 2011).

Assisted reproductive technologies (ART), artificial insemination (AI), embryo transfer and in vitro fertilization (IVF) enhance breeding efficiency. These technologies enable the rapid dissemination of superior genetics across livestock populations. For example, AI allows the widespread use of high-performance bulls that accelerate genetic improvement. IVF combines elite genetic materials that optimize to offspring performance (Gore, 2021). ART also supports the conservation of endangered breeds with single climate-adaptive traits. They reduce the need for animal transportation that minimize stress and disease risks. These technologies enhance breeding precision and genetic diversity. They accelerate adaptation to climate challenges enhanced productivity and resilience. ART Integrates with genomic selection to improve breeding products. Moreover, it strengthens food security and agricultural sustainability. ART is a powerful tool for sustainable livestock breeding (Menchaca, 2023).

Genomic selection uses DNA markers to identify animals with desirable traits on an early age. Furthermore, this approach enhances breeding accuracy and speed to accelerate genetic improvement. Marker-assisted selection (MAS) targets specific genes linked to climate elasticity in heat tolerance and disease resistance. For example, genomic studies have identified markers associated with tick resistance in cattle (Sinha et al., 2023). They select animals with these markers that breeders develop livestock with enhanced parasite resistance. Moreover, genomic tools also identify markers for efficient feed conversion and low methane emissions. Therefore, this precision breeding reduces environmental impact and improves productivity. They minimize trial-and-error in conventional breeding. Furthermore, genomic selection is cost-effective and enhances sustainability. They represent significant advancement in livestock breeding technology.

Community-based breeding programs (CBBPs) involve resident farmers in the breeding process to ensure traditionally appropriate and climate-resilient livestock (Recha et al., 2024). These programs influence indigenous knowledge for selection of animals best suited to resident conditions. They enhance food security and rural livelihoods to increase productivity and profitability. They maintain genetic diversity to preserve adaptive traits unique in resident environments. Moreover, this participatory approach ensures long-term adaptation and social acceptance. They reduce dependency on imported genetics and external inputs. They empower farmers and promote social equity. They are cost-effective and sustainable for smallholder systems. Therefore, this inclusive strategy enhances breeding success and community resilience. They contribute to sustainable livestock breeding and rural development (Kaasschieter et al., 1992).

For instance, breeding for disease resistance reduces reliance on chemical treatments to promote animal welfare and environmental sustainability. Selective breeding targets genetic traits linked to immunity and disease resistance. For example, certain sheep breeds possess genetic resistance to gastrointestinal parasites (Stear & Murray, 1994). Therefore, selection of these animals, breeders reduce the need for anthelmintics. Moreover, this approach minimizes chemical residues in food products and environmental contamination. They enhance livestock health and productivity. Furthermore, breeding for disease resistance also reduces veterinary costs. They support sustainable livestock systems to enhance resilience in disease epidemics. In addition to, this strategy contributes to food safety and public health. They are essential for sustainable livestock breeding.

Nutritional strategies complement sustainable breeding to enhance genetic potential. Moreover, balanced diets improve growth rates, reproductive performance and climate resilience(Hoffmann, 2010). For example, high-protein feeds enhance heat tolerance and immune function. For instance, selection of animals with efficient feed conversion ratios reduces resource use. Therefore, nutritional adjustments minimize methane emissions and environmental impact. Furthermore, this dual approach optimizes productivity and sustainability. They align breeding objectives with environmental conservation. In addition to, integration of nutrition with breeding enhances animal health and welfare. They reduce costs and improve profitability. This holistic strategy supports sustainable livestock systems (Gheorghe-Irimia et al., 2023).

4. Heat Stress and Livestock Productivity

Heat stress increases to concern in livestock production that significant in tropical and subtropical regions (Carvajal et al., 2021). Climate change driven temperature rise disrupts animal physiology and metabolism that lead to reduce health and productivity. Livestock have less ways to regulate their body temperature compared to humans that exit them very disposed in heat stress. The impact is not only in animal welfare and also on farm productivity and food security. The temperature rise beyond an animal's comfort zone initiates physiological responses,

e.g. increased respiration and sweating to promote cooling. Moreover, these mechanisms are often insufficient and lead to excessive heat accumulation. Furthermore, this hormone causes imbalances, oxidative stress and metabolic disorders to damage complete productivity (Chainy & Sahoo, 2020).

Moreover, heat stress has a major impact on reproductive efficiency in livestock. In dairy cattle, high temperatures disrupt hormonal cycles that make it difficult for cows to conceive (Sammad, Umer, et al., 2020). Furthermore, heat stress in pregnant cows significantly raises embryonic mortality to calve reduce success. Therefore, heat stress poses a serious threat to male fertility in bulls that leads to reduce sperm motility and viability in turn of reduces their reproductive effectiveness. In poultry, heat stress leads a higher incidence of early embryo mortality and reduced hatchability that ultimately decrease overall flock productivity (Biswal et al., 2022).

The significances of heat stress don't affect farmers. They also influence consumers to find in supermarkets. Animals that tolerate prolonged heat stress often produce lower-quality meat, affect its texture, taste and shelf life (Zhang et al., 2023). For example, heat-stress in pigs often yield pale, soft and exudative (PSE) meat that is watery and unappealing. Meat is a common in cattle and poultry that undergo heat stress before slaughter. These changes lead to financial losses for meat processors and lower consumer satisfaction.

Moreover, selective breeding offers a long-term solution to heat stress. Bos indicus breeds e.g. Brahman cattle have evolved physical traits that enhance heat tolerance (Naskar et al., 2012). Therefore, their short hair, loose skin and highly functional sweat glands allow them to regulate body temperature efficiently in hot climates. Genetic research has identified specific heat-tolerant genes in cattle to include the slick hair gene that enhance thermoregulation in reduce hair length and increase heat tolerance. Furthermore, advances in genomic selection allow scientists to incorporate these heat-resilient traits into commercial breeds without compromise of productivity (Cheruiyot et al., 2022). For instance, crossbreeding programs have already shown success Holstein cows bred with slick-haired genetics maintain higher milk production in tropical climates.

In addition to, livestock diet plays a crucial role in heat stress management. In hot weather, animals tend to eat less that result in weight loss and decreased productivity (West, 1999). Therefore, farmers modify feeding strategies to enhance digestibility and lower metabolic heat production. Furthermore, these diets help regulate body temperature more efficiently to minimize excess heat production in metabolism. Antioxidants are vitamin E and selenium that combat oxidative stress caused in heat. Electrolyte enhancements are potassium and sodium that help replace minerals lost through sweat to maintain hydration and support muscle function (Haftek et al., 2022). They recommend feeding in the cooler parts of the day to enhance food consumption. Hydration is equally important. Cows drink over 100 liters of water per day in extreme heat to make clean and cool water access essential for maintain their health and productivity.

Modern agriculture relies on shade that a primary strategy for heat stress management to integrate innovative approaches (Renaudeau et al., 2012). Moreover, evaporative cool pads and mist systems effectively lower heat stress in farm animals. These farms have implemented water-cooled parquet to enhance cattle comfort. Cross-ventilation and tunnel ventilation effectively regulate temperature to ensuring a cooler environment for livestock (Kuczynski et al., 2011).

Furthermore, a silvopasture system provides shade and improves animal comfort to promote environmental benefits (De-Sousa et al., 2023). Trees provide natural shade and cooling to reduce the heat load on animals that also improve soil moisture retention. Moreover, this approach has been mainly successful in regions of Latin America that farmers use silvopasture report for higher livestock productivity and better land sustainability. Carbon sequestration provides the additional benefit of contribute to climate change mitigation (Powlson et al., 2011). Technology plays an important role in livestock heat stress management. Moreover, these technologies improve agricultural resilience to predict heat waves and guide management decisions. Therefore, farmers use wearable sensors to monitor animal comfort and take preventive action against heat stress. Furthermore, farmers rely on automated cooling systems to regulate barn temperatures efficiently. These innovations make heat stress management more efficient and cost-effective (Namdeo et al., 2025).

Moreover, heat stress isn't a biological issue that has significant economic consequences. In India alone, heat stress leads an annual loss of 1.8 million tonnes of milk to cost the dairy sector over ₹2,661 crore ($320 million). In the U.S. and Europe, heat stress-related losses in cattle, poultry and pigs run into billions of dollars annually (Key et al., 2014). They risk financial instability of their productivity drops to a small margin. They rely on heat adaptation strategies not for sustainability and financial survival.

Therefore, heat stress is a major animal welfare concern that affect productivity and economics. For instance, heat stress in livestock causes exhaustion to reduce productivity and dehydration that lead to severe health complications or even death (Sammad, Wang, et al., 2020). Heat stroke result from prolonged exposure to high temperatures thar lead to serious health complications. Ethical farming practices require ensuring that animals have adequate cooling, hydration and shade. Consumer awareness rise and farms to prioritize animal welfare that gain stronger market support. Scientific research concretes the way for new solutions to heat stress (Bozzo et al., 2021).

In addition to, researchers explore heat-resistant microbiomes gut bacteria help animals digest food more efficiently to reduce internal heat production. CRISPR-based gene editing offers the potential to accelerate the development of heat-tolerant livestock without genetic trade-offs (Hill & Li, 2022). Additionally, thermal imaging and AI-assisted diagnostics are being developed to detect early signs of heat stress before visible symptoms appear.

Moreover, one of the biggest challenges in heat stress management the lack of knowledge among smallholder farmers. Many farmers are unaware of the early signs of heat stress and affordable adaptation techniques (Mertz et al., 2009). Therefore, investment in education, training programs and extension services is critical to equip farmers with the skills needed to protect their animals and sustain their operations. Governments and agricultural organizations essential prioritize knowledge sharing initiatives to ensure that all farmers regardless of their resources adapt to rise temperatures (Stathers et al., 2013).

Furthermore, heat stress in livestock is not limited to one country or region it's a global issue. For instance, countries with extensive experience in supervision extreme temperatures e.g. Australia, Brazil and parts of Africa that serve models for other regions (Rogers & Tsirkunov, 2013). International collaboration in research, policy-making and technology development will be essential for building a resilient global livestock industry. Heat waves and prolonged high temperatures will become extra common to climate change accelerates. The livestock industry essential take practical steps to invest in heat-tolerant breeds, climate-smart nutrition, advanced cooling technologies and improved management strategies. Future success in livestock farming will depend on fine we adapt today. Therefore, the combination of science, technology and traditional knowledge create an extra-resilient, sustainable and ethical livestock industry that thrives even in a warming world (Schuster, 2023).

5. Resilient Breeds and Genomic Approaches

Climate change has significant impacted in livestock production to necessitate the development of resilient breeds through advanced genomic approaches (Hoffmann, 2010). Temperature fluctuations, pathogen evolution and ecological shifts drive the need for cutting-edge approaches to sustain animal productivity and welfare. Breeders influence genetic perceptions to identify and enhance traits that improve livestock adaptation to harsh climates (Naskar et al., 2012). Moreover, these traits include heat tolerance, disease resistance, efficient feed conversion and reproductive robustness. Sustainable breeding programs must integrate both traditional selection methods and modern genomic technologies to maintain genetic diversity enhance resilience (Lammerts van Bueren et al., 2018). The success of these programs relies on interdisciplinary collaboration among geneticists, veterinarians and livestock producers. A comprehensive empathetic of climate adaptability mechanisms is essential to ensure long-term sustainability. The application of genomic tools significant to accelerate the breeding process that offer a target and efficient approach (Fatima et al., 2024). Scientists utilize genetic research to identify traits that support livestock survival in harsh climates. Recent studies have emphasized the importance of identify genetic markers connected with climate adaptation in livestock species. For example, research on beef cattle has revealed genes linked to thermotolerance, cellular physiology, DNA repair mechanisms, immune function and fertility (Worku et al., 2023). Furthermore, these genetic markers enable breeders to select animals with superior adaptation traits to ensure productivity below extreme environmental conditions.

Genome-wide association studies (GWAS) and whole-genome sequencing (WGS) have played a pivotal role in finding these adaptation-linked genetic variants (Gadji et al., 2024). Moreover, transcriptomic and epigenetic studies provide perceptions into gene expression changes in response to environmental stressors. Genetics provides perceptions that allow the development of precision breeding strategies for adaptation. The integration of high-throughput genotype and bioinformatics tools additional enhance the accuracy of selection. Therefore, these advancements emphasize the transformative potential of genomic approaches in sustainable livestock breeding (Gadji et al., 2024).

In addition to, one of the most advancements in livestock genomics is the application of genome editing technologies of CRISPR/Cas9 (Jabbar et al., 2021). Moreover, this powerful tool allows for precise modifications of specific genes to introduce desirable traits e.g. disease resistance, heat tolerance and improved productivity. For instance, scientists have effectively used genome editing to introduce the polled (hornless) trait in cattle that reduce the need for painful dehorning procedures (Mueller et al., 2021). Additionally, genome editing has been explored to enhance resistance to viral and bacterial infections in livestock species. Therefore, these genetic modifications hold great potential, ethical considerations and regulatory challenges sensibly addressed. Public perception and consumer acceptance also play a crucial role in determined of future adoption of genome-edited livestock (Sadikiel Mmbando, 2024). Researchers ensure transparency and engage stakeholders to promote the responsible use of these technologies. Moreover, genome editing represents a innovative approach to enhance livestock resilience and sustainability despite these challenges.

In developing countries, breeding programs increased focus on improve indigenous cattle breeds to enhance their adaptation in change of climates. for instance, Ethiopia has initiated genetic improvement programs target resident cattle populations (Hoffmann, 2010). Moreover, these programs aim to conserve genetic diversity select for traits that confer resilience to environmental stresses. In addition to, indigenous breeds often possess exclusive genetic adaptations that make them well-suited for specific climatic conditions (Naskar et al., 2012). Researchers identify and preserve these adaptive traits to leverage genomic tools. Furthermore, this approach not only ensures the survival of resident breeds that enhances their productivity and economic value. Sustainable livestock breeding in developing regions requires a balance between genetic conservation and performance improvement (Kaasschieter et al., 1992). Policymakers support researchers and farmers in implementation of effective breeding strategies through collaboration. Countries enhance food security and strengthen rural economies to prioritize local breed improvement (Rege et al., 2011).

In addition to, genomic selection has emerged a powerful tool for improve of livestock adaptability to climate change. Moreover, genetics provides the foundation for genomic selection to allow breeders that assess an animal's potential (Gutierrez-Reinoso et al., 2021). Furthermore, this approach significant increases the accuracy for the selection of low-heritability traits in heat tolerance and disease resistance. These values serve a powerful tool for breeders that help them

optimize selection strategies and maximize genetic improvements. The application of genomic selection in dairy cattle has demonstrated substantial improvements in milk yield, fertility and resilience to environmental stressors. Additionally, genomic selection allows for the early identification of superior breeding candidates to reduce the reliance on time-consume progeny testing. The genomic databases continue to expand that the predictive power of genomic selection improve. This innovation emphasizes the need for integration of genomics into livestock breeding programs worldwide (Husien et al., 2024).

In addition to, the conservation of livestock genetic resources is critical for long-term sustainability. Traditional breeds possess valuable genetic traits that allow them to thrive in specific environmental conditions. Moreover, the rapid increase of livestock production has led to the loss of genetic diversity (Notter, 1999). For instance, genomic tools offer a viable solution to identify and preserve these unique genetic resources. The genetic makeup of indigenous breeds provides essential information for researchers to design conservation strategies that preserve their adaptive potential (Frankham, 2010). Semen and embryos preserved through cryopreservation offer valuable genetic resources for breed restoration efforts. Additionally, genomic-assisted breeding programs help reintroduce lost genetic diversity into commercial populations. This preservation supports sustainable livestock farming to enhance adaptability in environmental changes (Pasipanodya et al., 2022). Governments need to establish joint initiatives to preserve these valuable genetic resources.

Moreover, livestock breeding strategies also address the emerging disease threats exacerbated to climate change. Global temperatures rise to contribute in the emergence of new infectious diseases and the resurgence of old ones (Zell, 2004). Therefore, genomic research has identified genetic variants associated with disease resistance in various livestock species. For example, certain cattle breeds exhibit natural resistance to tick infestations that a major issue in tropical and subtropical regions. Breeding programs that integrate genetic perceptions allow livestock producers to enhance disease resilience in their herds (Bishop & Woolliams, 2004). Vaccination strategies also complemented to genetic selection for immune robustness. The combination of genomics and veterinary sciences presents a holistic approach to disease management in livestock. Climate-related disease risks continue to evolve genomic research that play a crucial role in emergent of proactive solutions (Leal Filho et al., 2025).

In conclusion, genomic approaches revolutionize in livestock breeding by to enhance resilience of climate change. They offer practical solutions for improving adaptation traits that from genome editing to genomic selection (ASLAM et al., 2024). Sustainable livestock breeding integrates genetic conservation, ethical considerations and technological advancements. It ensures a long-term sustainability in the face of environmental challenges to prioritize resilience and productivity. They are essential to attach the full potential of genomic tools for climate adaptation. They contribute to global food security to modify the impacts of climate change with a proactive approach (Ingram, 2011).

6. Future Perspectives in Climate-Smart Breeding

Climate change continues to challenge global food systems that livestock sector adapts to ensure both sustainability and productivity. Moreover, climate-smart breeding emerged a game-changing approach, leveraging scientific innovations and traditional wisdom to create resilient, efficient and environmentally friendly livestock (Serageldin). They are shaping the future of livestock breeding to integrate advanced genetics, artificial intelligence and sustainable practices. Moreover, climate change brings extreme temperatures, new diseases and impulsive weather patterns that threaten of livestock productivity. Scientists are now identifying and selecting genes associated with heat tolerance, disease resistance and reduced methane emissions. This approach allows us to develop herds that thrive in harsh environments to reduce their environmental footprint (Herrero et al., 2015).

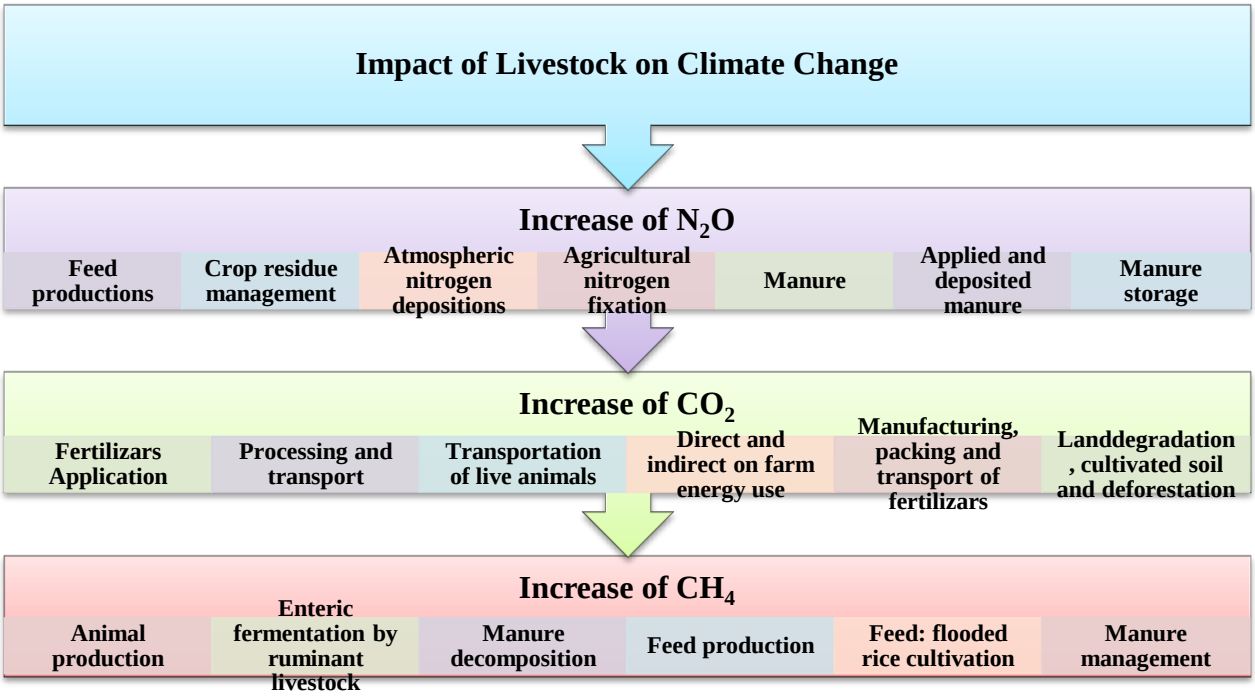
AI is revolutionizing the livestock industry analyze massive datasets to identify optimal breeding combinations. Moreover, AI-powered algorithms predict an animal's ability to survive heat stress or efficiently convert feed that help farmers make data-driven decisions to enhance resilience and productivity (Rebez et al., 2024). Traditional breeding methods rely on observable traits and genomic selection goes deeper to analyze DNA markers. Furthermore, this approach allows breeders to select animals with the best genetic potential for climate adaptation that ensure faster progress toward more resilient livestock populations (Hoffmann, 2010). Indigenous and local farmers have long tacit that breeds perform best in their regions. The combination of practical knowledge and modern genetic tools enables the creation of tailored breeding strategies that respect cultural wisdom to enhance livestock adaptability (Naskar et al., 2015). Policies are essential to encourage investment in climate-smart breeding. Governments worldwide are introducing new frameworks to facilitate responsible genetic advancements in gene-editing regulations that ensure both safety and sustainability (Wray-Cahen et al., 2022).

The success of climate-smart breeding depends on its adoption of the farm level. They help farmers to understand and implement these innovations. Moreover, climate-smart breeding economically viable for widespread adoption. They encourage farmers to invest in resilient breeding (Steenwerth et al., 2014). Modern consumers are more conscious of their food comes from. Public education on the benefits of climate-smart livestock drive demand. Therefore, climate-smart breeding is not just a local solution it's a global necessity. International research partnerships and cross-border breeding programs allow countries to share expertise and resources to accelerate progress toward resilient livestock worldwide (Thongoh-muia, 2022). For instance, effective monitoring and evaluation systems ensure that climate-smart breeding programs deliver concrete results. They continuously refine strategies for better products.

Sustainability should be the foundation of all breeding objectives to ensure long-term environmental health, resource efficiency and resilience to change climatic conditions (Lammerts van Bueren et al., 2018). Breeders can create animals that not only survive that thrive

in change of climates to prioritize traits are drought resistance, efficient feed conversion and lower methane emissions (Hoffmann, 2010). Moreover, genetic innovations hold great promise that ethical considerations remain central to breeding programs. Animal welfare, genetic diversity and public trust are key to sustainable and responsible advancements in livestock breeding (Wray-Cahen et al., 2022). Blockchain for traceability and precision farming tools that automated climate-controlled shelters to enhanced the efficiency of climate-smart breeding. These innovations ensure that animals receive optimal care to reduce the environmental impact of livestock farming (Vlaicu et al., 2024) as shown in figure 1.

Figure 1. Impact of Livestock on Climate Change



Furthermore, sustainable livestock breeding is only effective the entire supply chain from farm to fork supports it. Moreover, it ensures that consumers have access to responsibly bred livestock products in strengthening climate-friendly processing, distribution and retail networks (Neethirajan, 2023). Research into areas of epigenetics, microbiome science and alternative protein sources refine climate-smart livestock breeding. These innovations hold immense potential to improve resilience that reduce emissions and enhance food security in the years ahead (Rao et al., 2020). The future of livestock breeding lies in innovation, sustainability and collaboration. This approach helps mitigate climate change effects to secure the future of animal farming. The path forward requires a balance of cutting-edge science, economic incentives and ethical considerations (Neethirajan, 2024).

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Molecular Genetics and their Advances

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Mehwish IQBAL

Nighat MUSHTAQ

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1. Introduction

Add to it that molecular genetics and DNA marker technologies have recently changed the whole face of genetic research, breeding programs, and diagnosis applications in the last few years. The latest genomic tools provide a high throughput for developing accurate genetic analysis to discover novel markers associated with the desired traits. Next-generation sequencing (NGS) and single nucleotide polymorphisms (SNPs) are among the highly promising and disruptive technologies that have brought revolutionary improvements related to resolution and efficiency in genetics. The present chapter introduces up-to-date DNA marker technologies with special focus on their fundamental principles, diverse applications, and influences over the changing face of genetics today.

1.1 Introduction to Molecular Genetics and Genomics

Molecular genetics is a discipline dealing with the study of gene structure and function at the molecular level, including DNA sequencing, gene expression, and genetic variation (Griffiths et al., 2020). With molecular genetics allowing scientists to investigate genetic materials with high precision, the paradigm of heredity, evolution, and disease mechanisms have been changed with significant insights. Genomics, being an extension of molecular genetics, penetrates the complete assessment of entire genomes, both coding and non-coding regions, for deciphering their roles in biological processes (Watson et al., 2014). NGS technologies have further helped in expediting genomic research by performing genome-wide studies in a fast and economical way.

1.2 Evolution of DNA Marker Technologies

The advent of DNA marker technologies has brought about many changes since the first-generation marker was developed in the 1980s (Botstein et al., 1980). Among the earliest were the RFLPs, which are DNA-based polymorphism markers based on their detection by restriction enzyme digestion and Southern blotting. Although being laborious, RFLPs provided the basis of genetic linkage mapping and disease association studies (Jeffreys et al. 1985). List of markers are herewith (Figure 1).

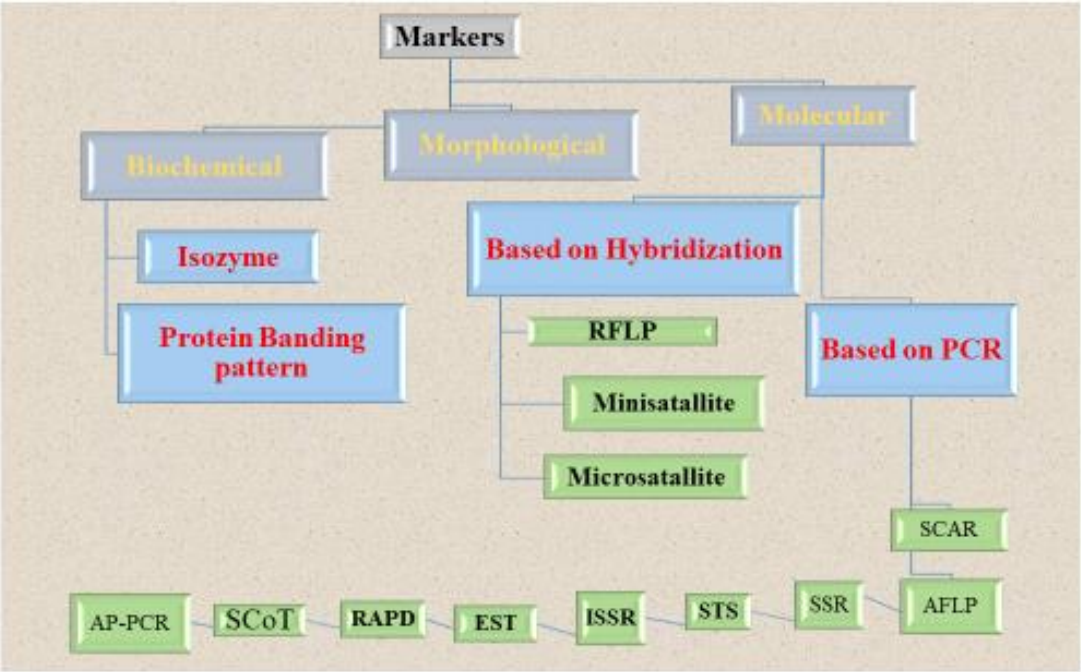

Figure. 1 Molecular Marker Systemic Roadmap

New developments in technologies such as genotyping-by-sequencing and CRISPR-based genotyping are helping to refine these DNA marker technologies further (Elshire et al., 2011). The combination of NGS and marker analysis allows ultra-high-throughput genotyping, thus permitting the screening of markers in the thousands (Davey et al., 2011). These recent advances have diversified applications in precision agriculture, with MAS aiding rapid crop breeding, and clinical genetics, where they play a role in the discovery of variants linked to diseases (Gupta et al., 2017). The future of DNA marker technologies is real-time sequencing, portable genomic devices, and AI-based genetic prediction models to further improve their utility across a variety of fields (Shendure et al., 2017).

2. Cutting Edge DNA Marker Technologies

2.1 Next-Generation Sequencing (NGS) and its Impact on Genetic Markers

Next-generation sequencing has been a blessing in the field of genetic research, as it has turned the procedure into a very rapid, economical, and high-throughput method. Contrary to Sanger sequencing, parallel sequencing of millions of fragments of DNA is enabled using various platforms like Illumina, Ion Torrent, and Oxford Nano pore. This allowed the discovery of innumerable markers and genomic studies (Metzker 2010). With the new technology, newly developed genetic markers, including SNPs, InDels, and structural variations, have also been detected and are useful in gene association studies (GWAS) and marker-assisted selection (MAS) for crop and livestock improvement (Varshney et al. 2014) as shown in Figure 2.

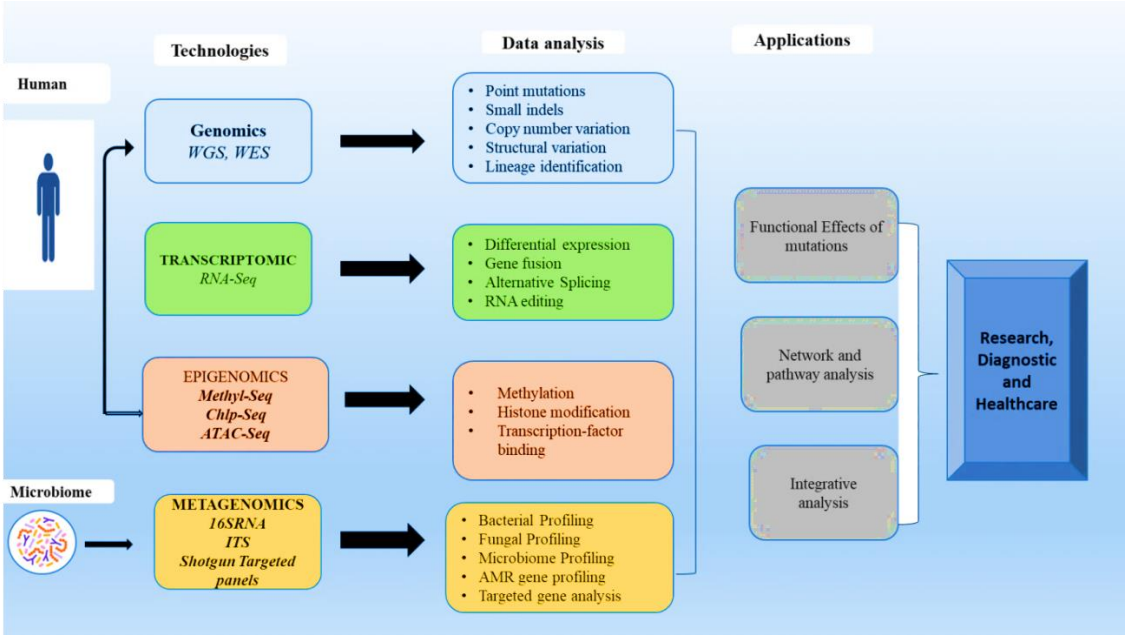

Figure. 2 Various Approaches Used for Genome Analysis and Applications of NGS

With the advances in next-generation sequencing (NGS), high-density genetic mapping using genotyping-by-sequencing (GBS) methods, or genome-wide resampling, are saving time and enhancing the efficiency of QTL mapping (Elshire et al., 2011). NGS also allows targeted, low-complexity marker discovery with good genomic coverage for RAD-seq (restriction-site associated DNA sequencing) and exome sequencing (Davey et al., 2011). Integrating bioinformatics tools with NGS have assisted in data analysis and enabled the identification of functional markers for disease resistance, yield, and stress tolerance in plants and animals (Goodwin et al., 2016).

2.2 Single Nucleotide Polymorphisms (SNPs) and High-Throughput Genotyping

Single nucleotide polymorphisms (SNPs) are the most popular of all genetic markers because of their high density, stability, and ease of use, especially in association with phenotypic variation (Rafalski, 2002). SNPs are often regarded as a single base change at some specific genomic locus that can be easily detected by high-throughput genotyping platforms, such as SNP arrays, Competitive Allele-Specific PCR (KASP), and TaqMan assays (Syvanen, 2005). Such technologies establish extensive SNP screening which allows the genome-wide scans for trait-marker associations in different species (Gunderson et al., 2005). New bioinformatics and statistical applications have further advanced SNP data analysis for exact haplotype mapping of rare variants (Browning & Browning, 2011). SNP genotyping combined with machine learning algorithms also enhances predictive breeding improvement for the faster development of superior genotypes (Crossa et al., 2017) as shown in Figure 3. As more and more affordable high-throughput genotyping systems become available, it can be expected that SNP-based markers will remain at the forefront of molecular genetics and precision agriculture activities.

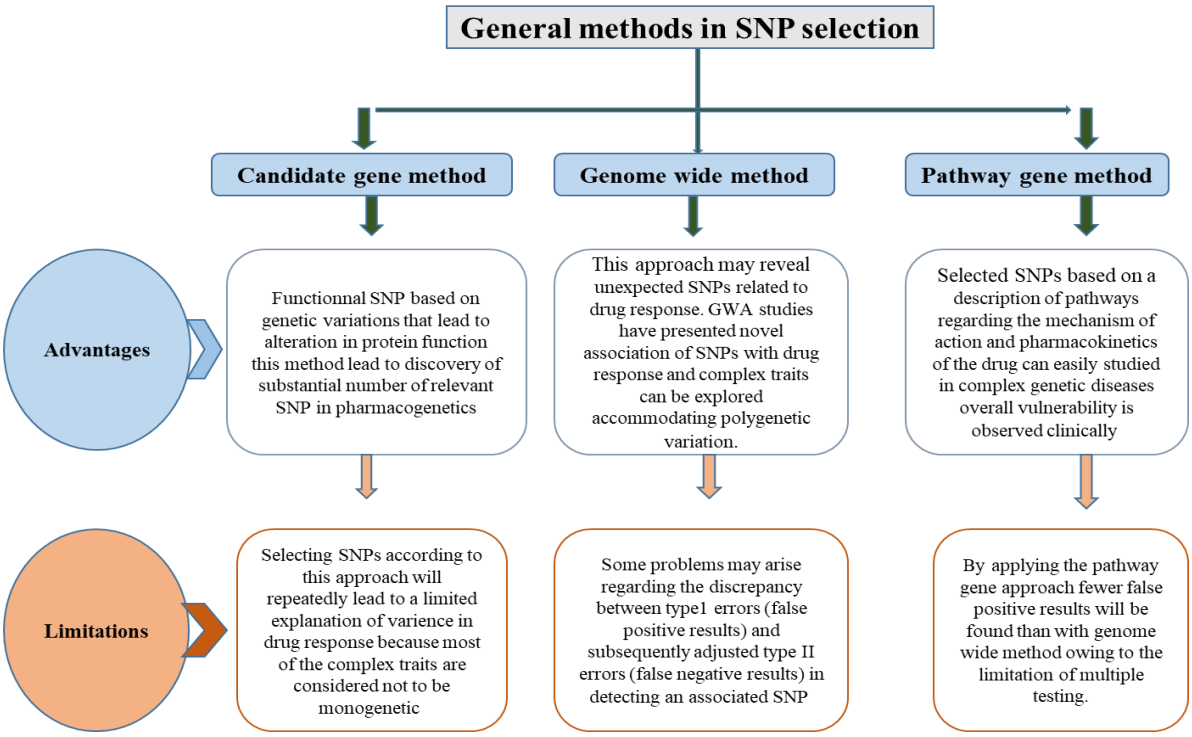

Figure. 3 Selection of SNPs and Their Different Pathways

3. Innovations in Molecular Genetic Techniques

Molecular genetics seems to have changed the face of the understanding of genomic architecture, gene regulation, and ultimately phenotypic expression. State-of-the-art technologies such as whole-genome sequencing (WGS), epigenetic profiling, and multi-omics approaches enable researchers to draw inferences on the very complicated genetic mechanisms underlying complex traits and diseases. Developing techniques create wide new applications for them in medicine, agriculture, and evolutionary biology from precision breeding to personalized medicine and to biomarker discovery. This section explores major advances in molecular genetic technologies, focusing on the principles, applications, and potential transformation they provide in genetic research.

3.1 Whole Genome Sequencing (WGS) and Its Applications

Whole-genome sequencing (WGS) has emerged as a cornerstone of modern genomics whereby genetic variation, structural rearrangements, and functional elements across the whole genome can be studied in an integrated manner. In contrast to targeted approaches, WGS provides a high-resolution assessment of the genome for detecting single nucleotide polymorphisms (SNPs), insertions and deletions, and copy number variations (CNVs) (Goodwin et al., 2016). The falling costs of next-generation sequencing (NGS) technologies have made WGS accessible for large-scale population studies, rare disease diagnostics, and cancer genomics (Shendure et al., 2017) as shown in Figure 4.

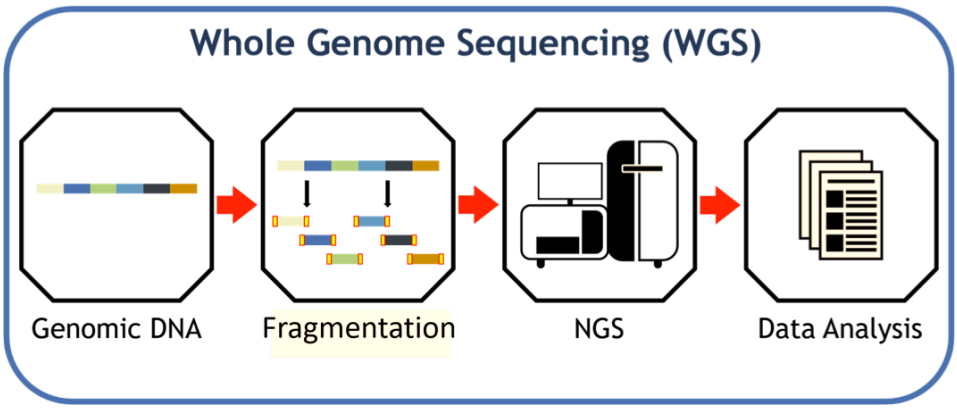

Figure. 4 *Whole Genome Sequencing (WGS) Comprehensive Method*

A major use of whole-genome sequencing (WGS) is in precision medicine, where it assists in the identification of disease-causing mutations and the consequent tailoring of therapeutic interventions. For example, WGS has provided formidable evidence towards the identification of novel genetic variants involved in single-gene disorders and in complex diseases like diabetes and cardiovascular disorders (Biesecker and Green, 2014). Current day, WGS is also significantly involved in agricultural genomics through its roles in the supporting of marker-assisted selection (MAS) and genomic prediction for crop and livestock improvement (Varshney et al., 2021).

3.2 Epigenetic Markers: DNA Methylation and Histone Modifications

Under histone modifications and DNA methylation, epigenetic modifications are instrumental in gene regulation without changing the DNA sequence. These are inheritable changes, causing cellular differentiation, development, and disease susceptibility by changing the chromatin structure and transcriptional activity (Bird, 2007).

Epigenetics have acquired importance as cancer markers for early detection, prognosis, and therapeutic targeting. Abnormal DNA methylation patterns, including hyper methylation of tumor suppressor genes, present biomarkers for various cancers (Baylin & Jones, 2016). In like manner, HDAC inhibitors are being tested as epigenetic drugs to reactivate silenced genes in cancers (Dawson & Kouzarides, 2012). Outside neoplastic studies, epigenetic studies have correlated environmental exposures, including dietary and stressors, with transgenerational inheritance of disease risk (Heard & Martienssen, 2014).

3.3 Proteomics and Metabolomics in Genetic Marker Development

Proteomics and metabolomics complete genomics through offering associated functional insight into proteins and metabolic pathway activity thus, providing the bridge between genotype and phenotype. Mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy-form high-throughput technologies that are crucial for identification of proteins and metabolites

(Aebersold & Mann, 2016). These approaches contributed to the development of biomarkers for diabetes, neurodegenerative diseases, and cancer (Patti et al., 2012) as shown in Figure 5.

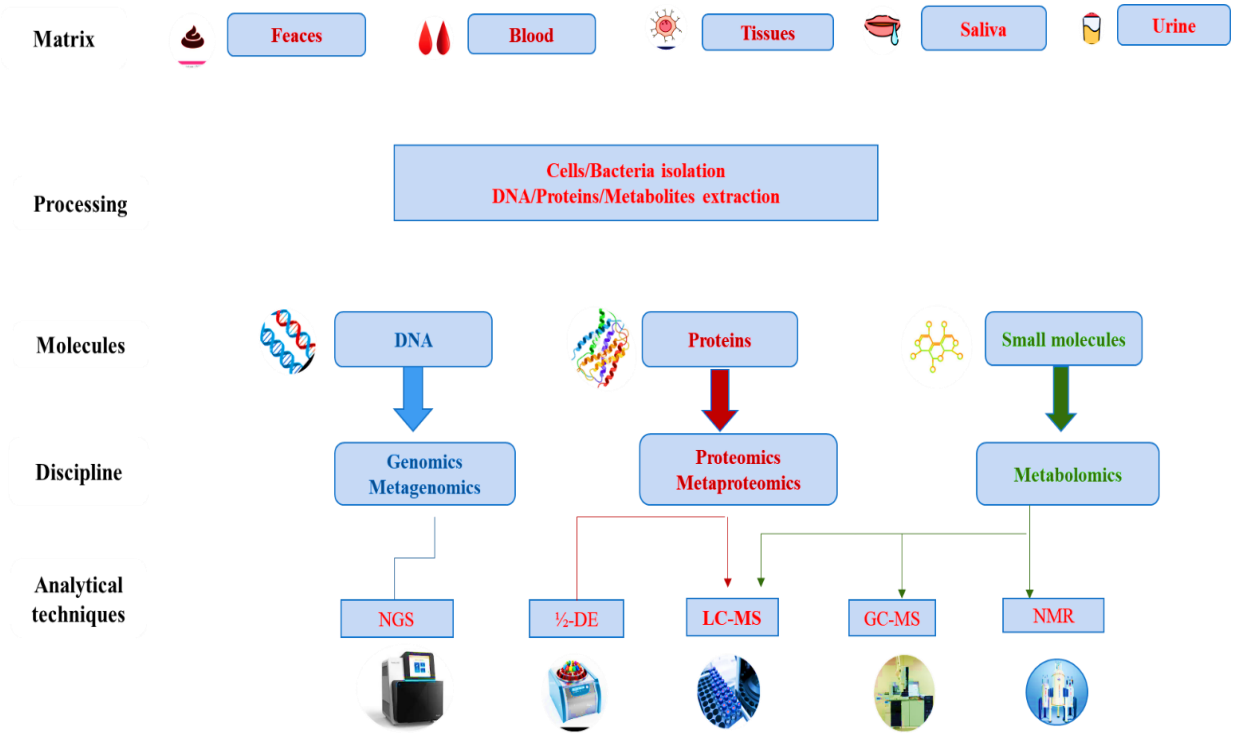

Figure. 5 *Proteomics and Metabolomics Approaches*

In the future, single-cell proteomics and spatial metabolomics promise resolution unparalleled for studying cellular heterogeneity (Kelly et al., 2019). With the further development of these technologies, their integration with AI-driven analytics will expedite biomarker discovery and applications in precision medicine.

4. Applications of DNA Markers in Research and Industry

The research and industrial horizon in many disciplines is a result of the technological improvements that have occurred in molecular genetics and DNA marker technology. DNA markers, such as SNPs, SSRs, or AFLPs, are some mighty tools in genetic analysis that allow precise genome mapping, trait association, and biodiversity analyses. Their applications range from agriculture and conservation biology to forensics and medicine, with unmatched abilities to improve molecular genetics diagnostics and breeding programs. This section discusses how DNA markers contribute to crop improvement, agricultural biotechnology, and conservation genetics, which are some of the main pillars of the developments in modern science and industry.

4.1 DNA Markers in Crop Improvement and Agricultural Biotechnology

So, the DNA marker studies speak volumes in the development of crops intended for production of new varieties with high yield, disease resistance, and resilience against climate. Marker

assisted selection (MAS) thus dubs the selection of best individuals on the progeny with easy introgression of traits into the chosen cross to minimize breeding time and costs while comparing it with traditional methodologies. For example, SSR and SNP markers play key roles in the identification of the stress tolerance traits in major crops: rice, wheat, and maize (Collard & Mackill, 2008). Another dynamic approach is called genomic selection (GS), which makes use of data from high-density markers to predict breeding values, thus accelerating genetic gain in crops such as soybean and barley (Meuwissen et al., 2016).

All the more, next-generation sequencing (NGS) and eventually other DNA marker technologies invigorated precision breeding purports to case-in-point like genome-wide association study (GWAS). That is, SNP arrays could identify novel QTLs (Quantitative Trait Loci) for drought tolerance in crops-like sorghum and chickpea (Varshney et al., 2014). This shows how radical the future is concerning DNA markers and global food security under climate change and population pressure.

4.2 Conservation Genetics and Biodiversity Studies Using DNA Markers

The whole structure of conservation genetics has been revolutionized by DNA markers which have provided a set of accurate tools for assessing genetic diversity, population structure, and evolutionary dynamics in relation to the endangered species. Microsatellite and mitochondrial DNA (mtDNA) markers are commonly used in the assessment of genetic bottlenecking and inbreeding depression in respect to wildlife populations so as to allow for the formulation of effective conservation strategies (Frankham, 2010).

One of the promising techniques in biodiversity studies is DNA barcoding using either cytochrome oxidase I (COI) or other short standardized gene regions for rapid species identification and discovery. Most importantly, it has helped in the cataloging of cryptic species in tropical rainforests and marine ecosystems (Hebert et al., 2003). Environmental DNA(eDNA) markers that may function in non-invasive monitoring of the presence of species in aquatic and terrestrial habitats can also be a part of baseline ecosystem management and the detection of invasive (Thomsen & Willerslev, 2015) as shown in figüre 6.

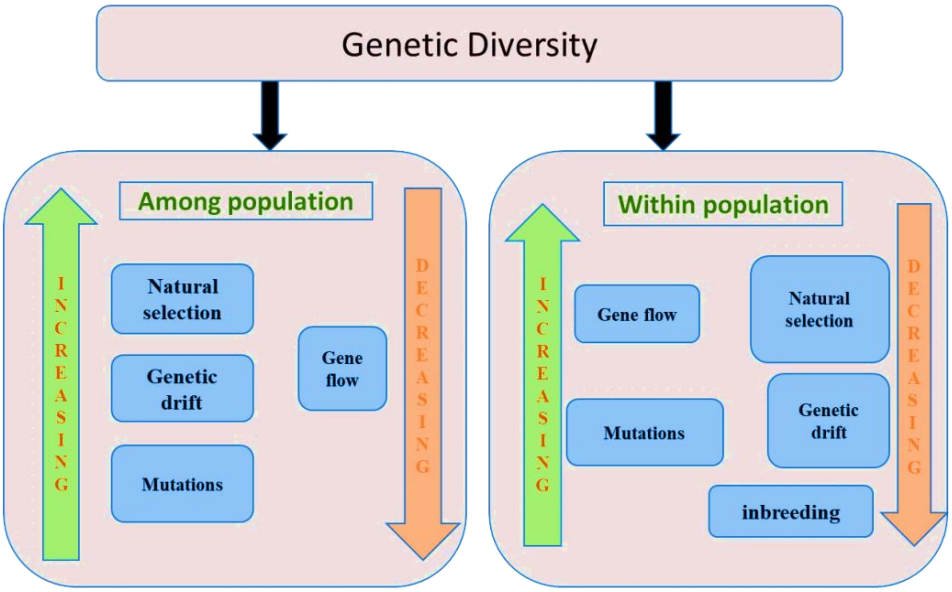

Figure. 6 *Genetic Diversity: Sources, Threats and Conservation*

5. Challenges and Future Perspectives

5.1 Ethical and Legal Issues in Genetic Marker Technologies

The swift rise of genetic marker technologies evokes serious ethical and legal problems with regard to privacy, consent, and discrimination based on genetics. Their evident usage of genomic data in research and clinical sectors calls for strict regulation to protect individuals from the misuse of their genetic information. For example, currently, direct-to-consumer genetic testing companies facing scrutiny regarding the security of their data and the potential of sharing sensitive information without consent (Hazel & Slobogin, 2018). Moreover, the use of DNA markers in forensic investigations raises ethical issues such as familial searching and the risk of false conviction (Samuel & Prainsack, 2019). In the U.S., the Genetic Information Nondiscrimination Act (GINA) is an example of a legal framework that aims at preventing genetic discrimination in employment and health insurance; however, gaps in enforcement remain worldwide (Rothstein, 2021).

Yet another issue that begs for an answer is the ethical reputation of gene-editing technologies such as CRISPR-Cas9 that use such DNA markers for precise fixing. Though these technologies are expected to offer cures for genetic disorders, their application for germline editing raises fears of unforeseen effects and designing of babies (Doudna & Sternberg, 2017). The genetic data commercialized from indigenous peoples, without proper consent, are often contested, reinforcing calls for inclusive and equitable policies (TallBear, 2013). To manage these challenges in ethics and law, there is a need for international cooperation, funding, and open public policy to secure responsible innovation in genetic marker technologies.

5.2 Emerging Trends: Nanopore Sequencing and Quantum Genomics

One such example of futuristic trend is quantified genomics in which quantum computing is employed to genetic analysis in order to solve complex biological problems. Quantum algorithms can speed processes like genome-wide association studies and protein-folding prediction unlike anything before them (Cao et al., 2018). Yet, problems concerning quantum decoherence and error correction remain as significant hurdles toward real applications (Arute et al., 2019). These technologies once matured will form boundary lines into which genomic research and precision medicine will traverse.

5.3 Future Prospects of DNA Marker Technologies

DNA marker technology is poised to develop with greater accuracy and cost-effectiveness and advanced applications under artificial intelligence (AI). Currently, next-generation sequencing (NGS) platforms are getting faster and cheaper to favor large-scale efforts of population genomics such as the All of Us Research Program in the U.S. (Denny et al., 2019). An attractive alternative is the application of environmental DNA (eDNA) markers in biodiversity monitoring and conservation. By harvesting tiny pieces of genetic materials from ecosystems, researchers can infer where in that ecosystem a species was present without having seen it in person (Thomsen & Willerslev, 2015). Advances in single-cell genomics, on the other hand, allow investigators to obtain insights into genetic heterogeneity within tissues, thus providing clues to cancer evolution and stem cell dynamics (Navin, 2015). The implementable interdisciplinary approaches will certainly help advance DNA marker technologies that would in turn stimulate innovation across different scientific disciplines, thus defining the future of genetics and biotechnology.

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Livestock Breeding Systems and Strategies

Muhammad ASIM

Fakhar-un-Nisa

Faisal RAMZAN

Improving livestock is always a strong desire of animal breeders. Animal breeding involves selecting male and female individuals within a breed or species based on their desired genetic traits and encouraging them to reproduce sexually to produce offspring that express these traits. Various breeding systems are used to promote and conserve the desired genetic traits within a breed or species. In these breeding systems, humans breed animals to produce offspring with the desired phenotypic traits and characteristics. Different mating systems are practiced worldwide, such as random mating, inbreeding, line breeding, outbreeding, crossbreeding, grading up, and phenotypic assortative mating, including positive assortative mating (like likes) and negative assortative mating (opposite individuals mating), etc. Additionally, artificial insemination (FTAI), sexed semen, and multiple ovulation and embryo transfer (MOET) techniques have revolutionized livestock breeding.

The aim of livestock breeding systems and strategies is to enhance livestock productivity by producing more and higher quality animals. There are three phases to establishing a logical genetic strategy for animal production. First, determine the conditions for production and marketing, and match the applicable performance levels of the animals to those conditions. Second, select the breeding system, and third, select genetic types, breeds, and individuals within breeds to align with the first two points. To address the growing food desires of the human population, livestock breeders now must choose breeding strategies that bring greater accuracy, efficiency, save time, and utilize genomic information to improve livestock breeding. Genomic selection can be highly accurate during the first several generations when using a large reference population. Genome editing is another technology used in livestock breeding to obtain desired traits and eliminate undesirable traits in livestock populations.

1. Systems of Breeding

The breeding systems for livestock include various methods of mating and selecting animals to improve genetic traits. Key systems include inbreeding, outbreeding, and crossbreeding, each with specific objectives and outcomes. These systems are used to enhance productivity, disease resistance, and other desired traits in livestock populations. Breeding systems can be divided into two main groups: inbreeding and outbreeding (Hammack, 2011; Ahmad, 2013; Falconer & Mackay, 1996).

1.1. Inbreeding

Inbreeding is mating of animals that have a closer relationship to each other than average relationship within relevant population. The paired individuals must have common ancestors (one or more) in their lineage up to 4-6 generations. Inbreeding includes the mating of parents' offspring, siblings, and similar relationships (Bourdon, 2013).

Inbreeding has been classified into two types: line breeding and close inbreeding (Falconer & Mackay, 1996). Close inbreeding is the mating among siblings or with parents and offspring to get relatively more uniform inbred lines. Mostly full sib mating method is commonly used. This effect can also be attained by continuously crossing the offspring of young parents. Line breeding is the milder form of close inbreeding in which relationships of multiple individuals are kept as close to an ancestor as possible. In generals, sires do not mate with their daughters, but half-sib mating is done among the offspring of a specific sire. The line breeding was extensively used in past for the development of British livestock breeds such as Hereford, Angus, and Shorthorn (Parekh & Srivastava, 2002). After identifying outstanding individuals, line breeding should be practiced in a high-quality purebred population and can be advocated for the formation of new generations.

Inbreeding is when it creates more homozygous gene pairs in a population regardless of the type of gene action involved. It does not increase alleles frequency in the breeding population but reduce it through increased homozygosity. When individuals are homozygous at multiple traits, the inheritance of the traits is ensured. Inbreeding reduces breeding power or results in inbreeding depression. In spite of some clear inbreeding disadvantages, there are chances where it can be used to improve livestock production. It can be used to keep genetic transparency and hence improve the pre-potency. Inbred lines are also developed by inbreeding to eliminate undesirable anomalies in animal population. When a bull sire is used to bred a minimum of twenty daughters and does not produce any unstable traits in the offspring, it can be concluded that the sire is not heterozygous for contrasting traits.

Inbreeding practice should only be done when herd is better than the average, meaning when frequency of the desired genes is higher, the herd has an outstanding head, the breeder is aware of breeding traits and flaws, and animal herd is not raised for commercial purposes (Marleen et al., 2014).

1.2. Crossbreeding

Crossbreeding begins with the mating of two purebred strains of different species. This results in the production of the first cross offspring, referred to as F1. There are three possible crossbreeding advantages namely: favorable combinations of traits, heterosis and complementation.

1.2.1. Heterosis

Heterosis is also known as hybrid vigor, which comes when performance of a crossbred offspring is changed (mostly better) from the average of its parent breeds, for example, if Breed A is 400 lb and Breed B is 450 lb, then the average of both breeds is 425 lb. But when we crossed these two breeds, the act of both shows 445 lb (445 - 425 = 20 lb heterosis, 20 ÷ 425 = 4.7% heterosis) (figure.1). This example directly explains heterosis, its effect on the performance of the crossbred individual due to the combination of genes from the parents. Maternal heterosis can also occur, or indirect effect of the crossbred dam's gene combination that influences the performance of its calf through the maternal environment it provides.

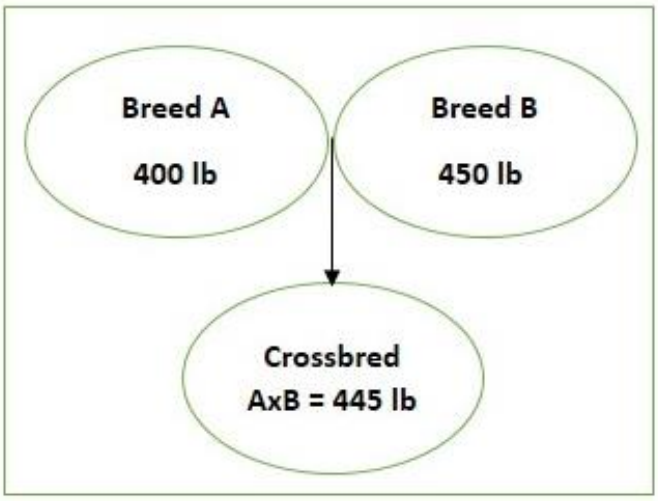

Figure 1. Hetrosis

Heterosis is the opposite of inbreeding depression, so it is greatest in the offspring of the least related parents. Heterosis decreases when both parents belong to the same breed. If the parents have not same breed, either the parents are purebred or crossbred, there is no detriment to heterosis. If an F1 is raised within one of its parent breeds (known as backcrossing), its offspring may exhibit nearly half of the heterosis present in F1. If this F1 is not crossed back but instead raised with a 3rd breed, or a non-related crossbreed, there should be no detriment to heterosis in its offspring since there is no shared breed like in backcrossing the sire and dam. If two animals from F1 of same cross are mated, the offspring, referred to as F2, should retain half of the heterosis of F1 generation. However, if the F2 generation of this cross and succeeding generations are mated together, their offspring should not experience any additional average heterosis loss excluding for any inbreeding that may develop over the time (Tomar, 2010).

The characteristics of heterosis are expressed in various ways. Heterosis is highest in traits like fertility, viability, and longevity. While the weight gain, milk production, feed efficiency, and the size of body are in the medium range; and carcass traits are the lowest. Even with benefits of the heterosis, crosses from average parents generally do not perform as good as high pure breeds. Therefore, most productive offsprings are produced by the genetically superior sire and dams.

1.2.2. Favorable Breed Combinations

Without heterosis, simply crossing generations could be beneficial. For example, high-quality strains of maize generally do not adapt well in tropical climate, and those with better tropical adaptability tends to produce relatively lower quality maize. Crossing generations of these two types can yield offspring which have moderate level of both traits. These favorable combinations of breeding could be beneficial for crossbreeding. These are main motivation for producing new generations by mixing old ones.

1.2.3. Complementarity

Completion needs different parents. Complementary results are not only obtained from favorable combinations of diverse traits but also from the ways they are paired. As mentioned, offspring of high carcass quality breed crossed with a tropically adapted breed is a mixture of those traits. However, most efficient and effective way to carry out these crosses is to use bulls of good carcass value and cows which are suited to tropical environment. The opposite cross is not more effective since the cows are not adapted well, and those are the cows that should function year-round under current conditions. Another example of completion is the use of large sires on small dams. The result is that the dams produce a higher percentage of their weight, and they do so more efficiently than if they were bred with sires of similar size. This type of completion can also be perceived directly in breeding by using genetic variations within the breed. However, it is completed more efficiently with crossbreeding (Simm, 1998).

1.3. Out Breeding

Outbreeding is mixing of the animals which have low genetic relationship to each other as compared to the average population. Outbreeding increase heterozygosity of individuals. The extreme practical utility of the breeding system is production of animals for the market. Outbreeding is a form of breeding where selection of mates is based on them not being related. Types of outbreeding include selective breeding, outcrossing, top crossing, and upgrading (Parekh & Srivastava, 2002; Warwick & Legates, 1979).

1.3.1. Selective Breeding

The use of selective breeding aims to maintain breed purity as well as in those selected areas or regions where a specific breed's population is predominant (Hammack, 2011).

1.3.2. Out Crossing

This generally applies to mating within a well-defined purebred breed. If two lines of same breed are separated for 4 to 5 generations and one herd is use sire from another herd counted as outside crossing. This is used when there is a lack of genetic variation and response to selection (Parekh & Srivastava, 2002).

1.3.3. Top Crossing

It refers to extreme use of racial sires in dams of base population or non-ethnic dams within same race. It generally refers to best individual of a lineage. It also refers to continuous practice of sires within a pure breed, the same race, or different families within different breeds (Parekh & Srivastava, 2002; Tomar, 2010).

1.3.4 Up-Grading

Categorizing or upgrading involves the repeated use of purebred sires on non-descriptive population females. If sires of a specific breed A are repeatedly crossed with another breed/non-descriptive animals B, improvements are observed in crosses. Five generations are enough to increase the inheritance level of breed A to 96.9% in 5^{th} generation. After repeatedly crossing a specific breed for five generations, animals become eligible for registration as purebred at the end of the fifth generation. After 7 to 8 generations of continuous categorization, the non-script population will be transitioned into a well-defined purebred (Parekh & Srivastava, 2002; Marleen et al., 2014).

1.4. Cross Breeding for New Breed Development

Cross breeding refers to breeding of two individuals from separate breeds. Cross breeding has been utilized to develop new breeds or synthetic strains in recent years. For example: Jamaica Hope, Norwegian Red and White, Santa Gertrudis, Hissardale, Karan Swiss, Australian Milking Zebu, Taylor, Sundanini breed, etc. The animals cross from different breeds results in progeny whose production exceeds from average of parents. This increased production can be attributed to higher fertility, enhanced viability before and after birth, faster growth, better maternal ability, etc. (Simm, 1998; Bourdon, 2013).

1.5. Types of Breeding Systems in Beef Cattle

For the improvement of commercial beef production, two types of breeding systems are commonly implemented. In production systems where replacement heifers are produced in own herd, system continues indefinitely. If cows' replacements are brought, the system is terminal. In continuous systems, calves are kept for two purposes: some are preserved for replacement and are sent back to herd, but the rest grow up and/or are finished for beef production. However, all calves in a terminal system have only one purpose, which is beef production. Continuous and terminal systems can be combined (Hammack, 2011).

1.5.1. Continuous Breeding System

A continuous breeding system produces its alternative material; it only needs sires from outside to avoid inbreeding. Since alternative cows are maintained, both sires and dams have genetic contributions in the herd. If sires express traits which are not desirable in calves, and these traits are existing in herd of cattle. Consequently, without any undesirable traits in both sires and dams, there should be a uniform expression of key traits. Extreme genotypes usually do not fit well. A continuous system could be straight breeding or crossbreeding (Hammack, 2011).

1.5.2. Terminal Breeding System

In a terminal system, alternative female terminal crosses come from outside. Changes can either be bought or developed in some other herd. As cows are not kept for the breeding, there is greater flexibility in selecting both dams and sires. Genetics are irrelevant for maternal capacity in terminal cross bulls because their daughters are not preserved for replacement. Still, the genetics for maternal capacity is important in those bulls whose daughters are used for terminal crossing. The terminal crossing is the only system where complementation can be realized. However, simply applying the terminal system does not guarantee complementation. Sires and dams must be different and must possess complementary traits. Specific breeds can be used in terminal systems to maximize benefits by utilizing their strengths while minimizing or removing weaknesses. Terminal systems usually composite crossbreds but can also be straightbred (Hammack, 2011).

1.5.3. Continuous Crossbreeding Systems

1.5.3.1. True Rotations

In a true rotation system, two or more breeds and a similar number of breeding groups are used. The simplest form of true rotation is also referred as crossbreeding as it uses only two breeds. In each of the two breeding groups sires of different breeds are used. Alternate calves are transferred from one group to the other, where they are mated with animals of a different breed to minimize the heterosis loss. Females remain in this breeding group for their entire lives. Over time, females in the two groups attract towards forming two-thirds of their lineage's breed and one-third of another breed's lineage. If there are three breeds or more in the rotation, a cow is moved to breeding group least related to sire's breed to ensure the minimize heterosis loss. True rotations breeding systems are complex since they require multiple breeding groups. Moreover, a tradeoff should be made between the complementary integration and group uniformity. Due to these limitations, real circulations are unusual (Hammack, 2011; Bourdon, 2013).

Table 1. Average Gene

Parents Breeds 1st Sire Breed, 2nd cow breed	Proportion in offsprings	
	Breed X	Breed Y
X x Y	0.5	0.5
X x (XY)	0.75	0.25
Y x [X(XY)]	0.375	0.625
X x (Y[X(XY)])	0.688	0.313
Y x [X(Y[X(XY)])]	0.344	0.656

1.5.3.2. Sire Rotations

Sire rotations are referred to as time rotations. Instead of roaming outside their breeding groups, as in actual rotations, sires of certain breeds are periodically rotated within the same breeding group. The duration for which a sire is used partially depend on how often the cow is returned to

herd. The extreme inbreeding should be avoided; therefore, sires should be replaced whenever necessary to prevent them from breeding their daughters. Compared to actual rotations, sire rotations have lower heterosis, although in planned systems this decrease is minor. The loss of heterosis is minimized by keeping alternative sires away from the dams, thus reducing the relationship of the sire to the heifer's breed, and minimizes breeding from the sire's breed (Hammack, 2011).

Some producers instead of known genetic compositions, use visible genetic traits and other physical characteristics to determine either to retain a cow as an alternative or not. It reduces phenotypic variation but can decrease heterosis also. It is easy to rotate sires compared to actual rotations as there is one genetic group only. This is a common method of crossbreeding (Bourdon, 2013).

1.5.4. Terminal Crossbreeding Systems

1.5.4.1. Static Terminal

In static terminal system, replacement heifers are sometimes purchased from different herd. The purchase of females facilitates the operations of this system as terminal crosses require only breeding. A straight-bred terminal is possible, but there is generally no reasonable justification for doing so (only if a producer wants to continuously preserve and manage cows in a straight-bred system) as the advantages of crossbreeding are not obtained. A two-generation static system, using sire and dam from different breeds, heterosis is produced in crossbred offsprings. But this system significantly loses maternal heterosis benefits from the crossbred dam (Hammack, 2011).

A more efficient form of this system is three-generation terminal system. In this system two generations with good maternal traits are mated to produce more productive daughters in F1 generation, which are then bred with third-generation sires in the terminal cross. In this complete system, approximately one-quarter of the dams should be straight, about one-quarter is needed to produce F1, and only half is available for terminal cross. This system can achieve maximum heterosis, favorable genetic combinations, and a complement bonus in both cows and calves. However, these benefits are influenced by the need for alternative females purchasing or producing them outside the terminal cross.

1.5.4.2. Rotation-Terminal

A rotation terminal integrates terminal and continuous systems. It provides a method for cross-bred alternative females for static terminals. The rotation system, whether real rotation or sire rotation, generates alternative females to sustain itself and be used in separate terminal herds. Middle-aged dams are moved to out-of-rotation terminals because if the terminal sires are relatively large, the risk of calf issues is reduced. Multiple breeding groups must be kept for rotation terminal systems; one or more for rotation, depending on whether it is true rotation or bull rotation, and one for the terminal crossing. The rotation requires about half of the dams to provide sufficient alternatives for entire system, with half of them being set aside for terminal

crossing. About two-thirds to three-quarters of calf terminal offspring come from terminal sires, with remaining majority being bull calves from the rotation (Thakur, 2022).

There is relatively more heterosis in rotating terminals as all offspring and dams interbreeding with each other. However, since it is necessary to maintain a high percentage of females in the terminal due to rotation, there is very small opportunity to select individual females for reproduction (Simm, 1998; Ahmad, 2013).

1.5.5. Combination Breeds

Modern livestock populations are often crossbred to produce hybrid breeds that combine desirable traits from multiple sources. Because these hybrids originate from crossbreeding, they retain a certain level of residual heterosis, the extent of which depends on factors such as the number of parent breeds involved, their genetic contribution, and the degree of inbreeding during breed stabilization. Consequently, even when a single hybrid breed is used in a straight-breeding system, some heterosis can still be expressed.

A notable example is the American Brahman, one of the earliest hybrid breeds, developed by combination of several *Bos indicus* breeds imported from Brazil and India. In regions like South America and Texas, numerous other hybrid breeds have emerged through crosses between Brahman cattle and either British or Continental European breeds, collectively known as American breeds. More recently, hybridization between British and Continental breeds has also begun, and although these combinations are still relatively uncommon, their prevalence is steadily increasing (Tomar, 2010; Simm, 1998).

1.6. Other Systems

All breeding systems that have been discussed so far use the same sire breed. Alternatives are available.

1.6.1. Multiple Sire Breed

In a single breeding season, it is possible to use sires from multiple breeds simultaneously. For instance, two British sire breeds may be incorporated within a continuous breeding system. To limit the reduction of heterosis and control phenotypic variability, the replacement heifers retained should generally display intermediate physical characteristics. Alternatively, sires from both British and American breeds may be utilized. In such cases, females exhibiting stronger American breed traits should be selected as replacements if this type shows better adaptation to the prevailing production environment. This method closely resembles a two-breed rotational crossing system (Warwick & Legates, 1979; Thakur, 2022; Bourdon, 2013).

A sire breed known for favorable maternal qualities may also be used alongside a terminal-sire breed, with the offspring of maternal-type sires retained provided they can be clearly distinguished phenotypically. Multiple maternal-type sires can be employed either concurrently or in rotation across years to maintain a degree of maternal heterosis. These strategies are like rotation-terminal or static terminal crossbreeding systems.

However, the multiple-sire breeding system has certain drawbacks. Compared to more structured systems, it tends to retain a lower level of heterosis (Bourdon, 2013). The use of large terminal sires can increase the risk of mating with younger females, potentially leading to calving difficulties. While this system introduces greater genetic variability among individuals, careful selection of replacement females can help minimize visible variation. Additionally, the number of calves produced from each sire breed may fluctuate annually, causing variations in the proportion of available replacement stock. Such systems are best suited for large, extensively managed herds, which are better equipped to implement multiple-sire breeding strategies effectively (Hammack, 2011).

1.6.2. Hybrid Sires and Composites

Just as females exhibit heterosis for reproductive performance, males can also show hybrid vigor in traits such as semen quality, fertility, mating ability, and lifespan. The use of hybrid sires provides a convenient approach to combine desirable characteristics that may not coexist within a single established breed. Certain seedstock producers specifically focus on developing hybrid bulls, typically by crossing breeds such as British–Continental or British–American types. In fact, many breed associations now offer formal registration programs for hybrid animals (Hutu et al., 2020).

However, obtaining a consistent and dependable source of high-quality hybrid bulls can be difficult. A common belief is that hybrid sires produce offspring with greater variability, though studies—particularly from the U.S. Meat Animal Research Center (Clay Center, Nebraska)—have questioned this assumption, especially for measurable traits like body weight. Comprehensive and reliable genetic evaluations are often unavailable for hybrid bulls, though some breed organizations have begun to include them in their performance databases.

The distinction among composite breeds, hybrids, and crosses is often unclear, as these categories frequently overlap. In practice, all three can be incorporated into breeding programs using similar principles. Table 1 provides a comparison of the major features of these breeding systems.

1.7. Efficiencies of Breeding Systems

A common benchmark for evaluating the production efficiency of a cattle breeding system is the total weight of calves weaned per cow, which reflects the combined effects of fertility, calf survival, and weaning weight. The baseline for comparison is typically straight breeding within traditional breeds, where no retained heterosis is present. Implementing a continuous crossbreeding system involving only a single breed rotation can improve overall performance by 10–15%. More complex crossbreeding programs—those using multiple breeding groups or incorporating external females in three-breed terminal crosses—can enhance productivity by 15–30%. In challenging tropical and subtropical regions, the inclusion of locally adapted breeds, particularly *Bos indicus*, often yields even higher efficiency due to increased heterosis and

superior adaptability. These benefits represent significant genetic and production advantages (Thakur, 2022; Simm, 1998).

A sound genetic strategy for cattle production can be outlined in four steps:

- Assess production conditions such as climate, nutrition, and market requirements, and define the appropriate level of animal performance for those conditions.
- Select a suitable breeding system aligned with those production goals.
- Identify biological types and breeds that are compatible with both the production environment and the chosen breeding system.
- Choose superior individuals within those breeds to serve as parents for the next generation.

Optimal production and profitability are achieved when these components are balanced effectively, rather than emphasizing one at the expense of the others.

Summary

Once a breeding system has been selected, the next step for the producer is to identify the breeds and the individual animals within those breeds that are best suited to the prevailing climatic conditions, forage resources, management practices, and market demands. The most common approach, regardless of herd size, remains the use of a single breeding group. Consequently, many breeding systems rely on the purchase of replacement females for both straight breeding (traditional and composite breeds) and terminal crossbreeding, often incorporating sire rotation or the use of multiple sires to maintain genetic diversity and productivity.

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Mendelian and Quantitative Genetics in Livestock

Muhammad Mujeeb ULLAH

1. Introduction

1.1 Historical Perspectives and Modern Advances

The study of livestock genetics has evolved significantly from traditional breeding practices to modern genomic technologies. Historically, farmers relied on selective breeding to enhance desirable traits like milk yield, growth rate, and feed efficiency. The foundation of this genetic understanding was laid by Gregor Mendel's principles of inheritance, which explained how traits pass from parents to offspring. In the early 20th century, quantitative genetics emerged, integrating Mendelian principles with statistical models to study polygenic traits such as growth and fertility (ASLAM et al., 2024) as shown in Figure 1.

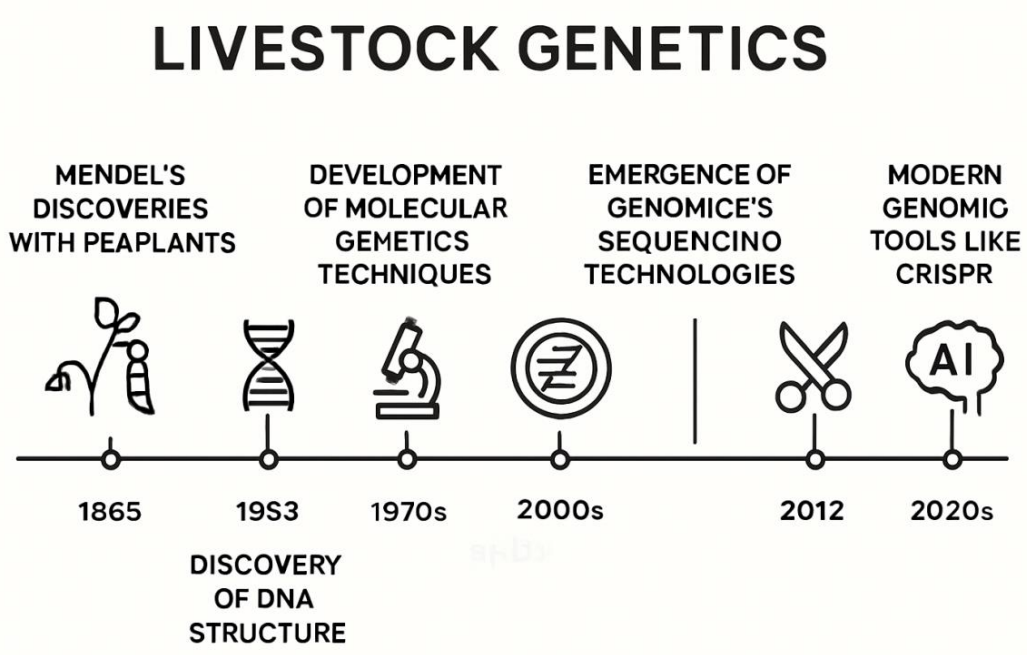

Figure 1. Key Milestones in Livestock Genetics: From Mendel's Foundational Discoveries to Modern Genomic Technologies Including CRISPR and Artificial Intelligence.

For example, if a herd has an average daily weight gain (ADG) of 1.2 kg and a sire's progeny averages 1.4 kg, with a heritability (h^2) of 0.4, the genetic gain (ΔG) is calculated as $\Delta G = h^2 \times S$, where the selection differential (S) is 0.2 kg. Thus, $\Delta G = 0.4 \times 0.2 = 0.08$, predicting a next-generation ADG of 1.28 kg.The advent of genomic technologies like SNP arrays and Genome-Wide Association Studies (GWAS) in the 21st century enabled the identification of genes such

as DGAT1, which influences milk fat yield in cattle. Genomic selection has reduced generation intervals, accelerating genetic improvement. Gene-editing technologies like CRISPR-Cas9 have further revolutionized breeding by introducing traits such as disease resistance in pigs and hornlessness in dairy cattle for better welfare. Additionally, artificial intelligence (AI) and machine learning are now applied in breeding programs to predict genetic potential more accurately. For instance, predicting the progeny of a bull with a genomic predicted transmitting ability (PTA) of +1,500 lbs and a cow with +1,200 lbs is calculated as 1500+1200/2 = 1350 lbs of expected milk yield. These modern advancements, combined with traditional principles, continue to drive efficiency, productivity, and sustainability in livestock genetics.

1.2 Importance of Genetics in Livestock Improvement

Genetics plays a crucial role in livestock improvement by enabling the selection and breeding of animals with desirable traits, ultimately enhancing productivity, efficiency, and sustainability. Through genetic selection, farmers can improve traits such as growth rate, milk yield, feed efficiency, disease resistance, and reproductive performance. For instance, the introduction of high-yielding Holstein cattle has significantly increased milk production worldwide due to selective breeding for genes associated with higher lactation performance. Quantitative genetics, which examines traits influenced by multiple genes, allows breeders to predict performance more accurately using heritability estimates and genetic correlations (Mackay, Stone, & Ayroles, 2009).

For example, if the heritability of milk yield is 0.35 and the selection differential is 1,000 liters, the expected genetic gain is calculated using the formula $\Delta G = h^2 \times S$, which equals $0.35 \times 1000 = 350$ liters. Modern tools like genomic selection and Genome-Wide Association Studies (GWAS) have revolutionized livestock breeding by identifying genetic markers linked to performance traits, reducing the reliance on phenotypic selection alone. Additionally, advanced technologies like CRISPR-Cas9 gene editing have enabled the development of disease-resistant breeds, such as PRRS-resistant pigs, contributing to animal health and welfare. The application of artificial intelligence and machine learning in genetic evaluation has further improved prediction accuracy, helping farmers make informed breeding decisions. As the global demand for animal products increases, the strategic use of genetics remains essential for meeting production goals while ensuring environmental sustainability and animal welfare.

2. Foundations of Mendelian Genetics in Livestock

2.1 Laws of Inheritance: Dominance, Segregation, and Independent Assortment

The laws of inheritance, formulated by Gregor Mendel through his experiments with pea plants, provide the foundation for understanding genetic transmission in livestock. These laws—dominance, segregation, and independent assortment—help explain how traits are passed from

parents to offspring, aiding breeders in selecting animals with desirable characteristics. The law of dominance states that when two alleles for a gene are different, one may mask the expression of the other. For instance, if the allele for black coat color (B) is dominant over the allele for red coat color (b) in cattle, crossing a homozygous black bull (BB) with a homozygous red cow (bb) will result in all heterozygous offspring (Bb) with black coats. The law of segregation explains that during gamete formation, the two alleles for a gene separate so that each gamete receives only one allele. For example, if a heterozygous rooster (Aa) for comb shape is crossed with a hen (Aa), the offspring will follow a 1:2:1 genotypic ratio (AA, Aa, aa) and a 3:1 phenotypic ratio if A is dominant. The law of independent assortment states that genes for different traits segregate independently during gamete formation if they are not linked. For instance, when crossing cattle heterozygous for coat color (Bb) and horn presence (Pp), the offspring show a phenotypic ratio of 9:3:3:1, demonstrating how different traits can combine in various ways. These laws underpin modern breeding practices, facilitating the prediction of genetic outcomes and improving livestock productivity through selective breeding (Strome, Bhalla, Kamakaka, Sharma, & Sullivan, 2024).

2.2 Mendelian Traits in Livestock Breeding

Mendelian traits in livestock breeding are those traits governed by a single gene with distinct dominant and recessive alleles, following Mendel's principles of inheritance. These traits are typically easy to track and predict, making them valuable for selective breeding programs.

Examples of Mendelian traits in livestock include coat color, horn presence, and certain genetic disorders. For instance, in cattle, the polled (hornless) trait is governed by a single gene, where the allele for polled (P) is dominant over the allele for horns (p). Crossing a heterozygous polled bull (Pp) with a horned cow (pp) results in offspring with a 1:1 phenotypic ratio, where 50% are polled and 50% have horns. Similarly, in poultry, the rose comb trait is dominant over the single comb trait, and breeders can predict the offspring's comb type based on the parents' genotypes. Genetic diseases like bovine leukocyte adhesion deficiency (BLAD) in Holstein cattle also follow Mendelian inheritance, where the disease manifests in individuals with two copies of the defective allele. Understanding these traits helps breeders make informed decisions, improve productivity, and manage genetic disorders, ensuring healthier and more productive livestock populations. Modern genetic tools, such as molecular markers and DNA testing, have further simplified the identification of Mendelian traits, enhancing the efficiency of breeding programs (Scheper et al., 2016).

2.3 Extensions of Mendelian Genetics: Epistasis, Pleiotropy, and Polygenic Traits

Extensions of Mendelian genetics, including epistasis, pleiotropy, and polygenic traits, provide a more comprehensive understanding of genetic inheritance beyond the simple dominant-recessive patterns described by Mendel.

Epistasis occurs when the expression of one gene is influenced by one or more other genes. For example, in Labrador Retrievers, coat color is controlled by two genes: one gene determines pigment color (B for black, b for brown), while another gene (E) controls pigment deposition. A dog with genotype ee will be yellow regardless of its B/b genotype, demonstrating epistasis where the E gene masks the effect of the B gene.

Pleiotropy refers to a single gene affecting multiple traits. For instance, the myostatin (MSTN) gene in Belgian Blue cattle influences both muscle growth and fat deposition. Mutations in MSTN lead to increased muscle mass (double muscling) but can also cause calving difficulties due to larger birth weights.

Polygenic traits involve multiple genes contributing to a single characteristic, often resulting in continuous variation rather than discrete categories. Milk production in dairy cattle is a classic example, influenced by many genes, each with a small effect. For instance, if the heritability of milk yield is 0.4 and the selection differential is 1,000 liters, the genetic gain can be calculated using the formula $\Delta G = h^2 \times S$, yielding $0.4 \times 1000 = 400$ liters. Understanding these genetic extensions helps breeders make more accurate predictions about complex traits, ultimately enhancing livestock performance and health (Mackay & Anholt, 2024).

3. Quantitative Genetics: Principles and Applications

3.1 Concept of Quantitative Traits and Polygenic Inheritance

The concept of quantitative traits and polygenic inheritance is fundamental in livestock genetics, as most economically important traits, such as milk yield, growth rate, and egg production, are controlled by multiple genes and influenced by environmental factors.

Quantitative traits exhibit continuous variation rather than distinct categories, resulting from the combined effects of many genes, each contributing a small, additive effect. For instance, milk yield in dairy cattle is influenced by numerous genes, along with environmental factors like nutrition and management practices.

Polygenic inheritance describes this genetic architecture, where multiple gene pairs collectively determine the phenotype. The distribution of these traits often follows a normal distribution due to the additive effects of many genes (Merrick et al., 2023).

For example, if a cow has a milk yield of 6,000 liters and a bull has a predicted transmitting ability (PTA) of +500 liters, the expected milk yield of their offspring can be calculated by averaging the parental values. If the heritability of milk yield is 0.35, the genetic gain (ΔG) from selection is calculated as $\Delta G = h^2 \times S$, where the selection differential (S) is 500 liters. Thus, $\Delta G = 0.35 \times 500 = 175$liters, indicating that the next generation is expected to produce 6,175 liters

on average. Understanding the genetic and environmental components of quantitative traits helps livestock breeders implement more effective selection strategies, utilizing tools like BLUP (Best Linear Unbiased Prediction) and genomic selection to achieve genetic improvement more efficiently (Figure 2).

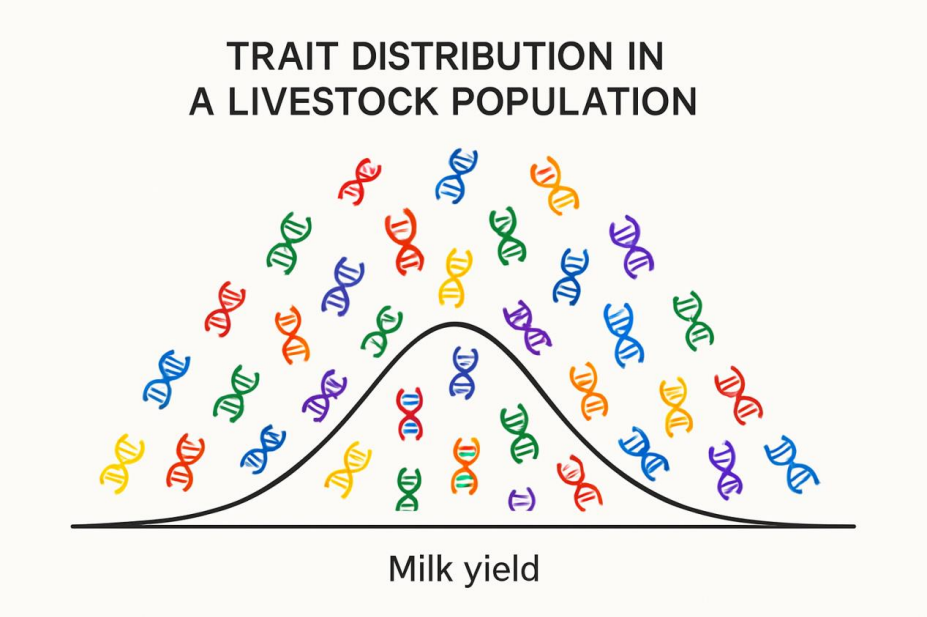

Figure 2. Trait Distribution of Milk Yield in a Livestock Population Illustrating the Polygenic Inheritance Pattern with Multiple Genes Contributing to Quantitative Variation.

3.2 Additive and Non-Additive Genetic Variance

Additive and non-additive genetic variance are key components of the genetic variation observed in livestock populations, particularly for quantitative traits like growth rate, milk yield, and fertility.

Additive Genetic Variance (Va) refers to the cumulative effect of individual alleles, where the genetic contribution of each allele can be summed to predict the offspring's performance. This variance is the primary focus of selective breeding because additive effects are consistently passed from parents to offspring. For example, if a bull has a predicted transmitting ability (PTA) of +500 liters for milk yield and a cow has a PTA of +300 liters, the expected PTA of their offspring is 500+300/2=400 liters, assuming only additive effects.

Non-additive Genetic Variance includes dominance and epistatic interactions. Dominance variance occurs when allele interactions within a single gene influence the phenotype, such as when a heterozygous genotype (Aa) expresses a trait differently than the average of the homozygous genotypes (AA and aa). Epistatic variance involves interactions between genes, like

in coat color inheritance in Labrador Retrievers, where the E gene influences the expression of the B gene. While non-additive genetic effects can enhance performance in crossbreeding programs through heterosis (hybrid vigor), they are less predictable and less heritable than additive effects. Understanding the relative contributions of additive and non-additive genetic variance helps breeders design more efficient selection and crossbreeding programs to optimize genetic gain and performance in livestock populations (Ebrahimi, Dashab, Faraji-Arough, & Rokouei, 2019).

3.3 Heritability, Genetic Correlation, and Breeding Value

Heritability, genetic correlation, and breeding value are essential concepts in livestock genetics, helping breeders predict and improve animal performance across generations.

Heritability (h^2) measures the proportion of phenotypic variance in a population that is attributable to genetic differences. It ranges from 0 to 1, where a higher value indicates a stronger genetic influence. For instance, milk yield in dairy cattle typically has a heritability of around 0.30, meaning 30% of the variation in milk production is genetic, while the rest is environmental. If the average milk yield is 6,000 liters and the selection differential (S) is 500 liters, the genetic gain (ΔG) is calculated as $\Delta G = h^2 \times S$. For $h2=0.30$, $\Delta G = 0.30 \times 500 = 150$ liters, predicting an average yield of 6,150 liters for the next generation.

Genetic correlation (rG) describes the relationship between two traits that are influenced by shared genetic factors. For example, milk yield and feed intake often have a positive genetic correlation, meaning selecting for higher milk yield may also increase feed consumption. If rG = 0.6, selecting for milk yield is expected to simultaneously increase feed intake.

Breeding value (BV) is an estimate of an animal's genetic potential to pass traits to its offspring. BV is calculated by doubling the difference between the offspring's performance and the population mean, assuming both parents contribute equally. For example, if a calf's weight gain is 1.2 kg/day compared to a population average of 1.0 kg/day, and the heritability is 0.4, its BV is $BV = 2 \times (1.2 - 1.0) \times 0.4 = 0.16$ kg/day. Understanding these parameters helps breeders make informed decisions to improve livestock performance through selective breeding and genetic evaluation programs (El-Bayoumi, El-Tarabany, Abdel-Hamid, & Mikaeil, 2015).

4. Genetic Evaluation and Selection in Livestock

4.1 BLUP (Best Linear Unbiased Prediction) and Genomic Selection

BLUP (Best Linear Unbiased Prediction) and genomic selection are powerful tools in modern livestock breeding, enabling more accurate predictions of genetic merit for economically important traits.

BLUP is a statistical method used to estimate breeding values by separating genetic effects from environmental influences. It considers information from an animal's performance, relatives, and progeny, providing unbiased predictions even in unbalanced data structures. For instance, if a dairy cow produces 7,000 liters of milk, exceeding the herd average of 6,000 liters, and the heritability of milk yield is 0.30, BLUP uses this data along with related animals' performance to predict the cow's breeding value. The breeding value (BVBV) can be estimated as

$$BV=h2\times(\text{individual performance-population mean})$$

$$BV=0.30\times(7000-6000)=300\ \text{liters}$$

This indicates the cow has the genetic potential to pass on an additional 300 liters of milk yield to its offspring.

Genomic selection, introduced with advances in molecular genetics, enhances BLUP accuracy by incorporating genomic information. It uses thousands of genetic markers (SNPs) spread across the genome to predict an animal's genetic merit without waiting for phenotypic performance. For example, in dairy cattle breeding, SNP data can identify genes like DGAT1 associated with milk fat content. By applying genomic selection, breeders reduce generation intervals and accelerate genetic gains. The prediction accuracy increases, especially for low-heritability traits like fertility. Combining BLUP with genomic selection has revolutionized livestock breeding, improving productivity, disease resistance, and overall genetic progress in animal populations (Abdollahi-Arpanahi, Lourenco, & Misztal, 2021).

4.2 Genetic Markers and QTL (Quantitative Trait Loci) Mapping

Genetic markers and quantitative trait loci (QTL) mapping are fundamental tools in modern livestock genetics, providing insights into the genetic architecture of complex traits.

Genetic markers are identifiable DNA sequences with known locations on the genome, used to track inheritance patterns. Markers can be classified into various types, including microsatellites and single nucleotide polymorphisms (SNPs). SNPs are widely used due to their abundance and ease of detection. For instance, in dairy cattle, SNP markers linked to the DGAT1 gene have been associated with variations in milk fat content.

QTL mapping involves identifying genomic regions associated with quantitative traits, such as growth rate or milk yield, which are influenced by multiple genes. This process typically uses marker-trait associations in a population to locate QTLs. For example, if a study finds that animals with a specific SNP variant produce 500 liters more milk than others, this SNP may be near a QTL for milk production. The mapping process usually involves statistical models like interval mapping or composite interval mapping.

The equation for estimating the genetic effect of a QTL is often based on the linear model:

$$Y = \mu + g + e$$

where:

a) **y** is the phenotypic value,
b) **μ** is the population mean,
c) **g** is the QTL effect, and
d) **e** is the residual environmental effect.

For instance, if the average milk yield is 7,000 liters, with a QTL effect of +300 liters and an environmental effect of -100 liters, the predicted yield for animals with the favorable allele is:

$$Y = 7000 + 300 - 100 = 7200 \text{ liters}$$

By integrating QTL information with genomic selection, breeders can enhance selection accuracy, especially for traits that are difficult or costly to measure, such as disease resistance or meat quality. This approach significantly accelerates genetic progress in livestock populations (Xu, Li, Yang, & Xu, 2017).

4.3 Accuracy and Response to Selection

Accuracy of selection and response to selection are critical concepts in livestock breeding, influencing the effectiveness of genetic improvement programs.

Accuracy of selection refers to the correlation between an animal's true genetic value and its estimated breeding value (EBV). Higher accuracy ensures more reliable predictions, leading to better breeding decisions. Accuracy depends on factors like the amount of performance data, heritability of the trait, and the use of genetic markers in genomic selection. For example, if the heritability of milk yield is 0.35 and data from multiple relatives are available, the accuracy of selection can reach 0.7 or higher with genomic information.

Response to selection (R) measures the genetic gain achieved per generation due to selective breeding. It is calculated using the formula:

$$R = h2 \times S$$

where:

a. **h2** = heritability of the trait,

b. **S** = selection differential (the difference between the mean of selected parents and the population mean).

For instance, if the average milk yield is 6,000 liters, the selected parents have an average yield of 6,500 liters, and the heritability of milk yield is 0.35, the selection differential is:

$$S = 6500 - 6000 = 500 \text{ liters}$$

The expected response to selection is:

$$R = 0.35 \times 500 = 175 \text{ liters}$$

This indicates that the next generation's average milk yield is expected to increase to 6,175 liters. The accuracy of selection directly influences the response to selection, as higher accuracy increases the effectiveness of selecting genetically superior animals. Modern breeding programs employ genomic selection, which uses dense SNP marker data to enhance accuracy, even for low-heritability traits like fertility and disease resistance. Consequently, combining high selection accuracy with a strong selection differential can significantly accelerate genetic improvement in livestock populations (Goddard, 2009).

5. Modern Tools in Livestock Genomics

5.1 Whole Genome Sequencing and SNP Arrays

Whole Genome Sequencing (WGS) and Single Nucleotide Polymorphism (SNP) arrays are transformative tools in livestock genetics, offering high-resolution insights into genetic variation and its impact on economically significant traits.

Whole Genome Sequencing (WGS) involves determining the complete DNA sequence of an organism's genome at a single time. It provides comprehensive information about all genetic variants, including SNPs, insertions, deletions, and structural variations. In livestock, WGS has been used to identify genetic variants associated with disease resistance, production efficiency, and reproductive performance. For instance, sequencing the genomes of high-yielding Holstein cows has revealed mutations in the DGAT1 and ABCG2 genes that significantly influence milk fat and protein content. WGS is particularly valuable for discovering novel variants and understanding the genetic architecture of complex traits.

SNP arrays, on the other hand, are cost-effective genotyping tools that detect known SNPs across the genome. These arrays come in different densities, such as 50K, 150K, or 770K SNPs, depending on the species and breeding objectives. SNP arrays are widely used in genomic selection to estimate breeding values more accurately by capturing genetic variation associated

with performance traits. For example, in beef cattle, SNP arrays help predict feed efficiency, carcass quality, and growth rates.

The relationship between genetic markers and phenotypic traits is often modeled using linear regression:

$$\mathbf{Y = \mu + \sum xi\ \beta i + e}$$

where:

a) y = phenotypic value (e.g., milk yield),
b) μ = population mean,
c) xi = genotype of the i-th SNP ,
d) βi = effect size of the i-th SNP ,
e) e = residual error.

For instance, if an animal has SNP genotype x1=1, x2=2 with effect sizes β1=50 and β2=30, and a mean performance (μ) of 6,000 liters, the predicted yield would be:

$$Y = 6000 + (1\times50) + (2\times30) = 6110 \text{ liters}$$

By integrating WGS and SNP arrays, researchers can conduct Genome-Wide Association Studies (GWAS) to identify genetic markers linked to important traits, enabling more accurate selection and accelerating genetic improvement in livestock populations (Jiang, Song, Gao, Zhang, & Ding, 2022).

5.2 Genome-Wide Association Studies (GWAS)

Genome-Wide Association Studies (GWAS) are a powerful tool in livestock genetics for identifying genetic variants associated with complex traits. GWAS analyzes the relationship between genetic markers, typically single nucleotide polymorphisms (SNPs), and phenotypic traits across the genome in a population. This method helps breeders' pinpoint genes influencing economically important traits such as milk yield, growth rate, feed efficiency, and disease resistance. The GWAS process involves genotyping a large group of animals using high-density SNP arrays and collecting corresponding phenotypic data. Statistical models then test for associations between SNPs and trait variation. The fundamental equation in GWAS is

$$Y = \mu + x\beta + e$$

Where

a. y = phenotypic value (e.g., milk yield),

b. μ = population mean,
c. x = SNP genotype (coded as 0, 1, or 2 for homozygous reference, heterozygous, and homozygous alternative alleles),
d. β = SNP effect size,
e. e = residual error.

For instance, if the mean milk yield is 6,000 liters, an animal has a genotype x=2 for a SNP with β=100, and the residual e=50, the predicted yield is:

$$Y = 6000 + (2 \times 100) + 50 = 6250 \text{ liters}$$

GWAS typically uses statistical tests like the chi-square test or linear mixed models to determine the significance of SNP-trait associations. Significant associations are visualized in a Manhattan plot, where SNPs that pass a genome-wide significance threshold suggest potential Quantitative Trait Loci (QTLs).In livestock, GWAS has identified key genes such as DGAT1 (affecting milk fat content in cattle), MC4R (influencing growth rate in pigs), and PRLR (affecting egg production in chickens). Integrating GWAS findings with genomic selection helps breeders enhance selection accuracy and accelerate genetic progress in livestock populations (Uffelmann et al., 2021).

