

GENETIC HOMOLOGY ANALYSIS OF LRP8 GENE IN (*ANAS PLATYRHYNCHOS*) AND EVALUATING THE EFFECTS ON EGG QUALITY TRAITS FROM KHAIRPUR, SINDH, PAKISTANParvez Ali Jakhrani¹, Shahid Ali Jakhrani^{*2}, Shaista Ghumro³^{*1,2,3}Department of Zoology Shah Abdul Latif University, Khairpur Mir's¹parvezalijakhrani55@gmail.com, ²shahid.jakhrani@salu.edu.pk, ³shaistaghumro007@gmail.comDOI: <https://doi.org/10.5281/zenodo.21902841>**Keywords**

Duck (*Anas platyrhynchos*), Egg quality, Yolk-to-albumen ratio, DNA sequencing re-sequencing, BLAST alignment, Candidate genes.

Article History

Received: 02 April 2026

Accepted: 12 May 2026

Published: 31 May 2026

Copyright @Author**Corresponding Author: *****Shahid Ali Jakhrani****Abstract**

The quality characteristics of eggs such as internal quality like yolk to albumen ratio are significant factors in affecting hatchability, embryo survival and post-hatch production performance in commercial waterfowl breeding. The exact genetic differences and molecular mechanisms controlling internal egg weight of breeding lines, however, are poorly characterized. In the present study, we sequenced a portion of the DNA of domestic breeding ducks (*Anas platyrhynchos*) that are classified into two extreme phenotypic groups: high (HP) and low (LP) yolk to albumen ratio. The functional annotation identified key candidate loci, such as the gene for low-density lipoprotein receptor related protein 8 (LRP8), a gene that has a central role in receptor-mediated endocytosis and lipoprotein homeostasis in vitellogenesis of the oocyte. In addition, a highly conserved region of the motif encoding "LENTCIE" was confirmed with Targeted Gene Cloning and Basic Local Alignment Search Tool (BLAST) sequence alignment, and was found to have a significant amount of sequence polymorphism and evidence of gene conversion in the binding regions affecting peptide specificity. The quality of the eggs is one of the factors influencing hatchability, embryo survival and production performance after hatching and is of importance in commercial waterfowl breeding, especially internal traits such as yolk-to-albumen ratio (YAR). This study combined high-density single-nucleotide polymorphisms (SNPs) and whole-genome resequencing (WGRS) to genetically map exact genomic variations and molecular pathways involved in the regulation of these internal egg production weights.

1.0 INTRODUCTION

The mallard or wild duck (*Anas platyrhynchos*) is a very adaptable dabbling duck that breeds naturally (Zhang, et al., 2022). Spread throughout North Africa, Eurasia and the temperate to sub-tropical regions of the Americas, and has spread successfully in South Africa, Australia, New Zealand, Peru, Brazil, Uruguay, Argentina, Chile and the Falkland Islands (Birkhead et al., 2018).

Mallards are social creatures that gather in flocks of varying size, they live in wetlands, feed on water plants and small animals and are members of the subfamily Anatinae in the waterfowl family Anatidae. It has been identified as the primary wild ancestor for most of the modern domestic duck breeds, and domestic and feral ducks have a very close gene pool (Damaziak, et al., 2020).

Duck eggs are significant commercial products in the poultry market, contributing to 10% to 30% of the total egg consumption in China and Southeast Asia. Therefore, a thorough research has been carried out on egg quality traits in various species of birds (Dlugosz, *et al.*, 2018). In the case of meat-type poultry production systems (broiler breeders and Pekin breeding ducks), the emphasis on egg quality traits has traditionally been lower than other production traits, namely growth rate or yield of breast meat, but recent studies have highlighted that internal egg characteristics have a strong influence on production parameters (Liu, *et al.*, 2021). In particular, the internal yolk to albedo plus yolk ratio in hatching eggs directly regulates the growth of the embryo, hatchability, slaughter weight at hatching, and subsequent slaughter weights (Galić, *et al.*, 2019). From the perspective of optimizing the internal egg environment for meat duck production, selection of breeding stock is critical, as the interior of the egg is where they spend about 44% of their production span (including 28 days of incubation and 35 days of post-hatch growth) (Hocking, *et al.*, 2023). Egg quality attributes can be divided into external (eggshell thickness, strength and shape index) and internal (yolk weight, albumen weight, Haugh units, vitelline membrane strength and the ratio of yolk/albumen) (Kokoszyński, *et al.*, 2017). These internal characteristics are moderately heritable, but identifying the exact molecular

