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Genetic Selection for Disease Resistance in Commercial Poultry Breeds

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Abstract | Outbreaks of disease in commercial poultry flocks result in significant economic losses, either through acute mortality or reduced productivity due to chronic infection. Although biosecurity and vaccination remain crucial elements of disease control, genetic selection to provide disease resistance has proven to be a long-term and sustainable measure, particularly for commercial poultry breeds. Historically, attempts to breed disease-resistant chickens started almost a century ago. Still, new fears over emerging and re-emerging diseases, such as Avian Influenza, have driven recent renewed scientific interest in the area. Experiments have demonstrated that commercial chickens possess inherent genetic resistance to several key diseases, including Marek's disease, avian leukosis complex, Newcastle disease, and salmonellosis. Molecular genetic progress has provided the tools for identifying genes and genetic markers associated with resistance, enabling more precise and practical selection. Technologies such as genomic selection with high-density SNP chips, RNA sequencing, and marker-assisted selection are transforming breeding programs by providing new options for improving disease resistance in commercial lines. Incorporation of these new tools with conventional breeding techniques is opening the way for more productive and durable commercial poultry populations.

Keywords | Genetic selection, Disease resistance, Commercial poultry, Molecular breeding, Genomic tools

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INTRODUCTION

Poultry production is a significant contributor to global food security, providing a cost-effective source of protein in the form of meat and eggs (Chatterjee and Rajkumar, 2015). The industry has long been subject to the scourge of infectious diseases like avian influenza, salmonellosis, and Marek's disease (Hartcher and Lum, 2020). Apart from undermining animal welfare and productivity, these diseases cause significant financial losses (Padhi, 2016). Historically, disease control has been particularly dependent on vaccination and biosecurity; however, the rise

of resistant strains and constraints on vaccine effectiveness have created an imperative for complementary methods to be sought (Wolc *et al*., 2016).

One of these approaches, which has been receiving significant attention (Kaiser, 2010), involves genetic selection for disease resistance, where naturally healthier immune systems or resistance to specific pathogens in individuals are identified and bred (Calenge *et al*., 2010). Recent breakthroughs in molecular genetics and high-throughput genotyping technologies have enabled scientists to identify single-nucleotide polymorphisms (SNPs) in genes associated with immunity (Miller and Taylor, 2016). The inclusion of this information within breeding programs is likely to create poultry lines which are more resilient to infection (Dawkins and Layton, 2012), less reliant on drugs, and more sustainable over the long term (Hocking, 2014).

This study investigates the genetic variation of four key immune-related genes Mx, TLR4, NRAMP1, and BF2—in commercial chicken breeds, specifically broilers and layers. Based on SNP genotyping data, allele frequencies (Vallejo *et al*., 2017), genotype distributions, and population differentiation, the paper elucidates the genetic structure of disease resistance as well as the application of these markers in selective breeding schemes (Thiruvenkadan *et al*., 2011). The findings aim to help develop gene-strong bird populations, with the ultimate goal of optimizing poultry industry efficiency and resistance.

MATERIALS AND METHODS

Improvements in molecular genetics have enabled the detection of specific genetic markers associated with disease resistance in poultry, allowing for the selective breeding of healthier and more productive flocks (Hoerr, 2010). The objective of this study was to investigate and establish the use of main genomic tools in detecting disease-resistant characteristics in commercial broiler and layer populations. The methodology combines standard molecular methods with population-scale genotyping to examine the distribution and frequency of alleles responsible for resistance.

Research design

A mixed-methods exploratory study design was employed, incorporating knowledge from the background literature alongside empirical molecular validation. The research aimed to identify resistance-associated genes and assess their usefulness in breeding programs through genotypic analysis of a set of selected commercial poultry breeds (Hoffmann, 2010).