5.3 CRISPR and Gene Editing in Livestock Improvement

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and gene editing technologies have revolutionized livestock genetics by enabling precise, targeted modifications in the genome to improve productivity, health, and welfare. CRISPR-Cas9, the most widely used system, allows researchers to add, delete, or modify specific DNA sequences, offering a faster and more efficient alternative to traditional breeding methods. The CRISPR-Cas9 mechanism works by guiding the Cas9 enzyme to a target DNA sequence using a guide RNA (gRNA). Once Cas9 introduces a double-strand break at the target site, the cell repairs the break through one of two pathways: non-homologous end joining (NHEJ), which may cause gene disruptions, or homology-directed repair (HDR), which can introduce precise genetic changes.

In livestock, CRISPR has been successfully applied in various species to enhance desirable traits. For instance:

1. **Disease Resistance**: Researchers have edited the CD163 gene in pigs to confer resistance to Porcine Reproductive and Respiratory Syndrome (PRRS), a devastating viral disease.
2. **Productivity**: In cattle, editing the myostatin (MSTN) gene increases muscle mass, improving beef yield.
3. **Animal Welfare**: Polled (hornless) cattle have been developed by editing the POLLED gene, eliminating the need for painful dehorning.

The efficiency of gene editing can be modeled using the equation:

$$\mathbf{P = G + E}$$

where:

a) P = Phenotype,
b) G = Genetic contribution (including edited genes),
c) E = Environmental influence.

For example, if gene editing increases growth rate by introducing a beneficial allele with an additive genetic effect of +200 g/day and environmental factors contribute 300 g/day to the baseline growth of 500 g/day, the new growth rate would be

$$\mathbf{P = 500 + 200 + 300 = 1000\ g/day}$$

While CRISPR and gene editing hold immense potential for livestock improvement, ethical considerations, regulatory frameworks, and public acceptance remain critical challenges. Nevertheless, these technologies offer promising avenues for enhancing productivity, animal welfare, and sustainability in modern livestock production systems (Raza et al., 2022).

6. Epigenetics and Its Role in Livestock Genetics

6.1 DNA Methylation and Histone Modification

DNA methylation and histone modification are key epigenetic mechanisms regulating gene expression without altering the underlying DNA sequence. These modifications play a crucial role in livestock genetics by influencing growth, reproduction, immune response, and other economically significant traits.

1. DNA Methylation

DNA methylation involves the addition of a methyl group ($-CH_3$) to cytosine bases, typically at CpG sites (cytosine followed by guanine). This process often suppresses gene expression by preventing transcription factors from binding to DNA. In livestock, DNA methylation affects traits like milk yield, meat quality, and disease resistance. For instance, methylation of the IGF2 gene in pigs influences muscle growth and fat deposition. The level of methylation can be measured using bisulfite sequencing, which differentiates methylated cytosines from unmethylated ones.

2. Histone Modification

Histone proteins package DNA into chromatin, and chemical modifications to histone tails influence gene accessibility. Key modifications include:

1) **Acetylation**: Addition of an acetyl group to lysine residues, generally associated with gene activation by loosening chromatin structure.
2) **Methylation**: Can either activate or repress gene expression depending on the specific histone and amino acid involved.
3) **Phosphorylation**: Involved in DNA repair and cell cycle regulation.

For instance, increased histone acetylation near the DGAT1 gene promoter enhances milk fat synthesis in dairy cattle.

Practical Implications in Livestock

Epigenetic modifications like DNA methylation and histone acetylation are influenced by environmental factors such as nutrition, stress, and developmental stages. Early-life nutrition can induce long-lasting epigenetic changes, a phenomenon known as epigenetic programming**.** For example, feeding methyl donor-rich diets (e.g., methionine and choline) to pregnant cows can enhance offspring growth and productivity by modulating methylation patterns. Understanding these epigenetic mechanisms helps breeders develop strategies to optimize livestock performance, improve animal health, and adapt to environmental changes without altering the genetic code (Stoll, Wang, & Qiu, 2018).

6.2 Epigenetic Inheritance and Environmental Effects on Gene Expression

Epigenetic inheritance refers to the transmission of gene expression patterns across generations without changes to the underlying DNA sequence. This inheritance occurs through mechanisms such as DNA methylation, histone modification, and non-coding RNA interactions, which can be influenced by environmental factors. In livestock, these epigenetic changes play a critical role in determining traits like growth, reproduction, milk production, and disease resistance.

Mechanisms of Epigenetic Inheritance

1. **DNA Methylation**: Methyl groups added to cytosine residues in CpG sites can silence or activate genes. For instance, methylation of the IGF2 gene influences muscle growth in pigs.
2. **Histone Modification**: Histone acetylation generally promotes gene expression by loosening chromatin, while methylation can either activate or repress gene activity depending on the site.
3. **Non-Coding RNAs**: MicroRNAs (miRNAs) regulate gene expression post-transcriptionally, affecting traits like immunity and growth.

Environmental Effects on Gene Expression

Environmental factors, including nutrition, temperature, stress, and toxins, significantly impact gene expression through epigenetic modifications. Examples in livestock include:

1) **Nutritional Programming**: Supplementing pregnant cows with methyl donors like choline and methionine can alter offspring growth performance via DNA methylation changes.

2) **Thermal Stress**: Heat stress during gestation in pigs can cause epigenetic changes that affect piglet growth and immune function.

3) **Microbiome Interaction**: Gut microbiota can influence gene expression through metabolites that impact histone modification and DNA methylation.

Practical Implications in Livestock

Understanding epigenetic inheritance allows breeders to design strategies for improving performance and resilience. For example, animals exposed to optimal prenatal nutrition often exhibit better growth and productivity due to favorable epigenetic modifications passed on to subsequent generations. Epigenetics thus serves as a bridge between environmental factors and genetic potential, helping optimize livestock production in response to changing environmental conditions (Lind & Spagopoulou, 2018).

7. Applications of Artificial Intelligence and Machine Learning in Livestock Genetics

7.1 Predictive Modeling for Genetic Traits

Predictive modeling for genetic traits in livestock involves using statistical and computational methods to estimate the genetic potential of animals for economically important traits like milk yield, growth rate, feed efficiency, and disease resistance. These models leverage genetic, phenotypic, and environmental data to guide selection decisions, improve breeding efficiency, and accelerate genetic gains.

Key Approaches in Predictive Modeling

1. **Quantitative Genetic Models**
   These models use phenotypic and pedigree data to estimate genetic parameters such as heritability, genetic correlations, and breeding values. The classic equation for phenotypic expression is:

P = G + E

where:

a) P = Phenotype
b) G = Genetic component
c) E = Environmental influence

For example, if a cow's milk yield is 6,000 liters, with a genetic contribution of 4,000 liters and environmental factors contributing 2,000 liters, the model helps predict the yield of its offspring when paired with another cow with similar characteristics.

2. **Best Linear Unbiased Prediction (BLUP)**

   BLUP uses mixed linear models to estimate breeding values by separating genetic and environmental effects. It is particularly useful in animal breeding programs for traits with moderate to high heritability.

The BLUP model can be represented as

$$\mathbf{Y = X\beta + Zu + e}$$

where:

a) y = vector of phenotypic observations
b) X = fixed effects matrix (e.g., farm management practices)
c) β = fixed effect estimates
d) Z = random effects matrix (e.g., animal genetics)
e) u = breeding values
f) e = residual error

3. Genomic Selection (GS)

GS uses high-density SNP markers distributed across the genome to predict genetic merit without direct phenotypic measurements. Machine learning algorithms like Random Forest, Support Vector Machines (SVM), and Bayesian models improve prediction accuracy.

4. Machine Learning and AI Models

Advanced models integrate multi-omics data (genomics, transcriptomics, and epigenomics) to predict complex traits. For instance, neural networks can capture non-linear relationships between markers and traits, enhancing prediction performance in polygenic traits like feed conversion ratio.

5. QTL Mapping and GWAS

Quantitative Trait Loci (QTL) mapping and Genome-Wide Association Studies (GWAS) identify genomic regions associated with specific traits, enabling more precise predictions of genetic potential (Guo et al., 2014).

Practical Applications

1) **Dairy Cattle**: Predicting milk yield, fat content, and mastitis resistance.
2) **Beef Cattle**: Forecasting growth rates and carcass characteristics.
3) **Poultry**: Estimating feed efficiency and egg production potential.
4) **Sheep and Goats**: Identifying markers for parasite resistance.

7.2 Data-Driven Breeding Programs

Data-driven breeding programs utilize advanced computational tools, genomic data, and statistical models to optimize genetic selection and improve livestock productivity. These programs integrate vast datasets, including phenotypic records, pedigree information, genomic profiles, and environmental factors, to make more informed, accurate, and efficient breeding decisions.

1. Phenotypic Data Collection

Accurate measurement of traits such as growth rate, milk yield, feed efficiency, fertility, and disease resistance forms the foundation of any breeding program. Automated systems, like electronic identification tags and precision livestock farming technologies, facilitate real-time phenotypic data collection.

2. **Genomic Data Integration**

   Modern breeding programs rely heavily on genomic information obtained through techniques like SNP arrays and whole-genome sequencing. Genomic selection enables breeders to assess the genetic potential of animals at an early age, reducing generation intervals and accelerating genetic progress (van Etten et al., 2023).

Statistical Models for Genetic Evaluation

1) **BLUP (Best Linear Unbiased Prediction)**: Estimates breeding values by separating genetic and environmental influences.
2) **Genomics BLUP (GBLUP)**: Extends BLUP by incorporating genome-wide marker data to predict genetic merit more accurately.

The GBLUP model is expressed as

$$\mathbf{Y = X\beta + Zg + e}$$

where:

a) y = phenotypic records,
b) X = matrix of fixed effects,
c) β = vector of fixed effect estimates,
d) Z = marker genotype matrix,
e) g = genomics breeding values,
f) e = residual errors.

Machine Learning and AI Integration

Advanced machine learning algorithms like Random Forest, Support Vector Machines (SVM), and Artificial Neural Networks (ANN) analyze complex, non-linear relationships between genetic markers and phenotypes, improving prediction accuracy for traits influenced by multiple genes.

Decision Support Systems (DSS)

DSS tools assist breeders by providing real-time insights and predictions based on integrated datasets. These systems help optimize mating plans, avoid inbreeding, and maximize genetic gains while maintaining genetic diversity.

Applications in Livestock Breeding

1. **Dairy Cattle**: Optimizing milk production, fertility, and udder health.
2. **Beef Cattle**: Enhancing growth rates, carcass quality, and feed efficiency.
3. **Poultry**: Improving egg production, feed conversion, and disease resistance.
4. **Sheep and Goats**: Selecting for parasite resistance and wool quality.

Benefits of Data-Driven Breeding

1) **Improved Accuracy**: Genomic and phenotypic integration increases the accuracy of genetic predictions.
2) **Shorter Generation Intervals**: Early genomic evaluation reduces the time required to identify superior breeding animals.
3) **Sustainability**: Data-driven insights promote resource-efficient production and genetic diversity preservation.
4) **Economic Efficiency**: More precise selection improves profitability through enhanced productivity and reduced costs.

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Bioinformatics and Computational Genomics in Livestock

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Bioinformatics and computational genomics have fundamentally reshaped livestock research by providing the means to analyze, interpret, and apply extensive genomic database, thereby plying a pivotal role in genetic enhancement, disease resistance, and precision breeding; this review delves into essential facets of bioinformatics within livestock genomics, encompassing data acquisition through next-generation sequencing (NGS), genotyping techniques, and transcriptomics, while also examining computational tools and databases such as Esembl, NCBI, and Animal QTLdb, which facilitate efficient genomic data management and analysis. Furthermore, it highlights genome assembly and annotation approaches, including de nova and reference-based assembly, alongside functional gene annotation strategies, and emphasizes the transformation of genomic selection and breeding through computational approaches, including machine learning (ML) and artificial intelligence (AI), which enhance predictive models for economically important traits. The review also explores the identification of genomic variations, including single nucleotide polymorphisms (SNPs) and structural variations, and their functional impact on livestock traits, while examining the role of metagenomics and microbiome analysis in animal health, with a specific focus on computational tools used to study gut microbiota. Additionally, it addresses epigenomics and systems biology approaches, including DNA methylation and histone modifications, which are increasingly utilized to understand gene regulation in livestock species, and discusses advances in disease resistance through host pathogen interaction studies genomic markers for disease resistance. Finally, the review underscore future trends, including the integration of big data, cloud computing, and AI in livestock genomics, along with the ethical and regulatory challenges, all of which will continue in shape the trajectory of sustainable livestock breeding and production.

Introduction to the Application of Bioinformatics in Livestock Genomics

Bioinformatics, a field bridging biology, computer science, and information technology, is essential for the analysis and interpretation of biological data. Within livestock genomics, it is pivotal for managing and analyzing extensive genetic datasets, thereby optimizing animal breeding, health, and productivity. By integration bioinformatics into livestock genomics,

researchers can identify genetic markers linked to desirable traits, enabling more precise selection in breeding programs. This precision leads to enhanced disease resistance, increased productivity, and improved animal welfare, all of which are critical for meeting the escalating global demand for animal derived food products(Adebayo, Popoola et al. 2024).

Bioinformatics has fundamentally transformed livestock genomics by seamlessly merging computational tools with biological data, facilitating a deeper understanding of genetic mechanisms, refining breeding programs, and significantly boosting productivity. As sequencing technologies and data analytics rapidly advance, bioinformatics has become an essential component for effectively managing and interpreting the vast quantities of genomic data generated(Khalid, Abdullah et al. 2021).

Livestock genomics aims to unravel the genetic basis of economically significant traits, including disease resistance, growth rate, reproductive capabilities, and milk production. By utilizing bioinformatics, researchers can pinpoint markers, conduct genome-wide association studies (GWAS), and apply genomic selection techniques to expedite genetic progress(Garcia, Do Carmo et al. 2012).

Essential bioinformatics tools for livestock genomics encompass alignment algorithms, genome annotation pipelines, and machine learning models that predict phenotypic outcomes based on genetic variations. Furthermore, databases like Ensembl, NCBI GenBank, and the Animal QTLdb serve as invaluable resources for comparative genomics and functional analysis. Combining omics technologies, such as transcriptomics, proteomics, and metabolomics, significantly deepens our understanding of intricate genetic traits(Arya, Gautam et al.). Additionally, systems biology strategies that utilize multi-omics data provide a holistic view of gene-environment interactions and epigenetic changes that influence livestock performance(Wani, Ganie et al. 2018).

While the ongoing evolution of bioinformatics, its application in precision breeding, genetic disease diagnostics, and conservation genetics will be crucial for achieving sustainable livestock production. Looking ahead, future developments in artificial intelligence, cloud computing, and blockchain technology are anticipated to future optimize genomic data management and utilization within the livestock sector(Ali, Awan et al. 2023).

Transformative Impact of Bioinformatics on Animal Science

Over the last few decades, bioinformatics has dramatically changed how we approach animal science. In the beginning, livestock genetic studies depended on traditional breeding practices and observations of physical traits. The emergence of molecular genetics brought techniques like marker-assisted selection, which were the first steps towards using computers in genetic analysis(Elrashedy, Mousa et al. 2025). However, it was the development of high-throughput sequencing and sophisticated computational algorithms that truly transformed livestock

genomics, enabling in-depth analyses of complex traits and the widespread use of genomic selection in breeding programs(Scarpa and Casu 2024).

Animal science has witnessed a remarkable transformation through the evolution of bioinformatics, which has fundamentally changed how genetic data is analyzed and applied to enhance livestock health, productivity, and sustainability. The past few decades have been marked by significant progress in computational tools and sequencing technologies, leading to a revolution in the field and providing unparalleled insights into animal genomes and their functional processes(Biswas, Kumari et al. 2023).

In its early stages, bioinformatics in animal science primarily concentrated on aligning sequences and identifying genetic markers. However, the introduction of high-throughput sequencing and genome-wide association studies (GWAS) broadened its scope, enabling researchers to map complex traits with increased precision. The subsequent development of whole-genome sequencing (WGS) and transcriptomics has further amplified our capacity to investigate gene expression patterns, epigenetic modifications, and the regulatory networks that shape phenotypic traits(Ahmed 2024). Modern bioinformatics now combines data from multiple “omics” fields, like proteomics and metabolomics, to give a complete picture of how genetics and environment interact. Cutting-edge computational techniques, such as artificial intelligence (AI) and machine learning (ML), are currently being used to forecast disease risk, improve breeding strategies, and refine precision livestock farming practices(ASLAM, MASOUD et al. 2024).

Publicity accessible genomic databases, such as Ensembl, NCBI GenBank, and the Animal QTLdb, have significantly aided comparative genomics and functional annotation, offering crucial insights into evolutionary biology and adaptations specific to different species. Furthermore, cloud computing and blockchain technology are increasingly recognized as indispensable tools for streamlined data management and secure information exchange within the livestock sector(Kim and Pathak).

Techniques for Livestock Genomic Data Collection

Advanced DNA Sequencing Techniques: Next-Generation and Whole Genome Sequencing

The progress made in DNA sequencing technologies has been crucial for advancing livestock genomics. Next-generation (NGS) platforms allow for the quick and affordable sequencing of complete genomes, providing detailed information on genetic variations and structural characteristics. Whole genome sequencing (WGS) offers a complete picture of an organism’s genetic composition, aiding in the identification of genes linked to valuable economic traits. These technologies have been fundamental in developing reference genomes for numerous livestock species, which are vital resources for both genetic research and breeding initiatives(Ghosh, Sharma et al. 2018).

Next-Generation Sequencing (NGS) has fundamentally changed livestock genomics by enabling the swift and simultaneous sequencing of DNA. Platforms like Illumina and Ion Torrent produce extensive amounts of highly accurate sequencing data, allowing researchers to pinpoint genetic variations, single nucleotide polymorphisms (SNPs), and structural variations linked to economically valuable traits. NGS is broadly used for transcriptome analysis, genome-wide association studies GWAS), and epigenetic investigations in livestock(Pareek, Smoczynski et al. 2011).

Whole Genome Sequencing (WGS)

Whole Genome Sequencing (WGS) delivers a complete picture of an organism's full genetic makeup, revealing insights into genetic diversity, evolutionary connections, and functional genomics. WGS is essential for mapping complex traits, detecting uncommon genetic variations, and identifying the mutations that cause disease resistance and performance traits. Thanks to the declining cost of sequencing, WGS has become a highly valuable tool in both precision breeding and conservation genetics(Price-Carter, Brauning et al. 2018).

Combining these sequencing technologies with bioinformatics tools facilitate efficient data processing, genome annotation, and predictive modeling, which in turn enhances genetic improvement programs in livestock. The ongoing advancements in sequencing methodologies, along with the integration of artificial intelligence and cloud computing, are anticipated to significantly accelerate discoveries in animal genomics and promote sustainable livestock production(Georges, Charlier et al. 2019).

Methods for Genotyping: SNP Chips and RAD-Seq

Genotyping techniques are critical for identifying genetic differences between individuals of the same species. Single Nucleotide Polymorphism (SNP) chips enable the simultaneous analysis of numerous SNPS, helping to pinpoint genetic markers associated with particular traits. Restriction site-associated DNA sequencing (RAD-Seq) is another method that facilitates the discovery and genotyping of SNPs throughout the genome, even without a pre-existing reference genome(Guppy, Jones et al. 2020). These techniques have significantly improved the efficiency of genetic research and the use of genomic selection in livestock breeding. Specifically, SNP chips are commonly used to genotype livestock populations by detecting genetic variations at predetermined locations. These microarray-based platforms offer quick and affordable screening of thousands to millions of SNPs across the genome. SNP chips are widely employed in genomic selection, parentage verification, and breed characterization, making them indispensable for livestock improvement initiatives(Andrews, Adams et al. 2018).

Restriction site Associated DNA Sequencing (RAD-Seq) Method

RAD-Seq is a streamlined sequencing method that allows for detailed genotyping without needing a pre-existing reference genome. By focusing sequencing efforts on DNA fragments close to restriction enzyme cut sites, RAD-Seq offers an economical way to discover genetic variations and create comprehensive genetic maps. This technique is especially valuable for investigating population genetics, genetic diversity, and evolutionary processes in livestock populations(Lowry, Hoban et al. 2017).

Combining these advanced sequencing and genotyping technologies with powerful bioinformatics tools for allows streamlined data processing, accurate genome annotation, and robust predictive modeling, significantly improving genetic enhancement programs for livestock. The ongoing progress in sequencing techniques, along with the integration of artificial intelligence and cloud computing, promises to further accelerate in animal genomics and contribute to sustainable livestock production(Mao, Zhang et al. 2021).

Methods of Transcriptomics and Proteomics in Livestock Studies

The evolution of omics technologies has dramatically reshaped the landscape of livestock genetics, physiology, and disease resistance research. Of these, transcriptomics and proteomics are indispensable for deciphering gene expression and protein function within animal health and production. Transcriptomics examines the full array of RNA transcripts generated by the genome under particular circumstances, whereas proteomics scrutinizes the complete protein composition of a cell or a tissue. Combining these methodologies yields critical knowledge about the molecular processes that govern growth, reproduction, immunity, and adaptation in livestock(Kadarmideen and Reverter 2007).

Transcriptomics examines the complete collection of RNA transcripts produced by a genome under specific conditions, while proteomics focuses on the full set of expressed proteins. In livestock, transcriptomic analyses are vital for understanding gene expression patterns related to key areas like growth, reproduction, and disease resistance. Simultaneously, proteomic studies offer insights into protein functions and interactions, contributing to a more profound understanding of physiological processes and the identification of biomarkers for health and productivity traits(Suravajhala, Kogelman et al. 2016). Proteomics, as the comprehensive study of proteins, has become a cornerstone in livestock research, offering critical insights into the molecular mechanisms that control growth, reproduction, immunity, and disease resistance(Lippolis, Powell et al. 2019).

In contrast to genomics and transcriptomics, which center on genetic and RNA expression, proteomics directly investigates protein expression, modification, interaction, and functions. Because proteins are the workhorses of cells, proteomics offers a more profound understanding of the physiological and pathological processes occurring in livestock(Chakraborty, Sharma et al. 2022). While proteomics holds immense potential in livestock research, challenges persist,

including the complexity of samples, the intricacies of data interpretation, and the high costs involved. Future progress in bioinformatics, machine learning, and high-throughput proteomic techniques will significantly enhance our capacity to utilize proteomics for precision livestock farming. Furthermore, integrating proteomics with other omics technologies-genomics, transcriptomics, and metabolomics-will lead to improved genetic selection, more effective disease management, and enhanced overall productivity in livestock(Mullins, Keogh et al. 2021).

Essential Tools and Databases for Livestock Genomic Research

Commonly Used Bioinformatics Software

The field of livestock genomics has undergone a significant revolution in recent years, propelled by breakthroughs in high-throughput sequencing technologies, computational biology, and bioinformatics. As genomic data accumulates, bioinformatics tools and databases have become essential for the storage, analysis, and interpretation of this extensive information. The incorporation of computational methodologies has fundamentally changed livestock breeding practices, disease resistance research, and efforts to improve productivity. This overview details the most frequently used computational tools and databases in livestock genomics, emphasizing their important in genetic analysis, genome annotation, functional genomics, and comparative studies(Hu, Koltes et al. 2012, Pandey, Singh et al. 2025).

Databases Facilitating Livestock Genomic Research

1. National Center for Biotechnology Information (NCBI)

The NCBI offers an extensive collection of genomic data, encompassing resources like the GenBank sequence database, the sequence Read Archive (SRA), and the Gene Expression Omnibus (GEO). These platforms enable researchers to access livestock genomes, transcriptomes, and epigenomic data, which in turn allows for the analysis of genetic variations, gene expression patterns, and evolutionary connections(Coordinators 2012).

2. Ensembl Genome Browser

Ensembl provides a comprehensive genome annotation platform that includes livestock genomes like cattle, sheep, pigs, and chickens. This platform offers access to gene models, transcript isoforms, regulatory elements, and comparative genomics data, which helps researchers pinpoint genomics regions with functional significance(Coordinators 2012).

3. Animal Genome Database (AGD)

The Animal Genome Database (AGD) acts as a central hub for livestock genome projects, providing curated genomic sequences, SNP data, and Quantitative Trait Loci (QTL) information. This platform assists researchers in identifying genetic markers associated with traits of economic importance(Gobikrushanth, Purfield et al. 2019).

4. Livestock Genomics Toolkit (LGT)

The livestock Genomics Tools (LGT) platform is specifically designed to combine various livestock-related datasets, making genome-wide association studies (GWAS) and functional annotations easier. It allows researchers to investigate genetic variations that impact productivity, health, and reproductive capabilities in livestock(Hosseini, Perello et al. 2023).

5. UCSC Genome Browser

The UCSC Genome Browser offers a visual interface that allows researchers to explore livestock genomes overlaid with annotated tracks, such as gene locations, single nucleotide polymorphisms (SNPs), and conserved elements. This tool is particularly useful for those investigating gene regulation and evolutionary conservation (Karolchik, Baertsch et al. 2003) as shown in table 1.

Table 1. Databases Used in Livestock Genomics Research

Database Name	Species Covered	Key Features	Data Types Available
Ensembl Genome Browser	Multiple livestock species (Cattle, Sheep, Pig, Chicken, etc.)	Genomic annotations, comparative genomics, variation data	Whole-genome sequences, SNPs, gene annotations, protein-coding genes
NCBI GenBank	All livestock species	Public repository for nucleotide sequences	Genomes, mRNA, protein sequences, functional annotations
UCSC Genome Browser	Cattle, Pig, Chicken, Horse, etc.	Visualization of genome assemblies, sequence alignment tools	Reference genomes, SNP data, gene expression datasets
Animal QTLdb	Cattle, Sheep, Pig, Chicken, Horse	Quantitative Trait Loci (QTL) database for livestock	QTL data for economically important traits, GWAS results
FAANG (Functional Annotation of Animal Genomes)	Cattle, Sheep, Pig, Chicken, Horse, and more	Epigenetic and functional genomic studies	Transcriptomics, chromatin accessibility, histone modifications
LGS (Livestock Genomics Sequencing Database)	Cattle, Goat, Sheep, Pig, Chicken	Sequencing and variant discovery in livestock genomes	Whole-genome sequencing, transcriptomics, epigenomics
dbSNP (NCBI)	All livestock species	SNP database for genome-wide	Single Nucleotide Polymorphisms (SNPs),

		association studies (GWAS)	genotype frequency
Databases of The International Sheep Genomics Consortium	Sheep	Sheep-specific genomic research data	Whole-genome sequences, genotyping array data, QTL
Bovine Genome Database (BGD)	Cattle	Functional and structural annotations of the bovine genome	SNPs, QTLs, gene expression data
Porcine Genome Database	Pig	Genomic information relevant to pig breeding and research	SNP data, QTL mapping, functional annotations
Avianbase	Chicken	Chicken genome assembly and gene annotation	Whole-genome sequences, SNPs, epigenetic modifications
Horse Genome Project	Horse	Research and annotations related to equine genomics	Whole-genome sequences, gene expression, disease markers
Goat Genome Database	Goat	Comprehensive genome data on goat breeds	SNP arrays, QTL mapping, transcriptomic data

Software Tools Frequently Used for Livestock Genomics Analysis

1. Tools for Variant Identification and Genomic Analysis

a. BWA (Burrows-Wheeler Aligner) and Bowtie

These computational tools are extensively employed to align short reads produced by high-throughput sequencing technologies against reference genomics, a critical step that facilitates the precise detection of single nucleotide polymorphisms (SNPs) and the analysis of structural variations, both of which are indispensable for effective genomic selection strategies in livestock breeding programs(Gao, Wu et al. 2017).

b. GATK (Genome Analysis Toolkit)

GATK provides a robust platform for variant discovery and genotyping in livestock, utilizing advanced algorithms to ensure highly accurate identification of genetic polymorphisms through precise variant calling, filtering, and annotation(Biscarini, Cozzi et al. 2018).

c. SAMtools and Picard

SAMtools and Picard are indispensable tools in genomic analysis, providing essential functionalities for processing sequence alignment files, including crucial tasks such as sorting, indexing, and quality control, thereby enabling effective downstream analysis pipelines(Yaro, Munyard et al. 2017).

d. PLINK

PLINK is a cornerstone software for researchers investigating genetic associations, offering capabilities for genome-wide association studies (GWAS), linkage disequilibrium analysis, and population genetics, ultimately empowering the identification of genetic markers linked to desirable traits within livestock populations(Husien, Saleh et al. 2024).

e. SnpEff and ANNOVAR

Functional annotation tools are crucial for deciphering the biological significance of genetic variants by predicting their impact on gene function and ultimately, phenotype; they serve as powerful aids in prioritizing candidate variants for targeted breeding programs and studies focused on disease resistance (Figure 1).

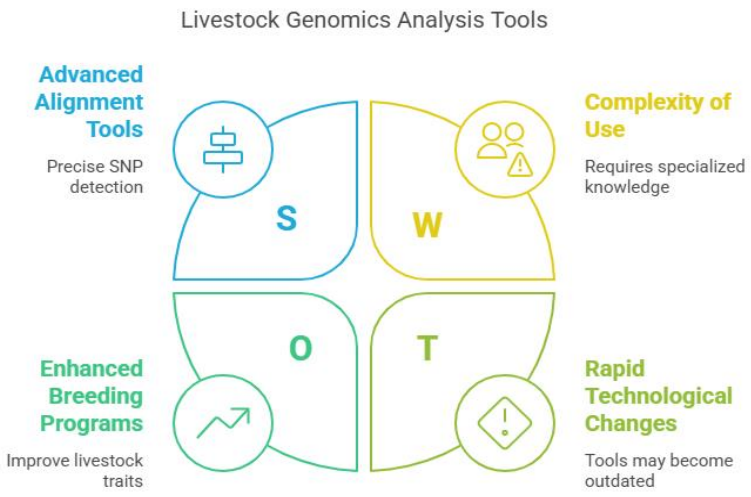

Figure 1. A Conceptual Diagram Illustrating Key Factors Influencing the Adoption of Advanced Genomic Tools in Livestock Breeding.

2. Tools for Transcriptomic and Functional Genomic Analysis

a. HISAT2 and STAR

HISAT2 and STAR, both powerful RNA-Seq aligners, are essential for transcriptome analysis, allowing researchers to delve into gene expression patterns, investigate alternative splicing events, and characterize transcript isoforms within livestock populations.

b. Cufflinks and DESeq2

Cufflinks and DESeq2 are critical tools for differential gene expression analysis, enabling the identification of genes whose expression levels are significantly altered, whether increased (upregulated) or decreased (downregulated), in response to diverse environmental or genetic influences.

c. EdgeR

EdgeR provides a robust framework for RNA-Seq data analysis, particularly valuable for comparative gene expression studies across various livestock breeds or in response to differing environmental conditions.

d. DAVID (Database for Annotation, Visualization, and Integrated Discovery)

DAVID aids researchers in understanding the biological context of gene expression changes by performing functional enrichment analysis, which identifies significant biological pathways and Gene Ontology (GO) terms linked to differentially expressed genes (Figure 2).

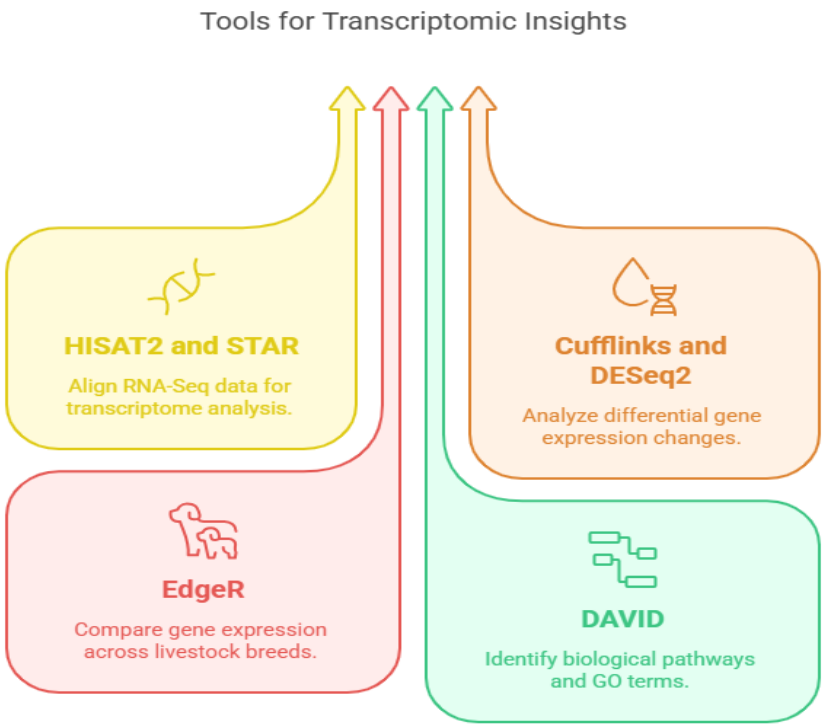

Figure 2. A workflow of Bioinformatics Tools used for Transcriptomic Analysis in Livestock Genomics. The Process Begins with Aligning RNA-Seq Reads to a Reference Genome Using Aligners such as HISAT2 or STAR.

3. Tools for Structural and Comparative Genomic Analysis

a. BLAST (Basic Local Alignment Search Tool)

BLAST stands as a cornerstone in bioinformatics, providing essential functionally for comparing nucleotide and protein sequences. This powerful tool directly assists researchers in the identification of homologous genes, revealing shared ancestry and function. Furthermore, it enables the detection of conserved domains, highlighting regions of functional importance. Ultimately, BLAST facilitates the exploration of evolutionary relationships, shedding light on the genetic connections among diverse livestock species.

b. OrthoMCL

OrthoMCL plays a vital role in comparative genomics by identifying orthologous and paralogous genes across multiple species. This capability enables researchers to conduct in-depth evolutionary analyses and gain insights into gene family evolution.

c. RepeatMasker

RepeatMasker is an essential tool for the identification and characterization of repetitive elements within livestock genomes. This process is critical for accurate genome annotation and provides valuable insights into structural variation, contributing significantly to our understanding of genome architecture and evolution.

d. COG (Clusters of Orthologous Groups)

COG facilitates the annotation of newly sequenced livestock genomes by categorizing proteins into functional groups based on their evolutionary relationships. This classification provides valuable insights into the biological roles of proteins and helps to understand the functional organization of genomes (Figure 3).

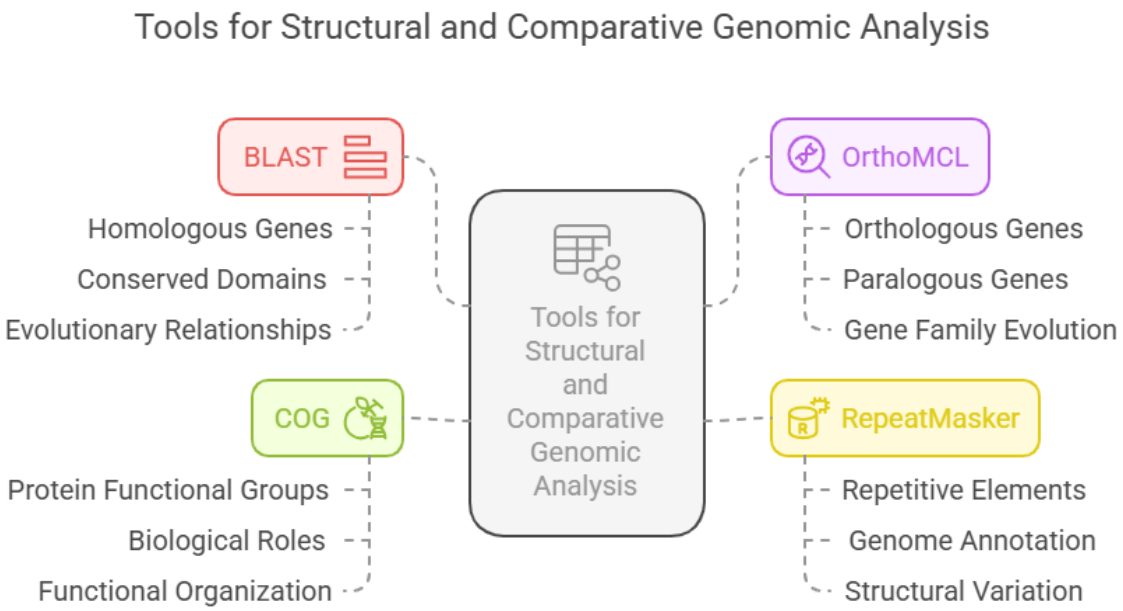

Figure 3. A Summary of Key Bioinformatics Tools for Structural and Comparative Genomic Analysis.

4. Genomic Tools for Regulatory and Epigenetic Studies

a. Bismark

Bismark is a key tool for deciphering DNA methylation patterns in livestock, enabling researchers to understand how epigenetic mechanisms control gene expression.

b. HOMER

HOMER enables the identification of transcription factor binding sites and regulatory elements within livestock genomes, thereby supporting focused on gene regulation mechanisms.

c. MEME Suite

The MEME Suite is a powerful tool for motif discovery and analysis, enabling researchers to pinpoint conserved regulatory sequence within livestock genomes. By revealing these key DNA patterns, it facilitates a deeper understanding of gene regulation mechanisms (Figure 4).

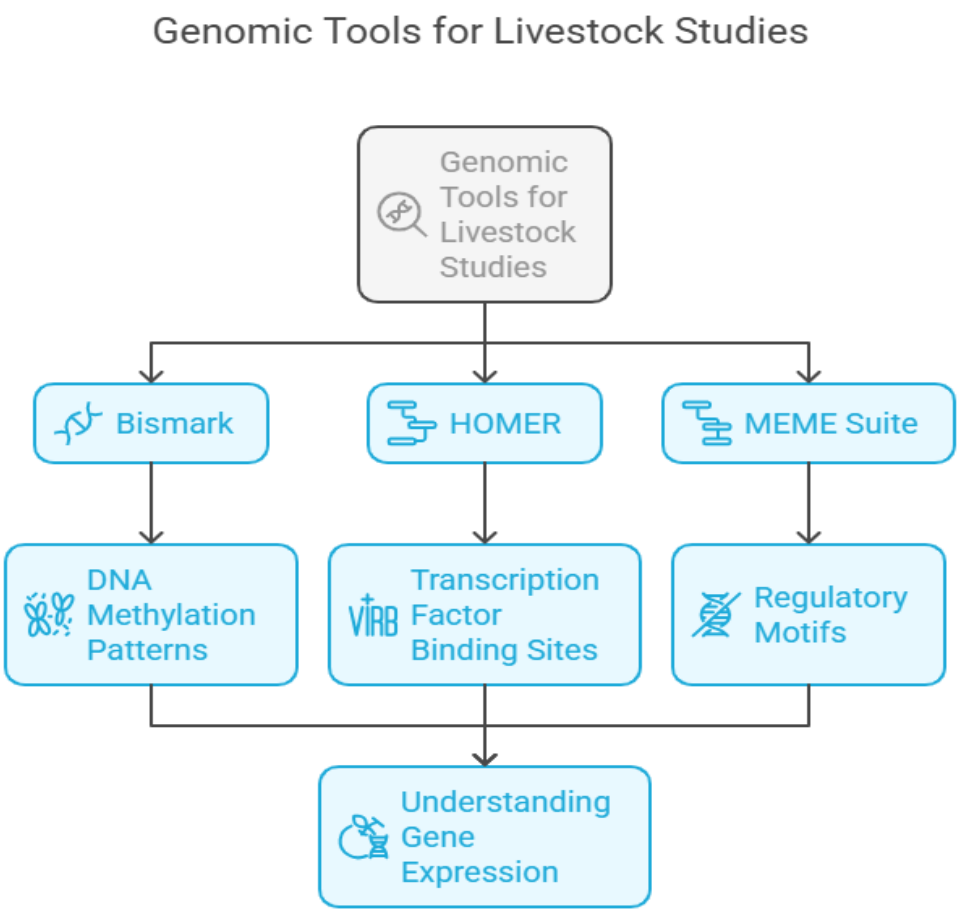

Figure 4. A Schematic of Genomic Tools used to Study Gene Regulation in Livestock.

Comprehensive Genomic Strategies for Livestock Improvement

The convergence of systems biology and multi-omics integration has revolutionized livestock genomics, yielding computational frameworks that synthesize diverse-genomics, transcriptomics, proteomics, and metabolomics. This holistic approach significantly deepens our comprehension of complex traits and paves the way for precision breeding strategies. Network-based analyses, facilitated by tools like Cytoscape, STRING, and iPathwayGuide, allow researchers to visualize pathways and regulatory networks(ASLAM, MASOUD et al. 2024).

While computational tools and databases have propelled livestock genomics forward, significant obstacles persist. Polygenic trait complexity, data standardization inconsistencies, and the demand for robust computing infrastructure pose substantial challenges(Xu, Liu et al. 2020). To address these hurdles, future advancements in artificial intelligence machine learning, and cloud-based bioinformatics are essential (Table 2). Furthermore, promoting open-access initiatives for livestock genomic resources will foster global collaboration and data sharing, accelerating research progress(Qureshi, Sabir et al. 2014).

Table 2. Essential Software for Livestock Genomic Analysis

Category	Tool	Function	Application in Livestock Research
Variant Calling and Genomic Analysis	BWA, Bowtie	Read alignment to reference genome	SNP discovery, structural variation analysis
	GATK	Variant calling, filtering, annotation	Genotyping, genome-wide studies
	SAMtools, Picard	Sequence alignment processing	Quality control, variant analysis
	PLINK	GWAS, population genetics	Identifying trait-associated markers
	SnpEff, ANNOVAR	Functional variant annotation	Predicting impact of mutations
Transcriptomics and Functional Genomics	HISAT2, STAR	RNA-Seq read alignment	Gene expression, alternative splicing
	Cufflinks, DESeq2	Differential expression analysis	Identifying genes influenced by environmental factors
	EdgeR	RNA-Seq analysis	Breed comparison, stress response studies
	DAVID	Gene enrichment analysis	Functional annotation of expressed genes
Structural and Comparative Genomics	BLAST	Sequence similarity search	Homology detection, evolutionary studies
	OrthoMCL	Ortholog detection	Evolutionary and functional studies
	RepeatMasker	Repeat element identification	Genome annotation and structure studies
	COG	Functional protein classification	Genome-wide functional annotation
Epigenomics and Regulatory Genomics	Bismark	DNA methylation analysis	Studying epigenetic modifications
	HOMER	Transcription factor binding site analysis	Regulatory element identification
	MEME Suite	Motif discovery and regulatory sequence	Characterizing promoter elements

		analysis	
Integrative and Systems Biology	Cytoscape	Network visualization	Protein interaction and pathway analysis
	STRING	Protein-protein interaction analysis	Studying functional networks
	iPathwayGuide	Pathway enrichment analysis	Functional genomics interpretation

Applying Genomics in Livestock Breeding Programs

Genomic selection (GS) has transformed livestock breeding by utilizing extensive genomic data to enhance the precision that depend on pedigree and observable characteristics, GS employs genome-wide markers to forecast an animal's genetic potential. By incorporating both additive and non-addictive genetic variations, this methodology significantly improves the reliability of estimated breeding values (EBVs). The application of whole-genome sequencing and SNP chips empowers breeders to identify and select genetically superior animals early in life, thereby shortening generation intervals and accelerating genetic advancements(Qureshi, Sabir et al. 2014).

A major benefit of genomic selection lies in its ability to improve traits that are challenging to measure directly, including disease resistance, fertility, and feed efficiency. This is vital for sustainable livestock production, enabling the selection of animals better equipped to withstand environmental pressures and diseases. Furthermore, GS reduces inbreeding by offering detailed genetic insights, thereby preserving genetic diversity within livestock populations. Developed livestock industries in countries like the United States, Canada, and European nations have extensively implemented genomic selection in dairy cattle, pigs, and poultry, resulting in substantial gains in productivity and enhanced animal welfare(Qureshi, Sabir et al. 2014, Burrow and Goddard 2023).

While genomic selection offers significant advantages, it also poses certain challenges. The upfront expense of genotyping and maintaining extensive reference populations can be considerable, especially for smaller agricultural operations. Moreover, the reliability of genomic predictions is contingent on the size and variability of reference populations, necessitating ongoing updates to genomic databases. As genomic technologies advance, the incorporation of multi-omics data, including transcriptomics and metabolomics, will further refine the precision of genetic assessments(Kadarmideen and Reverter 2007).

AI and Machine Learning for Genomic Insights in Livestock

AI and machine learning (ML) are revolutionizing livestock genomics by providing powerful methods to analyze extensive and intricate datasets, thereby enhancing genetic selection, disease forecasting, and production efficiency. Conventional statistical models often struggle to capture the complex interplay between genes and environmental variables. Machine learning algorithms, including deep learning, support vector machines, and random forests, offer a data-centric approach, enabling more accurate predictions of phenotypic outcomes(Chafai, Hayah et al. 2023).

AI-driven genomic selection models excel at analyzing vast datasets, revealing subtle patterns and genetic markers linked to economically significant traits. For example, deep learning algorithms have demonstrated superior accuracy in predicting breeding values from SNP data compared to traditional methods. Furthermore, machine learning techniques are being employed to refine mating strategies, effectively balancing the preservation of genetic diversity with the maximization of desirable traits within livestock populations(Kumar, Panigrahi et al. 2024).

AI is also significantly advancing disease prediction and surveillance in livestock genomics. By combining genomic, environmental, and health data, machine learning models can forecast an animal's vulnerability to diseases, enabling proactive interventions and focused breeding programs. Furthermore, AI-powered image recognition technologies are being deployed to evaluate animal health, behavior, and physical condition in real time, leading to more streamlined and effective herd management practices. Implementing AI and machine learning in livestock genomics faces several hurdles. These include the requirement for large, accurately labeled datasets, access to substantial computational resources, and specialized bioinformatics expertise. Moreover, although AI models improve prediction accuracy, their lack of interpretability poses a challenge, as complex algorithms can obscure underlying biological mechanisms. Future efforts will likely prioritize the development of explainable AI models and the integration of multi-omics data to achieve even more precise genomic selection(Adebayo, Popoola et al. 2024).

The combined power of genomic selection and AI-driven analytics promises to reshape livestock breeding (Table 3). By merging genomic information with advanced predictive models, breeders can make significantly more informed choices, accelerating genetic improvement and enhancing the long-term sustainability of animal agriculture. As these technologies continue to advance, they will be vital in tackling global issues such as food security, climate change adaptation, and the promotion of animal welfare (Pedrosa, Chen et al. 2024).

Table 3. Machine Learning and AI Tools in Livestock Genomics

Category	Tool	Function	Application in livestock research
Genomic Selection and Prediction	GBLUP (Genomic Best Linear Unbiased Prediction)	Estimates breeding values using genomic data	Improves accuracy of genetic merit predictions
	BayesB, BayesC	Bayesian regression models for genomic selection	Applied in trait prediction and genomic evaluations
	DeepGS (Deep Learning-based Genomic Selection)	Utilizes deep learning for genomic prediction	Enhances prediction accuracy for complex traits
Disease Prediction and Health Monitoring	Random Forest	Performs classification and regression	Identifies genetic susceptibility to diseases
	Support Vector Machine (SVM)	Classifies high-dimensional data	Assesses disease risk in livestock populations
	CNN (Convolutional Neural Networks)	Recognizes images	Analyzes body condition and detects diseases
Behavior and Welfare Monitoring	YOLO (You Only Look Once)	Detects objects in real-time	Monitors livestock movement and behavior
	LSTM (Long Short-Term Memory)	Analyzes time-series data	Predicts feeding behavior and stress responses
Multi-Omics Data Integration	XGBoost	Uses gradient boosting for feature selection	Enhances multi-omics-based genetic prediction
	AutoML	Automates machine learning model optimization	Identifies novel genotype-phenotype associations

Future Trends and Challenges in Livestock Bioinformatics

Livestock bioinformatics is undergoing rapid transformation, fueled by progress in sequencing, computing, and AI. Future directions emphasize the integration of diverse “omics” data, using blockchain for secure data handling, and implementing precision livestock farming. As research advances, there’s a critical need for scalable bioinformatics pipelines to manage the expanding

data volume. Personalized breeding, adapting genetic selection to specific environments, is anticipated to boost productivity and sustainability in livestock agriculture(Ahmad and Hamid 2023).

A significant hurdle in livestock bioinformatics is achieving data interoperability and standardization across diverse research and breeding initiatives. Integration varied datasets from genomics, transcriptomics, epigenomics, and metagenomics necessitates advanced computational tools capable of harmonizing and analyzing these complex data types. Furthermore, ethical concerns surrounding data privacy, ownership, and accessibility are paramount, especially as genomic information becomes a valuable commodity in commercial breeding(Patil, Kanthe et al. 2023).

The integration of high-throughput phenotyping, utilizing sensor and imaging systems, will refine genomic predictions and enhance livestock management. However, overcoming data accuracy, computational efficiency, and cost-effectiveness challenges is crucial for successful implementation. Continued interdisciplinary collaboration between geneticists, computer scientists, and veterinaries will be vital for translating genomics insights into practical breeding and management practices as bioinformatics progresses(Singh, Gautam et al. 2018).

Utilizing Cloud Computing for Big Data Challenges in Livestock Genomics

Bid data analytics and cloud computing are revolutionizing livestock genomics by changing how genetic information is managed. The explosive growth of genomic data from advanced sequencing technologies demands distributed computing and cloud infrastructures. Cloud platforms enable researchers and breeders to access extensive genomic datasets without needing substantial local computing resources, effectively broadening access to sophisticated bioinformatics tools. Big data analytics in livestock genomics combines various data types, such as genetic markers, phenotypic records, environmental factors, and health information. Machine learning algorithms are vital for extracting valuable insights, enabling predictive models for trait selection, disease resistance, and improved productivity. Cloud-based platforms like Google Cloud, AWS, and Azure support real-time data analysis, collaborative research, and secure data sharing among international partners(Koltes, Cole et al. 2019).

While big data and cloud computing offer numerous benefits to livestock genomics, their adoption is hindered by several challenges. Data security and privacy are top priorities, especially when handling sensitive genetic information. Moreover, the expenses of cloud services and the requirement for specialized bioinformatics and data science skills can restrict their use in resource-limited environments. To mitigate these issues, initiatives are focusing on creating open-access databases and intuitive bioinformatics platforms that can be seamlessly incorporated into breeding programs(Morota, Ventura et al. 2018).

The future of big data in livestock genomics on genomics hinges on automated data pipelines and AI-powered analytics. By integrating genomic, environmental, and management data,

researchers can build predictive models that optimize breeding and bolster livestock resilience to climate change. The ongoing development of cloud computing infrastructure will be crucial for enabling extensive genomic studies, driving advancements in precision livestock breeding and sustainable agriculture(Koltes, Cole et al. 2019, Perakis, Lampathaki et al. 2020).

Ethical Guidelines and Regulatory considerations for Livestock Genomics

The incorporation of cutting-edge technologies in livestock genomics brings forth crucial ethical and regulatory issues. The gathering and analysis of genetic data demand robust data privacy and security protocols to prevent unauthorized access and misuse. Furthermore, the application of AI and ML in decision-making must be transparent and understandable, fostering accountability and building trust among all involved parties(Banerjee and Mondal 2021).

Regulatory frameworks must keep pace with the evolution of genomics and bioinformatics. This requires setting standards for data sharing, intellectual property, and the ethical use of genomic data in breeding. Collaboration among policymakers, industry, and the public is vital to ensure technological advancements align with societal values and expectations(Kramer 2020).

Future Prospects in Computational Livestock Genomics

Driven by emerging technologies, computational livestock genomics is set for transformative advancements. The convergence of AI, Big Data analytics, and cloud computing will deepen our understanding of complex genetic traits and refine breeding strategies. A particularly promising area is the integration of multi-omics data, combining genomics, transcriptomics, proteomics area is the integration of multi-omics data, combining genomics, transcriptomics, proteomics, and metabolomics to achieve a holistic view of animal biology. This comprehensive approach will facilitate the identification enhancing animal health and productivity(Gutierrez-Reinoso, Aponte et al. 2021).

Adding to the transformative potential, the integration of genome editing technologies like CRISPR-Cas9 with AI opens avenues for precise genetic modifications, enabling the introduction of desirable traits or the correction of genetic defects. However, the responsible implementation of these advancements necessitates careful consideration of ethical implications and adherence to robust regulatory guidelines(Lamb, Hayes et al. 2020).

Conclusion

Bioinformatics and computational genomics have fundamentally changed livestock research, facilitating large-scale genomic analyses, precision breeding, and enhanced disease resistance. Through the integration of high-throughput sequencing, genome-wide association studies (GWAS), and machine learning, we`ve gained a much deeper understanding of genetic variations, complex traits, and the evolutionary history of livestock.

Computational tools in livestock genomics have been instrumental in pinpointing key genes linked to productivity, disease resistance, and environmental adaptation. Furthermore, functional genomics approaches, including transcriptomics, epigenomics, and metagenomics, have illuminated gene regulation and host-microbiome interactions. These advancements are crucial for enhancing animal health, refining breeding programs, and promoting sustainable livestock production. While significant strides have been made, challenges persist in livestock bioinformatics. These include the necessity for high-quality reference genomes, robust computational infrastructure, and standardized bioinformatics pipelines. Moreover, ethical considerations, data require further focus to fully realize the potential of bioinformatics in livestock research.

Looking ahead, sustained progress in artificial intelligence, cloud computing, and multi-omics integration will refine the predictive power of genomic models and facilitate livestock farming. Crucially, collaboration efforts among geneticists, computational biologists, and veterinarians will be essential to translate bioinformatics findings into actionable strategies that enhance animal welfare, productivity, and contribute to global food security. Leveraging bioinformatics and computational genomics positions the livestock industry achieve sustainable genetic advancements, leading to more efficient and sustainable animal production systems that can effectively address the increasing demands of the global population.

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CRISPR Technologies in Livestock

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CRISPR-Cas genome editing has dramatically changed livestock genetics, allowing for precise DNA alterations that improve production, disease resistance, and animal well-being. This technology enables the creation of livestock with faster growth, better feed use, and immunity to disease. Furthermore, it aids in allergen reduction and improved reproduction. While CRISPR opens doors for innovations like xenotransplantation and biopharming, ethical consideration, regulatory hurdles, and potential unintended genetic changes need careful attention. This overview examines CRISPRs current uses, benefits, and challenges in animal agriculture. Future progress will rely on combining extensive biological data, AI, and advanced gene delivery, alongside addressing ethical and regulatory concerns for responsible implementation. Optimizing genome for both efficiency and safety necessitates the integration of multi-omics data, artificial intelligence, and innovation gene delivery systems. Crucial to the responsible deployment of these technologies in animal breeding and biotechnology will be the resolution of ethical and regulatory challenges.

1. Introduction to Genome Editing in Livestock

Livestock production is fundamental to global agriculture, supplying vital products like meat, milk, wool, and with the growing world population, the demand for these high-quality animal products continues to escalate; consequently, sustainable genetic improvements are crucial to meet these rising needs, building upon the significant contributions of traditional breeding techniques such as artificial selection and crossbreeding that have shaped livestock for centuries. While traditional breeding has been vital, its slow pace and potential for unintended genetics changes have prompted the adoption of modern biotechnology, specifically genome editing, which offers precise and efficient enhancements in genetic research, notably the 1953 discovery of DNAs structure, which paved the way for contemporary genetic modification techniques(Petersen 2017, Bastiaansen, Bovenhuis et al. 2018). The progression of genetic modification in livestock began 1970s recombinant DNA technology enabling transgenic animal development, exemplified by the 1980s creation of the first genetically modified mouse. This expanded in the 1990s to include transgenic pigs and cattle engineered for the enhanced growth

and disease resistance, and was further revolutionized by the 1996 cloning of Dolly the sheep, demonstrating the power of these technologies in animal breeding. Despite the potential of transgenic livestock, ethical and regulatory hurdles hindered its widespread use in food production. The emergence of genome editing in the early 21st century, featuring technologies like ZFNs, TALENs, and CRISPR-Cas9, provided a solution by enabling precise genetic modifications without foreign DNA insertion, thus accelerating the development of improved livestock traits while mitigating the concerns surrounding the traditional genetic engineering. Genome editing offers a revolutionary approach to precisely modify an organism's DNA, enhancing traits like health, productivity, and adaptability(Raza, Hassanin et al. 2022). Distinct from traditional genetic engineering's insertion of foreign DNA, genome editing allows targeted changes without necessarily introducing external genetic material, a precision highly valued in animal breeding to minimize unintended alteration. Among the various technologies developed, CRISPR-Cas9 has become the most efficient and prevalent tool for this purpose. While zinc-finger nucleases (ZFNs) and transcription activator-like nucleases (TALENs) have played a role in genome editing, with ZFNs pioneering targeted gene modifications through engineered proteins that create DNA breaks, they have been largely surpassed by CRISPR-based systems due to CRISPRs superior ease and efficiency; the design complexity and restricted targeting flexibility of ZFNs have ultimately limited their widespread use in animal breeding(Ruan, Xu et al. 2017). TALENs, offering improved specificity and efficiency over ZFNs by utilizing distinct DNA-binding domains, still suffer from labor-intensive and costly procedures compared to the revolutionary CRISPR-Cas9 system, which, derived from bacterial immunity, excels in simplicity, precision, and efficiency through gRNA-guided Cas9 nuclease targeting for diverse genetic modifications like knockouts and insertions, thus making it ideal for livestock genome editing, this advancement builds upon millennia of animal genetic modification, beginning with the domestication and selective breeding of livestock, where early societies shaped breeds for desirable traits through centuries of selective pressure(Lee, Uh et al. 2020).

2. CRISPR-Cas9 and Alternative Genome Editing Techniques

2.1 CRISPR-Cas9: A Revolution in Genetic Engineering

The transformative power of the CRISPR-Cas9 system in the realm of genome editing stems from its elegant simplicity and remarkable efficiency, a characteristic inherited from its origin in bacterial adaptive immunity. At its core, this system relies on a guide RNA (gRNA), a meticulously designed molecule that acts as a navigational beacon, directing the Cas9 nuclease, a molecular scalpel, to a precise target DNA sequence. This targeted approach allows for unprecedented accuracy in gene editing, enabling researchers to make specific alterations with minimal collateral damage. Consequently, CRISPR-Cas9 has rapidly become an indispensable tool across diverse fields, from fundamental research to agriculture innovation and the burgeoning field of gene therapy(Ding, Li et al. 2016). The operational mechanics of the CRISPR-Cas9 system revolve around the interplay of two critical component: the Cas9 protein, which functions as the molecular scissors, and the gRNA, which serves as the GPS, guiding Cas9

to the desired DNA location. Upon successful binding, Cas9 initiates a double-strand break in the DNA, triggering the cells inherent DNA repair mechanisms. These mechanisms, such as non-homologous end joining (NHEJ) and homology-directed repair (HDR), determine the outcome of the edit. NHEJ, often resulting of small insertions or deletions, leads to gene disruption, effectively knocking out gene function. HDR, on the other hand, allows for precise gene corrections or insertions using a donor DNA template, enabling researchers to introduce specific genetic modifications. The versatility of CRISPR-Cas9 has led to its widespread adaptation in functional genomics, gene therapy, and synthetic biology. It has facilitated the development of disease-resistant crops, the creation of animal models for human diseases, and the exploration of gene therapies for inherited disorders. However, the widespread adoption of CRISPR-based therapies is tempered by concerns regarding off-target effects, potential immune responses, and the ethical implications of altering the human genome. Beyond Cas9, the CRISPR toolbox continues to expand with the discovery of alternative CRISPR-associated nucleases, such as Cas12, Cas13, and Cas14, each possessing unique properties. Cas12, for instance, targets single-stranded DNA, broadening the scope of editable genetic material. Cas13 acts on RNA, opening up possibilities for RNA editing and therapeutic interventions targeting RNA-based diseases. Cas14, a more recently identified enzyme, offers enhanced specificity and minimal off-target effects, making it a promising candidate for future genome editing applications(Ferreira and Choupina 2022). These emerging CRISPR systems not only expand the versatility of genome editing but also pave the way for innovative applications. Cas12 has been instrumental in diagnostic applications like SHERLOCK (Specific High-sensitivity Enzymatic Reporter unlocking), enabling rapid and sensitive detection of viral and bacterial infections. Cas14, with its enhanced specificity, holds significant promise for future genome editing applications, minimizing the risk of unintended genetic alterations(Zlobin, Lebedeva et al. 2020).

2.2 Zinc-Finger Nucleases (ZFNs): Early Genome Editing Technology

Zinc-Finger Nucleases (ZFNs) hold the distinction of being the pioneering programmable genome-editing tools, marking the initial foray into targeted DNA modification. Their operational mechanism, akin to that of TALENs, involves the fusion of zinc finger DNA-binding domains with a Fokl nuclease. Zinc finger proteins, by recognizing specific DNA triplets, enable ZFNs to bind to precise genomic locations and introduce double- strand breaks. These breaks, subsequently repaired through non-homologous end joining (NHEJ) or homology-directed repair (HDR), result in either gene disruptions or precise genetic modifications. While ZFNs represented a significant breakthrough in early genome editing, their usage has diminished due to inherent technical complexities(Yang, Yang et al. 2014). The design of custom zinc finger domains is an intricate process, demanding extensive protein engineering, which renders ZFNs less accessible compared to the more streamlined CRISPR or TALENs. Furthermore, ZFNs have been associated with off-target effects, a consequence of their specific binding characteristics. Nevertheless, they continue to be valuable in niche applications, particularly in therapeutic gene editing and foundational genetic engineering studies. Fundamentally, ZFNs are engineered

proteins comprising zinc-finger DNA-binding domains fused to a Fokl nuclease. They function by inducing site-specific double-strand breaks (DSBs) in DNA, which are then subject to cellular repair mechanisms, leading to the desired gene modifications. However, their effectiveness is counterbalanced by the requirement for extensive customization and the inherent complexity of their design, which collectively limit their widespread adoption. The specificity of ZFNs is achieved through the recognition of specific DNA sequences by their zinc-finger domains. Each zinc-finger domain typically binds to three nucleotides, and multiple domains are assembled in tandem to target a particular DNA sequence. Once bond, the Fokl nuclease cleaves the DNA, initiating the cellular repair pathways that can introduce insertions, deletions, or substitutions at the targeted site. ZFNs have found applications in diverse fields, including gene therapy, functional genomics, and agricultural biotechnology. However, the labor -intensive nature of their design, requiring extensive protein engineering, makes them less accessible compared to the newer, more user-friendly genome editing techniques(Singh, Braddick et al. 2017).

2.3 TALENs: Engineered for Precise Genome Modifications

Transcription activator-like effector nucleases (TALENs) represent a significant advancement in precision-based genome editing, operating by recognizing specific DNA sequences and inducing targeted modifications. Unlike CRISPR, TALENs utilize transcription activator-like effectors (TALENs), DNA-binding proteins derived from plant pathogens, fused with a Fokl nuclease domain. Each TALE repeat recognizes a single nucleotide, allowing for highly specific DNA binding. Upon binding, the FokI nuclease induces double-strand breaks (DSBs) at the target site, triggering cellular DNA repair mechanisms similar to those activated by CRISPR. This design offers high specificity and flexibility, making TALENs particularly valuable in applications demanding precise genome modifications, and they circumvent the RNA-guided targeting of CRISPR, mitigating off-target effects associated with mismatched guide RNAs. However, the requirement for complex protein engineering to design specific DNA-binding domains makes TALENs more labor-intensive and costly compared to CRISPR. Despite these challenges, TALENs have found successful applications in gene therapy, agricultural improvements, and functional genomics studies, including gene knockout studies, correction of genetic mutations, and the development of disease-resistant plants. Functionally similar to Zinc-Figer Nucleases (ZFNs), TALENs offer higher specificity and flexibility through the use of TALE repeats, but they also share the drawback of being labor-intensive and costly. The modular design, where each TALE repeat binds to a single nucleotide, allows for highly specific targeting of DNA sequences, and like ZFNs, TALENs induce DSBs, leading to genome modifications through cellular repair pathways. However, the extensive cloning and the protein engineering required for TALENs make them less efficient compared to the simpler and more streamlined CRISPR-based systems(Anzalone, Randolph et al. 2019).

Applications and Comparative Analysis in Genome Editing Technologies

The landscape of genetic research has been fundamentally altered by the advent of genome editing technologies, prominently featuring CRISPR-Cas0, Transcription Activator-Like Effector Nucleases (TALENs), and zinc Figer Nucleases (ZFNs), all of which facilitate precise modifications within DNA sequences(Gaj, Gersbach et al. 2013). Despite their shared goal, these technologies diverge significantly in their operational mechanisms, efficiencies, and application ranges, thereby rendering each uniquely suited for a diverse array of scientific and clinical endeavors. Researchers can optimize their experimental designs by conducting a comparative analysis of genome editing tools, revealing the individual strengths and limitations of each and facilitating informed decisions regarding the most suitable technology for specific applications(Jinek, Chylinski et al. 2012, Doudna and Charpentier 2014). Notably, CRISPR-Cas9, originating from bacterial immune systems, stands out as an RNA-guided genome editing tool, distinguished by its high efficiency, simplified design process, and cost-effectiveness when juxtaposed with TALENs and ZFNs. The operational simplicity of CRISPR-Cas9 is evident in its reliance on a single-guide RNA (sgRNA) to navigate the case Cas9 nuclease to the target DNA, a stark contrast to TALENs and ZFNs, which necessitate engineered protein-DNA interactions, resulting in more complex and labor-intensive design procedures(Mali, Yang et al. 2013). While CRISPR-Cas9 boasts remarkable efficiency, concerns regarding off-target effects, leading to unintended genetic alterations, have been raised. Conversely, TALENs and ZFNs, leveraging protein-based DNA recognition, exhibit higher specificity, but their inherit complexity and associated costs impede their broader implementation(Fu, Foden et al. 2013). Genome editing technologies have found diverse applications across medicine, agriculture, biotechnology, and environmental science(Kyrou, Hammond et al. 2018). Within the medical domain, CRISPR-Cas9 has become cornerstone for gene therapy, cancer treatment, and infectious disease research, with clinical trials showcasing its efficacy in correcting mutations associated with genetic disorders like sickle cell anemia and β-thalassemia(Sciences, Medicine et al. 2017). TALENs have pr0ven valuable in immunotherapy, particularly in the creation of chimeric antigen receptor (CAR) T-cell therapies for leukemia(Perez, Wang et al. 2008, Zhang, Liang et al. 2016). While less prevalent today, Zinc Finger Nucleases (ZFNs) played a crucial role in early gene therapy initiatives, notably I modifying T cells to resist HIV infection by disrupting the CCR5 gene. Within the agriculture sector, genome editing has been instrumental in creating genetically modified crops exhibiting desirable traits like drought resistance, increased yield, and enhanced disease resistance. Specifically, CRISPR-Cas9 has been employed to engineer rice, wheat, and maize variates, enhancing their stress tolerance and nutritional value(Burkard, Lillico et al. 2017). TALENs have found application in livestock breeding, notably in generating disease-resistant pigs through modifications of the CD163 gene. Although their use has decreased, ZFNs were foundational in early agricultural biotechnology initiatives, particularly in developing herbicide-resistant crops. Expanding beyond medicine and agriculture, genome editing technologies exert a profound influence on biotechnology and environmental sciences. CRISPR-Cas9 has become a versatile tool in synthetic biology, enabling the engineering of

microorganisms for diverse applications such as biofuel production, drug synthesis, and bioremediation. Furthermore, CRISPR-Cas9-based gene derived technology is being investigated for vector control, specifically in modifying mosquito populations to mitigate malaria transmission(Frangoul, Altshuler et al. 2021). TALENs have proven valuable in the creation of customized cell lines for pharmaceutical testing, While ZFNs, though less frequently used now, played a pioneering role in early environmental engineering efforts aimed at pollutant degradation. While genome editing holds immense promise, its revolutionary potential is tempered by significant ethical and safety concerns that demand careful consideration(Eyquem, Mansilla-Soto et al. 2017). Specifically, the potential for off-target effects with CRISPR-Cas9, along with the unknown long-term consequences of gene modifications, necessitate extensive research to guarantee safety in clinical settings. Moreover, the ethical dilemmas surrounding germline editing underscore the urgent need for robust regulatory frameworks. Although TALENs and ZFNs present a reduced risk of off-target effects, their complex design and higher costs restrict their widespread use(Doudna and Charpentier 2014).

Table 1. Comparative Overview of Genome Editing Technologies

Feature	CRISPR-Cas9	TALENs	ZFNs
Discovery	Originated from bacterial adaptive immunity (2012)	Engineered from plant pathogens (2010)	Developed from natural transcription factors (1996)
Targeting Mechanism	Uses RNA-guided recognition via guide RNA (gRNA)	Protein-based recognition through TALE repeats fused with FokI nuclease	Protein-based recognition using Zinc Finger DNA-binding domains fused with FokI nuclease
Target Recognition	Matches DNA sequences complementary to gRNA	Identifies DNA sequences via repeat-variable diresidues (RVDs)	Binds DNA sequences through zinc finger motifs
Editing Mechanism	Cas9 induces double-strand breaks (DSBs), repaired through non-homologous end joining (NHEJ) or homology-directed repair (HDR)	FokI enzyme creates DSBs, repaired by NHEJ or HDR	FokI enzyme generates DSBs, repaired via NHEJ or HDR
Efficiency	High due to simple design and rapid cleavage	Moderate to high, depending on target sequence	Moderate, influenced by zinc finger specificity

Specificity	High but prone to off-target effects	High precision with minimal off-target activity	High specificity, but complex zinc finger engineering is required
Ease of Design	Simple—only requires modifying the gRNA sequence	Complex—demands intricate protein engineering	Challenging—necessitates extensive protein modifications
Multiplexing Ability	High—can target multiple genes simultaneously	Low—difficult to edit multiple genes at once	Low—each target requires a separate ZFN construct
Cost	Low—affordable due to easy gRNA synthesis	Higher—costly due to complex protein engineering	Expensive—custom zinc finger design is resource-intensive
Delivery Methods	Introduced via plasmids, viral vectors, or ribonucleoproteins (RNPs)	Delivered through plasmids, mRNA, or viral vectors	Delivered via plasmids, mRNA, or viral vectors
Off-Target Effects	Possible, but reduced with high-fidelity variants like HiFi-Cas9	Very low—high precision in protein-DNA interaction	Possible, but generally lower than CRISPR
Therapeutic Potential	High—actively used in clinical trials for genetic disorders	High—applied in regenerative medicine and cell therapies	Moderate—early-stage therapeutic applications but less common today
Regulatory Approval	Under review, with clinical trials in progress	Limited approvals for therapeutic use	Approved for specific gene therapy applications

Operational Mechanism of CRISPR-Cas9 for Genome Editing

CRISPR-Cas9 stands as a potent instrument for genome editing, granting the ability to perform highly accurate alternations to the DNA of living organisms(Jinek, Chylinski et al. 2012). Its operational mechanism originates from the adaptive immune systems of bacteria and archaea, where it serves as a defensive strategy against viral invasion. This system utilizes a guide RNA (gRNA) to direct the case Cas9 endonucleases to a designated DNA sequence, thereby enabling targeted genome modifications(Doudna and Charpentier 2014). This CRISPR-Cas9 genome editing process is characterized by a series of crucial steps. Initially, a custom-designed single-

guide RNA (sgRNA) is created to be complementary to the desired target DNA sequence, ensuring precise binding. The single-guide RNA (sgRNA), a critical component, is constructed from two elements: the CRISPR RNA (crRNA), responsible for recognizing the specific target DNA sequence, and the trans-activating CRISPR RNA (tracrRNA), which facilitates the binding of the Cas9 protein(Cong, Ran et al. 2013). Upon successful target DNA recognition, Cas9 induces a double-strand break (DSB) at the precise genomic location, situated next to a protospacer adjacent motif (PAM) sequence, commonly represented as 5'-NGG-3'. This PAM sequence is indispensable for Cas9 activity, serving to prevent the cleavage of the bacterial hosts own genome while enabling the targeting of foreign DNA. Following the introduction of the DSB, the cells inherent repair mechanisms dictate the ultimate result of the gene edit. The two primary DNA repair pathways engaged are non- homologous end joining (NHEJ) and homology -directed repair (HDR)(Asta, Ricciardello et al. 2024). Non-homologous end joining (NHEJ), a repair pathway prone to errors, frequently results in small insertions or deletions (indels), effectively disrupting or knocking out gene function. This characteristic has been harnessed for gene function studies and therapeutic strategies, including the silencing of disease-causing mutations. In contract, homology-directed repair (HDR) offers a more precise repair mechanism, utilizing a homologous DNA template to introduce specific nucleotide changes or insertions(Hsu, Lander et al. 2014). This pathway is especially valuable for gene corrections and knock-in approaches, although it operates at a lower efficiency compared to NHEJ. The ongoing evolution of CRISPR-Cas9, which demonstrate a significant reduction in off-target effects and enhanced specificity. Furthermore, the development of base editing and prime editing has broadened the CRISPR-Cas9 toolkit, offering alternative methodologies for single-nucleotide modifications without the induction of double-strand breaks (DSBs). These advancements have significantly expanded the applicability of CRISPR-Cas9 in biomedical research, gene therapy, and functional genomics(Slaymaker, Gao et al. 2016). However, despite its substantial potential, CRISPR-Cas9 faces limitations, including the possibility of unintended genomic alterations, ethical dilemmas surrounding germline editing, and variable efficiency across different cell types. Current research efforts are dedicated to mitigating these challenges by refining CRISPR-associated nucleases, optimizing delivery mechanisms, and creating computational tools for precise target selection. As the technology progression, CRISPR-Cas9 is poised to be a key driver of innovation in genetic medicine and biotechnology(Gaudelli, Komor et al. 2017).

Advanced CRISPR Technologies: Cas12, Cas13, Base Editing, and Prime Editing

Cas12: A Highly Versatile Tool for DNA-Targeted Modifications

Cas12, a member of the type V CRISPR system, is an RNA-guided endonuclease that distinguishes itself from Cas9 through several unique characteristics. Notably, while Cas9 relies on a short protospacer adjacent motif (PAM) for target recognition, Cas12 demonstrates broader targeting capabilities by recognizing TTTV PAM sequences, where V can be A, C or G. Furthermore, Cas12 generates staggered, single-stranded breaks (SSBs) in SNA, producing sticky ends instead of the blunt-end cuts created by Cas9(Carlson, Lancto et al. 2016). This

attribute enhances its effectiveness in homology-directed repair (HDR), rendering it particularly advantageous for precise genome modifications. A further significant aspect of Cas12 is its collateral single-stranded DNA cleavage activity, which has been leveraged for groundbreaking diagnostic tools, exemplified by the SHERLOCK (Specific High Sensitivity Enzymatic Reporter UnLOCKing) system. The SHERLOCK system, leveraging Cas12 collateral activity, facilitates quickly and highly sensitive nucleic acid detection, proving invaluable in infectious disease diagnostics, cancer biomarker identification, and environmental monitoring. Given its inherent flexibility and enhanced specificity, Cas12 is also being actively investigated as a promising alternative to Cas9 for therapeutic genome editing applications(Cyranoski 2015).

Cas13: Targeting RNA for Editing and Diagnostics

Cas13, a Type VI CRISPR enzyme, distinguishes itself by targeting RNA rather than DNA. In contrast to Cas9 and Cas12, which induce DNA double-strand breaks, Cas13 operates as an RNA endonuclease, making it a valuable instrument for transcriptome engineering and RNA interference(Littlejohn, Henty et al. 2014). Guided by a CRISPR RNA (crRNA), Cas13 recognizes and cleaves RNA sequences without the necessity of a PAM sequence, offering extensive targeting flexibility. A key application of Cas13 lies in RNA editing, where it allows for the modulation of gene expression without permanent genomic alternations. This transient characteristic renders Cas13 particularly appealing for therapeutic interventions, especially for diseases arising from abnormal RNA transcript, such as neurological disorders and viral infections. Additionally, akin to Cas12, Cas13 displays collateral RNA cleavage activity, which has been leveraged to develop highly sensitive and specific diagnostic platforms, including REPAIR (RNA Editing for Programmable A to I Replacement) and DETECTR (DNA Endonuclease Targeted CRISPR Trans Reporter), designed for the detection of viruses and pathogens(Van Laere, Nguyen et al. 2003).

Advantage of Base Editing: Precise Changes Without DSBs

Base editing represents a sophisticated genome-editing technique that facilitates precise nucleotide conversion, bypassing the induction of double-strand breaks. In contrast to traditional CRISPR-Cas9 editing, which depends om non-homologous end joining (NHEJ) or homology-directed repair (HDR) for genetic alternations, base editing directly transforms one DNA base into another in a controlled, programmable fashion. This technology employes base editors, which are constructed by combining a catalytically inactive or nickase Cas9 (dCas9 or nCas9) with a DNA-modifying enzyme, such as cytidine deaminase or adenosine deaminase(Wallace, Rooke et al. 2015).

Base editing encompasses two primary categories:

1. **Cytosine Base Editors (CBEs):** which facilitate the conversion of cytosine (C) to thymine (T) without introducing double strand-brakes (DSBs)
2. **Adenine Base Editors (ABEs):** which enable the transition of adenine (A) to guanine (G) through targeted DNA sequence modifications.

This approach has significantly refined precision genome editing by minimizing off-target effects and circumventing the risks associated with DSB-induced extensive genomic rearrangements. Consequently, base editing has been employed in the therapeutic correction of single-nucleotide mutations in disease-related genes, addressing genetic disorders such as sickle cell anemia, Duchenne muscular dystrophy, and metabolic diseases. The high-fidelity DNA modification capabilities of base editors render them particularly suitable for therapeutic interventions in regenerative medicine and genetic correction(Carlson, Lancto et al. 2016).

Prime Editing: Achieving Versatile and Highly Precise Genome Modifications

Prime editing represents a groundbreaking advancement in genome editing, surpassing the precision of both conventional CRISPR-Cas9 and base editing. Designed to address the limitations of homology-directed repair (HDR)-dependent editing, prime editing allows for the direct insertion, deletion, and replacement of DNA sequences without inducing double-strand breaks (DSBs) or necessitating donor DNA templates. This innovative technique utilizes a fusion protein comprising a catalytically impaired Cas9 nickase (nCas9) and a reverse transcriptase enzyme, alongside a specialized prime editing guide RNA (pegRNA)(Cyranoski 2015). The pegRNA serves a dual purpose: guiding the prime editor to the target site and providing a template for the intended genetic modification. Following the nickase Cas9-induced single-strand nick in the DNA, the reverse transcriptase copies the desired sequence into the genome, facilitating precise genetic alternations. This method demonstrates remarkable versatility, capable of correcting a wide range of genetic mutations, including point mutations, insertions, deletions. Prime editing has shown significant potential in correcting pathogenic mutations associated with genetic disorders, such as Tay-Sachs disease, cystic fibrosis, and Huntington's disease. Given its flexibility and exceptional precision, prime editing is recognized as a significant leap forward in gene therapy and personalized medicine(Gao, Wu et al. 2017).

Table 2. Comparative Summary of CRISPR Variants

Feature	Cas9	Cas12 (Cpf1)	Cas13	Base Editing	Prime Editing
Target Molecule	DNA	DNA	RNA	DNA (single-base modifications)	DNA (insertions/deletions)
Cleavage Pattern	Produces blunt-ended DSBs	Creates staggered cuts	Cleaves single-stranded RNA	No cleavage; performs chemical base	No DSBs; utilizes reverse transcriptase

				conversion	
PAM Sequence	NGG (e.g., SpCas9)	T-rich sequences (e.g., TTTV)	Not required	Not required	Not required
Guide RNA Structure	Dual-RNA system (crRNA + tracrRNA) or single-guide RNA (sgRNA)	Single crRNA	Single crRNA	Cas9 fused with a deaminase	Cas9 fused with reverse transcriptase and pegRNA
Editing Efficiency	High	Moderate to high	High for RNA knockdown	High for precise single-nucleotide changes	Moderate (dependent on reverse transcriptase)
Specificity	High, but off-target effects possible	Higher specificity than Cas9	High for RNA with no DNA off-target effects	High for precise base conversions	High with reduced off-target effects
Repair Mechanism	NHEJ or HDR	NHEJ or HDR	Not applicable (RNA degradation)	No repair needed (direct base conversion)	No DSB repair required
Multiplexing Ability	High	High	High	Limited	Moderate
Applications	Gene knockout, insertions, disease modeling	Gene knockout, gene insertions, diagnostics	RNA knockdown, transcriptome regulation, RNA diagnostics	Precise correction of point mutations	Small insertions, deletions, and corrections
Therapeutic Potential	High—currently in clinical trials	Emerging—used in diagnostics and genome editing	High—applied in RNA-based therapies	High—effective for correcting point mutations	High—potential for gene therapy without DSBs
Limitations	Off-target effects, PAM sequence required	Larger enzyme, requires specific PAM sequence	Limited to RNA editing, risk of degradation	Restricted to specific base changes	Lower efficiency compared to Cas9

3. CRISPR's Role in Enhancing Livestock Production

CRISPR-Cas9 and its related technologies have fundamentally transformed livestock genetic engineering, offering precise, efficient, and affordable methods to enhance productivity, disease resilience, and animal well-being. This advancement has ushered in a new era of animal breeding, lessening the dependence on traditional, lengthy, and less accurate breeding methods. Scientists have successfully applied CRISPR for targeted genetic modifications across various livestock species, including cattle, pigs, goats, and poultry, showcasing its capability to improve key traits like growth rate, meat, quality, milk, production, disease resistance, and reproductive performance(Littlejohn, Henty et al. 2014).

1. Improving Resistance to Disease

A key application of CRISPR technology in livestock lies in bolstering resistance to infectious diseases. Numerous studies have centered on creating disease-resistant animals to alleviate the economic impact of livestock illnesses and reliance on antibiotics. For instance, CRISPR has been effectively employed to introduce a mutation within the CD163 gene in pigs, thereby conferring resistance to porcine Reproductive and Respiratory Syndrome Virus (PRRSV), a major threat to the swine industry. Similarly, scientist have modified the NRAMP1 gene in cattle to increase resistance to tuberculosis. These developments support sustainable livestock practices by limiting disease outbreaks and improving overall animal health(Van Laere, Nguyen et al. 2003).

2. Enhancing Growth and Feed Utilization

CRISPR technology has been utilized to improve growth performance and feed efficiency, both pivotal elements in meat production. For example, gene editing has been applied to disable the myostatin (MSTN) gene in cattle, sheep, and pigs, resulting in enhanced muscle growth and increased lean meat production. The MSTN gene controls muscle development, and its inactivation leads to a “double muscling” characteristics, thereby improving carcass yield. Additionally, research indicates that CRISPR-mediated alterations in IGF2 (Insulin-like Growth Factor 2) can improve feed conversion efficiency in pigs, contributing to a reduction in overall meat production expenses(Martínez-Álvaro, Auffret et al. 2020).

3. Improving Reproductive Performance

CRISPR technology has proven highly effective in enhancing reproductive efficiency. Genetic modifications have been implemented to increase litter size, fertility rates, and embryo survival. For example, the inactivation of the FSHR (Follicle-Stimulating Hormone Receptor) gene in pigs has been linked to improved ovulation rates and greater reproduction success. Likewise, CRISPR-based genome editing has enabled the production of sex-biased offspring by selectively modifying genes involved in sex determination. This capability holds significant economic

potential for the dairy and poultry industries, where specific sex preferences are economically advantageous(Whitworth, Lee et al. 2014).

4. Enhancing Milk Production and Quality

Researchers have investigated the use of CRISPR technology to increase milk production and enhance its nutritional quality. Genetic modifications targeting the CSN2 (Beta-casein) and CSN3 (kappa-casein) genes in cattle have been performed to increased protein content and alter milk composition, improving its processing and digestibility(Carlson, Lancto et al. 2016). Additionally, efforts have been made to reduce lactose levels in milk, aiming to produce lactose-free diary products for consumers with lactose intolerance. These developments not only improve the efficiency of diary production but also contribute to enhanced consumer health.

5. Promoting Animal Welfare While Reducing Environmental Impact

CRISPR technology is increasingly employed to enhance animal welfare by mitigating stress-related characteristics and eliminating the necessity for distressing management procedures. For instance, through genome editing, hornless cattle have been developed by introducing the POLLED mutation, thereby removing the need for dehorning, a routinely performed yet painful practice in the dairy sector. Furthermore, genes related to heat tolerance, like SLICK, have been targeted to create cattle breeds better adapted to warmer climates, reducing heat stress and boosting productivity. Importantly, CRISPR also offers potential for minimizing the environmental impact of livestock farming(Whitworth, Lee et al. 2014). Genetic modifications aimed at decreasing methene emissions from ruminants, by targeting genes that regulate the gut microbiome, are being explored. These strategies support sustainable livestock production and address the urgent challenges of climate change.

6. Strategies for Genetic Preservation and Conservation

CRISPR technology is playing a crucial role in safeguarding endangered livestock breeds and managing genetic resources. By identifying and preserving advantageous genetic traits, genome editing contributes to maintaining biodiversity within agricultural species(Khan, Heikal et al. 2023). Furthermore, gene-editing techniques can be utilized to restore traits that have been lost due to intensive selective breeding practices, thereby ensuring the long-term sustainability of heritage and rare breeds.

Table 3. Strategies for genetic preservation and conservation

Application Area	Targeted Traits/Genes	Examples/Outcomes	Benefits	Challenges/Considerations
Disease Resistance	CD163, NRAMP1	PRRSV-resistant pigs, tuberculosis-resistant cattle	Reduced disease outbreaks, decreased antibiotic use, improved animal health	Off-target effects, public perception, regulatory hurdles
Growth Rate & Feed Efficiency	MSTN, IGF2	Double-muscled cattle/sheep/pigs, improved feed conversion in pigs	Increased meat yield, reduced production costs	Potential for unintended physiological changes, consumer acceptance
Reproductive Traits	FSHR, sex-determining genes	Increased ovulation rates in pigs, sex-biased offspring	Enhanced reproductive efficiency, optimized livestock production	Ethical concerns regarding sex selection, long-term effects on animal health
Milk Composition & Yield	CSN2, CSN3, lactose-related genes	Increased protein content in milk, lactose-free milk	Improved milk processing, enhanced nutritional value, benefits for lactose-intolerant consumers	Potential alterations in milk properties, consumer perception of modified milk
Animal Welfare	POLLED, SLICK	Hornless cattle, heat-tolerant breeds	Reduced stress from management practices, improved animal comfort in extreme climates	Potential impacts on breed characteristics, long-term effects on animal adaptability
Environmental Impact Reduction	Gut microbiome-related genes	Reduced methane emissions from ruminants	Sustainable livestock production, mitigation of climate change	Complex gut microbiome interactions, long-term efficacy
Genetic Preservation & Conservation	Beneficial genetic traits in endangered breeds	Preservation of biodiversity, rescue of lost traits in heritage breeds	Maintenance of genetic diversity, conservation of valuable genetic resources	Ensuring genetic stability, ethical considerations regarding breed manipulation

4. CRISPR-Enabled Functional Genomics in Livestock Studies

The integration of functional genomics and CRISPR technology has revolutionized livestock research, providing precise tools for dissecting gene functions and enhancing economically significant traits. Functional genomics seeks to decipher the roles of genes and their interplay with environmental influences, while CRISPR enables targeted gene modifications for validating gene function and boosting livestock productivity(Zhang, Xu et al. 2024). Researches have adeptly employed CRISPR to unravel gene networks that control growth, reproduction, immunity, and metabolism, facilitating the identification and manipulation of critical genetic determinants. Through gene disruption or activation, scientists have gained valuable insights into disease resistance mechanisms, feed efficiency, and stress tolerance, thereby informing selective breeding strategies. CRISPR-based methodologies have proven instrumental in validating candidate genes linked to production traits. For example, the myostatin (MSTN) gene, a key inhibitor of muscle growth, has been targeted in cattle, sheep, and pigs to induce a double-muscling phenotype, resulting in increased meat yield and improved feed efficiency. Likewise, functional studies on genes such as IGF2 and LEPR have revealed their roles in growth performance and fat deposition in pigs. By precisely modifying these genes, researchers have developed livestock with desirable traits while minimizing unintended genetic variations. Disease resistance is another crucial area where CRISPR has been applied to validate gene function and develop genetically resilient livestock. Studies on the CD163 gene in pigs have demonstrated its essential role in Porcine Reproduction Respiratory Syndrome Virus (PRRSV) susceptibility(Wang, Cheng et al. 2025). Whitworth et al. (2016) successfully knocked out CD163 in pigs, making them resistant to PRRSV without adverse physiological effects. Similarly, genome editing of the NRAMP1 gene in cattle has enhanced resistance to bovine tuberculosis. These discoveries underscore CRISPRs potential to unravel host-pathogen interactions and expedite the creation of disease-resistant livestock breeds. Functional genomics has also been instrumental in understanding reproductive traits and fertility in livestock. CRISPR-mediated modifications to the follicle-stimulating hormone receptor (FSHR) gene have enhanced reproductive performance in pigs, resulting in larger litter sizes. Furthermore, targeted alterations to the SRY gene in cattle and poultry have explored sex determination and ratio manipulation, offering an innovation approach to optimize livestock production systems(Yuan, Liu et al. 2024). By combining functional genomics with CRISPR technology, researchers can analyze the genetic underpinnings of fertility and reproductive efficiency, ultimately improving breeding programs. Milk production and composition have also been central to CRISPR-based functional genomics research. Genes like CSN2 and CSN3, which code for beta-and Kappa-caseins respectively, have been modified in dairy cattle to improve milk quality and processing properties. Moreover, investigations into modifications of the LCT gene have explored the development of lactose-free milk, catering to consumer preferences and market demands. These advancements illustrate how CRISPR can be utilized within functional genomics to improve nutritional value and economic efficiency in dairy production. Environmental sustainability and animal welfare have also been positively impacted by CRISPR-based functional genomics.

Modifications to the POLLED gene have facilitated the creation of naturally hornless cattle, eliminating the need for the painful procedure of dehorning. Similarly, genome edits targeting heat-tolerance genes, such as the SILK variant, have enabled cattle to adopt to warmer climates, reducing heat stress and maintaining productivity. Additionally, functional genomics research on methane-related microbial genes in ruminants is aimed at reducing livestock methane emissions, thereby mitigating the environmental impact of animal agriculture. These discoveries underscore the extensive potential of functional genomics and CRISPR in fostering sustainable livestock production(Bertheau 2021). While these advancements are significant, challenges, persist in the application of CRISPR to functional genomics within livestock research. Issues such as off-target effects, mosaicism, and ethical considerations must be carefully addressed to guarantee precise and responsible genome editing. Moreover, the diverse regulatory frameworks governing gene-edited animals across different regions impact the commercialization of CRISPR-modified livestock products. Nevertheless, as CRISPR technology continues to advances, its integration with functional genomics is anticipated to provide deeper insights into the genetic regulation, enabling the development of genetically superior livestock breeds. Future research should prioritize refining genome-editing techniques, enhancing efficiency, and addressing public perceptions to fully realize the benefits of CRISPR in livestock research(Bártová 2024).

5. Modifying the Epigenome: Techniques and Regulatory Uses

Epigenome editing has become a significant too, for manipulating gene expression without damaging the underlying DNA sequence, offering valuable insights into gene function and potential advancements in livestock improvement. Differing from conventional genome editing, which causes permanent genetic changes, epigenome editing employs modified CRISPR systems to control chromatin accessibility and transcriptional activity. This is achieved mainly by fusing catalytically inactive Cas9 (dCas9) with epigenetic effectors, such as DNA methyltransferases, histone acetyltransferases, or repressor domains, enabling precise and reversible regulation of target genes. Epigenetic editing holds substantial promise for enhancing complex traits in livestock, including growth performance, reproduction, disease resistance, and environmental adaptation, as evidenced by recent research(Kim, Woo et al. 2023). A key application of epigenome editing lies in the regulation of economically vital genes related to livestock production. CRISPR-based epigenetic modifiers have been utilized to activate or repress gene expression by targeting promoter enhancer regions. For example, Liu et al. (2019) showed that dCas9 combined with the histone acetyltransferase p300 could boost gene expression by increasing histone acetylation at specific locations. Athis technique has been used to increase the activity of genes related to muscle development, such as MSTN and IGF1, in cattle and pigs, leading to increased lean meat production without making permanent changes to the DNA. Likewise, CRISPR interference (CRISPRi) systems have been used to suppress genes like FTO, which plays a role in fat deposition, thus improving carcass quality in livestock. Another important application of epigenome editing is the manipulation of genes that influence disease resistance. By changing DNA methylation and histone modifications, researchers have been able

to strengthen the immune response of livestock against infectious disease. A study by Tan et al. (2020) successfully used dCas9-DNMT3A, a fusion with a DNA methyltransferase, to silence the CD163 receptor genes in pigs, providing resistance to porcine reproductive and respiratory syndrome virus (PRRSV). This approach offers a promising alterative to direct gene knockout, minimizing concerns about unintended genomics changes while still achieving disease resistance(Cyranoski 2015). Epigenome editing is also vital in reproductive biology and fertility enhancement. Precise control of genes involved in ovarian function, spermatogenesis, and embryonic development has been explored using CRISPR-based epigenetic tools. Modifying the expression of FSHR and BMP15 through histone modification techniques has been suggested as a way to improve evolution rates and litter size in pigs and sheep. Furthermore, researchers are focusing on imprinted genes, which govern fetal growth and placental development, to enhance livestock reproduction efficiency and newborn survival. By manipulating these genes, they aim to optimize these critical aspects. Beyond its applications in production and health, epigenome editing holds substantial potential for advancing environmental sustainability. Specifically, by epigenetically modulating genes, involved in methane production within ruminants, such as MCR genes in the gut microbiota, scientists are developing methods to reduce greenhouse gas emissions from cattle. This reversible method allows livestock to maintain genetic diversity while lessening their environmental impact Despite its potential, epigenome editing in livestock is a nascent field, facing several obstacles. Off-target effects, the stability of epigenetic changes across generations, and regulatory complexities must be resolved before widespread applications(Kim, Woo et al. 2023). Additionally, ethical considerations and public opinion are paramount in the acceptance of epigenetically modified livestock. Future research should prioritize enhancing the precision and longevity of epigenetic modifications, studying the inheritance of edited trait, and establishing clear regulatory guidelines to promote the responsible use of epigenome editing in livestock breeding. As CRISPR-based epigenetic tools continue to advance, their integration into precision livestock farming offers significant potential for enhancing productivity, disease resistance, and sustainability without permanently altering the animal’s genetic code(Littlejohn, Henty et al. 2014).

6. Difficulties and Limitations of CRISPR in Livestock Improvement

While CRISPR technology offers significant promise for livestock improvement, its broad application is impeded by various challenges and limitations. A major concern is the occurrence of off-target effects, where unintended genetic changes happen at sites resembling the intended target. These unintended mutations can result in unpredictable physical traits, raising both safety and ethical issues. Although advancements like high-fidelity Cas9 variants and base editing have enhanced target specificity, completely eliminating off-target activity remains a difficult task. Furthermore, the effectiveness of CRISPR-based genome editing differs across livestock species and cell types, with some showing increased resistance to CRISPR components, thus lowering successful editing rates. Mosaicism also poses a considerable limitation in livestock applications of CRISPR. If genome editing is performed at the zygote stage, some cells in the developing

embryo might carry the intended modification, while others do not, resulting in mosaic animals with a combination of genetic makeups. This creates difficulties in establishing stable genetic lines and necessitates extensive screening and selective breeding to produce animals with consistent edits. Moreover, precise gene insertions using homology-directed repair (HDR) are inefficient in livestock because non-homologous end joining (NHEJ) repair pathways are prevalent, often leading to unpredictable insertions or deletions at the target site. Regulatory and ethical issues also impede the adoption of CRISPR technology in livestock breeding(Khan, Heikal et al. 2023). Regulatory frameworks for genome-edited animals vary significantly across countries, with some categorizing them as genetically modified organisms (GMOs), which subjects them to stringent approval procedures. For instance, the U.S. Food and Drug Administration (FDA) mandates thorough safety evaluations for gene-edited livestock, while regions like the European Union enforce restrictive regulations that impede commercialization. Public perception and consumer acceptance add another layer of complexity, as concerns about animal welfare, unforeseen ecological impacts, and food safety shape policy decisions and market success. Another CRISPR primarily targets DNA sequences, alternations in regulatory regions or chromatin structure can trigger unintended downstream effects on gene expression, potentially affecting animal health and productivity. Understanding the long-term consequences of these changes is a crucial area of ongoing research. Furthermore, the generation of gene-edited livestock through somatic cell nuclear transfer (SCNT) or microinjection techniques often suffers from low efficiency, high embryo loss, and developmental abnormalities, which limits the ability to scale up CRISPR applications in breeding programs. Even with these hurdles, continuous advancements in genome editing technologies, like prime editing and epigenome editing, are actively working to overcome many of these limitations. Enhanced delivery systems, including viral vectors, electroporation, and lipid nanoparticles, are improving CRISPR efficiency and reducing unintended effects. Moreover, the integration of CRISPR with genomic selection and advanced reproductive technologies is creating pathways for more precise and sustainable livestock breeding strategies. Future research must persist in refining these tools, navigating regulatory complexities, and fostering transparent communication with all involved parties to ensure the responsible implementation of CRISPR in livestock production(Kim, Woo et al. 2023).