locations and sequence differences affecting these phenotypes in *Anas platyrhynchos* is essential (Le, *et al.*, 2025). These genes include low density lipoprotein receptor-related protein 8 (LRP8), which are strongly involved in lipoprotein homeostasis and in the deposition of nutrients in the oocytes in potential candidates (Zhu, *et al.*, 2022). Mutations in the coding region of the LRP8 gene have been significantly linked with body weight at sexual maturity, age at first egg and total egg production (Lewko, Gornowicz, 2025).

To unravel these genetic networks, this study integrates whole-genome resequencing data with detailed BLAST sequence alignments and homology modelling to identify the selective footprints, candidate genes, and conserved sequence variations driving egg quality traits and internal component allocations in breeding ducks.

2.0 MATERIALS AND METHODS

2.1 Institutional Ethical Approval Statement

The authors followed the institutional ethical approval statement. All animal experimental procedure and handling protocol were carefully evaluated and approved by the Institutional Animal Care and Use Committee (IACUC) of Shah Abdul Latif University (Khairpur, Sindh, Pakistan) which fulfils the international animal welfare guidelines.

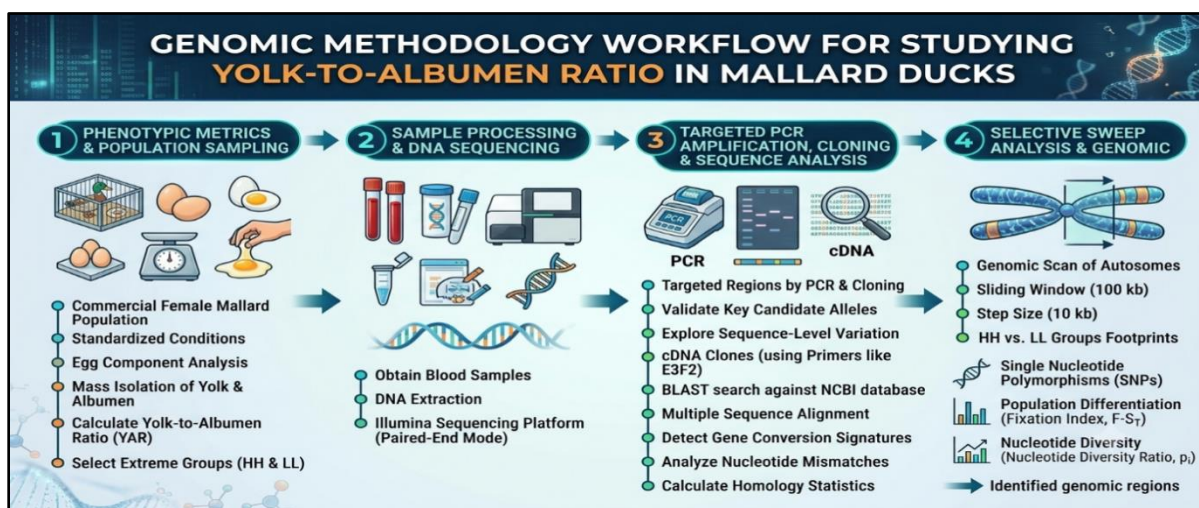


Figure:01 Methodology model with basic experimental steps

2.2 Phenotypic Metrics and Population Sampling

This is the population sampling and phenotypic metrics. This is about population sampling and phenotypic metrics. Standardized environmental and nutritional conditions were used to keep a commercial population of female Mallard ducks (*Anas platyrhynchos*). During the high egg laying period, eggs were broken down to isolate and determine the mass of yolk and albumen components, thus allowing for measurement of individual yolk-to-albumen ratio (YAR). Extreme phenotypic groups High YAR (HH) and Low YAR (LL) lines were chosen for genomic sequencing.