Background research and data sources

Literature in the field of science served as the basis for identifying relevant genes and molecular resources (Dana *et al*., 2010). Databases such as PubMed, Web of Science, ScienceDirect, and Google Scholar were queried for research articles from 2000 to 2024 with keywords such as:

- "Genetic selection for disease resistance in poultry"
- "SNP markers in poultry"
- "Genomic selection in chickens"
- "Molecular breeding in avian species"

There was a focus on English-language broiler and layer studies, particularly addressing diseases such as Marek's disease, avian influenza, Newcastle disease, and salmonellosis.

Selection of genetic tools

Based on the reviewed research findings and previous empirical work, the following genomic tools were chosen based on their accuracy and applicability in commercial breeding:

- High-Density Single Nucleotide Polymorphism (SNP) Chips
- RNA-Sequencing (RNA-Seq)
- Marker-Assisted Selection (MAS)
- Quantitative Trait Loci (QTL) Mapping
- Candidate Gene Analysis

The tools were evaluated on variables such as strength of association with traits, cost-effectiveness (Pawar *et al*., 2016), and viability for inclusion in commercial poultry breeding schemes.

Molecular validation through genotyping

In order to authenticate the existence and variation of disease-resistance alleles, 100 commercial poultry birds, i.e., 50 broilers and 50 layers, were taken randomly from an institutionally certified poultry research farm. Aseptically collected wing vein blood was processed following the Institutional Animal Ethics Committee-approved guidelines (Ajayi, 2010).

The phenol-chloroform procedure purified genomic DNA. DNA integrity and concentration were verified by UV spectrophotometry and agarose gel electrophoresis. Birds were typed for polymorphisms in resistance genes Mx, TLR4, NRAMP1, and BF2 using Illumina high-density SNP BeadChips.

Raw genotyping information was cleaned utilizing PLINK v1.9 to exclude SNP markers with:

- Call rate < 95%
- Minor Allele Frequency (MAF) < 5%

Data analysis and allele frequency estimation

Descriptive statistics were applied for allelic variation, heterozygosity, and distributions of genotypes (Gjedrem and Robinson, 2014). Frequencies of alleles of known resistance-associated variants were estimated employing the following basic formula:

$$p = \frac{2n_{AA} + n_{Aa}}{2N}$$

Where P is the frequency of the target allele, n_{AA} is the number of individuals who are homozygous for the allele, and n_{Aa} is the number of heterozygous individuals. N is the number of samples that were examined.

For estimating genetic variability and resistance capacity, the frequency distribution of beneficial alleles was compared among layers and broilers. To understand the breeding pattern and diversity of the populations under study, the Hardy-Weinberg equilibrium and other population genetic traits were also estimated.

RESULTS AND DISCUSSION

The present study aimed to identify and evaluate genetic variations associated with disease resistance in broiler and layer chickens using high-density SNP genotyping. With a focus on four immune-relevant genes Mx, TLR4, NRAMP1, and BF2 the study compared allele and genotype frequencies, levels of heterozygosity, and population differentiation in both populations. The findings provide valuable insights into the genetic structure of disease resistance, which is crucial for future applications in selective breeding programs.

DNA quality and SNP genotyping

High-quality genomic DNA was successfully extracted from all 100 sampled birds (50 broilers and 50 layers) (Nwogwugwu *et al.*, 2018).

Table 1: DNA quality and SNP genotyping summary.

Parameter	Broilers (n = 50)	Layers (n = 50)
Mean DNA concentration (ng/µL)	175.3±12.6	180.1±11.2
A260/A280 ratio range	1.75–1.88	1.76–1.90
SNPs before QC	650,000+	650,000+
SNPs retained after filtering	~620,000	~620,000
Average SNP call rate (%)	98.2	98.6

The findings presented in Table 1 confirm the effective extraction and high quality of genomic DNA from broiler and layer chickens (Hong *et al.*, 2012). The average concentrations of DNA were slightly elevated in layers (180.1 ± 11.2 ng/µL) than in broilers (175.3 ± 12.6 ng/µL), suggesting adequacy for subsequent genotyping. The A260/A280 ratios, which ranged from 1.75 to 1.90 for the samples, indicate high purity with negligible protein contamination. Illumina high-density SNP BeadChip genotyping generated over 650,000 SNPs per bird. Approximately 620,000 SNPs remain after quality control filtering using PLINK, ensuring high integrity for analysis (Berry *et al.*, 2011). Call rates were similarly high, averaging 98.2% for broilers and 98.6% for layers, demonstrating excellent data quality and dependability for additional genetic association and allele frequency analysis. These results confirm that the dataset is robust and suitable for studying genetic markers associated with disease resistance.