7. Future Research and Development in Genome Editing

The future of livestock genome editing is set for substantial progress, fueled by advancements in CRISPR-based technologies and supporting methods that improve precision, efficiency, and usability. New genome editing techniques, including prime editing and base editing, provide more sophisticated tools for targeted genetic alterations without causing double-strand breaks, thus minimizing the risk of off-target mutations and unintended genomic rearrangements(Miglani 2017). These technologies allow for precise single-nucleotide changes, enabling the correction of genetic flaws and the fine-tuning of complex livestock traits, such as disease resistance, feed efficiency, and reproductive performance. Furthermore, epigenome is

becoming increasingly popular as a less invasive way to control gene expression, avoiding permanent DNA changes. This helps alleviate worries about regulatory limitations and public concerns(Selin, Lambert et al. 2023, Dixit, Kumar et al. 2024). Improvements in delivery techniques are also essential for enhancing genome editing results. Traditional methods, like microinjection and electroporation, have drawbacks in efficiency and embryo survival. New delivery systems, such as lipid nanoparticles, viral vectors, and RNA-based platforms, are being researched to more effectively and safely deliver CRISPR components into livestock cells. Moreover, the creation of genome editing systems that target specific tissues or can be turned on and off will allow for precise modifications in particular organs or developmental phases. This will reduce unintended effects throughout the animal and expand the possibilities for genetic improvements in livestock(Mishra, Joshi et al. 2018). Combining genome editing with other cutting-edge technologies, such as artificial intelligence (AI) and genomic selection, offers tremendous potential for highly accurate breeding. AI-powered models can forecast the results of gene editing, improve the selection of target sites, and increase the accuracy of gene function analysis, thereby speeding up the identification of new genetic variations that are beneficial for livestock productivity and resilience. Furthermore, integrating CRISPR-based editing with high-throughput sequencing and multi-omics analyses, like transcriptomics and metabolomics, will offer deeper understanding of gene networks and regulatory pathways, leading to more refined genetic interventions for better animal health and performance(Pacesa, Pelea et al. 2024). Ethical and regulatory factors will continue to influence the direction of genome editing in livestock. While some nations have started to simplify approval processes for gene-edited animals, achieving global regulatory consistency remains a challenge. Continuous communication between scientists, policymakers, and consumers is crucial for ensuring transparent risk evaluations and building public confidence in gene-edited livestock products. In addition, progress in genome editing must be aligned with sustainability objectives, encompassing the reduction of livestock methane emissions, enhancement of disease resistance, and improvement of feed efficiency to tackle global food security challenges. With ongoing research and technological refinement, genome editing is poised to transform livestock breeding, providing innovative solutions to satisfy the needs of an expanding population while upholding ethical and sustainable agricultural practices(Carroll 2017, Metje-Sprink, Menz et al. 2019).

Conclusion

The advent of genome editing, with a particular emphasis on CRISPR-based technologies, has ushered in a transformative era for livestock genetics. This revolutionary approach has supplied researchers and breeders with highly precise, remarkable efficient, and economically viable tools designed to modify the genetic makeup of animals. These technological breakthroughs have significantly accelerated the pace of progress in crucial areas of livestock management, including the augmentation of productivity, the fortification of disease resistance, and the enhancement of adaptability to the ever-evolving challenges posed by environmental changes. The cornerstone of this revolution, the CRISPR-Cas9 system, alongside the ongoing development of emerging variants such as Cas12, Cas13, base editing, and prime editing, has dramatically expanded the scope of achievable genome modifications. These advanced techniques enable highly targeted genetic enhancements, allowing for precise alternations without the necessity of introducing foreign DNA into the animal's genome. The tangible and impactful application of CRISPR technology in livestock has already yielded remarkable results, evidenced by the successful development of animals exhibiting heightened resistance to a range of diseases. Furthermore, these advancements have led to substantial improvements in the production of meat and milk, addressing critical aspects of food security and agricultural efficiency. Additionally, the technology has facilitated the enhancement of reproductive efficiency, contributing to more sustainable and productive livestock breeding programs. Furthermore, genome editing has been instrumental in diminishing the dependence on antibiotics within livestock management, thereby bolstering animal welfare and fostering more sustainable agricultural practices. Nevertheless, the path to widespread integration of this technology into commercial breeding programs is not without its obstacles. Challenges such as off-target effects, which may lead to unintended genetic alterations, alongside ethical considerations and complex regulatory hurdles, necessitate careful deliberation and resolution. Future advancements in genome editing, particularly when synergized with the capabilities of artificial intelligence, integrated multi-omics approaches, and precision livestock farming methodologies, hold the promise of further optimizing genetic improvements while simultaneously mitigating the risk of unintended consequences. The collaboration efforts of geneticists, bioinformaticians, policymakers, and industry stakeholders are paramount in ensuring the responsible and ethical implementation of genome editing technologies. Through sustainable research and progressive regulatory advancements, genome editing possesses the transformative potential to revolutionize livestock breeding, significantly enhancing food security, animal health, and agricultural sustainability, thereby effectively addressing the growing demands of an expanding global population.

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Role of Epigenetics On Gene Expression in Livestock

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1. Introduction

Epigenetics describes the molecular processes and events around the DNA that can influence gene expression and genomic activity, despite the sequence of DNA. Developmental events are driven by epigenetic pathways, which are additionally linked to the emergence of disease. Livestock production and breeding optimization require a thorough understanding of the way the animal's environment impacts physiology, including its epigenetic, management and dietary exposures. Environmental issues affect livestock in several ways and have a detrimental impact on livestock's development, productivity and reproduction. The most significant factor is heat-stress which influences animal production in a constantly changing climate. The combined consequences of both temperature and humidity have a deadly effect on most of the livestock. In this chapter, the epigenetics and environmental influences on gene expression in livestock are discussed.

The word “epigenetics” was first used by Conrad Waddington in the year 1940 to describe the potential causal processes influencing the genes that control phenotypic consequences. Later, Huxley improved this description after realizing that differences in cellular phenotypic specification were not always caused by gene sequences. DNA methylation (mostly at CpG dinucleotides), PTMs (post-translational modifications) of histones, ncRNAs (non-coding RNAs) and re-modelling of chromatin are examples of epigenetic mechanisms (Triantaphyllopoulos et al., 2016). In general, epigenetics describes molecular changes associated with the activity of genes and mitotically persistent states that do not alter the sequence of DNA.

In order to ensure a more excellent supply of premium animal proteins with negligible environmental effects, the food livestock industry must develop sustainably in response to the challenges posed by global climate change and the growing demand for animal food from an ever-increasing population of humans. Dynamic alterations significantly influence individual development and growth in the epigenome brought about by the complex interplay of environmental and genetic variables (Wang and Ibeagha-Awemu, 2021). By serving as a bridge

between host genetics and environmental influences, epigenetic mechanisms help control several cellular functions and phenotypic expression. Moreover, epigenetic changes are dynamic during the lifespan since they are unique to particular stages of life or organs and change depending on the circumstances (Ibeagha-Awemu and Yu, 2021). Although epigenetic reprogramming, particularly DNA methylation, is most prominent during the development of embryos, epigenetic factors continuously alter cellular activity and phenotype display during development and growth in response to different environmental indicators (exposome) and the foundational genotype.

Knowing the function of epigenetics will allow for the quantification of some of the missing connection and inheritance of complicated live-stock well-being and production characteristics, as livestock undergo different developmental stages, are exposed to a variety of governing practices, and are subjected to a variety of environmental problems, pathogens, and nourishment throughout a production cycle (Ibeagha-Awemu and Yu, 2021). Environmental stressors, such as stress of heat, weaning, transportation, infections, dietary modifications, and so forth, affect gene expression, metabolism, immunological response, and epigenetic factors, which in turn affect welfare, well-being, and productivity in livestock (Corvo et al., 2021). Animal gene expression can be studied using a variety of methods or approaches. The fields of genomics and molecular biology, and the way an animal's genetic potential is influenced by its surroundings to assess performance have garnered more attention in recent years. The genomics techniques that have recently been designed will enable us to determine the way our production conditions, animal genetics, and the overall quality of the livestock products that we produce. The identification of certain genes, proteins, and/or regulatory pathways that are significant and can be altered or impacted to enhance the productivity, well-being, and financial success of grazing livestock production is one of the consequences of systems-level biology and genomics technologies (Thomson, 2016).

2. Epigenetics Process and its Impacts on Gene Regulation

Animal breeding could benefit greatly from epigenetics since it might cast insight on the inheritance of complex features and illnesses. This could, therefore, improve animal genetics and breeding methods. It is now clear that epigenetic variables affect the entire phenotype in along with DNA sequence data (Triantaphyllopoulos et al., 2016).

2.1 Epigenetic Processes

Methylation of DNA, chromatin re-modeling, the modification of histone non-coding RNA-mediated gene expression regulation, instability in genomic DNA, and any other element that alters an animal's phenotype are all examples of epigenetic processes that produce the epigenome (Figure 1) (Bannister and Kouzarides, 2011; Ibeagha-Awemu and Zhao, 2015).

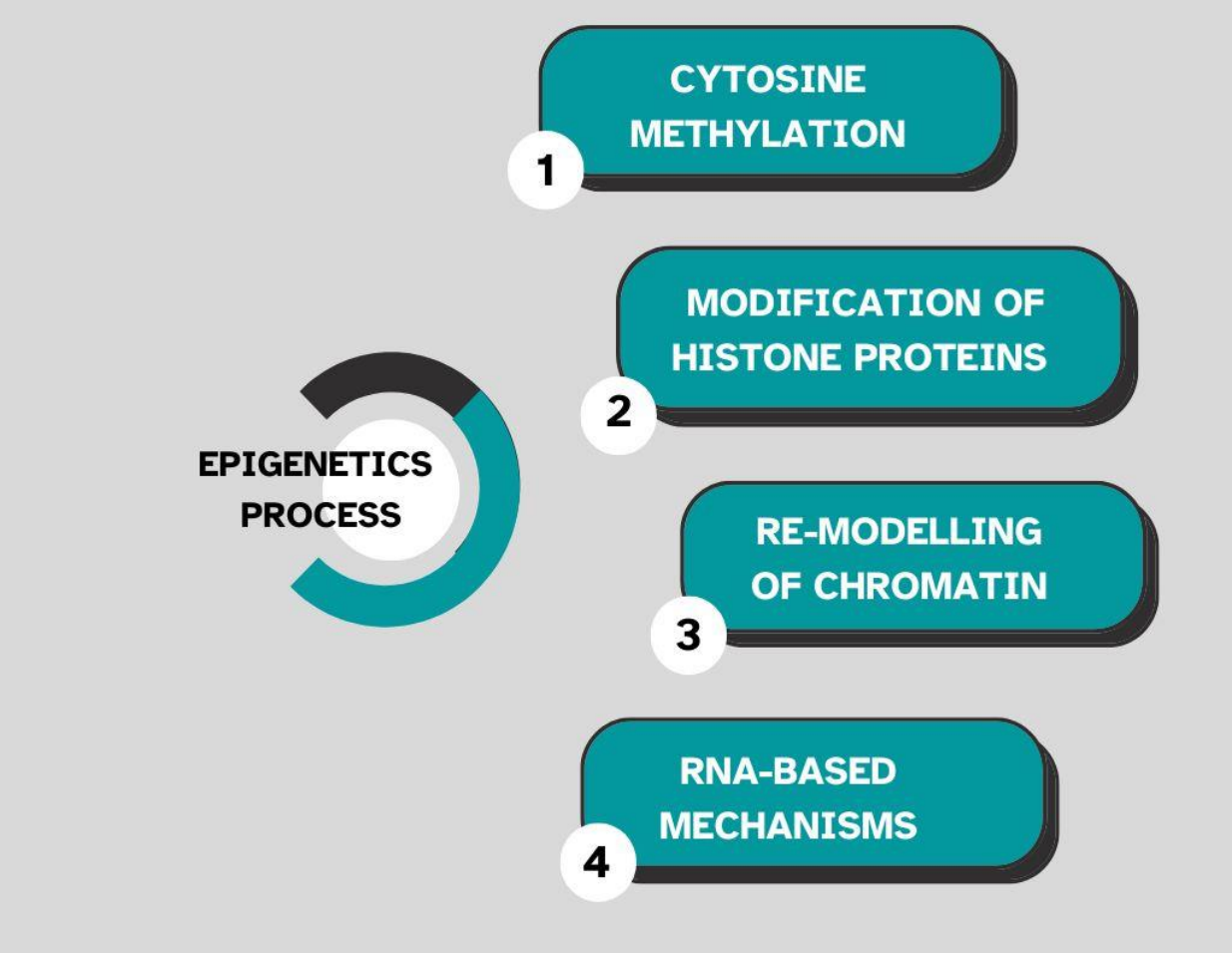

Figure 1: The Epigenetics Process

Since the epigenome controls the expression of genes, it plays a significant role in modifying the integrity and function of the genome. This includes regulation of non-coding RNA (ncRNA), remodeling of chromatin, histone modification, DNA methylation. It's interesting to note that these epigenetic aspects have been demonstrated to control animal attributes; as a result, they may be in charge of the extra diversity required for ongoing gains in cattle productivity and health (Ibeagha-Awemu and Yu, 2021).

2.2 Epigenetic Processes Impact on Gene Regulation

2.2.1 DNA Methylation

The genomic distribution of methylation of DNA is linked to its effect on gene expression. Frequently found in the promoter areas (generally expanding to 5UTR), CpG-rich areas (also called CpG islands) are normally non-methylated; nonetheless, their aberrant methylation may result in the suppression of related gene silencing and transcription (Smith et al., 2020; Deaton and Bird, 2011). DNA methylation may regulate the activity of the seventy-two percent of

promoters that are located inside CpG islands and are essentially unmethylated (Deaton and Bird, 2011; Saxonov et al., 2006). Methyl-binding domain or MBD proteins attract chromatin changes functions to methylated DNA or direct suppression of TF (transcription factor) binding are both the ways that DNA methylation may affect activities of gene expression (Yin et al., 2017; Greenberg and Bourc'his, 2019; Razin and Kantor, 2005; Zhu et al., 2016). TFs have been observed to have the potential to cause localized epigenetic re-modeling (Wapinski et al., 2013).

The binding selectivity of certain TFs has been altered due to DNA methylation in their identification sequences (Zhu et al., 2016). Methylation of DNA is linked to gene expression and chromatin structure, as demonstrated by the MBDs discovery like methyl-CpG binding protein 1 or MeCP1 and methyl-CpG binding protein 2 or $MeCP_2$. Exon-enriched methylation of DNA affects the expression and splicing of genes (Shayevitch et al., 2018; Gelfman et al., 2013; Yang et al., 2014). It was discovered that DNA methylation either promotes inclusion of exon by attracting splicing factors or $MeCP_2$, or it facilitates exclusion of exon by blocking binding of CCCTC-binding factor (CTCF) (Yearim et al., 2015; Shukla et al., 2011; Maunakea et al., 2013).

2.2.2 Histone Modifications

Epigenetics has another crucial mechanism that has a big influence on genome regulation and transcription process regulation the modification of histones (Adamczyk, 2019). A class of proteins known as histones (H1/H5, H2A, H2B, H3, and H4) park and arrange molecules of DNA into structural units known as nucleosomes. Histone methyl-transfers and demethylases control the dynamical variations of methylation of histone, that are often divided to mono, di and tri methylation of residues of lysine and mono methylation of arginine residues (Ye et al., 2017). Histone methylation has a complicated effect on transcriptional activity that is dependent on both the methylation status and altered residues (Jambhekar et al., 2019). Furthermore, transcriptional activity and DNA damage are regulated by phosphorylation of histone, ADP ribosylation and ubiquitylation (Alhamwe et al., 2018; Liu et al., 2017; Shanmugam et al., 2017).

2.2.3 Remodeling of Chromatin

During development, the instructional expression of genes pattern for differentiation of cells and lineage specificity is always correlated with the dynamics of chromatin structure (Quan et al., 2020; Kishi and Gotoh, 2018). Chromatin remodeling complexes control the intrinsic dynamic features of nucleosome development, which is essential for the condensation of genomic DNA into chromosomes and guarantees that genomic DNA operates in chromatin (Kobayashi and Kurumizaka, 2019; Zhou et al., 2016). It is well known that ATP-dependent remodeling of chromatin complexes uses the energy released during ATP hydrolysis to alter nucleosome shape, which in turn controls the accessibility of DNA to TFs (Hota and Bruneau, 2016). Possessing diverse functions during various phases of development of mammal, such as embryogenesis,

neurological development, cardiac development, musculoskeletal development, immunological cell development, etc., BAF complexes are made up of over fifteen distinct subunits (Sokpor et al., 2017; Sun et al., 2018; Hota and Bruneau, 2016; Nguyen et al., 2016).

2.2.4 Non-coding RNA Regulation

Non-coding RNA has significant roles for regulation in modification of chromatin and gene expression that affect cattle health and production, additionally to these well-known epigenetic events (DNA methylation, modification of histone, and chromatin remodeling) (Fomenky and Ibeagha-Awemu, 2017; Benmoussa et al., 2020). Non-coding RNA is a family of RNA species that primarily control transcriptional and post-transcriptional levels of gene expression and regulate their roles as RNA (not transcribed into proteins). siRNA or Small interfering RNA, piRNA or piwi-interacting RNA, miRNA or microRNA and lncRNA or long non-coding RNA are examples of ncRNAs with functions related to epigenetic that disrupt the transcription process, mRNA translation or stability (Wei et al., 2017; Kaikkonen et al., 2011). A feedback modification linked to methylation of DNA may be induced when miRNA binds to a particular target and degrades or blocks mRNA production (Lamouille et al., 2014). Non-coding RNA controls epigenetic modifications to histones and DNA (Sabin et al., 2013).

3. Epigenetics Impact on Livestock Reproduction, Growth and Development

Epigenetics is known as the study of modification of molecules that are heritable and are responsible for the gene expression and genome activity regulation that leads to phenotypic changes without altering the basic sequences of DNA (Merlin and Nicoglou, 2017; Greally, 2018). Processes of epigenetics that include regulation of ncRNA, remodeling of chromatin, histone modification and methylation of DNA regulates expression of gene and hence have an important role in genome stability and function (Do and Ibeagha-Awemu, 2017; Bird, 2002; Kouzarides, 2007; Morris and Mattick, 2014). Furthermore, the knowledge and use of mechanisms of epigenetics could be beneficial to comprehend traits that are quantitative and in attaining developments in the enhancement of disease resistance and productivity of livestock (Banta and Richards, 2018; Panzeri and Pospisilik, 2018; Ibeagha-Awemu and Zhao, 2015; Ibeagha-Awemu and Khatib, 2017).

3.1 Evidence of Epigenetic Processes Effects on Livestock Production and Health

Active modifications of epigenetics are important for standard development and growth by involving many processes of biology particularly in response to stimulus from the environment (Thomson et al., 2020; Corvo et al., 2020). The effects of epigenetic modifications on the production of livestock in response to various exposome or impact factor and phenotypic results are sum up in figure 2.

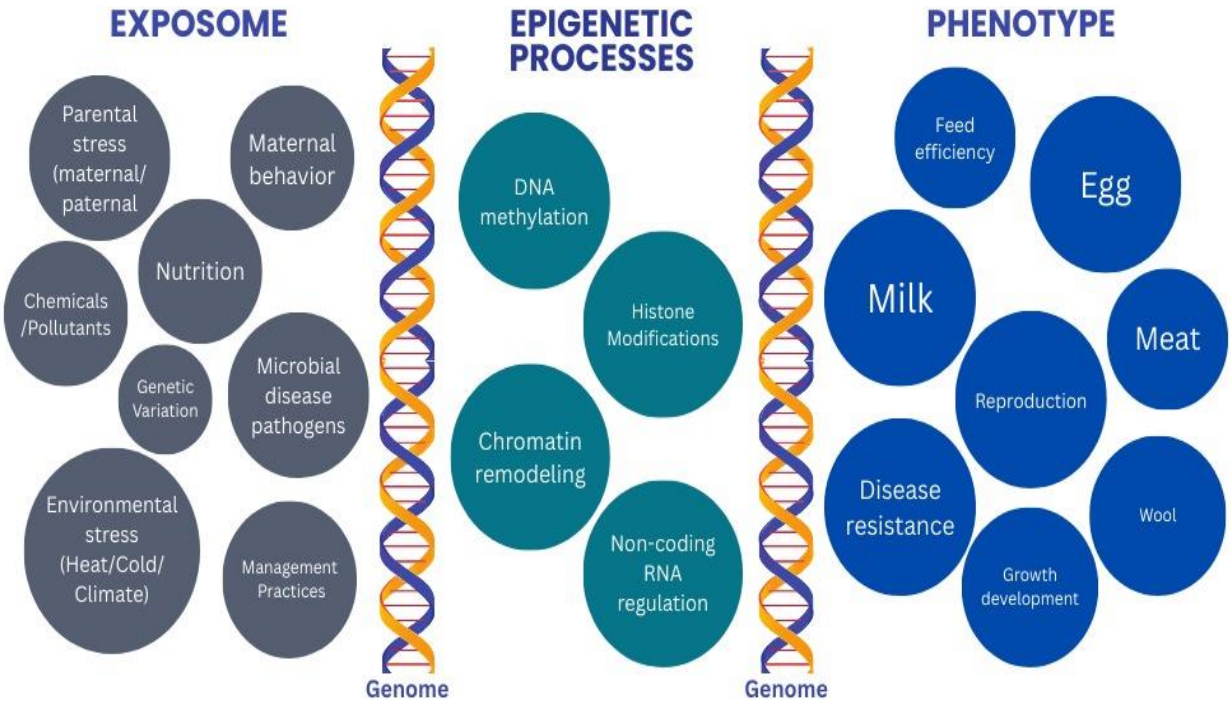

Figure 2. The Effects of Processes of Regulation of Epigenetic on Farm Animal's Production with Regard to Various Exposomes or Impact Factors.

The profiles of DNA methylation of genome of many tissues in various species of livestock including sheep, goat, swine, chicken and cattle etc. have been categorized (Liu et al., 2020; Wang et al., 2020). Pattern screening of methylation of DNA at different developmental embryonic stages in embryo, particularly at the beginning when two main reprogramming of epigenetics happens directed significant DNA methylation regulatory roles in embryo capability and development of fetus linked to many differentiation and metabolic processes (Ivanova et al., 2020; Cao et al., 2020). Changes in DNA methylation were ascertained to partially describe the offspring's bad performance triggered by maternal stressor i.e. negative energy balance, metabolic disorder and heat stress (Akbarinejad et al., 2017). Different methylated cytosine or DMCs in the calf's liver under the cooling treatment (water soakers and fans) or maternal heat stress were found in genes responsible for enzyme activity, development, cell cycle and immune function, whereas DMCs in the tissues of mammary gland were enhanced in biological functions i.e. cell signaling, cell and enzyme activation, phosphorylation and protein binding (Skibiel et al., 2018). However, DNA methylation dysfunction in sperm (Fang et al., 2019) and histone modification i.e. histone methylation and acetylation (Kutchy et al., 2017) have an impact on fertility of male and linked traits. The extremely varying methylated regions linked considerably with reproductive characters and were enhanced for genes that are glycosyltransferase and are

vital for fertilization and spermatogenesis whereas methylated regions that are variable were co-located with genes having roles of sperm motility (Liu et al., 2019).

Histone modification and DNA modification have been categorized in many stains of chicken over the past few years, pointing the possible role of processes of epigenetic in evolution and chicken's development (David et al., 2017). Analysis of epigenetic of many tissues i.e. muscles, liver, cornea, retina and brain potently discovered the connection to methylation of DNA in the development and chicken's growth (Liu et al., 2019). For instance, profiles of DNA methylation of layers and broilers at various stages of embryo discovered lower level of methylation in broilers and improved ontology of gene terms linked to development of muscles by linking genes indicate a possible DNA methylation contribution to development of muscles in embryo (Liu et al., 2019). MTTP and ACC indicate huge expression and were negatively interrelated with lower methylation of DNA at their promoters' areas with fatty liver syndrome in the chicken's liver (Liu et al., 2016). The universal profiling of methylation of DNA of weakly and strongly inbred chickens recognized many DMGs and DMRs improved in the pathway of reproduction s, pointing the DNA methylation regulatory role in the suppressed development of the system of reproduction of inbred chickens (Han et al., 2020). Conclusions of histone H1 subtype comparison between five species of avian i.e. pheasant, duck, quail, partridge, gray and chicken showed the possible connection of modification of histone in the function and structure in the chromatin development of poultry (Figure 3) (Kowalski and Palyga, 2017).

Furthermore, some data related to epigenetics have been described in other livestock species. For example, methylation of DNA was expected to be a process related to age in domestic horses (Andraszek et al., 2016). Honeybees have rare methylation pattern which are associated with gene expression and concentrated in gene bodies, unlike the general mammalian methylated genomes (Harris et al., 2019). Involvement of methylation of DNA in the memory and learning processes in honeybees have been indicated, these are the crucial production characters of honey (Li et al., 2017). In addition to epigenetic modification, i.e. phosphorylation, histone methylation and DNA methylation, signify novel mechanisms of determination process of caste and sex in honeybees (Yagound et al., 2019).

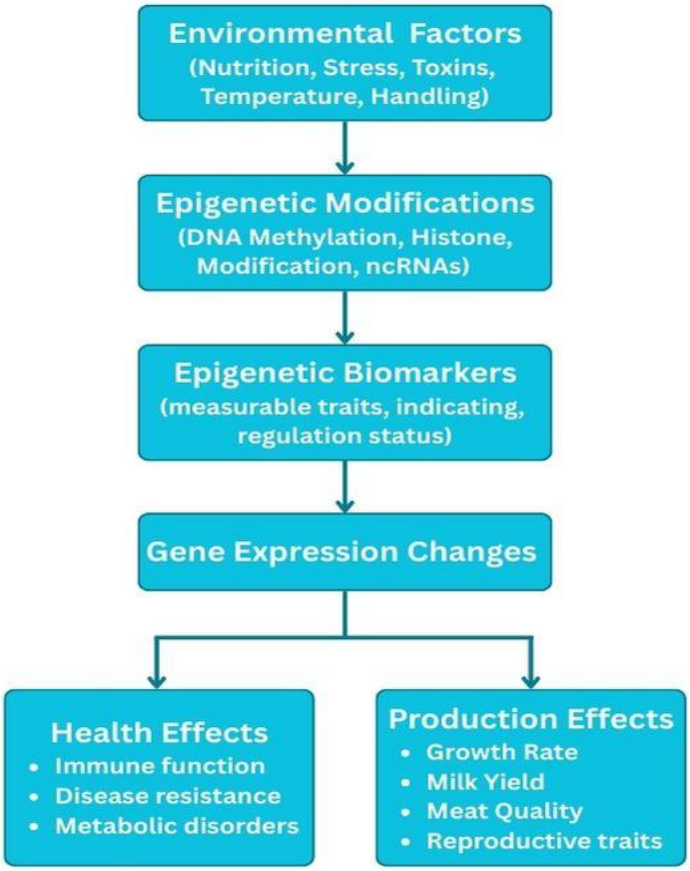

Figure 3. Environmental Factors That Influence the Epigenetic Modifications

4. Studies of Gene Expression: Techniques and Strategy

Typical study in livestock has pointed on supplies for fueling homeostasis, controlling performance, diet formulation, and specific nutrients for the purpose of optimizing production, feed efficiency, and the quality of product. For a long time, considerations diverted to particular rate-limited enzymes or pathways of metabolism in growth. In the recent 25 years, there has been a swing in the attention towards growth and nutrition research in molecular biology. Before genomics, molecular biology intended to study proteins or single genes and their function and structure in separation from a bigger background of other genes. Various procedures were adopted to quantify and identify the expression level of single genes i.e. real-time PCR, SAGE, differential display, subtractive hybridization, or northern blot and their products i.e. ELISA and Western blot. Expression of Gene relies on both environmental and factors of genetic. A useful approach when studying expression of gene is: (1) to manipulate the changeability of phenotype between breeds or individuals within and (2) to contrast animals with maximal characteristics i.e. quality of their products, management system, physiological state, growth rate, nutrition and divergence in their breed.

Research has shown a more detailed examination of the mechanism's elemental functions of metabolism, particularly in ruminants for the key digestion's end products are quantitatively and qualitatively diverse than in other mammals (carbohydrates, fats and amino acids in the latter vs. volatile fatty acids and amino acids in the former). For instance, research in typical gene expression is vastly adds to the interpretation of the fatty liver aetiology in dairy cows at calving (Gruffat et al., 1997; Bernabucci et al., 2004) enhanced capability for gluconeogenesis in early lactating cows (Greenfield et al., 2000) gene response to physiological or grade of nutrition in muscle (Hocquette et al., 2001), adipose tissues (Bonnet et al., 2000) and in the intestine in ruminant animals. Another research has analyzed the effect of genetic or breed polymorphism on gene expression. For example, a gene candidate investigation was led in Belgian, Texel sheep strain harboring a QTL or locus having quantitative traits for muscles' development. The early aim was to categorize the Texel gene and the myostatin was initial clear candidate, the muscle development's negative regulator in QTL. No differential myostatin mRNA expression was observed in sheep of Dutch Island of Texel. Though an alteration in the 30 untranslated gene region of myostatin was seen (Clop et al., 2006). In the cattle having double muscle, inactivation of myostatin signals throughout development is accountable for hyper muscularity (Grobet et al., 1997). In the recent years, the advancement in technologies in genome i.e. metabolomics, proteomics, array technology and large-scale DNA sequencing techniques has initiate analysis of hundreds of metabolites, genes or proteins in a sole experiment. By identify all the proteins or transcripts in tissues, researchers expect to point possibly gene of interest and mechanisms of molecules which can be biomarker for the quality of product and systems of management. This will affect majorly on complex traits characterization. The predicted results are the recognition of new vital genes from a specific signature of molecule and their application for the finding of farm animals with genetic selection or suitable characteristics. In contrast to the approach of candidate gene, the approach of genome gather data without any previous hypothesis on pathways of biology and in creating novel hypotheses. Between the available techniques, DNA microarrays i.e. function dedicated chips or tissue or pan genomic are effective tools to investigate the effect of different factors i.e. management of animal, type of diet, nutrition level, genetics and gene expression treatments in cells or tissues, particularly for exploring new genes that have not participated in nutritional or physiological response before (Lehnert et al., 2007). proteomics separates many protein isoforms with variance in isoelectric point value and molecular weight consistence to modification of post translational as proteolytic cleavages, glycosylation and phosphorylation. Isoforms of protein may also initiate from mRNA alternative splicing as demonstrated by the isoforms of troponin T that are involved in muscles' contraction (Bouley et al., 2005). Additional outcomes of genomics in the diagnostic tests that are basis of biotechnological tools that can be suitable for industry of farm animal for detection of animals with suitable traits.

5. Epigenetics Data Application in the Production of Livestock

5.1 Epigenetics Biomarkers in the Management of Health

Biomarker also known as characteristic or component that may be calculated and evaluated as a gauge or indicator of both pathological and normal biological processes. According to the Food and Agricultural Organization, a biomarker is any material, structure or mechanism that influences or forecasts the disease occurrence or its effects and can be measured (World Health Organization, 2001). There are numerous distinct categories of biomarkers such as risk, prognostic, predictive, therapeutic and diagnostic biomarkers (Bock, 2009). Biomarkers for clinical use should be stable, sensitive and specific and they should be able to be verified in a huge quantity of samples by several laboratories (Mishra and Verma, 2010).

The characteristics of biomarkers, an epigenetic modification biomarker is any altered epigenetic mechanism or epigenetic mark that can be detected in various bodily fluids or tissues. Prognostic biomarkers can predict the outcome of a disease, predictive biomarkers can indicate the response to therapy or treatment, therapy tracking biomarkers can monitor the response to therapy and risk, biomarkers can predict the likelihood of development of disease in future. Epigenetic biomarkers may reflect the development of single variations of phenotype and can help improve production and disease management because they react to various internal (such as the maternal environment) and external (such as diseased pathogens, management practices, nutrition, etc.) environmental cues as pointed by the underlying composition of genetics throughout a lifetime. Furthermore, one benefit of epigenetic biomarkers over stable (non-changing) genetic biomarkers based on sequence of gene is their ability to detect dynamic changes brought on by intra or extra-environmental conditions of cell as well as evolution or disease progression with regard to environmental stimuli (García-Giménez et al., 2016). While the correlation between genetic biomarkers and phenotypes is sometimes unclear across research, epigenetic markers show promise as stand-ins for prompt illness monitoring and diagnosis (Rahat et al., 2020). Moreover, the pattern of illness progression is reflected by particular to tissue epigenetic markers (Zeng et al., 2019). In addition, epigenetic markers particularly methylated DNA and miRNA are highly stable across a range of samples including milk, plasma, urine, blood, tissues, and so on. Additionally, it has been observed that the spontaneous epimutation rate in *Arabidopsis thaliana* is three orders of magnitude larger than the genetic mutation rate (Schmitz et al., 2011) suggesting that epimutations as opposed to genetic or nucleotide changes have a higher rate of spontaneous mutation and provide more raw materials for genetic improvement. A heritable alteration in gene activity associated with changes in chromatin or the increase or loss of DNA methylation is known as an epimutation which is distinct from a mutated DNA (Oey and Whitelaw, 2014). The two types of epimutations are primary (occurs without a change in the DNA sequence) and secondary (occurs as a result of a DNA mutation in a cis or trans-acting factor) (Horsthemke, 2006). Furthermore, epimutations have been characterized as constitutional

which means that since they come from the germline, they ought to exist in all of an individual's tissues or somatic (occur in cells in somatic tissues) (Hitchins and Ward, 2009).

5.2 Epigenetic Biomarkers for Breeding Purposes

There is increasing evidence that epigenetic changes contribute to phenotypic variance in livestock which aids the possible use of epigenetic biomarkers particularly DNA methylation in the breeding programs of livestock (González-Recio et al., 2015; Ibeagha-Awemu and Zhao, 2015; Triantaphyllopoulos et al., 2016; Ibeagha-Awemu and Khatib, 2017; Paiva et al., 2019). A further indication of the possible utility of epigenetic biomarkers in the breeding of livestock is the fact that expression of phenotype reflects not only an entity's sequence or composition of DNA but also how the epigenome copies and regulates the genome while accounting for both current and historical environmental influences or knowledge (Ibeagha-Awemu and Khatib, 2017). Additionally, any change in an offspring's phenotype that results from the transfer of variables other than information from the sequence of DNA from parents or grandparents is referred to as epigenetic inheritance (also known as transgenerational epigenetic effects or non-genetic inheritance) (Bonduriansky and Day, 2009). According to reports, phenotypic variance during one's own and offspring's development is significantly influenced by epigenetic inheritance (Nilsson et al., 2018; Triantaphyllopoulos et al., 2016). Consequently, the idea that environmental influences on founding generations at critical germline cell growth stages may contribute to individual phenotype changes is supported by epigenetic inheritance which includes both intragenerational and transgenerational transmission (Maamar et al., 2021; Skinner, 2011; Nilsson et al., 2018). Thus, livestock’s epigenetic inheritance involves the generational transfer of epigenetic biomarkers including ncRNAs, histone changes, and DNA methylation (Thompson et al., 2020; Triantaphyllopoulos et al., 2016). Only a percentage of variance of phenotype or characteristic heritability may be explained by the genetic data currently utilized in livestock breeding. Therefore, adding epigenetic indicators to genetic data could increase the accuracy of breeding value predictions (González-Recio et al., 2015; Ibeagha-Awemu and Khatib, 2017; Yakovlev, 2018).

In various tissues of various mammalian species, epigenetic changes that range from singular sites to epigenome-wide maps and their regulatory pathways for gene expression have been documented (Ahmad et al., 2021; Nebreda et al., 2019; Choi et al., 2020; Ibeagha-Awemu and Zhao, 2015; Palmer et al., 2018). Additionally, there has been evidence of a correlation between single nucleotide polymorphisms or SNPs and differential DNA methylation, suggesting that methylation changes result in varying associated gene expression and ultimately phenotype determination (Imgenberg-Kreuz et al., 2018; Banovich et al., 2014). The potential use of epigenetic biomarkers in livestock improvement breeding was proposed by the change in CpG sites brought about by SNP which may influence gene expression through altered epigenetic patterns (Maldonado et al., 2019; Zhi et al., 2013).

Nevertheless, there is currently little information to back up the investigation and use of epigenetic biomarkers in animal breeding. For example, there is no information on how epigenetic changes affect the heritability of livestock productivity and health characters. Furthermore, statistical techniques are desperately needed to enable the precise measurement of the role that epigenetic biomarkers play in phenotypic variance. Recently, it was suggested that while estimating trait heritability, the impacts of both non-genetic and genetic inheritance should be examined and taken into account (Danchin et al., 2011). Thus, the epigenetic of non-genetic variable components can be added to animal or mixed-effects models or statistical techniques that facilitate the instantaneous assessment of numerous variance mechanisms is the goal of livestock breeding (Ibeagha-Awemu and Khatib, 2017; Tal et al., 2010). In order to quantify the epigenetic contribution to breeding value prediction, new breeding techniques could incorporate the association between DNA methylation biomarkers and production qualities as determined by EWAS (Figure 4).

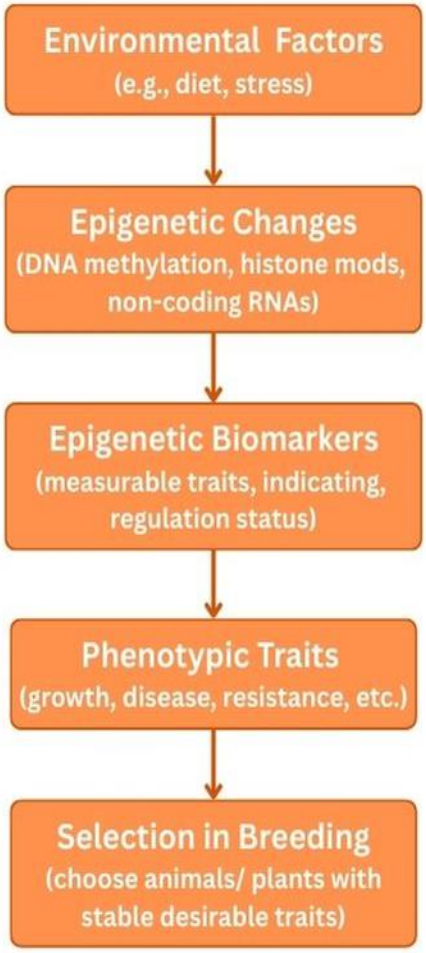

Figure 4: Environmental Factors That Influence the Phenotypic Traits Like Growth, Disease and Resistance.

6. Conclusion

Epigenetic research in domestic livestock is crucial because it can influence non-mendelian patterns of inheritance and varying performance of livestock. Environmental factors are the main determinants of livestock productivity in the scenario of climate change. Stressors in the environment lower cattle production metrics like development, dairy production and reproduction which results in significant financial limitations. Deeper insights into ways in which epigenetic changes, including DNA methylation, interact to establish phenotypic outcomes are anticipated to enhance the knowledge of genomic processes such as cellular, molecular, biological and immunological responses. The function of epigenetics and its fundamental processes to cattle reproduction, growth, development, productivity, quality of product, wellness and immunity are still not entirely understood. To better understand the process of epigenetics underpinning phenotypic variance in stock raising and well-being, more research is therefore required. Research of epigenetic regulatory systems could contribute to developing techniques for increasing livestock productivity with negligible environmental effects, given the high degree of human reliance on animal-based diets and the need to preserve the environment.

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Genetics, functional genomics and selective breeding in livestock

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1. Introduction

Genetics is the study of individual genes, their functions, and how they influence observable traits (phenotypes), including inheritance, gene expression, and variation within populations.

Genomics is the comprehensive study of an organism's entire genome, including genes, regulatory regions, and their interactions, enabling a broader understanding of genetic variation and complex biological processes. Genomics can be classified as structural genomics and functional genomics.

Structural Genomics focuses on sequencing, analyzing, and mapping the entire genome—the complete DNA content—of an organism. Unlike traditional genetics, which often studies individual genes, genomics takes a broader approach, examining gene interactions and genome-wide processes such as epistasis, pleiotropy, and heterosis.

Functional Genomics aims to understand how genetic information is translated into complex biological functions in livestock species. It involves investigating gene activity at multiple biological levels, including DNA, mRNA, proteins, and tissues. Unlike structural genomics, which focuses on mapping and sequencing genomes, functional genomics investigates gene expression, regulation, and interactions to determine how specific genes influence traits such as growth, reproduction, disease resistance, and milk production.

2. Importance of Genomics in Livestock Improvements

Ensuring a stable and sufficient food supply for a rapidly growing global population remains one of the most pressing scientific and ethical challenges of the 21st century. Despite progress in agriculture, hunger and food insecurity persist in many parts of the world, particularly in regions with limited resources and poor access to food. With the global demand for nutrient-rich, animal-based foods expected to rise sharply in the coming decades, especially in developing regions, there is an urgent need to enhance food production systems. While consumption in developed nations may grow more slowly, the global trend calls for sustainable, efficient, and innovative approaches to livestock production to address nutritional needs and reduce disparities in food access.

Advances in genomics offer powerful tools to meet this challenge by improving the genetic potential of livestock. Modern genomic technologies—such as Single Nucleotide Polymorphism (SNP) genotyping, high-density SNP arrays, Genome Wide Association Studies (GWAS), and whole-genome sequencing (WGS)—enable precise identification of genetic markers associated with key traits like milk yield, disease resistance, reproductive efficiency, and feed conversion. These tools have revolutionized animal breeding by allowing for more accurate and accelerated genetic improvement compared to traditional methods. In many developed countries, such genomic approaches are now standard in breeding programs, contributing to increased productivity, enhanced product quality, and better animal health. Additionally, these tools are invaluable in studying genetic diversity and population structure, which supports the long-term sustainability of breeding efforts.

3. History of Genomics

Genomics, though rooted in discoveries dating back to the late 19th century, emerged as a distinct scientific discipline in the 1970s. Early milestones included Friedrich Miescher's isolation of nuclein (DNA) in 1871, the proposal of the Chromosome Theory of Heredity by Sutton and Boveri, and Albert Kossel's identification of nucleotide bases. The mid-20th century brought key insights: Chargaff revealed base pairing rules, Hershey and Chase confirmed DNA as the carrier of genetic information, and Watson and Crick unveiled the double-helix structure. The genetic code was deciphered in the 1960s, and tRNA was first sequenced.

The era of DNA sequencing began in 1977 with Frederick Sanger's method, enabling the sequencing of the first complete genome. This paved the way for significant achievements, including gene mapping, development of Polymerase Chain Reaction (PCR) by Kary Mullis, and DNA fingerprinting by Alec Jeffreys, genome mapping, and bioinformatics in the 1970s and 1980s.

The term "genomics" was officially coined in 1986 by geneticist Tom Roderick. Over time, the field has evolved to include functional genomics, which focuses on understanding gene expression under various environmental conditions, further expanding the scope and impact of genomic research. The word 'omics' originates from the suffix '-ome', derived from a Greek word that means "whole", "all" or "complete". The suffix "-omics" is frequently used to refer to a field of study in life sciences that emphasizes large-scale, high-throughput data/information to understand life summed up in "omes".

The 1990s saw rapid progress with the Human Genome Project (completed in 2003), sequencing of various organisms (chicken, cow, pig, sheep, horse, goat), and the cloning of Dolly sheep. Advances continued with the sequencing of the mouse genome, the launch of the HapMap Project, and breakthroughs in sequencing technologies, culminating in large-scale efforts like the

1000 Genomes Project. These developments laid the foundation for modern genomics and its transformative impact on biology and medicine.

4. Genome Organization and Variation in Livestock Species

The genome organization of livestock species plays a fundamental role in shaping their biological functions, productivity, and adaptability. Livestock genomes are composed of diverse structural elements, including coding and non-coding regions, regulatory sequences, and repetitive DNA, all of which contribute to genetic diversity and trait inheritance. By integrating genomic insights with functional genomic approaches, researchers can explore gene expression patterns, regulatory networks, and molecular pathways that drive trait development. This knowledge enhances precision breeding, ensuring sustainable livestock production with improved efficiency, adaptability, and resilience to environmental challenges.

Genomic variation, such as SNPs, insertions and deletions (InDels), copy number variations (CNVs), and structural variants, influences key traits related to growth, reproduction, disease resistance, and environmental adaptability. Advances in sequencing technologies and bioinformatics have allowed researchers to decode these variations, facilitating GWAS and quantitative trait loci (QTL) mapping. These approaches help identify genetic markers for selective breeding and genetic improvement programs. An introduction of these variations, each contributing uniquely to genome complexity and function, is given below:

SNPs, the most common form of genetic variation, involve single-base-pair changes in the DNA sequence and serve as valuable markers in GWAS and selective breeding programs.

CNVs, which involve duplications or deletions of genomic segments, can impact gene dosage and phenotypic traits, influencing production efficiency and resilience.

Structural variations, including large insertions, deletions, inversions, and translocations, contribute to genome plasticity and can have profound effects on gene function and expression.

Understanding these genetic variations through advanced genomic technologies enhances livestock breeding strategies, enabling precision selection for desirable traits and improving overall herd performance.

5. Reference Genomes and Their Role in Livestock Research

Reference genomes serve as fundamental blueprints for understanding the genetic architecture of livestock species. These high-quality genome assemblies provide a standardized framework for studying genetic variation, gene function, and evolutionary relationships. By comparing individual genomes to reference sequences, researchers can identify mutations, structural variations, and gene expression patterns that influence economically important traits such as growth rate, milk yield, disease resistance, and reproductive efficiency.

The availability of species-specific reference genomes has revolutionized livestock research, enabling GWAS, marker-assisted selection (MAS), and genomic selection (GS) strategies. These resources also facilitate comparative genomics, helping to uncover evolutionary adaptations and breed-specific genetic signatures. Continuous advancements, such as pangenomes and haplotype-resolved assemblies, are further refining our understanding of genetic diversity and its implications for precision breeding and sustainable livestock production.

6. Overview of Structural and Functional Genomic Advancements

Traditionally, farmers selected breeding animals based solely on observable phenotypic traits. This approach was later refined with the integration of quantitative genetic principles, such as heritability estimates and Estimated Breeding Values (EBVs), which considered phenotypic records alongside fixed and random environmental factors. The introduction of molecular genetics tools, such as microsatellite markers, further enhanced the accuracy of selection by providing insights at the DNA level. Today, the incorporation of advanced genomic tools—such as SNP chips, GWAS, and GS—has the potential to revolutionize animal breeding. With these technologies, it is now possible to assess the genetic merit of animals at a very early age—even at birth—enabling more precise selection or culling decisions. This reduces the need for prolonged management, feeding, and housing costs typically required to evaluate an animal's breeding potential over time, thereby improving efficiency and accelerating genetic progress.

These tools are already being applied to enhance key traits such as milk yield in cattle and feed efficiency in poultry, pigs, and cattle, and may also be extended to traits like methane emission reduction in ruminants. These advancements build upon years of foundational work in conventional breeding, supported by systems for animal identification, phenotype recording, and incentivized production schemes.

With the advent of functional genomics, researchers can understand gene expression and regulatory mechanisms, paving the way for targeted breeding strategies. The integration of genomic technologies like RNA sequencing, transcriptomics, and proteomics with modern breeding programs has significantly reduced production costs, improved efficiency, and promoted sustainable livestock farming. These advancements contribute to precision livestock breeding, improved animal health, and sustainable agricultural practices. With the continuous evolution of genomic tools, livestock breeding is becoming more data-driven and efficient, paving the way for improved productivity, adaptability, and resilience in animal populations.

7. Functional Genomics Tools and Approaches in Livestock

Understanding gene expression is fundamental to improving livestock production, as it reveals how genes are regulated and translated into proteins that influence traits such as growth, fertility, milk production, disease resistance, and stress response. Gene expression involves two main steps: transcription (DNA to RNA) and translation (RNA to protein) which is studied under the umbrella of transcriptomics and proteomics, respectively. Additionally, metabolomics helps in

understanding how metabolites contribute to phenotypic traits, further linking genotype to function.

In livestock, this process helps identify genes and regulatory pathways responsible for economically important traits. Technologies like real-time PCR, DNA microarrays, and Northern blotting are commonly used to quantify mRNA levels, enabling researchers to assess gene activity under different conditions, such as heat stress, nutritional variations, or disease challenge.

In eukaryotic livestock species, RNA undergoes splicing to remove non-coding introns and retain exons, which increases the diversity of proteins produced from a single gene. This mechanism allows animals to adapt to various environments without changes to the DNA itself. Advances in transcriptomic technologies have enabled the study of gene expression profiles across tissues and breeds, aiding in marker discovery and genomic selection. By integrating gene expression data with genomic tools, livestock researchers can accelerate the development of improved breeds that are more productive, resilient, and well-suited to specific production systems. Omics technologies can help identify functional SNPs and their prioritize to increase the accuracy of genetic selection.

7.1 Transcriptomics: Gene Expression Profiling in Livestock

Transcriptomic technologies, particularly RNA sequencing (RNA-seq), have revolutionized gene expression studies in livestock, enabling researchers to explore biological responses to environmental stimuli, assess genetic merit, and identify molecular pathways linked to productivity, health, and disease resistance. RNA-seq has replaced older sequencing methods by offering high-throughput, unbiased quantification of gene expression across tissues, revealing insights into transcript variants, gene regulation, and alternative splicing. It also enables the identification of differentially expressed genes (DEGs) under various conditions, which can inform breeding decisions and the development of disease-resistant livestock lines.

Recent advances, such as third-generation sequencing technologies (e.g., SMRT and Nanopore), have improved transcriptome analysis by producing longer reads and eliminating PCR-related biases. Integration of RNA-seq with GWAS and other omics approaches allows the construction of gene regulatory networks and deepens understanding of complex traits. For example, studies in cattle have linked transcriptomic data to immune responses and puberty-related gene expression, aiding in the early selection of elite animals. Similarly, in Creole goats, RNA-seq has identified key immune pathways distinguishing parasite-resistant from susceptible genotypes. Such findings highlight the growing role of transcriptomics in enhancing genetic selection, improving animal health, and increasing livestock productivity.

By integrating transcriptomic data with genomic and proteomic analyses, livestock researchers can refine breeding strategies, improve disease management, and enhance productivity through targeted genetic selection and management practices.

7.2 Proteomics: Protein-Level Insights into Biological Functions

Wilkins and Williams first described proteomics in the mid-1990s. Proteomics allows analysis of all proteins, including their isoforms, in a particular cell, tissue, or organ at a specific time in a single experiment. Advancements in proteomic technologies have significantly enhanced our ability to study the full complement of proteins expressed in livestock tissues, offering insights beyond what genomics and transcriptomics alone can provide. Proteomics enables the identification of protein isoforms, quantification of protein abundance, and analysis of protein–protein interactions. Although its application in livestock research was historically limited due to high costs and lack of optimized protocols, recent improvements in analytical and computational tools have expanded its use across species. Techniques such as one- and two-dimensional gel electrophoresis, liquid and gas chromatography, and advanced mass spectrometry methods (e.g., MALDI-TOF, LC-MS/MS) have become central to proteomic studies. These methods enable the precise measurement of mass-to-charge ratios of peptides, which are matched to protein databases for identification. Additionally, modern strategies like Stable Isotope Labeling by/with Amino acids in Cell culture (SILAC), Isobaric Tags for Relative and Absolute Quantitation (iTRAQ), and Absolute Quantification (AQUA) have facilitated both relative and absolute protein quantification, as well as the detection of post-translational modifications.

In livestock, proteomics has found valuable applications in health monitoring, reproductive efficiency, disease surveillance, and post-harvest product quality. For example, proteomic analysis of seminal plasma has identified fertility-related protein markers in pigs and bulls, aiding in the selection of highly fertile sires to improve reproductive success and reduce non-return rates. Proteomic profiling has also been applied to meat and dairy products to identify genetic variants linked to desirable traits for breeding. Moreover, proteomics has revealed dietary response markers and compensatory growth potential in cattle, providing a powerful tool for marker-assisted selection. As proteomic data becomes increasingly integrated with genomic and transcriptomic information, it holds great promise for accelerating genetic gain, enhancing livestock productivity, and optimizing breeding programs in a more precise and functional manner.

7.3 Metabolomics: Metabolic Pathways and Livestock Productivity

An emerging area in the application of omics tools is the interrogation of the metabolome. Metabolomics is a comprehensive, qualitative, and quantitative study of all the small molecules in an organism. Metabolomics is increasingly being applied in livestock research as a powerful tool to uncover the biochemical underpinnings of animal physiology, productivity, and health. By generating comprehensive metabolic profiles through untargeted or targeted approaches, researchers can evaluate systemic responses to diet, environment, and genetics. Techniques like

Liquid Chromatography–Mass Spectrometry (LC-MS), Gas Chromatography–Mass Spectrometry (GC-MS), and Nuclear Magnetic Resonance (NMR) have facilitated the identification of biomarkers linked to milk yield, feed conversion efficiency, carcass quality, and stress response. For instance, studies have shown that muscle metabolite profiles can accurately distinguish between grass- and grain-fed cattle, or high- versus low-growth Nellore cattle, indicating their potential in phenotype-driven selection. Similarly, fecal metabolomics has been employed to assess feed efficiency and gut health, providing a non-invasive method to identify high-performing animals. Tools such as MetaboAnalyst and Kyoto Encyclopedia of Genes and Genomes (KEGG) are used to interpret these datasets and map metabolic pathways, while metabolite data have also been incorporated into GWAS to link specific metabolite traits with genetic variation in pigs, cattle, and goats.

The integration of metabolomics with genomic tools like WGS and high-density SNP chips marks a significant advancement in precision livestock breeding. Unlike traditional genomic prediction models that rely solely on pedigree and phenotypic data, incorporating metabolomic profiles enhances prediction accuracy by reflecting the animal's actual physiological state, bridging the gap between genotype and phenotype. High-resolution mass spectrometry (HRMS) has enabled the detection of metabolites at extremely low concentrations, improving trait discovery across various biological samples, including semen, saliva, amniotic fluid, and urine. Studies have also linked metabolic fingerprints with fertility, disease resistance, and responsiveness to feed additives. For example, LC-MS profiling of pig feces has shown potential for selecting animals with superior feed conversion efficiency. Future directions involve combining metabolomics with other 'omics' platforms—such as fluxomics, ionomics, and imaging mass spectrometry—for a multi-dimensional understanding of livestock biology, ultimately leading to faster genetic gains and improved production efficiency.

8. Structural Genomic Tools and Technologies

Advancements in genomic tools and technologies have transformed livestock research, enabling precise genetic analysis and accelerated breeding programs. High-throughput sequencing technologies, such as SNP Chips, next-generation sequencing (NGS), and WGS, provide detailed insights into genetic variations, aiding in the identification of key genes associated with desirable traits.

Genotyping platforms, including SNP chips and array-based technologies, allow efficient screening of genetic markers for genomic selection. They have significantly advanced the routine application of genomic tools in livestock for assessing genetic diversity and conducting GWAS and GS. These high-throughput SNP-based methods provide much greater resolution than earlier low-density marker approaches, enabling comprehensive genome-wide analysis of genetic structure, diversity, and breed relationships. This has greatly improved comparative studies of livestock populations across diverse geographic regions—an approach often referred to as

landscape genomics. High-density SNP arrays are now commonly used to detect signals of both natural and artificial selection in various environmental and production contexts.

Additionally, SNP panels have proven useful in distinguishing the genetic profiles of animals from large-scale commercial herds versus smallholder systems. Collectively, these approaches hold promise for identifying genomic regions linked to enhanced productivity, particularly in indigenous breeds adapted to challenging conditions in developing regions. An initial example is preliminary work on Gir cattle from Brazil, where some signatures of selection were in regions of the genome known to contain genes that might be associated with adaptation. Use of genomics in other Latin American cattle production is also being well explored. Combined with information from similar discoveries using improved breeds, genomic solutions should be very useful for improving production efficiency and outputs, provided breeding systems can be developed to ensure application of the improved genetics.

WGS involves sequencing the entire genome through the deep sequencing of millions of fragmented pieces of the target genomic DNA. It provides comprehensive coverage of both coding and non-coding regions, circulating DNA, CNVs, and structural changes within the genome, including InDels and translocations. This broad, genome-wide sequencing strategy is valuable for estimating the total gene count in a species and is also instrumental in applications like personalized medicine. Exome Sequencing is another targeted sequencing method that focuses on the exons or protein-coding regions of the genome. It is particularly useful for identifying genetic variations or polymorphisms among individuals of the same species.

Genotyping and DNA sequencing are foundational molecular tools in livestock genomics, each serving distinct yet complementary purposes. Genotyping is widely used for marker-assisted selection, parentage verification, and breed characterization. In contrast, DNA sequencing enables the discovery of novel mutations, structural variants, and regulatory elements. As costs decline and automation improves, integrating sequencing and genotyping into routine livestock research will become increasingly feasible, enabling more precise genetic improvement strategies across diverse production systems. With the advent of high-throughput technologies like SNP Chips, NGS, WGS, genotyping-by-sequencing (GBS) allow researchers to screen thousands of genetic markers at once, making them ideal for GWAS, GS, and population genomics in livestock. These technologies are discussed one by one.

8.1 Genome Wide Association Studies (GWAS)

It is a powerful approach used to identify genetic variants associated with complex traits in livestock. By analyzing genetic markers across the entire genome, GWAS helps uncover SNPs and other variations linked to economically important traits such as milk yield, growth rate, disease resistance, and fertility. GWAS involves comparing the genomes of individuals with differing phenotypes to detect statistical associations between genetic markers and traits of interest. High-throughput genotyping technologies, such as SNP arrays and NGS, have significantly enhanced the efficiency and accuracy of GWAS in livestock populations.

The findings from GWAS contribute to the development of genomic selection strategies, marker-assisted breeding, and precision livestock management. However, the effectiveness of GWAS depends on factors such as population size, genetic diversity, and the accuracy of phenotype measurements.

Despite its benefits, GWAS has limitations, including the challenge of detecting rare variants and accounting for gene-environment interactions. The integration of GWAS with other genomic tools, such as WGS and transcriptomics, enhances its potential for improving livestock breeding programs.

8.2 Genomic Estimated Breeding Values (GEBVs) and Genomic Selection (GS)

Genomic Estimated Breeding Values (GEBVs) and GS have revolutionized livestock breeding by improving the accuracy and efficiency of selecting animals with desirable traits. Traditional breeding methods relied on pedigree and phenotype-based selection, but genomic selection leverages genome-wide markers to predict an animal's genetic potential early in life.

GEBVs are calculated using genome-wide SNPs to estimate the genetic merit of an individual for economically important traits such as milk production, feed efficiency, growth rate, and disease resistance. Unlike traditional EBVs, GEBVs incorporate genomic information, allowing for more precise and earlier selection decisions.

GS applies GEBVs to breeding programs, enabling faster genetic gains by reducing generation intervals and improving selection accuracy. It is particularly beneficial for traits that are difficult to measure, have low heritability, or manifest later in life, such as fertility and disease resistance.

The success of GS depends on factors such as the size of the reference population, marker density, and statistical models used for prediction. Continuous advancements in sequencing technologies and computational tools have enhanced the effectiveness of GS, making it a cornerstone of modern livestock breeding programs. Integrating GS with other genomic tools, such as WGS and functional genomics, further refines breeding strategies for sustainable livestock production.

8.3 Marker-Assisted Selection (MAS) for Trait Improvement

It is a powerful genetic tool used in livestock breeding to enhance the selection of animals with desirable traits. Unlike traditional selection methods that rely solely on phenotypic observations, MAS utilizes molecular markers closely linked to genes controlling economically important traits, such as growth rate, milk yield, meat quality, and disease resistance.

The effectiveness of MAS depends on the identification of reliable genetic markers, such as SNPs and microsatellites, that are strongly associated with the traits of interest. Once these markers are validated, they can be used to select animals carrying favorable alleles, even before the traits are physically expressed. This accelerates genetic improvement by increasing selection accuracy and reducing the reliance on long-generation testing.

MAS is particularly beneficial for traits that are difficult or expensive to measure, have low heritability, or are expressed later in life. It is commonly used in combination with other genomic approaches, such as GS and GWAS, to optimize breeding programs. By integrating MAS with advanced genomic technologies, livestock breeding becomes more efficient, leading to sustainable improvements in productivity, health, and adaptability of farm animals.

The integration of these genomic technologies has revolutionized breeding strategies, improving productivity, disease resistance, and adaptability in livestock species. As these tools continue to evolve, their application in precision breeding and conservation genetics will further enhance livestock sustainability and efficiency.

9. Role of Bioinformatics and Computational Approaches

Bioinformatics tools and computational approaches facilitate genome annotation, variant detection, and functional analysis, enhancing our understanding of gene regulation and expression. Additionally, transcriptomic and proteomic techniques help in decoding gene function and interaction networks.

9.1 Impact of Cloud Computing in Genomics

As NGS and other high-throughput technologies generate massive volumes of biological data, bioinformatics has increasingly relied on cloud computing to manage, analyze, and store these datasets efficiently. Cloud-based technologies enable the distribution of computational workloads across multiple virtual processors, significantly reducing analysis time. These tools are typically categorized into four main service models: Data as a Service (DaaS), Software as a Service (SaaS), Platform as a Service (PaaS), and Infrastructure as a Service (IaaS). Each offers varying levels of accessibility, scalability, and flexibility, allowing researchers to access genomic data, perform complex analyses without local installations, develop custom applications, or even simulate full computing environments—all through internet-based platforms.

The adoption of cloud computing in bioinformatics not only lowers the cost of infrastructure and maintenance but also enhances data sharing, collaboration, and accessibility. Tools such as CloudBLAST, CloudAligner, and Crossbow exemplify how cloud platforms support genome sequencing, alignment, SNP detection, and expression analysis. In proteomics, advanced applications like Cloud-PLBS leverage frameworks such as Hadoop to analyze protein-ligand interactions across large datasets. By democratizing access to powerful computational resources, cloud computing continues to transform genomics and biomedical research, enabling faster, more collaborative, and more precise scientific advancements.

9.2 Omics data analysis in livestock genomics

The explosion of omics technologies in livestock research—encompassing genomics, transcriptomics, proteomics, and metabolomics—has led to the generation of vast, complex datasets often categorized as "Big Data." These datasets, ranging from gigabytes to terabytes,

cannot be efficiently managed or interpreted using conventional tools. Effective analysis requires a multidisciplinary team of bioinformaticians, statisticians, computer scientists, and domain-specific biologists. The high-dimensional nature of omics data, influenced by multiple biological and experimental variables such as age, sex, physiological state, nutrition, and parity, necessitates the use of advanced statistical models and computational tools to avoid errors and misinterpretations. Specialized software platforms and pipelines—including R packages from Bioconductor, EMBOSS, Galaxy, Staden, Biopython, and Bioconda—are now widely used to process, filter, normalize, and scale omics data.

Data analysis workflows are critical for deriving meaningful biological insights from raw omics outputs, yet results can vary depending on the computational pipeline employed. Therefore, using multiple validated and well-documented pipelines is often recommended to ensure robustness and reproducibility. Several tools and algorithms have been developed specifically for analyzing transcriptomic, proteomic, metagenomic, and metabolomic datasets, and efforts are increasingly focused on integrating these layers in multi-omics approaches. Reviews and resources such as Frontiers in Genetics, GitHub repositories (e.g., "Awesome-Bioinformatics"), and communities like Biostars provide up-to-date protocols and support for omics data analysis. As multi-omics becomes more prevalent in livestock selection programs, a strong foundation in data management and computational interpretation is essential for advancing precision breeding and improving animal performance traits.

9.3 Key Concerns Regarding Omics Data

The rapid advancement of genomic technologies in livestock has enabled the generation and analysis of massive volumes of genomic data, presenting both opportunities and ethical challenges. Sharing livestock genomic data can significantly enhance breeding programs, improve animal health, and accelerate the identification of genetic markers for economically important traits. For instance, genome-wide data can support early selection for disease resistance, feed efficiency, or environmental adaptability. However, the use and sharing of this data must be handled responsibly, particularly when it involves indigenous breeds, private farms, or commercial breeding operations. Ensuring prior consent from data owners, such as farmers or institutions, is essential to protect their interests and maintain trust. Regulatory compliance, data transparency, and proper data-sharing agreements are critical to ensure the ethical use of genomic information in livestock research and breeding.

At the same time, risks such as data inaccuracy, misuse, or unauthorized access pose significant concerns. Contaminated samples or flawed sequence data can mislead genetic selection and compromise breeding decisions. Inaccurate genomic records can result in economic losses or misdirected genetic improvement efforts. Additionally, data breaches or unauthorized use of proprietary breeding information could lead to unfair commercial advantages or exploitation of genetic resources, especially in developing regions. Therefore, just as in human genomics, the

livestock sector requires standardized international regulations, secure data infrastructures, and clear ethical guidelines to govern the sharing and use of genomic information, ensuring that the benefits of such data are maximized while minimizing potential risks.

10. Latest technologies in genomic research

Recent technological advances in genomics have significantly accelerated genome sequencing, making it more accessible and cost-effective. Tools such as Zinc Finger Nuclease (ZFNs), Transcription Activator-Like Effector Nucleases (TALENs), and especially Clustered Regularly Interspaced Short Palindromic Repeats–CRISPR-associated protein 9 (CRISPR-Cas9) have revolutionized gene editing, allowing precise modifications in DNA. In livestock, these technologies hold great potential for improving economically important traits such as disease resistance, growth rate, reproductive efficiency, and adaptability to environmental stress. CRISPR-Cas9, for instance, can be used to remove deleterious alleles or introduce beneficial variants into breeding populations, thus enhancing productivity and animal welfare.

World-leading institutions have demonstrated how integrating cloud computing and advanced data infrastructures can support large-scale genomic research, approaches that can also be adapted for livestock breeding programs. Furthermore, the COVID-19 pandemic underscored the power of genomic surveillance, which is equally applicable in monitoring livestock diseases and zoonotic threats. The future of livestock genomics will likely involve faster, more efficient genomic profiling and editing tools, enabling targeted breeding and disease control strategies that are vital for global food security and sustainable animal agriculture.

11. Use of Artificial Intelligence (AI) in Livestock Genomics

The integration of AI into livestock genomics offers transformative potential for analyzing vast and complex genomic datasets, improving precision breeding, disease resistance, and productivity. AI—particularly through machine learning (ML) and deep learning (DL) models—can efficiently detect patterns, identify genetic variants, and predict phenotypic outcomes by analyzing genomic and multi-omics data. These tools help accelerate GS, enabling breeders to make faster and more accurate decisions on animal health, feed efficiency, growth traits, and environmental adaptability. Technologies like CRISPR-Cas9 also benefit from AI, with platforms such as DeepCRISPR improving the accuracy of gene-editing by predicting on- and off-target effects, thereby enhancing the development of disease-resistant livestock.

AI's applications in livestock genomics extend to early disease detection, trait prediction, and feed optimization by integrating data from genomics, environmental sensors, and health records. For instance, models like Random Forests and Extreme Gradient Boosting have shown promise in classifying complex traits and identifying genetic markers. As genomic datasets grow, the ability of AI to manage, interpret, and apply this information becomes increasingly critical. However, challenges remain, including data security, algorithmic bias, and the need for ethical

and transparent use of AI. Ensuring proper data governance and cross-disciplinary collaboration will be essential to fully harness AI's power for sustainable and ethical livestock improvement.

12. Applications of Livestock Genomics

The integration of genomics into livestock breeding and management has revolutionized animal agriculture, offering innovative solutions to enhance productivity, health, and sustainability. By decoding the genetic makeup of animals, researchers and breeders are now able to make precise and informed decisions that accelerate genetic progress, improve disease resistance, optimize resource use, and conserve biodiversity. These applications are particularly valuable in the context of global food security and climate resilience, especially in developing countries where traditional livestock systems often face limitations in productivity, infrastructure, and access to modern technologies.

12.1 Genomic Selection (GS) and Accelerated Breeding

GS leverages genome-wide marker information to estimate the genetic potential of animals early in life through GEBVs. This approach allows for the selection of superior animals with high accuracy, reducing generation intervals and enhancing the rate of genetic gain. Unlike traditional selection methods that depend on phenotypic records and pedigree, genomic selection provides a more powerful tool for improving traits such as milk yield, growth rate, reproductive performance, and carcass quality. It has already been widely implemented in dairy cattle and is increasingly adopted in beef, poultry, and pig breeding programs.

12.2 Disease Resistance and Health Management

Genomic tools enable the identification of genetic variants associated with disease resistance and susceptibility. Selecting animals with favourable immune-related genotypes reduces the incidence of hereditary and infectious diseases, minimizing the need for antibiotics and veterinary treatments. This not only improves animal health and welfare but also contributes to sustainable livestock production by lowering treatment costs and reducing antimicrobial resistance risks.

12.3 Feed Efficiency and Environmental Sustainability

Feed constitutes one of the largest expenses in livestock production. Genomic studies have identified key genes influencing feed conversion efficiency, enabling the selection of animals that utilize nutrients more effectively. By breeding animals with superior feed efficiency, producers can lower feed inputs while maintaining high productivity, thereby reducing the environmental footprint of animal agriculture. This is particularly relevant in regions where feed resources are scarce or expensive.

12.4 Parentage Verification and Product Traceability

Genetic markers are extensively used for verifying parentage and ensuring pedigree accuracy, which is crucial for genetic improvement and breed integrity. Additionally, genomic traceability

systems enhance food safety by linking animal products such as meat and milk back to their source. This is essential for maintaining consumer confidence, meeting regulatory standards, and protecting brand value in global markets.

12.5 Conservation of Genetic Diversity

Many indigenous and locally adapted livestock breeds possess unique genetic traits that contribute to their resilience in challenging environments, such as heat tolerance, disease resistance, and the ability to thrive on low-quality feed. Genomic tools are instrumental in characterizing, managing, and conserving these genetic resources, which are often at risk of extinction due to modernization and breed replacement. Preserving such biodiversity is critical for long-term sustainability and future breeding innovations.

12.6 Metagenomics: Exploring the Microbial World Within

Metagenomics, a key application of genomics, involves sequencing the combined genetic material of microbial communities—such as microflora and microfauna—found in the gastrointestinal tracts of animals. This approach bypasses the need for culturing individual species, allowing researchers to analyze the full diversity and function of these microorganisms. Gut microbes are known to influence vital physiological processes, including nutrient absorption, immune regulation, and drug metabolism. By studying their collective genomes, metagenomics provides valuable insights into host-microbiota interactions, with implications for improving animal health, productivity, disease resistance, and targeted veterinary treatments.

12.7 Applications in Developing Countries

In developing nations, livestock genomics offers transformative opportunities to improve food security, production efficiency, and climate adaptation. Areas of particular promise include:

- Characterization of indigenous breeds to support conservation and breeding efforts.
- Improvement of production traits tailored to local environments.
- Identification of genes for climate resilience, including heat and drought tolerance.
- Enhanced disease surveillance and vaccine development through genomic insights.
- Implementation of traceability systems for value chain integrity.

However, challenges such as limited funding, lack of infrastructure, and inadequate expertise remain significant barriers. Capacity building, collaborative research, and integration of genomics into national breeding programs are essential for realizing these benefits.

13. Challenges and Future Directions

While livestock genomics has brought transformative advances in breeding, health management, and productivity, several critical challenges must be addressed to fully harness its potential.

These challenges span data integration, technical limitations, ethical concerns, and the need for emerging technologies to be responsibly and sustainably implemented.

13.1 Data Management and Integration

One of the foremost challenges in applying omics technologies is the proper handling and integration of vast, complex datasets. Genomics, transcriptomics, proteomics, and metabolomics collectively generate "Big Data" in gigabyte to terabyte ranges. Effective analysis requires rigorous preprocessing, including filtering, normalization, transformation, and scaling. Additionally, setting appropriate thresholds for microbial abundance, gene expression, and metabolite relevance is essential to avoid analytical bias. Dedicated databases such as ASlive have been developed to support such efforts, but more comprehensive, livestock-specific data repositories are needed to streamline multi-omics research and breeding applications.

13.2 Lack of Comprehensive Phenomics Data

Although many organized farms maintain basic records for production, reproduction, and growth traits, detailed phenomics data—covering adaptability, health status, physiological responses, and behaviour—remain largely under-recorded. This data gap limits the accuracy of genomic predictions and the development of truly precision breeding strategies. Advanced livestock monitoring systems, including remote sensors and Internet of Things (IoT) devices, offer a promising avenue to capture real-time phenotypic data, such as estrus detection, rumination, and lameness. Integrating these phenotypic records with omics data will create a more holistic understanding of animal performance and enable data-driven selection decisions.

13.3 Bioinformatics and Human Resource Limitations

The effective utilization of omics data depends heavily on bioinformatics expertise. Advanced knowledge of statistics, programming, and computational biology is essential for interpreting complex biological datasets. However, a significant barrier in many livestock sectors, especially in developing regions, is the lack of trained personnel and institutional capacity. Misinterpretation of data due to inadequate skills may lead to poor selection outcomes. Thus, capacity building through interdisciplinary training of breeders, veterinarians, bioinformaticians, and data scientists is vital to support the long-term adoption of genomics in livestock improvement.

13.4 Ethical and Practical Considerations

Ethical concerns related to animal welfare, genetic diversity, and the potential consequences of genetic manipulation must be addressed. Equally, practical challenges such as the high cost of genomic testing, limited access to advanced tools in resource-limited settings, and social

resistance to new biotechnologies (e.g., gene editing) hinder widespread implementation. Ensuring equitable access, responsible innovation, and inclusive policymaking is essential for sustainable progress in livestock genomics.

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Communication and Information Technology (CIT) in livestock feeding

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1. Introduction

The global livestock industry currently faces significant challenges in meeting the expanding demand for animal products, whilst concurrently minimising its environmental impact and optimising its utilisation of resources. This is further compounded by forecasts that the global population will continue to rise and reach 9.7 billion by 2050, resulting in an anticipated increase in demand for animal protein of over 70% (FAO, 2021). This projected increase poses significant challenges for the livestock sector as it is expected to have to address a number of issues, including climate change, the scarcity of resources, environmental degradation. Livestock production has been determined to be a significant source of greenhouse gas emissions, with a contribution of approximately 14.5% to the global emissions inventory (Gerber et al., 2013). Notably, the production of animal feed contributes substantially to this emissions footprint. Additionally, livestock production is under heightened scrutiny regarding its utilisation of water, land, and feed resources, particularly in the context of competing demands for these resources from human food production and other industries.

In the context of modern livestock feeding operations, CIT has emerged (Figure 1) as a transformative force, enabling precise monitoring and automation, and facilitating data-driven decision-making (Meena et al., 2024; Vlaicu et al., 2024). Digital innovations, including intelligent feeding systems, cloud-based platforms, and artificial intelligence-driven analysis, represent a paradigm shift in feeding strategies, thereby enhancing animal health, productivity and overall farm efficiency (Vlaicu et al. 2024). For instance, smart feeding systems equipped with IoT sensors have the capability to monitor feed intake in real time, thereby enabling farmers

to adjust rations dynamically based on the needs of individual animals (Akhigbe et al., 2021). In a similar manner, AI-driven analytics have been shown to predict optimal feeding schedules and nutrient requirements, thereby reducing feed wastage and enhancing growth performance (Dayoub et al., 2024). These technologies enhance productivity and contribute to sustainability by minimising resource use and environmental impacts.

Further explanations reveal how the microbiome affects autoimmune diseases: SCFAs are related to the microbial activities and have the ability to exert immunomodulatory effects, influence the regulatory T cell balance (Ratajczak et al., 2019). The autoimmune reactions can also be attributed to the molecular mimicry, where microbial antigens are similar to host antigens. Secondly, if the gut barrier is compromised, it allows luminal microbial components to directly enter the blood stream and potentially worsen systemic inflammation and autoimmune processes (Wells et al., 2017).

This shift in the concept of microbiome, particularly with reference to autoimmune diseases has, significant implication for both fields of study. It offers the chance for a clinical focus in Phillipsburg, to emerge and establish pharmacological strategies based on the disruption of microbiota to correct immunological disorders (Duarte-Chavez et al., 2018). Possible solutions may include use of probiotics as well as prebiotics, diet change and a process called fecal microbiota transplantation (FMT). Subsequently, targeting of biomarkers with links to specific autoimmune diseases would mean early identification and diagnosis of the disease, and elevating the management of autoimmune diseases to the next level: the potential of biomarkers in autoimmune diseases (Giacomelli et al., 2019).

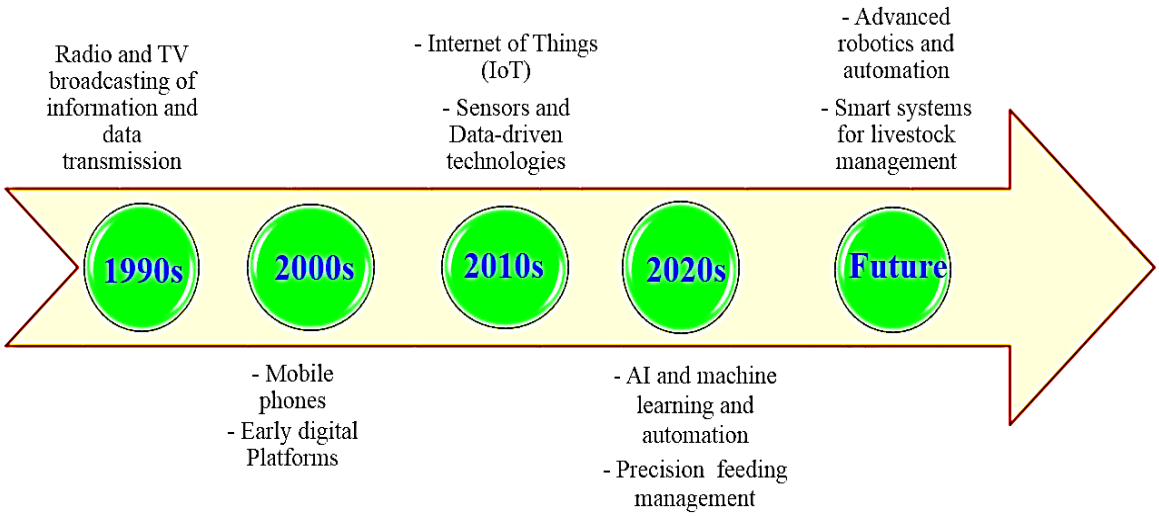

Figure 1. Timeline of CIT Evolution in Livestock Feeding

It is imperative to acknowledge the significance of feed costs in the domain of animal farming. A study conducted by Papadopoulos et al. (2025) has demonstrated that feed costs contribute to more than 60% of the total production costs, thereby underscoring its critical role in the realm of economic sustainability. Inadequate feeding strategies engender elevated costs and result in excessive nutrient excretion, thereby contributing to environmental pollution (Van Zanten et al., 2019). For instance, the presence of nitrogen and phosphorus from animal waste has been demonstrated to contaminate water bodies, resulting in phenomena such as eutrophication and biodiversity loss (Van Zanten et al., 2019). The employment of precision feeding systems, facilitated by CIT, has been identified as a solution to these challenges. These systems enable the delivery of the precise nutrients, in the optimal amounts and at the optimal time. Numerous studies have demonstrated the efficacy of this approach in reducing nitrogen excretion by up to 30%, thereby significantly mitigating environmental impacts (Domingos et al., 2024). Furthermore, these systems enhance feed conversion ratios, ensuring a greater proportion of feed is transformed into animal products rather than waste (Figure 2).

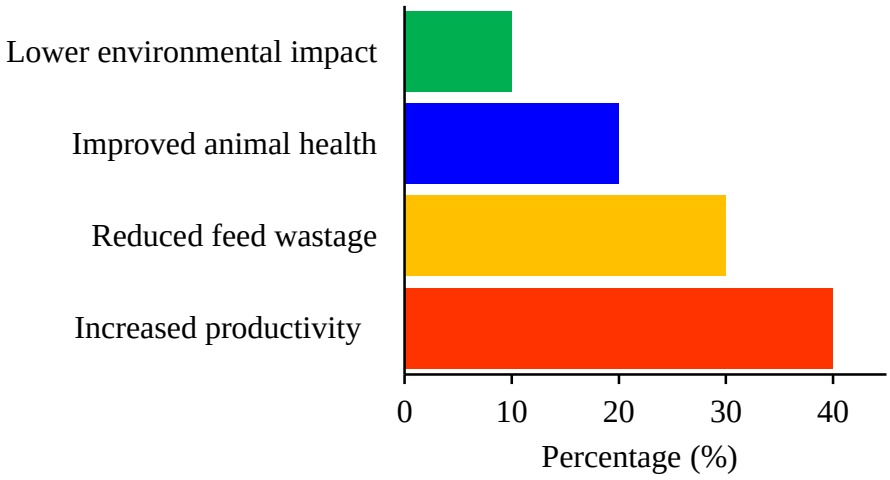

Figure 2. Benefits of CIT in Livestock Feeding

The integration of CIT into livestock feeding has been demonstrated to support animal welfare by facilitating the early detection of health issues and ensuring optimal nutrition is received by animals. Wearable sensors, for example, have been shown to monitor physiological parameters such as heart rate, rumination, and activity levels, thereby providing early warnings of metabolic disorders or stress (Neethirajan, 2020). This proactive approach to animal health has been demonstrated to reduce mortality rates and enhance productivity by maintaining animals in optimal condition. Furthermore, CIT facilitates traceability and transparency in the feed supply chain, ensuring that feed ingredients are safe, sustainable, and ethically sourced (Kamilaris et al., 2019). Blockchain technology, for instance, enables real-time tracking of feed ingredients from farm to fork, enhancing food safety and consumer trust (Neethirajan, 2020).

Notwithstanding the aforementioned advancements, the implementation of CIT in the domain of livestock feeding is confounded by several challenges. The initial costs are high, there is a paucity of technical expertise, and the infrastructure in rural areas is limited, which are significant barriers, particularly for small-scale farmers (Mahmud et al., 2021). Additionally, concerns about data security and privacy pose risks that must be addressed through robust cybersecurity measures and regulatory frameworks (Drape et al., 2021). Nevertheless, it is anticipated that the ongoing development of technology, in conjunction with favourable policies and educational programmes for farmers, will facilitate the overcoming of these obstacles and lead to the widespread adoption of CIT in the livestock feeding sector.

The present chapter offers a comprehensive exploration of the role of CIT in the field of livestock feeding, with a particular focus on recent advancements, prevailing challenges, and future prospects. It delves into the transformative impact of digital technologies, such as precision feeding systems, remote monitoring, decision-support tools, and blockchain, on the domain of livestock nutrition. The chapter further examines the economic, environmental, and social benefits of these technologies, while also addressing the barriers to their adoption. Finally, it outlines future prospects for CIT in livestock feeding, including the potential of emerging technologies such as quantum computing, edge computing, and robotics to further enhance efficiency and sustainability.

2. Precision Feeding and Digital Monitoring

2.1 Precision Feeding Systems

Precision feeding is defined as the process of administering the appropriate nutrients to livestock in the correct amounts and at the optimum times (Jiang et al., 2024; Su et al., 2024). CIT tools, including automatic feeders, smart scales, and real-time sensors, have been developed to ensure that feed composition and quantity are optimised (Wathes et al., 2008). Machine learning algorithms analyse feeding patterns and nutritional requirements, with the aim of reducing waste and improving growth performance (Wathes et al., 2008). Furthermore, precision nutrition minimises nutrient excretion, thereby contributing to environmental sustainability (Van Zanten et al., 2019).

Recent advancements in precision feeding systems have incorporated AI algorithms, which predict individual animal needs on the basis of real-time data (Assimakopoulos et al., 2025). For instance, Akintan et al. (2024) demonstrated the effectiveness of AI in optimising the formulations of animal feed for dairy cows, with results including improved milk yield and reduced feed costs. Furthermore, the integration of IoT devices with precision feeding systems facilitates seamless data collection and analysis, enabling farmers to make informed decisions promptly (Singh and Sharma, 2024).

2.2 Remote Sensing and Wearable Technologies

Advancements in sensor technology and the proliferation of IoT devices have facilitated the remote monitoring of livestock feeding behaviours by farmers. Wearable sensors have been employed to track various biological parameters such as rumination, feed intake, and digestion efficiency, thereby providing critical insights into animal health. These technologies have been shown to facilitate early detection of metabolic disorders and digestive issues, resulting in a reduction in morbidity and mortality rates (Neethirajan, 2020). The implementation of remote monitoring has not only led to a reduction in labour costs but also enhanced early disease detection through automated alerts, consequently leading to improved herd management (Bao and Xie, 2022).

Recent advancements in wearable technologies have given rise to the incorporation of biosensors that are capable of monitoring physiological parameters, including heart rate, body temperature, and stress levels. This development offers farmers a comprehensive view of their livestock's health status, facilitating timely intervention when issues are identified (Folarin et al., 2025). Furthermore, the integration of 5G technology has enhanced the reliability and the speed of data transmission from wearable devices, thereby further enhancing their utility in the field of livestock management (Raja et al., 2024).

A notable example of this is the use of GPS-enabled collars for the monitoring of cattle movements and feeding patterns during grazing in Australian agricultural settings (Menezes et al., 2024). These collars facilitate the analysis of movement and foraging data, which, in turn, provides farmers with the ability to optimise the utilisation of grazing land and to reduce overgrazing. The data collected via these collars is then subjected to analysis using AI algorithms, with the purpose of gaining insights into grazing behaviour patterns and the resulting environmental impacts.

3. Decision-Support Systems and Data Analytics

3.1 Role of Big Data in Livestock Nutrition

The integration of big data analytics into the realm of livestock feeding has empowered farmers to make decisions based on empirical data. The utilisation of cloud-based platforms facilitates the analysis of historical feeding data, the prediction of feed conversion rates, and the optimisation of rations. The employment of AI-powered analytics enhances feed efficiency, thereby promoting the sustainability of livestock production. Recent studies underscore the importance of predictive analytics in anticipating feed shortages and in the optimisation of supply chains (Can, 2024).

Emerging trends in big data analytics, as highlighted in the literature, include the application of machine learning models to predict the impact of climate change on feed availability and quality (Meena et al., 2024). This particular study utilised machine learning to forecast the effects of

drought on forage production, thus enabling farmers to adjust their feeding strategies proactively. Furthermore, the employment of blockchain technology, when combined with the analysis of voluminous data, has enhanced transparency and traceability within feed supply chains (Yang et al, 2025). This, in turn, has facilitated the assurance of food safety and quality. A case study undertaken on a poultry farm offers an illustrative example of the merits of big data analytics. Through the analysis of historical feeding data and market trends, it's possible to refine farm's feed formulations, reducing costs and maintaining production levels (Brill et al., 2024). The farm's implementation of blockchain technology for the tracking of feed ingredients serves to ensure compliance with food safety regulations.

3.2 Decision-Support Software for Feed Management

The utilisation of software solutions, such as feed formulation programmes and nutrient optimization models, has been instrumental in aiding farmers in the selection of cost-effective feed ingredients (Brill et al., 2024). The incorporation of real-time market data in such tools has led to a reduction in feed costs while ensuring the maintenance of nutritional equilibrium. Decision-support systems have also been found to facilitate enhanced farm management and strategic planning by integrating data on climatic, economic, and animal performance (Kazembe and Mkandawire, 2024). Furthermore, the implementation of blockchain technology has been shown to enhance feed traceability, thereby ensuring increased transparency and food safety (Kamilaris et al., 2019).

The development of mobile applications that provide farmers with real-time recommendations has also been an area of recent advancement, with these applications enabling farmers to make informed decisions in real-time, and with the potential to improve the efficiency of feed management (Brill et al., 2024). Furthermore, the incorporation of AI algorithms into decision-support software has led to enhanced accuracy of feed formulation models, consequently resulting in improved nutritional outcomes for livestock (Martinez et al., 2024).

4. Challenges and Limitations

4.1 Barriers to Technology Adoption

The utilisation of CIT in livestock feeding systems has been demonstrated to offer numerous benefits. However, the adoption of these technologies is found to be variable, with financial constraints, a paucity of technical expertise, and infrastructural deficiencies being cited as the primary factors hindering their integration into farming practices. This is particularly pronounced among small-scale farmers, who face significant challenges in incorporating advanced feeding technologies into their operations (Table 1).

Table 1. Challenges and Solutions for CIT Adoption in Small-scale and Rural Farms.

Challenges	Solutions
Poor Infrastructure: no internet, unreliable power…	Mobile-Driven Information Systems : SMS-base advisory services, …
High Costs: expensive devices, software, …	Government subsidies and low-cost solutions
Lack of farmer training: low digital literacy, …	Training programs: workshops, online courses,…
Limited access to advanced tools: no access to ToT, AI tools…	Integration with traditional media: radio, TV,…

Studies have underscored the pivotal role of government support and knowledge transfer initiatives in overcoming these barriers. Recent research has emphasised the imperative of collaborative endeavours between governments, technology providers, and farmers in fostering the adoption of CIT in livestock feeding systems. According to Le Hoang Nguyen et al. (2023), farmers' cooperatives and extension services play a crucial role in facilitating the adoption of precision feeding technologies among small-scale farmers. Additionally, Mishra et al. (2022) state that the development of low-cost, user-friendly technologies has the potential to increase adoption rates in resource-limited settings.

4.2 Data Security and Privacy Concerns

Concerns have been raised regarding the use of digital technologies in livestock feeding and the associated risks to data security and privacy (Drape et al., 2021). These risks have been attributed to cyber threats, unauthorised access, and the potential misuse of sensitive farm data. It is imperative that robust cybersecurity measures are implemented to address these issues.In addition, regulatory frameworks must evolve to ensure data protection while enabling innovation in digital livestock management (Akintan et al., 2024). Recent advancements in cybersecurity have included the development of blockchain-based solutions for securing farm data. The decentralised and secure nature of blockchain technology is particularly well suited to the storage and dissemination of data, thus reducing the risk of unauthorised access (Yang et al., 2025). Furthermore, the employment of AI-driven cybersecurity tools has led to improvements in the detection and prevention of cyber threats within digital livestock management systems (Akintan et al., 2024).

5. Future Perspectives

The future of livestock feeding is likely to be shaped by ongoing advancements in computerised information technology (CIT). The development of blockchain technology for feed traceability, artificial intelligence-driven predictive models, and enhanced automation will further optimise feeding efficiency, policy frameworks and farmer education programmes will play a crucial role in overcame adoption barriers and maximising the potential of CIT in livestock nutrition. Additionally, the integration of robotics in feeding operations may further streamline processes and reduce labour requirements (Uzedhe et al., 2023).

The application of emerging technologies, such as quantum and edge computing, holds considerable potential for a paradigm shift in livestock feeding. Quantum computing, notably, is poised to enhance the accuracy of predictive models by processing vast datasets with greater efficiency (Menezes et al., 2024). Furthermore, the integration of edge computing, in conjunction with IoT devices, has the capacity to mitigate latency and enhance the reliability of data transmission, thereby facilitating expedited decision-making processes (Folarin et al., 2025).

6. Conclusion

Recent advancements in communication and information technology have profoundly impacted the realm of livestock feeding, leading to significant improvements in terms of precision, efficiency, and sustainability. While ongoing challenges persist, the continuous development of technological innovations and the establishment of favourable policy frameworks are poised to catalyse sustained progress in this domain. The integration of intelligent feeding systems, data Analytics and automation holds considerable promise for shaping the future of animal agriculture. Consequently, the integration of communication and information technology in the field of livestock feeding is imperative in ensuring the attainment of sustainable and robust food production systems, particularly in the face of prevailing global challenges.

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Epigenetic Modifications in High-Performance Cows

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Introduction

The field of epigenetics has brought about significant changes in cattle research by concentrating on gene expression changes outside of the DNA sequence. In contrast to mutations, epigenetic modifications, which include DNA methylation, histone alterations, and the regulation of non-coding RNA, are very dynamic and changeable in response to environmental factors. Mutations, on the other hand, are static and cannot be reversed. These modifications affect essential characteristics such as growth, reproduction, milk production, and resistance to illness, which opens up new avenues for boosting cattle productivity and ensuring their long-term viability (NURYE & DEREJE, 2023). Recently conducted studies have shown evidence that epigenetics can effectively influence the productivity of animals. For instance, the Holstein cow population's DNA methylation of casein genes is responsible for around 15–20 % of the variation in milk protein synthesis (Giannuzzi et al., 2024). In cattle production, increasing histone acetylation boosts feed efficiency and marbling. MYOD1 methylation in pigs has been found to promote intramuscular fat for better meat quality. Epigenetic changes affecting both immunity and reproduction have been identified. Heat stress in chickens alters DNA methylation, significantly impairing immunological response and encouraging greater turndown mortality (Rebez et al., 2023).

The transgenerational impact of epigenetics is demonstrated by maternal stress in pigs has been proven to create alterations in histones, which impact the quality of sperm and fertility rates. The findings of a recent study indicate that epigenetic modifications are responsible for 20–35 percent of the phenotypic variation in cattle, highlighting the need to incorporate epigenetic elements into breeding methods (Wang & Ibeagha-Awemu, 2021). The field of epigenetics holds great economic promise for improving feed efficiency and maintaining resilience to heat stress. Therefore, epigenetic studies are essential for cattle production, well-being, and sustainability. This chapter discusses epigenetic processes, the environmental and nutritional factors that control them, and the vast potential for precision breeding.

Key Epigenetic Mechanisms

Epigenetic processes may alter gene expression, which do not modify the DNA sequence. This can lead to changes in cattle's growth, reproductive, and lactation performance, as well as their resistance to infection. Such alterations are reversible and dependent on environmental, nutritional, and management factors and are thus considered essential for improving productivity and sustainability. The primary epigenetic mechanisms include DNA methylation, histone modifications, and non-coding RNA regulation.

1). DNA Methylation: DNA methylation, illustrated in Figure 1, involves adding a methyl group to cytosine in CpG islands and results in the silencing of genes. It plays a role in regulating the livestock genes, such as casein genes in dairy cows and MYOD1 in pigs, affecting traits such as milk production and muscle development. DNA methylation patterns may also be altered by environmental stressors such as heat stress, which modifies immune-related genes and, therefore, affects resilience in livestock (Mullakkalparambil Velayudhan et al., 2023).

2). Histone Modifications: Histones chemically modify the packing of DNA to form chromatin, causing the effect of accessibility. Figure 1 illustrates how the acetylation of histones (for example, H3K9ac) results in the activation of gene transcription.

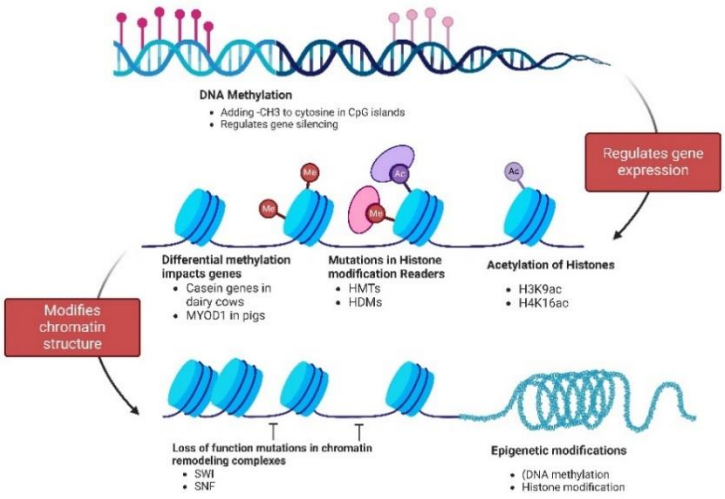

Figure 1. *Overview of Epigenetic Mechanisms in Gene Regulation*

On the other hand, methylation of histones can lead to activation and suppression of gene expression. Instead, specify the impact of histone modifications on production traits such as feed efficiency and reproductive performance. For example, histone acetylation in beef cattle affects feed efficiency and marbling, and histone methylation in pigs affects fertility (Osorio & Moisa, 2019).

3). Non-Coding RNA Regulation: Non-coding RNAs (ncRNAs), including microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), regulate gene expression at the post-transcriptional level. As shown in Figure 1, these molecules intervene in protein synthesis and immune responses. ncRNAs are well known to mediate traits such as immune function and muscle development (Luo et al., 2021). For example, miR-21 aids poultry disease resistance, whereas lncRNAs involved in regulating milk fat composition indicate possible applications for ncRNA-based intervention in livestock production.

