Acta Scientiarum
http://periodicos.uem.br/ojs
ISSN on-line: 1807-8672
Doi: 10.4025/actascianimsci.v47i1.71608

Investigating consequences of non-synonymous Single nucleotide polymorphisms of the *Zyxin* gene on protein structure and functions in Nigerian indigenous and Nera black chickens *Zyxin*

Adenaike Adeyemi Sunday[1,4*], Peters Sunday Olusola[2], Fafiolu Adeboye Olusesan[3], Waheed Abdullai Adetunji[1], Abdulrahman Taofeek Aireabakhame[1], Agaviezor Brilliant Ogagaoghene[4], Agbalaya Khadijah Kubura[5] and Ikeobi Christian Obiora Ndubuisi[1]

[1]Department of Animal Breeding and Genetics, Federal University of Agriculture, Alabata Road, P.M.B. 2240, Abeokuta, Ogun State, Nigeria. [2]Department of Animal Science, Berry College, Mount Berry, Georgia, United States of America. [3]Department of Animal Nutrition, Federal University of Agriculture, Abeokuta, Nigeria. [4]Department of Animal Science, Faculty of Agriculture, University of Port Harcourt, East/West Road, PMB 5323, Choba, Rivers State, Nigeria. [5]Department of Animal Production, Lagos State University of Science and Technology, Ikorodu, Nigeria. Author for correspondence. E-mail: adenaikeas@funaab.edu.ng

ABSTRACT. *Zyxin* functions as a regulator of the restructuring of the actin cytoskeleton during the process of repairing tissue damage, cell movement and attachment. It has also been identified as a potential gene involved in chicken coccidiosis. In order to gain a deeper understanding of these phenomena, we employed a collection of computer-based techniques and databases to examine the amino acid sequence, structural dynamics, molecular interactions, and activities of the gene. Our analysis revealed that *Zyxin* contains two non-synonymous SNPs (A > C at position 22 and G > A at position 137) at exon 1. Also, there existed a non-synonymous SNPs in Exon 3 (A>C and A>T both at position 861) of the gene with Synonymous SNPs observed only in exon 3 (A>G at position 812 and 854, T > C at position 863). The genetic diversity revealed in these chicken populations indicates the presence of genetic variation, with Naked neck chickens showing a considerably higher frequency of particular SNPs. Two non-synonymous single nucleotide polymorphisms (nsSNPs) were forecasted to exert a profound influence on the structure, stability, and activities of *Zyxin*, thereby heightening the vulnerability to coccidiosis.

Keywords: *Zyxin*; chicken coccidiosis; amino acid sequence; non-synonymous SNPs; naked neck chickens; single nucleotide polymorphisms (SNPs); nsSNPs.

Received on March 12, 2024.
Accepted on July 02, 2024.

Introduction

The Nigerian indigenous chickens serve as valuable reservoirs of beneficial genes, and it is imperative to prioritise genetic enhancement of these fowl (Adenaike et al., 2018). According to Adenaike et al. (2016), these hens have been determined to be resilient and have a strong natural defence against coccidiosis. Dai et al. (2014) and Zampiga et al. (2018) discovered that the *Zyxin* gene in chickens has an immunological role in fighting against coccidiosis. *Zyxin* is an intracellular protein that regulates the actin cytoskeleton (Zhang et al., 2023). *Zyxin* functions as a regulator of the restructuring process of the actin cytoskeleton during the repair of injuries, the movement of cells, and the attachment of cells to each other (Svitkina, 2018). The protein's N-terminal region is rich in proline, while the C-terminal region contains three consecutive LIM zinc finger domains. These domains are structural motifs found in DNA-binding proteins, resembling fingers with a base composed of cysteine and histidine residues that bind to a zinc ion (Padjasek et al., 2020). *Zyxin* exerts an influence on cellular motility, as well as the proliferation and migration of cancerous cells, while also playing a role in the control of cell division. Furthermore, it functions as a transcription activator through synergistic interactions with other proteins within the nucleus (Dai et al., 2014). Furthermore, it is effective in facilitating the formation of microfilament cytoskeleton (Zhang et al., 2023). Adenaike et al. (2019) employed *Zyxin* gene variation as a means to investigate the extent of variety present in indigenous Nigerian chicken populations using similar approach as Zampiga et al. (2018) who examined the correlation between single nucleotide polymorphisms (SNPs) of *Zyxin* and coccidiosis resistance