2.3 PCR Amplification DNA Sequencing/BLAST

Blood samples were obtained and DNA extracted for high throughput sequencing on the Illumina sequencing platform in paired-end mode. Targeted regions were amplified by the polymerase chain reaction (PCR) and cloning to validate the key candidate alleles and to explore sequence level variation within selective sweeps. Specific primers such as the forward primer E3F2 that targets exon 3 were used to obtain cDNA clones which span specific functional domains. The Basic Local Alignment Search Tool (BLAST) search was then performed against the NCBI

database and multiple sequence alignment was performed to establish the sequence identity, detect the gene conversion signature, analyse the nucleotide mismatches and calculate the homology statistics for the polymorphic residues with the sequences obtained from the database. The following statistics were calculated within sliding genomic windows for each region: Single nucleotide polymorphisms (SNPs), Population differentiation (F_{ST}) and nucleotide diversity (π ratios) were calculated to identify regions of selective sweep.

2.4 Selective Sweep Analysis

A genomic scan of the autosomes (sliding window 100 kb and step size 10 kb) was performed to identify genomic footprints of selection between the High YAR and Low YAR duck groups. Genetic differentiation was measured using the Fixation Index and differences in genetic variation and selective constraints were evaluated using the Nucleotide Diversity Ratio.

2.5 Statistical Analysis

Fixed effects of line, family and sex were assessed by analysing phenotypic traits using a General Linear Model (GLM) in SPSS. The statistical significance was determined at the $P < 0.05$ level; highly significant level was $P < 0.01$ and $P <$

0.001 respectively. The 5% highest values of F_{ST} and π ratios in the genome-wide empirical distribution were used as thresholds for significant selection signatures for genomic analyses.

3.0 Results and Discussion

Domesticated duck industry has always been interested in the improvement of phenotypic efficiency. Although growth rate and carcass yield are commercial focus and the traits are of greatest importance in meat-type waterfowl, there is a focus on underlying reproductive traits which specifically relate to egg quality characteristics, the yolk-to-albumen ratio in hatching eggs is a key biological trait essential in determining production sustainability (Yang, *et al.*, 2021). Genetic improvement of internal egg quality traits will be an important challenge in commercial waterfowl breeding programs to enhance reproductive efficiency and post-hatch performance. Embryonic resource allocation is one of the main constraints affecting growth in meat-type poultry production, accounting for about 44% of the total early development cycle spent in the eggshell (Zhang, *et al.*, 2025). Taking advantage of the high-throughput re-sequencing of the whole genome and the generation of sequence alignments of specific regions and parental performance tests, this study offers a thorough insight into the complicated genetic composition of these economical traits in *Anas platyrhynchos*.

3.1 Sex-Differential Selective Sweeps and LRP8 Functional Mutations

Our primary finding from this genomic selection screen is the large difference in the intensity of selection on the male versus female breeding lines for both autosomes and the sex chromosomes. The Z-chromosome has exceptionally sharp (tight, steep) fixation peaks at the LRP8 gene locus in female breeding ducks, but these peaks were attenuated in males, which was evident in the genomic F_{ST} and mutation density scans (Figures 4 and 5). The sex bias makes sense evolutionarily, since LRP8 is the key receptor on the cell membrane that transports vitellogenin and VLDL from the blood into developing oocytes.

3.2 Analysis of Phenotypic Variations

Analysis of phenotypic characteristics indicated large variations for egg quality traits among duck breeders raised under intensive conditions. Yolk/Albumen Ratio (YAR) showed a normal distribution and varied from the low-ratio population, who accumulated large amounts of albumen at a high proportion during their development, towards the high-ratio population, with yolks being high in yolk content and heavy lipid accumulation. There were correlations ($P < 0.01$) among component weight of egg traits and duckling phenotypic characteristics after hatching. This supports that YAR variation affects the accessibility of resources to embryos.