Role in Livestock Performance

Epigenetic modifications are essential in controlling key traits in livestock, such as growth, fertility, milk production, and disease resistance. These usually respond to environmental, nutritional, or management conditions, creating opportunities to improve livestock production and sustainability. Gene expression is influenced by different mechanisms such as DNA methylation, histone modification, and regulation by non-coding RNA, which have implications for essential traits and open further avenues in breeding and management. The factors above are viewed generally in Figure 2, which shows how epigenetic mechanisms affect livestock productivity and their reproductive performance under environmental stressors. Muscle growth and feed efficiency are regulated by DNA methylation, histone modifications control fertility and metabolic traits, and non-coding RNAs are essential to the immune system and stress resistance (Liu et al., 2022).

1). Growth and Feed Efficiency: Epigenetic processes significantly influence muscle growth, fat deposition, and feed efficiency. In beef cattle, histone acetylation correlates with better feed efficiency and increased marbling, while DNA methylation of the MYOD1 gene in pigs enhances intramuscular fat, thereby improving meat quality. Environmental stress can affect such modifications, necessitating the implementation of various epigenetic strategies for livestock growth under similarly changing conditions (Vogt, 2022a).

2). Reproductive Performance and Fertility: Epigenetic alterations affect fertility, sperm quality, and embryo viability. For example, histone methylation of pig fertility genes decreases sperm quality and ovulation rates under prenatal stress. In contrast, DNA methylation in cattle embryos affects implantation success, showing the role of epigenetics in reproductive efficiency (Zhu et al., 2021).

3). Immune Function and Disease Resistance: Epigenetic modifications are crucial in regulating immune responses and disease resistance. In poultry, miR-21 supported immune function and in overcoming diseases such as avian influenza (Ibeagha-Awemu & Khatib, 2023). The other example highlights how heat stress-induced methylation in genes associated with the immune response may lead to compromised immune responses and, hence, even greater death rates; these all speak to epigenetics' role in livestock health and disease prevention. Thus,

epigenetic modifications significantly influence livestock performance, making them avenues for enhancing productivity, fertility, and disease resistance, as described in Figure 2.

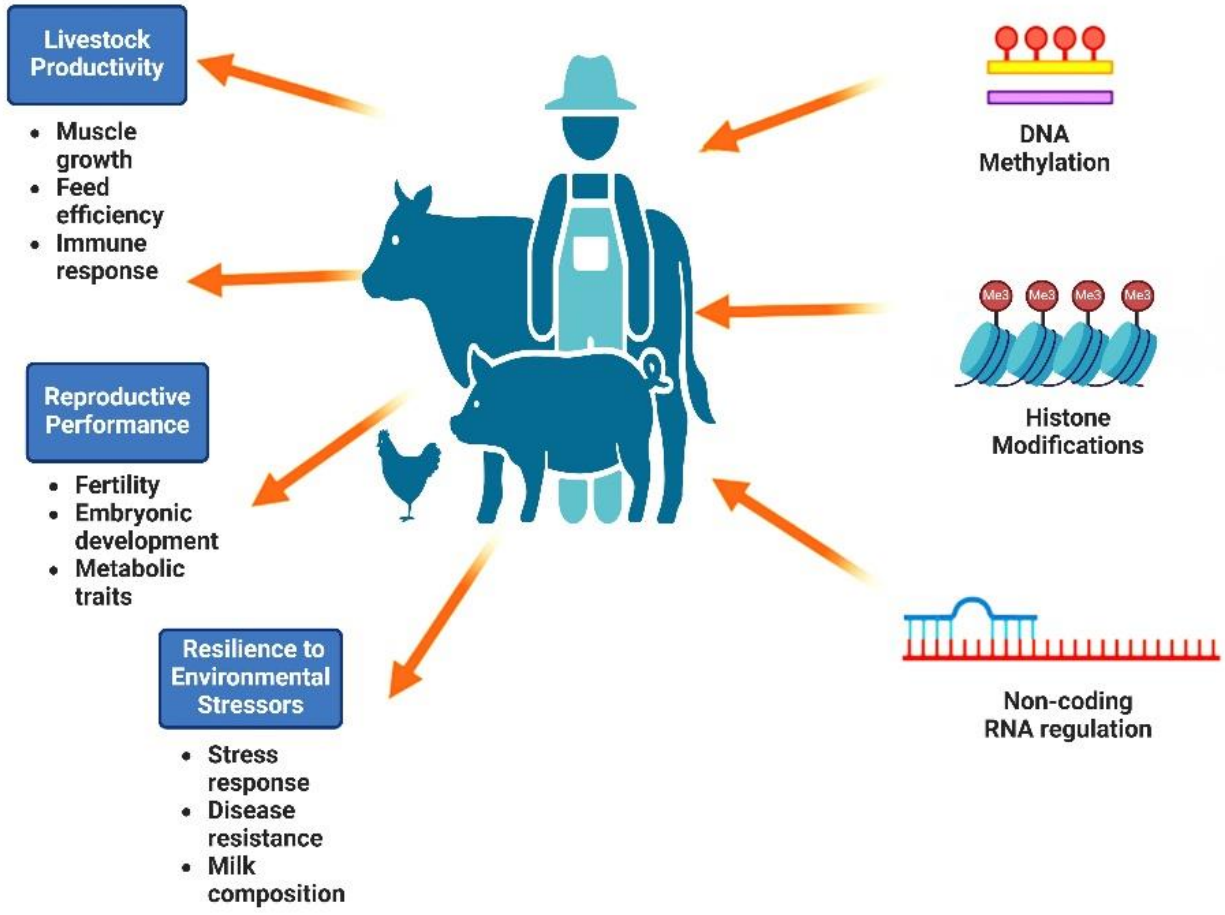

Figure 2. *Epigenetic Regulation of Key Livestock Traits*

4). Milk Production and Composition: Epigenetic regulation is vital for both the yield and composition of milk. DNA methylation of casein genes results in considerable variation in milk protein synthesis in dairy cows. Nutritional intervention, e.g., methyl donor supplementation, can also enhance histone modification, thus increasing milk yield, which highlights the use of epigenetics in dairy improvement (Ibeagha-Awemu & Yu, 2021).

Environmental and Nutritional Influences

Environmental and nutritional parameters play significant roles in the generation of epigenetic changes that control vital livestock characteristics such as growth, reproduction, milk production, immunity, and overall resilience. Unlike genetic mutations, epigenetic modifications are transient and reversible, thus facilitating adjustment by the animal under changing environmental factors, including climate, feed quality, and management (Vogt, 2022b). On the other hand,

adverse environmental and nutritional conditions can interfere with proper epigenetic regulation, leading to diminished productivity and health complications in the long run. Research has shown that environmental stressors, from heat stress to maternal nutrition and feed composition to exposures to environmental pollutants, induce DNA methylation, histone modifications, and modulation of non-coding RNAs, leading to permanent effects on gene expression and performance in livestock. For example, in poultry, studies have shown that methylation of immune-related genes caused by heat stress led to an increase in mortality by 17% (Chaudhary, 2023). Maternal undernutrition was also proven to alter fetal DNA methylation for pigs, resulting in lowered birth weights and decreased growth rates. Such findings emphasize the relevance of precision nutrition and environmental management in improving livestock productivity and adaptability.

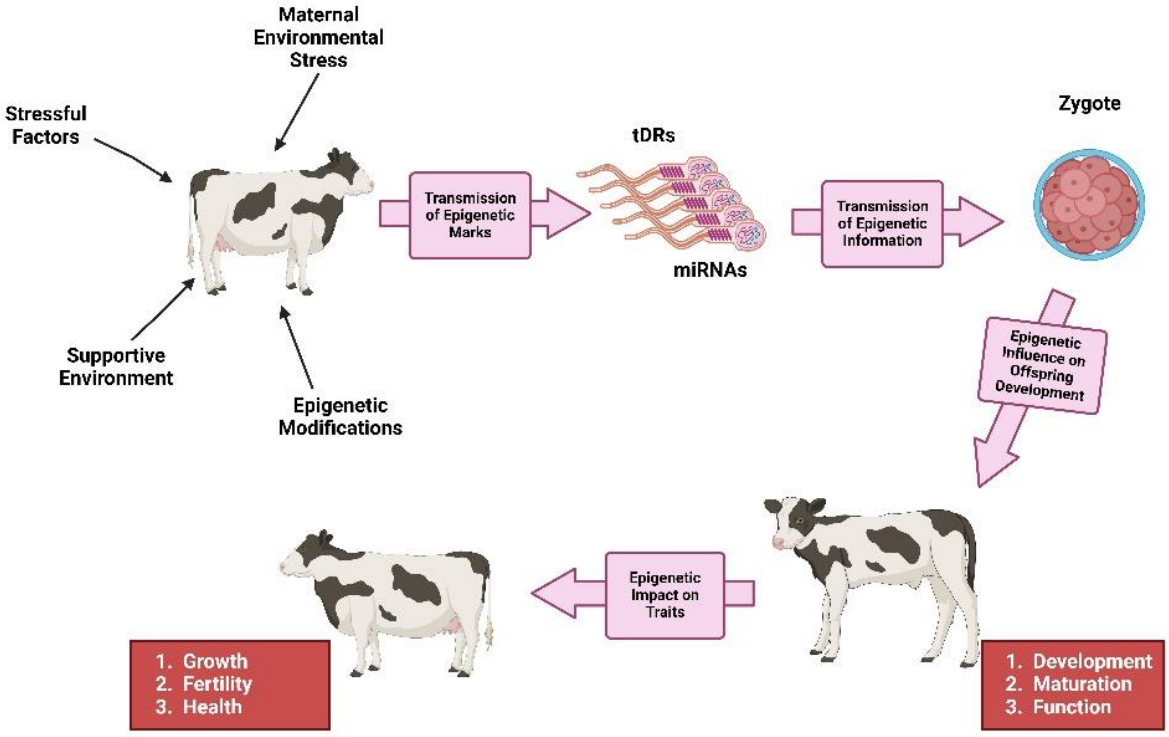

Figure 3. *Epigenetic Modifications and Transgenerational Effects*

The development of the epigenetic modifications can have transgenerational effects, as shown in Figure 3, whereby maternal environmental stress conditions cause epigenetic alterations transmitted to offspring through mechanisms like miRNAs and tDRs, thereby affecting traits such as growth, fertility, and health. Nutrition can modify epigenetic markers, resulting in performance improvement in livestock. For instance, methyl donors in folate, choline, and betaine increase DNA methylation in the mammary glands of dairy cows, thereby increasing milk yield by about 12% (Coleman, 2021). Diets rich in Omega-3 alter histone acetylation, improving milk fat composition and metabolic efficiency. Probiotics also target non-coding

RNAs to benefit pigs' gut health and nutrient absorption. Nevertheless, epigenetic modification has transgenerational effects whereby the offspring of heat-stressed dairy cows produce lower yields and exhibit reduced fertility, even in the best conditions (Cattaneo et al., 2022). Nutrient-deficient sow-born pigs exhibit poor feed conversion and poorer carcass quality, corroborating the maternal diet's permanent influence on the health and performance of the progeny. Epigenetic responses toward environmental and nutritional factors become strategic handles that should play a key role in livestock production sustainability to improve disease resistance, reproductive efficiency, and productivity in future breeding commitments.

Technologies for Epigenetic Analysis in Livestock

New epigenetic research technologies precisely characterize DNA methylation, histone modifications, and non-coding RNAs at individual loci for studying their critical functions in livestock growth, reproduction, milk production, and disease resistance potential. High-throughput sequencing, chromatin immunoprecipitation, and bioinformatics tools provide deeper insights into gene regulation in livestock applications toward precision breeding, feed optimization, and adaptation to future environments (Lu et al., 2024).

Table 1. Overview of Epigenetic Technologies in Livestock Research

Technology	Epigenetic Mechanism	Purpose	Applications in Livestock	Key Findings	References
Bisulfite Sequencing (BS-Seq)	DNA Methylation	Detects methylation at a single-nucleotide level	Used in cattle, pigs, and poultry for growth, milk yield, and stress adaptation studies	Methylation of casein genes in Holstein cows leads to 15–20% variation in milk protein synthesis, and MYOD1 gene methylation in Duroc pigs increases intramuscular fat by 10%.	(Kalaignazhal et al., 2024)
Methylated DNA Immunoprecipitation Sequencing (MeDIP-Seq)	DNA Methylation	Captures methylated DNA fragments for genome-wide	Used to study metabolism, feed efficiency, and immune response	Methylation of metabolism genes in beef cattle improves feed efficiency by 8%, and Heat stress-induced methylation in poultry immune genes increases	(Wang et al., 2017)

		analysis		mortality by 17%.	
Chromatin Immunoprecipitation Sequencing (ChIP-Seq)	Histone Modifications	Maps histone modifications genome-wide	Applied in reproduction, muscle growth, and lactation studies	Histone acetylation in beef cattle is linked to 8% higher feed efficiency and improved marbling, and Histone methylation in pigs reduces sperm quality by 22% and ovulation rates by 14%.	(Tan et al., 2021)
RNA Sequencing (RNA-Seq)	Non-Coding RNA Regulation	Identifies miRNAs and lncRNAs affecting gene expression	Used in immune response, milk composition, and stress adaptation	miR-21 enhances disease resistance in poultry, and lncRNA activity modulated by omega-3 diet improves milk fat quality in dairy cows.	(Bhargava & Srivastava, 2023)
Epigenome-wide association Studies (EWAS)	DNA Methylation, Histone Modifications, Non-Coding RNAs	Links epigenetic variations to production traits	Used in genome-wide studies for trait selection and stress resilience	35% of milk yield and composition variation in dairy cows is epigenetically regulated, and Epigenetic markers in pigs are associated with heat stress resilience.	(Wang & Ibeagha-Awemu, 2021)

Table 1 summarizes key epigenetic technologies and their applications in livestock. The DNA methylation patterns that BS-Seq and MeDIP-Seq have identified affect milk yield, muscle growth, and stress resilience, whereas ChIP-Seq has mapped the histone modifications regulating fertility and feed efficiency. However, RNA-Seq has revealed the roles of non-coding RNA in immune regulation and metabolism, feeding into strategies for disease resistance and nutritional enhancement. Aspects of EWAS have spearheaded the discovery of epigenetic markers for economically important traits and facilitated selection in livestock. Bioinformatics computational tools analyze vast sequencing data to detect epigenetic changes affecting productivity, disease resistance, and reproductive efficiency. Therefore, combining all these technologies into breeding programs and nutritional solutions contributes to better sustainability, resilience, and genetic adaptability in modern livestock.

Challenges and Future Prospects

1). Complexity and Variability of Epigenetic Modifications: Epigenetic changes are very dynamic and are subject to the influence of genetic background, environment, and developmental stages. Therefore, it is difficult to identify stable breeding and trait selection markers. The interrelationship among epigenetics, genetics, and environmental factors is indeed very complex and needs further research to ascertain their long-standing effects on livestock performance (Cassotta et al., 2021).

2). Aspect of Heritability and Stability: Epigenetic modifications are unlike genetic mutations; they are transient and reversible, causing difficulty in assessing which changes are inherited through generations (Ghai & Kader, 2022). This dampens any immediate usefulness in applied settings in breeding because, either by offspring, its beneficial modification may or may not last.

3). Technical and Economic Barriers: High-throughput sequencing and epigenetic profiling technologies are resource and capital-intensive and require specialized expertise and equipment (Ghamkhar et al., 2023). The limitations in access to those technologies, especially in low-resource agricultural settings, slow the widespread implementation of epigenetics in livestock breeding.

4). Future Visions and Developments: Emerging technologies such as CRISPR-based epigenome editing offer further possibilities for the precise regulation of gene expression without affecting the DNA sequence, thereby giving opportunities for targeted improvement of the trait. This suggested that the integration of epigenetic data would augur positively under genomic selection and AI-driven breeding programs to improve trait prediction, disease resistance, and productivity. Furthermore, the development of epigenetic biomarkers for feed efficiency, fertility, and climate resilience will also play a significant role in the sustainable and adaptive management of livestock (Wang & Ibeagha-Awemu, 2021).

5). A Step Towards Practical Applications: In the future, epigenetics for livestock production will become a reality only if research is geared toward identifying stable heritable markers, cost reduction for analysis, and implementation of epigenetics into breeding and nutritional programs (Ahmad & Hamid, 2023). Addressing these challenges will help solidly anchor epigenetics within the toolkit for enhancing productivity, animal welfare, and sustainability in 21st-century livestock systems.

Conclusion

Epigenetics has proved a crucial tool in understanding gene regulation in livestock, thereby influencing essential traits such as growth, reproduction, milk production, and disease resistance. Unlike genetic mutations, epigenetic modifications are dynamic and significantly impacted by environmental and nutritional factors, forming an essential basis for precision breeding and livestock management. Recent advancements in different technologies related to DNA methylation analysis, histone modification mapping, and non-coding RNA profiling have led to a greater understanding of livestock productivity and adaptability. Epigenetic markers associated with economically important traits have been identified by applying technologies such as BS-Seq, ChIP-Seq, RNA-Seq, and EWAS, aiding in targeted genetic selection and improving herd management strategies. Nonetheless, challenges with epigenetic modifications remain, such as their incredible complexity and variability, heritability, and the cost of high-throughput sequencing technologies. Still, emerging methods, including CRISPR-based epigenome editing and AI-supported genomic selection, give promise and hope for practical application in breeding and disease resistance. Future research should concentrate on the following steps: developing stable epigenetic biomarkers, combining epigenetics with genomic selection, and facilitating access to analytical tools. With continuous technological advancement, epigenetics will be critical in enhancing livestock production, sustaining the industry, and improving animal welfare toward a more efficient and resilient livestock industry.

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Advances in molecular genetics and selection of DNA markers in livestock

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Muhammad SAFDAR

1. Introduction

1.1 Evolution of Molecular Genetics in Livestock

The evolution of molecular genetics in livestock has transformed the way animals are bred, managed, and improved for production efficiency, disease resistance, and adaptability. Initially, livestock breeding relied on Mendelian genetics, where traits were selected based on observable characteristics. The discovery of DNA structure in the mid-20th century and the development of early molecular markers, such as RFLP (Restriction Fragment Length Polymorphism) and microsatellites (SSR), provided the first genetic tools to track inheritance patterns at the DNA level. The genomic revolution began with the identification of Single Nucleotide Polymorphisms (SNPs), leading to the development of high-density SNP chips that enabled genomic selection (GS)—a game-changer for improving livestock traits more accurately and efficiently. The introduction of Next-Generation Sequencing (NGS) and Whole Genome Sequencing (WGS) further accelerated genetic discoveries, allowing for the identification of genes responsible for key production and health traits. CRISPR-Cas9 gene editing has since emerged as a revolutionary tool, enabling precise modifications in livestock genomes, such as producing hornless cattle and disease-resistant pigs. Additionally, the rise of epigenetics and AI-driven genetic analysis is helping researchers understand how environmental factors influence gene expression, leading to precision livestock breeding. As molecular genetics continues to advance, it is reshaping livestock industries worldwide by enhancing sustainability, productivity, and genetic resilience in response to global challenges like climate change and disease outbreaks (de Camargo, 2018).

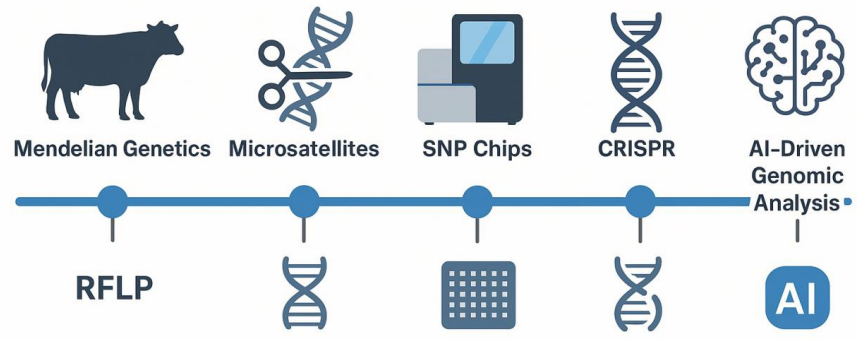

Figure 1. Evolution of Molecular Genetics in Livestock from Mendelian Breeding to AI-Driven Genomics

1.2 Importance of DNA Markers in Animal Breeding

DNA markers have revolutionized animal breeding by enabling precise genetic selection, parentage verification, disease resistance identification, and improved production efficiency. Unlike traditional breeding methods that rely on observable traits, DNA markers allow breeders to assess genetic potential at an early age, significantly accelerating genetic progress. Marker-Assisted Selection (MAS) and Genomic Selection (GS) utilize DNA markers such as Single Nucleotide Polymorphisms (SNPs) and Microsatellites (SSRs) to identify animals with superior genetic traits, leading to enhanced milk yield in dairy cattle, improved meat quality in beef cattle, and higher disease resistance in poultry, sheep, and goats. DNA markers also play a crucial role in conserving genetic diversity by identifying rare and valuable genetic variations, helping to maintain robust livestock populations. Furthermore, advancements in Next-Generation Sequencing (NGS) and genome-wide association studies (GWAS) have allowed for more accurate genetic evaluations, reducing reliance on time-consuming and costly phenotypic assessments. With the integration of AI and bioinformatics, DNA marker technology continues to refine breeding programs, ensuring that livestock are healthier, more productive, and better adapted to environmental challenges. This technology is essential for sustainable animal production, meeting the growing global demand for animal-based products while improving efficiency and animal welfare (Reshma & Das, 2021).

2. Technological Advances in Molecular Genetics

2.1 Next-Generation Sequencing (NGS) and Whole Genome Sequencing

Next-Generation Sequencing (NGS) and Whole Genome Sequencing (WGS) have revolutionized livestock genetics by providing high-resolution insights into the genetic makeup, disease resistance, production traits, and evolutionary history of farm animals. NGS is a powerful technology that enables rapid and cost-effective sequencing of entire genomes, allowing researchers to identify genetic variations, mutations, and structural changes with unprecedented accuracy. Whole Genome Sequencing (WGS), a specialized application of NGS, deciphers the entire DNA sequence of an organism, providing a comprehensive genetic blueprint. In livestock breeding, WGS has been instrumental in uncovering genes associated with milk production in dairy cattle, meat quality in beef cattle, egg production in poultry, and disease resistance in small ruminants. This technology has also enhanced Genomic Selection (GS) by enabling Genome-Wide Association Studies (GWAS), which link genetic markers with economically important traits.

Moreover, NGS has facilitated precision breeding, where animals with superior genetic potential can be selected at an early age, significantly reducing the reliance on traditional selection based on phenotype. It has also played a crucial role in livestock disease diagnostics, helping detect pathogens, mutations causing genetic disorders, and antimicrobial resistance genes, thereby

improving herd health management. As sequencing costs continue to decline and computational tools become more advanced, the integration of NGS and WGS in livestock breeding programs will continue to enhance productivity, sustainability, and genetic resilience in response to climate change and disease challenges. These technologies are paving the way for precision livestock farming, ensuring efficient and sustainable animal production in the future (Park & Kim, 2016).

2.2 CRISPR and Gene Editing in Livestock

CRISPR-Cas9 and other gene-editing technologies have revolutionized livestock genetics by enabling precise modifications to the animal genome, leading to improved productivity, disease resistance, and animal welfare. Unlike traditional selective breeding or transgenic methods, CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) offers a highly targeted approach to modifying specific genes without introducing foreign DNA, making it a more natural and acceptable method of genetic improvement. One of the most notable applications of CRISPR in livestock is the development of disease-resistant animals. For example, researchers have successfully used CRISPR to create pigs resistant to Porcine Reproductive and Respiratory Syndrome (PRRS), a disease that causes significant economic losses in the swine industry. Similarly, cattle have been genetically edited to develop resistance to bovine tuberculosis and other infectious diseases.

CRISPR has also been utilized to improve animal welfare traits, such as producing polled (hornless) cattle without the need for painful dehorning procedures. This not only enhances animal well-being but also aligns with ethical and sustainable farming practices. Additionally, gene editing has been explored to enhance growth rates, feed efficiency, and meat quality, such as modifying myostatin (MSTN) genes in beef cattle to increase muscle mass. Beyond productivity and disease resistance, CRISPR holds promise in reproductive technologies by improving fertility and embryo viability, which can optimize breeding programs and increase genetic gains. As research continues, CRISPR is expected to play a key role in climate resilience, enabling livestock to better adapt to heat stress and environmental changes. However, ethical concerns and regulatory challenges still shape the widespread adoption of gene editing in livestock. Nevertheless, CRISPR is set to redefine the future of animal breeding, offering precision, efficiency, and sustainability in global livestock production (Raza et al., 2022).

2.3 Genomic Selection and Precision Breeding

Genomic Selection (GS) and Precision Breeding have revolutionized livestock improvement by integrating advanced genetic technologies, bioinformatics, and artificial intelligence (AI) to achieve faster, more accurate, and efficient breeding outcomes. Unlike traditional breeding methods that rely on observable traits and pedigree records, genomic selection utilizes genome-wide DNA markers, such as single nucleotide polymorphisms (SNPs), to predict an animal's genetic potential early in life. This enables breeders to select superior animals for milk

production, meat quality, disease resistance, fertility, and feed efficiency with higher precision. Precision breeding, on the other hand, refers to using genomic tools, reproductive biotechnologies (such as artificial insemination and embryo transfer), and gene editing (e.g., CRISPR) to enhance desirable traits while reducing undesirable ones. This approach allows for tailored breeding strategies, ensuring that livestock populations are well-adapted to specific environmental conditions and production demands. For example, heat-tolerant dairy cattle have been developed to withstand rising global temperatures, and feed-efficient poultry are being selected to optimize nutrient utilization, reducing costs and environmental impact.

A key advantage of genomic selection and precision breeding is the ability to accelerate genetic progress by reducing generation intervals—meaning elite animals can be identified and used for breeding at a younger age, significantly increasing genetic gains over time. Additionally, these technologies enhance genetic diversity conservation, preventing the loss of valuable genes that contribute to livestock resilience and adaptability. As genomic databases expand and computational models improve, machine learning and AI-driven analytics are further refining genetic predictions, leading to personalized breeding programs tailored to individual farm needs. With the continued advancement of Next-Generation Sequencing (NGS) and big data analytics, Genomic Selection and Precision Breeding are set to transform sustainable livestock production, ensuring higher productivity, improved animal welfare, and greater adaptability to global agricultural challenges (Meuwissen, Hayes, & Goddard, 2016).

2.4 Multi-omics Approach

Integration of Genomics, Transcriptomics, and Epigenomics

The multi-omics approach is transforming livestock genetics by integrating genomics, transcriptomics, and epigenomics to gain a comprehensive understanding of complex traits, gene expression, and environmental interactions. This holistic strategy enables precise identification of genetic factors influencing growth, reproduction, disease resistance, and productivity in livestock species such as poultry, dairy cattle, beef cattle, sheep, and goats.

1) **Genomics** focuses on analyzing the complete DNA sequence of an organism, identifying genetic variations, single nucleotide polymorphisms (SNPs), and structural variations associated with economically important traits. High-throughput technologies like Next-Generation Sequencing (NGS) and Whole Genome Sequencing (WGS) have accelerated genetic mapping, allowing for genomic selection (GS) and marker-assisted breeding (MAS) to enhance desired characteristics in livestock.
2) **Transcriptomics** examines gene expression patterns by studying mRNA transcripts to determine how genes are activated under various physiological and environmental conditions. Using tools like RNA sequencing (RNA-Seq), researchers can assess how different genes contribute to muscle growth in beef cattle, milk production in dairy cows, or

immune responses in poultry. This information helps optimize breeding programs by selecting animals with superior gene expression profiles.

3) **Epigenomics** investigates heritable modifications in gene expression that do not involve changes in the DNA sequence, such as DNA methylation, histone modifications, and non-coding RNAs. Epigenetic mechanisms play a crucial role in livestock adaptation to environmental stressors, feed efficiency, and disease resistance. For example, epigenetic studies in heat-stressed dairy cows have revealed molecular pathways that help animals cope with extreme temperatures, leading to climate-resilient breeding strategies.

By integrating these multi-omics layers, scientists can develop a systems biology approach that provides deeper insights into how genes, gene expression, and environmental factors interact to shape livestock traits. This integration enhances precision breeding, accelerates genetic improvements, and ensures the sustainability of livestock production in response to global challenges such as climate change, emerging diseases, and food security. The future of livestock genetics lies in the continued advancement of multi-omics technologies, artificial intelligence (AI), and big data analytics, paving the way for a new era of data-driven, high-efficiency animal breeding (Kim & Kim, 2021).

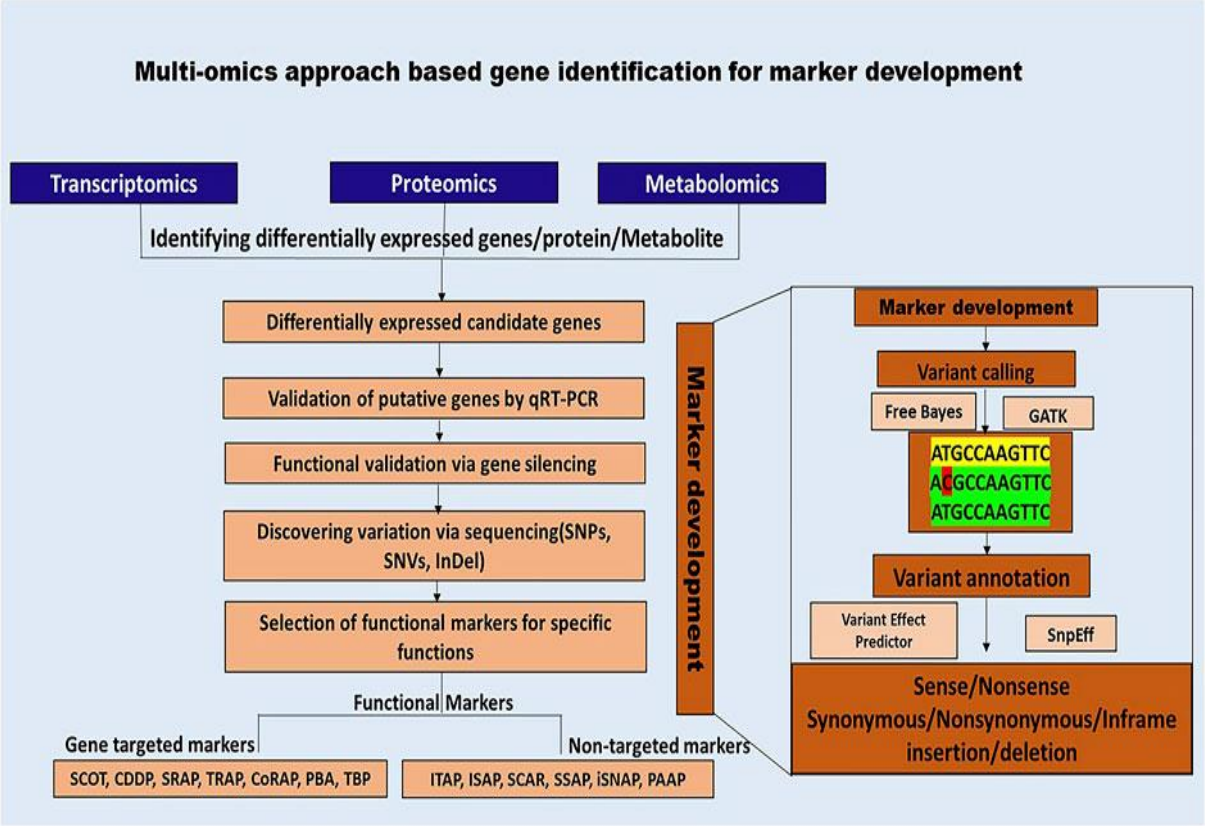

Figure 2. Multi-Omics Workflow Integrating Transcriptomics, Proteomics, And Metabolomics for Functional Gene Identification and Marker Development

3. Types of DNA Markers and Their Role in Animal Breeding

3.1 Restriction Fragment Length Polymorphism (RFLP)

Restriction Fragment Length Polymorphism (RFLP) is one of the earliest and most widely used DNA marker technologies in livestock genetics, allowing for the detection of genetic variations based on differences in DNA fragment lengths after enzymatic digestion. This technique relies on restriction enzymes, which cut DNA at specific sequences, generating fragments of varying lengths that can be analyzed using gel electrophoresis. Variations in fragment lengths arise due to mutations, insertions, or deletions at restriction sites, making RFLP a valuable tool for genetic mapping, parentage verification, disease gene identification, and breed characterization.In livestock breeding, RFLP has been used to identify genes linked to economic traits such as milk yield in dairy cattle, growth rate in poultry, and muscle development in beef cattle. For instance, the DGAT1 gene, which influences milk fat content in cattle, has been studied using RFLP analysis to distinguish between high- and low-fat-producing cows. Similarly, in poultry, RFLP has been applied to detect genetic variations associated with egg production, disease resistance, and feed efficiency.Although more advanced DNA marker technologies such as Single Nucleotide Polymorphism (SNP) genotyping, Next-Generation Sequencing (NGS), and Whole Genome Sequencing (WGS) have largely replaced RFLP due to higher throughput and precision, RFLP remains an essential tool in cost-effective, small-scale genetic studies, especially in regions with limited access to advanced sequencing technologies. Additionally, it continues to serve as a fundamental method in molecular genetics research, contributing to our understanding of genetic diversity, evolution, and the inheritance of complex traits in livestock (Schütte et al., 2008).

3.2 Microsatellites (SSR) and Their Applications

Microsatellites, also known as Simple Sequence Repeats (SSR), are short, repetitive DNA sequences consisting of 1–6 base pairs repeated multiple times throughout the genome. These regions are highly polymorphic due to variations in the number of repeat units, making them valuable genetic markers in livestock breeding, conservation, and evolutionary studies.

Applications of Microsatellites in Livestock Genetics

1) **Parentage Verification and Pedigree Analysis**
   Microsatellites are widely used in parentage testing to confirm genetic relationships in livestock species. By comparing microsatellite profiles of offspring and potential parents, breeders can accurately trace lineage and ensure genetic integrity in breeding programs.
2) **Breed Characterization and Genetic Diversity Studies**
   SSR markers are crucial for identifying and differentiating livestock breeds by assessing

genetic variation within and between populations. This helps in conserving indigenous breeds and managing breeding strategies to maintain genetic diversity.

3) **Marker-Assisted Selection (MAS) in Breeding Programs**
   Microsatellites linked to economically important traits such as milk production in dairy cattle, growth rate in poultry, and meat quality in beef cattle enable Marker-Assisted Selection (MAS). This helps in selecting superior animals with desired genetic traits for breeding.
4) **Disease Resistance Studies**
   SSR markers are used to identify genetic variations associated with disease resistance in livestock. For example, microsatellites linked to Major Histocompatibility Complex (MHC) genes in poultry and cattle help in breeding animals with stronger immune responses against infectious diseases.
5) **Population Genetics and Evolutionary Studies**
   Microsatellites provide insights into population structure, genetic drift, and evolutionary relationships among livestock species. They help researchers understand how different breeds have evolved and adapted to specific environmental conditions.
6) **Forensic Applications in Livestock Theft and Fraud**
   Microsatellite markers are used in animal forensics to trace stolen livestock and verify the origin of meat products, ensuring food safety and preventing fraudulent breed claims in the meat industry (Teneva et al., 2013).

3.3 Single Nucleotide Polymorphisms and Their Role in Genomic Selection

Single Nucleotide Polymorphisms (SNPs) are the most abundant and widely studied genetic markers in livestock genomics. SNPs refer to single base-pair variations in DNA sequences among individuals within a species. Due to their high density and stability across genomes, SNPs serve as powerful tools for genomic selection (GS), disease resistance studies, and precision breeding in livestock species such as poultry, dairy cattle, beef cattle, sheep, and goats.

Role of SNPs in Genomic Selection

1) **High-Resolution Genetic Mapping**
   SNPs provide detailed genetic maps that help identify genes associated with economically important traits, such as milk yield in dairy cattle, growth rate in poultry, meat tenderness in beef cattle, and wool quality in sheep. Genome-wide association studies (GWAS) use SNP markers to detect genetic loci influencing these traits, allowing for more targeted breeding strategies.
2) **Enhancing Accuracy of Breeding Programs**
   Unlike traditional selection based on phenotype and pedigree, Genomic Selection (GS) leverages SNP-based genomic estimated breeding values (GEBVs) to predict an animal's

genetic potential with greater precision. This enables early selection of superior animals, reducing generation intervals and accelerating genetic progress.

3) **Marker-Assisted Selection (MAS) for Disease Resistance**
   SNP markers linked to disease-resistant genes help in breeding livestock with improved immunity against bovine tuberculosis, foot-and-mouth disease in cattle, avian influenza in poultry, and parasite resistance in sheep and goats. This reduces disease outbreaks and enhances overall herd health.
4) **Feed Efficiency and Environmental Adaptation**
   SNP-based selection enables breeding for feed efficiency, optimizing nutrient utilization, and reducing feed costs. Additionally, SNP markers help identify animals with heat stress tolerance, ensuring better adaptation to climate change and improving livestock productivity in diverse environments.
5) **Reproductive Performance and Fertility**
   SNP markers assist in identifying genetic variations influencing fertility traits, calving ease, egg production in poultry, and prolificacy in small ruminants. This aids in selecting animals with superior reproductive efficiency, improving overall herd and flock management.
6) **Meat and Milk Quality Improvement**
   SNP analysis helps identify genes influencing fat composition, marbling, tenderness, and milk protein content, allowing breeders to produce high-quality meat and dairy products tailored to consumer preferences (Seidel, 2009).

3.4 Copy Number Variations (CNVs) and Structural Genomics

Copy Number Variations (CNVs) are large genomic structural variations where segments of DNA, ranging from 1 kilobase (kb) to several megabases (Mb), are duplicated or deleted in different individuals within a species. Unlike Single Nucleotide Polymorphisms (SNPs), which involve single base-pair changes, CNVs cause significant changes in the dosage and expression of genes, affecting key biological functions and traits in livestock.

Structural Genomics and CNVs in Livestock

Structural genomics focuses on understanding large-scale variations in genome organization, including CNVs, insertions, deletions, inversions, and translocations. CNVs play a critical role in livestock genetics, evolution, and adaptation, influencing growth, disease resistance, reproduction, and production traits (Li, 2023).

Role of CNVs in Livestock Genomics

1) **Impact on Growth and Meat Quality**

a) CNVs in genes like MYH3 (Myosin Heavy Chain 3) influence muscle growth, tenderness, and marbling in beef cattle and pigs.
b) In poultry, CNVs affecting IGF1 (Insulin-like Growth Factor 1) have been linked to body size and feed efficiency.

2) **Milk Production and Composition**

a) CNVs in CSN1S1 (Casein Alpha S1) and DGAT1 (Diacylglycerol O-Acyltransferase 1) influence milk yield and fat composition in dairy cattle and goats.

3) **Disease Resistance and Immunity**

a) CNVs in beta-defensin and MHC (Major Histocompatibility Complex) genes contribute to disease resistance in cattle, poultry, and small ruminants.
b) Structural variations in the NRAMP1 gene have been linked to tuberculosis resistance in cattle.

4) **Reproduction and Fertility**

a) CNVs in genes such as FSHR (Follicle-Stimulating Hormone Receptor) affect fertility and reproductive performance in sheep and goats.
b) Variations in ESR1 (Estrogen Receptor 1) impact litter size and reproductive efficiency in pigs and cattle.

5) **Environmental Adaptation and Heat Tolerance**

a) CNVs in HSP70 (Heat Shock Protein 70) genes help livestock adapt to heat stress, making them more resilient to climate change.
b) Adaptive CNVs in drought-resistant sheep and heat-tolerant cattle breeds are being studied for sustainable breeding strategies.

Impact on Different Livestock Species

4. Poultry Genomic Innovations

4.1 DNA Marker-Assisted Selection in Broilers and Layers

Marker-Assisted Selection (MAS) is a powerful tool in poultry breeding that utilizes DNA markers to identify and select birds with desirable genetic traits. In broilers and layers, MAS is revolutionizing breeding programs by enhancing growth rates, feed efficiency, disease resistance, egg production, and egg quality (Hocking & Hickey, 2017).

4.1.1 Marker-Assisted Selection (MAS) in Broilers

1. Growth Rate and Feed Efficiency

a) SNPs in genes like IGF1 (Insulin-like Growth Factor 1) and GH (Growth Hormone) are associated with faster growth and better feed conversion ratios (FCR).
b) CNVs in the myostatin (MSTN) gene contribute to muscle development and meat yield.

2. Meat Quality Traits

a) DNA markers linked to fat deposition, muscle texture, and oxidative stability help improve meat quality.
b) SNPs in the FTO (Fat Mass and Obesity-Associated) gene influence intramuscular fat content for better taste and tenderness.

3. **Skeletal Health and Disease Resistance**

a) MAS helps reduce skeletal disorders (e.g., tibial dyschondroplasia) by selecting for genetic markers associated with bone strength.
b) MHC-linked markers enhance resistance to viral and bacterial infections in broilers (Sodhi et al., 2013).

4.1.2 Marker-Assisted Selection (MAS) in Layers

1. Egg Production and Quality

a) SNPs in VIPR1 (Vasoactive Intestinal Peptide Receptor 1) are associated with egg production rate and persistency.
b) Genes like CALB1 (Calbindin 1) influence eggshell strength, reducing the risk of breakage.

2. Reproductive Performance

a) DNA markers help select layers with early maturity, high fertility, and optimal clutch size.
b) Genetic variations in ESR1 (Estrogen Receptor 1) affect egg-laying capacity and ovarian function.

3. Disease Resistance and Longevity

a) MHC markers aid in breeding layers resistant to Salmonella, E. coli infections, and coccidiosis.
b) SNP markers associated with longer laying periods and better health extend the productive lifespan of layers.

4.2 Genomics Approaches for Disease Resistance

Infectious diseases like Avian Influenza (AI) and Newcastle Disease (ND) pose significant threats to the global poultry industry, leading to severe economic losses and public health concerns. Traditional methods of disease control, such as vaccination and biosecurity, have limitations, making genomics-based breeding strategies crucial for developing disease-resistant poultry. Advances in genomics, transcriptomics, and molecular breeding offer precise tools for selecting and enhancing genetic resistance against these viral infections (Liu et al., 2014).

4.2.1 Identification of Genetic Markers for Disease Resistance

Genomic selection relies on identifying Single Nucleotide Polymorphisms (SNPs), Copy Number Variations (CNVs), and Quantitative Trait Loci (QTLs) associated with disease resistance.

1. **For Avian Influenza (AI)**

a) SNPs in genes such as MX1 (Myxovirus Resistance 1) and OAS (2'-5'-Oligoadenylate Synthetase) are linked to resistance against AI viruses.
b) Chickens with specific MX1 alleles exhibit stronger antiviral responses, reducing viral replication.

2. **For Newcastle Disease (ND)**

a) QTLs on chromosomes 1, 2, and 3 have been associated with immune response to NDV.
b) Genes like TLR3 (Toll-like Receptor 3) and IL6 (Interleukin 6) enhance innate immune responses against ND infection.

5. Dairy Cattle enhance Milk Production and Disease Resistance

5.1 Genomic Selection for High Milk Yield and Composition

Genomic selection has transformed dairy cattle breeding by enabling precise and early identification of superior animals for milk yield and composition (fat and protein content). Traditional selection methods relied on pedigree and progeny testing, which were time-consuming and expensive. With genomic selection, breeders can now use genome-wide markers to predict an animal's genetic potential, significantly improving the efficiency of dairy cattle breeding programs (Zhang et al., 2022).

5.1.1 Genetic Basis of Milk Yield and Composition

Milk production traits are polygenic, meaning they are influenced by multiple genes. Genomic selection identifies key Single Nucleotide Polymorphisms (SNPs) associated with milk yield, fat percentage, and protein content.

Major Genes Influencing Milk Production

1. DGAT1 (Diacylglycerol O-Acyltransferase 1)

a) Plays a crucial role in fat synthesis.
b) Cows with specific DGAT1 alleles produce higher milk fat content.

2. ABCG2 (ATP-Binding Cassette Transporter G2)

a) Regulates fat and protein secretion into milk.
b) A well-known mutation increases milk fat yield while slightly reducing volume.

3. CSN2 (Beta-Casein Gene)

a) Affects milk protein composition, especially A1 and A2 beta-casein variants.
b) Selection for the A2 variant is gaining popularity due to potential health benefits

4. GHR (Growth Hormone Receptor)

a) Influences milk yield and composition through metabolic regulation.
b) Mutations in GHR have been linked to increased milk production and protein yield (Pečiulaitienė, Miceikienė, Mišeikienė, Krasnopiorova, & Kriauzienė, 2007).

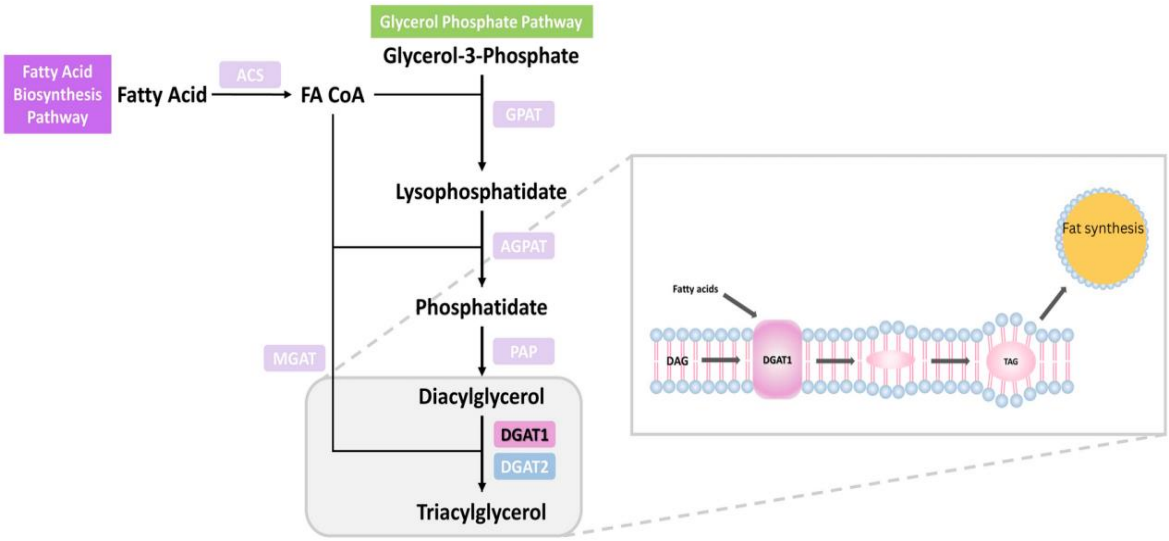

Figure 3. Role of DGAT1 in the Glycerol Phosphate Pathway, Catalyzing the Conversion of Diacylglycerol to Triacylglycerol for Fat Synthesis

5.1.2 Genomic Selection and SNP-Based Breeding

Genomic selection uses SNP markers across the genome to estimate the Genomic Estimated Breeding Value (GEBV) of an animal. This enables early selection of superior dairy cows and bulls based on their genetic potential for milk traits.

Advantages of Genomic Selection in Dairy Cattle

1. **Higher Accuracy:** More precise than traditional pedigree-based selection.
2. **Faster Genetic Gains:** Reduces the generation interval by selecting young bulls early.
3. **Improved Fat and Protein Content:** Allows selection for specific milk components (e.g., high-fat milk for cheese production).
4. **Better Adaptation to Climate & Feed Variability:** Selects cows with genes for higher efficiency and resilience.

5.1.3 Genomic Tools and Techniques for Selection

Several advanced genomic tools are used to identify the best animals for high milk yield and desirable composition.

1) **Genome-Wide Association Studies (GWAS)**

Identifies key SNPs linked to milk yield, fat percentage, and protein content.

2) **Genomic Estimated Breeding Values (GEBVs):**

Predicts an animal's genetic potential using SNP-based models.

3) **Marker-Assisted Selection (MAS):**

Uses specific DNA markers (e.g., DGAT1, GHR) to select high-performing cows.

4) **Whole Genome Sequencing (WGS):**

Helps discover novel mutations and structural variations affecting milk traits (Ponsart et al., 2014).

5.1.4 Role of Genomic Selection in Enhancing Fat and Protein Composition

Dairy industries often prioritize fat and protein content for processing efficiency and product quality.

1. Cheese-Making Efficiency

a) High casein protein content (CSN2, CSN3 genes) enhances cheese yield and texture.
b) High-fat milk improves butter and cream production.

2. Protein Variants and Consumer Demand

a) A2 milk selection (via the CSN2 gene) is increasing due to digestibility benefits.
b) High whey protein content enhances nutritional value for dairy-based supplements (Bovenhuis, Visker, & Lundén, 2013).

5.1.5 Future of Genomic Selection in Dairy Cattle

With the rapid evolution of genomic technologies, the future of dairy cattle breeding will focus on:

1) **CRISPR and Gene Editing:** Enhancing milk yield and composition through targeted modifications.
2) **Artificial Intelligence & Genomics Prediction:** AI-driven models for more accurate GEBV calculations.
3) **Climate-Resilient Dairy Breeds:** Selecting cows with superior feed efficiency and heat tolerance.
4) **Multi-Omics Approaches:** Integrating genomics, transcriptomics, and epigenetics for advanced selection (Gutierrez-Reinoso, Aponte, & Garcia-Herreros, 2021).

5.2 DNA Markers for Mastitis and Reproductive Efficiency

Mastitis and reproductive efficiency are two critical factors in dairy cattle productivity. Mastitis leads to economic losses due to reduced milk yield, increased veterinary costs, and culling rates, while reproductive inefficiencies result in extended calving intervals, reduced conception rates, and higher replacement costs. Advances in molecular genetics and DNA markers have enabled the identification of genes associated with disease resistance and fertility, improving selection for healthier and more productive dairy cattle (Pushpa et al., 2023).

5.2.1 Genetic Basis of Mastitis Resistance

Mastitis, primarily caused by bacterial infections (Staphylococcus aureus, Escherichia coli, and Streptococcus spp.), is influenced by immune response genes. Genomic selection and marker-assisted selection (MAS) allow breeders to select cows with genetic resistance to mastitis.

1. BoLA-DRB3 (Bovine Leukocyte Antigen-DRB3)

a) Plays a role in immune response and pathogen recognition.
b) Specific alleles (e.g., BoLA-DRB3.2 variants) are associated with lower somatic cell count (SCC) and reduced mastitis incidence.

2. TLR4 (Toll-Like Receptor 4)

a) Involved in pathogen recognition and activation of the immune system.
b) SNP variations in TLR4 influence susceptibility to bacterial infections.

3. LTF (Lactoferrin Gene)

a) Encodes lactoferrin, an antimicrobial protein found in milk.
b) Higher expression levels contribute to natural resistance to mastitis.

4. CXCR1 (CXC Chemokine Receptor 1)

a) Regulates immune cell migration to infection sites.
b) SNPs in CXCR1 are linked to enhanced resistance to clinical and sub-clinical mastitis.

5.2.2 DNA Markers for Reproductive Efficiency

Reproductive performance in dairy cattle depends on fertility traits such as conception rate, embryonic survival, and calving interval. Genomic selection helps identify animals with better reproductive efficiency, reducing infertility-related losses.

1. LHR (Luteinizing Hormone Receptor Gene)

a) Regulates ovarian follicular development and ovulation.
b) SNPs in LHR affect conception rates and estrous cycle regulation.

2. IGF1 (Insulin-Like Growth Factor 1)

a) Influences follicular growth, embryo survival, and pregnancy maintenance.
b) High IGF1 levels are associated with better fertility rates.FSHR (Follicle-Stimulating Hormone Receptor)
c) Control's ovarian function and follicle stimulation.
d) Variants in FSHR impact ovulation rate and reproductive success.

3. PAG (Pregnancy-Associated Glycoproteins)

a) Biomarkers for early pregnancy detection.
b) Genetic variations influence pregnancy maintenance and embryonic development.

4. STAT5A (Signal Transducer and Activator of Transcription 5A)

a) Regulates milk production and fertility.
b) Specific SNPs in STAT5A are linked to improved pregnancy rates (Safdar et al., 2024).

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Genomic Selection and Reproductive Biotechnology in Sheep and Goats

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1. Introduction

The world of livestock breeding suffers a revolutionary shift that significant in the management of small ruminants of sheep and goats (Delgado et al., 1999). Genomic selection, gene editing and reproductive biotechnology provide powerful tools to enhance genetic traits that improve disease resistance and increase complete productivity. Moreover, these innovations offer a hopeful solution to meet the increase of global demand for high-quality animal products that ensure sustainability in farming practices (Pulina et al., 2017). They have transformed traditional breeding methods to leverage a vast amount of genetic data to make precise predictions about an animal's potential. Furthermore, conventional approaches that rely on pedigree records and observable traits to genomic selection utilizes high-density molecular markers across the entire genome. Therefore, this allows for the early selection of animals with superior genetics that enable breeders to make informed decisions on improve meat quality, milk production, fertility and resilience in diseases (Pryce et al., 2023). A major breakthrough in livestock genetics is the use of gene editing that significant of CRISPR/Cas9 technology. For instance, this tool allows scientists to directly modify specific genes that lead a precise alteration that were previously impossible with conventional breeding. For example, gene editing has been used to knock out the myostatin (MSTN) gene in sheep and goats to result in enhanced muscle growth and better meat yield (Kalds et al., 2023). In addition to, other applications include to reduce allergenic proteins in milk and improve disease resistance that contribute to both economic gains and consumer health.

Moreover, reproductive biotechnology plays a crucial role to accelerate genetic improvement. Techniques of artificial insemination, embryo transfer and in vitro fertilization have been widely adopted in livestock breeding (Das et al., 2022). These methods combined with genomic selection and gene editing that enable the rapid distribution of desirable traits. For instance, gene-edited embryos implanted into surrogate mothers to ensure that improved genetics are rapidly and efficiently introduced into a population (O'Brien, 2018). The potential benefits of these advanced biotechnologies are vast. Furthermore, one of the most promising applications is enhancing disease resistance in small ruminants. Scientists target specific genes associated with susceptibility to diseases that scrapie or foot rot to create healthier livestock populations with reduced reliance on antibiotics. This not only improves animal safety and also contributes to

more sustainable farming practices. In addition to, another critical advantage is the improvement of feed efficiency and environmental adaptability (Bist et al., 2024). Sheep and goats are often raised in diverse and sometimes harsh environments that from arid regions to mountainous terrains. Therefore, genomic selection and gene editing allow breeders to select animals that thrive in these challenging conditions to ensure better productivity and minimize environmental impact (Table 1).

Table 1. Overview of Important Sheep and Goat Breeds in Pakistan

Sr.	Breed	Species	Origin (Region)	Physical Features & Characteristics	Average Daily Milk Yield
1	Beetal	Goat	Punjab	Large, golden brown/red body with white/black spots; long ears & horns	~2.2 liters
2	Daira Din Panah	Goat	Punjab, Sindh	Black, hairy coat; long ears; large head and strong body structure	~1.8 liters
3	Nachi	Goat	Punjab	Medium-sized, black with white patches; unique "dancing" gait	~1.1 liters
4	Teddy	Goat	Punjab, KPK	Small breed, fast-growing; white, brown, or black with droopy ears	~0.5 liters
5	Kamori	Goat	Sindh	Large breed; dark brown body with black patches; long ears	~1.8 liters
6	Tapri	Goat	Sindh	Hardy, high-growth potential; primarily used for meat	~3.5 liters
7	Kachhi	Goat	Sindh	Medium-sized, adapted to arid conditions; high meat production	~2.0 liters
8	Pateri	Goat	Sindh	Well-adapted to dry climates; white coat with dark markings	~2.9 liters
9	Bugi Toori	Goat	Sindh	Strong body structure; good milk and meat production	~2.0 liters
10	Jattan	Goat	Sindh	Medium-sized, known for resilience; moderate milk yield	~2.0 liters
11	Lohi	Sheep	Punjab	Large-sized; white body with tan, black, or brown head	~1.2 liters
12	Kajli	Sheep	Punjab	White fleece with black patches on the head; primarily for meat & wool	~1.3 liters
13	Kaghani	Sheep	Khyber Pakhtunkhwa (KPK)	Wool breed; white or light brown; known for high-quality fleece	~1.0 liters

In addition to these advancements, challenges still need to be addressed. Furthermore, one of the major hurdles is the relatively limited genomic resources available for sheep and goats compared to other livestock species of cattle and pigs (Jones & Wilson, 2022). The accuracy of genomic selection and gene editing remains a challenge without comprehensive reference genomes and genetic databases. They have responsibility efforts to sequence a wider variety of breeds in improve the genetic knowledge base. The significant challenge of ethical and regulatory landscape surrounding of gene editing in livestock (Zhang et al., 2020). Public perception of genetically modified organisms (GMOs) varies widely and policymakers strike a balance between scientific innovation and societal concerns. Gene editing in agriculture has gained approval in some countries that regulatory frameworks continue to evolve in impact the speed of these technologies adopted in small ruminant breeding. Research institutions and breeding organizations are actively working to overcome these barriers. They are crucial to ensure that genomic selection and gene editing applied responsibly and effectively (Tizard et al., 2016). These efforts contribute to build public trust and promote ethical applications of genomic technologies. The cost of implement these technologies is another factor to consider the beyond scientific and regulatory challenges. Therefore, it has become more affordable over time that gene editing and advanced reproductive technologies still require significant investment. Research progresses and techniques become more refined and costs expected to decrease that makes these innovations more accessible to small and medium-scale farmers (Begimkulov & Darr, 2023).

The integration of genomic selection, gene editing and reproductive biotechnology also requires a shift in traditional breeding programs. Farmers and breeders equipped with the knowledge and technical expertise to effectively utilize these tools (Rege et al., 2011). Workshops adoptive collaboration and knowledge exchange to ensure that theoretical concepts effectively translated into real-world applications. In addition to improve productivity, these technologies offer potential solutions to press global challenges that climate change and food security. The industry contributes in food security to develop livestock that survive the harsh environmental challenges (Ehrlich et al., 1993). Furthermore, reduce the environmental footprint of animal agriculture through improved efficiency aligns with global sustainability goals. The future of small ruminant breeding lies in the synergy between genetics, biotechnology and precision farming. Digital tools e.g. genomic databases, artificial intelligence, and big data analytics become increases integrated into breeding programs. These advancements enhance decision-making to allow farmers optimize their breeding strategies with unique accuracy. Researchers and industry leaders consider ethical, economic and practical aspects to ensure the effective implementation of these biotechnologies. The research continues to evolve, these technologies hold immense potential to revolutionize sheep and goat production that ensure a more efficient, sustainable and resilient livestock industry for the future (Silva et al., 2022).

2. Advancements in Genomic Selection for Improving Sheep and Goat Breeding

Traditional breeding methods for sheep and goats rely on phenotypic assessments and pedigree records to estimate breeding values (Rupp et al., 2016). These approaches have limitations that particularly for traits with low heritability or difficult to measure. Moreover, Genomic selection (GS) has revolutionized livestock breeding to incorporate high-density molecular markers across the genome that enable more precise genetic evaluations. It has transitioned from dairy cattle breeding to small ruminants to provide new opportunities for genetic enhancement in sheep and goats. A robust GS framework depends on comprehensive genomic resources. The significant progress has been made in identifying genetic variants associated with economically important traits, carcass quality, fertility and disease resistance (Davies et al., 2009). They are polygenic traits that mean influenced of numerous small-effect loci. Functional genomic data include transcriptomics and epigenomics that provide additional layers of information to refine genetic predictions to identify regulatory elements affect gene expression. Recent advancements have focused on integrate functional genomic data into GS models. For instance, genome-wide association studies (GWAS) have identified variants linked to complex traits and these findings are being incorporated into predictive models. Multi-omics approaches that combine genomics with transcriptomics and epigenetic to improve the accuracy of genomic predictions. The Farm GTEx project aims to generate tissue-specific gene expression data for sheep that further enhance the precision of genomic selection (Zhao et al., 2024).

The accuracy of genomic selection depends on several factors that include marker density, reference population size, genetic architecture of traits and heritability. High-density SNP arrays improve marker-QTL linkage disequilibrium that led to more precise genetic evaluations (Sorkheh et al., 2008). They enhance prediction accuracy to provide robust training datasets. Additionally, traits with higher heritability generally yield more reliable genomic breeding values (GBVs. Many statistical models for GS include Genomic Best Linear Unbiased Prediction (GBLUP), single-step GBLUP (ssGBLUP) and Bayesian regression approaches. GBLUP assumes equal variance across markers that Bayesian models assign variable weights to improve accuracy for traits influenced a few major loci (Misztal et al., 2021). The ssGBLUP method integrates pedigree and genomic data into a single evaluation to increase predictive power for genotyped and non-genotyped animals alike. Moreover, genomic selection is being employed to enhance key production traits in sheep that include growth rate, wool quality and reproductive performance. Studies in Norwegian White Sheep demonstrate that incorporate genomic selection into breeding programs improves selection responses that significant for maternal traits. The cost-effectiveness remains a crucial factor that genotype expenses balanced against the rate of genetic gain. Goat breeding programs are increases utilizing genomic selection to improve economically significant traits e.g. milk yield, disease resistance and fiber characteristics (Ncube et al., 2025). The development of high-density SNP chips that Illumina GoatSNP50 Bead Chip has facilitated genome-wide association studies (GWAS) and genomic evaluations. Therefore, genomic selection (GS) become an integral component of goat breeding strategies worldwide.

Cashmere production is a key economic driver for many pastoral communities. Furthermore, genomic selection has been applied to Inner Mongolia Cashmere goats to refine selection for fiber diameter and yield. They can achieve more rapid and accurate genetic gains to leverage genomic data that ultimately increase the economic value of their herds (Rexroad et al., 2019).

They require significant investment and data accuracy to effective in sheep and goat breeding. High genotype costs to limit a reference population sizes and the complex genetic architecture of certain traits pose hurdles to widespread adoption (Saleh et al., 2023). Additionally, the infrastructure for performance recording and genetic evaluation in small ruminants is less developed than in cattle that require further investment and research. The financial viability of genomic selection is a critical consideration for breeders. For instance, genomic selection accelerates genetic progress that its cost-effectiveness depends on factors to genotype expenses and trait selection intensity. Studies indicate that low-cost genotype strategies and imputation-based approaches help reduce costs to maintain selection accuracy. The future of genomic selection in sheep and goat breeding is promising, driven by advances in sequencing technologies, artificial intelligence, and bioinformatics (Adebayo et al., 2024). Machine learning techniques are being explored to refine genomic selection models and improve prediction accuracy. The technology costs decrease and data integration improves that genomic selection become more accessible and widely adopted. They offer new avenues for genetic improvement beyond genomic selection with CRISPR-Cas9 being a prominent example (Kumar et al., 2024).

In addition to, these modifications target genes linked to key traits that accelerate genetic progress in sheep and goats to combine with genomic selection strategies. The synergy between genomic selection and reproductive technologies that artificial insemination (AI) and embryo transfer (ET) enhances genetic progress (Mueller & Van Eenennaam, 2022). Breeders increase the distribution of superior genetics across populations to integrate genomic selection with advanced reproductive methods that accelerate genetic improvement. The adoption of genomic selection and gene editing raises ethical and regulatory concerns. Public acceptance, animal welfare considerations and vary international regulations influence the implementation of these technologies (Tizard et al., 2016). Transparent policies and responsible breeding practices crucial for ensure of sustainable and ethical genetic advancements. Therefore, genomic selection transforms sheep and goat breeding to improve genetic gain that refine trait accuracy and optimize breeding efficiency. Innovations in technology and research are driving improvements in genomic selection methodologies even cost and infrastructure challenges remain. The integration of functional genomics, machine learning and gene editing holds immense potential for the future of small ruminant breeding that ensure sustainable and productive livestock systems (Yousuf et al., 2024).

3. Gene Editing Technologies: Applications and Ethical Considerations in Small Ruminants

Gene editing technologies significant CRISPR-Cas9 have revolutionized genetic research and breeding in small ruminants of sheep and goats (Kalds et al., 2019). These tools enable precise modifications of the genome to allow for targeted improvements in traits of disease resistance, productivity and product quality. The efficiency and accuracy of these technologies have made them invaluable in modern animal husbandry that offer solutions to many challenges in livestock management. The significant application of gene editing in small ruminant the enhancement of muscle growth (Li et al., 2016). Researchers target the myostatin (MSTN) gene that a negative regulator of muscle development and produce animals with increased muscle mass. Studies have demonstrated that MSTN-modified goats and sheep exhibit significant higher lean meat production that led to improve meat yields (Ayuti et al., 2024). This application has the potential benefit farmers to increase productivity and profitability that ensure food security. It has been employed beyond muscle development to enhance fiber production in goats. The FGF5 gene known to regulate hair growth was targeted use CRISPR-Cas9 that led to goats with longer and more luxurious cashmere fibers. Therefore, this advancement not only boosts the economic value of these animals and also meets the growing demand for high-quality fibers in the textile industry. These genetic modifications pave the way for sustainable and efficient livestock farming practices (Gudi et al., 2024).

Disease resistance is another critical area of gene editing has shown promise. They have been used to enhance resistance to infectious diseases that scrapie and foot-and-mouth disease in sheep. They aim to develop healthier livestock populations that require fewer antibiotics and medical interventions to alter specific genes play a role in pathogen susceptibility (Aarestrup, 2015). This approach contributes to reduce the overall impact of diseases in the agricultural sector. The quality of livestock products has also been improved through gene editing. For example, milk from cows and goats naturally contains β-lactoglobulin (BLG)and a protein associated with milk allergies in infants. Researchers have developed livestock to produce milk with reduced allergenicity that knock out the BLG gene using CRISPR-Cas9 techniques (Koloskova et al., 2021). This advancement has the potential to create hypoallergenic dairy products, benefiting consumers with lactose intolerance or dairy allergies to expand market opportunities for farmers. Animal safety considerations have also been addressed through gene editing. They mitigated genetically to introduce polled (hornless) traits that reduce the pain associated with traditional dehorning in cattle and sheep. CRISPR-Cas9 has been successfully used to eliminate genes responsible for horn growth that reduce the need for dehorning procedures (Hennig, 2018). This significant application has been widely studied in cattle that its potential in small ruminants remains a promising area of research for improve in animal welfare. Ethical considerations surrounding gene editing in animals are paramount despite these advancements. They include potential environmental impacts that biodiversity imbalances and unforeseen ecological disturbances result from the release of genetically modified organisms

(Nawaz et al., 2020). Additionally, the use of certain toxic reagents in gene-editing processes raises questions about environmental sustainability. These issues necessitate thorough risk assessments and the development of mitigation strategies to prevent unintended consequences (Klinke & Renn, 2002).

Furthermore, animal welfare ethics are another significant concern. Gene editing improve traits that disease resistance and productivity to unintended off-target effects lead to health complications or deformities in animals. Moreover, the primary focus of some gene editing studies is on enhancing human benefits that inadvertently compromise animal welfare (Wright et al., 2022). It is crucial to balance technological advancements with ethical considerations to ensure that the health and natural behaviors of animals are not adversely affected. Regulatory frameworks for gene-edited animals vary global with many countries lacking clear guidelines. Industrialized nations have established policies for the responsible use and promotion of genetically modified animals that others remain cautious due to ethical and safety concerns. The European Union enforces stringent monitoring of gene editing procedures to ensure safety and public acceptance. Governments and scientific bodies play a crucial role in establish of regulatory standards for responsible gene editing worldwide (Shukla-Jones et al., 2018). Public perception and acceptance of gene-edited livestock products influenced in ethical, environmental and health considerations. Transparent communication about the benefits and risks associated with these technologies is vital to gain public trust. Consumers and stakeholders play a crucial role in discussions about gene editing that help to dispel misconceptions and foster acceptance of genetically improved livestock. Educational initiatives that provide clear evidence-based information on gene editing instrumental in address of public concerns (Garden & Winickoff, 2018).

In addition to, the economic implications of gene editing in small ruminants also noteworthy. They have high initial costs and their long-term benefits in disease resistance, productivity and product quality outweigh the expenses. Farmers stand to gain from increased efficiency that reduce medical costs and higher market value for genetically enhanced livestock (Barrows et al., 2014). Strategic investment in gene editing leads to a more sustainable and profitable agricultural sector. Moreover, gene editing also has potential applications in conservation biology. Scientists are exploring ways to use genetic modifications to preserve endangered breeds of sheep and goats. They enhance traits that improve survival rates and adaptability to change climates to play a role in maintain of genetic diversity within livestock populations (Table 2). This approach could help prevent the extinction of valuable breeds that support sustainable livestock management (Taberlet et al., 2008). They require long-term studies to fully understand the potential risks and benefits of gene editing in small ruminants. They continuously monitored and ethically reviewed to assess the impact of genetic modifications on animal health, reproduction and overall well-being. Scientists remain vigilant in address safety concerns to ensure that gene editing does not introduce unintended genetic defects or compromise the natural integrity of livestock species. In conclusion, gene editing technologies offer significant potential for advance

small ruminant breeding to enhance desirable traits that improve disease resistance and increase productivity (Liu et al., 2022). These scientific advancements balanced with ethical considerations, environmental sustainability and public acceptance. They are essential steps toward the responsible integration of gene editing in animal husbandry. Research advances, scientists continually assess the long-term effects of gene editing to ensure sustainable and ethical practices in the field.

Table 2. The Important Marker-Assisted Studies in Local Sheep and Goat Breeds of Pakistan.

Sr.	Study	Breeds Analyzed	Markers Used	Key Findings
1	Population Structure, Genetic Diversity, and Selection Signatures Within Seven Indigenous Pakistani Goat Populations	Bugti Toori, Bari, Black Tapri (variant), Kamori, Pateri, Tapri, and White Tapri (variant)	Goat 50K SNP chip	Bugi Toori goats exhibited the highest inbreeding levels, lowest diversity, and longest runs of homozygosity, highlighting the need for conservation efforts. Selection signatures were detected in genes related to coat color (*KIT*), reproduction (*BMPR1B, GNRHR*), body size (*SOCS2*), ear size (*MSRB3*), and milk composition (*ABCG2*, *CSN2*).
2	Genomic Analysis of Adaptation to Tropical Climates and Milk Production Traits in Native Goats	Beetal, Damani, Kamori	Whole-genome sequencing	Identified candidate genes linked to tropical climate adaptation (*KITLG, HSP70*) and milk production traits (*IGFBP3, LPL*). Population structure analysis revealed genetic relationships between Pakistani goats and samples from North-East Asia.
3	Molecular Investigations on the Rajanpuri Strain of Beetal Goat Breed Using mtDNA and Microsatellite Markers	Rajanpuri strain of Beetal goat	mtDNA displacement region sequencing, microsatellite markers	Genetic diversity and phylogeographic assessments of the Rajanpuri strain highlighted its unique genetic characteristics and the importance of conservation initiatives.
4	Genetic Diversity of the Endangered Kutta Sheep Breed of Valley Swat,	Kutta sheep	Microsatellite markers	The Kutta sheep breed exhibited high genetic diversity, with an average heterozygosity of 0.77. The study emphasized the importance of

	Pakistan, Based on Microsatellite Analysis			conserving this endangered breed, which has not faced a recent genetic bottleneck.
5	Microsatellite-Based Genetic Diversity and mtDNA Variation in Economically Important Goat Breeds of Pakistan	Beetal, Dera Din Panah, Teddy, Barbari, Kamori	Microsatellite markers, mtDNA D-loop sequences	This study provided insights into the genetic diversity and phylogeography of five major indigenous Pakistani goat breeds, emphasizing their economic significance and the need for conservation strategies.
6	Genetic Characterization of Balkhi and Hashtnagri Sheep Breeds of Pakistan	Balkhi, Hashtnagri	Microsatellite markers	High genetic diversity was observed in both breeds, with evidence of admixture. Findings supported breed differentiation and conservation measures.
7	Mitochondrial DNA Variation and Phylogeographic Analysis of Pakistani Goat Breeds	Beetal, Dera Din Panah, Kamori, Teddy	mtDNA D-loop sequences	Distinct haplotypes identified, indicating historical migration patterns and domestication events.
8	Whole-Genome SNP Analysis of Thalli and Kajli Sheep Breeds	Thalli, Kajli	SNP markers	Genetic distance analysis confirmed a closer relationship between these breeds, highlighting selection for meat production traits.
9	Genetic Diversity and Parentage Verification in Teddy and Barbari Goat Breeds Using Microsatellite Markers	Teddy, Barbari	Microsatellite markers	Parentage analysis confirmed high genetic diversity, with microsatellites proving useful for breeding program implementation.
10	Molecular Insights into Resistance Genes in Pakistani Goat Breeds	Beetal, Teddy, Kamori	Candidate gene analysis	Identified genetic variants associated with disease resistance, particularly in immune-related genes (*TLR2*, *TLR4*, *IL10*).

4. Reproductive Biotechnology: Artificial Insemination, Embryo Transfer, and Cloning in Sheep and Goats

Reproductive biotechnologies have revolutionized sheep and goat breeding to enable genetic improvement, disease control and the preservation of valuable traits (Yousuf et al., 2024). Artificial insemination (AI), embryo transfer (ET) and cloning are widely applied in breeding programs that offer unique benefits and facing distinct challenges. They rely on continued advancements in these technologies to enhance productivity and sustainability in small ruminant production (Salem & Smith, 2008). Moreover, artificial insemination is a widely used technique that involves collect and deposit semen into the female reproductive tract without natural mating. This method facilitates rapid genetic dissemination, disease prevention and cost-effective breeding management. Therefore, AI enables breeders to utilize superior genetics across large populations that minimize the need for extensive male herds to improve herd uniformity and complete productivity (Otoikhian et al., 2022).

The success of AI in sheep and goats depends on various factors that include semen quality, storage conditions and synchronization of estrus in females. Semen cryopreservation techniques have evolved that incorporate extenders e.g. Tris, citric acid, fructose, egg yolk and glycerol to enhance viability. Laparoscopic AI has significant improved conception rates in sheep to overcome cervical anatomical barriers that limit the efficiency of traditional AI methods (Spanner et al., 2024). Embryo transfer (ET) is a reproductive technology designed to amplify the genetic impact of superior females. It involves the superovulation of donor females, collection of embryos and subsequent transfer to synchronized recipient females. This technique accelerates genetic progress, preserves high-value genotypes and enhances reproductive efficiency in elite breeding programs. The ET process in sheep and goats struggles with its unpredictable superovulatory responses that costly hormonal treatments and **its** need for skilled personnel. The emergence of in vitro embryo production (IVEP) presents an alternative that allow embryos to develop outside the reproductive tract before implantation (Souza-Fabjan et al., 2021). IVEP not only reduces generation intervals and also maximizes the reproductive potential of genetically superior females to increase efficiency of breeding programs. Cloning, significant somatic cell nuclear transfer (SCNT) that has been explored a method to replicate genetically superior animals. The birth of Dolly the sheep in 1996 demonstrated the feasibility of cloning in mammals and paved the way for its application in livestock breeding. Therefore, cloning has potential applications in genetic conservation that the replication of high-performance individuals and the production of transgenic animals for biomedical research (Jindal & Sharma, 2010).

Moreover, cloning efficiency remains low due to challenges to implantation failures, abnormal fetal development and immunological rejection. These limitations, researchers have developed handmade cloning (HMC) that a simplified version of SCNT to reduce technical complexity and costs (Khan et al., 2018). They require further refinements in cloning methodologies and improvements in epigenetic reprogramming to enhance their practicality in commercial breeding

programs. Interspecies somatic cell nuclear transfer (iSCNT) has been investigated a tool for conserving endangered species. This approach involves transfer the nucleus of an endangered species' somatic cell into an oocyte from a closely related domesticated species that facilitate the production of embryos to implant into surrogate mothers. The iSCNT presents exciting possibilities for biodiversity conservation that its success rate remains low due to genetic incompatibilities, epigenetic abnormalities and implantation failures. They require continued research to refine its technique and enhance its efficiency that significant for the preservation of rare and endangered small ruminant species (Comizzoli & Holt, 2022). The advent of gene editing technologies of CRISPR/Cas9 has revolutionized genetic modification in sheep and goats. These tools enable precise alterations to the genome that allow the introduction of desirable traits e.g. disease resistance, enhanced productivity and improved reproductive performance.

Moreover, gene editing also holds promise for improve cloning efficiency to reduce developmental abnormalities and address reproductive disorders (Yuan et al., 2024). They hindered to ethical concerns; regulatory challenges and the high costs associated with embryo manipulation and molecular biology techniques to limit its widespread adoption. They essential for integration of gene editing into practical breeding strategies that focus on advancements in delivery methods, genome-targeting accuracy and regulatory frameworks. The significant advancements the several challenges continue to hinder the widespread adoption of reproductive biotechnologies in sheep and goats. They are influenced by semen quality, cryopreservation methods and precise estrus synchronization (Andrabi, 2009). They remain major obstacles due to variability in superovulatory responses and the high cost of hormone treatments. They restrict large-scale applications due to low efficiency, genetic abnormalities and ethical considerations. They impact the pace of gene editing adoption due to complex regulatory landscapes and public perception. Future research should focus on optimize superovulation protocols, refining in vitro fertilization techniques and developing cost-effective gene editing solutions. Integration of artificial intelligence and big data analytics into reproductive management could further enhance breeding efficiency and genetic selection. Additionally, ethical and regulatory considerations addressed through transparent policies and public engagement to facilitate the responsible application of these technologies (Li et al., 2023). Moreover, reproductive biotechnologies that include AI, ET, cloning and gene editing offer transformative potential for genetic enhancement and conservation in sheep and goats. They steadily improve the efficiency and feasibility of these technologies that despite remaining challenges. They play a crucial role in ensuring the sustainability and productivity of the sheep and goat industries for future generations through responsible implementation, regulatory support and continued innovation (Silva et al., 2022).

5. Integrating Genomic Selection and Gene Editing for Enhanced Productivity and Disease Resistance

The integration of genomic selection (GS) and gene editing represents a revolutionary approach to improve productivity and disease resistance in sheep and goats (ASLAM et al., 2024). These advanced breeding technologies enable precision in genetic enhancements to ensure superior livestock with optimized traits. They enhance breeding efficiency to leverage genetic predictions that gene editing allows for precise DNA modifications on the molecular level. These tools revolutionize small ruminant breeding programs, increasing efficiency and sustainability to address global challenges in animal agriculture (Dayoub et al., 2024).

Moreover, genomic selection a radical breeding technique that utilizes high-quantity DNA sequencing to estimate genomic estimated breeding values (GEBVs). They enable breeders to make informed selection decisions based on an animal's genetic profile on an early age and unlike traditional selection methods that rely on phenotypic and pedigree data (Flint & Woolliams, 2008). This approach is significant valuable in sheep and goats that economically important traits of meat quality, wool production, fertility and disease resistance improved more efficiently. They addressed to fully realize the benefits of genomic selection in small ruminants that include the need for robust genomic databases and the complexity of polygenic traits. Furthermore, gene editing particularly using CRISPR-Cas9 has revolutionized livestock genetics to enable precise DNA modifications (Jabbar et al., 2021). This technique allows breeders to introduce beneficial traits that remove undesirable mutations and enhance animal resilience. In sheep and goats, gene editing has been successfully applied to improve meat yield, fiber characteristics and disease resistance. A notable example is the knockout of the myostatin (MSTN) gene that leads to increased muscle mass in sheep to enhance meat production without negatively impacting overall health. They offer a powerful approach to accelerate genetic improvement in sheep and goats that combine genomic selection with gene editing (Lu et al., 2024). Furthermore, genomic selection identifies animals with superior genetic potential that gene editing enables targeted enhancements of these genetic traits. This integration allows breeders to achieve rapid genetic gains bypassing the slow generational cycles of conventional breeding. For instance, genomic selection identifies animals with high GEBVs for disease resistance that gene editing introduces or enhance resistance genes to create flocks with superior resilience to infections (Hu et al., 2020).

In addition to, one of the most significant applications of gene editing in small ruminants is the development of disease-resistant breeds. Scientists have successfully edited genes associated with resistance to major diseases of scrapie, Johne's disease, and foot rot (Nanda, 2023). Breeders, combining genomic selection to identify animals with inherent disease resistance and employ gene editing to introduce protective mutations that can enhance livestock health, improve productivity and reduce reliance on antibiotics and vaccines. This approach not only improves animal welfare and also addresses public concerns about antimicrobial resistance in livestock production. Reproductive efficiency is an important determinant of profitability in sheep and

goat farming. Moreover, gene editing has enabled targeted modifications in fertility-related genes that led to increase litter sizes and enhanced reproductive success (Hansen, 2014). For example, specific mutations in the BMP15 and GDF9 genes have been linked to higher ovulation rates in sheep and gene editing optimize these genetic factors. These interventions, combined with genomic selection allow for the selection and propagation of highly fertile breeding stock that significant to improve productivity. Sheep and goats are valuable not only for meat and also for their wool and fiber production. Moreover, genomic selection has helped identify superior animals for fiber traits and gene editing further refine these characteristics (Constable et al., 2015).

For example, researchers have successfully edited the FGF5 gene in sheep to result in longer and finer wool that meets the demand for high-quality textiles. Integration of genomic selection and gene editing ensures that desirable fiber traits are rapidly and reliably introduced into breeding populations (Constable et al., 2015). Therefore, integrating genomic selection and gene editing in sheep and goat breeding contribute to more sustainable livestock farming. Selecting animals that require fewer resources have improved feed efficiency and exhibit resilience to climate change help reduce the environmental footprint of livestock production. In addition to, gene editing also enhance traits of heat tolerance and reduced methane emissions that align with global efforts to make animal agriculture more environmentally sustainable. They face numerous challenges that including regulatory constraints, ethical concerns, technical limitations and the need for further research to optimize their application in livestock breeding (Dayoub et al., 2024). The acceptance of gene-edited animals varies to country and with some regions imposing strict regulations on genetic modifications in livestock. Ethical debates surrounding animal welfare, genetic diversity and unintended genetic consequences further complicate the adoption of these technologies. They ensure responsible implementation, transparent communication, ethical breeding practices and comprehensive regulatory frameworks essential.

Moreover, the future of integrating genomic selection and gene editing in sheep and goats looks promising and with advancements in sequencing technologies, functional genomics and bioinformatics expected to enhance their accuracy and efficiency (Husien et al., 2024). Emerging gene-editing tools of base editing and prime editing offer even more precise modifications with fewer off-target effects. They play a pivotal role in developing resilient and highly productive livestock breeds and also drive innovations in disease resistance, improved fertility and enhanced sustainability in livestock farming. A notable success story in gene editing involves the MSTN gene that regulates muscle growth. The use of CRISPR-Cas9 to knock out this gene has allowed scientists to develop sheep with increased muscle mass that led to enhanced meat yields. This case climaxes targeted genetic modifications significantly improve economically important traits in livestock to demonstrate the practical benefits of integrating GS and gene editing (Mafakheri & Kordrostami, 2020). Regulatory frameworks for gene editing in livestock vary worldwide. Some countries, such as the United States have adopted more flexible regulations that others impose strict guidelines may limit the commercial application of gene-edited animals. The global

Achieved harmonization of policies and ensure public trust in these technologies crucial for their widespread adoption in the livestock industry (Tizard et al., 2016). Therefore, gene editing in livestock raises ethical concerns related to animal welfare, genetic integrity and potential unintended consequences. The establishment of ethical guidelines and the involvement of key stakeholders including farmers, scientists, policymakers and the public in discussions about the benefits and risks associated with gene editing are essential to ensure responsible use. Transparency and education will play a crucial role in fostering public acceptance (Szabó & Tück, 2018).

6. Future Prospects and Challenges in Genetic Improvement of Sheep and Goats

The genetic improvement of sheep and goats has seen remarkable progress in recent years with genomic selection, gene editing and reproductive biotechnology leading the charge. These innovative technologies promise to enhance animal productivity, disease resistance and overall adaptability to change environments (Rege et al., 2011). These innovations bring enormous potential their widespread adoption is not without its challenges. These hurdles crucial to maximize the benefits of advancements across diverse farming systems. Moreover, genomic selection (GS) represents one of the most exciting advancements in animal breeding. High-density molecular markers, GS provides a more precise way of predicting an animal's genetic potential. They analyze the entire genome to identify superior animals early in life that rely solely on observable traits take years to express (Alvarez et al., 2015). This technique is a game-changer for improving traits of growth rate, wool quality and disease resistance in sheep and goats. The application of GS faces several barriers that particularly the need for comprehensive genomic data and robust infrastructure. In many parts of the world, access to such data is limited that make it difficult to implement GS on a large scale. Additionally, the integration of GS into traditional breeding programs requires substantial training and technical expertise that challenge for smallholder farmers (Gedil & Menkir, 2019). These obstacles that it holds great promise for speeding up genetic progress in these livestock populations. Gene editing technologies that especially CRISPR-Cas9 have opened new doors for targeted genetic modification. They allow for precise changes on the DNA level unlike traditional breeding methods that rely on selective mating to make it possible to directly modify traits associated with disease resistance, wool quality and reproductive efficiency. For instance, CRISPR has been explored to reduce susceptibility to diseases like scrapie that significantly improve herd health and productivity (Pal & Chakravarty, 2019). Ethical issues, regulatory challenges and the potential unintended consequences of gene modifications need careful consideration. The safety of gene-edited animals is still under intense scrutiny and a comprehensive regulatory framework is required to ensure that these technologies are applied responsibly. Furthermore, there is considerable debate about the long-term effects of gene editing on animal welfare and biodiversity. The research continues, addressing these concerns will be essential for broader acceptance of gene editing in livestock (Tizard et al., 2016).

Reproductive technologies, such as artificial insemination (AI) and embryo transfer (ET) are powerful tools that have already made a significant impact on the genetic improvement of sheep and goats (Yousuf et al., 2024). AI allows farmers to introduce superior genetics into their herds without the logistical challenges of moving animals that enable the spread of desirable traits of faster growth or higher disease resistance. Embryo transfer takes this one step further allow the propagation of high-value genetics that rapidly expanding the reach of elite animals. The effectiveness of these technologies is contingent on factors like semen quality that the timing of insemination and the skill of those performing the procedures. These techniques also require considerable investment in equipment and training. For small-scale farmers, the financial burden of reproductive technologies a major barrier that limit their ability to take full advantage of these innovations (Tripp, 2006). They represent a powerful way to increase reproductive efficiency and accelerate genetic improvement for larger-scale operations. They are clear in their benefits and several barriers remain in their path. One of the primary challenges is the high cost associated with these technologies. Genomic testing, gene editing and advanced reproductive techniques require significant financial investment that make them out of reach for many smallholder farmers (Ndudzo et al., 2024). Moreover, the successful application of these technologies relies on specialized knowledge and training. Farmers must be educated in managing advanced breeding techniques that a major obstacle in areas with limited access to technical resources. In addition, there is always the risk that narrowing the genetic focus to specific traits reduce overall genetic diversity that make populations more vulnerable to diseases and environmental stresses. These challenges, a holistic approach involving collaboration between researchers, governments and farmers is necessary to make these technologies accessible and sustainable for all types of farming operations (McCown, 2002).

The future of sheep and goat breeding lies in the integration of genomic selection, gene editing and reproductive biotechnology. These technologies, breeders achieve faster that more targeted genetic improvements. For example, genomic data guide gene editing decisions to ensure that changes are made in the optimal genetic background. Reproductive technologies then disseminate these improved genetics across populations efficiently that accelerate progress in desirable traits (Fleming et al., 2018). These technologies require overcome multiple obstacles. These include technical challenges in implementing and combining the technologies that financial and ethical considerations. It is also crucial to develop a regulatory framework that ensures the safe and responsible application of gene editing in livestock. Researchers, policymakers, and farmers work together to create an ecosystem that supports the responsible and equitable use of these advanced technologies. Moreover, genomic selection, gene editing and reproductive biotechnology offer tremendous potential to improve the genetic quality of sheep and goats that essential to take a balanced approach to their implementation (Lu et al., 2024). They applied in a way that considers economic, environmental and ethical factors with any technological innovation. It is crucial to ensure that smallholder farmers access these technologies and that their concerns are addressed. The integration of traditional breeding knowledge with new technologies will allow for a more inclusive and sustainable approach.

Additionally, maintaining genetic diversity within populations is key to ensuring long-term resilience against diseases and climate change. In conclusion, the future of genetic improvement in sheep and goats is incredibly promising with genomic selection, gene editing and reproductive biotechnology playing a central role (Menchaca et al., 2016). These technologies hold the potential to enhance productivity that improve disease resistance and make livestock more adaptable to climate change. They require overcoming significant challenges that include financial barriers, ethical concerns and the need for specialized knowledge and infrastructure to realize their full potential. A collaborative, multi-stakeholder approach essential to address these challenges and ensure that the benefits of these technologies are accessible to all farmers regardless of their size or location. They pave the way for more sustainable and resilient farming systems in the future with careful planning, responsible application and continued research in the genetic improvement of sheep and goats (Hotea et al., 2023).

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Role of genetics and epigenetics on growth traits in chicken

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1. Introduction

1.1 Overview of Poultry Industry and Economic Importance of Chickens

The poultry industry is one of the cornerstones of agricultural production by contributing to food security globally, economic growth, and rural development. Mostly, chickens are the most raised poultry species within these species, with regard to the majority of meat and egg production around the world. Data from the Food and Agriculture Organization (FAO) indicated that global chicken meat production surpassed 130 million metric tons in 2022, making it the most consumed meat on Earth (FAO, 2022). The reasons for this heavy consumption are the adaptation of chickens in various environments, cheaper production costs, and the highest feed conversion efficiency. So, chickens become easy and sustainable animal protein sources in developing countries where small scale poultry keeping is a vital activity in the rural economy (Mottet & Tempio, 2017) as shown in figure 1.

Figure 1. *Overview of Poultry Industry*

Chickens have great economic importance besides being a food source. Poultry provide employment through the production, processing, distribution, and retailing of poultry products. In developing countries, smallholder poultry farming constitutes a major source of income for millions of families, thus helping alleviate poverty and promote economic stability in these nations (Alders et al., 2018). In addition, the export of poultry products, especially poultry meat and eggs, contributes importantly to international trade, with Brazil, the USA, and Thailand being major players (Windhorst, 2019).

In summary, as an important part of the global agricultural economy, the poultry industry revolves around chickens. Food security, economic development and livelihoods have remained the foundation of the industry (Figure 2). The industry will continue to transform, and adapting to the growing challenges and developing opportunities will be imperative in ensuring its sustainability and resilience to global changes (FAO, 2022).

Figure. 2 *Isometric Chicken Production at Poultry Farm*

1.2 Importance of Growth Traits in Chicken Production

Growth traits are defining parameters affecting the profitability and sustainability of any chicken production system. These traits such as body weight, growth rate, and feed efficiency directly determine the broad economic feasibility of poultry farming. For instance, broiler chickens that manifest higher growth rates reach market weight quicker, thus lowering production costs and increasing turnover rates (Havenstein et al., 2003). This efficiency becomes increasingly relevant when met with the growing demand for poultry product supply around the globe due to fast population growth, urbanization, and increasing income levels (Alexandratos & Bruinsma, 2012).

Feed efficiency as another growth trait is highly relevant when the total production cost in the poultry sector is being considered (Figure 3). Chickens that convert feed into body mass more efficiently consume less feed to reach market weight, benefiting producers from reduced feed costs and lower environmental risk associated with poultry farming (Havenstein et al., 2003). This means even more when feed constitutes approximately 60% to 70% of total production costs in broiler farms (NRC, 1994). Improvement of feed efficiency enhances profitability while also ensuring the sustainability of poultry production through less resource utilization and waste generation (Leinonen et al., 2012).

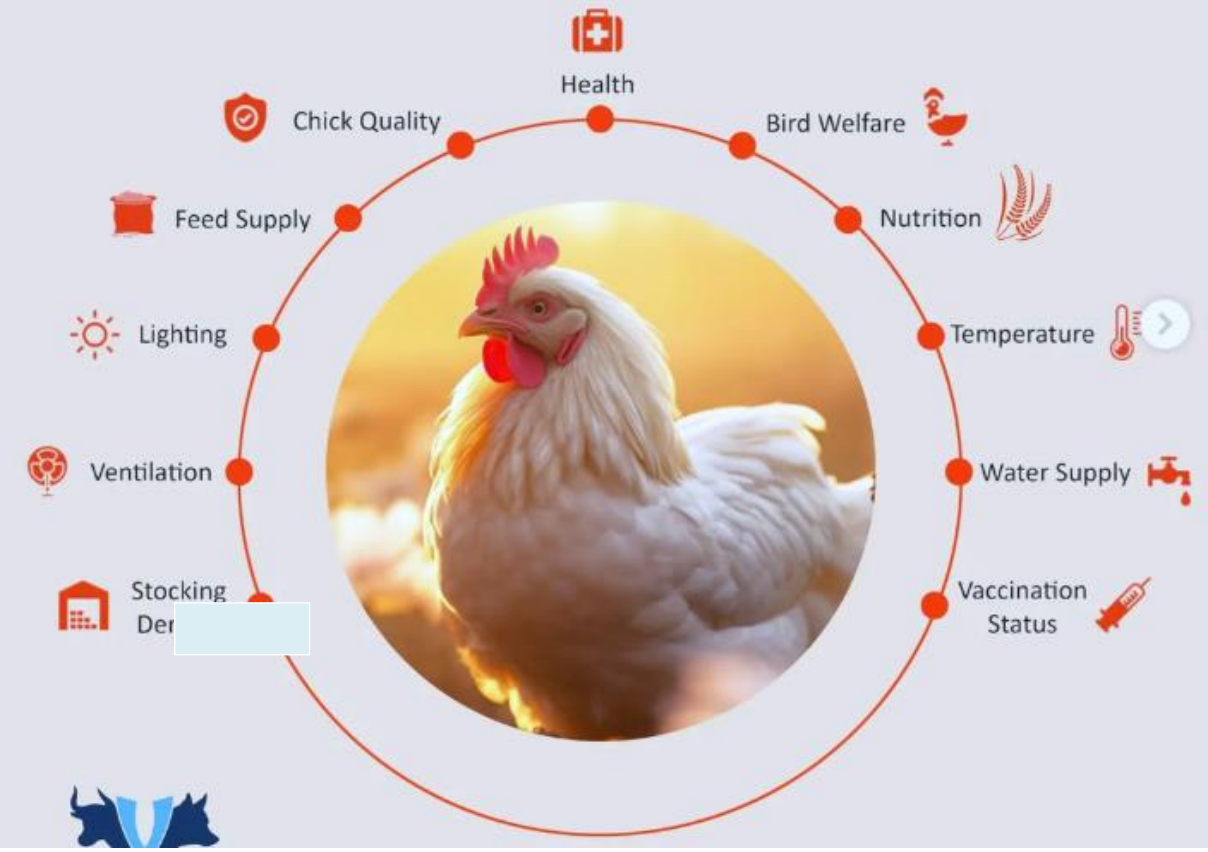

Figure.3 *Key Factors for Optimal and Healthy Growth of chicken*

The effectiveness of growth traits is not limited to their economic advantages. Improvement in animal welfare follows rapid and effective growth. It reduces the time in production systems and reduces health risks associated with long rearing durations (Dawkins & Layton, 2012). Another important factor is that growth performance must also be considered in relation to other traits such as skeletal health and immune function. This is to ensure the overall welfare of chickens. For breeding and management this would be a requirement for sustainable and ethical poultry production (Bessei, 2006).

All in all, growth traits give essentialness to chicken production because they are the condition sine qua non of economic feasibility, consumer acceptance, as well as sustainability. It is through

this that future research and innovation in this area will grant hope for dealing with future challenges and opportunities for the chicken-business sectors in the coming decades (Havenstein et al., 2003).

1.3 Role of Genetics and Epigenetics in Animal Breeding

Animal breeding has gained modern tools and insights into improving desirable traits in livestock, including chickens, from genetics and epigenetics, which have greatly changed the field. The science of heredity and variation became the backbone of traditional breeding programs long ago and has greatly improved over time. By selection of superior individuals for reproduction, successes have been achieved in improving growth, feed efficiency, and disease resistance over generations (Hill, 2014). But modern genomic technologies have today changed the whole scenario, offering tremendous precision and efficiency in the selection process.

The interplay between genetics and epigenetics gives a comprehensive understanding of the complex regulation of the growth traits of chickens. While genetics gives the blueprint for potential within an organism, epigenetics determines the manner in which such potential is achieved in response to environmental signals (Skinner, 2015). Genetic and epigenetic targeting should be exploited by breeders in developing healthier and hardy populations of chickens that can thrive under different environmental situations. Such integration in these times seems crucial with regard to climate change and other global challenges threatening the success of poultry production (Rosenfeld, 2017).