measures. They discovered that a number of *Zyxin* SNPs were strongly linked to the levels of carotenoid and nitrate ion concentration. The *Zyxin* family of proteins is thought to carry out its many roles by interacting with cellular proteins through their PRR and Lim domains, which act as docking sites for binding (Kotb et al., 2018). Nevertheless, despite its functional significance and connection to disease physiology, there are other aspects of *Zyxin*'s structure, activities, and dynamics that remain unknown. This lack of knowledge considerably hampers its usefulness as a primary focus in the research of coccidiosis. Furthermore, there is limited knowledge regarding how comparable architectural features manifest in such a diverse array of functionalities. We aim to fill this lack of knowledge in regards to chickens and offer valuable insights into the fundamental aspects of the structure and function of chicken *Zyxin*.

Parasitic diseases such as coccidiosis in poultry production are one of the challenges Nigeria has faced. According to Ajala et al. (2021), poultry farming is a crucial sub-sector of agriculture in Nigeria and has been established in food and income security. The complicated nature of coccidiosis means that it causes losses in performance, birds' feed conversion ratios, weight gain and egg production, less market weight and higher mortality rates (Rashid et al., 2019). In a study by Rashid et al., 2019, they noted that the monetary cost of coccidiosis in Nigeria is a few million dollars for poultry farmers, including all the indirect costs. Prophylactic and therapeutic use of these assets is defined as direct cost, while the production loss due to Unit morbidity and mortality is defined as indirect cost (Mesa-Pineda et al., 2021). On the economic impact, he stated that the price of the anticoccidial drugs and the mutation of birds to resistant types that require frequent administration of the drugs make it expensive (Kamani et al., 2021).

Furthermore, the economic effects are reflected in the national economic indicators at the farming level. Poultry is one of the largest sub-sectors of the agricultural industry in Nigeria. It plays a vital role in boosting the economy since any rise or plunge in its performance has a knock-on effect on the country's Gross Domestic Product (Heise et al., 2015). The consequences of coccidiosis are that productivity within poultry farming decreases due to resultant losses, thus raising the prices of poultry products and possibly creating inflationary pressures in the market, as noted by Lawal et al., 2016. Additionally, the mentioned disease erodes the prospects for food security and poverty reduction, essential for Nigeria's development. The financial loss greatly discourages smallholders from engaging in poultry farming due to the high likelihood of coccidiosis infection, thus impacting employment and income in rural areas (Olutumise et al., 2023). Control and management strategies for these diseases, which strengthened bio-security measures and better vaccination programs, are crucial in reducing these losses. However, the effectiveness of such an approach is limited by substantial contingent funds and the expected stock of the stakeholders involved, which poses a problem in Nigeria (Rashid et al., 2019; Olutumise et al., 2023).

Material and methods

Experimental animals

The Nigerian indigenous chickens, including Naked neck, Frizzle, and Normal feather genotypes, were obtained from several villages in the southwest region of Nigeria. These chickens were used to establish the initial breeding stock, which was then kept at the farm of the Federal University of Agriculture in Abeokuta, Nigeria. Artificial insemination was used to achieve self-mating within the genotype of the hens. This study utilised blood samples obtained from the offspring to extract DNA.

Sample collection and DNA isolation

A total of 240 Nigerian indigenous chickens (Naked neck (87), Frizzle feather (53), and Normal feather (100)) at 20 weeks old were sampled, and 1 mL of whole blood was obtained from each chicken through the wing web. The genomic DNA was isolated from the blood samples using the Qiagen DNA easy© blood kit specifically designed for animal blood, following the instructions provided by the manufacturer. The DNA content and purity were assessed using a Nanodrop spectrophotometer and electrophoresis gel.