Resistance-associated SNPs identified

Four candidate genes were studied: Mx, TLR4, NRAMP1, and BF2. They are associated with resistance to avian influenza, salmonellosis, Marek's disease, and other diseases (Vandana *et al.*, 2021).

Table 2: Identified SNPs in resistance-associated genes

Gene	SNP ID	Chromosome	Associated disease	Type of variation	Detected alleles
Mx	rsMX-1023	1	Avian influenza	A/G	A, G
TLR4	rsTLR4-234	4	Salmonellosis	C/T	C, T
NRAMP1	rsNR-101	7	Bacterial infections	G/A	G, A
BF2	rsBF2-311	16	Marek's disease	T/C	T, C

Table 2 describes the particular single-nucleotide polymorphisms (SNPs) that were found for four main candidate genes—Mx, TLR4, NRAMP1, and BF2—that individually have been linked to resistance to major poultry pathogens. The Mx gene (rsMX-1023) on chromosome 1 is associated with avian influenza resistance, exhibiting an A/G variation between alleles. The TLR4 gene (rsTLR4-234) on chromosome 4, which plays a role in the defence against salmonellosis, had a C/T polymorphism. Similarly, NRAMP1 (rsNR-101) on chromosome 7, associated with resistance to bacterial infection, exhibited a G/A polymorphism (Esatu *et al.*, 2022). Finally, the BF2 gene (rsBF2-311) on chromosome 16, which is associated with resistance to Marek's disease, showed a T/C polymorphism. The detection of these SNPs in the surveyed population suggests genetic variation at loci associated with resistance, laying a basis for further allele frequency analysis and selective breeding to promote disease resistance in commercial poultry strains.

Allele frequency distribution

Distinct allele frequency differences were found among broiler and layer populations for TLR4 and BF2 (Oltenacu and Broom, 2010), indicating different selection histories.

Table 3: Allele frequencies in broiler and layer populations.

Gene	SNP ID	Broilers (p)	Layers (p)	Chi-Square (HWE)	Significance (p < 0.05)
Mx	rsMX-1023	0.62	0.74	3.45	No
TLR4	rsTLR4-234	0.55	0.68	5.02	Yes
NRAMP1	rsNR-101	0.48	0.51	2.10	No
BF2	rsBF2-311	0.66	0.81	7.88	Yes