Hence, genetics and epigenetics are two avenues that open avenues to consider wrestling with previously unknown fronts in animal breeding and give novel chances for poultry production improvements. Through the synergies between traditional breeding and modern technology, the research and breeding communities will be able to address the ever-widening gaps in poultry production in a sustainable way for generations to come (Li et al., 2019).

2. Genetic Basis of Growth Traits in Chickens

Traits of growth like body weight, growth rate, and feed efficiency significantly found their economic impact in poultry production, which is controlled by an interplay of genetic factors and the environment. So, it becomes necessary to understand the genetic basis of growth traits for their selective breeding to improve poultry production. The major key genes, quantitative trait loci (QTL), and genetic markers have been unearthed by advances in genomics and molecular genetics, directly associated with growth performances in chickens. This section deals with heredity of growth traits, major candidate genes, and how genomic studies may contribute to unfolding the genetic architecture of growth in chickens.

2.1 Key Growth Traits: Body Weight, Growth Rate, and Feed Efficiency

The body-weight growth rate and feed efficiency are those principal growth traits with direct bearing on the profitability of poultry farming. Body weight is one of the major selection criteria for broiler breeding because it is directly related to meat yield (Aggrey et al., 2010). Growth rate-the increase in body weight over a period of time-affects the time required for chickens to grow to market size. Feed efficiency is defined as the ratio of gain in weight to feed consumed, which is critical to minimizing production costs (Zerehdaran et al., 2004). Some improvements in these traits have greatly enhanced poultry productivity over the last several decades.

2.2 Heritability of Growth Traits

Heritability of growth traits in chickens differs by population, environment, and conditions. The studies give moderate to high heritability estimates for body weight (0.3-0.6) and growth rate (0.2-0.5), thereby implying a very strong genetic component, as suggested by Wolc et al. (2011). Feed efficiency measure which usually uses feed conversion ratio (FCR) also exhibits moderate heritability (0.2-0.4), suggesting that selective breeding could improve such trait (Mebratie et al. 2019). The heritability knowledge allows a breeder to design an appropriate selection scheme to improve growth performance.

2.3 Major Genes Associated with Growth in Chickens

Several candidate genes have been implicated in regulating growth traits in chickens. These genes influence muscle development, metabolism and hormone signaling.

2.3.1 IGF-1 (Insulin-like Growth Factor 1)

The IGF-1 was vital in the growth and differentiation of skeletal muscles and thus also in the proliferation of cells (Ankra-Badu et al., 2010). Polymorphisms in the IGF1 gene were associated with body weight and growth rate in chickens (Zhou et al., 2005).

2.3.2 GHR (Growth Hormone Receptor)

The impact of growth hormone on growth and metabolism has been mediated through the Gene Encoding Growth Hormone Receptor. Mutations of GHR were shown to be associated with differences in growth performance and feed efficiency of broilers (Feng et al., 2007) as shown in figure 4.

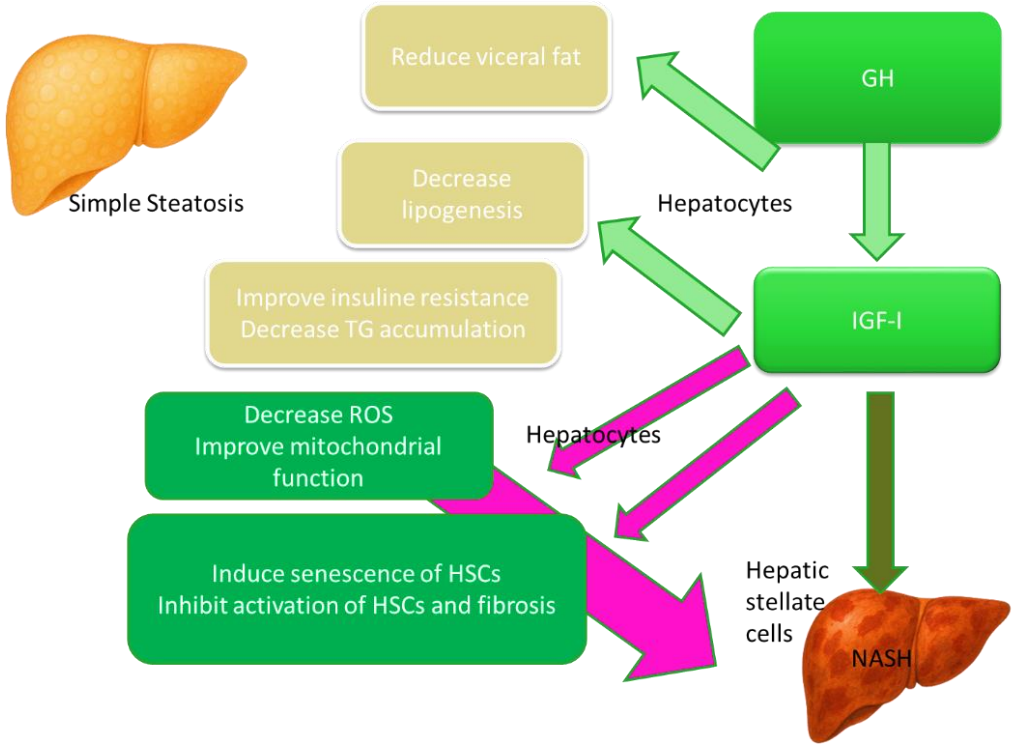

Figure. 4 *The role of GH and IGF-I in the Liver*

2.3.3 MYO1D (Myosin 1D)

MYO1D is implicated in muscle development and considered a candidate gene for body weight regulation in chickens (Gu et al., 2011). Its role in growth trait variation has been reported in genome-wide association studies (GWAS).

2.4 Quantitative Trait Loci (QTL) and Genome-Wide Association Studies (GWAS)

One of the important tools for QTL mapping and GWAS is growth traits analysis in chickens. Several QTLs for body weight and feed efficiency have been reported on chromosome 1, 4, and 27 (Abasht et al., 2009). Effective use of GWAS further fine-mapped these loci-down to single nucleotide polymorphisms (SNPs) which are associated with growth performance (Xie et al., 2012). The genomic tools would enrich marker-assisted selection in poultry breeding programs.

3. Epigenetic Regulation of Growth Traits

Epigenetic mechanisms affect gene expression and development in chickens without modifying the DNA sequence. Influencing growth and development, these mechanisms including DNA methylation, histone modification, and non-coding RNAs govern the extent to which phenotypic

variance responds to genetic and environmental cues. The emerging focus in epigenetics is immensely important to the poultry industry, specifically in elucidating the influence of epigenetic modifications on growth traits such as muscle development, metabolism, and overall body composition (Li et al., 2018).

3.1 Overview of Epigenetics: DNA Methylation, Histone Modification and Non-Coding RNAs

There are three main kinds of epigenetic regulation: one is DNA methylation; next, there is one related to histone modifications; and finally, there is associated with non-coding RNAs. DNA methylation is defined as an addition of methyl groups to cytosine residues and it has been characterized by repression of gene expression. This is particularly relevant for genomic imprinting and X-chromosome inactivation (Zhang et al., 2020). The histones undergo significant modification such as acetylation and methylation, thereby resulting in alteration in the structure of chromatin which then brings about gene activation or silencing (Wang et al., 2019). They imply that noncoding RNAs, such as microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), regulate post-transcriptionally the expression of genes by degrading some mRNAs or inhibiting their translation (Chen et al., 2021) as shown in figure 5. These three epigenetic processes regulate gene expression patterns responsible for growth-related traits in chickens.

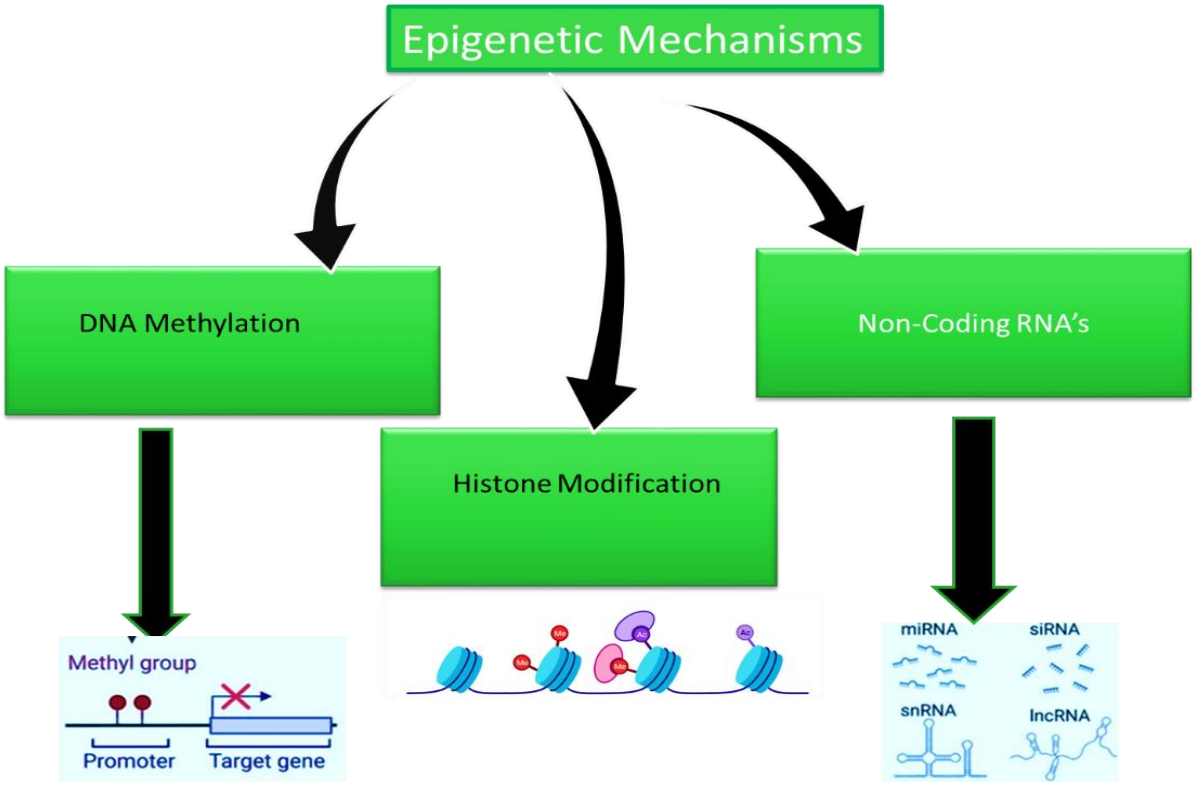

Figure. 5 *Epigenetic Mechanisms in Gene Expression*

3.2 Epigenetic Mechanisms Influencing Growth and Development

Epigenetic modifications play a significant role in development traits regulating key genes involved in myogenesis, adipogenesis, and metabolism. For example, methylation of the IGF2 (Insulin-like Growth Factor 2) gene can affect muscle development, and histone acetylation of MYOD1 (Myogenic Differentiation 1) enhances skeletal muscle growth (Nie et al., 2020). Other miRNAs that may modulate muscle proliferation and differentiation include miR-206 and miR-133a (Gu et al., 2022). Collectively, they form epigenetic patterns of phenotypic plasticity that alter and control the patterns of growth in chickens with respect to their maturation and environmental stimuli.

3.3 Environmental Factors Affecting Epigenetic Modifications

3.3.1 Nutrition and Diet

Nutrition greatly contributes to the shaping of the epigenome, especially during early development. Dietary components, including methionine, folate, and vitamins, influence DNA methylation patterns, while caloric restriction can change histone modifications (Jiang et al., 2021). For instance, maternal diet affects the growth of offspring through methylation of growth-related genes such as GH (Growth Hormone) and GHR (Growth Hormone Receptor) (Zhao et al., 2020).

3.3.2 Stress and Housing Conditions

Environmental stressors such as heat stress and packing density influence changes in epigenetic mechanisms and thereby growth performance as well. On prolonged exposure to stress, glucocorticoids promote aberrant DNA methylation in genes that regulate metabolism and immune functions (Li et al., 2019) as shown in figure 6. Moreover, sub-standard housing also alters the patterns of histone acetylation, which ultimately impacts muscle development and feed efficiency (Dong et al., 2021).

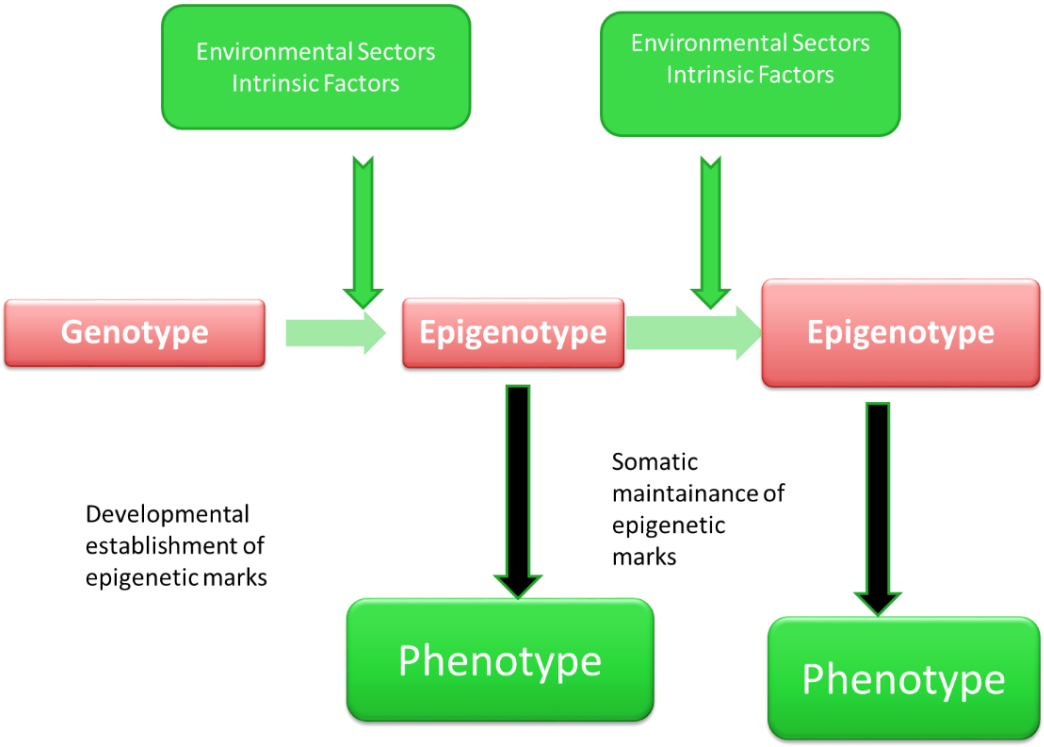

Figure. 6 *Environmental and Nutritional Effects on the Epigenetic Regulation*

3.4 Transgenerational Epigenetic Inheritance in Chickens

Moreover, epigenetic modifications can be passed on to progeny ancestry and have an implication on the growth attributes of progeny. Studies have shown that parental exposure to nutritional sources or the environment leads to heritable alterations in DNA methylation and miRNA expression in their offspring (Xing et al., 2020). For example, methylation changes in Peroxisome Proliferator-Activated Receptor Gamma (PPARγ) induced by paternal diet modify adipogenesis in chick embryos (Hu et al., 2021). Transgenerational inheritance of epigenetics is an important factor for selective breeding programs for improving the growth and productivity of poultry.

4. Interplay between Genetics and Epigenetics in Growth Traits

The growth attributes in chickens are influenced by genetic and epigenetic interplay. Genetic setup gives the basic blueprint for growth processes, while epigenetic modifications modulate gene expression in relation to environmental and developmental cues. The understanding of such interactions would help unravel the molecular basis of variation in growth and improve poultry breeding strategies. All such interactions relate to genetics and epigenetics in terms of the growth traits under study, denoting genes, epigenetic regulation of pathways for growth, and even empirical evidence from case studies.

4.1 Gene epigenome Interactions

Genetic variations such as SNPs and structural variants directly or indirectly modulate the epigenetic marks, thereby controlling gene expression involved in growth. In contrast, these epigenetic mechanisms-DNA methylation, histone modifications, and noncoding RNAs-incorporate genetic variants in determining functional outcomes. Examples include those where mQTLs were identified in chickens as genetic variants affecting methylation patterns proximally to growth-associated genes such as IGF2 and GHR (Li et al., 2019). They reveal such bidirectional relationships between genome- and epigenome-induced phenotypic outcomes.

4.2 Epigenetic Modulation of Gene Expression in Growth Pathways

It is epigenetics that intricately tune the expression of genes involved in metabolism and growth. DNA methylation can silence the transcription of growth inhibitors, while histone acetylation can elevate the expression levels of anabolic genes. According to Zhang et al. (2020), fast-growing chicken breed lines have decreased promoter methylation of the MYOSTATIN gene (a negative regulator of muscle growth), thereby permitting greater muscle mass. miR-206 and other microRNAs exhibit post-transcriptional regulation of myogenic factors, thereby exercising further epigenetic regulation over growth pathways.

4.3 Case Studies: Genetic and Epigenetic Insights into Chicken Growth

Some case studies demonstrate how the combination of genetic and epigenetic data provides an impressive insight into growth traits in chickens. For example, the combination of genome-wide association studies (GWASs) and epigenome-wide association studies (EWASs) has identified loci where the genetic variation and DNA methylation work together to influence body weight (Guo et al., 2021). In another study, breed-specific epigenetic signatures were found for differential methylation of the LEP gene, with consequences for feed efficiency and growth rates (Chen et al., 2022). These findings emphasize the strength of multi-omics approaches in poultry genetics research.

5. Future Directions and Research Opportunities

Almost excellent progress has been made in poultry genetics and epigenetics for the molecular understanding of the underlying working mechanisms of growth traits in chickens. Nevertheless, some challenges still exist, while future research needs to make good use of emerging technologies and integrative approaches to maximize breeding efficiency and improve productivity, with the greatest emphasis being on sustainability. This section, thus, discusses new trends, technological advancements, and novel strategies that are promising for the future of poultry production.

5.1 Advances in Genomic and Epigenomic Technologies

Recent advancements in genomic and epigenomic technologies - including single-cell sequencing, CRISPR-Cas9 gene editing, and whole-genome bisulfite sequencing (WGBS) - have revolutionary reliquaries poultry research by enabling accurate detection of genetic variants and epigenetic modifications associated factors such as growth, disease resistance, and feed efficiency (Smith et al., 2020). For instance, it is well-known that MSTN gene-targeted CRISPR-based genome editing improved muscle growth in chickens (Lee et al., 2021). Moreover, single-cell epigenomics can reveal methylation patterns specific to cell types, providing insights into gene regulation during development (Zhang et al., 2022). The combination of these technologies would enable the rapid finding of new biomarkers for selective breeding.

5.2 Integrating Omics Data for Precision Breeding

The bright of poultry breeding will probably be the application of combining multi-omic data approaches, i.e., genomics, transcriptomics, proteomics, and metabolomics, as precision tools for breeding strategies. Machine learning and artificial intelligence (AI) could be used to predict genetic merit and optimize breeding decisions from huge datasets (Wang et al., 2023). For example, epigenetic profiling, supplemented by genome-wide association studies (GWAS), captures phenotypic variation at the level of human DNA methylation that leads to gene expression variation concerning growth traits (Chen et al., 2021). Moreover, synergies in system biology will enhance the disintegration of complex gene networks, facilitating the selection of genotypes of predomination efficiency and resilience (Kaiser et al., 2022). Such integrated approaches will realize poultry breeding programs more accurately and efficiently.

5.3 Sustainable Poultry Production Through Genetic and Epigenetic Approaches

Being sustainable is a hot topic in modern poultry production, and genetically-epigenetic strategies are expected to contribute to these environmental-friendly practices. By employing selective breeding practices that allow for feed efficiency and a reduced environmental impact, in combination with epigenetic modulation to increase stress resilience, poultry welfare and productivity can be improved (Dunn et al., 2020). Thus, identifying epigenetic markers associated with heat tolerance can be useful to breed chickens that are more suited to climate change (Zhou et al., 2023). Another area for improvement would be using epigenetic memory to enhance disease resistance and reduce antibiotic use in a sustainable farming context (Li et al., 2022). Future research should develop eco-friendly breeding solutions in line with global food security requirements as shown in Figure 7.

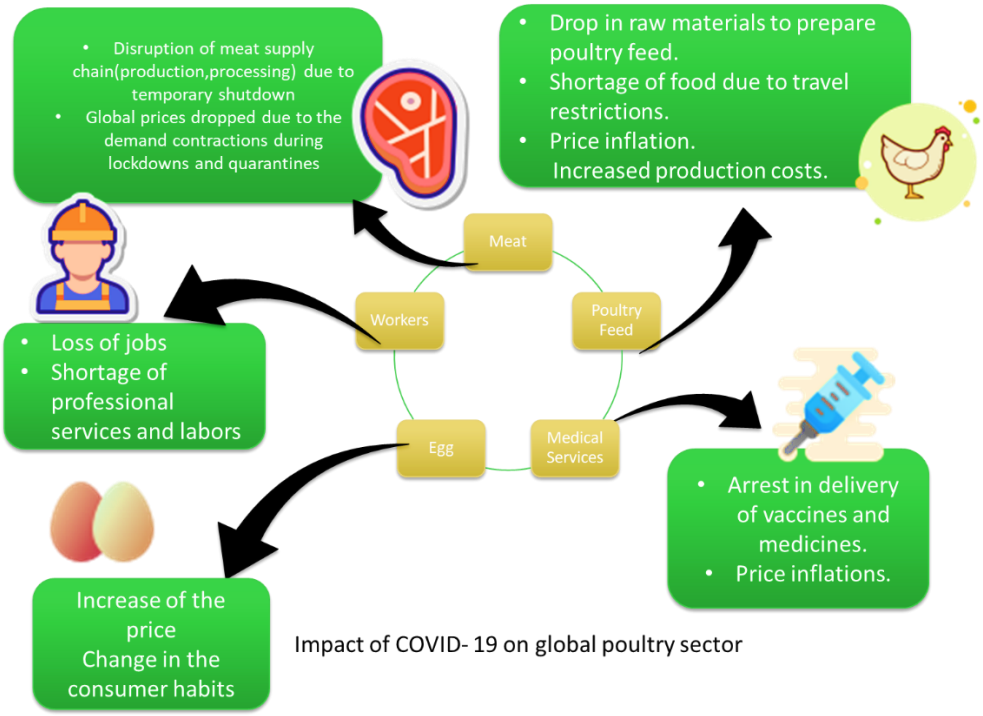

Figure 7. *Impact of the COVID-19 Pandemic on the Global Poultry Sector*

6. Conclusion

6.1 Summary of Key Findings

Research in genetics and epigenetics seems to have contributed mostly to understanding the growth traits in chickens. Notable genetic markers-the presence of polymorphisms in the IGF1 and GHR genes-are responsible for variation in growth rates and muscle development (Nie et al., 2022). Further, epigenetic mechanisms-in particular, modifications in DNA methylation patterns in genes related to growth-fine-tune these traits in response to environmental cues (Zhang et al., 2021). One example is early-life nutritional stress induction of long-lasting epigenetic changes affecting feed efficiency and body composition (Li et al., 2020).

6.2 Implications for the Poultry Industry

Highly efficient selection precision induced by the integration of genetic and epigenetic knowledge into poultry breeding programs should promote faster growth rates and feed-converting efficiency in chickens. Precision breeding strategies, such as marker-assisted selection (MAS), epigenome editing, and beyond heralds promise by optimizing growth performance while ensuring animal welfare (Biscarini et al., 2020); biomarkers for epigenetic engineering could give insight as to how chickens could respond to dietary change, stress, and disease, thus enhancing farm management practice.

6.3 Final Thoughts on the Role of Genetics and Epigenetics in Chicken Growth

The genetics-epigenetics interplay is a broad framework within which one will understand variability in growth traits in chicken. Future studies should extend to multi-omics approaches (genomics, epigenetics, transcriptomics)-i.e., unraveling dynamic interactions between the genes and their environment. Leveraging such insights would enable the poultry industry to make sustainable genetic improvements that secure food security under global challenges.

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Mechanisms of CRISPR-Cas systems in livestock

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1. Introduction

The innovations that are pursued in the development of genome editing technologies, particularly the CRISPR-Cas system, revolutionized genetic engineering in livestock. They now involve very precise alteration to an organism's genome, offering exciting new possibilities for the improvement of animal health, production, and resilience. While there are many approaches to genome editing, CRISPR-Cas has proved itself to be the most versatile and easy and cost-effective method. This chapter describes the basic mechanisms of CRISPR-Cas systems, all their tools, and their various applications for livestock improvement and biotechnology.

1.1 Overview of Genome Editing Technologies

The technique of genome editing modifies the genome of an organism with outstanding precision and has made great strides in biological research and biotechnology. Today with the rapid spread of application and versatility, the clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein 9 (CRISPR-Cas9) system stands high on the preference index of biotechnologists for its simplicity and efficiency and economic returns (Doudna et al., 2014). Essentially, CRISPRs act as an adaptive immune system for bacteria. To induce double-strand breaks (DSBs) at a specific genomic site, the CRISPR-Cas9 system relies on a guide RNA that brings along the Cas9 nuclease. The DSBs are then repaired through the cell's intrinsic repair mechanisms via either non-homologous end joining (NHEJ) or homology-directed repair (HDR) to achieve cleavage and targeted knockout, insertion, or modification of the target gene (Jinek et al., 2012).

The application of genome editing in livestock has potential consequences for productivity, disease resistance, and animal welfare. The CRISPR technique was also used to create pigs' resistant to porcine reproductive and respiratory syndrome by knocking out the CD163 gene (Whitworth et al., 2016). In another example, genetically modified cattle are to enhance heat tolerance and meat quality traits (Gao et al., 2017). Such advancements prove genome editing to be transformational on the spectrum of livestock breeding programs.

1.2 Importance of Genome Editing in Livestock Improvement

The rising population and increasing protein consumption are going to lift global demand for animal-derived products by 2050 (FAO, 2018). Traditional livestock breeding practices (Figure 1) would prove effective, but would be time-consuming and usually limit the range of genetic variation available. Through genome editing, traits associated with disease resistance and enhanced growth can be introduced straight into livestock genomes at a faster and more targeted pace than conventional methods could provide (Tait-Burkard et al., 2018).

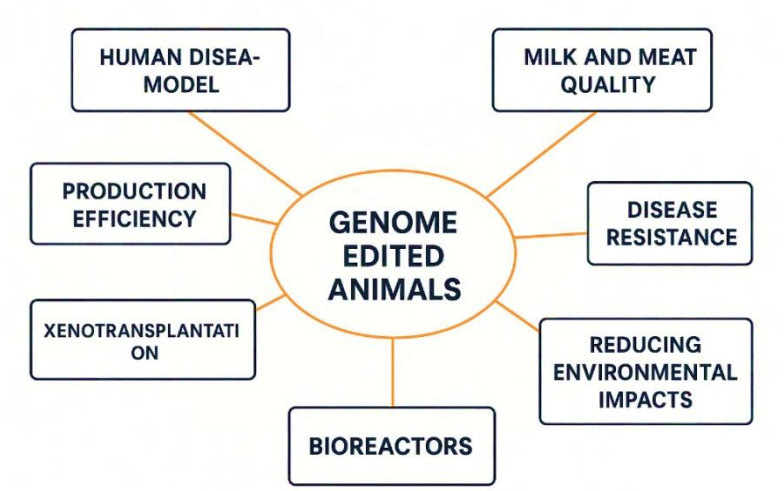

Figure. 1 *Applications of Genome Editing in Livestock*

Another essential area is animal welfare improvement through genome editing. One such example comes in the form of hornless dairy cows developed through CRISPR, thereby doing away with painful dehorning procedures (Carlson et al., 2016). Genes related to muscle growth such as MSTN (myostatin) have also been edited and livestock produced that yield leaner meat. This benefit, therefore, comes to both producers and consumers (Proudfoot et al., 2019). Genome editing can create environment-friendly opportunities beyond productivity and health. Livestock will constitute ruminants that have a great effect on greenhouse emissions since they generate huge amounts of methane gases due to their digestion processes. Gene editing strategies have been earmarked to change the gut microbiome of these cattle and therefore reduce their methane emissions (Broucek, 2018). Such innovations would really serve as beneficial ammunition in the war waged by climate change against food security.

That brings us to the conclusion that genome editing is a transformative tool for livestock improvement; it can provide solutions to the most pressing challenges in food security, animal health, and environmental sustainability. Responsible application of these technologies will be critical to realizing their full potential in the agricultural sector, as research will develop further into the future.

2. CRISPR-CASE System Mechanism and Tools

2.1 Basics of CRISPR-Cas9: How It Works

CRISPR-Cas9 is a technology that cuts specific DNA at defined genomic loci, and it is an adaptation of the bacterial adaptive immunity. The CRISPR-Cas9 system has two components: The Cas9 endonuclease and the single-guide RNA (sgRNA). The sgRNA uses base pairing to direct the Cas9 endonuclease to the DNA target, after which the Cas9 enzyme generates a double-strand break (DSB) into the sequence (as noted in Jinek et al., 2012). In response to a DSB, the cell can activate its DNA repair mechanisms like non-homologous end joining (NHEJ) or homology-directed repair (HDR), leading to knockouts and precise insertions, respectively, to either knock out or insert back genes (Figure 2).

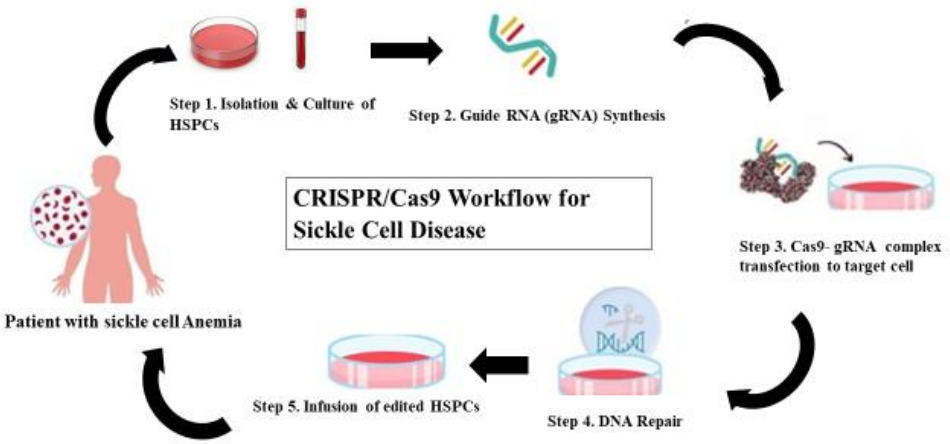

Figure. 2 *Steps in CRISPR/Cas9 Gene Editing Technology*

Due to its elegance and simplicity, CRISPR-Cas9 is recognized as a very useful genome-editing tool in livestock. For instance, CRISPR-Cas9 was used to give disease resistance to cattle (Gao et al., 2017) and improve muscle growth in pigs (Wang et al., 2015). Another plus is that gene-editing can be done on more than one gene at once, using multiplexing, hence greatly enhancing its application in the agricultural biotechnology arena.

2.2 Different CRISPR Systems (Cas9, Cas12, Base Editors, Prime Editors)

Unlike Cas9, other CRISPR-associated proteins bear these advantages. For instance, Cas12 recognizes a T-rich PAM and is capable of making staggered cuts through the DNA and is thought to promote gene editing, whereas Cas9 recognizes a G-rich PAM (Zetsche et al., 2015). Then Cas12 carries out the crRNA processing in a straightforward manner and allows multiplexing of editing events. All these combined intrinsic features make this enzyme an attractive candidate for applications in livestock, especially those dealing with the editing of AT-rich genomic regions (Figure 3).

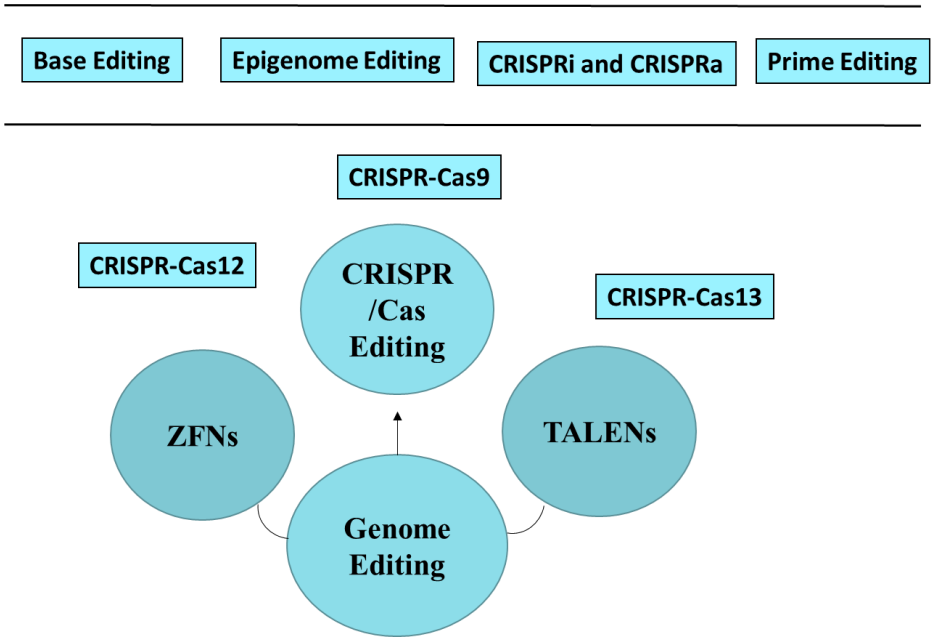

Figure. 3 *Various Aspects of GED.*

Prime editors (PE) and base editors (BE) are a new generation of CRISPR-derived tools to allow for precise changes in nucleotide without introducing double-strand breaks (DSB). Base editor tools consist of cytosine base editors (CBE) and adenine base editors (ABE), which enable conversion of C•G to T•A and A•T to G•C, respectively (Gaudelli et al., 2017). By using these precise systems, single nucleotide polymorphisms (SNPs) associated with desired traits in livestock can be deployed.

The newest is the prime editing mechanism that uses catalytically deficient Cas9; a pegRNA (Anzalone et al., 2019). This system affords very precise insertion and deletion and all 12 possible conversions from one base to another, without the need for donor DNA templates. Prime editing, with great accuracy and a low possibility for off-target events, holds promise as an invaluable tool for generating disease-resistant or high-yielding livestock. All these new developments in the CRISPR toolbox-from Cas9, Cas12, base editors, and prime editors-have indeed enabled researchers to use such very flexible strategies for livestock genome engineering. Further optimization of these systems will enhance their efficiency, specificity, and applicability in agriculture.

3. Applications of CRISPR in Livestock Breeding

3.1 Enhancing Disease Resistance in Farm Animals

Diseases of livestock bring about much economic loss and jeopardize food security worldwide. CRISPR-Cas9 has come onto the scene as a viable tool for enhancing disease resistance in farm animals by targeting genes that may be responsible for infection susceptibility. For example, in pigs, the CD163 gene was successfully disrupted by researchers with CRISPR, rendering the

pig's resistant to the Porcine Reproductive and Respiratory Syndrome Virus (a deadly pathogen in swine populations) (Whitworth et al., 2016). Similarly, genetic editing has enabled the resistance of cattle to bovine tuberculosis through modification of immune-related genes like NRAMP1 (Gao et al., 2017). These breakthroughs also show CRISPR's potential for reducing antibiotic use and enhancing animal welfare (Figure 4).

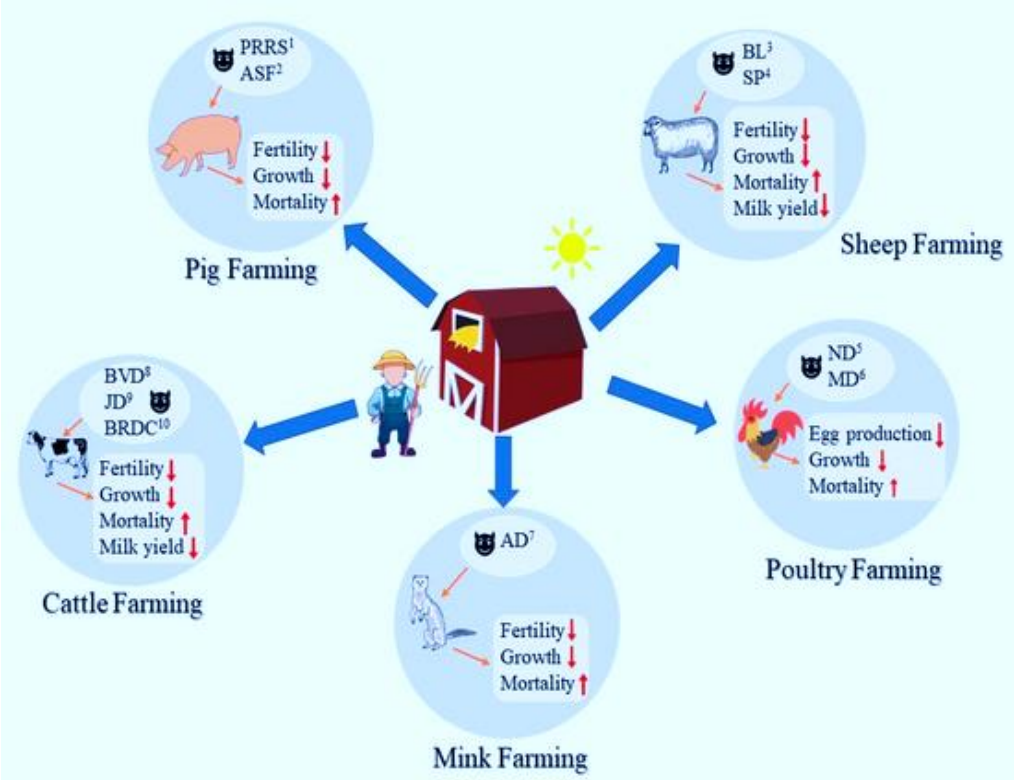

Figure. 4 *Economic Consequences of Common Diseases in Farm Animals*

Another interesting application is the production of pig's resistant to African swine fever virus (ASFV) by CRISPR-mediated editing of RELA, which plays a central role in innate immune response (Burkard et al. 2018). Poultry researchers, too, have used CRISPR to confer resistance to avian influenza by targeting the ANP32A gene critical for viral replication (Long et al. 2019). Such advances inch genome editing closer toward mitigating the adverse impacts of infectious diseases by creating healthier populations of livestock, thus promoting sustainable agriculture.

Apart from viral infections, CRISPR is also deployed to cause some sort of beneficence against parasitic maladies, such as editing involvement in immunity regulation genes related to girdle nematode infection in sheep (Smith et al., 2020). Not only this, but goat models have been gene edited to improve resistance to bacterial mastitis, an acute problem in dairy utilization, through the alteration of the LYZ gene encoding lysozyme, the antimicrobial enzyme (Zhang et al., 2021). This shows that CRISPR is relevant in developing disease-resistant livestock, resulting in decreased economic losses and increased food production efficiency.

3.2 Improving Meat, Milk and Wool Production Traits

In myriads possible, the CRISPR-Cas9 technology has taken an advantageous position in adjusting various economically important improving production traits in meats, dairy, and wool sheep. In beef cattle, muscle hypertrophy and lean meat production have targets under the MSTN (myostatin) gene editing with genes being realized that concentrate more on feed efficiency and high yield of meat in future breeds (Cyranoski, 2015). Such selections have also been proved effective in pig MUSCLE development using gene editing with reduced MSTN expression (Qian et al., 2018), thus opening another opportunity for the industry to satisfy the increase in global demand for high quality proteins. Wool production, too, had its benefits derived through CRISPR in sheep; by editing the FGF5 gene responsible for hair production, it has produced sheep with longer and thinner and finer fibers because their habitat has been improved for food materials (Wang et al., 2017). Further, metabolic genes are targeted to improve feed conversion efficiency by low production costs with high wool outputs (Li et al., 2019). Such examples show the possible optimization of livestock through CRISPR at better and economically more industrial use (Figure 5).

The integration of the CRISPR technology into livestock breeding holds great promise in tackling the global food security scenario. By imparting disease resistance and other useful production traits, genome editing has set up a pathway toward sustainable, efficient animal agriculture. Future definite research and ethical consideration are essential for fully exploiting CRISPR in transforming livestock production systems worldwide.

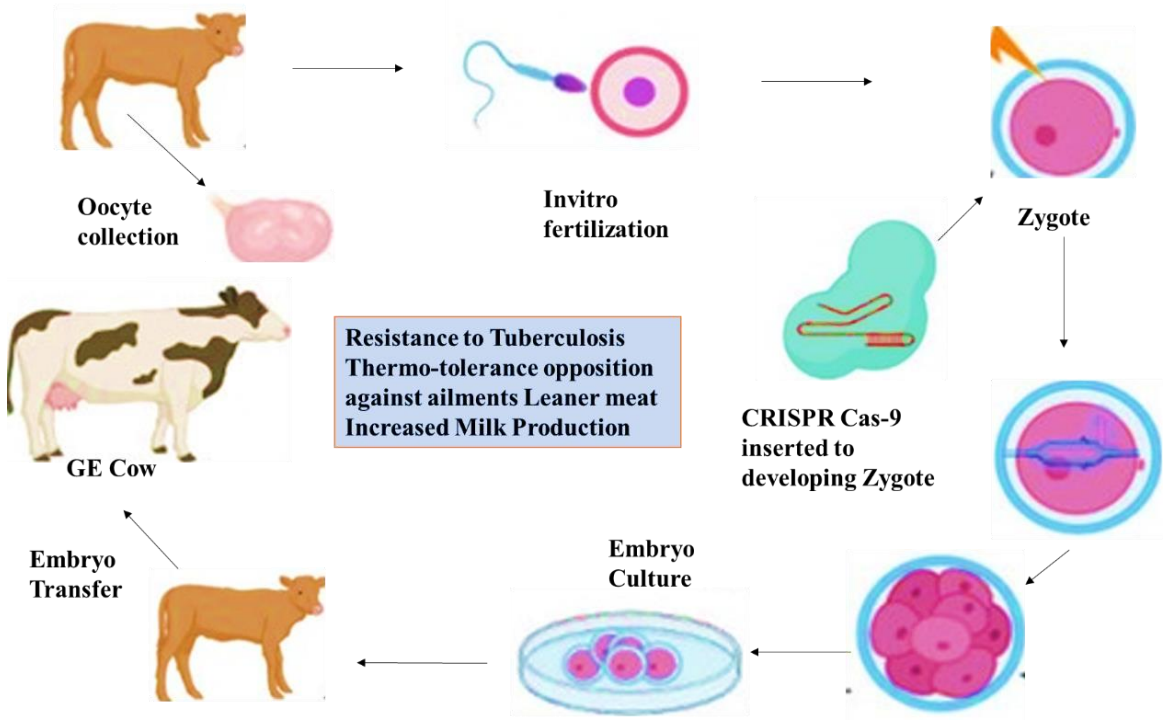

Figure. 5 *CRISPR-Cas9 in Cattle to Improve Milk Production and Meat Quality*

4. Ethical, Legal and Regulatory Considerations in Genome Edited Livestock

After a brief description of the events that gave rise to CRISPR-Cas9 and other genome-editing technologies and how they revolutionized livestock breeding by facilitating targeted genetic alterations for increased productivity, disease resistance, and animal welfare, we will discuss some important moral, legal, and regulatory challenges. These constraints need to be addressed so as to pave the way toward responsible innovation. This section will probe the ethical quandaries surrounding the genome-edited livestock and review the current global landscape of regulatory frameworks controlling CRISPR applications on animals.

4.1 Ethical Concerns in Genome-Edited Livestock

The ethical implications of utilizing CRISPR-Cas9 in livestock breeding are several and involve animal welfare, environmental, and socioeconomic aspects. While most putative unintended effects that can occur are expected to be off-target events, which could even result in health problems in the edited animals (Bishop et al. 2020). Some critics of this application argue that genetic modification can lead to unintended suffering unless very rigorous testing is taken, thereby calling for critical welfare reviews before commercialization.

Environmental impacts are also among the ethical arguments, such as the release of genome-edited animals into ecosystems. Gene drives, for instance, could disseminate alterations rapidly through wild populations and could lead to changing biodiversity (Esvelt et al. 2014). These are the ecological risks that require the same risk assessment and containment strategy for preventing uncontrolled ecological consequences. Socioeconomic equity is another very pertinent ethical concern. Genome-edited livestock will probably reflect an increased marginalization of smallholders without access to the advanced technologies promoted by industrial farm systems, which may push the developing world’s further behind in food production (Van Eenennaam, 2019). This pushes the global inequalities in food production further ahead where questions remain about biotechnological fair share of benefits.

4.2 Global Regulatory Frameworks for CRISPR-Edited Animals

Scattered and fragmented are the waters of the CRISPR-edited livestock regulatory framework, as are different ethical, legal, and economic underpinning priorities. In the United States, the FDA regulates gene-edited animals under the provisions of "New Animal Drug" that require an extensive evaluation of safety and efficacy (FDA, 2020). Thus, this creates good oversight but is said to be quite severe compared to mainstream breeding regulations. In contrast to this, Argentina has a more flexible and favorable model granting specific CRISPR-edited animals with exemption from GMO legislation if they have no foreign DNA (Whelan & Lema, 2019). Hence, the policy embraces the promotion of innovation under safe retention and could be an example in turn for other countries (Figure 6).

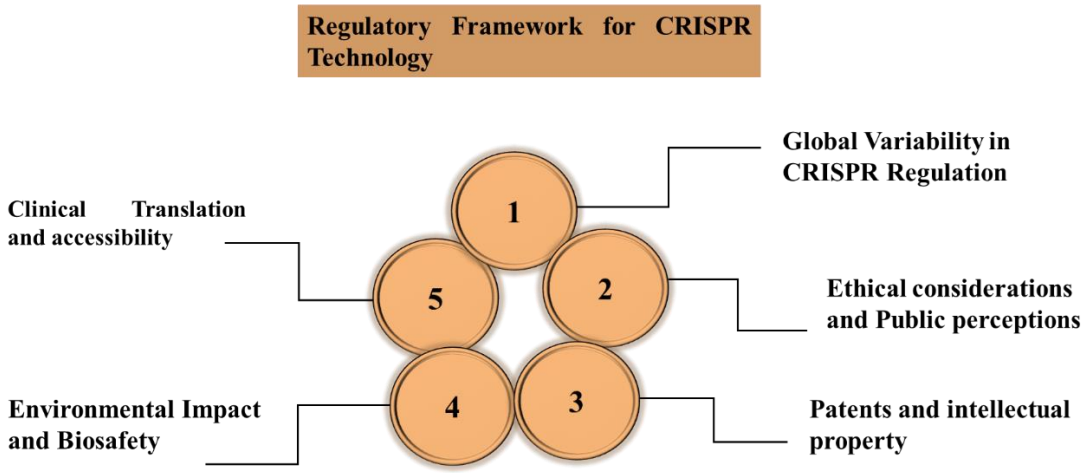

Figure. 6 *Regulatory Frameworks for CRISPR Technology*

The European Union has adopted a precautionary principle; instead of exempting genome-edited livestock from stringent regulation that applies to transgenic GMOs, they were subjected to the same strict regulations as transgenic GMOs (Court of Justice of the EU, 2018). In recent years, this has been a subject of debate; proponents argued in favor of precaution but opponents bemoaned the stifling effects on agricultural biotechnology advancements.

International organizations like the WHO and FAO take steps toward harmonization for genome-edited animals (FAO/WHO, 2022). Such an attempt has been established in safeguarding innovative research with an ethical scope in instituting international cooperation in livestock biotechnology governance.

5. Challenges and Future Perspectives in Genome Editing for Livestock

CRISPR-Cas9 and other genome editing technologies are new avenues to enhance genetic modifications with precision for cattle breeding, bolster productivity, enhance disease resistance, and guarantee animal welfare (Bhat et al., 2020). Despite the recent developments, however, some challenges need to be tackled for genome editing to be widely adopted. Such challenges comprise technical challenges, ethical dilemmas, regulatory barriers, and acceptance by the public-at-large (Tait-Burkard et al., 2018). The forerunning and compensation of these hindrances are important for the optimization of genome editing in agriculture. This chapter addresses the current limitations that exist within some specific genome-editing techniques and discusses future innovations that are expected to help in overcoming these impediments, thereby promoting sustainable and efficient livestock production.

5.1 Limitations of Current Genome Editing Techniques

Poor delivery efficacy of CRISPR-Cas9 in large animals remains a challenge to genome editing of livestock. Mosaic mutations are expressed; unlike in vitro cell cultures, some of the cells within the living organism carry the intended modification (Burkard et al., 2017). This creates a

limitation for the generation of uniformly edited animals and affects the breeding program's cost and duration. Another big concern is off-target effects, that is, unintended modifications occurring at genomic sites with sequences similar to those of the targeted sites (Zhang et al., 2021).

The regulation and ethics surrounding genome editing further deter scaling of the edited livestock. Here, social acceptance brings to play animal welfare, environmental and ecological concerns in case the edited animals enter wild populations (Van Eenennaam, 2019). A regulatory framework is being established for genome-edited animals, but varying approaches have been taken in different countries, with some equating edited livestock to GMOs while others are less rigorous with their regulations (Ishii, 2020). Such disparities complicate international trade and marketing of genome-edited livestock products (Figure 7).

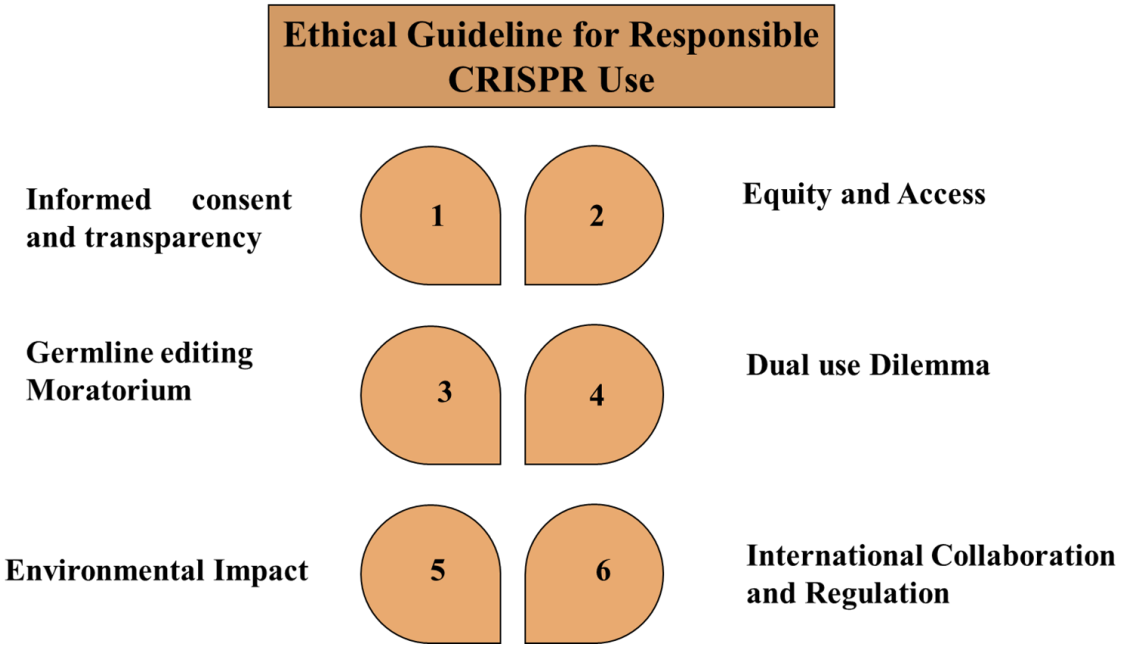

Figure. 7 *Ethical Guidelines for Responsible CRISPR Use*

5.2 Future Innovations: CRISPR and Beyond

To surmount the limitations of existing genome-editing techniques, researchers are investigating the next-generation CRISPR systems-bases and prime editing, which allow accurate modification without the induction of double-strand breaks (Anzalone et al., 2019). For example, base editors allow for subtle modifications at single nucleotide changes and do so with minimal off-target effects, making them useful for inducing small but important genetic changes in livestock. The more recent technology now allows for even greater precision by enabling small insertions, deletions, and conversions of bases, all of which are possible without HDR (Chen & Liu, 2021). These improvements hold promise for greatly enhancing the efficiency and safety of livestock genome editing.

Other than CRISPR, synthetic biology approaches such as gene drives and synthetic chromosomes can possibly revolutionize livestock breeding by facilitating large targeted genomic modifications or the introduction of completely novel biological pathways (Esvelt et al., 2014). In addition to these possibilities and in conjunction with genome editing, AI could assist in the elucidation of the best gene targets with reduced off-target effects, therefore fast-tracking the generation of superior livestock breeds that can ameliorate drought conditions and marginal lands (Libbrecht & Noble, 2015).

6. Conclusion

Genome editing and most immediately CRISPR-Cas9 technology has revolutionized livestock breeding by the precision alteration of the animal genome. These endowed researchers with the possibility of improving certain traits, developing disease resistance, and enhancing the productivity of livestock species. The future application of CRISPR techniques in agriculture carries a great promise toward addressing global challenges for food security, climate change, and zoonotic disease outbreaks. The increasing demand for sustainable and ethical livestock production would mean that CRISPR-based interventions are becoming a golden path to deviating from time-consuming and inefficient conventional breeding methods. The present chapter discusses the advances, challenges, and future opportunities for CRISPR technology in livestock farming, focusing on its application in sustainable agriculture.

6.1 Summary of Key Findings

The advent of CRISPR-Cas9 has provided a new dimension for livestock genetic enhancement. These gene-editing interventions in cattle and pigs-and the myostatin gene in particular-delete or alter certain traits, such as muscle growth, disease resistance-for example, PRRSV-resistant pigs-and feed efficiency improvement (Van Eenennaam et al., 2021). Another application of CRISPR was the generation of hornless dairy cows, thus bypassing painful dehorning (Carlson et al., 2016). Such innovations illustrate how genome-editing technology can improve animal welfare while also increasing agricultural productivity. Another exciting finding is CRISPR in the prevention of zoonotic diseases. By conferring genetic resistance to pathogens including African swine fever virus and avian influenza, genome editing may offer a method for curtailing economic losses in livestock and antibiotic use (Tait-Burkard et al., 2018). Furthermore, CRISPR-assisted development of livestock with lower environmental footprints, such as low-methane-emitting cattle, is another quantum leap in attaining climate-smart agriculture (Brouns et al., 2021). The successful interventions notwithstanding, challenges-such as off-target effects, regulatory hurdles, and public acceptance-stand in the way of bringing these technologies to the marketplace.

6.2 The Future of CRISPR in Sustainable Livestock Farming

Transparent animal breeding involves CRISPR into gene drives and synthetic biology applications for faster genetic gains in livestock farming. In this area, studies are focused on multiplex genome editing, the process of combining changes within multiple genes toward improving traits such as heat tolerance and reproductive efficiency (Bishop et al., 2020). Besides, CRISPR can facilitate the production of hypoallergenic milk and bioengineered livestock to produce pharmaceuticals: farming. Thus, biotechnology will surpass the traditional agriculture applications (Cyranoski, 2015) as shown in Figure 8.

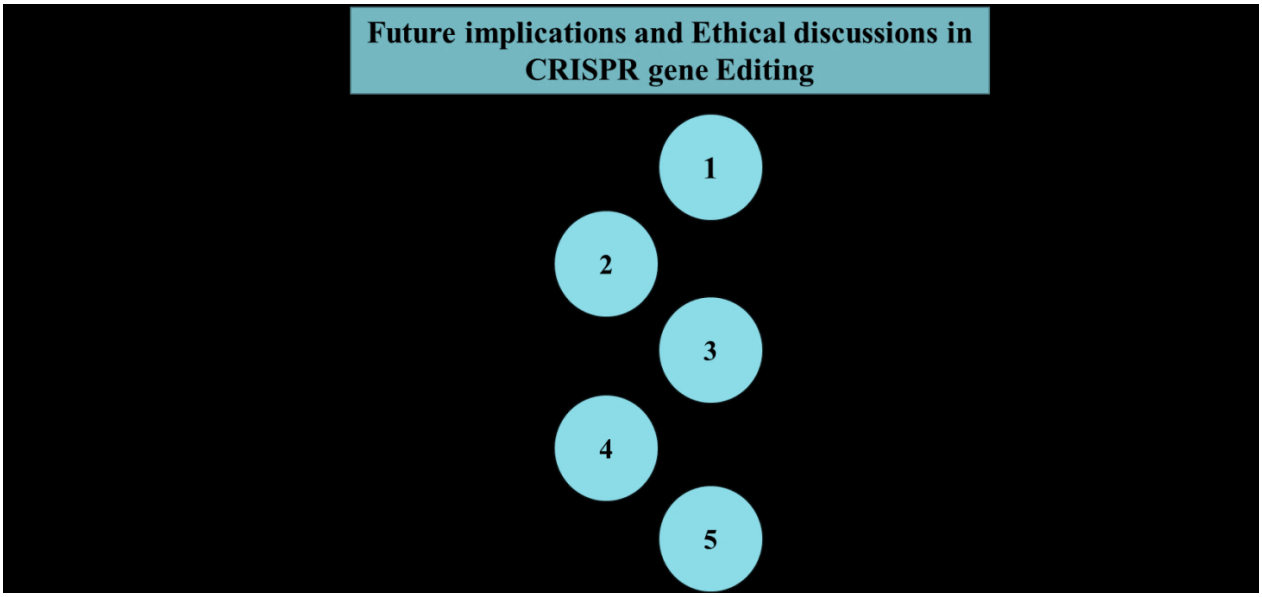

Figure. 8 *Future Implications and Ethical Discussions in CRISPR Gene Editing*

Sustainable livestock production systems can also capitalize on CRISPR innovations as they relate to global food security goals. For example, the genome editing of animals potentially answers some of the increasing calls for environmental sustainability in animal protein production by lowering animal disease susceptibility and improving resource-use efficiency (Godfray et al., 2018). However, the ethical and regulatory landscape should change so that equitable access to CRISPR technology is ensured and concerns such as biosafety and animal welfare are treated. Only with the input of scientists, policymakers, and stakeholders, joining forces across countries, will there be a brighter scenario of CRISPR enhancing resilience and sustainability of livestock systems.

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Role of epigenetics on gene expression in livestock

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Aneesa RIAZ

Zulfiqar KHURSHEED

Muhammad SAFDAR

1. Introduction

The term epigenetics refers to the study of modifications that are inherited and not made at the level of the DNA sequence but are responsible for their gene expression (Bird, 2007). These changes include DNA methylation, histone modification, and gene silencing by non-coding RNA; all of them are known to participate in the regulation of cell differentiation, development, and susceptibility to disease (Jaenisch & Bird, 2003). In comparison to genetic changes, epigenetic modifications are plastic and reversible, in which cells can modulate their gene expression as per the requirement based on environmental cues, diet, stress, and toxins (Feinberg, 2007). This therefore results in epigenetics being one of the most important mediators in this gene-environment interaction that will ultimately determine the final phenotypic response in the life of an individual.

1.1 Gene-Environment Interactions

Gene-environment interactions (GxE) describe how genetic predispositions and environmental exposures jointly influence an individual's traits and disease risk (Rutter, 2006). While more traditional genetics focused on the inherited DNA sequences, GxE courses feature the environment as a modifier of gene expression by epigenetic mechanisms inducing different health outcomes (Meaney, 2010). For example, identical twins-fairly similar genetically-acquire epigenetic differences through their life trajectory that often lead to differences in susceptibility towards certain diseases (Fraga et al., 2005) as shown in Figure 1&2.

These environmental factors can be nutrient intake, different toxins, psychological stress, and even physical activity, all of which can cause epigenetic modifications that will not wane even long after the initial exposure. It was proven that conditions during pregnancy, such as maternal diet and stress, relate to the epigenetic programming of the fetus with life-lasting impacts on the health of the offspring in their adulthood (Gluckman et al., 2008).

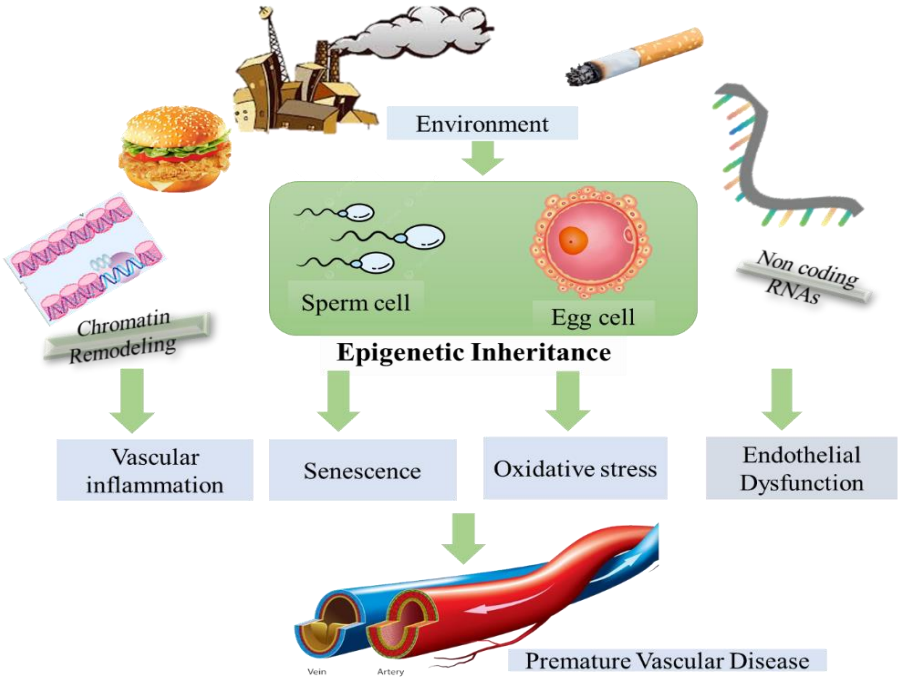

Figure. 1 *Epigenetic Changes and Environmental Factors. Exposure to Different Environmental Factors induces Several Epigenetic Modifications*

2. Mechanisms of Epigenetic Regulation

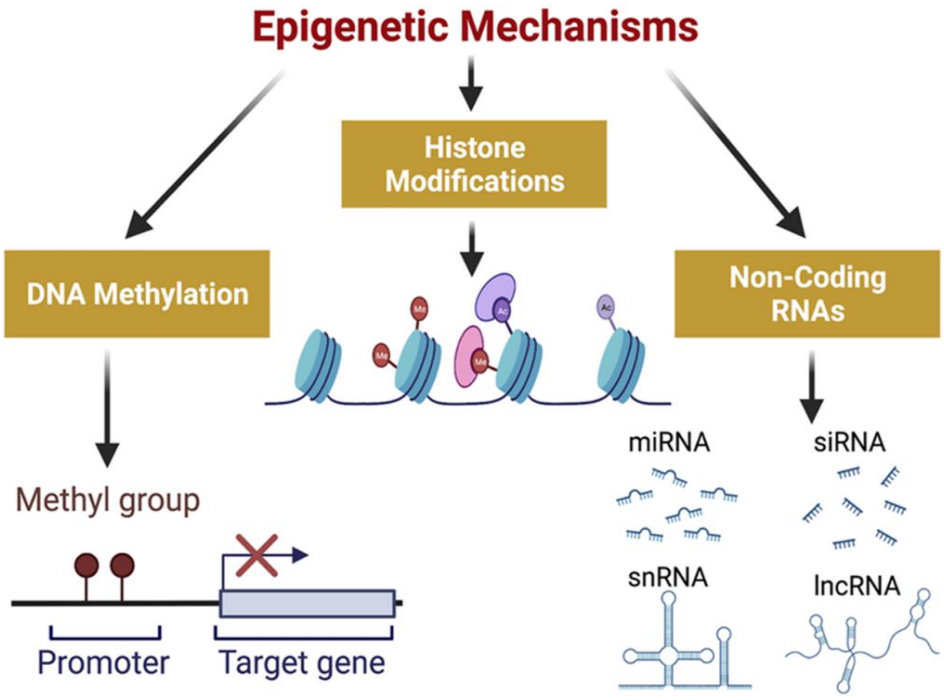

Figure. 2 *Mechanisms of Epigenetics*

2.1 DNA Methylation and Environmental Influences

DNA methylation - the modification that covalently adds a methyl group to the fifth carbon of cytosine (5-methylcytosine) - is primarily found in CpG dinucleotides where it carries out an important role in gene silencing, genomic imprinting, and X-chromosome inactivation (Jones, 2012). DNA methylation can also be affected by environmental aggressors, such as diet, toxins, and stress, thus impacting gene expression and having some consequences throughout life (Figure 3). This was highlighted in the study of Dutch Hunger Winter, which proved that maternal nutrition during gestation impacts methylation of genes that are metabolically active in offspring and also involved in stress responses (Heijmans et al., 2008).

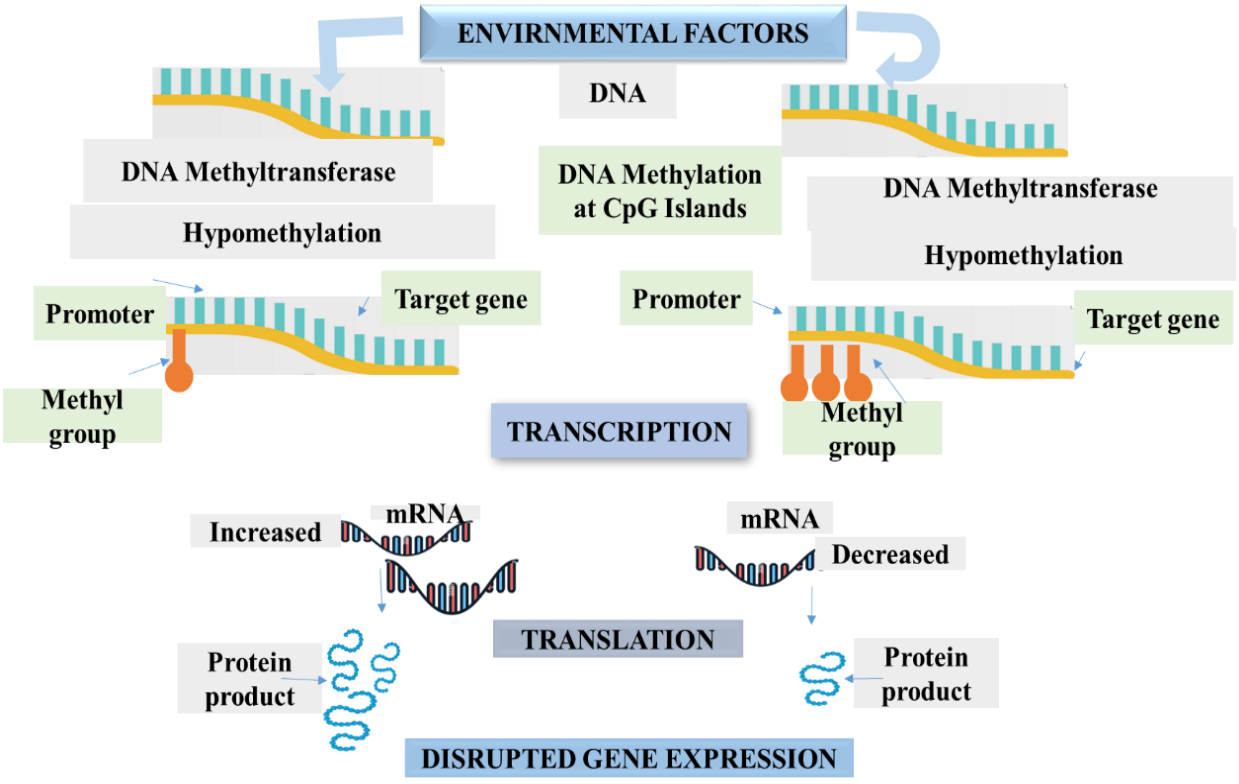

Figure. 3 *Epigenetic Modifications of DNA Methylation and its Influence on Gene Expression*

Stressful experiences in early life, including childhood trauma, have been correlated to lasting effects on DNA methylation in some stress-related genes, such as the glucocorticoid receptor (or NR3C1) and brain-derived neurotrophic factor (BDNF) (McGowan et al., 2009). Such epigenetic modifications may promote the risk of psychiatric disorders in those who experienced adversities. Moreover, lifestyle factors such as exercise and smoking change the methylation pattern of genes pertaining to aging and inflammation, showing that epigenetic marks are reversible (Zhang et al., 2015). These studies collectively emphasize the far-reaching effects of environmental factors on DNA methylation and health and disease.

2.2 Histone Modifications in Response to External Stimuli

Extensions of histones including acetylation, methylation, phosphorylation, and also ubiquitylation affect chromatin structure and gene expression changing access to transcription factors on DNA (Bannister & Kouzarides, 2011). Different environmental stimuli such as stress, diet, and chemical exposure can induce specific changes in histone modifications that switch the transcriptional activity. For example, the histone acetylation normally associated with activation of gene expression is under the opposing actions of histone acetyltransferases (HATs) and histone deacetylases (HDACs), which respond rapidly to cues from metabolism (Kurdistani et al., 2004). In fact, histone acetylation would be expected to increase with caloric restriction, especially in genes associated with longevity, thereby linking diet to epigenetic change and lengthened lifespan (Figure 4).

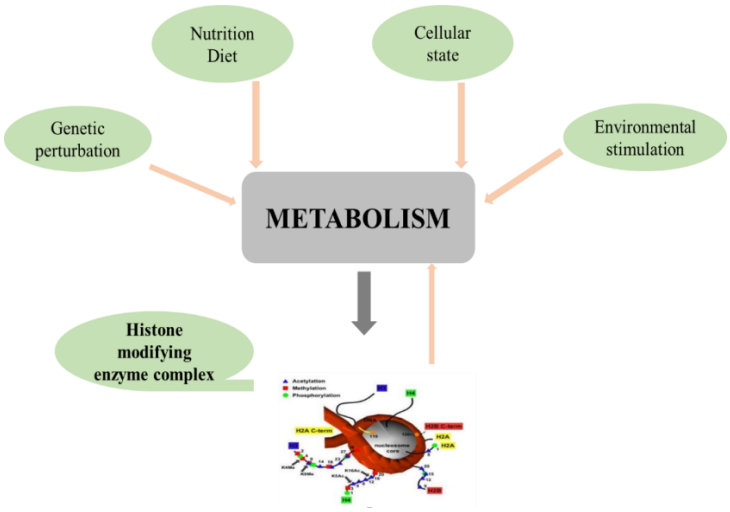

Figure. 4 *Metabolic Regulation of Histone Post-Translational Modifications*

The patterns of histone modifications may be changed by the presence of environmental toxins, consequently switching on or off gene expression. Heavy metals, including cadmium, were shown to reduce global histone acetylation, contributing to transcriptional repression and cell toxicity (Cantone et al., 2011). Air pollution may induce changes in histone methylation on pro-inflammatory genes, thereby complicating respiratory and cardiovascular diseases (Dai et al., 2016). The findings here illustrate how agents from the environment can sculpt the epigenetic landscape, thereby linking this to disease.

3. Environmental Factors Influencing Epigenetic Modifications

Epigenetic modifications refer to heritable gene expression changes occurring without alterations of the original DNA sequence. Some changes involve DNA methylation, histone modification, and non-coding RNA-mediated gene silencing, and these are important for cellular differentiation and development, disease susceptibility, and the cell's response to various environmental cues. Contemporary studies in this particular area reveal that a plethora of

environmental factors, such as diet, exposure to toxins, and physical conditions, may modify epigenetic change that, in turn, navigates into pathways of enduring health ramifications. Thus, knowing how these exogenous variables influence the shaping of the epigenome will be of pivotal importance in explaining disease mechanisms and, therefore, preventive strategies.

This chapter looks at three of the most important environmental factors that regulate the epigenetic machinery: diet and nutrition, exposure to toxins and pollutants, and physical parameters, whereby temperature and radiation are represented.

3.1 Diet and Nutritional Epigenetics

Nutrition is one of the most researched environmental factors in relation to epigenetic regulation. For example, micronutrients, such as folate, vitamin B12, and choline, as well as bioactive compounds, such as polyphenols and omega-3 fatty acids, are substrates and cofactors for epigenetic enzymes that are vital in epigenetic processes, especially at the level of DNA methylation-a mechanism in which gene expression is controlled by their presence. There are methyl donors in the process, such as folate (Figure 5). Therefore, nutrient deficiency can cause hypo hypermethylation, resulting in the loss of normal gene silencing and an increase in disease risk (Anderson et al., 2012).

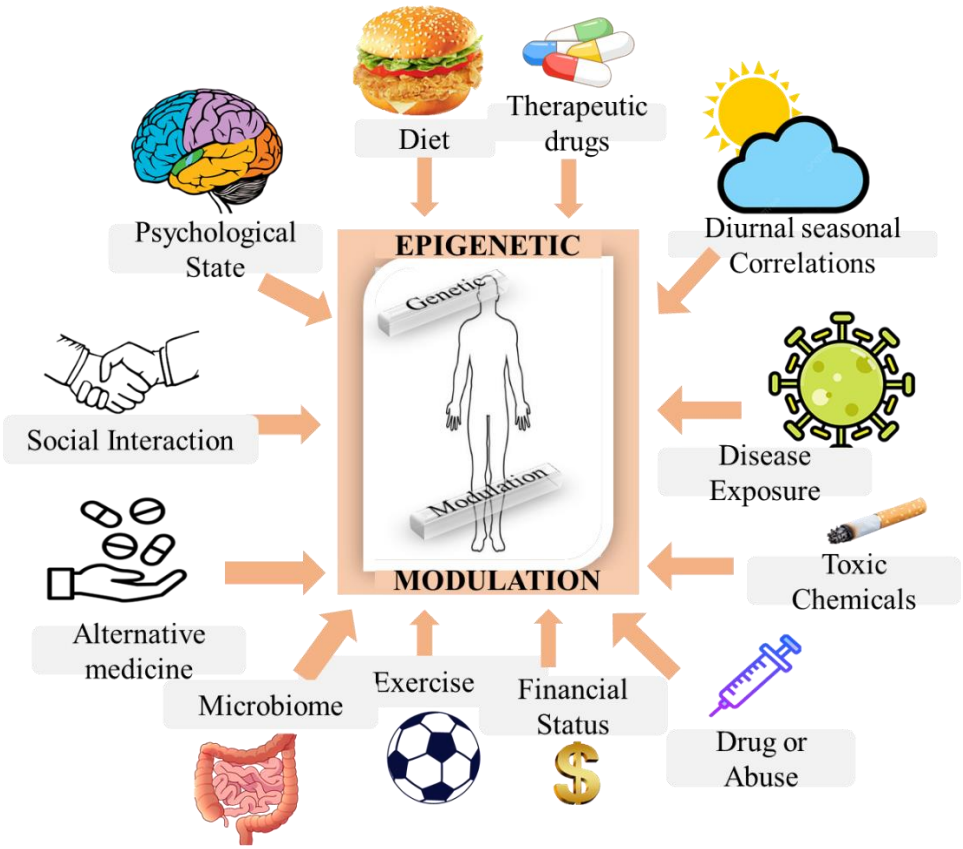

Figure. 5 *Epigenetic Modifications*

3.2 Exposure to Toxins and Pollutants

There have been toxic and environmental pollutants-having the likes of heavy metals, pesticides, and air pollutants-found to inhibit epigenetic regulation. Heavy metals among which arsenic, cadmium, and lead comprise are widely studied epigenome-altering metals. An example of them is that arsenic exposure causes global DNA hypomethylation and hypermethylation of tumor suppressor genes, which leads to cancer (Reichard & Puga, 2010).

The last pollutants that have very impressive epigenetic action are those endocrine-disrupting chemicals (EDCs), such as bisphenol A (BPA) and phthalates. For example, exposure to BPA leads to disturbed methylation of imprinted genes, which may subsequently modify growth and metabolic functions (Dolinoy et al., 2007). This shows a strong call for better environmental regulation on toxicants in curbing epigenetic risks that might arise from extensive exposure.

3.3 Physical Factors (Temperature, Radiation)

There is high probability for epigenetic influences to be caused by external environment conditions like temperature and radiation. For instance, effects of temperature stress on the alteration of DNA methylation during development in various especies have been documented. Cold stress brings about dynamic changes in DNA methylation and enables plants to adapt to the environments that are regarded as harsh (Zhang et al., 2018). In mammals, extreme temperature exposure during development is known to induce epigenetic difference in metabolic genes so that their progeny are more prone to metabolic disorders (Yan et al., 2014).

There is also emerging evidence regarding the possibly epigenetically active non-ionizing radiation, such as that from electromagnetic fields (EMFs). Although research in this area is still unfolding, some studies show long exposure to EMF affecting DNA methylation patterns in neuronal cells, raising fears regarding possible neurological consequences (Liu et al., 2015). More details are required before one could establish fully the epigenetic effects of physical environmental factors.

4. Epigenetics in Developmental and Transgenerational Effects

4.1 Early Life Environmental Exposures and Developmental Programming

The beginning stages of life-they begin when an organism is conceived and last until it becomes an infant-provide an especially vital window of developmental plasticity within which environmental exposures have lifelong implications on health and disease susceptibility. The epigenetic mechanisms which constitute non-coding RNAs, DNA methylation, and histone modifications, are pivotal mediators of developmental programming by modulating gene expression in response to environmental signals (Gluckman et al., 2008). Modifications of these kinds will have an impact on cellular function and will affect tissue formation, which in turn can

predispose individuals to metabolic, neurological, and cardiovascular disorders in later life (Barker, 2007).

Stress and psychosocial adversities in early life get epigenetic modification, especially with respect to hormones that regulate the hypothalamic-pituitary-adrenal (HPA) axis. Studies in both humans and animal models have shown that childhood trauma or maternal stress result in hypermethylation of the glucocorticoid receptor (NR3C1) gene, creating a dysregulated response to stress and vulnerability to mental disorders (McGowan et al., 2009) as shown in Figure 6. They also point to an embedding of biological memories by early life stressors through epigenetic mechanisms that will ultimately influence long-term physiological and behavioral outcomes.

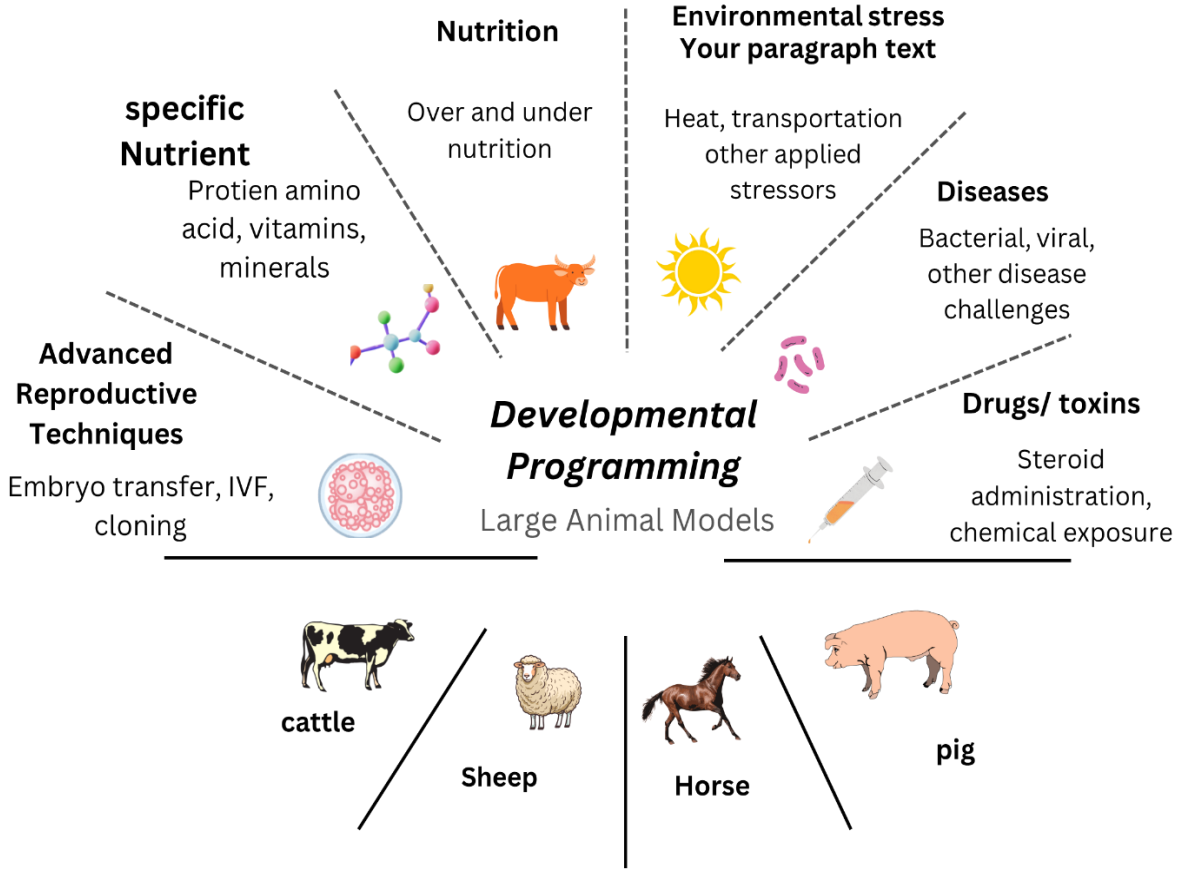

Figure. 6 *Large Animal Models of Developmental Programming*

Despite much advancement, there are still major challenges to unravel the exact manner by which early-life exposures convert into stable epigenetic changes. Longitudinally designed studies using multi-omics approaches (epigenome, transcriptome, and metabolome) will be required to study the dynamic interactions between the environment and epigenetic regulation (Tobi et al., 2018). Anti-reduction also exists about the clinical translation of epigenetic biomarkers for early disease prediction and personalized interventions.

To conclude, early-life environmental exposures have very profound and persistent effects on epigenetic regulation, operational and developmental pathways, and disease susceptibility. Understanding this mechanism will be the key to targeted research to achieve health across generations.

4.2 Transgenerational Epigenetic Inheritance

Such changes are found to be persistent through generations of organisms and termed as transgenerational epigenetic inheritance (Heard & Martienssen, 2014). Epigenetic modifications are traditionally thought of as reversible and to respond to environmental cues. This inheritance pattern challenges classical genetic paradigms by showing that acquired traits influenced by environmental factors may be passed on in progeny without any alterations in DNA sequence (Daxinger & Whitelaw, 2012).

Compelling evidence of transgenerational epigenetic effect stems from animal studies. For instance, in gestating female rats, the fungicide vinclozolin causes alterations in sperm DNA methylation that are propagated for at least three generations and renders the exposed rat offspring susceptible to kidney disease and behavior abnormalities (Anway et al., 2005). Similarly, nutritional interventions, such as protein-restricted diets in pregnant mice, were found to induce metabolic disorders in subsequent generations due to hereditary epigenetic changes (Radford et al., 2014). Indications from these studies are that environmental insults can cause stable epigenetic changes in germ cells, through which progeny inherit features of their environment.

Transgenerational epigenetic inheritance as an idea proposes a paradigm shift with major consequences for evolutionary biology, medicine, and public health. Future research should interrogate the molecular mechanisms, validating the result in the human population and seeking interventions to erase further harmful epigenetic legacies.

5. Epigenetics in Disease Susceptibility

Epigenetics refers to heritable changes that occur in gene expression and are not due to a change in the DNA sequence itself. Such modifications including DNA methylation, histone modifications, and regulation by non-coding RNAs, are fundamentally important in cell differentiation, development, and the susceptibility to diseases (Bird, 2007). More and more support is given to the view that environmental factors, including diet, toxins, or stress, might also promote epigenetic changes to various diseases, including cancer, metabolic disorders, and neurodegenerative disorders (Feinberg, 2007). Knowledge of these mechanisms will provide insight into the prevention, early diagnosis, and targeted therapies for diseases. The present chapter will examine the epigenetic contribution to disease susceptibility with an emphasis on cancer and metabolic disorders, as well as the mechanisms by which environmental exposures influence epigenetic regulation.

5.1 Cancer and Environmental Epigenetics

Genetic mutation and epigenetic change define cancer as a multifaceted disease in its etiopathogenesis. Epigenetic alterations, which could be hypermethylation of tumor suppressor genes or hypomethylation of oncogenes, have been internalized as one of the major hallmarks of many cancers (Jones & Baylin, 2007). Environmental agents such as tobacco smoke, heavy metals, and endocrine-disrupting chemicals can bring about such epigenetic changes associated with increased predisposition to cancers. For instance, one such exposure to the benzene has been linked to an alteration of DNA methylation in blood cells which is associated with the development of leukemia (Bollati et al., 2007). Diet forms an important part of cancer epigenetics. Folate deficiency entails global DNA hypomethylation (Figure 7), promoting increased genomic instability and risk toward cancer (Kim, 2005).

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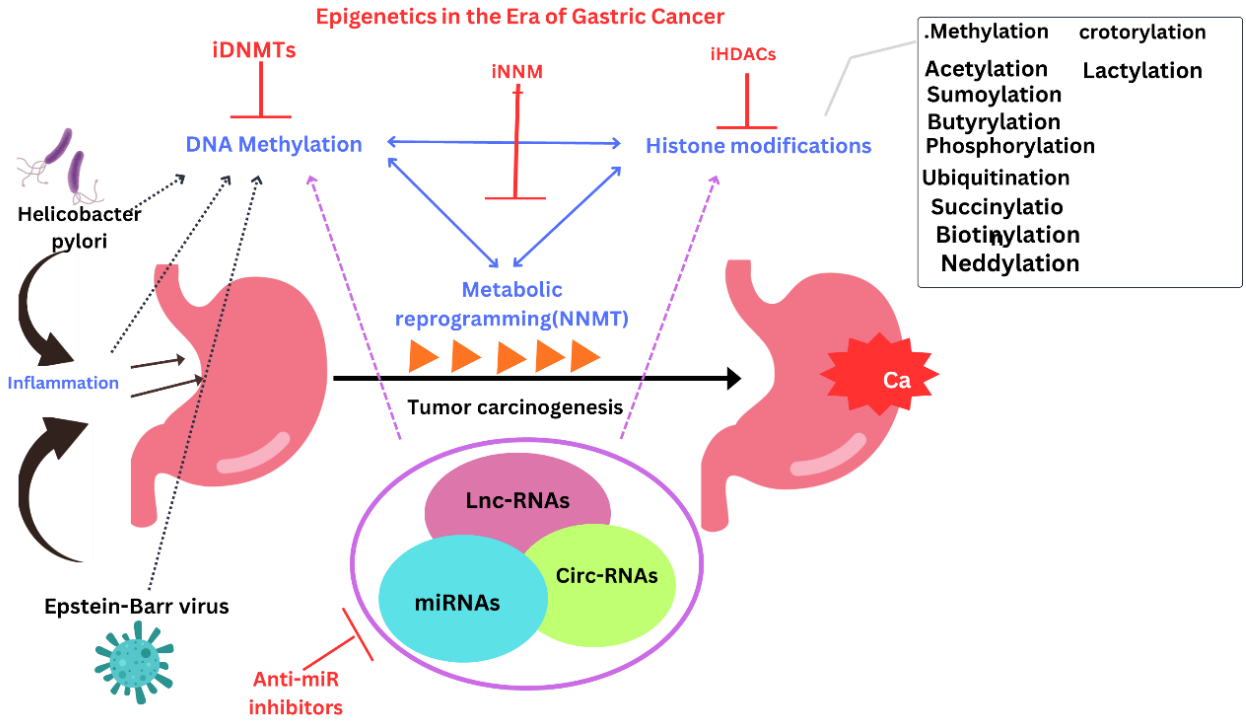

Figure. 7 *The Main Epigenetic Mechanisms and Environmental Factors That Mediate During Gastric Carcinogenesis*

Several forms of epigenetic therapy, such as DNA methyl transferase inhibitors (e.g., azacitidine) and histone deacetylase inhibitors (e.g., vorinostat), are currently being used to reverse cancer-associated epigenetic changes (Issa & Kantarjian, 2009). Nevertheless, it is difficult to achieve specificity and thereby limit off-target effects. Future research should focus on discovering new epigenetic markers relevant for the early detection of cancers and designing targeted epigenetic drugs that could improve treatment outcomes.

5.2 Metabolic Disorders (Obesity, Diabetes) and Epigenetic Changes

Both heredity and environment may be responsible for metabolic disorders like obesity and type 2 diabetes mellitus (T2D) by modifying the epigenetic control. Research proved that maternal nutrition while being pregnant could invoke epigenetic changes in adolescent offspring, increasing the risk of metabolic diseases in later life (Gluckman et al., 2008). For instance, children born to mothers who experienced famine during pregnancy show changes in DNA methylation in glucose metabolism-relevant genes (Figure 8), available to predispose them for insulin resistance (Heijmans et al., 2008).

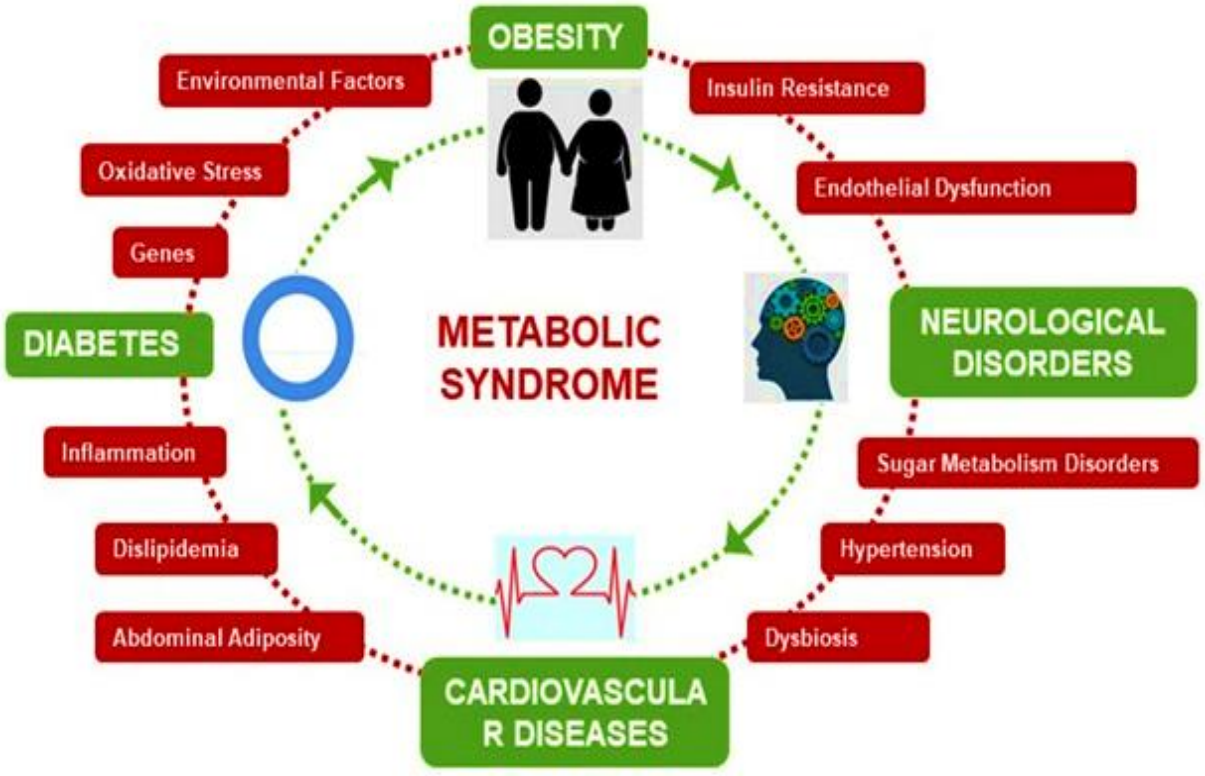

Figure. 8 *Metabolic Disorders*

These epigenetic changes due to a high-fat diet are associated with most widespread epigenetic changes and affect other adipogenesis and inflammation-related genes. At the same time, most studies indicate that the effects of perimenopausal women hyper methylation of the PPARγ gene mark important regulators of fat-cell differentiation, hence deteriorating an obese individual's metabolic function (Milagro et al., 2013). The LEP (leptin) gene also exhibits altered DNA methylation, contributing to leptin resistance characterizing obesity (Barres et al., 2012). High-fat diets and endocrine-disrupting chemicals (e.g., bisphenol A) are among environmental factors worsening the negative effects imparted by these epigenetic changes regarding metabolic dysfunction (Vandenberg et al., 2012).

6. Ethical and Societal Implications

6.1 Ethical Concerns in Epigenetic Research

New and specific kinds of ethical dilemmas in epigenetic research have arisen because of the kind of information that can be gained about an individual's environment, disease susceptibility, and even ancestral trauma. Unlike genetic data, which is unchangeable, epigenetic modifications change from time to time; hence they reflect dynamic interaction between an individual's life and the surrounding environment. Such occurrences raise privacy concerns because epigenetic profiling could be exploited by employers or insurers to discriminate among individuals based on perceived health risk (Rothstein et al., 2017). For example, if an insurance company knows that DNA methylation is related to smoking or some stressful event, it could deny coverage or employment, necessitating close legal scrutiny on the matter.

These areas of research in epigenetics also converge with those of social justice; as it has been the case, the population in marginalized communities has been a major victim of more environmental toxins that have these epigenetic changes as harmful effects. The dislocation of low-income populations and racial minorities with respect to air pollution, lead exposure, and stress-related epigenetic changes has been shown by studies (Juarez et al., 2014). Above all, the researchers need to ensure that the findings do not further stigmatize already vulnerable populations but provide further insight into policies that would serve to reduce health disparities. An ethical framework under which scientific advancement balances equity and epigenetics would ensure that there will be no beneficial advances in epigenetics that would exacerbate the already existing inequalities within or between populations.

6.2 Public Health Policies and Environmental Regulations

Integrating epigenetics with public health policies has opened incredible avenues toward the disease prevention targeted at changing or modifiable environmental risk factors. For example, the population would identify a very high risk due to environmental pollution, malnutrition, toxic chemical exposure, etc., with epigenetic biomarkers and provides early intervention possibilities (Vineis et al., 2020). The government or health agencies can use the evidence for enforcing stricter environmental regulations by reducing industry waste emission and prohibiting specific harmful pesticides that have also been suggested to be linked with the epigenetic disturbances.

Public health strategies should also address environmental exposure inequalities by focusing on vulnerable communities. Here, epigenetic research demonstrates how socioeconomic factors, such as the pollution in neighborhoods and access to healthy foods, create health inequities (Bollati & Baccarelli, 2010). Such environmental injustice-reducing policies, urban green spaces, clean water initiatives, and occupational safety can alleviate these epigenetic hazards. Moreover, public campaigns should make people aware of how lifestyle choices, smoking, and poor nutrition can cause epigenetic changes whereby future generations might be affected.

In conclusion, while epigenetics holds out great promise for public health and environmental medicine, it has to be navigated carefully through its ethical and societal implications. Strong legal protections, equitable policies and interdisciplinary collaboration are necessary to harness epigenetic knowledge, ensuring that scientific advance reaches meaningful improvement in human health.

7. Conclusion and Future Perspectives

7.1 Summary of Key Findings

The last 20 years of scientific study have confirmed environmental factors can induce epigenetic modifications that regulate gene expression (Feil & Fraga, 2012). The most studied epigenetic event is DNA methylation, whereby exposures to agents such as smoking, air pollution, and nutritional deficiency may affect health throughout life (Jirtle & Skinner, 2007). For example, during pregnancy, the maternal diet has the ability to change methylation in the offspring influencing metabolism and immune function (Waterland & Michels, 2007). Response in the form of histone modifications, including acetylation and methylation, to environmental stressors varies in a dynamic concert with chromatin structure and transcriptional activity (Bannister & Kouzarides, 2011). Non-coding RNAs, especially micro-RNAs, have long been postulated to mediate environmental effects to gene expression (Bartel, 2009). These small RNA molecules post-transcriptionally modulate gene expression, and dysregulation of their expression has been implicated in diseases, including cancer and cardiovascular diseases (Esteller, 2011). Furthermore, it has been noted that some epigenetic changes can be transmitted from generation to generation, thereby raising the question of what might be the effects of such exposure on subsequent generations (Daxinger & Whitelaw, 2012).

7.2 Unanswered Questions and Emerging Research Areas

There are major questions that need to be solved despite advances occurring. The first and probably most important is distinguishing between causal epigenetic modification and mere correlation (Jones et al., 2015). Although multiple studies have reported environment-induced epigenetic changes, there is a need for mechanistic studies to corroborate their functional effects. Another hot topic in research is that of reversibility, especially concerning therapeutic applications (Baylin & Jones, 2016). The use of epigenetic drugs, including DNA methyltransferase inhibitors, has gone a long way toward effective treatment of cancer, although their use for other diseases is still being explored (Issa & Kantarjian, 2009).