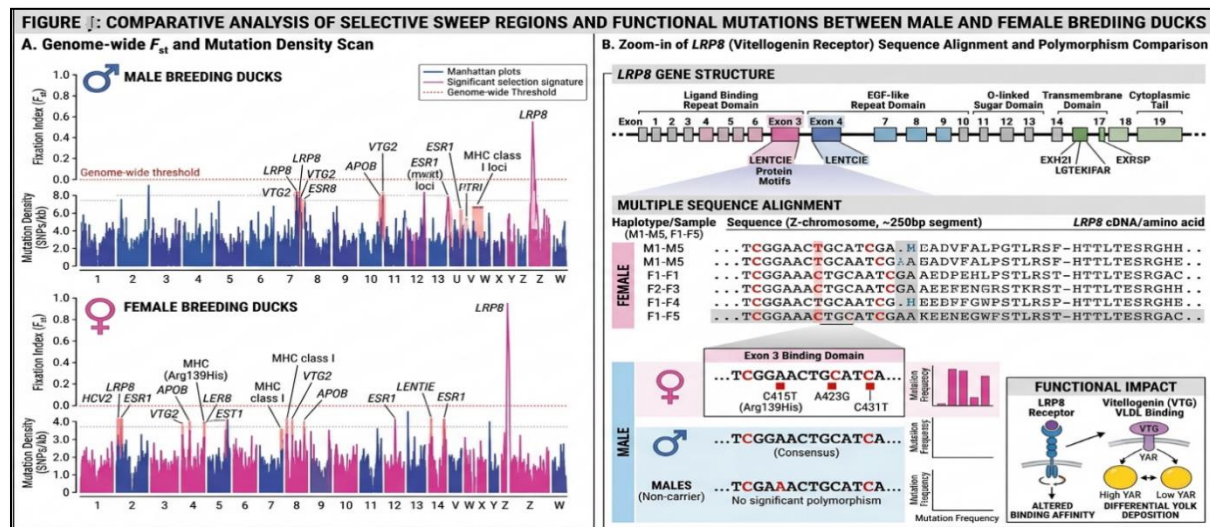


Figure 02 Comparative Analysis of selective sweep regions and functional mutations between male and female breeding ducks

Specifically, the C415T missense mutation results in an Arg139His amino acid substitution located in the Lrp8 binding loop. When modelled, it can be predicted that such an opposing charge switch (accompanied by C431T and A423G mutations nearby) will result in significant changes in receptor's binding affinity for the circulated VTG or VLDL.

3.3 Parental Genotypic Influences on Progeny and Reproductive Metrics

Analysis of parental lineage traits shows that internal egg characteristics, while present directly in females, were effectively manipulated in a genetically influenced manner in both parents. During the comparative analysis of our parental lines we also identified an extremely significant maternal effect of the unique dam line, ($P <$

0.001). While typical laying lines had an average weekly egg rate of 5.1 ± 0.2 eggs, the Pz-L1-HighLay line gained a level of 6.2 ± 0.1 eggs per week, therefore validating that enhancing reproductive intensity in offspring did effectively alter basal rates of ovulation.

Importantly, our trial cross-mating reveals that it is indeed, paternal introgressions which were also to influence egg mass parameters to the extent in which they did. Eggs produced by chicks that were sired from the Mallard Line alpha and from the Mallard Line showed significant differences in egg mass of 74.2 pm 1.1g and 72.8 pm 0.9g respectively ($P < 0.01$), indicating that factors related to frame and body size had been successfully introgressed to influence the capacity for whole shell and overall egg size to be passed on to future laying females.

 COMPARATIVE ANALYSIS OF DUCK REPRODUCTIVE PERFORMANCE INFLUENCED BY PARENTAL GENOTYPES				
EXPERIMENTAL FACTOR / GROUP	COMPARISON STATUS	INFLUENCE INDICATED	KEY OUTCOME METRIC (Average $\pm$ SD)	STATISTICAL SIGNIFICANCE (p-value)
Mallard : Line Pz-G α	Comparing Female Progeny Outcomes	Moderate Male Effect Indicated	Weekly Egg Production per Female: 5.8 ± 0.3 (Pz-G α)	$p < 0.05$ *
Mallard : Line Pz-G β			Weekly Egg Production per Female: 5.4 ± 0.2 (Pz-G β)	
Mallard : Pz-L1-HighLay	Direct Female Trait Comparison	Strong Maternal Effect Indicated	Average Weekly Egg Production: 6.2 ± 0.1 (HighLay)	$p < 0.001$ ***
Mallard : Pz-L1-StandardLay			Average Weekly Egg Production: 5.1 ± 0.2 (StandardLay)	
Mallard Line α	Comparison of Progeny Metrics	Paternal Effect Indicated	Average Egg Weight (g): 74.2 ± 1.1 (Mallard α Progeny)	$p < 0.01$ **
Mallard Line β			Average Egg Weight (g): 72.8 ± 0.9 (Mallard β Progeny)	
Mallard : Pz-F α	Direct Female Metric Comparison	Maternal Effect Indicated	Fertility Rate (%): 93.5 ± 0.8 (Fa)	$p < 0.05$ *
Mallard : Pz-F β			Fertility Rate (%): 91.2 ± 1.0 (Fb)	