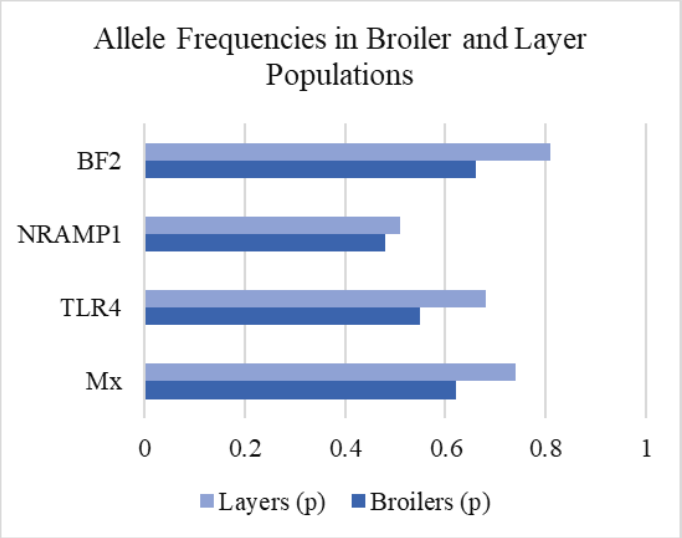

Figure 1: Allele Frequencies in Broiler and Layer Populations

Table 3 presents the pattern of allele frequency of SNPs associated with resistance in broiler and layer populations. Interestingly, alleles for the TLR4 (rsTLR4-234) and BF2 (rsBF2-311) genes were more frequent in layers (0.68 and 0.81) than in broilers (0.55 and 0.66). The Chi-square test for both genes (5.02 and 7.88) indicates statistically significant deviations from the Hardy-Weinberg Equilibrium ($p < 0.05$), suggesting possible selection pressures or non-random mating at these loci (Abdallah *et al*., 2023). The frequencies of alleles of Mx and NRAMP1 varied little between the two groups. They did not differ significantly from HWE, suggesting a more equilibrated state of resistance allele distribution among populations. These results suggest possible differences in genetic selection history and disease resistance strategies used in broiler versus layer breeding programs.

Genotype distribution and heterozygosity

Layers had increased levels of heterozygosity for disease-resistance alleles compared to broilers (Hayes *et al*., 2013), indicating greater genetic diversity.

Table 4: Genotype frequencies and heterozygosity estimates.

Gene	Population	Homozygous (AA)	Heterozygous (Aa)	Homozygous (aa)	Observed Het (Ho)	Expected het (He)
Mx	Broiler	18	26	6	0.52	0.47
Mx	Layer	12	32	6	0.64	0.55
TLR4	Broiler	20	24	6	0.48	0.49
TLR4	Layer	14	30	6	0.60	0.53
BF2	Broiler	22	20	8	0.40	0.49
BF2	Layer	10	33	7	0.66	0.61

Table 4 presents the genotypes and estimates of heterozygosity for the genes of interest related to resistance in broiler and layer lines (Meuwissen *et al*., 2016). For all three genes—Mx, TLR4, and BF2—layers had greater observed heterozygosity (Ho) than broilers. For example, in Mx, observed heterozygosity was 0.64 in layers and 0.52 in broilers. Likewise, in the case of TLR4, layers had a value of 0.60, whereas broilers had a value of 0.48. The BF2 gene exhibited the most remarkable contrast, with an observed heterozygosity of 0.66 in layers and a mere 0.40 in broilers.

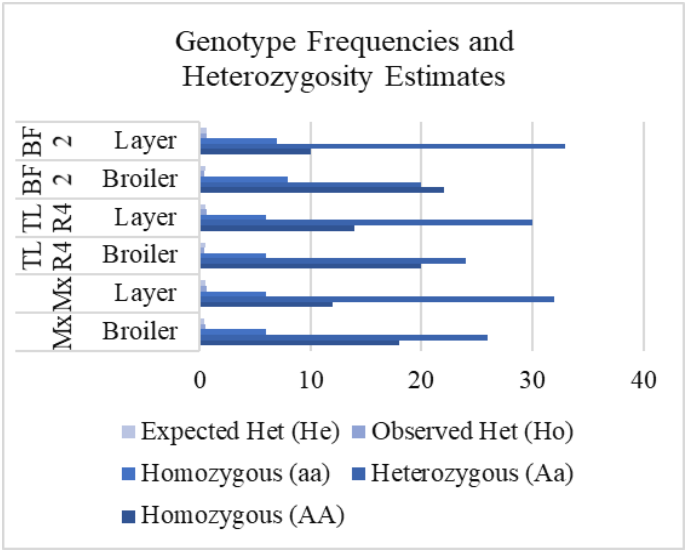

Figure 2: Genotype frequencies and heterozygosity estimates.