Further research should investigate personalized epigenetic interventions, where lifestyle or pharmacological strategies are individual-tailored according to the person’s epigenetic profile. Longitudinal studies documenting the dynamics of epigenetic change as it occurs over time in response to controlled manipulations will be an essential prerequisite in establishing causality and preventive and therapeutic applications (Relton & Davey Smith, 2012).

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Molecular Biotechnology and Its Pivotal Role in Advancing Animal Genomics

Muhammad TARIQ

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1. Introduction

Molecular biotechnology is important in advancing animal genomics through its ability to manipulate and study genetic materials at the molecular level. This combination of molecular biology with technological advancements continues to transform animal genetics and provides surprising benefits in agriculture and medicine in addition to conservation. Cutting-edge technology, e.g., gene editing (e.g. CRISPR-Cas9), polymerase chain reaction (PCR), next-generation sequencing (NGS) has given researchers the ability to promote animal health and productivity as well as welfare. Molecular biotechnology is used in agriculture which uses selective breeding, which enhances features such as resistance to diseases and high reproductive efficiency. In medicine, it helps to create better vaccines and cures especially to zoonotic diseases. In addition, it has been invaluable in the protection of the endangered species by conservation of genetic diversities. Still, there are some ethical issues of genetic modifications and animal welfare despite great potential. With the future evolution of these technologies, their use will play a critical role in facilitating the worldwide solutions to other issues that include food security and conservation of biodiversity, and sustainable ways of animal farming, as well as being ethically and responsibly utilized.

Molecular biotechnology is the discipline where molecular biology meets technological advances to alter the molecular parts of living objects. It encompasses the practices of genetic engineering, genomics, and gene editing to analyze and alter organisms on the molecular level (Kozielska, 2025). It has transformed a variety of fields such as agriculture, medicine, and environmental studies. In animal genomics, molecular biotechnology is central in allowing the identification, alteration, and investigation of traits and behaviors contributing to genes. The importance of molecular biotechnology in animal genomics cannot be underestimated because it has created new horizons of enhancing animals' health, productivity, and conservation (Jones & Wilson, 2022).

Animal genomics is a science dealing with analysis of the entire genetic makeup (genome) of animals. It includes the study of genomic structure, functionality, and behavior of genes, and their interaction with environmental factors. Genomics reveals how genetic variations lead to certain traits, diseases and behaviors of animals (Spangler, 2022). Examples of key advancements in the field are the completion of the initial draft genomes of different organisms, including human beings, mice, cows, and chicken, among others. The current age of genomic

discovery has enabled researchers to learn more about genetic diversity and inheritance patterns, and there has been much information gathered that is useful to medicine, agriculture, as well as conservation (Olschewsky & Hinrichs, 2021).

Modern science has given a lot of significance to animal genetics because of possibilities to solve some of the most serious challenges in many areas. Genomics has been a major aspect in advancing the quality and productivity of livestock in agriculture. Molecular biotechnology can also help selective breeding of animals that have more desirable characteristics by finding genes that have these desired traits, such as growth rate, disease resistance and reproductive efficiency (Hossein-Zadeh, 2024). It has the potential to increase the productivity and viability of animal farming, resulting in reduced environmental impact of livestock farming. Furthermore, by accommodating the increasing trend in the demand for animal-based products, genomics is eminent in ensuring that the industry can handle the global demands of food security, as well as upholding ethics and environmental practices (Dayoub et al., 2024).

Animal genomics in medicine has provided new opportunities in the development of veterinary practice as well as the development of knowledge concerning zoonotic diseases that can be transmitted between animals and humans. Molecular biotechnology can contribute to the development of stronger vaccines and treatment by determining genetic aspects of susceptibility. Such as, genetics of resistance to disease such as avian influenza, foot-and-mouth disease, or mad cow disease, can be studied using the genomics of livestock species (Monath, 2013). In addition, the value of genetically modified animals, which are created to produce drugs or make a certain protein, cannot be overestimated in terms of the development of biomedicine. As an example, transgenic animals have been induced in production of human proteins, such as insulin, growth-hormones, and antibodies and therefore have played a role in medical therapy (Shepelev et al., 2018).

The conservation of the endangered species is also one domain of molecular biotechnology on which it is important. This sequencing of the genomes of threatened species allows scientists to find genetic diversity among populations, a very essential part of management in conservation. The loss of diversity or genetic bottlenecks caught by scientists will allow them to develop more focused conservation efforts, like breeding programs which maintain genetic diversity and enhance the odds of survival to this species. Besides, genetic technologies can also be applied to check and manage the fitness of endangered species in nature, to avoid extinction and maintain sound populations (Rossetto et al., 2021).

In animal genomes, molecular biotechnology also plays a role in the ethics of animal genome modification. The issues are ethical in nature as using genomic technologies like CRISPR-Cas9, there has been the ability to have a precise editing of animal DNA. Although gene editing could repair genetic deformities and improve good qualities in animals, it has raised concerns on how far people should alter the natural genetics of animals (Ayanoğlu et al., 2020). As an example, the well-being of genetically modified animals and potential environmental impact of

introducing genetically changed life forms into the ecosystem has been raised as a concern. All these arguments on ethics point out the importance of strict regulation and consequences of the long-term effects of molecular biotechnology on animal genomics (Lorenzo et al., 2022).

Animal genomics molecular biotechnology has developed through the use of various state of the art technologies including polymerase chain reaction (PCR) and DNA sequencing, and tools to edit genes, like CRISPR-Cas9. An example is PCR, which enables researchers to amplify parts of the DNA of their choice so that they can carry an analysis to identify certain genetic markers that can be attributed to specific characteristics. Over the years, DNA sequencing technologies have drastically advanced and thus facilitating an easy and quick sequencing of the entire genomes. In addition, the introduction of next-generation sequencing (NGS) has also increased the speed of research in genomics, as more than one genome can be sequenced at single time hence finding the variations in the large animal numbers is easy (Mushtaq et al., 2021). CRISPR-Cas9 has proved to be a breakthrough in this respect since it enables editing of genes of high specificity, which can be applied to correct genetic alteration or genetically enrich animals with desirable traits. Not only are these developments in molecular biotechnology changing our comprehension of the nature of animal genomes but these implications are vast in the advancement of animal health, agriculture and conservation (Khalil, 2020).

The ability to advance complex biological questions and practical problems has been enabled by the incorporation of molecular biotechnology into animal genomics. Considering the example of identifying the genetic causes of animal diseases, it is possible to more accurately diagnose and develop individual ways of treating pets, farm animals, and rare species. In animal agriculture, molecular biotechnology allows the selection of genetic markers determining traits such as milk production, resistance to diseases, and growth rates, and therefore, makes breeding programs more efficient and selective. Consequently, it enables farmers and breeders to breed animals with enhanced traits and thus resulting in better animal welfare and productivity (Domrazek & Jurka, 2024).

Molecular biotechnology has revolutionized the study of animal genomics, giving strong tools to comprehend, modify, and enhance animal genetics. Molecular biotechnology has become central in the future of animal science in terms of improving agricultural methods, medical science, protecting rare and endangered animal species and so on (Ghildiyal et al., 2023). The further evolution and use of these technologies can provide an opportunity to overcome pressing global problems, including food safety, animal health, and biodiversity conservation (Figure 1). With the improved knowledge of genomics and the ongoing developments in technology, the future remains bright in terms of increased applications of molecular biotechnology in animal genomics, which provide innovative solutions to many scientific and social challenges.

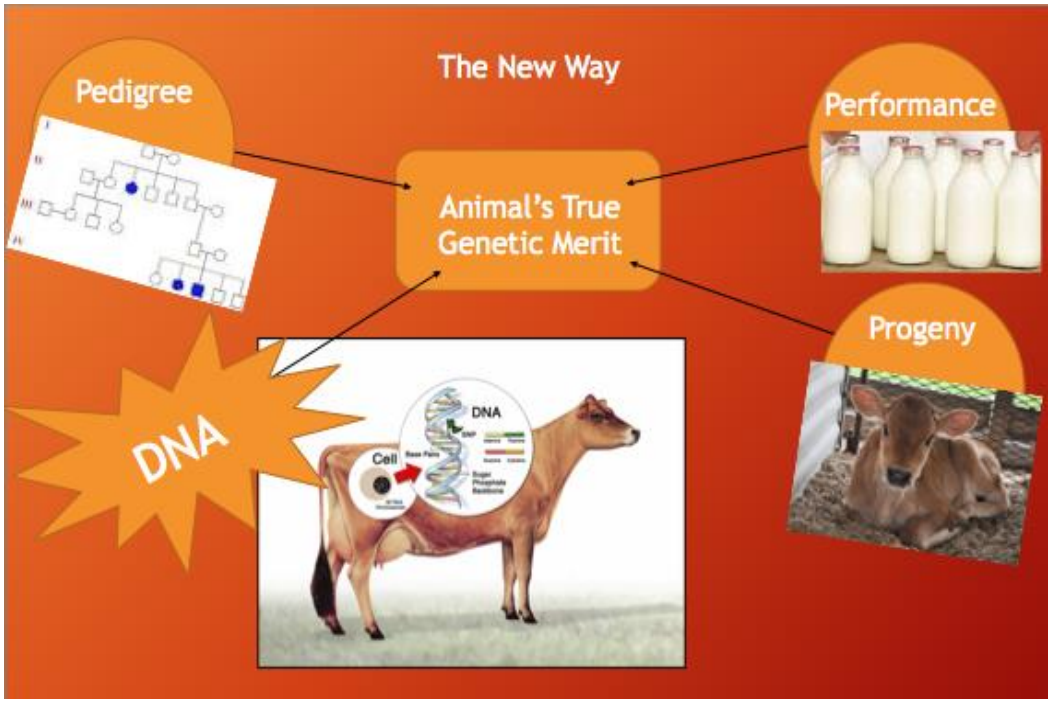

Figure 1. Overview of Molecular Biotechnology and Animal Genomics

1. Historical Background

Animal genetics has developed greatly throughout the last several decades, and the practice originated with simple investigations into genetic content and has developed highly advanced methods to manipulate. The initial progress of animal genomics was severely marked by pioneer discoveries in the field of genetics and molecular biology. Genetics has been in study since the mid 19 the century when Gregor Mendel developed a science of heredity by using pea plants. His ideologies formed the basis of the way in which the traits were inherited and much later, the molecular basis of these traits was pursued, especially in case of animals (Jones & Wilson, 2022). Early efforts to study animal genetics were largely informed by phenotypic traits like coat color or resistance to disease. Modern animal genomics has its roots in these early studies of the selective breeding of animals to accentuate certain traits, long before the molecular basis of inheritance was well understood (Hill, 2014).

Animal genomics made its real breakthrough when James Watson, Francis Crick, and Maurice Wilkins discovered the structure of DNA in 1953. This discovery preconditioned the examining of genomes in molecular aspects. It became established the genetic information was contained within the DNA as genes and this brought new opportunities in understanding the element of genes behind animal traits and behaviors. Soon after this discovery, scientists started coming up with methods to analyze DNA. The discovery of the genetic code and the capacity to read small fragments of DNA was one of the first great strides. In the initial years however, animal genomics was confined to the knowledge about a few major genes such as those influencing the apparent traits such as the coat color, size or disease resistance (Friedmann, 2025).

The actual shift in animal genomics occurred in the 1970s when recombinant DNA technology was discovered, allowing scientists to work and analyze DNA in ways never thought possible before. This enabled transfer of genes of one species to another hence leading to the advantage of creating transgenic animals. This was the first real-life practical approach on how animal genomes could be altered using molecular biotechnology since such animals are commonly engineered to create proteins of interest. Using an example, the development of genetically modified mice in 1980s offered good models to understanding of the human diseases like cancer and Alzheimer. These transgenic animals contribute both to science and to illustrating the potential biotechnology that must increase the productivity of animals and agriculture (Mattei et al., 2022).

Another major advancement was polymerase chain reaction (PCR) developed by Kary Mullis in 1983 during this period. PCR changed the scenario of the study of specific genes since it enabled scientists to quickly produce large amounts of targeted portions of the DNA. This marked a turning point on genomic studies as it allowed researchers to work on individual genes and that of their interaction in a biological process. The easier genetic markers due to PCR became also identifiable in animals based on traits, most commonly disease resistance, growth rate etcetera, and these phenotypes became a key issue in selective breeding and genomics-assisted breeding programs (Madadelahi et al., 2024).

During the 1990s, interest in genome sequencing increased and genomic science received a significant boost when the Human Genome Project (HGP) was completed in 2003. Although the HGP was mainly concerned with the human genome, its completion has significant implications to the animal genomics. Within a short span after the methods that were discovered during the HGP, they were extended to species of animals with the sequencing of the animal genomes starting soon thereafter. An example is the sequencing of the bovine genome that was finished in the year 2009 which was a major milestone in livestock genomics. The project offered a lot of information about the nature of traits including the milk, disease resistance, meat quality in cows, which have genetic foundation (Hofker et al., 2014).

Meanwhile, genetics was further being boosted by the rapid growth in technological aspects. In the mid-2000s, however, the emergence of next-generation sequencing (NGS) technologies enabled sequencing complete genomes to be much quicker and significantly cheaper than in the case of traditional sequencing technology. On the one hand, NGS technologies turned out to be a revolution in how researchers tackled animal genomics since it was able to create large datasets of data within a short duration. This made it possible to sequence genomes of a wide variety of species, including domesticated animals all the way to wild species at risk. The potential to map full genomes also resulted in an information bubble, and as such bioinformatics was created to assist in handling and processing this complex data in genomics (Barba et al., 2014).

More recently, other technological advancements like genome editing (ex: CRISPR-Cas9) have made their way into the animal genomics domain. CRISPR-Cas9 has the advantage of enabling

the genome to be edited by aiming at a particular gene that may be removed or inserted or edited. Technology has influenced significantly genomic research as it has resulted in the development of genetically modified animals more accurately than ever before (Pacesa et al., 2024). One example is that scientists have modded or modified animals, using CRISPR, to make them have an increased resistance to the disease, or create models of genetic disarray that they can then study. This is because this new capability of being able to edit genes specifically has created an opening to a host of possible outcomes such as enhancing agricultural efficiency to advancements in healthcare (Kolanu, 2024).

The animal genomics field is becoming more exact and potent as the technologies of the genome keep advancing. Since the dawn of genetic selection to the elaborate genome-editing capabilities of today, we have seen the cosmic nature of genomic technology transform the way in which scientist investigates and manipulates animal genome (Ansori et al., 2023). The capacity to sequence and edit animal genomes has not only contributed to our knowledge of animal biology but has also found such practical use as in agriculture, medicine and conservation (Bruford et al., 2015). With advances in technology, it appears the future of even greater breakthrough in animal genomics might be endless, as further development in sequencing, editing and computation programs investigate developing even more interesting findings of animal genomics (Table 1).

Table 1. Summary of Key Milestones in Molecular Biotechnology and Animal Genomics

Year	Milestone	Description
1953	Discovery of DNA Structure	James Watson, Francis Crick, and Maurice Wilkins discovered the double-helix structure of DNA, laying the groundwork for modern genomics.
1970s	Development of Recombinant DNA Technology	Early work on recombinant DNA technology paved the way for genetic manipulation and transgenic animals.
1983	Invention of PCR (Polymerase Chain Reaction)	Kary Mullis developed PCR, enabling the amplification of specific DNA sequences, a key tool in genomics.
1980s	Creation of Transgenic Mice	Transgenic mice were created by inserting foreign genes, providing models for studying human diseases.
1990s	First Animal Genome Sequenced (C. elegans)	The roundworm *Caenorhabditis elegans* was sequenced, marking the beginning of animal genome sequencing projects.
2003	Completion of the Human Genome Project	The first complete sequence of the human genome was published, influencing animal genomics by refining sequencing techniques.
2009	Bovine Genome Sequencing	The complete genome of cattle was sequenced, offering insights into livestock improvement and disease resistance.

2012	CRISPR-Cas9 Genome Editing Technology	The discovery of CRISPR-Cas9 enabled precise genome editing, revolutionizing the modification of animal genomes.
2016	First CRISPR-edited Animals	Researchers successfully edited the genomes of animals, including pigs and mice, for various traits like disease resistance.
2020	Sequencing of the Pig Genome	The pig genome was fully sequenced, offering opportunities for enhancing livestock breeding and disease management.

2. Key Technologies in Molecular Biotechnology

Molecular biotechnology has been greatly advanced with the invention of several technologies that have enabled scientists to accurately manipulate and deconstruct or interpolate genetic material. The impact of these technologies on the advance of animal genomics has allowed greater efficiency in breeding programs, methods of dealing with diseases and a stronger knowledge of animal biology (Bhat et al., 2017). Some of the most life-changing technologies have been Polymerase Chain Reaction (PCR), methods of DNA sequencing, CRISPR-Cas9 genome editing, RNA interference, and genomic mapping. These techniques have transformed research into the fact that it is now simpler to discover, change and follow genetic variations of animals (Quintyn, 2020).

Polymerase Chain Reaction (PCR) forms central technology in the field of molecular biotechnology. Kary Mullis invented PCR in 1983 and it enables scientists to create millions of copies of a target gene or sequence; it can be used to amplify targeted portions of the DNA. This is most appreciable in the analysis of genes that might be present in low amounts in the genome of an organism. The use of PCR spans animal genomic fields including finding genetic markers of traits, diagnosis or management of genetic diseases, and population genetic variation management. It has found a wide application in animal breeding where desirable genetics such as disease resistance and milk production are being tracked (Kadri, 2019).

The methods used in DNA sequencing have been of great importance in unraveling the information contained in the genomes of animals. Sanger sequencing method -The Sanger sequencing method developed by Frederick Sanger in the 1970s was the first approach that was used to sequence the DNA widely. Sanger sequencing is the technique of finding the exact sequence of nucleotides (A, T, C, G) in strand of DNA by making labeled nucleotides to extend a growing strand of DNA. Sanger sequencing is both time-wasting and costly; thus, it is not effective in sequencing a large genome, although it was revolutionary during its release (Eren et al., 2022). Consequently, the next generation sequencing (NGS) technologies have been developed to drop these shortcomings. NGS methods, including Illumina sequencing can be used to sequence collections of DNA fragments in parallel, enabling throughput many-orders-of-magnitude greater than sequencing done one fragment at a time and orders-of-magnitude less expensive. It has made whole-genome sequencing of animals possible and has allowed

researchers to explore genetic variation in entire genomes efficiently and in small amounts of time. NGS has significantly revolutionized the field of animal genomics and allowed sequencing of animal genomes across many species, including livestock and endangered species, and created new opportunities in genetic investigations and custom breeding approaches (Park & Kim, 2016) as shown in table 2.

In recent years, CRISPR-Cas9 may be the most revolutionary-yet set- up technology in the field of molecular biotechnology and animal genomics. This useful genome-editing tool was first found in bacteria as a protection tool against viruses and makes it possible to achieve a more exact change in this or that gene within the genome of an organism by scientists. CRISPR-Cas9 functions by having a guide RNA to inform the Cas9 enzyme to present a specific DNA arrangement followed by bringing about a twofold chain gap (Asif et al., 2024). The cell can then patch up this break with either adding a new sequence or removing the already existing sequence. CRISPR-Cas9 has been used as a game-changer because of its accuracy and effectiveness in genetic studies as well as breeding animals. CRISPR has also generated genetically modified animals in animal genomics including disease-resistant pigs or improved traits cows. It has additionally enabled investigators to observe the role that genes play, and they have done this by creating mutations in model organisms so they can get a good deal with regards to gene role, disease mechanism, and therapeutic manipulation (Parrington, 2016).

Another strong method in molecular biotechnology is RNA interference (RNAi) which is used to silence or knockdown the expression of specific genes. RNAi is a process in which small RNA molecules are added, including small interfering RNA (siRNA) or microRNA (miRNA), which attach to messages RNA (mRNA) that block their conversion into proteins. This has enabled researchers to learn the outcomes of silencing specific genes in animals, and this is particularly good in coming up with the functions of genes as well as finding out possible targets in the treatment of diseases. In animal genomics, RNAi has been used in the study of genes engaged in avoiding diseases, growing, and reproducing as well as having potential in treating animal diseases and treating classic ailments in animals with gene therapy procedures (Lennox & Behlke, 2016).

Whole genome sequencing and genomic mapping are the two technologies that have eased researchers to examine whole genomes. Genomics mapping is the preparation of a detailed graph of genetic material in an organism, in which genes, control areas, etc. can be drawn up. The method has proven essential in isolating genes with favorable attributes in animals like disease resistance, growth and milk production. Sequencing the entire genome of an organism gives scientists the big genetic picture of a given organism; this is enabled by whole genome sequencing which is enabled by next-generation sequencing technologies (Quainoo et al., 2017). It has allowed the sequencing of genomes of a broad range of animals, including livestock (cattle, pigs, chicken) and led into more efficient breeding programs, disease control schemes. Another definitive role of the whole genome sequencing in conservation is that genetic diversity

of endangered classifications can hold up and next breeding can be directed using whole genome sequencing to sustain diversity (Blasco & Toro, 2014).

Technology has also revolutionized animal genomics, giving researchers potent means with which to analyze, and change the genetic composition of animals, and to better it. They have created opportunities to increase the health of animals, agricultural productivity, and biomedical research and made possible exact genetic analysis and modification (Figure 2). With the continued evolution of these technologies, it is bound to result in future discoveries in animal genomics and allow more creative processes to be built in animal breeding, disease management, and conservation (ASLAM et al., 2024).

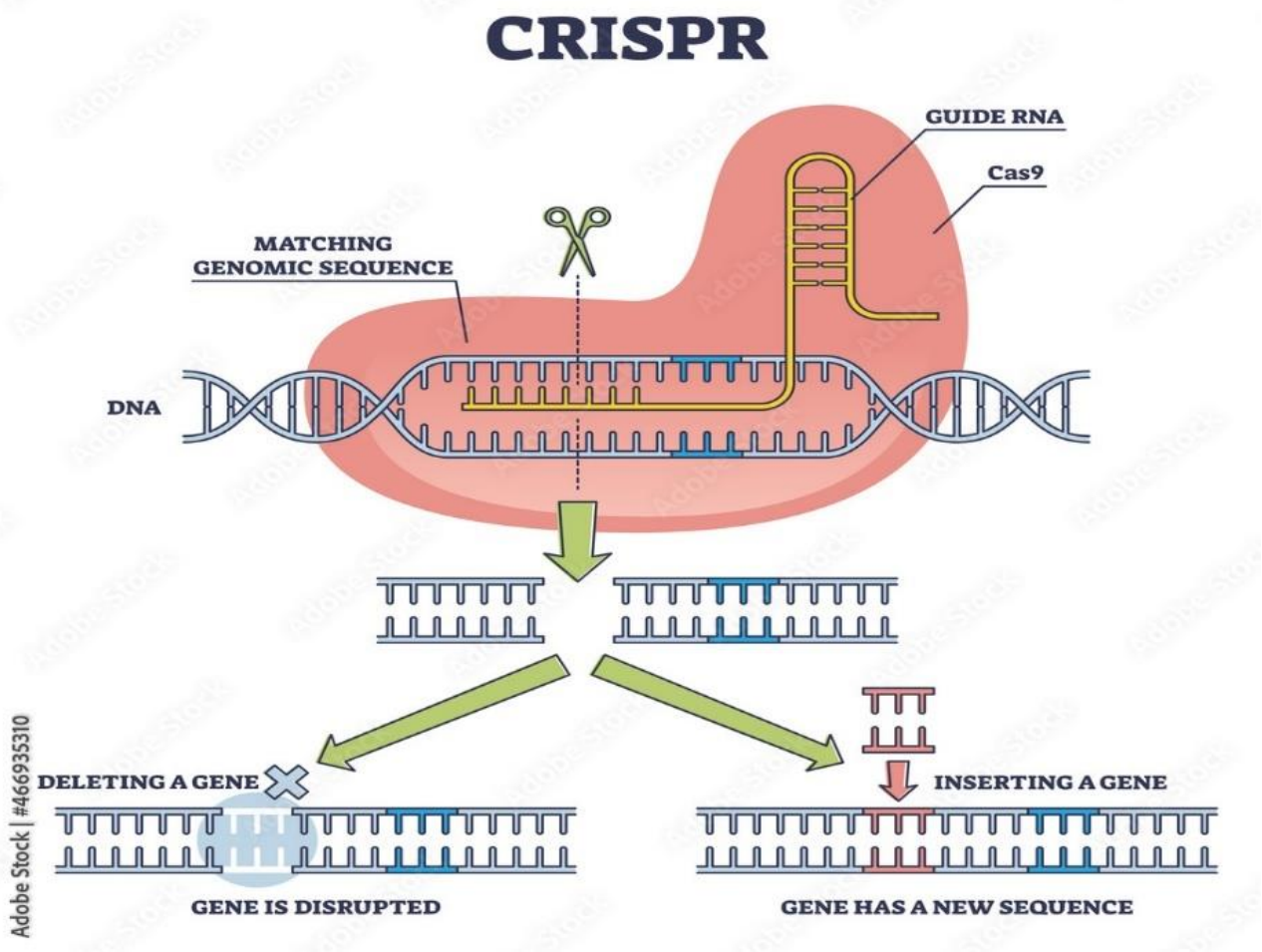

Figure 2. CRISPR-Cas9 Mechanism in Genome Editing

Table 2. Comparison of DNA Sequencing Technologies (Sanger vs. NGS)

Features	**Sanger Sequencing**	**Next-Generation Sequencing (NGS)**
Sequencing Method	Chain-termination method using dideoxynucleotides	Massively parallel sequencing of millions of DNA fragments simultaneously
Throughput	Low; typically sequences one DNA fragment per reaction	High; sequences millions to billions of DNA fragments per run
Read Length	Long; up to ~1,000 base pairs	Short; typically, 50–300 base pairs
Accuracy	Very high (~99.99%)	High; depends on depth of coverage and platform, but generally >99%
Cost	Higher per base; cost-effective for small-scale projects	Lower per base; cost-effective for large-scale projects
Speed	Relatively slow; hours to days per sample	Fast; hours to days for entire projects
Applications	Targeted sequencing of specific genes or regions	Whole-genome sequencing, transcriptome analysis, metagenomics, etc.
Data Analysis	Straightforward; less computationally intensive	Complex; requires advanced bioinformatics tools
Sample Throughput	Low; suitable for small numbers of samples	High; suitable for large numbers of samples
Error Rate	Low; minimal base-calling errors	Slightly higher; mitigated by deep sequencing and bioinformatics correction

3. Applications of Molecular Biotechnology in Animal Genomics

Molecular biotechnology has presented many opportunities in terms of promoting animal genomics, providing much hope of enhancement in genetic improvement, better remedies concerning diseases and conservation of animals. Scientists can tackle the issues of animal agriculture, wild animal conservation, and biomedical research using molecular resources to innovate genomic sequencing, gene editing, and marker-assisted breeding. Its applications of technologies are far-reaching as these technologies have transformed how animals are raised, researched, and conserved (Rothschild & Plastow, 2008).

Genetic improvement and breeding programs are one of the most visible uses of molecular biotechnology. The use of genomics tools, such as genomics-assisted selection (GAS) and genomic prediction have enabled breeders to base their decision on breeding animals of interest on genetic rather than visible traits. Molecular biotechnology has increased the effectiveness of breeding plans with greater efficiency and sensitivity by knowing certain genes that produce desirable traits such as growth rate, disease resistance or milk production characteristics (Crossa

et al., 2025). In livestock, e.g., genetic markers affecting traits such as meat quality in cattle or disease resistance in pigs can be found using genomic techniques. The result is healthier, more productive animals grow in less time which increases the sustainability and profitability of animal farming (Hu et al., 2020).

Molecular biotechnology is important in the field of disease control and resistance in livestock because it is used to generate animals which are more resistant to infectious diseases. With the application of genomic technologies, they may find genetic variants that make them resistant to diseases, e.g., foot-and-mouth disease or avian flu. The knowledge has enable animals to be bred that are naturally resistant to these diseases and need less antibiotics and vaccines, leading to animal welfare (Pal & Chakravarty, 2019). Moreover, genetic testing may allow farmers to target genetic diseases in their herds and cure them; so, the hereditary disease does not spread among the other individuals, and the overall health of the population increases. Transgenic animals have also been developed with the help of genome editing technologies, including disease-resistant animals, e.g. genetically modified pigs which are resistant to the porcine reproductive and respiratory syndrome (PRRS) (Shakweer et al., 2023).

Molecular biotechnology is also referred to as being crucial in conservation of endangered species. Through the genome sequencing of the endangered animals, scientists will be able to capture insight into genetic variety of the population, which is important in the conservationist efforts. When genetic variety is lost, it may result in inbreeding and the inability to adapt to any environmental changes thus making its possible extinction harder. Using genomic analysis, researchers can find genetically valuable individuals in the breeding programs so that the species does not develop an unhealthy gene pool (McMahon et al., 2014). Moreover, molecular applications enable genetic health to be checked in wild populations to enable the implementation of conservation measures such as habitat restoration and reintroduction programs. The conservation application of genomics has also been used to find species that are at risk of extinction and ensure focused protection of the species (Hohenlohe et al., 2021).

A vast application of molecular biotechnology is these animals and products of biotechnology which are transgenic. Transgenic animals refer to animals which are genetically altered to express genes of other species mainly to produce valuable materials or compounds. As in the case with goats, genetically modified goats can produce human therapeutic proteins known as antithrombin in their milk which is applied to treat blood clotting conditions (Shakweer et al., 2023). Likewise, genetically engineered salmon grow faster increasing their time-to-market compared to their wild specimen and is a more sustainable source of protein supply. These new biotechnological achievements hold great potential as solutions to international problems such as food and health care. But when it came to the making of animals alike, then the major issue that prevailed on this was the topic of animal welfare and strong regulatory mechanisms are being employed to guarantee safe and humane animal usage (Fajardo et al., 2025).

Moreover, molecular biotechnology has also achieved a lot in studying both complex traits and animal behavior. Genetic causative factors of a complex trait (such as intelligence, aggression or animal social behavior) can be found using methodologies such as genome-wide association studies (GWAS) (Guo, 2024). The studies have expanded our knowledge base of the role of genetics in behavior of domesticated animals and wildlife. As an example, in dogs, GWAS has been used to characterize genes correlated with temperament and trainability which is useful in breeding. Within the study of wildlife, genetics has contributed to the formation of a better understanding of animal behavior in terms of mating, migration, and survival, which can be used in advancing developments in conservation (Kazantseva et al., 2025).

4. Advancements in Animal Genomic Research

Research on animal genomics has seen a massive boost in the recent past due to technological advancements as well as incorporation of other fields of science. Such advancements have widened knowledge about the biology of animals, illnesses, and evolution. The new developments and emergence of bioinformatics and incorporation of epigenetics as a part of genomics have also led to the advancement of animal genomic studies (Rexroad et al., 2019).

Advancements in animal genomics in the recent past have been because of advancement in the level of sequencing. The due to next generation sequencing (NGS) has transformed our way we are thinking genome sequencing and has led to whole-genome sequencing in many species, including livestock, endangered species, and model organisms. As a point of illustration, the sequencing of the genome of major cultivated animals such as cow, pig, and chicken has revealed more about their genomics, hence breeding programs can now be conducted more efficiently. In addition, the development of sequences of wild animals, like tigers and elephants, has improved conservation measures because it provides a deeper insight regarding their genetic diversities and evolution (Akintunde et al., 2024). Moreover, technology such as CRISPR-Cas9 has made it possible to edit individual genome substantively, therefore, researching on the specific number of genes and how they work. The developments have increased our understanding of pursuing research of complicated characteristics, including disease resistance and reproduction, and have much value when it comes to elevating animal wellness and output capacity (Pereira, 2017).

Bioinformatics and data analysis in the field of animal genomics have become essential roles of animal science research. High-throughput sequencing technologies produce huge amounts of data that need advanced tools and algorithms to process, store and analyze genomic data (Rexroad et al., 2019). Bioinformatics helps scientists to store this information, discover gene variations and how genes interact with the environment. Such tools as genome-wide association studies (GWAS) potentially allow genetic markers associated with certain traits, such as disease resistance or growth rate, to be determined that may be of value in breeding and the management of disease. Also, bioinformatics tools combine information about various genomic, transcriptomic and proteomic data sets leading to the ability to study animal biology in an

integrated manner. Bioinformatics will become more essential as the repositories of genetic information are growing (Suravajhala et al., 2016).

The combination of epigenetics and animal genomics has also become an important field of research. Epigenetics can be defined as variations in the expression of the genes which are not based on a switch of the DNA sequence. Environmental factors that may trigger these changes include Diet, stress and exposure to toxins and these changes may affect the development, behavior and susceptibility to diseases in an animal. Knowledge of the epigenome is vital to animal genomics, whereby one can understand how genetic information is changed and the influence of environmental agents in expressing particular genes (Turner, 2009). Epigenetics offers a potential explanation in farm animals of the life-long health and productivity effect of early-life environment, including nutrition or stress. Application of epigenetics to genomics offers comprehensive insight into how genetic and environmental influences contribute to the determination of animal traits and provides novel methods of breeding, health care and conservation (Wang & Ibeagha-Awemu, 2021).

5. Impact on Animal Agriculture and Biotechnology

Molecular biotechnology has had far-reaching effects on animal biotechnology and animal agriculture through which livestock breeding, rearing and management practices have been changed. Biotechnology has enabled higher productivity, welfare and more sustainable use of animals, by using more advanced genomic methods. Such developments have greatly affected agricultural productivity as well as food security in the world (Singh).

Molecular biotechnology has made some of the greatest improvements to the productivity and quality of livestock. Multiple new technologies, including genomics-assisted selection (GAS), give breeders the ability to see and then select animals with better traits based on faster growth rates, disease resistance, enhanced feed conversion, and advances in reproductive success (Mueller & Van Eenennaam, 2022). An example is that genomic technologies helped to increase milk production in dairy cows and meat quality production in beef cattle and swine through identification of certain sets of genetic markers linked with these traits. The use of genetic data in breeding choices can enhance productivity and the quality of the animals as it results in improved economic returns as well as efficiency in food production. Molecular biotechnology is also essential in minimizing environmental footprints of animal agriculture (Singh et al., 2014). Through breeding of animals with improved feed efficiency, one can decrease feed consumption to produce meat or milk and hence reduce resource consumption, and greenhouse gases emissions caused by production of livestock. Furthermore, through genomic technologies, it is possible to breed livestock that are resistant to diseases hence limiting encounters in which antibiotics or other veterinary measures are needed, which might be associated with environmental effects (Strandén et al., 2022). The adoption of sustainable practices including ensuring that livestock breeding is optimized to minimizing the level of methane that is produced in the cattle is on the rise due to the emergence of issues related to climate change.

Biotechnology provides an opportunity to produce more nature-intelligent and resource-saving forms of agriculture (Tade & Melesse, 2024).

But there is also the moral issue of the use of biotechnology in animal agriculture, and this is the issue of genetic modification in animals. Ethical concerns have been raised over the development of transgenic animals or through gene editing (such as CRISPR-Cas9) as a means of altering the genetic components of an animal to have a better phenotype or resist disease (Ormandy et al., 2011). Issues of animal welfare, problems with unintended effects of genetic modifications, and impacts on the ecosystem in the long-term should a genetic modification of animals go wild are the object of concern. Moral debates also border on the issue of whether we should use animals as bioreactors to produce pharmaceuticals to treat human beings or genetically modify their animals to enhance farming. Though these technologies obviously have certain positive values, moral reasons require tight maintenance of them to protect the lives of animals and preserve natural biodiversity (Yunes et al., 2021).

The existence of regulatory structures and literature on international standards is important in the proper application of molecular biotechnology in animal production. With technology still in its development process, national and global regulatory organs should come up with regulations that answer to the security, animal wellbeing, or environmental consequences of genetically modified animals (Wray-Cahen et al., 2024). The existence of regulatory frameworks, which have grown in popularity through standards set by the U.S. Food and Drug Administration (FDA), European Food Safety Authority (EFSA), among others, is important in the assessment of the safety of genetically modified organisms (GMOs) before they get in the market. International policies and standards also assist in creating consistency in the application of biotechnology within the countries in the sense that the products produced with the application of genetically modified animals, follow safety concerns, ethical and environmental aspects (Liu et al., 2024).

There have been significant impacts of the integration of molecular biotechnology into animal production and biotechnology in terms of the productivity of livestock, sustainability of the environment and animal welfare. As innovative as these developments may seem in terms of the future, they also demand deliberations involving ethics and regulatory issues that will change the face of these developments in a manner that they are beneficial to the entire society (Sharma et al., 2022).

6. Challenges and Limitations

Although molecular biotechnology and animal genomics have marked a revolution in the field of agriculture and animal science, there are some concerns and setbacks that come with them. These are some of the challenges that should be undertaken to maximize the potential of these technologies (Council et al., 2002).

One of the clearest controversies in animal genomics concerns technical and ethical implications. When it comes to the technical application, one of the primary difficulties consists in the

complexity of animal genomes. Although our knowledge of gene and environmental relationships has improved with the rise in the development of sequencing technologies, this type of information is still challenging to uncover. Also, it is important to notice that such exact gene editing tools as CRISPR-Cas9 are not free of inaccuracy and efficiency limitations with off-target effects and unwanted genetic edits being the issue (Eriksson et al., 2018). Ethically, the modeling of the animal genomes, particularly in the production of transgenic animals, is subject to the issue of animal safety, the effect in the long run on the ecology and the implications of the introduction of genetically modified organisms into the wild (de Graeff et al., 2019).

Limitations to the existing genomic technologies are also a problem. Although their use has seen major improvements in next-generation sequencing and genome editing, the technologies are still costly particularly when large scale is involved. The sheer quantity of genomic data produced is demanding a level of sophisticated bioinformatics tools to analyses, so this may be a barrier to smaller scale researchers or farmers. Also, although this has made some advancement, our knowledge of such complex traits as animal behavior or animal intelligence is still rudimentary (Abbasi & Alexandrov, 2021).

Genetic modifications among the common challenges include public perception and acceptance. There are a lot of individuals who are doubtful or against the use of genetically modified animals, especially in food production. The safety aspects of genetically modified organisms (GMOs) used as food and the well-being of animals stay some of the elements subject to debate that have implications in regulations and in consumer preferences (Karalis et al., 2020).

Moving forward, the challenge of precision livestock farming presents tantalizing opportunities, whereby farmers can better manage breeding, health, and productivity based on genetic information. Widespread adoption on the other hand will need to address the technical, ethical and society inhibitions to make sure that the use of these technologies in the future are applied responsibly and sustainably (Monteiro et al., 2021).

7. Summary

Molecular biotechnology provided revolutionary achievements in the world of animal genomics, and the detailed analysis of animal genetics has been transformed in a large way. Such technologies as PCR, DNA sequencing, CRISPR-Cas9 and genomic mapping have enabled more efficient breeding programs, more effective disease management and more effective conservation strategies. The developments have seen a serious improvement in the livestock productivity and animal health as well as the conservation of the endangered species. As much as has been discussed, genomic research of animals looks as bright as can be as far as the future is concerned, with new developments such as precision livestock farming and advanced genome editing opening new possibilities of improving both agricultural sustainability and animal welfare. Yet, together with these developments, moral and feasible implications related to animal well-being, environmental considerations, as well as social acceptance, must be thoroughly considered. Maintaining technological advancements and balancing ethical and responsible practices will be important so that animal genetics can produce the benefits of molecular technology without interfering with the animals and society.

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Microbiome Analysis for Precision Livestock Health Management

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Introduction

Gut microbiomes are essential for animal health as they facilitate digestion, strengthen immunity, and enhance disease resistance. Advancements in precision animal husbandry indicate that microbiome research provides a sustainable alternative to antibiotics for combating antimicrobial resistance (Kumar et al., 2021). Microorganisms that are helpful to the body contribute to enhanced nutrition and resistance to illness. The advancements that have been made in sequencing technologies, multi-omics, and artificial intelligence have made microbiome profiling more accurate, which has led to the early detection of illnesses and the implementation of targeted interventions. Microbiological imbalance, also known as dysbiosis, is believed to be the underlying cause of most severe diseases affecting animals. For instance, dairy cows who suffer from ruminal acidosis experience a loss of 30% of their microbial diversity, leading to inflammation and metabolic issues. Birds with an unbalanced gut exhibit varying feed efficiency and susceptibility to illness. The microbiome affects vaccine efficacy, stress resilience, and productivity. Mastitis may be predicted with 85% accuracy using profiling, facilitating early intervention and reducing antibiotic consumption (Haxhiaj et al., 2022).

The microbiome in pigs acts as a health indicator since piglets with a high prevalence of *Lactobacillus* species have enhanced resistance to post-weaning diarrhea. Microbiome-based strategies enhance cattle health and sustainability, including probiotics, prebiotics, and precision nutrition. Fowl-administered probiotics exhibited a 25% reduction in digestive ailments and an 8% enhancement in feed efficiency compared to those treated with antibiotics (Suvorov et al., 2023). Butyrate-producing bacteria enhance animal intestinal health and reduce methane emissions from cattle, helping livestock and the environment. However, artificial intelligence modelling and real-time microbiome monitoring are making it more feasible to address the difficulties that continue to exist, which include fluctuating bacteria, inconsistent environments, and high intervention costs. Precision livestock farming will enter a new age of disease

prevention, efficiencies in feed conversion, and sustainability due to advancements in microbiome science. Microbiome-based treatments, which have been shown to have a transformative influence on animal health, are the subject of this chapter's analysis. Microbiome composition, host interactions, and disease resistance are also discussed.

Livestock Microbiome Composition

Microbiomes comprise bacteria, fungi, archaea, and viruses, which metabolically and immunologically regulate digestion. Core microorganisms are distinct from species yet provide essential functions. *Firmicutes* and *Bacteroidetes* are the principal agents of fiber digestion in ruminant animals like cattle, with the rumen generating the bulk of volatile fatty acids, contributing around 70% of the potential energy. The metabolism of carbohydrates and the functioning of the immune system in monogastric animals are predominantly affected by the actions of *Lactobacillus*, *Prevotella*, and *Methanobrevibacter*, the latter being particularly significant in ruminants concerning digestion and methane emissions (Huaiquipán et al., 2023).

1). Influence of Diet: Nutrition fundamentally influences the diversity of the microbiome. Rumen bacteria, like *Ruminococcus flavefaciens*, flourish on elevated forage levels for fiber digestion; yet, high-grain diets foster the proliferation of *Streptococcus bovis*, leading to acidosis. High-fiber diets in swine augment the population of butyrate-producing bacteria, therefore fortifying the gut barrier and diminishing infections by around 30% (Hu et al., 2023). Poultry, especially broilers, may justify the investment of time and effort owing to elevated levels of *Bifidobacteria and Lactobacilli*, decreased pathogen burden, and enhanced feed efficiency.

2). Environmental Impact: The stability of the microbiome is affected by its environment. In addition to boosting immunity and reducing methane emissions by twenty percent, sheep grown in pasture have a greater diversity of microorganisms. Thermal and other seasonal stressors adversely impact digestion and immune function. Climate change and pollution disrupt microbial habitats, leading to reduced fiber digestion and disease resistance, while elevated temperatures constrain microbial diversity and productivity in cattle. Figure 1 illustrates the environmental impact on bacterial diversity when comparing pasture-raised and conventionally farmed cattle.

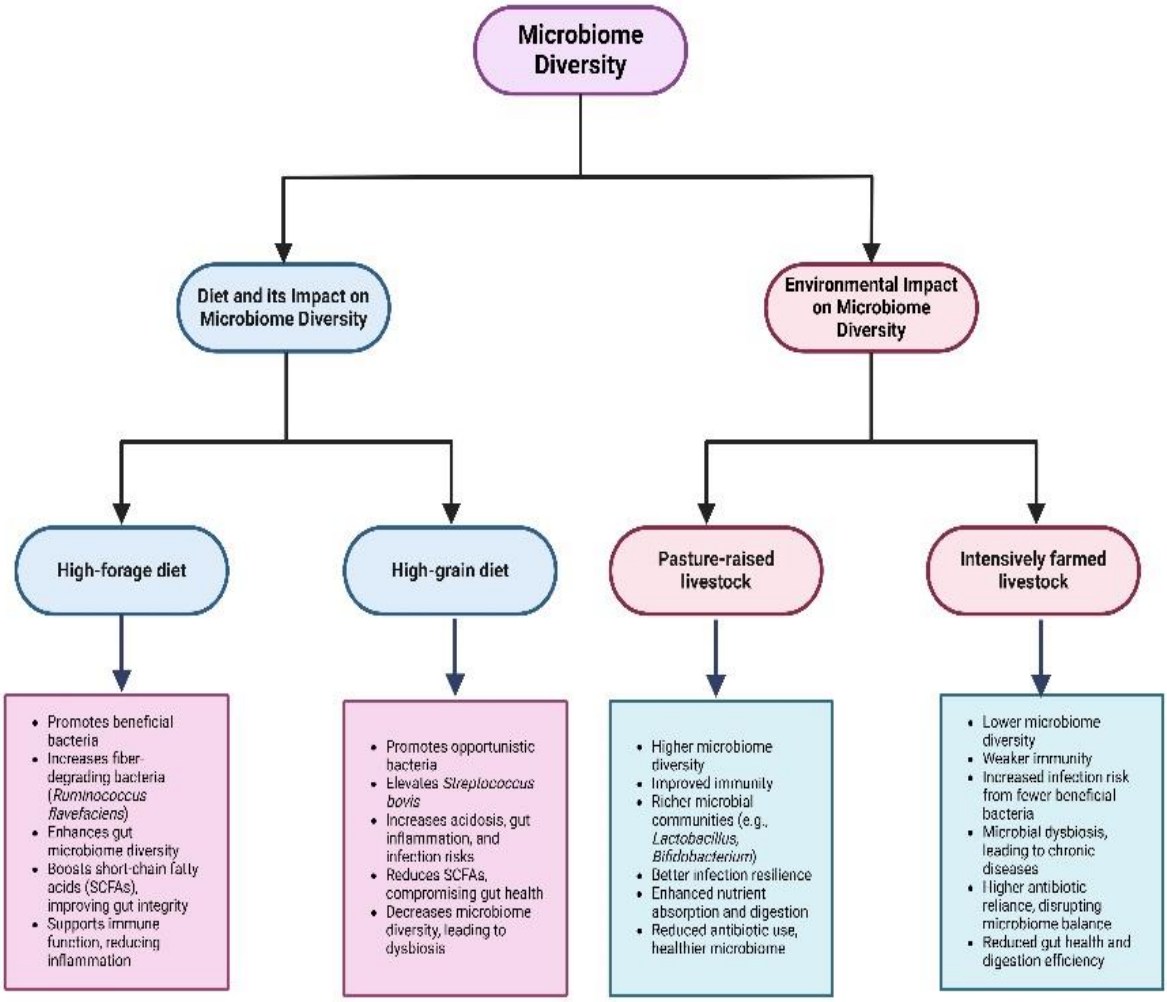

Figure 1. *Impact of Diet and Environment on Microbiome Diversity in Livestock*

3). Variation in Microbiome and Its Impact: Distinct species, agricultural settings, and geographical locations possess diverse microbiomes, rendering a universal microbiome intervention difficult. A specific probiotic may be effective on one farm but not on another due to variations in bacterial populations, impacting the treatments' dependability. Researchers are creating standardized microbiome databases to provide customized probiotics and use AI-driven microbiome modelling to predict microbial changes and dietary customization therapies. These strategies broaden the range of microbiome-based interventions for enhancing cow health and productivity.

Host-Microbiome Interactions

The relationship between cattle and their microbiome is dynamic and mutually beneficial. It governs digestion, immune function, metabolism, and general health. The gut microbiota contributes to nutrient absorption, pathogen protection, and immunological control. The host, in return, sustains the bacteria via mucus secretion, immunological signals, and nourishment. Dysbiosis arises when this balance is disturbed, leading to metabolic irregularities, increased vulnerability to diseases, and diminished productivity. Comprehending these relationships is essential for enhancing animal health and livestock management.

1). Microbiome and Nutrient Metabolism: The microbiome facilitates nutrient degradation and energy extraction in ruminants through microbial fermentation of plant fibers into short-chain fatty acids (SCFAs) or volatile fatty acids (VFAs), predominantly propionate, acetate, and butyrate, which achieve approximately 70% of the host's energy needs. They facilitate the conversion of dietary fibers into short-chain fatty acids in monogastric animals, hence enhancing gut health. Piglets exhibiting higher levels of butyrate-producing bacteria showed a 15% enhancement in feed efficiency and a significantly reduced occurrence (20%) of post-weaning diarrhea (Uddin et al., 2021).

2). Microbiome and Immune System Regulation: The gut microbiome significantly influences immune function, since over 70% of the body's immune cells are in the gastrointestinal tract and engage with microbial antigens. Beneficial microbes stimulate gut-associated lymphoid tissue (GALT), enhancing immune tolerance and resistance to pathogens. *Lactobacillus* and *Bifidobacterium* species and their metabolites inhibit several infections, including *Salmonella* and *Escherichia coli*. A robust microbiome regulates inflammation, and cattle with anti-inflammatory bacteria such as *Faecalibacterium prausnitzii* saw a 30% decrease in mastitis (Zhao et al., 2024).

3). Microbiome and Pathogen Defense: An intact microbiome protects against infections through competitive exclusion, which occurs when helpful microbes outcompete harmful pathogens. In hens, elevated Clostridiales in the stomach reduced Campylobacter colonization by 40% (Valeris-Chacin et al., 2021). Microbiota-based therapies, including fecal microbiota transplantation (FMT) in swine, have been employed to mitigate outbreaks of porcine epidemic diarrhea virus (PEDV).

4). The Gut-Brain Axis and Livestock Behavior: According to findings from recent studies, the gut microbiota can alter cattle's behaviour and stress responses through the gut-brain axis. Brain function and stress resistance are affected by microbial metabolites, including short-chain fatty acids (SCFAs) and neurotransmitter precursors. The administration of probiotics resulted in a reduction in cortisol levels and stress-related behaviours in pigs, which suggests that the maintenance of a healthy microbiome may benefit animal welfare and productivity (Joung et al., 2024).

Microbiome and Disease Resistance

Comprehending how alterations in microbial community composition may bolster disease resistance may facilitate the advancement of microbial therapeutics that reduce antibiotic consumption while enhancing animal wellness. Figure 1 illustrates several microbiome-based strategies for disease resistance, encompassing microbial alteration, transplantation, and targeted therapy for gastrointestinal, cutaneous, and urinary tract infections.

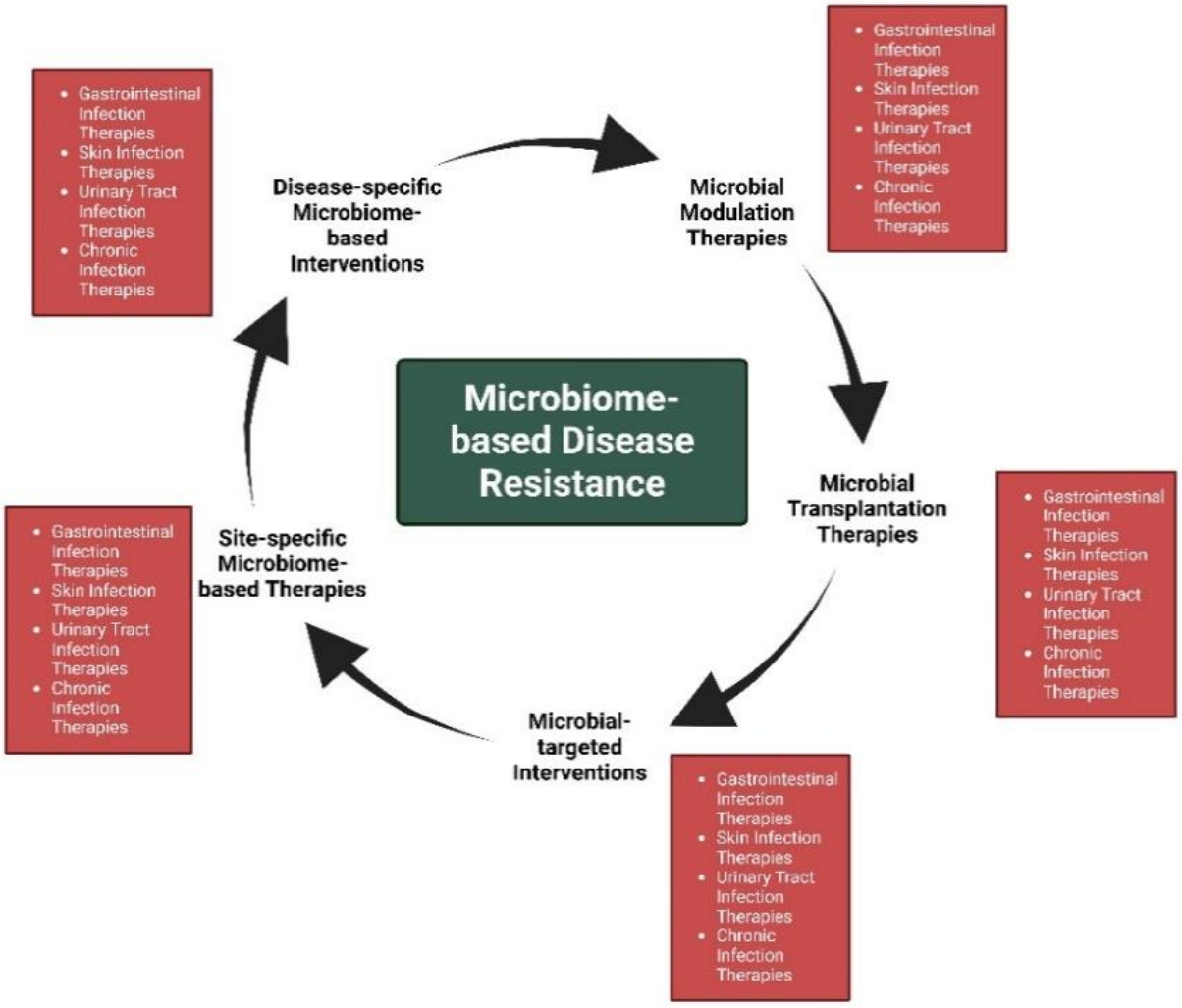

Figure 2. *Pathways of Microbiome-Based Interventions for Enhancing Disease Resistance*

1). Microbiome and Disease Resistance: Through a process known as competitive inhibition, the stable microbiota ensures that animals are protected against diseases. The frequency of *Salmonella* and *Campylobacter* infections was reduced by 40% in chickens with diverse gut microbiota. On the other hand, piglets that had increased levels of *Lactobacillus* and

Bifidobacterium revealed a 30% reduction in the risk of diarrhea caused by *E. coli*. Certain beneficial bacteria are capable of producing antimicrobial substances. For example, *Butyrivibrio fibrisolvens* in cattle produces butyrate, which helps to strengthen the gut and reduce the likelihood of invasion (Munteanu & Schwartz, 2024).

2). Microbiome and Immunity: The gut contains 70% of immune cells, and the microbiome influences immunity through many mechanisms. These beneficial microorganisms decrease inflammation, hence preventing disease. In dairy cows, *Faecalibacterium prausnitzii* is associated with a 30% reduction in mastitis incidence. Butyrate-producing bacteria in swine have demonstrated the ability to enhance vaccination responses and augment immunity to *porcine circovirus* (Wang et al., 2022).

3). Dysbiosis and Disease Risk: Inadequate diet, antibiotics, or stress can all contribute to an imbalance that makes a person more susceptible to disease. In ruminants, diets that are high in grains encourage the development of Streptococcus bovis, which leads to acidosis and inflammation (Chako, 2014). In chickens, antibiotics diminish the variety of microorganisms, promoting infections resistant to antibiotics and raising the risk of *necrotic enteritis*.

4). Microbiome-Based Solutions: Microbiome-based treatments enhance animal health by decreasing antibiotic use and augmenting immunity. Probiotics, namely Lactobacillus reuteri, diminished Clostridium perfringens infections in broilers by 35%, whilst prebiotic mannan-oligosaccharides mitigated diarrhea in pigs by 25%. Fecal microbiota transplantation (FMT) enhances gut resilience, resulting in a 10% increase in weight gain and a decrease in E. coli-induced diarrhea in calves (Beyi et al., 2022). As mentioned earlier, all approaches enhance disease resistance, promote gut health, and render cow management more sustainable.

Microbiome-Based Interventions

Interventions based on the microbiome are transforming animal health by offering sustainable alternatives to antibiotic usage. Probiotics, prebiotics, postbiotics, and fecal microbiota transplantation (FMT) all enhance gut health, disease resistance, and nutrient absorption, while maintaining a healthy microbiome and preventing dysbiosis. Their utilization decreases antibiotic consumption, hence enhancing cow welfare and agricultural profitability. Figure 3 illustrates various treatments, encompassing probiotics to improve gut health and reduce sickness risk, postbiotics to augment immunity, prebiotics to promote beneficial bacteria, and fecal microbiota transplantation (FMT) to restore microbial equilibrium and increase disease resistance.

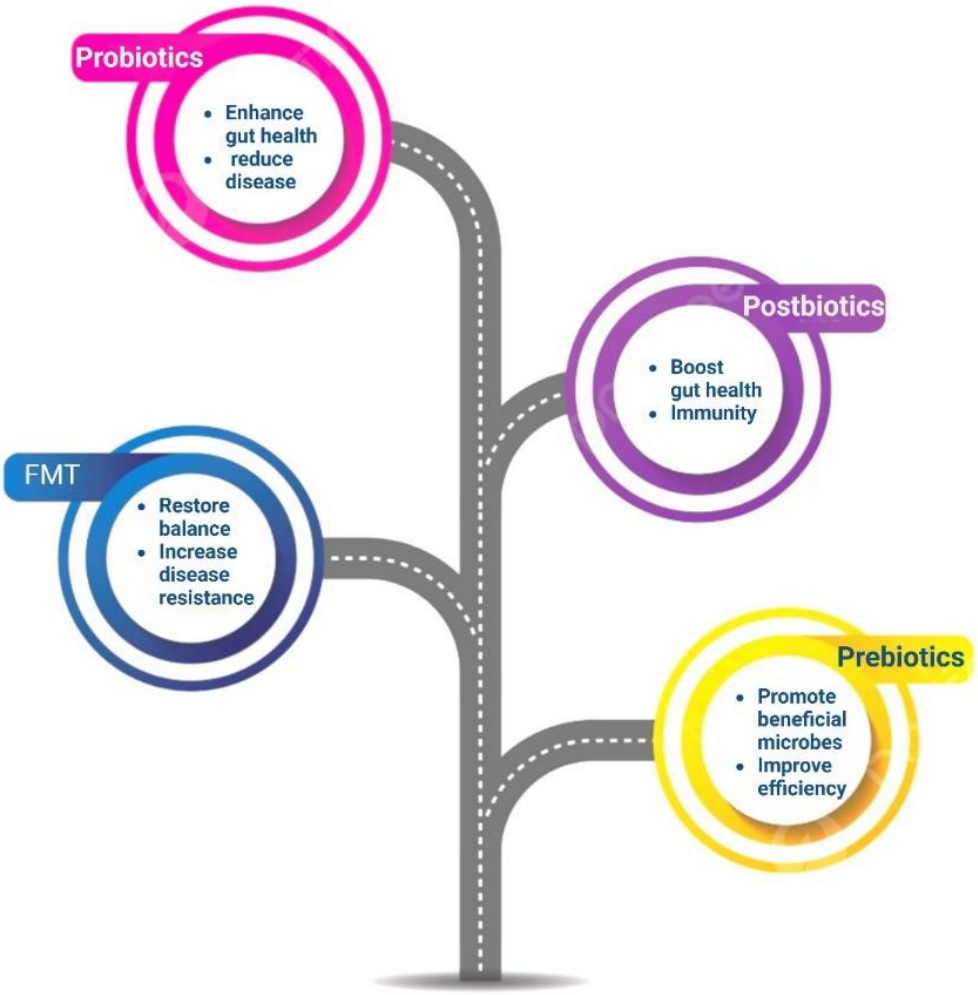

Figure 3. *Microbiome-Based Interventions for Livestock Health*

1). Probiotics: The gut microbiota is altered by these live bacteria, which benefits the gut's health and the immune system. *Lactobacillus* supplementation in poultry can diminish *Clostridium perfringens* infections by 35% and enhance feed conversion efficiency. *Bacillus* subtilis strains in dairy cattle enhance rumen pH stability and reduce the incidence of acidosis (Arthur, 2023). Multi-strain probiotics in swine reduced post-weaning diarrhea by 25% and improved weight gain.

2). Prebiotics: Probiotics are non-digestible fibers that promote the growth of advantageous intestinal microorganisms. Mannan-oligosaccharides (MOS) reduced the colonization of *Salmonella* in avian species. In swine, fructooligosaccharides (FOS) enhanced butyrate-producing bacteria and decreased enteric infections by 18% (Rodríguez-Sorrento et al., 2022). Prebiotics in cattle enhance the balance of rumen bacteria, facilitating fiber digestion and diminishing methane emissions, hence promoting sustainability.

3). Postbiotics: Probiotics generate beneficial metabolites termed postbiotics, including short-chain fatty acids (SCFAs) and bacteriocins, directly enhancing gut health. A study utilizing broiler chicks showed that short-chain fatty acids (SCFAs) generated by postbiotics reduced the occurrence of necrotic enteritis by 22% and enhanced development rates. Postbiotics in dairy cows diminish mastitis incidence by 28% through the enhancement of gut-immune interactions (Kober, 2024).

4). Fecal Microbiota Transplantation (FMT): Microbiota transfer from healthy organisms to species with impaired microbiota is referred to as FMT, an abbreviation for the method. Faecal microbiota transplantation was found to lessen post-weaning diarrhea in pigs by 30%, according to research conducted in 2023 (Drobot et al., 2024)FMT resulted in a 15% increase in feed efficiency and weight gain in dairy calves. Despite its early stages of development, FMT shows considerable promise as a targeted microbiome therapy for animal health.

Advancements in Microbiome Analysis

Recent advancements in microbiome research encompass high-throughput sequencing, multi-omics technology, and artificial intelligence (AI) to enhance animal health. Next-generation sequencing (NGS) facilitates precise microbial profiling, whereas metabolomics and proteomics elucidate microbial interactions with immunity and digestion. AI-enhanced modelling enhances disease forecasting and refines medicines based on the microbiome, hence augmenting livestock resilience and productivity. Real-time microbiome monitoring tools, including portable sequencing, will facilitate early disease detection and precise dietary treatments. These innovations facilitate sustainable cattle management without the need for antibiotics, laying the groundwork for future precision agriculture. Table 1 delineates significant future research themes, challenges, methodologies, and applications in microbiome studies.

Table 1. Future Research Directions in Microbiome Analysis for Precision Livestock Health Management

Research Area	Key Focus	Challenges	Future Research Methods	Potential Applications	References
Microbiome Standardization	Creating universal microbiome profiles for livestock	Variability across species and environments	High-throughput sequencing, meta-analysis of microbiome datasets	Personalized nutrition and disease prevention	(Tegegne & Savidge, 2024)

Microbiome Engineering	Modifying beneficial microbes for improved livestock health	Ethical and regulatory concerns	CRISPR-Cas9 gene editing, synthetic biology	Enhanced probiotics for gut health and disease resistance	(Parvin & Sadras, 2024)
AI-Driven Microbiome Modeling	Predicting disease risk and optimizing microbiome interventions	Large, high-quality datasets are needed	Deep learning, real-time microbiome monitoring	Early disease detection, AI-driven veterinary decision-making	(Ali, 2024)
Microbiome-Resilient Breeding	Selecting livestock with stable, beneficial microbiomes	Complex host-microbiome interactions	Genome-wide association studies (GWAS), microbiome heritability analysis	Disease-resistant livestock with improved immunity	(Farrant et al., 2020)
Fecal Microbiota Transplantation (FMT)	Restoring gut health using donor microbiota	Donor selection, long-term microbiome stability	Clinical trials, microbiome tracking post-FMT	Reduced antibiotic use, improved gut resilience	(Singh et al., 2024)
Postbiotics & Metabolomics	Identifying beneficial microbial metabolites	Limited knowledge of metabolite-host interactions	Mass spectrometry, bioactive compound analysis	Functional feeds for enhanced immunity and gut health	(Gueniche et al., 2022)
Microbiome-Based Vaccines	Enhancing vaccine efficacy through microbiome modulation	Variability in immune responses	Microbiome-immune interaction studies, RNA sequencing	Development of microbiome-optimized vaccines	(Kamel et al., 2024)

Climate Change & Microbiome Stability	Understanding how environmental stress affects microbiota	Microbial shifts due to climate change	Longitudinal microbiome studies, heat stress adaptation trials	Climate-adaptive feeding strategies for resilience	(Chan)
Microbiome-Based Antibiotic Alternatives	Reducing antimicrobial resistance (AMR) using microbiome-targeted therapies	Regulatory approval, consistency of results	Antimicrobial peptide identification, competitive exclusion trials	Probiotic and postbiotic-based disease prevention	(Niedźwiedzka et al., 2024)
Precision Microbiome-Optimized Feeds	Developing microbiome-based customized diets	High cost of personalized feed	Metagenomics-guided diet formulation, probiotic feed trials	Improved feed efficiency, lower methane emissions, and optimized growth	(Obembe et al., 2022)

Conclusion

Microbiome research transforms precision cattle health management by advocating for sustainable and evidence-based approaches to disease prevention, nutritional improvement, and antibiotic stewardship. Gut microbiomes facilitate digestion, regulate the immune system, and defend against pathogens, all impacting livestock productivity and well-being. Recent methodologies, including high-throughput sequencing, multi-omics technology, and artificial intelligence, have yielded significant insights into microbial communities, facilitating illness prediction and targeted therapeutics to alter the microbiota. Probiotics, prebiotics, postbiotics, and fecal microbiota transplantation (FMT) may enhance microbial equilibrium, illness resilience, and feed efficiency. Nevertheless, challenges including microbiome variability, substantial implementation expenses, and regulatory impediments must be emphasized to encourage adoption. Future research should focus on standardizing microbiome therapies, assessing microbiome engineering, and integrating real-time microbiome monitoring with innovative agricultural technologies. The livestock business may adopt more sustainable and precision-oriented management approaches, while microbiomes have significant potential for reducing antibiotic reliance, enhancing animal welfare, and raising farm profitability. The sustainable viability of precision livestock farming, enhanced by advanced microbiome research, will support food security in a resource-constrained society.

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AI-Driven Genomic Selection for Disease Resistance in Livestock

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Introduction

The heightened demand for sustainable livestock production has boosted the need for resistant animals with minimal dependency on antibiotics to improve herd health. Conventional breeding methods have improved traits over generations, but are slow and affected by complex interactions between genes and their environments. Genomic selection and AI have transformed livestock breeding from slow to fast, and AI-assisted genomic selection will enhance the application of AI in faster data-based genetic improvement for disease resistance (Vidanagamachchi & Waidyarathna, 2024). The breeding advances with AI-accompanied genomic selection combine machine learning with high-throughput genetic sequencing, thus providing unprecedented accuracy and efficiency in predicting disease susceptibility. In 2023, a fascinating account was presented of AI implementation in the dairy industry for bovine tuberculosis (bTB), a disease. The machine-learning algorithms to identify bTB-resistant cows from genomic data derived from more than 50,000 dairy cattle crossed with different genetic markers identified with bTB resistance. Making their AI model capable of improving bTB prediction value by up to 30%, provided information to enhance selective breeding arrangements for resistance to bTB without affecting milk production efficiency (Skuce et al., 2011). The same AI combination with genomic selection has proven valuable in the battle against PRRS, pigs' most crucial viral disease. The developed genomic deep learning models for identifying PRRS-resistant pigs reduced mortalities by 20% while improving productivity in herds.

AI-driven genomic selection simultaneously optimizes heat stress resilience in cattle, an important factor given global warming. In Brazil, heat-tolerant dairy cows were identified through AI-based genomic selection, allowing milk production to continue under extreme conditions. Poultry genome-wide association studies, empowered by AI, have associated resistance to Newcastle disease with specific genetic variants, facilitating the effort of selective breeding towards stronger immunity (Lane et al., 2024). Many advantages have been cited for using artificial intelligence in genomic selection. Still, the complexity of data, computational

requirements, and ethical concerns account for the various inhibitions against progress in this area. Nevertheless, speed is a great ally, and very soon, artificial intelligence, cloud computing, and precision livestock farming will find ways to scale over these setbacks. This chapter is about how AI would be applied in genomic selection concerning disease resistance and its possible applications that may influence future livestock breeding.

Genomic Selection in Livestock

Genomic selection has significantly changed livestock breeding through its ability to select animals with a strong genetic basis for disease resistance, productivity, or adaptability to environmental stress. Genomic selection differs from traditional breeding methods that entail measuring phenotypic traits and pedigree records; it is based on high-throughput DNA sequencing and genome-wide association studies (GWAS) that accurately estimate an animal's genetic value. This method speeds breeding, minimizes disease incidence, and increases herd health.

1). Advancements in Dairy Cattle Breeding: Genomic selection has sensibly improved disease resistance and the efficiency in milk production in dairy cattle. Breeders can select cows for increased immunity against mastitis, bovine respiratory disease (BRD), and lameness using genomic estimated breeding values (GEBVs). A recent study conducted (Barasa, 2020). Genome-wide selection of Holstein cows demonstrated a 25% reduction in mastitis cases, confirming that it is a powerful tool in promoting animal health and farm profit.

2). Genomic Selection for Disease-Resistant Swine: Genomic selection is a brilliant decision against porcine reproductive and respiratory syndrome (PRRS), one of the significant economic diseases for pig farming in the pig industry. For these reasons, it can breed pigs to produce stronger immunity against the disease by using genetic markers mapping resistance to PRRS in the breeding programs. The work of (Lunney et al., 2016) reported the effects of genomic selection in reducing PRRS-related mortality by 20% and thus playing a role in better herd resilience.

3). Enhancing Livestock Health Through Genomic Selection: Such improvements, genomic selection, and animal disease resistance are applied to poultry and beef cattle. Newcastle disease and avian influenza are examples of genomic tools for disease resistance. An AI-based GWAS has ensured genetic markers associated with stronger immunity, allowing the breeder to build more robust birds that require reduced dependence on antibiotics, improving flock health. Genomics directly targets bovine respiratory disease (BRD), a much broader concern in the feedlot of beef cattle. Studies have indicated that better immune response genes reduce the susceptibility of animals to BRD by about 30%, thus improving their survival and productivity rates. Indeed, genomic advances will be indispensable for sustainable and efficient livestock production (Flippin, 2023).

AI in Genetic Data Analysis

With technology and changes in genomic data, artificial intelligence is a significant change agent in livestock breeding. It hastens and increases the accuracy of identifying the genetic markers associated with traits of interest, in this case, traits such as disease resistance, productivity, and ADAPTABILITY. The larger implications of machine learning are in the analysis of data from genomics and their subtle illumination of gene-environment interactions, which are the kernels for increasing selection accuracy. Artificial intelligence and genome-wide association studies (GWAS) improve both herd health and production. AI models analyze genetic data to identify traits of interest toward accelerating animal genetics selection and continuous improvement methodologies, as shown in Figure 1.

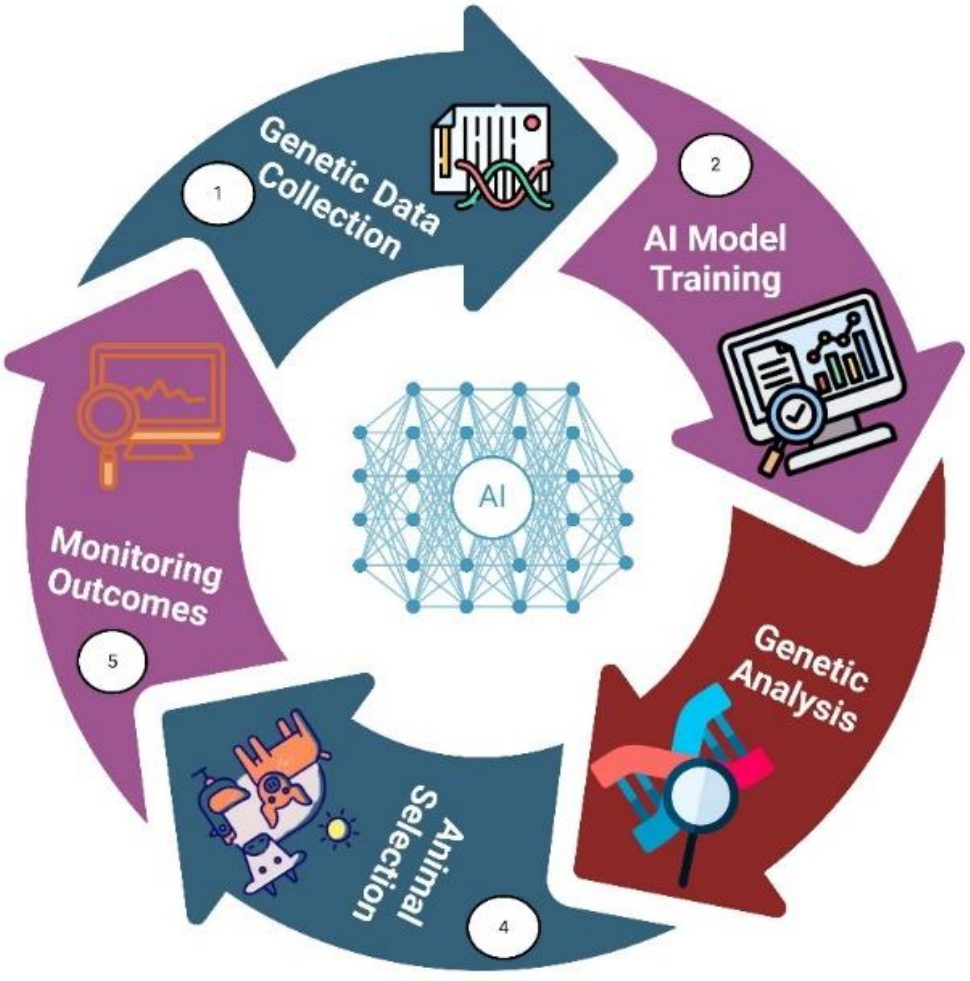

Figure 1. *AI-Driven Genomic Selection Process*

1). Artificial Intelligence in Genome-Wide Association Studies (GWAS): AI-powered GWAS increases the efficiency of validating genetic markers related to beneficial traits. Machine learning algorithms explore massive genomic databases for qualities such as disease resistance and yield that conventional procedures might overlook (Grinberg et al., 2020). The AI-supported GWAS identified indicators for heat tolerance in dairy cattle and markers for PRRS resistance in pigs, enhancing disease protection in livestock.

2). Letting Machine Learning Enter Predictive Genomic Selection: Machine learning employs genetic, phenotypic, and environmental data to enhance the prediction of traits, including diseases and growth patterns. AI models for predicting bovine respiratory disease (BRD) in cattle enhanced selection accuracy by 35%, hence reducing sporadic disease outbreaks and economic losses (Grace et al., 2023). AI genomic selection enhanced longevity in chickens by discovering genetic variants that confer resistance to Newcastle disease.

3). Deep Learning for Genomic Pattern Recognition: Deep learning, encompassing convolutional and recurrent neural networks, is utilized on genomic data to reveal concealed genetic patterns related to traits of interest, such as disease resistance. Techniques have identified distinct variations associated with *Salmonella* resistance in poultry, opening new pathways for disease management and precision breeding (Lamichhane et al., 2024).

4). AI for Genomic Data Processing in Real-Time: Real-time genetic analysis, facilitated by AI-enhanced sequencing, accelerates breeding decisions. On-farm investigations indicated that AI-driven genetic screening could decrease the detection time for mastitis in cows from days to hours, facilitating timely intervention to save economic losses (Michelena et al., 2024).

Machine Learning for Disease Prediction

Machine learning facilitates the early detection of animal diseases by utilizing genetic, phenotypic, and environmental factors to identify risks more promptly than conventional veterinary practices. It uses genetic markers instead of nonspecific immune responses or microbial alterations to enhance many pharmaceuticals in health management. Diverse AI models, including ANN, CNN, and LSTM, enhance prediction accuracy by 35%, 34%, and 30%, respectively. In contrast, models such as SVM, XGBoost, and RF facilitate AI-driven advancements in disease detection for BRD, PRRS, mastitis, and gastrointestinal disorders, enabling farmers to make swifter and more precise decisions to enhance animal health.

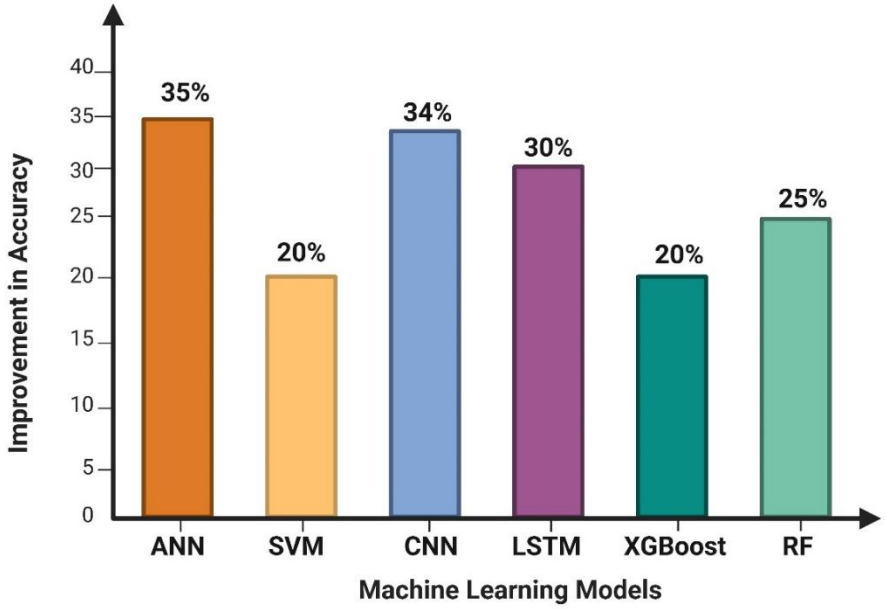

Figure 2. *Improvement in Disease Prediction Accuracy by Machine Learning Models*

Detection of AI-Driven Diseases

Artificial intelligence models have markedly accelerated the speed of early intervention for diseases such as bovine respiratory disease (BRD) in cattle, porcine reproductive and respiratory syndrome (PRRS) in swine, and infectious bronchitis (IB) in poultry. Table 1 illustrates the propensity of ANN and SVM models to enhance accuracy and breeding decisions for each species. Mastitis detection, gastrointestinal condition assessment, and real-time health monitoring for parasite management through microbiome analysis are all achievable with machine learning. For instance, AI forecasts worm infestations in cattle, coccidiosis in poultry, and metabolic lactation problems in dairy cows, reducing piglet mortality and improving herd health and production.

Table 1. *Machine Learning Models for Disease Prediction in Livestock*

ML Model	Application	Livestock Species	Accuracy Improvement	Recent Studies
Artificial Neural Networks (ANNs)	Predicting bovine respiratory disease (BRD) and mastitis	Cattle	35% higher accuracy	(Zhang et al., 2021)
Support Vector	Identifying PRRS-resistant	Swine	20%	(Huang et al.,

Machines (SVMs)	pigs, reproductive disorders		improvement	2018)
Random Forest (RF)	Early detection of avian infectious bronchitis (IB), coccidiosis	Poultry	25% faster diagnosis	(Yogi & Yadav, 2024)
Gradient Boosting Machines (GBM)	Predicting ruminal acidosis (SARA), metabolic diseases	Cattle	30% better prediction	(Giannuzzi et al., 2022)
Long Short-Term Memory Networks (LSTMs)	Analyzing real-time sensor data for disease detection	Cattle, Swine, Poultry	40% improved early detection	(Yajie et al., 2023)
Convolutional Neural Networks (CNNs)	Image-based parasitic disease detection (e.g., coccidiosis)	Poultry, Cattle	35% more accurate	(Luong & Nguyen, 2024)
XGBoost	Predicting heat stress resilience, disease susceptibility	Dairy Cattle, Poultry	20% better adaptation prediction	(Liu et al., 2023)
Recurrent Neural Networks (RNNs)	Monitoring post-weaning diarrhea (PWD) via microbiome data	Swine	30% higher precision	(Sherstinsky, 2020)
Bayesian Networks	Disease risk assessment using genomic and phenotypic data	Cattle, Swine	25% better accuracy in complex disease interactions	(Morgan Bustamante et al., 2024)
K-Nearest Neighbors (KNN)	Predicting foot-and-mouth disease (FMD) outbreaks	Cattle, Sheep, Goats	30% better outbreak forecasting	(Nazari Ashani et al., 2024)
Deep Reinforcement Learning (DRL)	Adaptive disease control and vaccination optimization	Poultry, Swine	35% optimized treatment strategies	(Teymoori, 2023)

Challenges and Ethical Considerations

Despite the potential of advanced genomics to enhance animal breeding, substantial technological, economic, and ethical limitations hinder its broad adoption. Case examples from diverse locations underscore the issues and the imperative for sustainable solutions.

1). Technical and Data Barriers: AI models required extensive datasets and costly computational resources, which are generally inaccessible to smallholder farms. Artificial intelligence improved the prediction of bovine tuberculosis resistance by 30% in the UK; yet, expenses have impeded its extensive implementation. AI algorithms often neglect to predict resistance in native species and reduce genetic diversity by prioritizing the creation of high-yield commercial varieties (Das et al., 2022).

2). Economic Barriers to Adoption: The high cost of sequencing and the expensive implementation of AI represent challenges for small farms. Artificial intelligence has detected heat stress indicators in Brazil; yet, testing has proved excessively costly for smallholders. Despite lower costs, inadequate adoption is intensified by farmers' skepticism towards AI and insufficient AI literacy, as exemplified by Australia's mastitis prevention project (Borelli, 2024).

3). Ethical and Regulatory Issues: AI-assisted selection may reduce genetic diversity, introducing unforeseen trade-offs. Increased resistance to foot-and-mouth disease was observed, along with lower fertility rates. Resolution has already been hampered by regulatory inconsistencies, with Europe enforcing tight genetic policies in contrast to the United States' generally deregulated environment (Hamburger, 2019).]. Data ownership is another issue, because generating insight through AI on the spot is mainly of interest to corporations or governments.

4). Ensuring Sustainable AI Adoption: Expanded genetic datasets will mitigate bias and subsidization, while collaboration can decrease costs. Enhancing farmer competency will facilitate the reduction of the disparity in AI adoption. A comprehensive legal framework should be established to regulate AI usage, ensuring the protection of genetic diversity and equitable data ownership. Therefore, meticulous attention to these issues will be essential for the long-term sustainability and accessibility of AI-assisted breeding.

Future Applications

Artificial intelligence has improved livestock through genetic selection, disease resistance, and climate adaptation. Figure 3 illustrates that substantial advancements occurred in 2018 in AI-assisted gene editing and disease resistance, and in 2019, in real-time health monitoring, which improves illness detection and agricultural management. By 2020, AI-facilitated climate

adaptation will have equipped livestock to withstand environmental stressors, while precision breeding aims to improve genetic traits for enhanced output and sustainability.

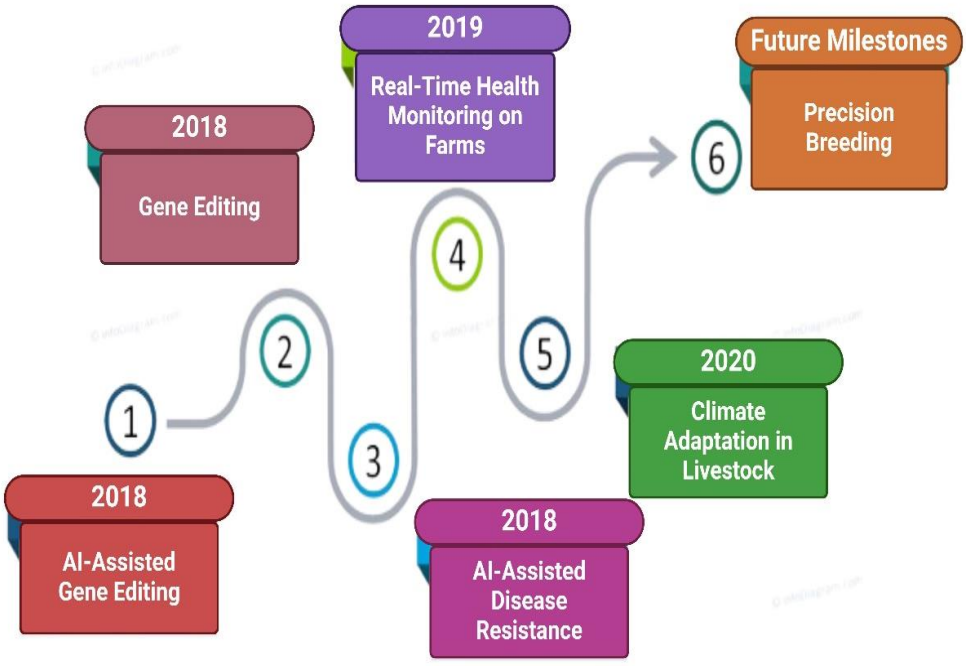

Figure 3. *Roadmap of AI Technologies in Livestock Breeding*

1). AI in Gene Editing: AI CRISPR technology will revolutionize disease-resistant animal breeding by identifying and modifying immune-related genetic markers. AI-assisted gene editing has been effectively utilized to enhance PRRS resistance in pigs (Assavacheep & Thanawongnuwech, 2022). Future-oriented applications will facilitate improvements in genetic traits while mitigating adverse effects, decreasing antibiotic consumption, and enhancing overall herd health.

2). Precision Breeding: Machine learning will enhance genomic selection methodologies, facilitating personalized breeding strategies tailored to individual genetic profiles. AI-driven models will optimize trait selection for disease resistance, productivity, and adaptability, enabling cattle populations to be bred for optimal genetic potential and resilience (Harfouche et al., 2019).

3). Real-Time Monitoring: Artificial intelligence will employ wearable and ingestible biosensors for continuous health surveillance and early disease detection. AI-driven sensors are now used to assess Newcastle disease in chickens during the preclinical phase, leading to significant decreases in mortality rates (Hsiung et al., 2024). The proposed method would facilitate automated disease intervention and prognosis, hence preventing economic losses.

4). Climate Adaptation: Artificial intelligence will be essential in developing climate-resilient animals as temperatures escalate and environmental stresses intensify. Genomic selection models are being formulated to incorporate heat tolerance, drought resistance, and adaptability in animals, hence ensuring the sustainability of livestock production under extreme conditions (Silpa et al., 2021).

5). AI in Synthetic Biology: Artificial intelligence is expected to play a part in the manufacturing of lab-grown meat and in the engineering of animal cells. Genetic alterations will be simulated, and cell growth processes will be optimized, yielding lab-grown animal cells that are disease-resistant, thereby offering a sustainable alternative to conventional breeding (Xu et al., 2020). Consequently, AI-driven genomic selection will revolutionize cow breeding via precision genetics, real-time health monitoring, and climate adaptation by enhancing disease resistance and productivity. Biosensors will be amalgamated with CRISPR and synthetic biology to enhance sustainable and resilient animal production.

Conclusion

AI-driven genomic selection revolutionizes animal breeding by improving disease resistance, productivity, and environmental adaptation. Artificial intelligence integrates machine learning, gene editing, and real-time livestock health monitoring to provide significantly more accurate and efficient selection, presumably reducing reliance on antibiotic treatments and improving overall herd health. AI-facilitated PRRS resistance in swine and heat stress resilience in dairy cattle are two studies that illustrate how AI can tackle the most urgent issues in animal agriculture. The selection of AI has advanced; yet, it continues to encounter numerous technological, economic, and ethical hurdles, such as computational constraints, data biases, and a range of concerns. The financial challenge for small and medium-sized farms continues to be a substantial obstacle to the use of AI-driven genomic methods. Moreover, legislative inconsistencies and challenges with data ownership provide ethical dilemmas concerning the application of AI in animal breeding. Enhanced investment and accessibility to AI, alongside standardized legal frameworks, will be necessary to fully actualize this potential. Addressing these challenges will facilitate the sustainability, equity, and advantages of AI genomic selection in worldwide livestock production. Artificial intelligence will play a vital role in ensuring the sustainability of animal agriculture, enhancing global food security, and promoting ethical breeding practices.

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Crude Glycerın in Poultry Nutrıtıon: A Pathway to Sustaınable and Affordable Meat Productıon

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M. ADNAN

Introduction

Poultry production is a major contributor to global meat supply, providing a highly efficient and cost-effective source of animal protein for human consumption. As the poultry industry grows, the demand for economical and sustainable feed ingredients continues to rise. According to Elahi et al. (2022), feed expenses account for approximately 60 to 80 percent of the total production costs in different types of chicken farming. Managing a chicken enterprise has become increasingly challenging due to rising costs and limited availability of traditional ingredients. Traditionally, poultry diets have been based on grains such as corn and wheat, supplemented with soybean meal as a primary protein source. However, fluctuations in grain prices and competition with human food industries have driven researchers and feed formulators to explore alternative feed ingredients that are not only economical but also sustainable. One such alternative that has attracted attention in recent years is crude glycerin, a byproduct of biodiesel production.

Crude glycerin, a by-product derived from biodiesel production, has garnered considerable attention as a cost-effective and sustainable feed ingredient in animal nutrition (Alvarenga et al., 2012). With the burgeoning global demand for biodiesel, the surplus production of crude glycerin has created opportunities for its utilization in livestock and poultry diets (Terré et al., 2011; Alvarenga et al., 2012). As a by-product, crude glycerin contains substantial amounts of energy and has the potential to replace conventional feed ingredients, reducing feed costs while supporting sustainable agricultural practices (Thompson & He, 2006). The rising emphasis on eco-friendly practices and efficient resource utilization in agriculture has made crude glycerin a promising candidate for inclusion in animal diets.

Several studies have explored the inclusion of crude glycerin in poultry diets, demonstrating its impact on growth performance, carcass traits, and overall health. For instance, Cerrate et al. (2006) reported that crude glycerin could effectively replace corn in broiler diets without compromising growth performance. Similarly, Dozier et al. (2008) highlighted the potential of crude glycerin to maintain feed efficiency and carcass yield in broilers when included at moderate levels. However, the responses of meat chickens to dietary glycerin have been variable, influenced by factors such as the level of inclusion, the source and purity of glycerin, bird age,

and overall diet composition. This chapter encompasses the effect of crude glycerin on the growth and performance of meat chickens.

Crude Glycerin Supplementation

According to Swiatkiewicz & Koreleski (2009), crude glycerin can be used as an energy source in poultry diets, potentially replacing some cereal grains. When used correctly, crude glycerin is generally considered safe. It is a combination of glycerol, water, and ash (Code of Federal Regulations, 2004; EFSA, 2010). The Apparent Metabolizable Energy content of glycerin for chickens varies from 3,254 to 4,134 kcal/kg (Lammers et al., 2008; Dozier et al., 2011; McLea et al., 2011). These values represent nearly 96% of the total energy provided by the glycerol in commercial glycerin. It has been estimated that broilers need a maximum of 5 to 10% glycerin in their diet to achieve optimal growth performance (Simon et al., 1996; Cerrate et al., 2006; Dozier et al., 2008).

Glycerol is found in both the gastrointestinal tract of mammals and birds. It is easily absorbed and serves as a crucial component of phospholipids and triglycerides. Glycerol can be used for energy production through pathways such as glycolysis and the tricarboxylic acid cycle. It also plays a role in lipogenesis and gluconeogenesis by acting as a precursor to glyceraldehyde 3-phosphate (Brisson et al., 2001; Lammers et al., 2008). Kerr et al. (2011) concluded that glycerol metabolism in non-ruminant animals primarily takes place in the livers. Cryer & Bartley (1973) observed significant physiological, biochemical, and morphological changes in the livers of rats that consumed high amounts of glycerol. However, Lin et al. (1976) found no impact on the relative weight of chicken livers or liver fatty acid synthesis when glycerol was included in their diet.

Effect on Growth Performance

Research suggests that incorporating glycerin into poultry diets can influence broiler performance. Mandalawi et al. (2014) conducted a study to evaluate the impact of glycerin supplementation on broiler performance. They formulated eight diets that included two glycerin sources and four inclusion levels (2.5%, 5.0%, 7.5%, and 10%). The study found that the average daily gain remained consistent from day 1 to day 21, while the feed conversion ratio showed a linear improvement (L, $P \leq 0.01$) with increasing glycerin content in the diet. Topal & Ozdogan (2013) conducted a study to investigate the effects of different concentrations of crude glycerol on broiler performance. The researchers fed the birds diets enriched with 0%, 4%, or 8% glycerol for a period of 42 days. The results showed that adding either 4% or 8% glycerol to the diet on day 21 led to a significant improvement in body weight gains. However, the diet with 8% glycerol on day 42 exhibited the highest body weight gains ($P < 0.05$). Although the addition of crude glycerol increased the feed conversion ratio from 0 to 21 days and from 0 to 42 days of age ($P < 0.05$), there were no statistically significant changes in feed consumption. In another study

by Cerrate et al. (2006), the impact of different concentrations of glycerin on the performance of broiler chickens was examined. Glycerin was added to the diets at three levels: 0%, 5%, and 10%. The researchers concluded that although glycerin supplementation enhanced average daily gain, it had no effect on the feed conversion ratio.

In another study, De Souza et al. (2020) examined how different levels of crude glycerin affected various aspects of broiler performance. This included blood parameters, carcass traits, carcass chemical composition, and meat quality. The diets during phase 1 (1–21 days) were supplemented with six levels of glycerin (0%, 1%, 3%, 5%, 7%, and 9%). These same glycerin levels were maintained during phase 2 (22–42 days). During phase 1, it was observed that adding 5%, 7%, and 9% glycerin led to a significant improvement in weight gain than the diet with 0% glycerin (control). Moreover, birds that received glycerin throughout both phases (1–42 days) showed improvements in feed consumption, body weight gain, and feed conversion ratio compared to those that only received glycerin during phase 1 ($P < 0.05$). Silva et al. (2012) also examined how dietary glycerin affected broiler performance. The diets were supplemented with four levels of glycerin (0%, 2.5%, 5.0%, 7.5%, and 10%). It was found that feed intake and body weight gain increased linearly with the addition of up to 10% crude glycerin from 1 to 7 days. However, from 1 to 21 days, these variables did not follow any regression model. Henz et al. (2014) conducted a study to investigate how different proportions of crude glycerin ranging from 3% to 15% affected live performance (feed intake, weight gain, and feed conversion ratio) of broiler from 1 to 21 day. They found that there was a quadratic relationship between these parameters, with the optimal inclusion level of glycerin being 6.06%. Sehu et al. (2013) also examined the impact glycerin levels (0%, 5%, and 10%) on broiler growth, economic performance, carcass yield, organ weights, and meat moisture content. Their findings showed that birds receiving 5% and 10% glycerin had higher body weight, weight gain, and improved feed conversion ratio compared to those who did not receive glycerin in their diet (0%).

In another study, Sehu et al. (2012) investigated the effects of adding methanol-free glycerol, a byproduct of biodiesel production, to broiler chicken diets at levels of 0%, 5%, or 10%, on weight gain and carcass traits. The chickens that received these treatments showed significant improvements in weight gain, especially those supplemented with 5% glycerol. Additionally, there was a slight improvement in feed efficiency with glycerol supplementation, particularly noticeable at the 5% level ($P<0.05$) during the fourth week. Simon et al. (1996) conducted a study to investigate the effects of glycerol supplementation at varying levels (0%, 5%, 10%, 15%, 20%, and 25%) on the live performance of broiler chickens in terms of feed intake, weight gain, and feed conversion ratio. Their findings revealed that supplementing with 5% and 10% glycerol significantly improved the growth (body weights and weight gains) of the birds. In a study, Farrapo et al. (2017) examined the effects of incorporating vegetable and mixed glycerin into broiler quail diets on performance, carcass characteristics, and meat quality. The diets were supplemented with five different levels of either vegetable or mixed glycerin (0%, 5%, 10%, 15%, and 20%). It was observed that mixed glycerin enhanced feed intake and feed conversion. However, neither the type of glycerin nor the levels tested had an impact on weight gain.

Interestingly, the use of mixed glycerin resulted in a reduction in both feed conversion and weight gain. Guerra et al. (2011) conducted a study to determine how the inclusion of varying proportions of crude glycerin mixture in broiler feed affects performance, carcass yield, and carcass composition. The researchers supplemented the diets with glycerin mixture at six different levels: 0%, 2%, 4%, 6%, 8%, and 10%. They found that as the proportion of glycerin mixture increased, feed intake also increased in a linear fashion. However, weight gain remained unaffected, which led to a decrease in feed conversion.