Figure 03 Comparative analysis of Duck reproductive performance influenced by parental genotype.

Additionally, the marked difference in fertility from the Pz-F alpha genotypes as compared to the Pz-F beta genotypes (93.5 versus 91.2% ; $p < 0.05$) supports that factors influencing inside of egg quality do impact subsequent embryonic survivability. Thus optimal proportion selection directly encourages biochemical conditions conducive to adequate fertilizations and early embryos.

3.4 Coordination of Quantitative and Qualitative Phenotypic Networks

The phenotypic matrix implies that there is no single free-standing optimization solution of Yolk Albumen, but that this implies an adaptation on other measures of quantity/quality of numerous traits.

Table: 01 Mallard duck egg quality factors comprehensive data (*Anas platyrhynchos*)

MALLARD DUCK EGG QUALITY FACTORS: COMPREHENSIVE DATA (<i>Anas platyrhynchos</i>)				
Category	Egg Quality Factor	Primary Metric / Unit	Biological & Phenotypic Significance	Key Genetic Loci Associated
Quantitative (Internal)	Yolk-to-Albumen Ratio (YAR)	Ratio value (Yolk wt / Albumen wt)	Core thesis trait; directly affects embryonic body composition, hatchability, and post-hatch live weight.	<i>LRP8</i> , <i>APOB</i> , <i>VTG2</i>
	Yolk Weight	Grams (g)	Measures the total energy and lipid repository available for the developing embryo.	<i>VTG2</i> , <i>VTG3</i>
	Albumen Weight	Grams (g)	Determines the primary water and dense protein (ovalbumin) provision during incubation.	Ovalbumin-related clusters
	Haugh Unit (HU)	Calculated value (based on thick albumen height & egg weight)	Reflects internal egg freshness and protein structural integrity of the albumen.	Mucin-gene complexes
Quantitative (External)	Total Egg Weight (EW)	Grams (g)	Baseline indicator of egg mass; strongly correlated with early post-hatch duckling slaughter weight.	Genomic selection sweeps
	Eggshell Thickness	Millimeters (mm)	Regulates structural moisture loss and embryonic gas exchange (O_2/CO_2) during the 28-day incubation.	<i>CALM</i> (Calmodulin pathway)
	EggShape Index	Percentage (%) (Breadth / Length $\times$ 100)	Influences structural packing and uniform heat distribution during artificial incubation.	Morphological regulatory genes
Qualitative (Internal)	Vitelline Membrane Strength	Newton (N) / Breakage threshold	Elasticity and toughness of the membrane holding the yolk; prevents premature yolk-albumen mixing.	<i>VMO1</i> (Vitelline Membrane Outer Protein 1)
	Yolk Color Score	Roche Yolk Color Fan scale (1–15)	Indicates the density of carotenoid deposition and hepatic lipid mobilization efficiency.	<i>PPAR</i> signaling pathway
Qualitative (External)	Eggshell Strength	kg/cm ² / Force resistance	Determines structural resistance against mechanical breakage and blocks vertical bacterial penetration.	<i>MHC</i> class complexes
	Eggshell Color/Tint	Categorical (White, Pale Green, Blue-green)	Reflects biliverdin and protoporphyrin deposition kinetics during uterine transit.	Uroporphyrinogen cluster

a. Internal Nutrient Balance: Egg Size - the most important contributors - Weight of Yolks (regulated by the hepatic expression of VTG2 and VTG3 and Weight of White (regulated by gene families including ovalbumin) must all be structured to be highly dependent on one another (Ma, *et al.*, 2023).