These trends suggest that layers exhibit greater genetic diversity at disease resistance loci, possibly due to less intense or wider selection practices compared to broiler lines, which tend to be more narrowly selected for growth rate (Heba *et al*., 2017). In addition, the high correlation between observed heterozygosity (Ho) and expected heterozygosity (He) in most instances indicates that the populations are predominantly in Hardy-Weinberg equilibrium, with minimal inbreeding or genetic drift at these loci. This genetic variation can give layers a wider

adaptive ability to withstand new and current pathogens.

Population differentiation

Moderate genetic differentiation was observed between the two populations for BF2 and TLR4 (Nawab *et al.*, 2018), as indicated by Fst values.

Table 5: Population genetic differentiation (Fst) for selected loci.

Gene	SNP ID	Fst value
Mx	rsMX-1023	0.11
TLR4	rsTLR4-234	0.15
NRAMP1	rsNR-101	0.09
BF2	rsBF2-311	0.18

Table 5 presents genetic differentiation among broiler and layer populations based on Fst values for SNPs associated with disease resistance. Fst values represent the proportion of genetic variation due to population divergence, with the larger the value, the higher the differentiation (Ogada *et al.*, 2016).

Of the genes tested, BF2 (Fst= 0.18) and TLR4 (Fst= 0.15) were the most differentiated, indicating considerable divergence in allele frequency between layers and broilers at these loci (Bishop and Woolliams, 2014). This may be the result of different selective pressures on these genes in the two populations, possibly due to variations in disease challenges or breeding goals. The Mx gene (Fst = 0.11) and NRAMP1 (Fst = 0.09) were found to have moderate to low differentiation, suggesting relatively comparable allele frequencies between the populations for these loci.

CONCLUSIONS AND RECOMMENDATIONS

The work highlights the potential of using genetic selection as a long-term strategy to enhance disease resistance in commercial broiler and layer fowl. Screening for important resistance genes Mx, TLR4, NRAMP1, and BF2 revealed remarkable genetic differences between broiler and layer populations. Layers had elevated allele frequencies and heterozygosity at these critical loci, particularly BF2 and TLR4, indicating increased genetic diversity and thereby potentially improved innate disease resistance. Moderate population differentiation (Fst) also confirmed differential selection history in the two groups. These results support the combination of molecular breeding tools, such as SNP genotyping and marker-assisted selection, with commercial breeding programs. Poultry breeding industries should prioritize routine screening of resistance-associated markers to create genetically resistant lines. Moreover, prioritizing genetic diversity in breeding populations is crucial for maintaining adaptive capacity against novel pathogens, ultimately leading to a healthier and more productive poultry industry.

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NOVELTY STATEMENT

This research presents a novel combination of high-density SNP genotyping with focused analysis of key immune-related genes—Mx, TLR4, NRAMP1, and BF2 among commercial broiler and layer chickens to assess genetic resistance to important poultry diseases. In contrast to existing studies that have mainly emphasised individual gene characteristics or experimental lines, the current research offers a comparative examination of natural allele frequency fluctuations, genotype frequencies, and population differentiation in commercially important poultry breeds. The results not only show breed-specific genetic signatures but also illustrate the practical applicability of molecular markers in selective breeding schemes. By confirming resistance-related loci by population-scale analysis, the work presents a strategic model for integrating genomic selection into actual poultry breeding operations, representing an important advance toward sustainable disease management via genetic resistance.

AUTHOR'S CONTRIBUTION

All of the trials were designed by Aya Yaqoub Jasim and Maryam Tareq Dahham. Ali Bashir Alwan, Ghadir Kamil Ghadir and Shaima Abd conducted all of the tests, gathered the data, and composed the manuscript draft. Ola Kamal A. Alkadir, Nooruldeen Ali Abdulhussein and Heba A. Abd-Alsalam Alsalame helped with the data analysis that was done to prepare the work for submission to the journal. The final draft of the work was reviewed and approved by all authors for publication in the Journal of Animal and Health Production.

Ethical consideration

Not applicable.

Generative AI or AI-assisted Technology Statement

The authors declare that no Genrative AI was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of Interest.

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