Broch et al. (2018) studied the impact of glycerin supplementation on broiler performance by incorporating it into diets at six different levels: 0%, 3%, 6%, 9%, 12%, and 15%. Their findings revealed a quadratic effect ($P<0.05$) on feed intake, weight gain, and feed conversion ratio between 21 and 42 days of age. The increased levels of glycerin in the diets resulted in decreased bird viability ($P<0.05$). Arif et al. (2017) added glycerin to the diets of growing Japanese quails at various levels: 0%, 2.5%, 5%, 7.5%, and 10%. The researchers found that the supplementation of glycerin did not have a significant impact on the birds' feed intake and body weight ($P>0.05$). However, birds fed diets containing 2.5% or 5% glycerin exhibited an improved feed conversion ratio compared to those on the control diet. Erol et al. (2009) carried out a study on laying quails, where they provided varying levels of glycerol supplementation (0%, 2.5%, 5%, 7.5%, and 10%). Their findings revealed that the dietary glycerol treatment showed no impact ($P>0.05$) on feed intake and body weight gain. In another study, McLea et al. (2011) provided broiler chickens with crude glycerol obtained from biodiesel production for a period of 21 days. The diets were supplemented with four different concentrations of glycerol (0%, 3.3%, 6.7%, and 10%) derived from two different sources. The inclusion of glycerol did not exhibit any noticeable effect on feed intake. Abd-Elsamee et al. (2010) conducted a study to examine how various levels of crude glycerin supplementation (0%, 2%, 4%, 6%, and 8%) affected broiler performance. Their findings indicated that adding either 6% or 8% glycerin to the broiler diets did not affect feed consumption, body weight gains, or feed conversion ratio throughout the 42-day period.

Gasparino et al. (2012) examined the effects of dietary glycerol on the mRNA expression of growth hormone and insulin-like growth factor I in 35-day-old Japanese quails. The study involved three groups of quails who were fed different levels of glycerol in their diets: 0%, 4%, and 8%. The research results indicated that the body weight gain of the Japanese quails was not affected by the amount of glycerol in their diets. Bernardino et al. (2015) fed broiler chicks diets containing varying levels of glycerin (0%, 1.75%, 3.5%, 5.25%, and 7%). The results showed that glycerin increased the feed conversion ratio in quails, but there was no statistically significant impact on feed intake. Garcia et al. (2018) conducted a study where they added glycerin to broiler diets at concentrations of 0.25%, 2.5%, 5.0%, and 7.5%. The researchers found that the different levels of glycerin concentration did not have any significant impact ($P>0.05$) on the performance variables. In their study, Simon et al. (1997) incorporated glycerol into broiler chicken diets at different levels, ranging from 5% to 25%. They found that

incorporating glycerol did not have any detrimental effects on feed intake or feed conversion ratio, regardless of the amount added. However, previous research by Lin et al. (1976) suggested that glycerol supplementation might have adverse effects on specific growth characteristics of broiler chickens. Cerrate et al. (2006) examined the impact of glycerin inclusion on broiler growth performance. Three different levels of glycerin supplementation were used: 0%, 5%, and 10%. The researchers concluded that the inclusion of 10% crude glycerin had a negative effect on broiler feed conversion ratio and body weight from 1 to 42 days. Similarly, Carole et al. (2009) found that broilers consuming diets with 10% crude glycerin for 1 to 35 days showed a decrease in feed intake.

Effect on Carcass Characteristics

Several studies have shown that supplementing broiler chickens with glycerin can affect their slaughter performance. In a study conducted by Mandalawi et al. (2014), broiler chickens were given different doses of glycerin (2.5%, 5.0%, 7.5%, and 10%) from two sources, starting from 1 to 21 days of age. The results indicated that as the glycerin content in the feed increased, there was a significant increase ($P<0.01$) in the relative weights of the digestive tract and liver by day 21. In their study, Sehu et al. (2012) supplemented the diet of broiler chickens with glycerol over a period of 42 days. The chickens were divided into three groups, receiving diets with concentrations of 0%, 5%, or 10% glycerol. Their findings indicated that the inclusion of glycerol had a positive impact on the carcass characteristics, specifically in the organ weights (heart, liver, and gizzard). However, the relative weight of the spleen or the overall carcass yield remained unchanged ($P > 0.05$). Suchý et al. (2011) conducted a study to examine how broiler chickens' production parameters and slaughter characteristics were affected by the provision of pure and untreated glycerol. The researchers found that when the glycerol content in the feed was reduced to 3%, there was a noticeable decrease in the liver weight of 40-day-old broilers. Cerrate et al. (2006) conducted a study to investigate how glycerin at different levels affected the growth performance of broiler chickens. The researchers added glycerin to the diets at three different levels: 0%, 5%, and 10%. The results showed that chickens provided with either 2.5% or 5% crude glycerin experienced a 3% increase in breast yield.

In a study by Bernardino et al. (2015), broiler chickens were given diets with varying levels of glycerin sourced from three different origins (0%, 1.75%, 3.5%, 5.25%, and 7%). The researchers found that glycerin had a significant impact on the leg quarter and breast yields of the broiler chickens. As per Coşkun et al. (2007), broilers that were given a diet supplemented with 5% glycerol had lower weights of the gizzard, liver, and heart compared to the control group. Conversely, broilers fed with 10% glycerol showed an increase in the weights of these organs. Martínez (2012) conducted a study to evaluate the carcass yield of broiler chickens fed a diet supplemented with soy glycerin. The study included two groups: one group received a 2% supplementation from ages 1 to 21, and the other group received a 4% supplementation from ages 22 to 40. The results showed a significant variation in the yield of meat cuts, specifically the

breast and leg, between the two groups. However, the yield of abdominal fat remained consistent. A study conducted by Faria et al. (2013) examined how different amounts of crude glycerin in broiler chicken diets affected the characteristics and quality of the carcass. They added glycerin to the broiler diet at four different levels: 0%, 3%, 6%, 9%, and 12%. The study results indicated that the glycerin levels had an impact on various carcass parameters, such as the weights of the legs and wings, the overall carcass yield, and the slaughter weight.

Garcia et al. (2018) examined the effects of incorporating glycerin into broiler diets. They investigated various parameters of carcass and part yields. The diets were enriched with four different levels of glycerin (0%, 2.5%, 5.0%, and 7.5%) on an as-fed basis. The study revealed that the concentration of glycerin did not have a significant ($P > 0.05$) impact on carcass and part yields. A study conducted by Sehu et al. (2013) examined the effects of glycerin addition in broiler diets at various levels (0%, 5%, and 10%) on various aspects of broiler performance. The study revealed that, with the exception of the gizzard, glycerin supplementation did not have a significant impact on organ weights and carcass attributes, such as moisture content of drumstick and breast meat ($P >0.05$). However, a notable decrease ($P <0.05$) in the gizzard weight was observed with the addition of glycerin, particularly at the 10% level of supplementation. A study conducted by Farrapo et al. (2017) investigated how glycerin affected the live and carcass characteristics performance of of broiler quail. The diets were supplemented with varying levels of vegetable or mixed glycerin (0%, 5%, 10%, 15%, and 20%). The study found that the yield of hearts, livers, gizzards, thighs + drumsticks, and carcasses remained consistent across all treatment groups.

Batista et al. (2013) conducted a study where crude and semi-purified vegetable glycerin was provided to quails aged between 15 and 35 days. The glycerin was given at concentrations of 4%, 8%, 12%, and 16%. The results showed that carcass, breast, and thigh yields remained consistent across all treatments, whereas abdominal fat showed significant variation among the treatment groups. In a study by Freitas (2012), broiler chickens were fed a glycerin-mixed diet. The study found that this diet did not affect the yield of carcass, breast, thigh, back, or abdominal fat. Silva et al. (2012) evaluated the impact of glycerin supplementation on the live and slaughter performance of broiler chickens. They incorporated five levels of glycerin (0%, 2.5%, 5.0%, 7.5%, and 10%) into the diets. The study concluded that carcass, breast, thigh, and wing yields remained unaffected by the varying glycerin levels. Arif et al. (2017) examined how the incorporation of glycerin into the diet of quails affected their slaughter performance. They incorporated varying levels of glycerin (0% to 10%) into the diets. The study found that dietary glycerin had no impact on carcass, breast, and thigh yields, as well as organ weights, including the gizzard, liver, spleen, and bursa of Fabricius. Guerra et al. (2011) investigated how different concentrations of a crude glycerin mixture affected the performance, carcass yield, and composition of broiler chickens. The diets were enriched with the crude glycerin mixture at six levels: 0%, 2%, 4%, 6%, 8%, and 10%. Their findings revealed no variations in the carcass,

thigh, breast, or wing yield among chickens fed up to 10% crude glycerin. However, the percentage of abdominal fat showed a consistent decrease in a linear fashion.

In a study conducted by Topal & Ozdogan (2013), broiler chickens were given diets containing either 0%, 4%, or 8% glycerol for a duration of 42 days. They found no differences in the carcass yield or organ weights. Broch et al. (2018) evaluated how various inclusion levels of glycerin (0%, 3%, 6%, 9%, 12%, and 15%) impacted the live and slaughter performance of broilers. The researchers found that the amount of crude glycerin in their diet did not show any significant effect ($P > 0.05$) on dressing percentage and breast yield, as well as liver weight. However, there was a decrease (7.05%, $P < 0.05$) in drumstick yield, while thigh yield increased ($P < 0.05$). Additionally, a quadratic effect ($P < 0.05$) was observed for glycerin supplementation in wing yield and abdominal fat. In their study, Sopian et al. (2020) examined how various concentrations of glycerin (0%, 5%, or 10%) impact the characteristics of Betong chicken carcasses and internal organs. The results showed that the addition of glycerin did not have a significant impact on the measurements of internal organs or the features of the carcasses ($P > 0.05$).

Effect on Meat Quality

Several studies have explored the impact of glycerin on meat quality attributes. De Souza et al. (2020) examined how different levels of crude glycerin affected the meat quality characteristics of broiler chickens. Glycerin was incorporated into the diets at levels ranging from 0% to 9% during phase 1 (1–21 days), and these inclusion levels were maintained during phase 2 (22–42 days). The results showed that adding glycerin up to 9% did not affect the color of broiler meat. However, the water retention capacity of the carcass was influenced by the level of glycerin used. Garcia et al. (2018) examined the impact of mixed semi-purified glycerin derived from both vegetable and animal sources on the meat quality of broiler chickens. They added four different levels of glycerin (0%, 2.5%, 5.0%, and 7.5%) to the diet. The concentration of glycerin had no significant impact ($P > 0.05$) on most broiler meat-quality characteristics, except for the color of thigh meat. As the level of glycerin inclusion increased, there was a linear decrease ($P < 0.05$) in the yellowness value of thigh meat. Sopian et al. (2020) conducted a study to assess how varying levels of glycerin incorporation affected the carcass characteristics and meat quality of Betong chicken. Birds were given three experimental diets, which contained 0%, 5%, or 10% glycerin. After being chilled for 24 hours, the pH of the meat decreased in all groups. Similarly, varying glycerin levels influenced the color of the meat ($P < 0.05$). However, the shear force and cooking loss were consistent among the diets ($P > 0.05$).

Farrapo et al. (2017) investigated how incorporating glycerin into the diet of broiler quails would affect their performance and the physical characteristics of their meat. The birds were given diets containing varying percentages of glycerin, ranging from 0% to 20%. The researchers evaluated the impact of these different diets on water retention and post-cooking weight loss and found that there were no significant changes in meat quality. Martínez (2012) conducted a study to examine

how including soy glycerin at different levels (2% for ages 1-21 and 4% for ages 22-40) in the diet of broiler chickens would affect the physical characteristics of their meat. The findings of the study showed that adding mixed glycerin to the chickens' feed did not have any impact on the physical characteristics of their meat. Faria et al. (2013) investigated the impact of dietary glycerin on carcass characteristics and the quality of broiler chicken meat. The study consisted of five treatments: T1, which consisted of the basal diet without any glycerin addition; T2, which included 3% glycerin; T3, which included 6% glycerin; T4, which included 9% glycerin; and T5, which included 12% glycerin. Despite increasing the glycerin content in the feed to 12%, the researchers did not notice any significant changes in the color of the broiler chicken meat. Furthermore, there were no observed alterations in cooking-induced weight loss or final pH levels. Bernardino et al. (2014) conducted a study where broiler chickens were given different levels of glycerin. The researchers noticed a consistent reduction in water loss during cooking, with a decrease of 0.0457% for each gram of glycerin addition to per kilogram of feed.

Effect on Serum Biochemistry

Several studies have shown that adding glycerin to poultry diets can affect their performance and blood profiles. De Souza et al. (2020) conducted a study to examine how different levels of glycerin can influence the performance and blood parameters of broiler chickens. The diets were supplemented with six levels of glycerin (0%, 1%, 3%, 5%, 7%, and 9%) during phase 1 (1–21 days), and these same levels were maintained during phase 2 (22–42 days). The blood levels of alanine aminotransferase and cholesterol were higher in birds that were fed glycerin until 21 days of age, as opposed to those that were fed glycerin until 42 days of age. In contrast, Arif et al. (2017) examined the impact of glycerin inclusion levels on the growth and blood cholesterol levels of Japanese quail. They added five different levels of glycerin (0%, 2.5%, 5% 7.5%, and 10%) to the diets. The study results indicated that dietary glycerin at any level did not affect the birds' overall blood cholesterol level. Topal & Ozdogan (2013) conducted a study to examine how different concentrations of crude glycerol affect the blood profile of broiler chickens. The birds were fed diets with varying levels of glycerol (0%, 4%, or 8%) for a period of 42 days. The researchers found that the total cholesterol levels did not change with varying glycerin levels in the diet. Yalçin et al. (2010) conducted a study to examine the impact of incorporating glycerol derived from soybean oil biodiesel into laying hen diets. The study aimed to determine the effects on blood profile. Glycerol was added to the diets at concentrations of 0%, 2.5%, 5%, and 7.5%. The results indicated that varying glycerol levels did not have a significant impact on the blood cholesterol levels of the laying hens.

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Recent Advances in Animal Biotechnology

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1. Introduction

Animal biotechnology is a fast-evolving field that brings positive changes to agriculture, animal health and medicine (Selvakumar et al., 2023). Recent advances in biotechnology that enables the breeding of stronger, healthier animals and the production of safer to more nutritious food. These new technologies help us to face global challenges of food shortages, animal diseases and climate change. Farmers, scientists and veterinarians are now working together to use biotechnology in real-world situations. This progress is no longer limited to laboratories. It is now changing lives. They improve animal welfare; reduce disease, and protect natural resources. The applications reach farms, hospitals and research centers worldwide. These progresses build a safer and more sustainable future for all.

Moreover, one of the most important recent advances in animal biotechnology is gene editing through CRISPR-Cas9 technology. Gene editing allows scientists to make precise changes in an animal's DNA to improve traits of disease resistance and growth. For example, pigs have been successfully edited to resist deadly viruses to save thousands of animals. Cattle are also being edited to grow naturally without horns that prevent painful dehorning procedures. These real-world applications reduce animal suffering and improve farming safety. Therefore, gene editing is fast, affordable and widely used today. They bring hope for healthier animals and safer food. Furthermore, another valuable advancement in animal biotechnology is cloning technology that has become more accurate and efficient. Scientists now safely clone animals with excellent genetic traits of high milk production or strong disease resistance. They help farmers to preserve top-quality animals for future generations without restarting breeding cycles. They also being used to help and save endangered animals of the Przewalski's horse. Recent improvements have made cloning more accessible for farmers and scientists. Therefore, cloning is not only a farm tool that is now a lifeline for saving animals. This technology is reshaping agriculture and biodiversity.

In addition to, the development of transgenic animals is another exciting breakthrough in recent animal biotechnology. These animals are specially designed to produce useful human medicines in their milk, blood, or eggs. For instance, genetically modified goats now produce a life-saving protein that helps prevent blood clots in humans. Transgenic animals are helping patients around

the world receive affordable treatments. Moreover, scientists continue to improve these techniques for higher success rates. The use of transgenic animals is now moving from the lab to real-life medical factories. Animal biotechnology is truly saving human lives.

Firstly, recent progress in animal biotechnology has also improved animal nutrition and feed efficiency. Moreover, scientists have developed specialized feeds and supplements that help animals grow faster and stay healthier naturally. These improvements reduce the need for antibiotics that important for preventing antibiotic resistance in humans. Modern feeds also help animals digest food better to lower waste and pollution. This means farmers can produce more meat, milk and eggs with fewer resources. Therefore, these nutritional advancements give benefits to both small and large farmers worldwide. Animals grow stronger with less harm to the environment. It is now evident that better nutrition is a key success of animal biotechnology.

Secondly, another major achievement is the creation of fast, effective animal vaccines and diagnostic tools. Furthermore, new vaccines mixed into animal feed or drinking water to make vaccination easier and safer. Moreover, rapid disease tests are helping farmers detect deadly diseases within minutes instead of days. They allow early detection and fast treatment that protect animals and prevent farm-wide outbreaks. Diseases of avian flu and foot-and-mouth disease can be controlled more quickly. These advances save both animal lives and farmer livelihoods. Farmers can protect large herds with less effort. Therefore, modern vaccines and tests a big win for animal biotechnology.

Thirdly, environmental sustainability a key focus in recent animal biotechnology advances. Moreover, scientists are working to develop animals that naturally produce less methane; a gas to harm the environment. Therefore, new animal breeds can now grow use less land, water, and feed that make farming more eco-friendly. Researchers also create ways to manage animal waste better to prevent farm pollution. These green technologies reduce the environmental impact of livestock farming. Animal biotechnology offers real solutions to climate change problems. Farmers produce more food with fewer resources. This balance makes animal agriculture more sustainable and planet-friendly.

Finally, recent advances in animal biotechnology have had a big impact on human medicine and biomedical research. Scientists are using genetically modified animals to study serious human diseases of cancer and diabetes. Moreover, researchers also grow pig organs that may soon be used for human transplants that offer hope to patients. These animals help doctors test new drugs safely before human use. Biotechnology is speeding up the search for cures and improves medical safety. Animals are now partners in saving human lives. Ethical and safety concerns remain an important part of animal biotechnology discussions despite the progress. Some people are worried about the safety of eating products from genetically modified animals. Moreover, others worry about animal welfare and the effects on natural ecosystems. There are also concerns that big companies may control biotechnology to leave small farmers behind. Governments and

scientists are working together to make sure biotechnology is used safely and fairly. Furthermore, the recent advances in animal biotechnology and their growing applications are transforming animal farming, medicine and conservation. They are improving animal health and human life through breakthroughs in gene editing, cloning, vaccines, nutrition, and organ research. Therefore, animal biotechnology is now shaping a future of humans and animals can thrive together (Council et al., 2002).

2. Genomic Technologies in Livestock

Genomic selection has revolutionized livestock breeding in using DNA markers to predict animal performance. This technology allows farmers to identify the best calves, lambs or piglets from birth. Moreover, breeding cycles are now faster and desirable traits can be selected with great accuracy. Dairy farmers have significantly improved milk yield and disease resistance using this method. In beef cattle, genomic selection enhances meat quality and feed efficiency. This DNA-driven approach reduces guesswork to save farmers time and money. They support food security in accelerate genetic progress. It helps create healthier and more productive animals for future generations. CRISPR-Cas9 gene editing is widely used to improve animal health and productivity. For instance, one of major success is the development of pigs resistant to PRRS and a costly viral disease. They naturally resist infection and require fewer antibiotics in removing the CD163 gene.

Furthermore, in cattle, CRISPR has been used to create hornless animals to improve welfare and safety. They explore CRISPR to protect poultry and sheep from common diseases. This technology offers a humane alternative to painful farm practices. Gene-edited animals are healthier, more resilient and easier to manage. CRISPR is shaping the future of disease prevention in livestock. They can change single DNA letters without cutting the genome, unlike CRISPR. They minimize risks of unwanted mutations to make them safer for livestock applications. Scientists use these tools to improve heat tolerance and disease resistance in animals. They also are explored to correct inherited defects in farm animals through base editing. These precise techniques support ethical and welfare-friendly genetic improvements. They gained farmer confidence to reduce off-target effects through base and prime editing. They considered the next frontier in safe and responsible animal biotechnology. They threatened for livestock health and productivity as climate change intensifies. Scientists have developed "slick coat" cows with shorter, glossier hair for heat resistance.

These cows can maintain milk production and stay comfortable in hot climates. Genomic tools are also targeting water-use efficiency and feed intake during heatwaves. Heat-adapted animals will become essential equally global temperatures continue to rise. This research helps protect both animal welfare and farm profitability. Climate-ready livestock will help secure future food supplies in challenging environments.

Genomics provides fast and practical solutions to climate-related farming problems. In livestock, particularly cattle that they are significant contributors to greenhouse gas emissions. Moreover, new genomic studies are identifying low-methane-producing animals using DNA markers. Scientists are also exploring ways to edit rumen microbes to cut methane output.

Therefore, Genomic technologies now enable rapid on-farm disease detection in livestock. CRISPR-based diagnostics identify viruses of African swine fever within hours. This quick detection helps farmers isolate infected animals before outbreaks spread and faster responses reduce animal suffering and prevent large-scale economic losses. These portable tools become essential for modern disease management. It reduces dependency on antibiotics and significantly cuts mortality rates. Moreover, genomic diagnostics bring lab-level accuracy directly to the farm environment. This innovation help safeguard animal health more effectively than ever. Farmers benefit from smart farming in use of genomic data and AI-driven sensors for better outcomes. They provide real-time data on temperature, behavior and feeding patterns around the clock. AI analyzes this data alongside DNA profiles to predict health issues early. This system allows farmers to provide individualized care to each animal. For instance, smart farms improve animal welfare and boosting efficiency and productivity. These technologies also help reduce stress and disease across entire herds. Omics approaches combine genomics, proteomics, metabolomics and more for a complete animal profile. This integration reveals complex biological networks that control key traits. Farmers select animals based on both genetic potential and biological performance. For example, omics data helps target genes linked to better digestion or immune strength. This holistic view improves selection accuracy and animal welfare outcomes. They ensure that breeding decisions support overall health and productivity. Furthermore, genomic technologies advance; ethical concerns gain attention worldwide. Consumers generally support gene editing and it improves animal welfare. Countries of Argentina and Japan have approved gene-edited animals without foreign DNA. Transparency, safety testing, and clear labeling are key to public acceptance. Farmers and scientists must work together to ensure responsible use of these tools. Ethical breeding focuses on animal health, sustainability, and consumer confidence. This principle climaxes the equal importance of trust and genetic progress. In addition to, genomic technologies transform livestock farming from the ground up. They enable the development of healthier, eco-friendly livestock and revolutionize animal management through technology. The focus shifts from pure productivity to animal welfare and sustainability (Dwyer, 2020) as shown in table 1.

Table 1. Genomic Technologies in Livestock

Sr#	Technology / Approach	Key Advances & Applications
1	Structural Variation Analysis (SV & CNVs)	Detects large-scale genomic variants impacting growth, immunity, adaptation traits.
2	Copy Number Variation (CNVs)	CNV screening reveals associations with reproductive, pigmentation, growth, disease traits in herbivorous livestock.
3	Third-Generation (long-read) Sequencing	Enables high-quality assemblies, structural variant detection, isoform and methylation profiling.
4	CRISPR/Cas9 and Base/Prime Editing	Precise edits for polled cattle, disease resistance, milk/meat quality, fertility regulation.
5	Genomic Selection & GWAS (including ML integration)	Dense SNP panels + machine learning enhance prediction of complex traits (e.g. fertility, disease).
6	Portable Nanopore Sequencing (e.g. MinION)	Field-based, rapid on-site genomic surveillance and outbreak response.
7	Multi-omics Integration	Combines genomics, transcriptomics, metabolomics, epigenomics for systems understanding of trait regulation.
8	Wearable & Environmental Sensors	Real-time monitoring of activity, health, feeding, welfare with sensor networks and predictive analytics.
9	Tiny ML-based Multimedia Behaviour Recognition	Embedded real-time sensor fusion (vision + accelerometer) for behaviour recognition in livestock.
10	Computer Vision for Individual Identification	AI models (e.g. muzzle-ID, YOLO) achieve >96% accuracy in identifying individual cattle for traceability.
11	Precision Livestock Farming (PLF) Computer Vision Datasets	Public datasets enable AI systems for welfare monitoring, behaviour, and disease detection.
12	Methane / Emission Phenotyping Platforms	Combines Green Feed-type sensors with genetics to identify low-emission cattle.
13	Circular RNA (circRNA) Profiling (eQTL analysis)	Investigates regulatory circRNAs linked to meat quality, health and performance traits.
14	Transgenic & Biopharming Livestock	Livestock engineered to produce pharmaceutically valuable proteins (e.g. human proteins in milk).
15	Functional Genomics for Disease Resistance	Identifies key alleles for innate resistance e.g. PRRS in pigs integrated into breeding selectio

3 Advancements in Reproductive Biotechnologies

Reproductive biotechnologies have evolved rapidly over the past two decades that reshape humans address infertility, genetic disorders and family planning. They once relied on natural conception but now include a spectrum of tools ranging from in vitro fertilization (IVF) to lab-grown gametes and AI-guided embryo selection. They bring real hope to individuals and couples and thought they could never have children. Moreover, reproductive possibilities expand that it's a young cancer survivor freezing ovarian tissue or a same-sex couple envisioning biological parenthood through in vitro gametogenesis (IVG). Traditionally, IVF has experienced a revolution with the integration of technology. For instance, advanced culture media now closely mimic the conditions of the female reproductive tract that allow embryos to thrive in vitro. Vitrification (ultra-fast freezing) has dramatically increased embryo survival rates. AI is now being used to monitor embryos through time-lapse imaging to provide real-time perceptions into developmental quality. This enables doctors to select embryos not just based on appearance that on data-backed potential for success to give couples better odds of pregnancy with fewer cycles. Preimplantation genetic testing (PGT) allows screening embryos for chromosomal abnormalities and inherited diseases before implantation. For example, families with a history of conditions like cystic fibrosis or thalassemia now have the opportunity to prevent transmission to future generations. Therefore, thanks to next-generation sequencing (NGS) that tiny sample from an embryo can now reveal detailed genetic maps. They now find reassurance and success through PGT-A or PGT-M and many couples previously faced repeated miscarriages or failed IVF. This not only increases the likelihood of a healthy baby that also reduces emotional and financial strain.

Furthermore, hormonal stimulation affects each body differently that no two individuals respond the same way. Historically, patients experienced trial and-error protocols to retrieve eggs that often resulting in under or over-stimulation. Machine learning models predict ovarian response using patient-specific data like age, hormone levels, and ultrasound images. This leads to customize treatment plans that maximize egg yield that minimize side effects of ovarian hyperstimulation syndrome (OHSS). This shift means fewer injections, fewer risks and more confidence in their treatment journey. Moreover, for women facing cancer or other life-altering diagnoses of reproductive biotechnologies that offer a more than hope and they offer options. In vitro maturation (IVM) now allows immature eggs to be matured outside the body to reduce the need for hormone-heavy treatments. They considered an experimental procedure and ovarian tissue cryopreservation is now a validated and effective fertility preservation method of with successful pregnancies reported even years after cancer treatment. This is life-changing for young girls and women that want the chance to build a family after surviving serious illness. These techniques affirm the importance of fertility a part of long-term quality of life. IVG is still in early stages and its implications are profound. They created to convert adult skin or blood

cells into induced pluripotent stem cells (iPSCs) that allow scientists to potentially produce lab-made sperm or eggs. Animal studies are progressing rapidly, that even though no human child has yet been born using this method. In vitro gametogenesis (IVG), proven successful and safe in humans that allow same-sex couples, individuals with infertility, and even postmenopausal women to have genetically related children.

In addition to, one of the most futuristic trends in reproductive biotechnology the use of nanoparticles and microrobots. These "spambots," guided in magnets or electric fields can help immobile sperm reach the egg. In lab settings, they've even been used to deliver medication directly to reproductive tissues to minimize systemic side effects. Nanomedicine holds the promise of treating uterine infections or endometriosis with pinpoint precision. Moreover, these advances are still being tested; they represent the blending of engineering and biology in solving deeply human problems. Scientists are now growing miniature versions of reproductive organs called organoids in the lab. These 3D models of the uterus, ovary, and fallopian tubes can n add useful in empathetic unexplained infertility or testing new fertility drugs without using human subjects. For example, researchers can simulate an embryo's implantation in an artificial uterus that help solve cases of recurrent implantation failure. For instance, these organoids lead to bioengineered uterine transplants that offering new hope to women born without a uterus.

Therefore, gene editing tools like CRISPR-Cas9 allow scientists to correct genetic mutations in embryos that theoretically prevent inherited diseases before birth. In laboratory settings, researchers have repaired genes linked to conditions of hypertrophic cardiomyopathy. Germline editing carries profound responsibility that affects not just one child but future generations. Some countries cautiously movie forward under strict regulation that others have banned practices. Global scientific communities agree: rigorous oversight, long-term safety studies, and public dialogue essential before clinical applications proceed. Furthermore, reproductive biotechnologies no longer just about infertility. They're about autonomy, choice, and the right to build a family on one's own terms. AI powered IVF to the possibility of lab-grown gametes that these advancements reflect both scientific ingenuity and deep human desire (George et al., 2025).

4. Transgenic and Cloned Animals

In the last few decades, science has found ways to do things once thought impossible like giving animals new abilities or even creating genetic copies of them. These two innovative approaches of transgenesis and cloning that transform the way we raise animals, treat diseases and protect endangered species. Transgenic animals carry genes from another species to perform useful tasks. Cloned animals are perfect genetic replicas of a donor. Moreover, they form the heart of modern animal biotechnology. This is not merely about advanced science about develops practical and innovative solutions to real-world challenges in agriculture, medicine, and environmental sustainability that also engage with critical ethical considerations. A transgenic animal that carries a foreign gene inserted into its genome to express a novel trait not naturally

present to enhance growth or therapeutic protein production. Gene insertion naturally performed on the embryonic stage that utilizes techniques of microinjection or the more precise CRISPR-Cas9 gene-editing system. For example, a gene from a jellyfish added to make a mouse glow, that fair to track changes in its body. Biotechnological intervention may initially seem personal that often pursues critical goals to include disease prevention, pollution reduction and nutritional improvement. The fundamental objective remains clear to utilize biological advancements for the advancement of animal and human health.

Moreover, in cloning a scientist takes the complete DNA from one animal and places it into an egg cell that has had its nucleus removed. The outcome a new animal that an exact genetic replica of the original and comparable to an identical twin born on a later time. A prominent example of Dolly the sheep was the first mammal cloned from an adult somatic cell in 1996. Today, cloning used to preserve elite farm animals, beloved pets and even endangered species. Many cloned animals suffer from health problems or fail to develop properly. The application of cloning in wildlife conservation is valuable that a species reduced to a minimal number of individuals. Farmers and scientists alike are working to meet the demands of a growing global population. The insertion of genes that enhance growth rates, improve disease resistance or increase feed efficiency enables farmers to produce greater quantities of food to utilize fewer resources. For instance, transgenic pigs that digest phosphorus better help reduce pollution from farms. Cloned animals ensure that the best-performing animals copied and preserved. This means more consistent quality in meat or dairy products. In short, biotechnology allows us to produce healthier and safer food. Moreover, one of the most heartening uses of transgenic animals is in medicine. These animals can be engineered to produce proteins in their milk or eggs that are used as life-saving drugs. An illustrative case is that of a genetically modified goat capable of producing antithrombin to a therapeutic human protein used to prevent harmful blood clot formation. The milk collected and purified into a medicine now used by patients. Some animals also modified to mimic human diseases that help scientists understand conditions of cancer or Alzheimer's. These animals aren't just test subjects that they're partners in the effort to improve human health and their contributions to medical science have already saved lives.

Furthermore, a species disappears due to habitat loss and climate change that cloning offer a glimmer of hope. Scientists have already cloned the black-footed ferret and the Przewalski's horse and both species once nearly extinct. Moreover, researchers have employed decades-old frozen DNA to generate living animals that now able to reproduce and aid in the recovery of dwindling populations. This is particularly useful for a species has very few individuals left and natural breeding is no longer enough to sustain genetic diversity. While cloning alone won't save wildlife that plays a powerful support role in broader conservation strategies. Researchers were surprised to find that genetically engineered animals contribute meaningfully to ecological sustainability. Therefore, farming large numbers of animals produces a lot of waste to use massive amounts of water and emits greenhouse gases like methane. Transgenic animals designed to grow quickly or digest food better reduce this environmental load. For example, fast-

growing salmon reach market size in half the time that need less feed and energy. Pigs that digest phosphorus more efficiently help protect rivers and lakes. These small changes, applied on a large scale that lead to big environmental improvements to show biotechnology isn't just smart and sustainable too. The application of advanced genetic technologies carries significant ethical responsibility that particularly in the context of animal alteration and cloning. Many scientists agree that the welfare of animals must always come first. The introduction of genetically modified or cloned animals to beneficial still raises significant ethical and societal concerns. Public education and strong regulation are so important. Institutions like the FDA and EFSA are responsible for conducting comprehensive safety reviews of biotechnology products to ensure they meet established standards before market release. Transparent research practices, accurate labeling and open communication are essential for fostering public trust in biotechnology. Public acceptance of innovative technologies necessitates transparent communication, education, and gradual acclimatization that particularly in areas concerning food safety and environmental integrity. Some scientists are even working on "de-extinction" bring back extinct animals of woolly mammoth that these advances come with responsibility (Shapiro, 2020).

5. Nanobiotechnology in Animal Health

Nano biotechnology advance field that merges nanotechnology with biological sciences to improve health outcomes. In animal health, it transformed diagnose, prevent and treat diseases in both livestock and companion animals (Council et al., 2005). Researchers utilize nanobiotechnology at the molecular and cellular scale to deliver high targeted therapeutic interventions. Antimicrobial resistance, emerging pathogens and concerns over animal welfare present significant challenges to veterinary medicine that underscore the importance of advanced interventions. The nanoscale approach offers precision tools that minimize waste and maximize therapeutic benefit. Applications range from vaccines and drug delivery to diagnostics and biosensing. Animal agriculture swells meet raised to global demand that benefits significantly from nanobiotechnology's role to enhance productivity through sustainable means. It holds immense potential to redefine the future of veterinary care. Moreover, one of the major benefits of nanobiotechnology is its ability to improve drug delivery in animals. Nanoparticles act as carriers that transport drugs directly to affected tissues or cells to improve their absorption and reduced side effects. This is particularly useful in treatment of chronic or hard-to-reach infections of lungs or gastrointestinal tract. For example, chitosan or polymer-based nanoparticles can slowly release antibiotics over time to ensure steady therapeutic levels. This reduces the frequency of dosing to important for animals that are difficult to handle. Additionally, targeted delivery means fewer drugs are needed overall that lowers the risk of residues in meat or milk. Nanocarriers are also being explored for antiparasitic, antiviral and antifungal agents in livestock and pets. This technology enhances treatment outcomes to support food safety. It is crucial to diagnose diseases quickly and accurately to prevent outbreaks and reduce economic losses in animal agriculture. Nano-diagnostics uses nanomaterials of gold nanoparticles, carbon nanotubes and quantum dots to detect pathogens at very low levels. These nano-sensors can identify

viruses, bacteria, or disease markers within minutes that even before clinical symptoms appear. For instance, nano-diagnostic strips or kits can detect avian influenza or foot-and-mouth disease at the farm level to avoid delays in lab testing. Therefore, portable devices use nanotechnology become more accessible to field veterinarians and farmers. These tools are particularly valuable in remote or resource-limited areas that traditional labs unavailable. They contribute to both early treatment and the reduction of excessive antibiotic use that promote more responsible veterinary practices. The integration of nanotechnology into animal health systems has led to significant improvements in speed, efficiency, and precision.

Furthermore, nanotechnology also revolutionized vaccine development through the creation of nanovaccines. These vaccines use nanocarriers e.g. liposomes, nanoemulsions or polymeric particles to deliver antigens in a more controlled and targeted manner. Nanovaccines generate stronger immune responses compared to traditional vaccines and often require fewer doses. They can also be designed to mimic natural infections to train the immune system more effectively. In animals, nanovaccines have shown promise against diseases of Newcastle disease in poultry and brucellosis in cattle. Some formulations can even be administered orally or intranasally that reduces the need for injections and stress to animals. Nanovaccines are also more stable and easier to store that particularly important in rural or tropical regions. They offer a robust and scalable solution equally the need for herd immunity and disease control grows.

In addition to, animal nutrition another area of nanobiotechnology that make an impact. Nano-sized minerals and supplements can improve the absorption and bioavailability of nutrients in animal feed. For example, nano-zinc and nano-selenium are more easily absorbed than their conventional counterparts that lead to better growth and immunity in livestock. Nanoparticles can also be used to encapsulate vitamins or probiotics that protect them from degradation in the digestive system. This means animals get more consistent benefits from their feed. In poultry, cattle, and aquaculture, nano-nutrition strategies have been linked to improved weight gain, feed efficiency, and disease resistance. Moreover, better nutrient utilization reduces environmental pollution from animal waste. These benefits contribute to healthier animals and more sustainable farming practices.

For instance, they gain attention as antimicrobial agents due to rise concerns about antibiotic resistance. They possess inherent antibacterial, antiviral, and antifungal properties that particularly nanoparticles of silver, copper oxide, and zinc oxide. They disrupt microbial cell walls to generate reactive oxygen species or interfere with DNA replication. In veterinary medicine, these nanoparticles are being explored as alternatives or complements to antibiotics. They applied a coating on animal housing that injected into infected sites or added to wound dressings. For example, silver nanoparticles have shown effectiveness in treating skin infections and surgical wounds in pets and livestock. These materials reduce pathogen load without promote resistance of conventional drugs. They offer a safer and longer-lasting solution to infectious disease management equally a result of their antimicrobial properties. It is vital for

early disease detection and management in farm animals to have continuous health monitoring. Nanobiosensors developed through nanobiotechnology that capable to detect physiological changes of stress, fever or infection in real-time. These sensors often use nanostructured surfaces that respond to specific biomarkers of hormones or metabolites. They placed on animals to track health parameters of body temperature, respiration rate or glucose levels. The data can then be transmitted wirelessly to farm managers or veterinarians for quick action. In dairy cattle, biosensors already used to detect mastitis or metabolic disorders before clinical signs appear. This not only improves animal welfare then also prevents production losses. They will be integral to smart animal health management as precision livestock farming continues to grow.

Moreover, nanotechnology is being applied in animal reproduction and breeding to enhance fertility and reproductive success. Nanoparticles can deliver hormones or fertility drugs more efficiently to reproductive organs. This helps in synchronizing estrus to improve artificial insemination outcomes or manage reproductive disorders. Additionally, nanosensors can detect hormone levels in real-time to add in precise breeding decisions. In male animals, nanotechnology can improve semen preservation and sperm quality in reducing oxidative stress. In vitro fertilization techniques also benefit from nanomaterials that create better microenvironments for embryo development. These advancements contribute to more successful breeding programs that particularly in high-value livestock and endangered species. Nanobiotechnology accordingly supports both genetic improvement and biodiversity conservation in animal populations. It carefully regulated despite its potential in improve animal health through nanobiotechnology.

Therefore, they include concerns about the possible toxicity of nanoparticles to their environmental impact and residues in animal-derived products of milk, meat, and eggs. They are working to develop guidelines for the safe use of nanomaterials in veterinary applications. Research is ongoing to assess long-term safety and biocompatibility in different species. Ethical questions also arise regard the use of advanced technology in animals that particularly involves genetic manipulation or invasive monitoring. Public acceptance of nanobiotechnology in the food chain depends on transparency, safety, and proven benefits. Policymakers are collaborating with scientists to create evidence-based regulations for responsible nanotechnology deployment in veterinary care. The future of nanobiotechnology in animal health is full of possibilities. We expect more sophisticated nanodevices for diagnostics, smart drug delivery systems and bio-interactive feed solutions of research continue. It may soon become a reality personalized veterinary care that treatments tailored to individual animals based on nano-diagnostic feedback. They will further enhance decision-making in animal farming through integration with AI and data analytics. They will depend on affordability, safety validation and ease of use in field settings for widespread adoption. Nanobiotechnology is not a replacement but a powerful complement to exist veterinary tools. They can significantly improve animal health, productivity, and welfare with proper development and regulation that make it a key pillar in the future of sustainable animal agriculture (Buller et al., 2018) as shown in Figure 1.

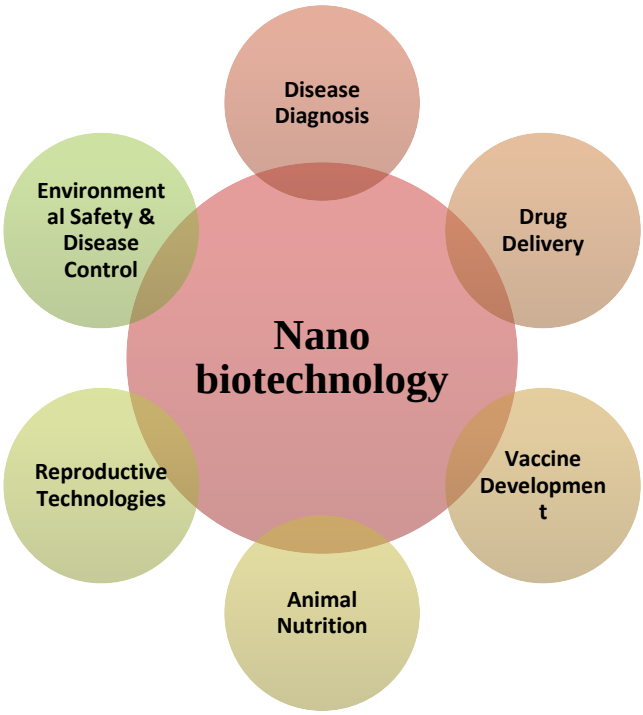

Figure 1. Nanobiotechnology in Animal Health

6. Omics Technologies (Genomics, Transcriptomics, Proteomics, and Metabolomics)

Moreover, the advent of omics technologies that encompass genomics, transcriptomics, proteomics and metabolomics has revolutionized the landscape of animal biotechnology to enable comprehensive and multi-dimensional insights into biological systems. These high-throughput platforms facilitate a transition from reductionist approaches to a systems-level empathetic of cellular and organismal functions. They have accelerated innovations in precision livestock breeding, reproductive efficiency, nutritional optimization, disease resistance, and animal welfare to elucidate the molecular mechanisms behind complex phenotypes. They represent transformative trajectory toward predictive, preventive and personalized veterinary and agricultural practices through their synergistic integration. They offer unique resolution and reliability for data-informed to decision-making in animal science that particularly in the context of increasing global demands for sustainable and ethical animal production. Genomics, the comprehensive study of the entire genome, serves as the cornerstone for molecular breeding and genetic improvement programs. They enable the identification of quantitative trait loci (QTLs) and genomic variants associated with key economic and adaptive traits through high-resolution approaches of whole genome sequencing (WGS), single nucleotide polymorphism (SNP) genotyping and genome-wide association studies (GWAS). The implementation of genomic selection (GS) in livestock has substantially enhanced selection accuracy and genetic gain in leverage genomic estimated breeding values (GEBVs). Additionally, genome editing technologies and most notably CRISPR-Cas9, offer precise manipulation of functional genes,

facilitating the development of animals with improved disease resistance, thermotolerance, or productivity. Moreover, comparative genomics serves as a cornerstone in evolutionary biology in revealing phylogenetic relationships and conserved genomic architecture that simultaneously informing cross-species trait prediction essential to advancements in animal biotechnology and breeding. Transcriptomics entails the global profiling of gene expression through RNA sequencing (RNA-Seq) that enable quantification of messenger RNA (mRNA) levels, long non-coding RNAs (lncRNAs), and alternative splicing events under physiological or pathological stimuli. For instance, transcriptomic data capture the dynamic regulatory responses of cells and tissues to provide vision into transcriptional reprogramming in response to infection, thermal stress, or nutritional shifts. This layer of omics is indispensable for unraveling gene regulatory networks (GRNs) associated with development, metabolism, immunity, and reproduction. For example, transcriptome analysis of ovarian follicles or early embryos provides mechanistic empathetic of gametogenesis, folliculogenesis, and embryo viability. Moreover, differential expression analysis between phenotypic extremes enhances the functional annotation of candidate genes identified through GWAS that bridg the gap between genotype and phenotype.

Furthermore, proteomics provides a high-input platform for the systematic identification, quantification, and functional annotation of proteins and their post-translational modifications (PTMs) (Koenig et al., 2022). Proteins serve the direct effectors of cellular processes that proteomic analyses offer indispensable visions into phenotypic plasticity, stress responses, immune mechanisms, and metabolic regulation. Techniques of liquid chromatography–tandem mass spectrometry (LC-MS/MS) and isobaric tagging (iTRAQ, TMT) allow for the identification of differential protein abundance and interaction networks. In animal biotechnology, proteomics has been instrumental in discovering biomarkers for early disease detection that kind of vaccine-induced immunity and characterize the proteome of reproductive fluids of seminal plasma and follicular fluid. These analyses provide a systems-level empathetic of protein function and interaction to reveal key nodes and pathways vulnerable to stress or mutation and guiding evidence-based intervention planning. For instance, these metabolites serve dynamic indicators of cellular function that capture the cumulative effects of upstream molecular processes. NMR spectroscopy, GC-MS and LC-MS serve as powerful tools for capturing dynamic metabolic changes that enhance our kind of organismal health and stress resilience. Biochemical patterns revealed through metabolomics enable the detection of condition-specific markers associated with disorders, stress adaptation and nutrient-related deficiencies. In reproductive biology, analysis of embryo-conditioned media has identified key metabolites predictive of embryo competence and implantation potential. Moreover, metabolomic describe of ruminal fluid, plasma, and milk supports the formulation of precision diets and early detection of subclinical disease. This omics layer offers high-resolution visions into physiological states to enable real-time monitoring of disease progression, stress responses, or metabolic shifts. The integration of genomics, transcriptomics, proteomics, and metabolomics constitutes a multi-omics systems biology framework that allow for the deconvolution of complex traits through hierarchical molecular layering. Integrative approaches that combine computational modeling, machine

learning, and network inference algorithms enable the reconstruction of causal gene-to-phenotype pathways and the prediction of biological behavior under defined conditions. Multi-omics integration, through data fusion techniques of canonical correlation analysis (CCA), partial least squares regression (PLS-R), and Bayesian network modeling that enables the identification of regulatory hubs, molecular modules, and pathway bottlenecks. These integrative analyses are crucial for precision phenotyping that enable robust trait prediction and targeted interventions in breeding, nutrition, and health management. The expansion of omics datasets, coupled with their integration under standardized ontologies and adherence to FAIR (Findable, Accessible, Interoperable, Reusable) data principles that will enhance reproducibility and translational potential. The application of omics technologies in veterinary science has revolutionized the diagnosis, surveillance, and control of infectious and non-infectious diseases. Therefore, genomic epidemiology facilitates the tracking of pathogen evolution, virulence markers, and antimicrobial resistance genes. Transcriptomic and proteomic analyses uncover host-pathogen interaction dynamics to enable the development of molecular diagnostics and immune-response-informed vaccines. The high temporal resolution of metabolomics allows for the timely identification of shifts in immunometabolic status and early-stage pathological processes. For example, multi-omics approaches have elucidated molecular mechanisms underlying bovine mastitis, porcine respiratory disease, and avian coccidiosis. The integration of omics with point-of-care diagnostics, biosensors, and wearable devices promises a future of real-time animal health monitoring, reducing morbidity, mortality, and production losses while enhancing biosecurity.

In addition to, omics platforms have significantly advanced reproductive biotechnology; improve our awareness of the molecular determinants of fertility, embryo viability, and gestational success. Genomic selection facilitates the identification of alleles conferring superior reproductive traits that transcriptomic and proteomic analyses of gametes, zygotes, and reproductive tissues reveal biomarkers for fertility potential and developmental competence. Non-invasive metabolomic outline of spent embryo culture media allows for the evaluation of embryonic developmental competence without compromise embryo integrity. These technologies have enhanced the efficiency of assisted reproductive technologies (ART) include artificial insemination (AI), in vitro fertilization (IVF), and embryo transfer (ET). Moreover, omics-based assessments of sperm proteome and seminal plasma metabolome provide critical markers for male fertility diagnostics that enable personalized breeding strategies. The field of nutrigenomics investigates the interactions between dietary components and gene expression to enable the development of precision feeding strategies. Omics technologies elucidate the molecular basis of nutrient-gene interactions, enabling the identification of diet-responsive transcripts, proteins, and metabolites that mediate growth, immune competence, and stress resilience. Transcriptomic analyses of intestinal mucosa, for instance, reveal pathways modulated by prebiotics, enzymes, and phytochemicals. Proteomic outline detects stress-related proteins associated with dietary imbalances that metabolomics evaluates ruminal fermentation profiles, nutrient bioavailability, and feed efficiency. The integration of omics data informs the

formulation of species-specific and even individualized diets to optimize nutrient utilization, production metrics, and environmental sustainability in minimize nitrogen and methane emissions. The trajectory of omics in animal biotechnology increased to orient toward real-time, on-farm applications that driven to the convergence of artificial intelligence, edge computing, robotics, and cloud-based data platforms. The deployment of portable sequencers, AI-powered diagnostic tools, and automated phenotyping systems is set to redefine livestock management. These technological advancements necessitate rigorous ethical considerations that particularly with regard to genome editing, data ownership, biocontainment, and animal welfare. Transparent governance frameworks, stakeholder engagement, and interdisciplinary collaboration are imperative to ensure equitable and responsible innovation (Lescrauwaet et al., 2022).

7. CRISPR-Cas Systems and Precision Animal Breeding

CRISPR-Cas systems, once a microbial immune mechanism against invade viruses that have evolved into the most powerful genome editing tool in modern biotechnology (Javed et al., 2018). The movement of these biological systems from native bacterial contexts to controlled laboratory settings underscores their utility in driving bio-based innovation. In animal biotechnology, CRISPR has opened a new era of "precision breeding," that traits can be designed and directed rather than randomly inherited. This genome-editing approach offers a rapid and targeted alternative to conventional breeding in allow specific modifications at the nucleotide level. It allows scientists introduce to remove or alter genes in controlled manners that bring predictability to livestock genetics. The integration of CRISPR into livestock production systems holds promise for optimizes traits related to growth, immunity, and welfare. The rise of CRISPR signals not only a technological advancement and also a profound shift in our scientific kind of heredity and gene function. This system relies on a simple yet highly effective mechanism that guide RNA binds to a complementary DNA sequence to enable the Cas9 protein to cleave the genome at an exact location. The outcome of genome editing determined in the cellular response and error-prone NHEJ seals the break, typically inactivating the target gene, or high-fidelity HDR integrates a donor template to introduce specific genetic changes. These natural repair process scientists have learned to harness. Genome editing with CRISPR-Cas9 are similar to molecular microsurgery that allowing researchers to cut, alter and reassemble DNA with unprecedented precision. Today, we are not merely observing genetics; we are actively rewriting it. In animal systems, this capability enables the purposeful design of traits with a high degree of molecular precision and predictability. In traditional livestock breeding, improve a trait of muscle growth or milk production can take decades of selective mating and still come with unintended genetic baggage. CRISPR changes that equation. For example, researchers have successfully disabled the *myostatin* gene in pigs and cattle that result in significantly increased muscle mass and leaner meat without harm the animal's welfare. Genes involved in fat deposition, nutrient utilization, and milk protein synthesis now amenable to targeted editing that enable fine-tuned control over economically important traits. These aren't just hypothetical uses that they are already being demonstrated in experimental herds. Moreover, CRISPR gives us the

ability to improve animal productivity without compromising health or biodiversity. In effect, we're moving from chance to choice that designing traits with full knowledge of their genetic substructures. Diseases in farm animals not only reduce productivity and also cause immense suffering and lead to the overuse of antibiotics. CRISPR presents a way to prevent disease at its genetic root. For example, pigs have been engineered to lack the CD163 receptor a key entry point for the PRRS virus that make them immune to one of the most devastate diseases in swine farming. Researchers are also exploring gene-editing strategies to enhance disease resistance in livestock that engineering cattle resistant to bovine tuberculosis and chickens immune to avian influenza. These interventions don't just benefit the animal that they also protect the food chain and reduce the risk of zoonotic disease transmission. This proactive model could redefine animal health management in the 21st century.

Furthermore, the use of CRISPR in animals and other transformative biotechnologies that brings ethical concerns to the forefront, particularly regarding welfare, naturalness, and long-term consequences. There's also the matter of public acceptance will consumers embrace meat, milk, or eggs from gene-edited livestock. The answers require transparency, ethical deliberation, and global dialogue. Scientists, ethicists, farmers, and policymakers must collaborate to establish clear, science-based regulatory systems. Therefore, CRISPR can support ethical breeding goals of eliminate hereditary diseases or improve resilience without genetic manipulation that compromises animal integrity. The societal acceptance of genome editing hinges on building trust through open dialogue, ethical responsibility, and regulatory oversight. Traditionally, animal breeding has given us many of the productive breeds we know today. This process is inherently slow, imprecise, and subject to the randomness of inheritance. Traits are often polygenic controlled in multiple genes and confounded by environmental factors. And importantly, CRISPR doesn't require the introduction of foreign DNA, differentiating it from transgenic approaches. It offers a clean, fast, and effective alternative that complements—rather than replaces—conventional breeding. For complex traits, CRISPR can target key regulators, increasing the efficiency of traditional programs. Moreover, One of CRISPR's most remarkable capabilities multiplex the ability to edit several genes at once. In animals, this opens doors to solve complex biological challenges in a single generation. This is no longer science fiction. Researchers are already experimenting with simultaneous edits to multiple loci in pigs and poultry. The potential is staggering and it also demands careful orchestration to avoid unwanted interactions between genes. These approaches allow us to sculpt entire physiological shapes rather than tweak traits in isolation an unprecedented leap in animal breeding science.

Therefore, it is not a one-size-fits-all task to apply CRISPR across animal species. Species diversity entails distinct reproductive quirks, embryonic development timelines, and genetic complexities. For instance, zygote microinjection works well in pigs and cows that edit birds remains technically difficult due to their hard-shelled eggs. Moreover, aquaculture species of salmon require entirely different delivery methods and ethical considerations. The progress is being made. Techniques, ranging from electroporation in sheep embryos to gene editing in

chickens via primordial germ cells that are being refined in scientists to suit each species' unique biology. These adaptations are keys to make CRISPR a truly universal tool in animal breeding. The potential of CRISPR extends far beyond agriculture. In conservation biology, genome editing may one day help rescue endangered species from extinction. For example, CRISPR used to restore genetic diversity lost through inbreeding or reintroduce traits needed for survival in changing climates. Scientists are also exploring and help eliminate invasive species or even revive extinct animals. These ideas come with enormous ecological, ethical, and technical challenges. Gene editing in one animal could trigger ripple effects throughout entire ecosystems Conservation applications must therefore be pursued with extraordinary caution, rigorous ecological modeling, and public consultation. The future may bring breeds customized not just for productivity that for climate adaptability, welfare optimization, and environmental compatibility. Integration of AI, omics technologies and real-time genome monitoring may soon guide in an era of fully digitized breeding programs that each animal engineered according to predictive models and practical demands. This future depends on more than technology. It will require international cooperation, regulatory clarity, public dialogue, and a strong commitment to ethical science (Roco, 2008).

8. Biotechnological Approaches to Vaccine Development

Furthermore, the evolution of biotechnology has revolutionized vaccine development that transitioning the field from empirical formulations to rational and targeted design (De Gregorio & Rappuoli, 2014). Traditionally, vaccines derived from whole inactivated or attenuated pathogens, although historically effective, often pose limitations in terms of biosafety, immune variability, and complex manufacturing processes. Modern biotechnological strategies offer sophisticated alternatives that allow for precision in antigen selection, customization for host species and rapid scalability. In the context of animal health that innovations are particularly vital to give the diversity of livestock, the urgency of zoonotic control, and the economic imperatives of the global animal agriculture industry. Recombinant subunit vaccine technology stands at the forefront of this transformation that enable the expression of selected antigenic proteins in heterologous system of *E. coli*, yeast, insect, or mammalian cells. This approach eliminates the need to cultivate the entire pathogen, thereby significantly improve biosafety and simplify regulatory compliance. For example, the recombinant glycoprotein G-based rabies vaccine exemplifies purified that structurally stable proteins can elicit robust immune responses in domestic and wild animal reservoirs. These subunit vaccines also offer flexibility in formulation that enable the combination of multiple antigens or fusion proteins to target complex pathogens. Genetic vaccines that include DNA and mRNA platforms to represent a paradigm shift in veterinary immunoprophylaxis. These vaccines encode antigenic sequences that are translated within the host cells to lead the endogenous antigen production and presentation via major histocompatibility complexes (MHC). DNA vaccines have shown notable success against infectious diseases in aquaculture (e.g., infectious hematopoietic necrosis virus) and livestock (e.g., foot-and-mouth disease). mRNA vaccines, although more nascent in veterinary

applications that offer distinct advantages of rapid development timelines, cell-free manufacturing, and potent stimulation of both humoral and cellular immunity to hallmarks of protective and durable responses.

Moreover, viral vector-based platforms utilize modified viruses as delivery vehicles to introduce foreign antigen genes into host cells that mimicking natural infection without causing disease. These vectors that include non-replicating adenoviruses, lentiviruses, and poxviruses, are engineered for safety and can induce strong mucosal, systemic, and cellular immunity. A prominent example in veterinary medicine is the recombinant canarypox-vectored vaccine, successfully used against equine influenza and canine distemper. The modularity of viral vectors enables multivalent constructs and prime-boost regimens to offer exceptional versatility in combating antigenically diverse or intracellular pathogens. The integration of reverse vaccinology that support in high-input genomics and immunoinformatics has fundamentally altered antigen discovery. This approach bypasses traditional culture-based methods to allow the computational screening of entire pathogen genomes to identify conserved, surface-exposed, and immunologically relevant proteins. This methodology, particularly useful against complex or unculturable pathogens has accelerated vaccine design for *Mycoplasma hyopneumoniae*, *Pasteurella multocida*, and *Lawsonia intracellularis*. These advancements streamline the identification of protective antigens to make vaccine development faster and more effective that even for emerging or re-emerging animal diseases. It is equally crucial to innovate adjuvant systems that they enhance vaccine immunogenicity in activating innate immune receptors and promoting efficient antigen presentation. Traditionally, aluminum salts are now complemented in advanced adjuvants of saponins (e.g., Quil-A), TLR agonists (e.g., CpG motifs), emulsions (e.g., Montanide™), and cytokine-based formulations. In veterinary vaccines, adjuvant choice is pivotal not only for efficacy and also for safety, cost, and species-specific immune modulation. Biotechnologically engineered adjuvants improve vaccine potency, reduce antigen dose requirements, and enable long-lasting protection that particularly in high-throughput livestock vaccination settings.

In addition to, nanotechnology further extends the reach of biotechnology in offer smart delivery vehicles of virus-like particles (VLPs), lipid nanoparticles (LNPs), biodegradable polymers, and liposomes. These nanosystems enhance antigen stability, facilitate mucosal uptake, and often act as self-adjuvanting platforms. VLPs mimic the architecture of native viruses without carrying genetic material that provide both safety and immunogenic fidelity. For instance, successful veterinary applications include VLP-based vaccines for porcine circovirus and bluetongue virus. These platforms support controlled release, targeted delivery, and scalable manufacturing to make them particularly suitable for modern livestock production systems. A pivotal advancement in veterinary vaccine biotechnology is the development of DIVA (Differentiating Infected from Vaccinated Animals) vaccines. These vaccines are genetically designed to include or exclude specific antigenic markers that allow diagnostic assays to distinguish between naturally infected and vaccinated animals. This is particularly important for notifiable diseases of pseudorabies,

classical swine fever, and bovine herpesvirus-1 that eradication and trade policies demand precise epidemiological surveillance. DIVA-compatible vaccines that combined with companion serological or molecular diagnostics to enable mass vaccination without compromising disease monitoring frameworks. Mucosal and edible vaccines are emerging as innovative alternatives for non-invasive immunization that particularly in species of individual injection is impractical. These vaccines stimulate both systemic and mucosal immunity at primary pathogen entry points. Biotechnology has enabled the production of antigens in transgenic plants, edible algae, and genetically modified lactic acid bacteria, offering cost-effective, stress-free, and scalable solutions. Edible vaccines for poultry diseases of Newcastle disease and infectious bursal disease are currently experience trials that show great potential for mass administration in hatcheries and aquaculture facilities. It is anticipated that the convergence of biotechnology with artificial intelligence, CRISPR/Cas genome editing, and systems biology will define the next generation of veterinary vaccines. AI algorithms enable predictive modeling of epitopes and immune responses that CRISPR allows for the rational attenuation of pathogens or targeted insertion of immune-modulating genes. Systems biology approaches provide holistic insights into host-pathogen interactions to facilitate the design of precision vaccines tailored to species-specific immune landscapes. These synergistic technologies will not only improve animal health and productivity and also play a central role in global as shown in figure 2.

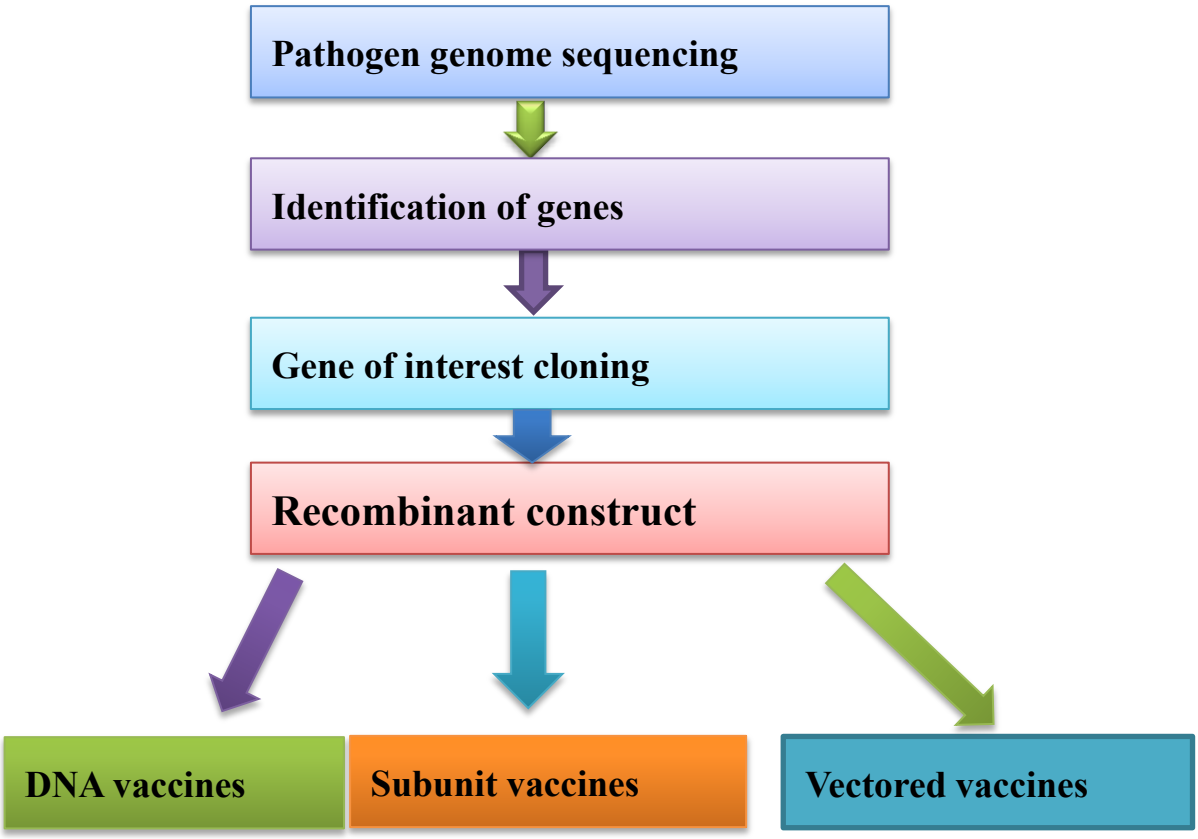

Figure 2. Biotechnological Approaches to Vaccine Development

9. Applications in Animal Nutrition and Feed Biotechnology

Moreover, in today's world of rapidly evolving agriculture to feed biotechnology has emerged as a powerful solution to the challenges faced in the animal nutrition sector (Den Hartog & Sijtsma, 2013). Livestock feeding today is no longer limited to fulfill basic nutritional needs that it now emphasizes maximizing feed efficiency, enhance animal health, and reduce environmental impacts. Biotechnology brings a scientific edge to feeding practices in introducing precision tools that enhance nutrient absorption, reduce waste, and even promote animal well-being. The industry has shifted its focus from quantity to quality and sustainability that through the development of smarter enzymes or the repurposing of agricultural by-products into high-value feed ingredients. In this landscape, feed is treated not just as fuel for animals and also as a strategic input for the future of livestock farming. Biotechnology becomes the bridge between traditional nutrition and smart eco-conscious farming. For instance, one of the most transformative applications of biotechnology in feed science is the use of tailored enzymes. These enzymes of phytases and xylanases to act like biological scissors that cut through tough plant fibers and release nutrients for animals would otherwise excrete unused. For example, monogastric animals of poultry and pigs cannot digest phytate-bound phosphorus on their own with the help of phytase enzymes that this vital mineral becomes available to reduce both supplementation costs and environmental pollution. These enzymes are not one-size-fits-all thanks to genetic engineering that they can be customized for different species, diets, and digestive environments. The result is a smarter, more targeted approach to feeding—one that ensures animals extract maximum nutritional value from every bite. The role of biotechnology goes beyond aiding digestion that it actively shapes and sustains the gut microbiome, a key driver of immunity and nutrient absorption. Probiotics beneficial live microbes and prebiotics compounds that feed those microbes are being used to promote a balanced gut flora. A healthy gut doesn't just support digestion that it builds immunity, reduces stress, and improves overall performance. This microbial management is particularly important as the livestock industry moves away from antibiotic growth promoters due to public health concerns. With biotech-driven feed additives, animals can achieve growth and health naturally that through strengthened biological defenses. These microbial allies offer a more humane, sustainable, and health-conscious approach to animal production one that aligns with both science and ethics.

Furthermore, in the field of crop biotechnology scientists are now engineering feed plants to be more nutritionally efficient and resilient. These advancements of maize varieties engineered for higher lysine levels and soybeans with enhanced protein bioavailability that are already being realized to transform feed quality at its source. Genetic engineering enables feed crops to be designed with specific animal needs in mind to reducing the need for costly supplements. The ability to embed bioactive compounds of omega-3 fatty acids directly into plant tissues represents a significant advancement that transform conventional feed into functional food. This not only supports animal health and also enhances the quality of animal products consumed by humans. The integration of plant and animal biotechnology represents a seamless farm-to-fork

innovation to aligning crop development with animal nutrition and food quality. For instance, waste in one system can be treasure in another and feed biotechnology thrives on this principle. Fermentation enables the transformation of low-value agricultural residues of sugarcane bagasse, rice husks, and fruit peels into high-protein feed through the metabolic activity of specialized microorganisms. Yeast and filamentous fungi serve as functional microbes that both fortify the substrate with vital nutrients and break down complex indigestible fibers. This process not only creates affordable feed alternatives and also helps reduce environmental waste. Single-cell protein (SCP) refers to microbial biomass that is particularly valuable in regions experiencing protein shortages for livestock. This approach creates a win-win scenario to deliver cost-effective and sustainable protein for livestock that alleviating pressure on conventional feed resources. Environmental sustainability is no longer a luxury and an essential requirement. Biotechnological tools are being used to reduce the environmental impact of feeding livestock that particularly concerning nitrogen and phosphorus waste and greenhouse gas emissions. Feed additives like methanogenesis inhibitors, precision enzymes, and specialized microbial cultures help animals utilize nutrients more efficiently that result in lower excretion levels. For example, methane emissions can be significantly reduced in altering fermentation pathways in the rumen. This not only makes feeding more eco-efficient and also helps producers meet tightening regulations on environmental performance. The complex microbial world of the rumen holds vast untapped potential, and biotechnology is the key to unlocking it. This means more energy from the same amount of feed to improve fiber digestion, and reduced methane output. Modulation of the microbial population enables a shift in fermentation pathways that benefits both animal health and environmental sustainability. This approach doesn't just improve feed efficiency it transforms the rumen from a black box into a controllable system.

Therefore, animal nutrition today extends far beyond growth that it encompasses fertility, immunity, stress resilience, and product quality. Biotechnologically produced feed additives of protected amino acids, peptides, and bioactive minerals support these broader physiological functions. Nano-encapsulation techniques are used to protect sensitive nutrients from degradation in the gut to ensure that they reach target tissues effectively. For instance, rumen-protected choline plays a vital role in liver health and reproductive success in dairy cows. Trace mineral that provided in bioavailable forms to significantly enhance eggshell quality in poultry and improve semen quality in breeding bulls. These specialized nutrients elevate the concept of feed from mere sustenance to a tool for targeted performance enhancement. Biotechnology has redefined that it means to feed animals well. It brings precision, sustainability, and scientific elegance to a practice that was once purely empirical. The field of animal nutrition has been revolutionized by biotechnology to encompass innovations of enzyme engineering, microbial supplementation, crop genetic enhancement, and nutrient tailoring.

10. Future Perspectives

Modern livestock breeding is now undergoing a revolution with site-specific nucleases of CRISPR/Cas9 and TALENs (Wani et al., 2023). These tools enable precise, efficient edits in embryos or stem cells that drastically reduce the time to develop animals with traits of disease resistance or enhanced feed efficiency. Researchers predict that in the end of this decade that multiplex editing targets multiple genes at once will become routine. It is essential to establish regulatory clarity and ethical oversight to ensure that advancements do not compromise safety or animal well-being. Scientists are engineering pigs already preferred over primates due to breeding traits and pathogen risks to become viable organ donors. Gene-editing removes endogenous retroviruses and histocompatibility antigens to reduce human immune rejection. These innovations have already led to the first pig-to-human heart and kidney transplants in clinical settings. The next 5–10 years are expected to bring more precise genetic modifications that enhance graft survival and enable the development of tailored disease models to marke a significant advancement in regenerative medicine. Cellular agriculture is taking shape through cultured meat a sustainable alternative to conventional protein. They combine stem cell lines, scaffolds, and AI-driven bioprocess optimization that teams are significantly shortening development cycles. AI now helps in predicting media formulation and scaffold texture that slash trial-and-error experiments. CRISPR/Cas9-driven edits are improving performance traits in fish like tilapia and carp targeting disease resistance, accelerated growth, sterility (to prevent escapees breeding), and climate resilience. In parallel, high-throughput phenotyping and biosensor networks will facilitate data-driven selection that enable farms to rapidly adapt fish stocks to evolve environmental conditions maintain biodiversity through eDNA surveillance. They are transforming animal health that field-ready diagnostics of portable PCR, biosensors, and rapid genomic sequencers enable immediate that on-site disease detection and surveillance." Now, vets and fish farmers can detect infectious agents, track zoonotic threats, and anticipate outbreaks in real-time. The next frontier integrates these tools with AI-driven predictive modeling and “lab-on-a-chip” platforms embedded in wearable collars or feed to create 24/7 health monitoring systems. This will enable proactive, rather than reactive, disease management. Reproductive technologies from IVF and embryo transfer to somatic cell cloning are increasingly integrating with genomic selection to enhance the productivity of livestock and support the conservation of endangered species. Cryopreservation of gametes and embryos is establishing global bio banking initiatives. A new frontier gene-edited surrogate sires carrying donor germ cells is projected to revolutionize genetic dissemination to helping remote farmers access elite genetics and wildlife researchers restore genetic diversity in dwindling populations.

Therefore, the future of breeding lies in melding genomics, transcriptomics, proteomics, and metabolomics. They are decoding critical traits of fertility, stress resilience, and disease resistance through cutting-edge research in cattle, buffalo, and fish. AI-powered algorithms will fuse omics and environmental data to forecast animal performance to minimize blind selection methods. This approach shortens breeding windows, turbocharges genetic gains, and fosters

climate-adapted breeds. Livestock farms of the future will be highly instrumentation-rich, featuring IoT sensors, robotics, and smart cameras monitoring behavior, feeding, and health in real-time. They will detect lameness, fever, appetite loss, and even emotional stress that prompt and compassionate treatment through AI-driven monitoring. Robotics for feeding, milking, and cleaning are set to minimize human error, reduce animal stress, and enhance labor efficiency in livestock operations. Genomic technologies accelerate from gene-edited animals to de-extinction efforts transparent and inclusive governance is critical. Policymakers must navigate welfare risks, ecological balance, and fairness that harmonize international regulations. Public engagement, digital traceability, and participatory policy design will be vital in building consumer trust and socially responsible biotech deployment. Animal biotechnology is increasingly aligned with climate and sustainability goals. Examples include capturing low-methane cattle genes, engineering disease-resistant poultry, and refining aquaculture to minimize environmental footprints. Life-cycle assessments and carbon accounting will guide future developments that particularly essential in nations of Pakistan livestock is a key income source. It will ensure equitable and resilient food systems for all to bridging access gaps to emerging biotech across the Global South. These ten refined, human-centric paragraphs draw upon recent literature to tell a compelling story that animal biotechnology is transitioning from its experimental roots into everyday tools for food, medicine, conservation, and welfare provided society shapes its governance and ethics accordingly (Zeshan, 2019).

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Antimicrobial Resistance: Exploration of Multiple Superbugs in Asia

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Introduction

Antibiotic resistance, antiviral resistance, and resistance to antiparasitic medications are all major concerns related to the rising danger of antimicrobial problems across Asia, particularly Pakistan. Antibiotic, antiviral, and antiparasitic medicine abuse and misuse are major problems in many Asian countries, including Pakistan. This may happen in agriculture as well as in the healthcare of people and animals. One factor contributing to the improper use of antibiotics is the absence of strong regulatory restrictions on their distribution and sale. Common issues include the availability of substandard or counterfeit medications and unrestricted access to medicines. Antimicrobial overuse occurs in some areas due to inadequate healthcare infrastructure, unavailability of high-quality diagnostics, and inappropriate prescribing practices. Antibiotic usage in agriculture, especially raising animals, may lead to the emergence of bacterial strains that are resistant to treatment and may make their way up the food chain and into human bodies. Asiatic nations, such as Pakistan, often have a high prevalence of infectious illnesses. Resistance may arise because of the extensive use of antibiotics to treat illnesses. Antimicrobials are necessary because poor sanitation and hygiene habits may cause illnesses to spread. This adds to the total amount of antibiotic resistance. The transmission of resistant bacteria across borders is facilitated by increased worldwide travel and commerce, making it a global challenge requiring international cooperation.

Superbugs are types of microorganisms that are resistant to the actions of antibiotics, antivirals, or other treatments. They might be bacteria, viruses, parasites, or other microbes. The phrase is often used about bacteria that have developed resistance to many antibiotics, making the treatment of diseases brought on by these organisms difficult. Antibiotic resistance develops because of natural selection. When susceptible bacteria are treated with antibiotics, resistant forms of the bacteria may persist and proliferate. Antibiotic abuse or misuse may cause these resistant strains to proliferate over time, decreasing or even eliminating the effectiveness of traditional therapies (Mancuso et al., 2021). Superbugs are a danger to public health because they may make illnesses that were previously curable more challenging to treat. This may result in protracted sickness, more medical expenses, and a higher chance of infection transmission. The

emergence of superbugs highlights the need to use antibiotics responsibly, maintain good hygiene, and conduct continuing research to create novel therapies and countermeasures against antibiotic resistance. Superbugs are microbes that are hard to cure because they have become resistant to many treatments. Superbug bacteria include:

Methicillin-intolerant *Staphylococcus aureus* (MRSA)

Human nasal passages and skin are frequently host to the bacteria *Staphylococcus aureus*. It can cause a variety of diseases, from mild skin infections to potentially fatal disorders including bloodstream infections, pneumonia, and endocarditis, even though it can harmlessly reside in many people. The antibiotic family known as beta-lactams, and more especially the penicillinase-resistant penicillins, includes methicillin. Penicillin-resistant strains of *Staphylococcus aureus* were the source of the infection for which it was first created. On the other hand, MRSA emerged when certain strains of *Staphylococcus aureus* developed the *mecA* gene, making them resistant to methicillin and other related medications. This strain of the bacterium has become resistant to methicillin and other widely used medications. Both the general and hospital environments may harbor MRSA infections (Ippolito et al., 2010).

***E. coli* That is Resistant to Many Antibiotics**

Common bacteria *E. coli* is responsible for several diseases, including foodborne illnesses and urinary tract infections. The ability of *E. coli* to withstand the effects of antibiotics is often referred to as resistance, and it is a serious problem in both clinical and environmental settings. Like many other bacteria, *E. coli* may become resistant in a variety of ways. It may be difficult to treat some strains of *E. coli* since they have become resistant to certain drugs (Reygaert, 2017).

***Klebsiella pneumoniae* Carbapenemase (KPC) Generating Bacteria**

An enzyme called *Klebsiella pneumoniae* carbapenemase (KPC) is synthesized by some bacteria, most notably *Klebsiella pneumoniae,* and it gives them resistance to the antibiotic carbapenem. A class of antibiotics known as carbapenems is frequently utilized as a final option to treat severe infections brought on by bacteria that are resistant to most drugs. But because KPC-producing bacteria can cause illnesses that are hard to cure and enhance mortality as well as morbidity, their rise presents a serious problem for healthcare systems. Pneumonia and other illnesses may be brought on by the bacterium *Klebsiella pneumoniae*. Certain strains secrete an enzyme known as carbapenemase, which degrades and renders useless carbapenem antibiotics (Fattouh et al., 2015).

Vancomycin-resistant Enterococci (VRE)

Enterococcal strains known as vancomycin-resistant enterococci (VRE) are resistant to the drug vancomycin, which is frequently prescribed for treating infections brought on by Gram-positive bacteria, including Enterococcus species. Both the environment and the human gut are habitat to

enterococci by nature. Although they often do not affect healthy people, they can lead to infections, especially in those who have had invasive medical operations undertaken or have compromised immune systems. Since these germs can be difficult to cure, the rise of VRE poses a serious threat to healthcare environments. The development of certain genes, such as *vanA* or *vanB*, which encode enzymes that alter the structure of enterococci's cell walls and render them resistant to the effects of vancomycin, is frequently the cause of vancomycin resistance. These bacteria, which are often found in the intestines, may infect other regions of the body. A common antibiotic for treating severe infections, vancomycin, has caused resistance in some strains of the bacteria (O'Driscoll & Crank, 2015).

Producers of Extended-spectrum Beta-lactamases (ESBLs)

This category includes microorganisms that manufacture extended-spectrum beta-lactamases, including *E. coli* and *Klebsiella pneumoniae*. These enzymes can degrade a wide variety of antibiotics, including beta-lactams like cephalosporins and penicillins. These instances demonstrate the variety of superbugs and the significance of combating antibiotic resistance through infection control strategies, prudent antibiotic usage, and continuous investigation and creation of novel therapeutic approaches (Paterson & Bonomo, 2005). Most people identify the term "superbugs" with bacteria, specifically antibiotic-resistant forms of the organism. Nevertheless, fungal strains that are resistant to antifungal drugs might potentially become resistant to these drugs. The term "superbugs" is also sometimes used to describe this resistant fungus. A few examples of fungi that are resistant to drugs are as follows:

Candida auris

A particular kind of yeast (fungus) called Candida auris can lead to serious infections, especially in hospitalized patients with compromised immune systems. High death rates have been linked to Candida auris, particularly in individuals who are very ill or have severe medical conditions. The yeast species Candida auris has drawn attention because it may lead to serious infections, particularly in medical environments. Numerous antifungal medications have shown resistance to Candida auris, making treatment difficult (Chowdhary et al., 2017).

Aspergillus fumigatus

A filamentous fungus called Aspergillus fumigatus is frequently found in the environment, especially in soil and decomposing organic materials. As one of the most common species in the Aspergillus genus, it can cause invasive infections and allergic responses among other human disorders. Aspergillus fumigatus spore exposure usually does not result in sickness in immunocompetent persons (those with healthy immune systems). In patients with reduced immune systems, such as those afflicted with HIV/AIDS, organ transplantation, or specific cancer therapies, Aspergillus fumigatus can result in invasive aspergillosis, an extremely serious and potentially fatal illness. Aspergillus species are widespread molds that give rise to

respiratory illnesses, especially in immunocompromised people. Some strains of Aspergillus fumigatus have developed resistance to azole antifungal drugs (Wadhwa & Kaur, 2022).

Cryptococcus neoformans

People with weakened immune systems, such as those with HIV/AIDS, organ transplant patients, or those on immunosuppressive therapy, are more susceptible to the fungus pathogen Cryptococcus neoformans. The fungus Cryptococcus neoformans is linked to a potentially dangerous illness called cryptococcal meningitis, which affects the brain and spinal cord. In some circumstances, resistance to antifungal drugs like fluconazole has been documented (Perfect, 2017). Contrary to antibiotic resistance, antifungal resistance is less often addressed, but it nevertheless presents a serious risk, particularly for those with weakened immune systems or those having invasive medical operations. Antifungal-resistant strains, like bacterial superbugs, highlight the need to use antifungal drugs carefully, take precautions against infections, and continue research to provide new treatment options. Resistance to antiviral medications is not as often associated with viruses as it is with "superbugs." Nevertheless, certain viruses may show resistance to antiviral drugs, making treatment difficult. Here are a few instances:

Influenza Virus

Viruses that predominantly affect the respiratory system are very infectious and known by the name influenza, or flu. Influenza viruses may be classified into four basic categories: types A, B, C, and D. Seasonal flu outbreaks in humans are typically caused by influenza A and B viruses, whereas influenza C viruses often cause milder respiratory illnesses. Cattle are the main target of influenza D, which is not known to infect humans. Oseltamivir (Tamiflu) and other antiviral medications may cause influenza viruses, including strains of the seasonal flu and avian influenza (bird flu), to become resistant to them. This resistance may make controlling influenza epidemics more difficult (Hatakeyama et al., 2007).

HIV (Human Immunodeficiency Virus)

The lentivirus known as the human immunodeficiency virus (HIV) targets CD4+ T cells, which are essential for organizing the body's immunological response to infections. HIV affects the immune system. HIV infection develops into acquired immunodeficiency syndrome (AIDS), which is marked by a compromised immune system and greater susceptibility to opportunistic infections and certain malignancies. Antiretroviral medications used to treat HIV/AIDS may cause HIV to become resistant to them. The virus's tendency for mutation and poor adherence to treatment plans are two possible causes of this resistance. HIV strains that are widely and multidrug-resistant provide management problems (Gupta et al., 2012).

Herpes Simplex Virus (HSV)

Humans are frequently infected with the Herpes Simplex Virus (HSV), which can lead to a range of clinical symptoms. The two primary forms of HSV are HSV-1 and HSV-2. Oral herpes is usually caused by HSV-1 and presents as fever blisters or cold sores on the lips and surrounding area of the mouth. Genital herpes, which is mostly caused by HSV-2, is characterized by uncomfortable sores or blisters in the genital region. The oral and genital herpes viruses, or Herpes simplex, are susceptible to developing resistance to antiviral medications such as acyclovir. HSV strains that are resistant to drugs may cause outbreaks that are more severe and persistent (Bacon et al., 2003).

Hepatitis C Virus (HCV)

The blood-borne Hepatitis C Virus (HCV) mostly damages the liver by inflaming it and possibly causing severe liver damage. It is among the main factors that cause chronic liver disease throughout the globe today. Although not often known as a "superbug," some hepatitis C virus strains have the potential to become resistant to direct-acting antiviral (DAA) drugs. Treatment plans for hepatitis C may become less successful as a result (Wyles, 2013). It is not as usual to use the term "superbugs" to describe parasites. Nevertheless, parasites can become resistant to antiparasitic medications, which makes treating and managing parasitic illnesses difficult. Examples of parasites that exhibit medication resistance include the following:

Epidemiology

The development and spread of antibiotic resistance are two major variables in the epidemiology of superbugs, or antibiotic-resistant microbes, in Asian nations. Superbugs are a complicated and dynamic problem that might differ throughout Asian nations and areas. The following instances illustrate the epidemiological features of superbugs in various Asian nations:

India

Several Asian nations, notably India and Thailand have reported cases of HIV antiretroviral resistance. There have been documented cases of drug-resistant leishmaniasis in the Middle East and the Indian subcontinent. Bacteria that produce New Delhi Beta-Lactamase (NDM-1) have been documented in India which has resulted in resistance to many medicines, including carbapenems (Kumarasamy et al., 2010).

China

Antiviral medications known as nucleoside/nucleotide analogs, which are often used to treat hepatitis B, may sometimes cause chronic hepatitis B infections to develop resistance. Antiviral resistance in hepatitis B cases has been documented in several Asian nations, including South Korea and China. Enterobacteriaceae that produce extended-spectrum beta-lactamases (ESBLs),

such *Klebsiella pneumoniae* and *E. coli*, have been found in Chinese healthcare settings (Hu et al., 2019).

Pakistan

A variant of typhoid fever that is extensively drug-resistant (XDR) is one of the superbugs that Pakistan has to be concerned about. Since its emergence, this strain has proliferated throughout the nation. It is exceedingly challenging to treat since it is resistant to several of the most widely used medicines for typhoid. *E. coli* and Klebsiella pneumonia are two examples of carbapenem-resistant Enterobacteriaceae (CRE) pathogens that have been identified in Pakistani healthcare settings (Hasan et al., 2015).

South Korea

Healthcare environments in South Korea have been shown to have Methicillin-resistant *Staphylococcus aureus* (MRSA) strains, which are recognized for their resistance to beta-lactam medicines. Cases of CRE infections have been documented in South Korea, mostly in medical settings, which presents difficulties for infection management and treatment. Like other Asian nations, South Korea has had typhoid fever epidemics brought on by strains of Salmonella Typhi resistant to fluoroquinolone drugs (Park et al., 2009; Shin et al., 2021).

Japan

Antiviral resistance in influenza cases has been documented all over the world, including in many Asian nations, including China and Japan. Limited treatment options are available for infections caused by vancomycin-resistant Enterococci (VRE) strains that have been documented in Japanese healthcare settings. In Japan, MRSA is a significant superbug (Mizuno et al., 2018).

Thailand

Thailand and India, has reported high rates of HIV antiretroviral drug resistance. Drug-resistant malaria cases have been reported from Thailand, Myanmar, Cambodia, and other nations. There have been reports of extensively drug-resistant (XDR-TB) and multi-drug-resistant (MDR-TB) strains of tuberculosis in Thailand, which presents difficulties for attempts to manage the disease (Disratthakit et al., 2015; Palittapongarnpim et al., 2018).

Table 1. Major Issue Reported in the World

Country	**Major Issue Reported**	**Findings**
Pakistan	High prevalence of CRE, MRSA, VRE, and MDR/XDR strains	A high percentage of samples contained these isolates
Bangladesh	Environmental and clinical	64.28% of *Salmonella* and 36% of *E. coli*

	presence of MDR	isolates showed MDR
India	Overuse and misuse of antibiotics	85% of residents and 72% of interns believed antimicrobials would not help recovery
Nepal	High use of antimicrobials irrespective efforts to control	12.1% of prescriptions were found irrelevant
Philippines	Quick emergence of AMR due to improper use	A huge number of non-prescribed antibiotics about 66%
Thailand	Excessive use of antimicrobials	100 deaths have been reported due to AMR-associated infections

Antimicrobial Resistance Mechanisms (including Evolution of Superbugs)

Antibiotic-resistant bacteria can arise via a variety of methods, including acquired, adaptive, and innate resistance. The term "intrinsic resistance" describes a bacteria's innate ability for defense against specific drugs. Gram-negative bacteria are an example of inherent resistance; since their outer membrane in the cell envelope is impenetrable these bacteria are immune to glycopeptide antibiotics. When a bacteria that was previously sensitive to antibiotics acquires resistance due to a mutation in its genome or through the introduction of additional genetic material from an outside source, this is known as acquired resistance. The term "adaptive resistance" describes a bacterial tendency to get resistant to one or more antibiotics because of particular external factors. Adaptive resistance is intermittent in contrast to intrinsic and acquired resistance. When bacteria are exposed to antibiotics, it helps them react fast, and after the inciting signal is removed, it usually goes back to its natural state (Rizi et al., 2018).

Destruction of Antibiotic

Bacterial enzymes are one of the methods that can alter the antibiotics. These enzymes render the antibiotic ineffectual by targeting particular regions of the molecule. Enzymes that carry out this activity include β-lactamases and extended-spectrum β-lactamases (ESBL). ESBLs pose a serious risk to health as they are frequently the cause of infections contracted in hospitals. From minor infections like infections of the urinary tract to serious, potentially fatal diseases like sepsis, these infections can take a variety of forms. The mobile genetic elements (MGEs) or chromosomes include the β-lactamase encoding genes, which allow the bacteria to proliferate extensively. In the 1960s, plasmid-encoded β-lactamase TEM-1 was discovered in Gram-negative bacteria. The identification of novel β-lactamases that might neutralize these freshly synthesized chemicals coincided with the finding of new β-lactam antibiotics. Among these

enzymes are ESBLs, which confer resistance to penicillins and certain cephalosporins, while maintaining susceptibility to inhibitors of β-lactamase. β-lactamase inhibitors do not block AmpC enzymes, which impart resistance to a variety of penicillins and cephalosporins, including third-generation cephalosporins. A broad class of enzymes known as carbapenemases confers resistance not just to carbapenem antibiotics but also frequently to other β-lactams (Jacoby, 2017; Queenan & Bush, 2007) (**Figure** 1).

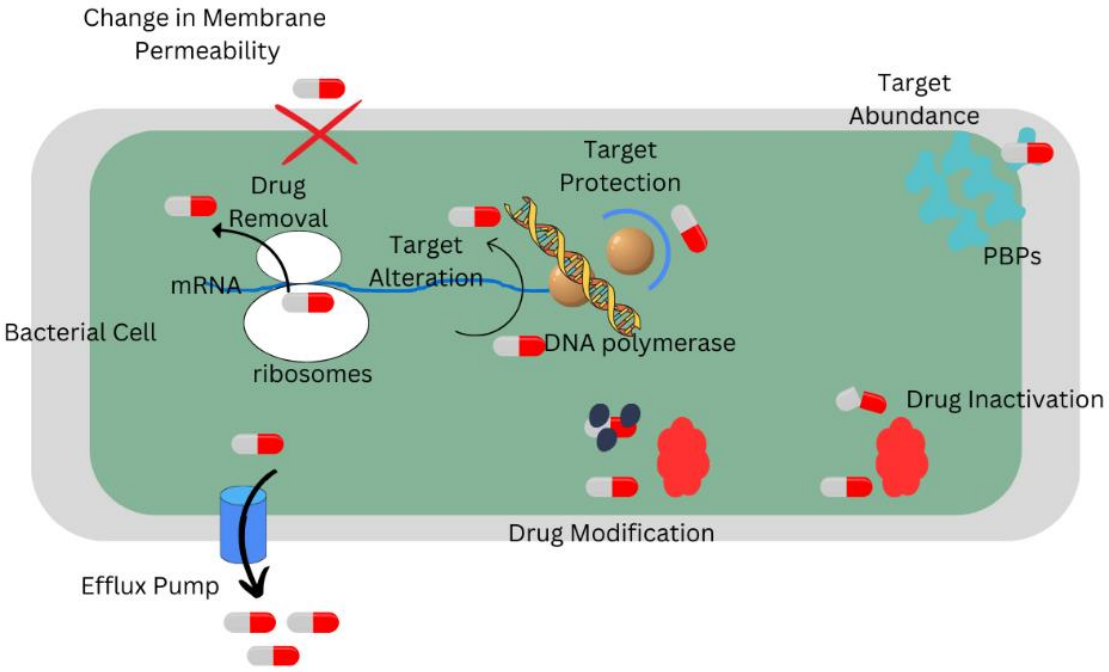

Figure 1. Antibiotic Resistance Mechanisms in Bacteria

Modifying Antibiotics

Modification of antibiotics with the help of enzymes is the most common method which is clinically significant towards aminoglycosides. For example, aminoglysides targeting enzymes such as AMEs (Aminoglycoside Modifying Enzymes) reduce the target specificity of antibiotics by altering it through acetylation, phosphorylation or adenylation. The AMEs encoding genes are located in Mobile Genetic Elements (MGEs) which help them spread among bacteria more easily. In all clinically important bacteria aminoglycoside resistance can be demonstrated with the help of this mechanism. For developing chloramphenicol resistance, acetylation of antibiotic molecules by enzymes is a well-known method. In a number of bacterial species, multiple Chloramphenicol Acetyltransferases (CATs) have been detected (Schwarz et al., 2004).

Antibiotic Activating Enzymes Alterations

Bacterial reductases play an important part for the activation of nitrofurantoin as they help produce toxic metabolites that are helpful for the antibacterial activity of nitrofurantoin. When mutation occurs in genes encoding reductases, such as *nfsA* and *nfsB*, is mainly responsible for the development of resistance against nitrofurantoin. Lumazine synthase, an enzyme responsible for the production of riboflavin, is encoded on *ribE* gene. Any mutation that occurs in this gene also results in the development of resistance against nitrofurantoin (Osei Sekyere, 2018).

Modification of Target or Replacement

In this mechanism, bacteria alter the composition or structure of the drug target site in a way that the binding or attachment of the drug is prevented. For example, in *Staphylococcus aureus* PBPs (Penicillin-binding proteins), the target site for beta-lactam antibiotics, is modified due to which antibiotics cannot attach to these targets and bacterial growth is unrestricted even at higher concentrations of antibiotics. The production of mosaic PBP genes in *S. aureus* results in the development of resistance against beta-lactams. These genes are produced due to the recombination of foreign DNA from beta-lactam-resistant streptococci and native DNA. When *mecA* gene is acquired and incorporated into the bacterial chromosome of *S. aureus*, it develops resistance against methicillin. This gene is located on an MGE known as *Staphylococcal* chromosomal cassette *mec* (SCCm). PBP2a, which is a different form of PBP has low binding affinity to all beta-lactam ring-containing antibiotics, is encoded on *mecA* gene. In this way, the synthesis of cell wall continues even in the presence of beta-lactam antibiotics (Moellering Jr, 2012).

Van gene clusters, a set of genes acquired by enterococci to develop resistance against glycopeptides, are involved in the replacement of glycopeptide targets thus decreasing the affinity of antibiotic molecules to bind them. The acquisition of van gene clusters from vancomycin-resistant enterococci to *S. aureus* has also been reported to develop VRSA (*Vancoycin-resistant Staph. aureus*). Trimethoporim-resistant DHFR enzymes and sulfamethoxazole-resistant DHPS enzymes have been reported for transferable resistance against these antimicrobials due to the acquisition of DHFR (dihydrofolate reductase) and DHPS (dihydropteroate synthase) genes (Eliopoulos & Huovinen, 2001).

Reduction in Drug Permeability

Bacteria use a variety of strategies to avoid the effects of antibiotics, which adds to the severity of antibiotic resistance. Lowering the membrane permeability is a common strategy that blocks the entry of drugs into the cell. Inhibition of porin synthesis, one of the major pathways that allow antibiotic access, is how this is completed. Bacteria that are resistant to drugs like β-lactams, tetracyclines, and fluoroquinolones protect themselves from danger by mutations in

genes that govern porins, such as *OmpK35*, *OmpK36*, and *OprD*. Further, changes in the chemistry of lipids and porin proteins limit the effectiveness of antibiotics. For example, *OprD* promotes the uptake of certain antibiotics, such as carbapenems; its loss or dysfunction results in resistance to drugs, such as imipenem and meropenem. Gram-negative bacteria like *Enterobacteriaceae* become more resistant when mutated porins and carbapenemases are combined (Livermore, 2001).

Along with membrane changes, bacteria use sequestration processes to manufacture drug-binding proteins that trap antibiotics before they reach their intended site. For example, bacteria that produce the bleomycin antibiotic family produce binding proteins such as BlmA, TlmA, and ZbmA, which sequester the antibiotic and counteract its effects. Additionally, certain bacteria suppress antimicrobial peptide expression, strengthening their resistance to drugs. Bacterial ability to bring together various resistance mechanisms highlights the complicated nature of this problem. To reduce the disastrous impact of antimicrobial resistance on public health, a multimodal strategy is required, comprising sensible antibiotic usage, the discovery of innovative therapies, and the improvement of prevention and treatment methods (Paterson, 2006).