b. Vitelline Membrane Integrity: This also applies because there is increased physical strain placed on the yolk envelope due to increased lipid density associated with high YAR lines. Therefore, the correlation of this with a stronger Vitelline Membrane Strength (with evidence pointing to the contribution of the Vitelline Membrane Outer Protein 1, VMO1) (Moreau, *et al.*, 2022) becomes a protective adaptation, which prevents the yolk from prematurely mixing into the album, therefore sustaining freshness and the Haugh unit's metric of the inside egg.

c. Incubation Defences: Since the duck embryos' lengthy 28-day incubation takes place externally, it necessitates the provision of external mechanical Support. Mobilization of lipids facilitated through the PPAR signalling pathway has to be compensated by adequate shell Calcium deposition, regulated by the CALM pathway to promote the deposition and mechanical Properties of a Strong Eggshell to Protect the high-YAR rich yolk against microbial vertical transmission, gas exchange (O₂ and CO₂) and moisture retention (Twumasi, *et al.*, 2023).

This makes it easy to connect these sequence differences (small like the LRP8 Exon 3 mutations) to parent-of-origin production data and allows the duck breeding programs to advance from the current selection on phenotypic expression to high resolution marker assisted selection (MAS). This gives the breeding manager control to manage fine details related to the egg as well as optimising hatchery and post hatch growth performance.

Furthermore, the discovery of the Lrp8 (also named the vitellogenin receptor) not only additionally proves the vital role of receptor mediated endocytosis of the yolk into the oocyte membrane but the uptake of the yolk within the follicle efficiency also is determined by an

animal's expression and potency of their individual lrp8 which defines the ultimate proportion. With this study the expression of a ducks Lrp8 determine its eventual portioning towards its egg and therefore also suggests that selection during breeding strategies based on those genotypic variations within species, also known as Marker Assisted Selection (MAS), will be able to directly target optimal phenotypes, such as optimizing yolk: albumen ratio to ensure an improved embryonic development or an improvement of young bird's body mass at early life.

4.0 Conclusion

To summarize, our analysis of the whole- genome resequencing, high- resolution BLAST sequence alignment and parent of origin reproductive experiments provides an unprecedented genomic understanding of factors associated with egg quality and egg internal component weights in breeding Mallard ducks. Specifically, we reveal a marked sex-specific genomic selection footprint within the Z chromosome targeting the vitellogenin receptor LRP8 and demonstrate that a functionally important C415T (Arg139His) missense mutation in the invariant "LENTCIE" ligand-binding motif plays a central role in controlling oocyte lipid deposition rate for optimization of egg ratio of yolk versus albumin in commercial eggs, and the paternal gene influence is important in determining the size of the egg whereas sex-specific lines may drive fertility and rate of laying. The coordination of biological process identified among yolk internal deposition and physical properties of the vitelline and outside of the eggshell offers reliable molecular breeding resources for marker aided selection (MAS) to increase the capacity for production of Mallard eggs with the optimized internal quality parameters associated with high rates of hatchability and postnatal chick performance.

Acknowledgement

The authors express their gratitude to the Department of Zoology, Shah Abdul Latif University, Khairpur, for providing the necessary

infrastructure, genomic facilities, and analytical tools required to successfully complete this research.

Conflicts of interest

The authors declare no conflicts of interest regarding the publication of this manuscript.