Efflux Pumps

Efflux pumps have been a major factor in the development of superbugs and are essential to the processes behind antibiotic resistance. Numerous bacterial cells, both Gram-positive and Gram-negative, have such pumps. They work by forcefully removing antimicrobials from the bacterial cell, such as antibiotics and antimicrobial peptides (AMPs), decreasing their intracellular accumulation down to levels below lethal thresholds. Bacterial chromosomes and plasmids both include efflux pump encodes that enable horizontal transmission of the pumps across bacterial cells. The main facilitator superfamily (MFS), ATP binding cassettes (ABC), small multidrug resistance (SMR), resistance nodulation division (RND), and multidrug and toxic compound extrusion (MATE) are some of the families to which they belong (Goessens et al., 2013). Because each member of these families has unique substrate specificities, bacteria can tolerate a broad spectrum of antimicrobial treatments.

ATP hydrolysis and the membrane's electrochemical potential are two energy sources that are used by the efflux pump mechanisms to propel antimicrobial drugs out of the cell. To remove antibiotics from the cell, for example, the RND tripartite pump seen in Gram-negative bacteria is made up of outer membrane channels, periplasmic adaptor proteins, and inner membrane transporters. In bacterial cells, efflux pumps play a role in resistance that is inherent. Their amplification can result in acquired resistance, which frequently happens as a result of mutation or outside stimulation. Furthermore, the regulation of antibiotic production and efflux pump genes indicates a synchronized approach ensuring the survival and adaptability of bacteria (Kabra et al., 2019).

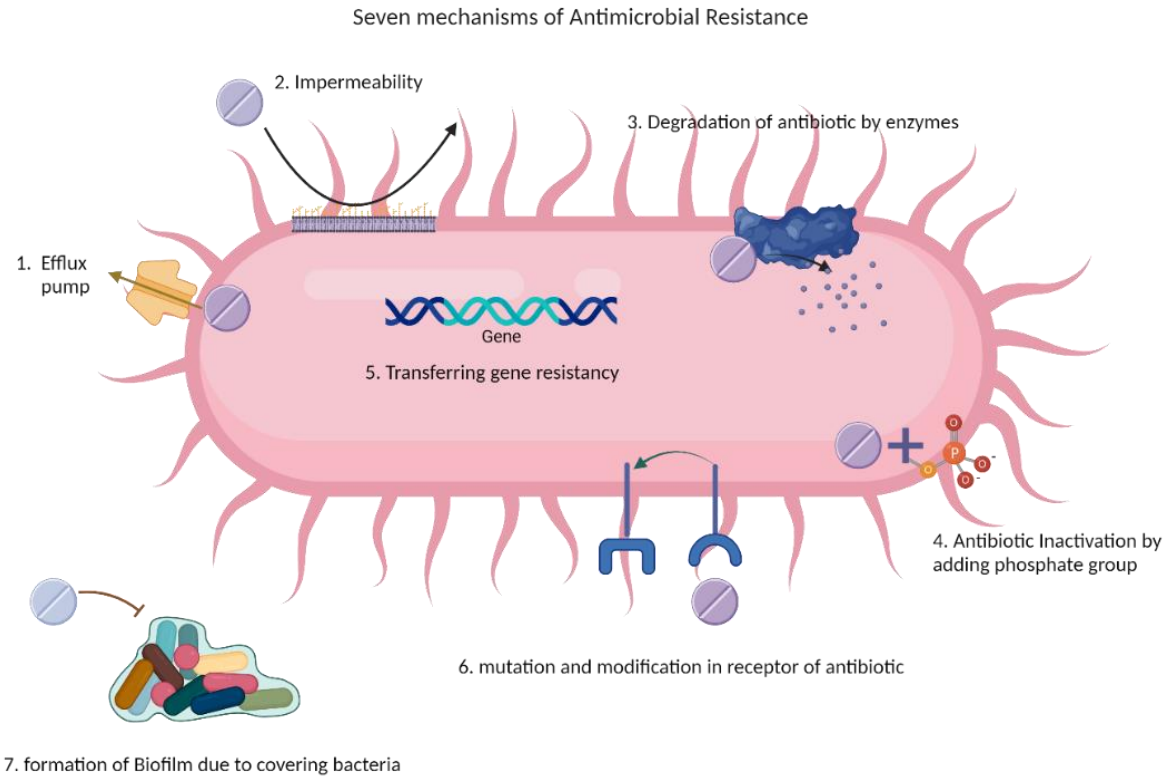

Figure 2. Represents the Seven Different Mechanisms for Antimicrobial Resistance.

Public Health Implications (Impact and Consequences of Multiple Superbugs in Asia)

Antimicrobial resistance (AMR) causes a 2–3.5% decline in the world gross domestic output, which in turn causes a 3–8% decrease in livestock and an estimated cost of USD 100 trillion (O'neill, 2014). Emergence and the spreading of antibiotic resistance bring serious consequences for public health, especially in areas such as Asia where superbugs are widespread. AMR affects medical facilities, economic growth, and global health stability in addition to individual health findings. The National Action Plan is one of the strategies that the Indian government approved in order to combat the alarming AMR situation (Taneja & Sharma, 2019). Still, other initiatives must be approved so as to successfully address this problem. AMR has been designated as a high-priority topic by the World Health Organization, requiring immediate action, due to its significant global rise in death rates. Hospital ventilation systems and discharges of wastewater from these medical institutions include antibiotic-resistant genes (ARGs) and antimicrobial-resistant bacteria (ARBs) due to nosocomial infections and sick individuals. Therefore, it can be shown that hospitals cover 33% of ARBs. To make conditions worse, during floods in Pakistan and India, sewage from cities affects both surface and groundwater, bringing hazardous

substances into our water supplies. Additionally, the air surrounding treatment facilities contains aerosols that carry antibiotic-resistant genes (ARGs) and bacteria (ARBs) (Hu et al., 2018).

The spread of many superbugs throughout Asia has serious consequences for public health. The efficiency of conventional antibiotics is quickly declining due to the evolution of antimicrobial resistance (AMR) in a variety of bacterial infections, such as *E. coli*, *Klebsiella pneumoniae*, and *Enterococci*. The likelihood of treatment failure, persistent sickness, higher healthcare expenses, and greater mortality are all enhanced in these circumstances. This problem is further aggravated by the widespread occurrence of *Extended Spectrum Beta-lactamases (ESBLs)* and other mechanisms that promote resistance. These strains are resistant to several kinds of antibiotics, which limits the range of available treatments and forces the use of antibiotics as the final option. The combined effect of several reasons in Asia makes the AMR issue worse. Antimicrobial-resistant bacteria thrive in hospital environments due to insufficient cleanliness and sanitation, which increases the risk of nosocomial infections. Furthermore, the issue is worsened by deficient institutional guidelines for the administration of antibiotics, which permit both abuse and misuse of the drugs. As a result, superbugs like *vancomycin-resistant enterococci (VRE)* and *methicillin-resistant Staphylococcus aureus (MRSA)* may grow and spread more easily (O'neill, 2014; Van Wamel et al., 2007).

Surveillance and Monitoring (Strategies for Tracking Superbug Proliferation in Pakistan)

An increasing threat for morbidity, mortality, and costs internationally is AMR, particularly in countries with low and middle incomes (LMICs) such as the nation of Pakistan. In 2019 approximately 1.29 million (M) people died due to AMR rates are predicted to rise more if preventive measures are not taken AMR develops in nations due to a variety of factors. These are the inappropriate prescription & administration of antimicrobial agents, particularly for diseases that run self-limiting naturally, and a shortage of reliable monitoring programs that measure the use of antibiotics (Sartelli et al., 2020).

Hospital Surveillance

It is critical to track drug resistance in healthcare facilities. Frequent examination of patient samples such as cultures taken from blood or urine helps to diagnose superbug incidence in clinics. AMR is made worse by a lack of infection, prevention, and control (IPC) teams in hospitals, issues with infrastructure and hygiene, physicians' misunderstanding of AMR and antibiotics, and excessive use of antibiotics for treatment in growing crops and the agriculture sector (Shaikh et al., 2022).

National and International Surveillance Networks

The World Health Organization (WHO) can take control of this emerging issue via the establishment of an international action plan to promote national capability against superbugs. Monitoring certain bacterial pathogen's resistance to various antibacterial drugs is the aim of the

Global action plan (GAP). Now, there are significant gaps in our understanding of the national, regional, and local levels of antibiotic resistance in addition to designs of present antimicrobial usage within areas. Based on the national action plans (NAPs) of each country, the Global Antimicrobial Resistance Surveillance System (GLASS) was created in response to the WHO Global Action Plan. To determine the scope and effects of AMR on populations, this surveillance system integrates the patient's laboratory and epidemiological data. Applying the National Action Plan in Pakistan creates serious queries regarding facilities skills, and availability of assets (Saleem, Godman, et al., 2022).

This applies to Pakistan because there are lots of serious issues related to the surveillance that is presently established to thoroughly record AMR patterns throughout the nation, which is being rendered worse by hospitals not requesting culture and susceptibility screening, but changes are currently being recorded (Jabeen et al., 2022).

Community-Acquired Surveillance

One possible explanation for this high resistance could be the widespread practice of prescribing antibiotics without a doctor's recommendation in the majority of acquired from community health issues. The current knowledge revealed *E. Coli's* severe ability to resist the quinolone, the macrolide, cephalosporin, and antibiotic penicillin medicines. The researchers found that 82% of the total 93 *E. coli* isolated strains studied in private medical facilities in Lahore, Pakistan, were resistant to beta-lactam antibiotics. Co-infections (1.14% of the individuals that were admitted) or repeated infections in 3.14 percent of people were reported in private hospitals in Pakistan. This reveals that the major cause of this incidence is insufficient data about infected people and there is concern about the authentic diagnosis of infected individuals (Shaikh et al., 2022).

Factors That Impact

Insufficient data on antibiotic sensitivity and diagnostic facilities lead to careless consumption of antibacterial medicines. This issue should be continuously checked at primary, secondary, and tertiary hospital levels & national levels. To control the resistance of bacteria there is a need to implement extra antibiotic control programs that are coordinated with healthcare departments in Pakistan, but given many issues before applying to this program should first control. One of them is the cost of diagnosis, culture, and susceptibility testing of a patient’s specimen. It should be added as a routine testing of CST, the Pakistani authorities should add this CST and make a part of the national action plan to accommodate the patient’s specimen to overcome the cost which he can’t afford (Saleem, Haseeb, et al., 2022).

Utilizing and Abusing Antimicrobials

Human Medical Care

The worldwide rise of resistant bacteria to antibiotics has been greatly exacerbated by the excessive and improper use of antimicrobial agents for human treatment. Nevertheless, its excessive and erroneous usage promoted the spread of germs resistant to drugs, making some formerly successful therapies no longer work. Furthermore, individuals may neglect to finish the entire course of antibiotics recommended to them, thus permitting the remaining bacteria (superbugs) to grow resistant (Haseeb et al., 2023).

Farming

AMR is largely caused by the excessive and inappropriate use of antibiotics in farming, which poses a major risk to human wellness as well as the well-being of animals. Animals that employ these non-therapeutic applications develop pathogens that are resistant to pharmaceuticals. The creation of alternative methods and a cautious intake of medicines to protect animal health and efficiency (Hazards et al., 2021).

Absence of Development of Novel Antibiotics

One major obstacle in the field of AMR and medical care is the absence of new antibiotic research. There often exist financial obstacles that make it difficult for drug companies when it comes to funding the research and production of antibiotics (Assar et al., 2020).

International Business and Tourism

More than ever, individuals, items, and wildlife travel over countries in our increasingly interconnected globe. Furthermore, the global exchange of food items, such as vegetables and cattle, can spread resistant germs to previously uninfected areas. It emphasizes how important it is to have uniform policies on the use of antibiotics and surveillance (Aijaz et al., 2023).

Natural Ingredients

The abuse and inadvertent of antimicrobials in a variety of industries, such as farming, medical care, and pharma production, may allow the medications to be released into the atmosphere along with resistant microorganisms. More specifically, soil and water sources may become contaminated with resistant microorganisms and antibiotic residues due to effluent from pharmaceutical manufacturing companies and healthcare facilities. This natural resource can act as an ideal environment for the growth of microorganisms and genes that are resistant, which can subsequently be spread to wildlife, individuals, and other organisms (Hendriksen et al., 2019).

Antibiotic Residues in Water and Food

The extensive use of antimicrobial agents in livestock husbandry, farming, and aquaculture can often be the cause of the residues' existence in our environment and food supply. The use of antibiotics can cause resistant bacteria and their traces to enter water supplies when they are given to animals. This can happen through runoff and the disposal of animal waste. Similarly, dietary items that come from animals that produce meat, milk, and shellfish may have antibiotic residues and be given these medications as treatment. This emphasizes how agriculture, the environment, and human health are all impacted by antibiotic resistance (AMR), underscoring the significance of responsible antibiotic use and all-encompassing initiatives to slow the spread of AMR down the food chain (Hanna et al., 2018).

Both Public View and Patient Consciousness

A lot of people are ignorant of the effects of AMR, which might result in unwarranted requests for antibiotics. and an absence of understanding of the risks connected with the abuse and misuse of these medications. Furthermore, promoting a more comprehensive comprehension of AMR's influence on public health, including the possibility of incurring incurable diseases and elevated medical expenses can enable people to stand up for the responsible use of antibiotics in their communities and make more informed decisions (Abushaheen et al., 2020).

A One Health Perspective

AMR includes medicine for animals, farming, medical care, and the natural world; it isn't restricted to any particular sector. The idea of One Health highlights how important it is for veterinary doctors, environmental scientists, human health specialists, and microbiologists to work together in a multidisciplinary manner. It highlights the prudent use of antibiotics for the health of humans and animals, promotes strict infection prevention strategies, and encourages studies and surveillance to monitor resistance patterns in the environment and between humans and animals. In the end, the One Health strategy provides an all-encompassing framework for successfully combating AMR by recognizing that the well-being of humans, animals, and ecosystems (Rousham et al., 2018).

Observational Study of Antibiotics in Pakistan

According to a study conducted in 2018 a total of 2523 were diagnosed among them *Staphylococcus aureus* (gram-positive bacteria 866 (34.3%), and gram-negative bacteria 65.4% isolated, from different samples like pus (1464, 58.0%), sputum (81, 3.2%), blood (164, 6.5%) and urine (718, 28.5%). From urine, *E. coli* (497,61.10%) was diagnosed (Table 2). *Staphylococcus* and *Pseudomonas aeruginosa* were isolated from pus samples (Saleem et al.,

2023). Healthcare institutions and the appropriate authorities are additionally consulted by surveillance operations when establishing methods for actual therapy.

Table 2. This table represents the bacteria type with sample percentage observed in Pakistan

Number	**∑ cultures report**	**G. positive, negative bacteria**	**Percentage %**	**Type of sample**	**Sample + Percentage**
1	2523	*Staphylococcus aureus*	866 (34.3%)	Pus	704 (81.30%)
2	-	E. coli	814 (32.2%)	Urine	497 (61.10%)
3	-	p. aeruginosa	454 (18%)	Pus	307 (67.60%)
4	-	Klebiella spp	269 (10.7%)	Sputum	81 (3.2%)

Thus, on behalf of a One Health policy, monitoring activities need to be performed on occasion in Pakistani clinics to monitor the prevalence of bacteria that are multidrug-resistant inside in addition to in the supply chain of food.

International Spread of Antibiotic Resistance

Europe has several of the highest AMR rates in the entire globe, and numerous infections caused by bacteria are becoming resistant. Like methicillin *Staphylococcus aureus*, vancomycin enterococcus and multi-drug-resistant tuberculosis. In the European Union (EU), some 33,000 people who get antibiotic-resistant bugs pass away each year. The antimicrobial-resistant bacteria that are widespread in Europe include the following: *A. baumannii*, pneumonia-causing *E. coli*, and MRSA. A sizeable percentage of infections in the UK are brought on by the *E. coli* bacteria. According to recent studies, an increase in resistance is caused by bacteria like *Aeruginosa* and *Klebsiella pneumonia*. German people were diagnosed and there was a carbapenem antibiotic resistance in their medical healthcare facilities. Many different species have been reported in different countries like Italy, Spain, France, etc. Another concern incidence of antimicrobial resistance occurs in Africa and the spread of antibiotic resistance happened due to the lack of limited health facilities and the increasing rate of AMR. For example, in Africa, third-generation antibiotics were used routinely and caused resistance to pneumonia and some reports showed that there was *multidrug-resistant mycobacterium tuberculosis* prevalence too (Ouedraogo et al., 2017).

Interventions and Strategies (Addressing the Antimicrobial Challenge in Asian Nations)

AMR-related public health concerns are particularly prevalent in Asia. Recent findings suggest that certain Asian pathogenic strains *Klebsiella pneumoniae*, a strain of Acinetobacter baumannii, and *E. coli* in particular are showing increased tolerance levels. Asia faces a serious threat from MDR-TB about thirty percent of people TB diagnosis, whereas India has emerged as the source of about 6% of MDR-TB infections worldwide. AMR is also influencing other infectious diseases that are common in Asia, like malaria and dengue fever. Statistics indicate an increasing predisposition to drug resistance in dengue fever therapy. AMR poses a serious threat to Pakistan's healthcare system. Among these are inappropriate use of prescription antibiotics, poor use of infection control protocols, and limited access to appropriate antimicrobial drug therapies (Lim, 1994). According to estimates, a significant percentage of newly diagnosed TB patients in the area roughly 27% have developed resistance to any of the first-line medications in Pakistan. Reports state that the antiviral drugs used to treat hepatitis C are seeing an increasing trend of resistance in Pakistan. Improving surveillance, promoting advisable consumption of antibiotics, and implementing successful infection-control procedures are all strategies that could be used to control AMR in the country (Torumkuney et al., 2022).

There is limited information available about the prevalence of AMR in Antarctica because there aren't many people living there. Every isolate, according to the study, showed resistance to at least one antibiotic, with tetracycline resistance predominating widely. Compared to other geographic locations, Australia has lower rates of antimicrobial resistance. Substantial amounts of resistance to antibiotics have been discovered, observed in several Australian-found microorganisms, including *Klebsiella pneumoniae*, *E. coli* and the bacteria *Staphylococcus aureus* (Wozniak et al., 2022).

Strategies To Monitor Superbugs Proliferation and Challenges

New and innovative vaccine approaches have become in greater supply because of the emergence of AMR. Vaccines made from conjugates function via connecting bacterial polysaccharides to protein carriers to improve the immune system's reaction. Combo vaccinations offer a lot of effectiveness for treating antimicrobial-resistant bacteria. The efficiency of combo vaccinations in avoiding diseases is driven by the two primary agents that generate AMR, Streptococcus pneumoniae and it is proven that Haemophilus influenzae also exists in the category of superbugs. The recombinant vaccinations have proven to be highly effective in preventing pneumococcal disease, which is caused by *Streptococcus pneumoniae*. The ongoing need for vaccination updates to keep up with the evolving bacterial strains is a significant barrier. A further barrier is the high cost of developing and producing these vaccinations (Rosini et al., 2020).

Vaccines of the shape of virus-like components or VLCs are produced by self-assemblage and mimic the molecular makeup of viruses without including any genetic data. The effectiveness of VLPs in enhancing immunization against the virus that causes human papillomavirus (HPV) is now established. VLP vaccines activate the body's immune system to combat illnesses by using non-contagious parts that mimic viruses. VLP immunizations have produced positive findings from preliminary research especially when it involves controlling immunizations against *Clostridium difficile*, *Salmonella*, and *E. coli*, three pathogens which greatly support AMR. Choosing the right bacterial indicators to include in the immunization is a significant challenge. Vaccines that employ VLP give an encouraging approach to tackling the problem of AMR. Using plasmids that have the genetic code for antigens, DNA vaccines are produced. Studies conducted on animals have demonstrated the effectiveness of DNA vaccines in treating bacterial infections, such as *Salmonella* and *E. coli*. There has been proof of a DNA vaccination working to protect mice from a fatal MRSA infection. One concern is the possibility that certain individuals may have compromised immune systems, which could result in a decreased effectiveness of the vaccination. Live attenuated vaccines use less potent viruses to elicit an immune response without causing disease. The *Salmonella* vaccine is an example of a live attenuated vaccination that targets AMR. In trials with animals, the vaccination was effective in protecting against *A. baumannii*. The potential for the vaccination strain to return to its harmful form and cause sickness is one potential barrier (Deiana et al., 2022).

Nanotechnology is the use of tiny particles as well as additional tiny particles in the identification and treatment of illnesses. It has been shown that tiny silver particles may hinder the growth of many bacterial strains. The antibacterial characteristics of various kinds of small particles, including liposomes and dendrimers were additionally studied. The use of nanotechnology to identify specific bacterial species or genes that contribute to antibiotic resistance. The application of AI has the potential to have a major effect on the fight against AMR. The use of AI technology may facilitate the quicker creation of novel antibiotics. AI technologies can help physicians make decisions at the moment. Artificial intelligence algorithms can be used to analyze patient data and prescribe the best medications. AI can be used in tracking and surveillance methods for AMR monitoring and supervision. In therapeutic contexts, AI tech is used to tackle types of germs that are immune to antibiotics and other drugs. AI can assist in identifying microorganisms that are challenging to treat (Ali et al., 2023).

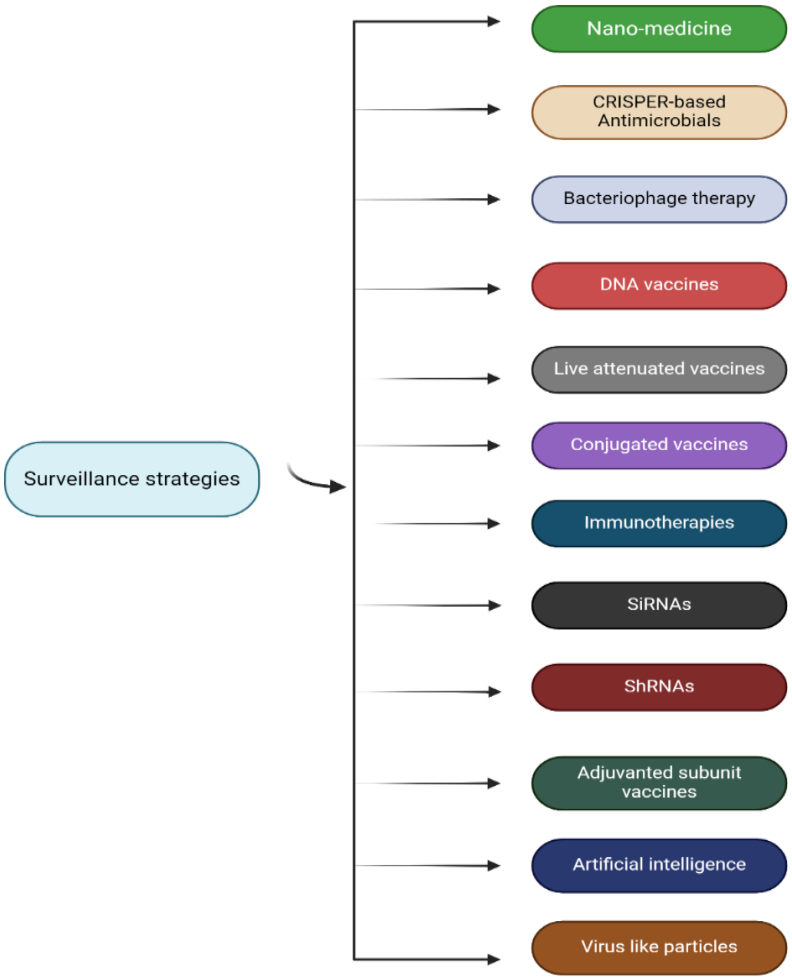

Figure 3. Represents The Surveillance Strategies to Monitor Superbug Proliferation.

Moreover, monitoring systems powered by AI can keep an eye on the spread of germs resistant to several drugs and antibiotics. Enabling a moral and effective implementation of AI technology, in addition to protecting patient data. Genetic methods for editing are employed in CRISPR-mediated antimicrobial agents to concentrate on and destroy kinds of bacterium. The phages are viruses that possess the capacity to colonize and eliminate bacteria (Bikard & Barrangou, 2017).

Scientists are looking into the possible use of depolymerases and endolysins. Researchers are trying to apply microbiome techniques as a source of bacterial infection. Using monoclonal antibodies as a possible substitute for antibiotics is another strategy. To tackle this coming danger, international collaboration is not just vital but also morally required (Mayorga-Ramos et al., 2023).

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Cultivated Ancient Wheat Species: A Potential for Wheat-Based Products and Crop Trait Improvement

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Introduction

Wheat (*Triticum* spp.) constitutes Turkey's most extensively cultivated cereal crop in terms of both planted area and production. Globally, it ranks first in cultivation area and second in production quantity after maize (FAO, 2022). This strategic crop serves as a fundamental pillar of global food security, supplying approximately 20% of the world population's daily calories and protein intake (Sharma & Sharma, 2025). In Türkiye, grain products-including bread, pasta, bulgur, biscuits, and yufka (thin flatbread)-provide approximately 40% of daily dietary energy (Kalkan & Özarık, 2017). Wheat's indispensability stems from its high starch (60-75%) and carbohydrate content, extended storability, and adaptability to fully mechanized agriculture (Shiferaw et al., 2013).

Origin and evolution of wheat species

Türkiye harbors a unique genetic diversity pool encompassing hundreds of local populations comprising 28 wild wheat taxa, alongside ancient and modern cultivated varieties (Özberk et al., 2016a). Archaeological evidence indicates wheat cultivation commenced 10,000–12,000 years ago within the Fertile Crescent, Southeastern Anatolia (Maron, 2019). Research confirms that tetraploid wheats (the *T. turgidum* group) originated from wild *Triticum dicoccoides* (AABB genome), which was derived from natural hybridization between *Aegilops speltoides* (BB genome) and *T. urartu* (AA genome) (Levy & Feldman, 2022). This wild species was domesticated into hulled *T dicoccum* (emmer), subsequently evolving into free-threshing durum-type wheats (*T. durum*, *T. polonicum*, *T. turanicum*), cultivated in Anatolia since the Neolithic period (Gökgöl, 1969). Hexaploid bread wheat (*T. aestivum*, AABBDD) emerged from natural hybridization between diploid *Ae. tauschii* (DD genome) and tetraploid *T. dicoccum*, initially forming hulled *T. spelta* before assuming its free-threshing form (Dvorak et al., 2012). Other hexaploid species includes free-threhing *T. sphaerococcum* (indian dwarf wheat) and hulled *T. spelta* (spelt) (Özberk et al., 2016b). *T. polonicum* is adapted to the Mediterranean basin, while *T. ispahanicum* represents a landrace endemic to the Iranian Plateau (Waines & Lukaszewski, 2017). Originally considered a form of *T. polonicum* (Mackay, 1963), *T. ispahanicum* was later reported to derive from a mutated *T. dicoccum* (Watanabe, 1999). Three hypotheses exist regarding the origin of the Chinese landrace, *T. petropavlovskyi*: hybridization between *T. polonicum* and *T. aestivum*, hybridization between *T. polonicum* and *Ae. tauschii*, or a mutation of *T. aestivum* (Chen et al., 2016).

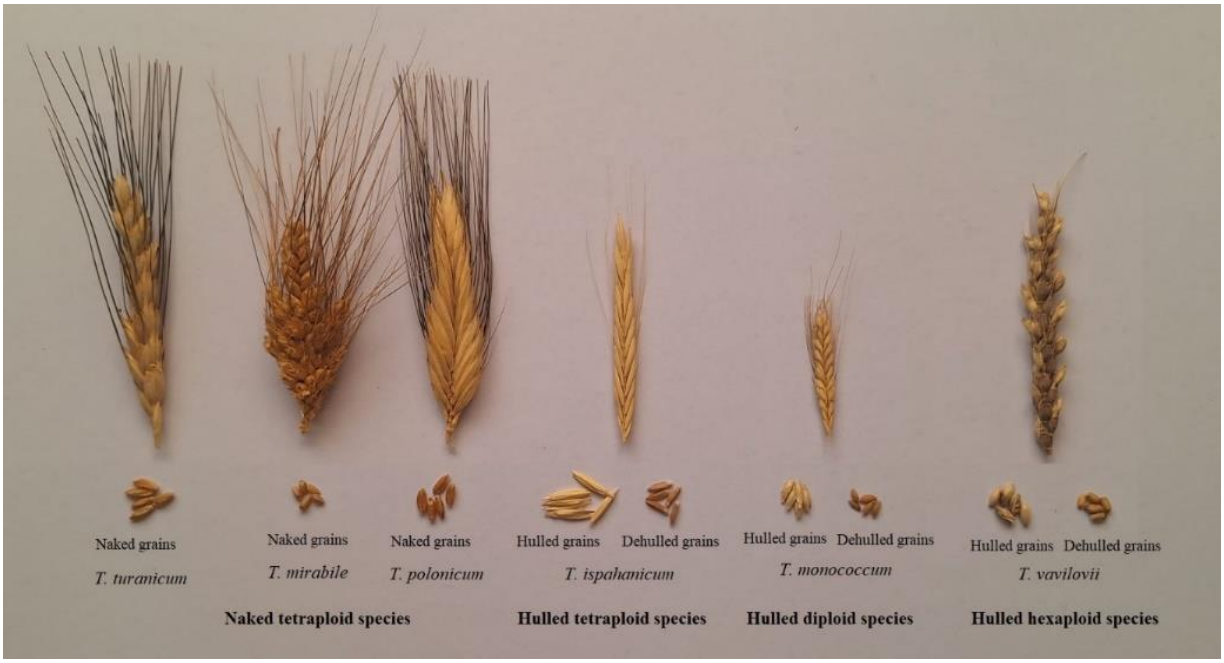

Figure 1. *Diploid, Tetraploid, and Hexaploid Ancient Wheat Species*

The ancient hulled hexaploid species *T. vavilovii*, a branching mutant, was discovered as an admixture in a bread wheat landrace northeast of Lake Van, Türkiye (Feldman & Levy, 2023). *T. mirabile* is another branching ancient wheat species locally cultivated by farmers in Türkiye (Figure 1). The only cultivated diploid and hulled ancient wheat species, *T. monococcum*, originating from the wild species *T. boeoticum*, was first domesticated in the Karacadağ Mountain region of southeastern Türkiye (Peng et al., 2011).

Ancient wheat species for climate change and future challenges

Future projections indicate that feeding a global population of 9.7 billion by 2050 necessitates increasing current cereal production by 30–62% (Alexandratos & Bruinsma, 2012; UN-DESA, 2019; van Dijk et al., 2021). Achieving this target requires the development of high-yielding, nutritious cultivars that exhibit resilience to abiotic stresses-driven by rising temperatures, erratic precipitation patterns, and elevated CO_2 concentrations (e.g., drought, thermal stress, salinity)- and emerging biotic threats such as novel pathogen races (Luck et al., 2020; FAO, 2022). In the future, climate change is anticipated to cause yield reductions (Zhu et al., 2022). The narrow genetic base (genetic bottleneck) of modern wheat varieties renders them vulnerable to diseases and environmental stress while constraining phytochemical diversity and micronutrient content (Reynolds & Langridge, 2016; Nasim et al., 2025). Breeding cultivars with desired traits is intrinsically linked to access to broad genetic diversity (Borlaug, 2002; Godfray et al., 2010). For this reason, ancient wheat species offer unique genetic variability for breeding of climate-resilient cultivars. These species serve as critical genetic reservoirs for enhancing resilience to stressors (including valuable resistance to powdery mildew (*E. graminis*), leaf rust (*P. recondita*), and yellow rust (*P. striiformis*) provided by einkorn and certain emmer accessions

(Grausgruber et al., 2004), adaptive capacity, and yield potential (Jaradat, 2011; Özbek, 2014). The tetraploid wheat *T. turanicum* thrives in Mediterranean-like regions due to its tolerance to drought and heat stress factors (Sanfilippo et al., 2025). The ancient wheat species, *T. carthlicum* (Persian wheat), has been suggested for bread wheat breeding due to its combined abiotic and biotic stress tolerance (Saleh, 2020). Indian dwarf ancient wheat, *T. sphaerococcum*, has been reported to have drought and heat stress tolerance (Gaikwad et al., 2025). Emmer and einkorn wheat species demonstrate tolerance to salinity, drought, high temperature, and wheat rust (Ullah et al., 2018).

Nutritional superiority of ancient wheats

Driven by growing improvements in consumer priorities, including personal health, and environmental sustainability, interest in ancient wheat varieties has revived due to their distinctive traits. The agronomic and nutritional value of ancient wheat is evidenced by its suitability for organic farming, its tolerance to various environmental stresses, and its enhanced concentrations of beneficial compounds like dietary fiber, phenolics, and micronutrients (Sanfilippo et al., 2025). Ancient wheat species are documented to possess elevated levels of protein, resistant starch, phytochemicals, fiber, and microelements (Arzani & Ashraf, 2017; Patijn et al., 2018). Diploid einkorn (*T. monococcum*), tetraploid Khorasan (*T. turanicum*), as well as emmer (*T. dicoccum*), and spelt (*T. spelta*) wheat species, have gained popularity due to their unique functional and nutritional merits such as supporting health-focused dietary approaches targeting cardio-metabolic diseases (Dinu et al., 2018; Škrobot et al., 2025). Einkorn wheat (*T. monococcum*) boasts high phenolic compounds, carotenoids, B vitamins, and antioxidant activity, coupled with the advantages of a lower glycemic index and reduced cholesterol impact (Vallega, 1996; Fogarasi et al., 2015; Emeksizoğlu, 2016). Notably, einkorn wheat surpasses polyploid wheats in carotenoids-per-meal density and exceeds bread and durum wheat specifically in total tocol (Vitamin E) content, reaching a maximum of 115.85 mg kg^{-1} and averaging 77.96 mg kg^{-1} (Mahdavi et al., 2024). Emmer and einkorn exhibit high protein and lutein, along with high Fe and Zn content (Ullah et al., 2018). Ancient and wild wheat species constitute vital genetic reservoirs for combating "hidden hunger" (micronutrient deficiency). CIMMYT screening of >3000 wheat genotypes showed minimal difference in Zn and Fe content among adapted modern cultivars, whereas progenitor ancient and wild wheat species (*Ae. tauschii, T. dicoccoides*, *T. boeticum*, *T. monococcum*) and landraces exhibit substantially higher concentrations (Çakmak et al., 2000; Monasterio & Graham, 2000). Modern wheat breeding programs offer limited scope for enhancing Fe and Zn contents; therefore, ancient wheat species, including *T. dicoccum* and *T. spelta*, have been utilized in breeding Zn-enriched cultivars (Singh et al., 2017)**.** CIMMYT leveraged *T. spelta* in developing Pakistan's first Zn-biofortified cultivar 'Zincol-2016' and utilized *T. dicoccum* in breeding India's 'WB02' and 'HPBW-01' (Singh et al., 2017).

Ancient wheat products and technological quality

Global consumer demand for products derived from these species-such as biscuits, pasta, and bread-is experiencing sustained growth. The wheat species, *T. polonicum*, *T. turanicum*, *T. monococcum*, *T. dicoccum*, and *T. spelta* are the most widely preferred for cereal products compared to other ancient wheat species.

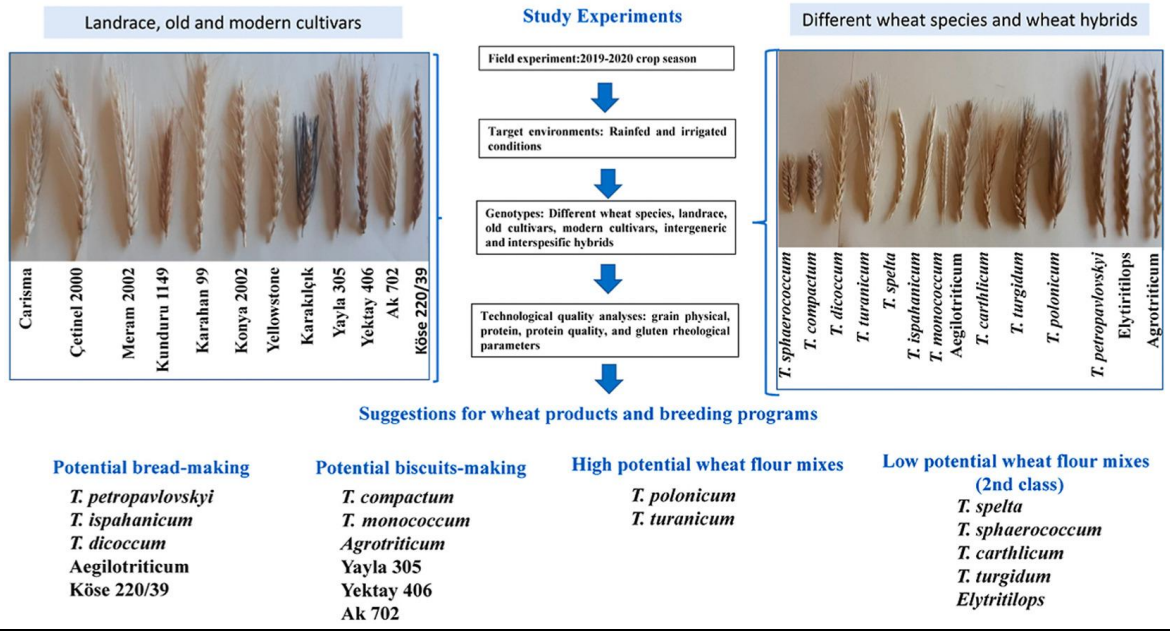

Figure 2. *Technological Quality of Neglected Ancient Wheat Species and Hybrids Compared to Landrace, Modern, and Old Cultivars (Akman & Karaduman, 2021)*

Khorasan wheat (*T. turanicum*) is notably preferred in bread, pasta, and couscous production due to its superior sensory attributes (with Egyptian participants rating kamut couscous [made from *T. turanicum*] as savory, buttery, crispy, and delicious (Abdel-Haleem et al., 2012) and bread loaf volumes comparable to modern wheats. Beyond couscous, kamut features in cereals, baked goods, and pasta (Brester et al., 2009). Similarly, *T. polonicum* has been identified as a viable alternative for high-quality bread production due to its exceptional organoleptic properties (Suchowilska et al., 2019). Spelt (*T. spelta*) wheat is utilized in breakfast cereals, breads, pasta, and crackers, leveraging advantages such as a robust bread aroma, excellent flavor profile, and extended retention of freshness and soft texture (Bojnanska & Francakova, 2002; Marques et al., 2007). Critically, when used in bread blends with spelt, ancient grains deliver comparable organoleptic and physical properties to those of regular wheat, underscoring their nutritional and sensory value (Angioloni & Collar, 2011). Among ancient wheats, *T. petropavlovskyi* and *T. ispahanicum* are distinguished by superior bread-making quality (Akman & Karaduman, 2021). It should be noted that these ancient wheat species are often recognized as regional landraces.

For instance, *T. monococcum* (cultivated in Türkiye) is called Siyez wheat, while *T. dicoccum* includes a landrace known as Kavılca. Numerous local wheat species are traditionally processed into thin flatbreads (yufka), unleavened flatbreads (bazlama), pasta, and bulgur using artisanal methods (Şanal, 2017). Despite often exhibiting inferior technological quality (farinograph, extensograph, alveograph) compared to common wheat, ancient wheat species including *T. monococcum*, *T. dicoccum*, and *T. turanicum* particularly einkorn with its high yellow pigment-offer elevated protein content, making them valuable sources for functional and health-promoting foods (Grausgruber et al., 2004**).** Consequently, ancient wheat species have been proposed as suitable for both bread and biscuit production (Akman & Karaduman, 2021).

Conclusion

The urgency is underscored by projections requiring substantial cereal production increases by 2050 amidst climate change threats. Modern wheat varieties, constrained by a narrow genetic base limiting resilience and nutritional value, highlight the indispensable role of ancient wheats like einkorn, emmer, spelt, Khorasan, and Polish wheat. These species serve as vital genetic reservoirs, offering enhanced tolerance to abiotic stresses (drought, salinity, heat) and resistance to key diseases. Crucially, they possess individually superior nutritional profiles, including significantly higher levels of protein, essential micronutrients (Fe, Zn), carotenoids (notably lutein), tocols (Vitamin E), phenolic compounds, and antioxidants compared to modern cultivars. This positions them as critical resources for combating micronutrient deficiencies ("hidden hunger") and developing biofortified varieties, as demonstrated by successful programs. While technological dough properties may sometimes lag behind those of common wheat, their exceptional compositional advantages drive growing consumer demand for health-promoting foods like pasta, bread, and bulgur. Consequently, conserving and characterizing ancient wheat species, particularly through research on understudied technological quality and processing traits for traditional products, is imperative. Leveraging these ancient genetic resources through breeding and direct utilization offers a sustainable pathway to enhance nutritional security, crop resilience, and the development of functional foods in a changing climate.

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Ethical, Social, and Policy Issues in Livestock Genetics

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Mehmet OZASLAN

1. Ethical Considerations in Livestock Genetic Modification

Genetic modification of livestosck is one of the most significant advancements in Genetic modification of livestock is one of the most significant advancements in agricultural science (Van Der Berg, Kleter, Battaglia, Groenen, & Kok, 2020). They alter the genetic makeup of animals and scientists aim to enhance traits of disease resistance, growth rate and environmental adaptability. The potential benefits increased food production, reduced environmental impact and improved animal health. Moreover, these scientific discoveries come with complex ethical questions. Research into genetic modification address concerns about animal welfare, environmental impact, public perception and social to follow scientific progress. Ethical decision-making in this field requires a balanced approach that considers both the benefits and the moral implications to change animal life. A major ethical concern revolves around animal welfare. They planned to boost productivity that sometimes harm the animals themselves. For example, breeding animals to grow faster or produce more milk led to skeletal problems, weakened immune systems and other health issues. They face a new vulnerabilities or chronic embarrassment that genes modified to make livestock more resistant to disease. Therefore, this raises ethical concerns about ensuring that improve productivity or disease resistance does not compromise the animal's inclusive welfare The process of improve productivity or disease resistance raises ethical concerns about the potential impact on the animal's welfare.

Furthermore, environmental impact is another significant consideration. The introduction of genetically modified livestock into the wild could disrupt ecosystems and threaten biodiversity to introduce new traits into natural populations (Snow et al., 2005). For instance, a genetically modified cow with enhanced disease resistance outcompetes wild relatives that lead to the decline of native species. Additionally, the reduced genetic diversity caused by selective breeding make livestock populations more susceptible to disease epidemics and environmental changes. The implementation of strict containment measures and systematic ecological risk assessments essential to maintain ethical standards that release genetically modified animals. Public perception and trust also key factors in the ethical debate Apprehension toward large biotech companies that control key genetic modification technologies delays acceptance. Transparency and public engagement are critical in addressing these concerns. Governance of ethical means involve the public in decision-making processes and ensure that independent bodies, not just corporations, oversee the development and regulation of genetically modified livestock.

Moreover, social justice is another critical dimension of this issue. They often hold patents on genetic modification technologies that creates barriers for small-scale farmers. These advantages for wealthy nations and large agricultural companies intensify global inequality. They should ensure that genetic advancements accessible to small farmers and developed countries. They ensure that small-scale farmers benefit more fairly from genetic advancements. These actions are necessary to avoid wide existing social and economic gaps through genetic modification. The ethical challenges adjacent livestock genetic modification is complex and multifaceted. Scientific progress in this field holds great promise that pursued with a strong moral compass. Farmers face the challenge of increase efficiency that conservation of animal welfare, environmental health and social justice to require thoughtful dialogue and sound policies. Equitable access to genetic technologies to support open communication and ethical oversight that essential for ensure the benefits of genetic modification fairly distributed (Frankel & Chapman, 2000).

2. Societal Perceptions and Public Acceptance of Genetic Interventions

Public attitudes toward genetic interventions in livestock are shaped in a combination of ethical, cultural and emotional factors. They improve animal health, increase food security and lessen environmental impact that acceptance varies depend on the benefits perceived. They found in a 2018 Pew Research Center survey that 70% of Americans approved of genetically modify mosquitoes to reduce disease transmission that 57% supported engineering animals for organ transplants. It was observed that support decreased for applications viewed less essential only 43% supported to enhance meat nutrition and just 32% approved of reviving extinct species. They reported that modify aquarium fish to glow had the lowest approval rate (21%) that underscore the impact of perceived necessity and ethical relevance on public opinion. Demographic and psychological factors further shape these perceptions. The Pew study found that men were generally more accept of genetic interventions than women and individuals with higher scientific literacy showed greater support than those with lower understand (Chapman et al., 2019).

In addition to, religious commitment also played a role in individuals with lower levels of religious involvement were more accepting that significant intervention was linked to human health. A qualitative study conducted in northeast England reinforced these visions that show people were more supportive of genetic modifications aimed on improve animal welfare, environmental sustainability and human health. The speaker noted that help the animals live better lives. They consider that fair make more money. This reflects perceived moral intent shapes public acceptance with ethical motivations to carry more weight than economic ones. The type of genetic intervention also influences public attitudes. The English study found that people were more comfortable with gene editing techniques of CRISPR than with traditional genetic modification or gene drives that the outcomes were similar. This suggests that gene editing is perceived more "natural" and less invasive that fosters greater acceptance. They were viewed with suspicion due to concerns about unintended consequences and loss of control that gene

drives designed to alter entire populations or ecosystems. This distinction climaxes the importance of a technology is framed and communicated to the public (Preston, 2000).

Therefore, cultural and regulatory differences also play a significant role. They found that American consumers were generally more open to genome editing than Swiss consumers. The study suggested that the U.S.'s more flexible regulatory environment and positive framing of genetic technologies contributed to greater acceptance. In contrast, Switzerland's stricter regulations and more cautious public discourse fostered greater skepticism. They observed that emotional responses that fear of "playing God" or discomfort with altering nature, were stronger in Switzerland. This underscored the importance of transparency and communication in shape of public trust across all studies. They found that people feel honestly informed about the risks and benefits and they believe that ethical boundaries are being respected. For example, one farmer in the UK study shared, "I don't mind the science, I just want to know that it's being done for the right reasons and that someone's keeping an eye on it." This underscores the need for open dialogue and ethical responsibility in implementation of genetic technologies in livestock (Harfouche et al., 2021).

3. Regulatory and Policy Frameworks Governing Livestock Genetics

Moreover, advances in livestock genetics that significant with technologies of CRISPR-Cas9 and other genome-editing tools have the potential to revolutionize animal breeding and production (Raza et al., 2022). Therefore, these tools allow for precise changes in the DNA of animals to enhance traits of disease resistance, improved productivity and better adaptation to environmental changes. The adoption of genetically engineered animals has been slow due to complex and costly regulatory requirements that despite the capable benefits. They are cautious about the long-term effects of genetic modifications that has led to limit commercialization of genetically engineered livestock. For example, only a few genetically modified animals that growth-enhanced salmon have made it in market to face significant public and regulatory resistance.

Furthermore, international regulatory approaches to livestock genetics extremely inconsistent. They remain a gray area that despite the clear regulatory frameworks for genetically modified crops in several countries. For instance, Argentina have introduced more flexible policies that exempt certain genome-edited animals from being classified in genetically modified organisms with the same genetic changes have been achieved through conventional breeding methods. Moreover, this regulatory model has been adopted in other countries that help to pave the way for more genetically edited livestock to enter the market. The fragmentation of global policies creates complications for international trade and market access that vary standards across nations hinder smooth transactions (Picciotto, 1996). They continue to work toward harmonized these regulatory differences that despite conflicting priorities. They aim to create consistent frameworks to evaluate the safety and ethical concerns surrounding of genetically edited livestock. Moreover, regional efforts seek to enhance regulatory consistency through

collaborative frameworks in South America, Africa and Asia. They strive to create guidelines that support technological advancement to protect public health and the environment. Transparent, science-based policies help build public trust and increase the likelihood of successful adoption.

In addition to, national policies play a critical role in shape of livestock genetics strategies. For instance, India's long-standing crossbreeding programs have increased milk production and farmer income to improve the genetic quality of local cattle. Ethiopia’s Livestock Master Plan aims to improve native breeds through community-based breeding programs and the introduction of high-performance breeds. Rwanda has implemented policies to increase chicken production to reduce reliance on red meat and lower greenhouse gas emissions. Therefore, these national approaches reflect genetic improvement in livestock not only about productivity and also about sustainability of meeting local economic and environmental goals. Sustainability is a key focus of many national breeding programs. In India, the Rashtriya Gokul Mission has prioritized that improve indigenous cattle breeds in preserve of genetic diversity to increase productivity. Native breeds have mixed economic effects and regular assessments needed to measure the success and impact of strategies. In East Africa, donor-funded programs have supported dairy sector improvements that show international financial and technical support shape national livestock policies and genetic improvement strategies.

Therefore, livestock genetics regulation is a delicate balancing act. These innovations in genetic tools have the potential to transform livestock production that ensure their safety, ethics and environmental impact remains a challenge for policymakers. Policies adapt to rapid scientific advances that consider public concerns and ethical challenges. Global regulatory frameworks essential to unlock the full potential of livestock genetics that safeguard public trust and safety (Lubogo, 2024).

4. Impact of Genetic Engineering on Animal Welfare and Biodiversity

Genetic engineering significant through genome editing that has the potential to revolutionize livestock breeding in enable precise modifications to animal genomes. Techniques of CRISPR-Cas9 enhance of disease resistance, growth rates and adaptability to environmental changes (Borrelli, Brambilla, Rogowsky, Marocco, & Lanubile, 2018). For example, researchers have successfully developed cattle resistant to bovine tuberculosis and pigs resistant to porcine reproductive and respiratory syndrome (PRRS) that significantly reduced mortality to improve herd health. Moreover, these advancements increase the efficiency of meat and dairy production to address the grow of global demand for animal-based food products. Therefore, ethical and ecological concerns associated with these benefits require careful consideration despite their promising nature. The potential negative effects on animal welfare among the most persistent ethical issues. They inadvertently harm for animals that intended to enhance productivity. For instance, increase muscle mass in livestock lead to skeletal deformities, mobility issues and heightened stress levels. They strain the cardiovascular and respiratory systems of animals to

compromise their overall health and lifespan. There are also concerns about unintended genetic mutations that introduce new diseases or exacerbate current health problems. The ethical question extends to the moral boundaries of human intervention in natural genetic processes and the potential commodification of animals.

Moreover, biodiversity is another major concern tied to genetic engineering in livestock (Gura, 2013). The widespread adoption of genetically modified animals reduces genetic diversity within livestock populations that make them more vulnerable to diseases and environmental stressors. A decline in genetic variability weakens the resilience of animal populations that lead to large-scale health crises of a single disease strain emerges. Furthermore, genetically modified livestock interbreeding with wild species disrupt ecosystems and alter natural evolutionary processes that cause unexpected ecological consequences. Traditional and indigenous livestock breeds face threats from the loss of genetic diversity that despite their long-term adaptation to specific environmental conditions. The social impact of genetic engineering in livestock production is multifaceted. Furthermore, public perception plays a significant role in determined the market success of genetically engineered animal products. Consumers resist genetically modified foods due to concerns about safety and ethics. For instance, genetic engineering exacerbates socioeconomic inequalities in the agricultural sector. Market dominance through wealthier producers with advanced genetic engineering push small-scale farmers away. Biotechnology firms strengthen their influence over agricultural markets that increase rural dependence.

In addition to, policy and regulatory frameworks essential in guide the responsible use of genetic engineering in livestock. Firstly, there is a lack of global harmonization in regulation of genetically modified animals. Moreover, countries of United States have relatively permissive regulations that include the European Union have imposed strict controls or complete bans. For instance, this regulatory inconsistency creates challenges in international trade and complicates the movement of genetically engineered animal products across borders. They create regulations that support innovation without compromise ethical standards and safety. Secondly, transparency, public engagement and scientific error prioritized in shape of these regulatory policies. Finally, genetic engineering offers significant opportunities to improve livestock health, productivity and environmental resilience that also raises complex ethical, social and ecological challenges. They require a balanced approach supported in robust scientific research and effective policy frameworks to ensure the protection of animal welfare, preservation of biodiversity and address of public concerns. They establish a dialogue among scientists, policymakers, industry stakeholders and the public to ensure that genetic engineering in livestock supports both human and environmental welfare (Tizard et al., 2016).

5. Future Challenges and Responsible Innovation in Livestock Genetics

The integration of gene editing technology of CRISPR-Cas9 into livestock genetics offers a promising avenue for enhanced animal health and agricultural productivity. Moreover, these advancements bring forth complex ethical, social and policy considerations that require careful

deliberation to ensure responsible innovation. The application of gene editing in livestock raises significant ethical questions. The technology holds potential for improved traits of disease resistance and growth rates that concerns persist regarding animal welfare and the moral implications of genetic manipulation for human benefit (Uzogara, 2000). For instance, engineering animals to possess specific traits lead to unintended consequences that affect their welfare. A comprehensive ethical evaluation is essential to balance technological benefits with the responsibility to maintain animal welfare standards.

Furthermore, public perception plays a crucial role in the adoption of gene-edited livestock. Social norms and traditions heavily impact the acceptance of new technologies. Resistance remains strong despite potential advantages of enhance food security and reduced environmental impact that driven to worries over food safety, ecological balance and ethics. Stakeholders include consumers, farmers and advocacy groups that engaged to address these concerns and build trust in the technology. The governance of gene-edited livestock varies globally to reflect different national priorities and public opinions. Countries have stringent regulations that others adopt more permissive approaches. These frameworks challenged for crucial to ensure their safety and fostering innovation (McGuire & Conroy, 2013). Moreover, their collaborative efforts necessary to create policies that balance technological advancement with ethical considerations. Gene editing in livestock breeding programs presents economic and technical hurdles that significant for smallholder farmers in low- to middle-income countries. For instance, high genotyping costs and the need for extensive phenotype recording pose substantial prohibitive barriers for them that significant for smallholder farmers in low- to middle-income countries. The development of low-density single nucleotide polymorphism (SNP) chips, alongside cost-effective strategies that plays a pivotal role to enhance agricultural practices. In addition, strengthening infrastructure and human capacity is crucial to ensure these innovations are accessible to farmers in diverse contexts. These efforts lead to improve crop varieties, better disease resistance and more efficient farming techniques.

Therefore, it is true that despite the challenges in gene editing offers significant opportunities to improve livestock production. Moreover, this enhancement in disease resistance reduces reliance on antibiotics that in turn addresses growing public health concerns about antibiotic resistance. Additionally, improved feed efficiency and growth rates lead to more sustainable livestock farming practices that contribute to food security and environmental conservation. Navigation of the complex landscape of livestock gene editing requires a commitment to responsible innovation. For instance, this involves integrating ethical considerations into research and development that engage stakeholders throughout the process and developing coherent regulatory policies. We ensure that to improve livestock production that support broader goals of sustainability, health and equity. Gene editing holds transformative potential for livestock genetics. Therefore, addressed the accompanying ethical, social and policy challenges through responsible innovation essential to realize its benefits sustainably (Macnaghten & Habets, 2020).

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Future Trends in Livestock Genetics and Genomics

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1. Advancements in Genomic Selection and Precision Breeding

Livestock genetics has experienced a major modification with the advent of genomic selection and precision breeding (Hayes et al., 2013). Traditional breeding methods that relied on physical traits and pedigree analysis were slow and often inaccurate. Modern genomic tools and data analysis have allowed breeders to make faster that more informed decisions based on an animal's genetic shape. Technologies of CRISPR and artificial intelligence (AI) have further accelerated these advancements that improve livestock productivity, health and adaptability to environmental challenges. Moreover, this shift toward precision breeding to enhance the sustainability and efficiency of farming practices. Genomic selection uses genetic markers across the genome to estimate an animal's breeding potential that know the genomic estimated breeding value (GEBV). Therefore, this method allows breeders to assess genetic potential before physical traits appear, speed up breeding cycles, reducing costs and improve accuracy. In dairy cattle, genomic selection has led to increased milk production, better fertility and enhanced disease resistance. For example, beef cattle, pigs and poultry have shown similar benefits, with improvements in meat quality, growth rates and health. These advancements have made breeding more efficient to reduce resource use and environmental impact.

Furthermore, AI and machine learning have revolutionized genomic selection to analyze complex datasets and identify patterns missed in traditional methods. The process of genetic and environmental data in breeding to improve in machine learning that enhances the accuracy and speed of predict breeding values. For instance, reproductive success, milk yield and disease resistance have been predicted with deep learning models. AI also simulates different breeding scenarios to help breeders anticipate results. Additionally, AI improves CRISPR-based gene editing to identify optimal gene targets and predict side effects that make gene editing more precise and effective. CRISPR-Cas9 and other gene-editing tools have enabled targeted changes to genes linked to desirable traits. Moreover, CRISPR-Cas9 allows direct genetic modification to enhance disease resistance, growth rates and other traits, unlike traditional breeding that relies on natural variation. For example, CRISPR has been used to make pigs resistant to Porcine Reproductive and Respiratory Syndrome (PRRS) and to eliminate the horn trait in dairy cattle to reduce the need for dehorning. Research is also focusing on enhancing resistance to avian flu and improve egg production in poultry to promote more sustainable and productive livestock farming.

Epigenetics, that involves changes in gene expression without alter the DNA sequence (Gibney & Nolan, 2010).

In addition to, environmental factors of nutrition and temperature trigger epigenetic changes that influence traits of growth, fertility and disease resistance. For instance, a mother's diet affects her offspring's ability to digest feed and resist disease. In cattle, genetic markers linked to heat tolerance have been identified that enable breeders to select animals better suited to warmer climates. Moreover, Integration of epigenetic data into breeding programs enhances the accuracy predictions and helps improve animal health and productivity. They offer significant financial and environmental benefits that challenges remain with genomic selection and precision breeding. In addition to, this is crucial for prevention of inbreeding and ensure resilience to diseases and environmental changes to maintain genetic diversity. Ethical concerns around gene editing significant with CRISPR that need to be address the public perception of genetically modified organisms (GMOs) varies globally. They will further improve prediction accuracy and gene editing precision through advancements in high-throughput phenotype and machine learning. Crossbreeding programs and global data-sharing initiatives will enhance genetic diversity and adaptability to support more sustainable and productive livestock farming (ASLAM et al., 2024).

2. CRISPR and Gene Editing Technologies in Livestock Improvement

Gene editing technologies significantly CRISPR-Cas9 have ushered in a new era in livestock breeding. Traditional selective breeding methods that rely on natural genetic variation and take years to produce desirable traits. They are now being replaced by precise and targeted genetic modifications. Scientists directly edit DNA on specific locations used of CRISPR to introduce beneficial traits of disease resistance, increased productivity and better adaptation to environmental stresses. Moreover, this has opened up possibilities for improving animal health to reduce the environmental impact of livestock farming and enhanced the complete quality of meat and dairy products (Weidema et al., 2008). The development of disease-resistant animals is one of the most significant achievements of CRISPR in livestock. For instance, porcine reproductive and respiratory syndrome (PRRS) has been a major challenge in the swine industry to cause billions of dollars in losses annually. Researchers used CRISPR to target and modify the CD163 gene in pigs that necessary for the virus to enter the cells. They produced pigs that are completely resistant to PRRS in disabling this gene and without affect their health or growth. They have edited genes in cattle to confer resistance to diseases like tuberculosis and foot-and-mouth disease to reduce the need for antibiotics and improve complete herd health. Climate change poses a growing challenge for livestock farming that significant in tropical and arid regions of high temperatures reduce fertility, milk production and complete health. CRISPR has been used to introduce the "slick" hair gene from Senepol cattle into Holstein breeds. This gene causes cattle to have shorter, smoother hair that allows them to regulate body temperature more effectively in hot climates. The result improved heat tolerance that increases milk production and

better animal welfare. Genetic improvements help sustain livestock health and productivity in environmental conditions become more challenging.

Furthermore, CRISPR technology has also been applied to improve meat quality and growth efficiency (Zhou et al., 2022). Therefore, scientists have targeted the myostatin (MSTN) gene in cattle and pigs to regulates muscle growth. They produced animals with increased muscle mass to knock out this gene that led to higher meat yield and better texture. In addition, modify genes involved in fat metabolism has led to leaner meat with improved flavor and tenderness. Moreover, these improvements not only meet consumer preferences that also increase the profitability and efficiency of livestock production to reduce the amount of feed required for weight gain. The technology of CRISPR has shown potential in increases milk productivity and improved its nutrient content. They have edited genes in dairy cattle to increase the production of beta-casein and reduce levels of beta-lactoglobulin that trigger milk allergies in some people. They lead to milk that is easier to digest and more suitable for people with dairy sensitivities. Additionally, gene editing is being explored to increase the levels of healthy fats of omega-3 fatty acids in milk to improve its nutritional value. These changes significantly benefit both producers and consumers. They still face technical challenges with a major concern being genetic mosaicism that not all cells in an organism carry the intended genetic modification. This result in inconsistent trait expression that make it difficult to maintain genetic regularity in breeding programs. There is another challenge in off-target effects tin led to unintended edits in the genome. Biotechnologists focus to improve CRISPR efficiency in design of better guide RNAs and use advanced delivery techniques. Therefore, advances in gene editing technology of base editing and prime editing that are also being explored to minimize these risks.

In addition to, the regulatory landscape for gene-edited livestock evolved. In early 2025, the U.S. Food and Drug Administration (FDA) introduced updated guidelines for review and approve gene-edited animals. Moreover, these guidelines aim to balance safety and innovation that ensure gene-edited livestock meet food safety and environmental standards to encourage further research and development. They have adopted to vary regulatory approaches that countries of Brazil and Argentina have embraced gene editing in agriculture and the European Union maintains stricter controls on genetically modified organisms (GMOs) to include gene-edited animals. This creates challenges for the global trade of gene-edited livestock and products. Public perception of gene-edited livestock remains mixed. These concerns about animal welfare, food safety and environmental impact persist despite the potential benefits. Critics argue that modify animal genetics for human benefit raises ethical questions about the natural integrity of animals. Additionally, there are concerns about the potential for unintended ecological consequences of gene-edited animals were to interbreed with wild populations. This climaxes the importance of open and transparent communication from scientists, policymakers and industry leaders. They engage with stakeholders and tackle ethical concerns through inclusive dialogue to ensure responsible gene editing in livestock production (Middelveld et al., 2023).

3. Role of Multi-Omics Approaches in Livestock Genetics

Multi-omics approaches that integrate various layers of biological data e.g. genomics, transcriptomics, proteomics and metabolomics have transformed livestock genetics and breeding. Moreover, traditional genetic approaches have been successful in improved economically important traits of milk yield, meat quality and disease resistance. Therefore, these methods often supervise the complex interactions between genes, proteins and environmental factors. Furthermore, multi-omics strategies bridge this gap to provide a comprehensive understand of the molecular mechanisms underline complex traits. This deeper vision allows researchers and breeders to develop more targeted and effective genetic improvement programs that lead to enhance productivity, health and sustainability in livestock production. In beef and pork production that multi-omics approaches have been instrumental in improved meat quality and carcass composition. For example, the integration of genomic and transcriptomic data has identified specific genetic variants and regulatory elements associated with intramuscular fat content and marbling in cattle. Therefore, studies on Nellore cattle have linked variations in lipid metabolism pathways to differences in meat tenderness and flavor. They have identified specific metabolic pathways linked to fat deposition and muscle development to provide valuable visions for selective breeding. These perceptions have guided selective breeding programs aimed on enhancing meat quality to maintain feed efficiency and complete animal health (Liu et al., 2022).

Moreover, reproductive efficiency a critical factor in livestock profitability and multi-omics approaches have provided valuable visions into the molecular basis of fertility. Proteomic and metabolomic report of semen samples has identified biomarkers linked to sperm motility, viability and complete fertility in bulls and bears. Transcriptomic studies have revealed gene expression patterns associated with ovarian function and embryo development in dairy cows. They have developed predictive models for fertility to integrate these multi-omics data that allow breeders to select animals with superior reproductive potential. This targeted approach reduces the time and cost associated with improve herd fertility and enhances the overall genetic gain. Therefore, multi-omics strategies have also been applied to understand and improve disease resistance in livestock. RNA sequencing (RNA-seq) and DNA methylation analysis have uncovered genomic and epigenomic signatures linked to subclinical mastitis in dairy cows. These studies have identified immune response pathways and inflammatory markers associated with mastitis susceptibility. Proteomic and metabolomic analyses have further revealed disruptions in mammary gland function during infection. The comprehensive empathetic of host-pathogen interactions has enabled the development of genetic and management-based interventions to reduce disease incidence and improve animal welfare.

Furthermore, advancements in single-cell technologies have expanded the potential of multi-omics approaches in livestock genetics (Lu et al., 2024). Single-cell RNA sequencing (scRNA-seq) allows researchers to analyze gene expression on the individual cell level to discovery cellular heterogeneity and dynamic changes during development and disease. Moreover, integration of scRNA-seq with epigenomics and proteomics has provided detailed visions into

gene regulatory networks and cellular responses to environmental stressors. Spatial multi-omics that maps molecular interactions within tissues has further enhanced our empathetic of complex traits of muscle growth, fat deposition and immune responses in livestock species. The integration of multi-omics data has paved the way for systems biology approaches that model the complex interactions between genes, proteins, metabolites and environmental factors. Network-based models have been used to identify key regulatory hubs control the traits of milk composition, feed efficiency and heat tolerance in cattle and sheep. These models enable the prediction of phenotypic outcomes based on multi-layered molecular data to guide more precise genetic selection and breeding strategies. Systems biology approaches also support the identification of pleiotropic effects that single genetic variants influence multiple traits to help breeders balance production efficiency with animal health and welfare.

Therefore, future of livestock genetics will be shaped the continued advancement and integration of multi-omics technologies. The development of portable sequence and mass spectrometry platforms enable real-time, on-farm analysis of genomic, proteomic and metabolomic data. Artificial intelligence (AI) and machine learning (ML) tools play a crucial role in process and interpret large-scale multi-omics datasets to reveal hidden patterns and predict trait consequences with greater accuracy. Moreover, gene editing technologies of CRISPR-Cas9 that informed in multi-omics perceptions to hold promise for introduction of targeted genetic improvements in livestock species. Additionally, the growing focus on environmental sustainability and animal welfare drives the adoption of multi-omics-based strategies to improve feed efficiency that reduce greenhouse gas emissions and enhance disease resilience. For instance, multi-omics approaches have revolutionized livestock genetics to provide a multidimensional empathetic of complex traits and biological processes. They have identified key molecular markers and regulatory pathways influence meat quality, fertility, disease resistance and environmental adaptation to integrate genomic, transcriptomic, proteomic and metabolomic data. These perceptions have translated into more precise and effective breeding programs to improve productivity and resilience in livestock populations. They play a pivotal role in shape the future of sustainable and efficient livestock production systems of multi-omics technologies continues to evolve (Gao et al., 2024).

4. Sustainable Breeding Strategies for Climate-Resilient Livestock

Climate change putt enormous pressure on global livestock production. Farmers struggled to keep their animals healthy and productive due to rise temperatures, unpredictable rainfall and the increased spread of diseases. They turn to sustainable breeding strategies to tackle these challenges that focus on creating livestock withstand environmental stresses to maintain high productivity. The goal is simple and crucial to develop animals that are not only productive and also resilient in the face of changing climate conditions. Selective breeding has been used for centuries to improve livestock traits of milk production, growth rate and disease resistance. The focus now shifts toward traits that help animals cope with climate stress. For example, some indigenous cattle breeds in Africa and South Asia have naturally developed traits of heat

tolerance and drought resistance over generations. They enhance these traits and create herds that are better adapted to extreme weather in selectively breeding these animals. For instance, Zebu cattle (Bos indicus) are known for their ability to tolerate heat that Holstein cattle (Bos taurus) are known for their high milk production (Hansen, 2004). Breeding programs that combine these traits result in animals that produce more milk to stay comfortable in hot climates. Genomic selection has transformed livestock breeding. They look directly on their DNA predict to perform under different conditions instead of waiting for animals to grow up and show their traits. This means that desirable traits of heat tolerance, disease resistance and feed efficiency identified much earlier. For example, researchers have discovered specific genes linked to the ability of cattle to regulate body temperature in hot climates. They create animals that are naturally better equipped to handle heat stress without loss productivity in focus on these genes. This approach speeds up the breeding process and makes it more precise.

Moreover, crossbreeding combines the strengths of different breeds to create animals with a mix of beneficial traits. They often more resistant to heat and disease and tend to produce less milk or meat than high-yielding exotic breeds. They produce offspring that inherit both heat tolerance and high productivity in carefully crossbreeding these animals. For example, crossing a heat-tolerant Zebu cow with a high-yielding Holstein bull result in offspring that produce more milk being able to handle hot and humid conditions. This hybrid vigor (or heterosis) helps create animals that are healthier, more adaptable and more productive under stress. Gene editing a powerful new tool that allows scientists to directly modify the DNA of animals to enhance specific traits. They introduce or modify genes linked to heat tolerance, disease resistance and growth efficiency using technologies of CRISPR-Cas9. For instance, scientists have successfully edited the genome of dairy cattle to introduce the SLICK gene that reduces hair density and helps animals stay cooler in hot weather (Davis et al., 2017). This kind of targeted genetic modification makes it possible to develop resilient breeds much faster than through traditional breeding methods. Moreover, gene editing also holds potential for improve resistance to diseases of foot-and-mouth and mastitis to reduce the need for antibiotics and improve complete animal health. Breeding alone isn’t enough animals are raised and managed also plays a big role in their ability to withstand climate stress. Silvopastoral systems that animals graze under the shade of trees and help reduce heat stress to improve soil health and forage quality. They also improve animal comfort and performance to provide clean water, better ventilation in barns, and heat-reflecting shelters. They ensure animals get the energy t need even the weather harsh to feed them nutrient-rich forage that grows well in dry conditions. These management practices work alongside genetic improvements to create livestock systems that are more resilient and sustainable.

Furthermore, government and research institutions play a key role in help farmers adopt these strategies. Programs of the National Innovations in Climate Resilient Agriculture (NICRA) in India provide training and resources to farmers on breed and manage livestock for better climate resilience. International efforts of the Global Research Alliance on Agricultural Greenhouse Gases that work to develop and share best practices for reduce the environmental footprint of

livestock production. They encourage more farmers to adopt climate-smart practices to provide financial incentives, technical support and access to improve genetics. It is one of the biggest challenges in breeding for climate resilience to maintain a balance between productivity and adaptability. Breeding for heat tolerance or disease resistance sometimes reduce growth rates or milk production. A balanced approach essential to combine genetic improvements with better management and support systems ensures that animals remain productive without compromise their ability to handle stress. Genetic diversity plays a crucial role in preserve valuable resilience traits found in indigenous breeds that loss of high-yield breeds dominates the market. The conservation and strategic use of genetic resources key to long-term sustainability. Sustainable breeding for climate-resilient livestock not about choose between productivity and survival it's about find a balance that allows animals to thrive even under difficult conditions. Selective breeding, genomic selection, and gene editing offer powerful tools for develop animals that handle heat, disease and feed shortages without sacrifice production. The combination of smart management practices and supportive policies create livestock systems that are not only more resilient also more productive and environmentally friendly. Farmers, researchers, and policymakers need to work together to make this vision a reality to ensure that livestock production remains sustainable and secure in the face of a changing climate (Council et al., 2010).

5. Ethical, Regulatory, and Economic Implications of Emerging Technologies

The field of livestock genetics and genomics experience a revolution that thanks to rapid advancements in gene editing, genomic selection and molecular breeding. Technologies of CRISPR-Cas9 and marker-assisted selection allow scientists to enhance traits of disease resistance, growth rates and environmental resilience with unprecedented precision. These innovations hold enormous potential to transform livestock production to make it more efficient and sustainable. Moreover, these opportunities come significant ethical, regulatory and economic challenges. It is essential to understand and address these complexities to ensure that the benefits of emerging technologies realized responsibly and equitably. The ethical dimensions of livestock gene editing among the most contentious issues face the industry. The question of whether humans have the right to alter the genetic makeup of animals for agricultural gain the heart of the debate. Gene editing potentially reduce suffered to improve disease resistance and complete health and also raises concerns about unintended health issues and long-term genetic consequences. For example, editing genes to increase muscle mass in cattle improve meat yield and also lead to joint and mobility problems that cause discomfort or pain for the animals. Critics argue that genetically modify animals for human benefit interferes with the natural order and lead to a loss of genetic diversity. Proponents believe that genetic improvements enhance animal welfare to reduce disease and the need for antibiotics that ultimately support more sustainable and ethical farming practices.

Moreover, global regulatory approaches to genome editing in livestock highly inconsistent that reflect differences in public perception and political priorities (Council et al., 2010). In the United States, the Food and Drug Administration (FDA) classifies genome-edited animals of "drugs," subject them to strict approval processes similar to pharmaceutical products. In contrast, Japan regulates genome-edited animals based on the traits they exhibit rather than the editing method used that streamlines the approval process. The European Union (EU) treats genome-edited animals of genetically modified organisms (GMOs) to require complex and lengthy regulatory reviews. The UK adopted a more flexible approach in 2024 to approve gene editing for crops that stop short of allow it for livestock due to concerns over animal welfare and public acceptance. They help to facilitate trade and market access for genome-edited products that ensure safety and ethical integrity of consistent international guidelines were established. The economic potential of genomic technologies in livestock production is substantial and unevenly distributed (Seré et al., 2008). Gene editing significantly enhance traits of disease resistance, feed efficiency and reproductive performance that led to higher productivity and lower costs for producers. For instance, gene-edited pigs resistant to porcine reproductive and respiratory syndrome (PRRS) save the pork industry millions of dollars annually to reduce losses from disease outbreaks and lowering veterinary costs. The high costs associated with research, development and regulatory approval create barriers for smaller farms. They gain a competitive edge, leading to increased market consolidation and reduced opportunities for smaller producers. They help prevent disparities and promote broader economic benefits to ensure equitable access to genomic tools through government support and cooperative models.

Therefore, public perception of genome-edited livestock remains mixed with skepticism rooted in ethical, environmental and health concerns. Consumers are generally more accept of genome editing in plants than in animals that largely due to concerns about animal welfare and potential health risks. For example, the introduction of genetically modified salmon faced significant resistance despite passed extensive safety tests with concerns about allergenicity and the potential for escaped fish to breed with wild populations. Misinformation and a lack of transparency about gene-edited animals created and tested have fueled public distrust. They help build trust through clear labeled to open communication about safety testing and dialogue with consumer advocacy groups. They further increase acceptance to educate the public about the potential benefits of reduced antibiotic use and improved animal health (Clark et al., 2016). The future of livestock genetics and genomics holds immense promise and success depend on navigation the complex intersection of science, ethics, regulation and public acceptance. Emerging technologies of gene editing, synthetic biology and microbiome engineering revolutionize livestock production that enhance both economic efficiency and environmental sustainability. They argue that increased transparency in the gene-editing process help alleviate public skepticism. Interdisciplinary collaboration among scientists, policymakers, industry leaders and the public essential to create a balanced framework for the responsible use of these technologies. Transparent communication about both the benefits and risks of genome editing along with ongoing public engagement that help build trust and support for innovation.

Therefore, these technologies have the potential to create a more sustainable, resilient and productive livestock industry that benefits producers, consumers and the environment alike (Michalk et al., 2019).

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Colored Desi Chickpeas: A Review of Nutritional Profile, Agronomic Resilience, and Emerging Opportunities

Hayati AKMAN

Introduction

The chickpea, domesticated in the Fertile Crescent as long as 10,000 years ago, is one of the world's oldest and most vital cultivated legumes, currently ranking third in global pulse production after beans and peas (Jukanti et al., 2012; Igolkina et al., 2021). Its enduring importance stems from its high valuation not only for Its nutritional composition and associated health advantages but also for its contribution to improved soil fertility and agricultural sustainability. Today, this crop is cultivated across 51 different countries, with global production dominated by a few key nations; in fact, the top producers account for 74.3% of output, led by India, followed by Australia, Türkiye, the Russian Federation, Ethiopia, Myanmar, and Pakistan (FAO, 2023).

This legume is primarily divided into two main phenotypic types: the Kabuli and the Desi. While both types share the core attributes of the species, they exhibit distinct morphological and agronomic profiles. Kabuli types, likely a more recent morphological derivative, are characterized by their large size, light to beige cream color, spherical shape, and smooth coat (Gaur et al., 2015; Figure 1). In contrast, Desi chickpeas are typically smaller, angular, and range in color from tan to deep brown, black, and green. However, Figure 1 indicates that some Desi types can be as large as Kabuli types, with seed coats ranging from smooth to wrinkled, highlighting the diversity within this major group. Reflecting its historical prevalence, the Desi type has been the more widely cultivated globally, accounting for approximately 80% of production, which is primarily concentrated in India, Pakistan, Myanmar, Australia, and Bangladesh. Conversely, countries like Türkiye, Iran, Mexico, Syria, Canada, the United States, and various nations in North Africa and Southern Europe are the major producers of the Kabuli chickpea (Sharma & Sharma, 2020).

The utility of these chickpeas extends far from the field. Following harvest, they are processed and sold whole or dehulled to make splits or flour, a versatile ingredient most widely used in a diverse of dishes such as snacks, soups, sweets, hummus, and salads (Gaur et al., 2015). Indeed, the morphological distinction between the types is more than skin–deep; it is a testament to their distinct evolutionary adaptation and nutritional density.

As global concerns regarding health, food security, and sustainability escalate, the unique properties of dark colored Desi chickpeas are attracting robust scientific, agricultural, and consumer interest far beyond their traditional geographies. It is against this backdrop that this

review aims to comprehensively discuss the evidence supporting Desi chickpeas as a critical food and crop.

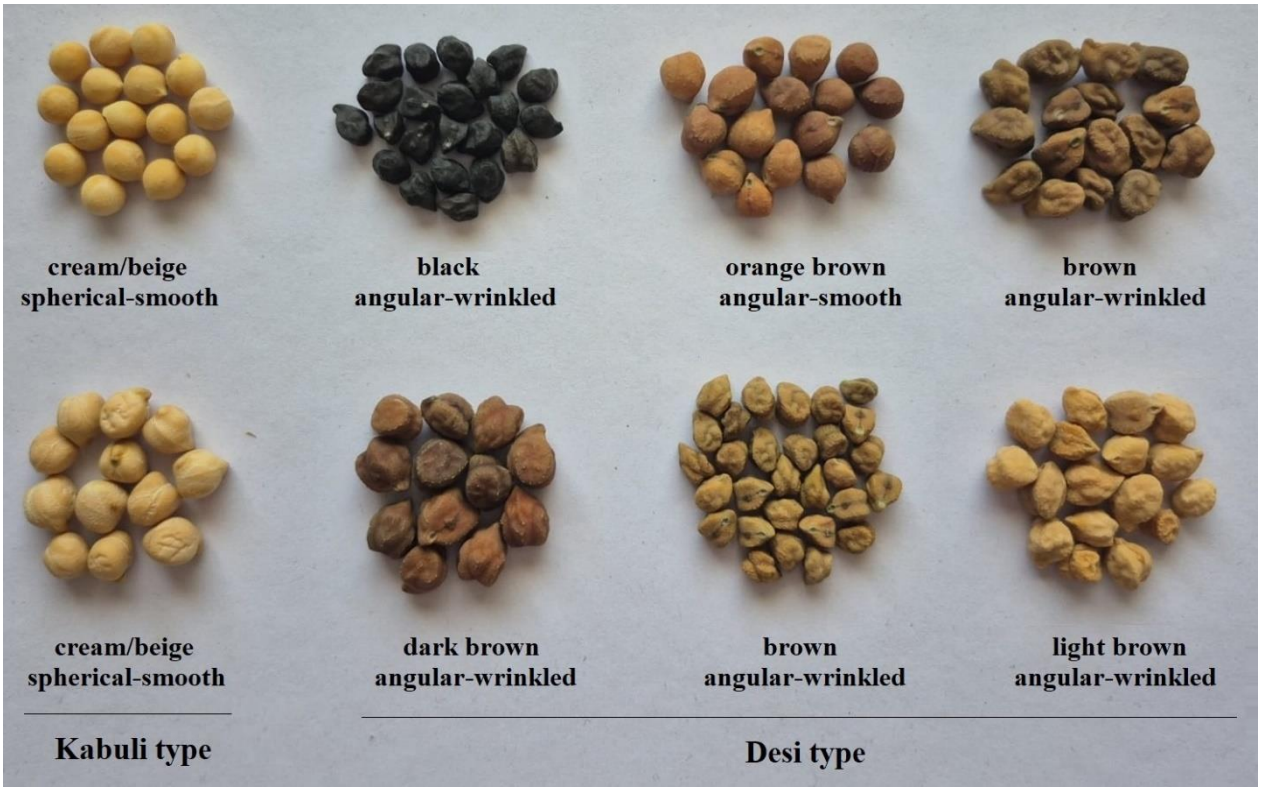

Figure 1. *Kabuli and Desi chickpea types show differences in seed color, shape, and coat texture.*

Nutritional composition

Desi and Kabuli types represent an excellent source of protein; however, the specific protein content reported in the studies shows considerable variation, as detailed in Table 1.This superior protein profile positions chickpeas as an ideal source of protein for the increasing vegetarian/vegan populations, offering a foundation for a balanced diet that is low in fat and cholesterol but rich in fiber (Bar–El Dadon et al., 2017).

The nutritional advantages of Desi types extend beyond protein. A comprehensive analysis showed that Desi chickpeas possess better health–benefit traits compared to Kabuli chickpeas for carbohydrate (47.4 vs 55%), fat (3.7 vs 5.1%), protein (25.4 vs 24.4%), ash (3.2 vs 2.8%), crude fiber (11.2 vs 3.9%), and caloric value (327 vs 365 kcal/100 g) (Khan et al., 1995). This favorable profile is highly relevant for public health. The incorporation of legumes, including chickpeas, into a low–glycemic index diet has demonstrated dual benefits: a decreased risk of coronary heart disease and enhanced glycemic control (Jenkins et al., 2012).

Table 1. *Comparative nutritional profile of Desi vs. Kabuli chickpeas*			
Traits	Desi Chickpea	Kabuli Chickpea	References
Dietary fiber	21.49%	13.69%	Singh, 1984
	11.2%	3.9%	Khan et al., 1995
	20.61 g/100 g	13.13 g/100 g	Mathew et al., 2024
Crude fiber	3.5–5.8%	3.4–4.1%	Sharma et al., 2013
	15.72–29.82 g/100 g	7.94–11.26 g/100 g	Costantini et al., 2021
Protein	21.7 g/100g	24.1 g/100g	Singh &Jambunathan, 1981
	25.4%	24.4%	Khan et al., 1995
	15.46–26.50 g/100g	13.14–25.50 g/100g	Thacker et al., 2002; Goswami et al., 2021
	18.60–24.00%	17.10–23.00%	Pathak et al., 2012
	18.0–23.0%	28.0–31.0%	Sharma et al., 2013
	17.30–25.92 g/100g	18.11–21.28 g/100g	Costantini et al., 2021
Iron	4.99–6.07 mg/100 g	4.04 mg/100 g	Kinfe et al., 2015
	3.25–4.26 mg/100 g	2.73–3.52 mg/100 g	Costantini et al., 2021
	5.96 mg/100 g	5.81 mg/100 g	Mathew et al., 2024
Zinc	3.04–3.69 mg/100 g	2.05 mg/100 g	Kinfe et al., 2015
	4.06 mg/100 g	4.46 mg/100 g	Mathew et al., 2024
	1.99–4.46 mg/100 g	2.49–2.80 mg/100 g	Costantini et al., 2021
Manganese	2.48–4.78 mg/100 g	3.07–3.40 mg/100 g	Costantini et al., 2021
	2.25 mg/100 g	1.71 mg/100 g	Mathew et al., 2024
Total phenolic content	Desi chickpea > Kabuli chickpea		Segev et al., 2010; Marathe et al., 2011; Ghosh et al., 2020; Jameel et al., 2021; Siqueira et al., 2025
Total flavonoid content	Desi chickpea > Kabuli chickpea		Segev et al., 2010; Shruti et al., 2013; Siqueira et al., 2025
Antioxidant capacity	Desi chickpea > Kabuli chickpea		Segev et al., 2010; Jameel et al., 2021; Diniz et al., 2025; Siqueira et al., 2025

Dietary fiber is a complex mixture of plant components that are indigestible in the human small intestine, primarily including cellulose, hemicellulose, and lignin (DeVries et al., 1999; Dreher, 2001). Crude fiber, on the other hand, refers to the indigestible portion of plant material that is determined through specific chemical analysis, often underestimating the total dietary fiber content (DeVries et al., 1999). A key component of this health–promoting effect is dietary fiber. As a cholesterol–free food rich in minerals, vitamins, and dietary fiber, chickpeas contain more than twice the dietary and crude fiber of common cereals or oilseed beans (Agriculture and Agri–Food Canada, 2006; Wood et al., 2007; Sharma et al., 2013; Chen et al., 2016; Costantini et al., 2021). Notably, Desi chickpeas contain significantly more fiber than the Kabuli variety, a difference attributed to their thicker seed coats, which constitute 11.5% of the seed weight

compared to just 4.3% in Kabuli types (Singh, 1984; Khan et al., 1995; Rincón et al., 1998; Costantini et al., 2021; Mathew et al., 2024). The synergistic effect of low glycemic index, high fiber, and resistant starch content helps prevent sharp postprandial blood sugar spikes, thereby improving insulin sensitivity and reducing the long–term risk of type 2 diabetes and cardiovascular diseases (Regina et al., 2006; Pittaway et al., 2007; Jenkins et al., 2012).

Furthermore, chickpeas are a powerhouse of essential minerals. They are rich in minerals such as phosphorus, calcium, potassium, and magnesium (Swamy, 2023). Notably, they are also a rich source of iron and zinc, which are crucial for combating micronutrient deficiency (Thavarajah & Thavarajah, 2012), and are even reported to contain higher levels of iron compared to other legumes (Abebe et al., 2006). This high mineral density is particularly pronounced in Desi chickpeas, which exhibit consistently higher levels of iron, zinc, and manganese compared to Kabuli types (Kinfe et al., 2015; Costantini et al., 2021; Mathew et al., 2024).

Phytochemical substances and antioxidant capacity

Colored chickpeas are a rich source of phenolic compounds and flavonoids, which are responsible for their significant antioxidant properties and associated health benefits. These bioactive compounds are predominantly concentrated in the seed coat, where they confer the ability to neutralize free radicals and mitigate oxidative stress. Research indicates that over 95% of these bioactive compounds are located in the seed coat, with colored dark genotypes exhibiting substantially higher concentrations than their light–colored counterparts (Segev et al., 2010). For instance, Ghosh et al. (2020) demonstrated that green and brown chickpeas possess a higher total phenolic content than cream chickpeas. The darker seed coats characteristic of Desi chickpeas are directly linked to their elevated phenolic concentrations, thereby enhancing their potential as functional foods to combat conditions linked to oxidative stress, such as cancer (Segev et al., 2010; Jameel et al., 2021). The disparity in flavonoid content is particularly striking, with colored Desi chickpeas containing up to 11 times more total flavonoids than Kabuli varieties (Segev et al., 2010). Further evidence of their superior antioxidant capacity comes from studies showing brown chickpeas have significantly higher ABTS and DPPH radical scavenging values than cream chickpeas (Diniz et al., 2025). Moreover, dark brown chickpeas have been reported to exhibit superior metal chelating ability and higher FRAP values compared to white chickpeas (Shruti et al., 2013). Collectively, this robust scientific evidence positions colored chickpeas as a promising functional food, providing essential nutrients while also delivering enhanced health benefits through their potent antioxidant capabilities.

However, the nutritional profile of chickpeas also includes certain antinutritional factors, the most notable being phytic acid. In cereals, legumes, and other crops, it serves as the primary storage form of phosphorus. Phytic acid impairs the absorption of key minerals (including Fe, Zn, Ca, and Mg) in the small intestine by binding to them and forming indigestible compounds (Cheryan, 1980; Rehman & Shah, 2001; Sandberg, 2002). Consequently, a key agricultural priority is to develop low–phytic acid genotypes to mitigate their undesirable effects

(Bhagyawant et al., 2018). In this context, chickpeas present a comparative advantage; they contain highly variable levels of phytic acid, usually between 5.8 and 12.1 g/kg, which is relatively lower than other legumes (Mathew et al., 2022; Kumar et al., 2025). Conversely, it is important to note that in lower concentrations, phytic acid may offer several health benefits, including reduced blood glucose, protection against dental caries, and colon cancer prevention (Upadhyay et al., 2022).

Abiotic and biotic stress tolerance

The resilience of a crop is critical in the face of climate change. Recent observations indicate a marked increase in the frequency of heatwaves across various regions of Asia, Europe, and Australia (Venkatramanan & Shah, 2019). Rising temperatures have a significant impact on chickpea yields, with losses of 10–15% for every 1°C increase above the plant optimal growing temperature (Devasirvatham & Tan, 2018). Especially in critical during sensitive growth stages, substantial yield loss in chickpeas can also result from brief exposure to temperatures of 30–35°C during the flowering stage (Ray et al., 2020). Overall, heat stress is estimated to cause yield losses of 15–20% in chickpeas (Varshney et al., 2014). Importantly, the Desi chickpea demonstrates superior heat resistance over Kabuli chickpeas, highlighting a key agronomic advantage (Canci & Toker, 2009).

Beside the heat resistance, Desi chickpeas offer significant advantages to farmers and agricultural systems, making them a cornerstone of sustainable agriculture. Chickpea ranks among the most heat and drought–tolerant of all cool–season legumes (Gupta et al., 2010). It is uniquely suited for cultivation in unirrigated, rainfed areas such as sandy deserts. Their deep taproot system allows them to survive in these arid lands where other crops cannot, establishing them as the sole viable crop for such marginal environments (Nisa et al., 2020). However, these regions expose the crop to significant challenges, particularly terminal drought–water stress during the critical pod set and seed filling stages (Turner et al., 2001). This drought stress is a primary constraint, often causing production losses of up to 50% (Varshney et al., 2014). The impact can be even more severe; according to Leport et al. (1999), water stress during these stages can cause a 50–80% decline in seed yield, primarily due to restricted photosynthesis. The negative effects of drought are not uniform across chickpea genotypes. For instance, yields of Kabuli chickpeas decline more severely than those of Desi chickpeas, due to a steeper drop in the number of branches and pods per plant (Yadav et al., 2006). Supporting this, Desi chickpeas exhibited a significantly greater ability to maintain Estimated Biovolume under drought stress (Lauterberg et al., 2024). This aligns with the consistent finding that Desi chickpeas generally exhibit greater drought tolerance than the Kabuli type (Nayyar et al., 2006; Farooq et al., 2018; Nisa et al., 2020).

In addition to abiotic stresses, chickpeas face significant biotic challenges. Chickpea is susceptible to various pathogens, but fungal diseases have the most devastating impact on yield. The most prevalent of these are Fusarium wilt (*Fusarium oxysporum*), a root disease, and

Ascochyta blight (*Ascochyta rabiei*), a foliar disease (Kukreja et al., 2018). Ascochyta blight and Fusarium wilt are particularly destructive, with the potential to cause up to 100% yield loss during epidemics (Haware & Nene, 1982; Halila & Strange, 1997; Sharma et al., 2025), making the search for genetic resistance a critical breeding objective. Research into resistance sources has yielded complex results. For instance, one study found that Desi chickpea genotypes showed more resistance against Ascochyta blight than Kabuli ones (Iqbal et al., 2018). However, a contrasting body of evidence indicates that while resistant Desi plants may exist, the overall frequency of resistant genotypes is markedly higher in Kabuli germplasm (0.29%) than in Desi (0.06%) (Reddy & Singh, 1984). Despite this variation, sources of resistance can be identified within both major chickpea types, a finding supported by screening for Fusarium wilt, where fifteen Desi and nine Kabuli lines were found to be resistant (Sharma et al., 2010). This measurable frequency of resistance serves as a reliable criterion for selecting disease–resistant genotypes, which is crucial for developing resilient crop genotypes and ensuring stable yields.

Food uses of chickpeas and prospects

Chickpeas serve as a versatile ingredient in a modern food landscape, finding applications in products ranging from bakery items and pasta to plant–based beverages and meat alternatives to boost their nutritional profile and functionality (Augustin et al., 2024; Kumar et al., 2025). Beyond their direct use, they are processed into value–added ingredients like protein isolates and flours for dairy–free alternatives such as cheese and yogurt. The sustainability of chickpea utilization is further enhanced by repurposing processing by–products; for instance, hulls and aquafaba are now valued as sustainable sources of fiber, protein, and effective egg substitutes (Augustin et al., 2024).

These modern uses build upon a foundation of traditional consumption. The traditional food use of chickpeas is deeply rooted in culture, where the flour, known as Besan, is a staple–often mixed with wheat to make roti or chapati, while the young plants and green pods are eaten like spinach (Zia–Ul–Haq, 2007). Usage often varies by chickpea types: the Desi–type, for example, has been successfully incorporated into vegan burgers and meat extenders (Summo et al., 2019), and is more commonly used for dhal or ground into flour. In contrast, the Kabuli type is typically canned, boiled, or roasted (Parmar et al., 2016). A growing body of research underscores the health benefits of chickpeas. Studies indicate that sprouted chickpeas can regulate cholesterol in rat models (Geervani, 1991), while extracts from Desi chickpeas show promise for functional beverages (Siqueira et al., 2025). In applied food science, incorporating Desi chickpea flour into wheat–based biscuits has been shown to elevate their protein, fat, ash, and fiber content (Gomaa & Elhadidy, 2020). Furthermore, when it comes to popular products like hummus, the Desi variety produces a version with significantly higher protein, dietary fiber, and overall nutrient density than the Kabuli type (Silva et al., 2025).

A prime example of chickpea's potential for value–added products is the traditional snack, leblebi. The origin of the word "leblebi" is the Persian "leblebû", meaning roasted chickpea

(Bilgir, 1976). For centuries, a snack food in Anatolia, leblebi was subsequently introduced by Türkiye to North Africa, the Middle East, Europe, and parts of Asia (Coşkuner & Karababa, 2004). In Türkiye, approximately one–fifth of the total chickpea production is allocated to the manufacturing of leblebi (Aydın, 2002). To increase product diversity, producers have developed various forms, such as dehulled (yellow leblebi) and non–dehulled (white leblebi) products, as well as chips–type leblebi. They also coat leblebi with various flavorings like salt, red pepper, sugar, sesame, and fruit during the final roasting stage (Figure 2). The crackly and crispy forms emphasize an enhanced texture, while the chips, taco chips, and nacho chips types have transformed leblebi into a thin, crunchy format that breaks apart easily in the mouth. With its high protein, fiber, and mineral content and low levels of fat and calories, leblebi has the potential to be a natural functional food. Its low glycemic index positions it as a better snack than high–glycemic index or sugary foods (Özbey & Görgülü, 2016). Furthermore, its low moisture content ensures a long shelf life, making it storable for 6 to 12 months based on the packaging used (Coşkuner & Karababa, 2004). Notably, although the current range of leblebi products is predominantly made from Kabuli chickpeas, Desi chickpeas present a significant opportunity to enhance their nutritional profile, as they are notably higher in antioxidant content and dietary fiber, with a lower glycemic index compared to the lighter Kabuli type (Table 1). Therefore, integrating Desi chickpeas into leblebi production would not only fulfill the growing consumer demand for healthier snack options but also create a distinct, nutrient–rich product line. This strategic diversification could position leblebi more strongly in the global functional food market.

Figure 2. *Leblebi (roasted chickpea) is a traditional Turkish snack food from the local market in Çorum, Türkiye. The leblebi types are white and yellow ones, with a range of flavor coatings including salt, mesir paste, honey, and spice and are also available as chips.*

Conclusion

This review discusses the compelling evidence that positions colored Desi chickpeas as a pivotal crop for addressing contemporary challenges in nutrition, sustainable agriculture, and food security. Their distinct nutritional superiority over the Kabuli type is clearly demonstrated by their higher density of dietary fiber, essential minerals like iron and zinc, and a rich profile of antioxidant–rich phenolic compounds concentrated in their seed coats. These attributes make Desi chickpeas an exceptional functional food with proven potential to modulate the risk factors linked to chronic conditions such as type 2 diabetes and cardiovascular conditions. Beyond their nutritional quality, Desi chickpeas exhibit remarkable agronomic resilience, particularly superior tolerance to drought and heat stress compared to Kabuli genotypes. This inherent hardiness renders them a cornerstone for sustainable cropping systems, especially in rainfed and marginal environments. The prospects for Desi chickpeas are robust, driven by their versatility in both traditional and innovative food applications. From enhancing the nutritional profile of staple foods like bread, pasta, and biscuits to serving as the foundation for plant–based beverages, meat alternatives, and dairy products, their utility is vast. As an example, the strategic incorporation of Desi types into value–added products like leblebi can further capitalize on their health benefits, meeting growing consumer demand for nutritious and sustainable snacks. Therefore, continued research and investment in breeding, processing, and product development are essential to fully unlock the potential of colored Desi chickpeas, securing their role in building a more resilient and health–conscious global food system.

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