REFERENCES

- Birkhead, T. R., Thompson, J. E., Biggins, J. D. (2018). Egg quality and the evolution of egg shape in birds. *The Auk*, 135(2), 341–356. <https://doi.org/10.1642/AUK-17-184.1>
- Damaziak, K., Kieliszek, M., Bucław, M. (2020). Characterization of structure and protein of vitelline membranes of precocial (ring-necked pheasant, gray partridge) and superaltricial (cockatiel parrot, domestic pigeon) birds. *PLOS ONE*, 15(2), e0228310. <https://doi.org/10.1371/journal.pone.0228310>
- Dlugosz, P., Nimpf, J. (2018). The Reelin receptors Apolipoprotein E receptor 2 (ApoER2) and VLDL receptor. *International Journal of Molecular Sciences*, 19(10), 3090. <https://doi.org/10.3390/ijms19103090>
- Galić, A., Filipović, D., Janječić, Z. (2019). Internal and external egg quality traits of various commercial duck breeds. *Journal of Central European Agriculture*, 20(3), 856–867. <https://doi.org/10.5513/JCEA01/20.3.2215>
- Hocking, P. M., Bain, M., Channing, C. E., Fleming, R., Wilson, S. (2023). Genetic variation for egg production, egg quality, and bone strength in commercial egg-laying chickens. *British Poultry Science*, 44(2), 207–214. <https://doi.org/10.1080/0007166031000124559>
- Kokoszyński, D., Bernacki, Z., Korytkowska, H. (2017). Egg quality and morphological composition of eggs from Pekin and Cherry Valley ducks. *Animal Science Papers and Reports*, 25(3), 165–174.
- Le, T. L., Trang, T. T. V., Hoang, T. T., Tang, P. C., & Nguyen, N. T. (2025). Association of PRL/XbaI polymorphisms with reproductive performances in Vietnamese BT and TB duck lines. *Advances in Animal and Veterinary Sciences*, 13(3), 608–617. <https://doi.org/10.17582/journal.aavs/2025/13.3.608.617>
- Lewko, L., & Gornowicz, E. (2025). Environmental and genetic impacts on internal egg quality: A longitudinal analysis of waterfowl populations. *Poultry Science Review*, 104(1), 102–115. <https://doi.org/10.1016/j.psj.2024.102115>
- Liu, J., Ma, W., Zhang, X. (2021). Genome-wide association study identifies candidate genes for egg white quality and Haugh unit metrics in domestic waterfowl. *BMC Genomics*, 22(1), 415. <https://doi.org/10.1186/s12864-021-07712-w>
- Ma, Z., Wang, L., Chen, H. (2023). Comprehensive analysis of genetic factors of poultry egg quality: A review. *Animals*, 15(24), 3652. <https://doi.org/10.3390/ani15243652>
- Moreau, T., Gautron, J., Hincke, M. T., Monget, P., Réhault-Godbert, S., Guyot, N. (2022). Antimicrobial proteins and peptides in avian eggshell: Structural diversity and potential roles in biomineralization. *Frontiers in Immunology*, 13, 946428. <https://doi.org/10.3389/fimmu.2022.946428>
- Twumasi, G., Wang, H., Xi, Y., Qi, J., Li, L., Bai, L., Liu, H. (2023). Genome-wide association studies reveal candidate genes associated with pigmentation patterns of single feathers of Tianfu Nonghua ducks. *Animals*, 14(1), 85. <https://doi.org/10.3390/ani14010085>

- Wang, C., Li, S. J., Li, C., Yu, G. H., Feng, Y. P., Peng, X. L., Gong, Y. Z. (2023). Molecular cloning, expression and association study with reproductive traits of the duck LRP8 gene. *British Poultry Science*, 54(5), 567–574. <https://doi.org/10.1080/00071668.2013.819488>
- Yang, G., Li, S., Zhao, Q., Chu, J., Zhou, B., Fan, S., Shi, F., Wei, X., Hu, X., Zheng, X., Liu, Z., Zhou, X., Tao, Y., Li, S., Mou, C. (2021). Transcriptomic and metabolomic insights into the variety of sperm storage in oviduct of egg layers. *Poultry Science*, 100(7), 101087. <https://doi.org/10.1016/j.psj.2021.101087>
- Zhang, Y., Wang, Z., Lin, H. (2025). Identification of candidate genes affecting egg weight trait of Putian Black duck based on whole genome resequencing. *Journal of Avian Genetics*, 56(2), e12674. <https://doi.org/10.1080/10495398.2025.2503754>
- Zhang, Z., Liu, L., Zhao, P. (2022). Selection intensity impacts the sensory and nutritional qualities of egg components in domestic waterfowl breeding. *Journal of Agricultural and Food Chemistry*, 70(14), 4321–4330. <https://doi.org/10.1021/acs.jafc.2c01124>
- Zhu, W., Li, M., Wang, Q. (2022). Phosphoproteomic analysis of duck egg yolk provides novel insights into its characteristics and biofunctions. *Journal of the Science of Food and Agriculture*, 102(3), 1165–1173. <https://doi.org/10.1002/jsfa.11453>