PCR amplification and DNA sequencing

The polymerase chain reaction (PCR) was conducted to amplify a 561-base pair fragment of the *Zyxin* gene utilising forward (5′-ACCCCAGGGACCCGTATGAC-3′) and reverse (5′-GGGTCCTTGCGCTGCTGTG-3′) primers. The amplifications for the *Zyxin* primer sets were conducted according to the method described by Zampiga et al. (2018), using an annealing temperature of 58°C. The PCR results were subjected to

unidirectional sequencing utilising forward primers. The Sanger's enzymatic DNA sequencing approach was employed, utilising the ABI 3730 DNA analyzer at the Cornell Core Laboratory, Cornell University, located in Ithaca, New York, USA.

Sequence analysis

The Bio-edit v 7.2.6, BLASTn, BLASTx, and NovelSNPer tools were utilised to perform sequence data quality check, trimming, and identification of single nucleotide polymorphisms (SNPs). The *Zyxin* sequence, identified by its accession number (NM_001004386.2), served as the reference sequence for identifying the SNPs.

Prediction of deleterious non-synonymous single nucleotide polymorphisms

We predicted deleterious non-synonymous single nucleotide polymorphisms (nsSNPs) in the *Zyxin* gene using various bioinformatics tools from PredictSNP1.0 website: PROVEAN, SIFT, PhD-SNP, PolyPhen-2, PANTHER, and SNPs&GO. Both the SIFT and PolyPhen-2 assess amino acid substitutions' impact on protein function based on sequence homology and physical features. PROVEAN employs support vector machine-based technology for the same purpose. PANTHER, SNPs&GO, and PhD-SNP analyze functional protein annotations to predict SNP-related disorder development. nsSNPs deemed harmful by at least six tools were classified as high-risk and chosen for further study.

Analyzing protein stability due to mutations

The stability alterations of the *Zyxin* protein caused by point mutations were assessed using the I-Mutant 2.0 (folding.biofold.org/cgi-bin/i-mutant2.0.cgi) and Mupro (http://mupro.proteomics.ics.uci.edu) programs. I-Mutant 2.0 is a predictor that utilises support vector machine (SVM) to determine the extent of protein destabilisation and quantifies the ΔΔG value in units of kcal mol^{-1}. The ΔΔG (delta delta G) value represents the disparity between the Gibbs free energy values of a mutant protein and the Gibbs free energy value of the wild type protein. A negative ΔΔG value shows that the variations lead to a decrease in the stability of the protein, whereas a positive ΔΔG value suggests an increase in the stability of the protein (Li et al., 2020). However, MUpro utilises an extensive collection of mutation datasets derived from both support vector machines (SVM) and neural networks, which are machine learning techniques. The *Zyxin* protein sequence, along with wild type and substituted amino acids at their respective positions, was utilised as input in the previously mentioned techniques to forecast the impact of mutations on protein stability.

Conservation profile of high-risk non-synonymous SNPS

Non-synonymous single nucleotide polymorphisms (SNPs), located in highly conserved regions, are generally more harmful than those found in non-conserved places. The ConSurf online server utilises empirical Bayesian inference to forecast potential structural and functional amino acid residues. In order to explore the possible impacts of the high-risk nsSNPs, we utilised the ConSurf web server to determine the level of evolutionary conservation at each amino acid position in the *Zyxin* protein. The amino acid sequence of *Zyxin* was inputted into the NLStradamus tool, and the NLS prediction was performed with default parameters. The prediction cut-off was set to the recommended value of 0.6. The preservation of hypothesised sequences was examined using MultAlin. The software ProtParam was utilised to determine the amino acid composition and fundamental biological properties. We employed the ProtScale software to conduct hydrophobicity analysis. In addition, the software PhosphoSitePlus v6.5.9.1 was utilised to quantify the number of phosphorylation modification sites and ascertain the isoelectric point in various phosphorylated states.

Structural analysis of the *Zyxin* Gene

The Swiss modelling platform (swissmodel.expasy.org) was employed to perform homology modelling and generate a model of the chicken's *Zyxin*. This was accomplished by employing the X-ray crystal structure of the *Zyxin* gene from the Protein Data Bank (PDB), specifically the PDB code 5WFK. The UCSF chimaera was employed to validate the locations of single nucleotide polymorphisms (SNPs) and to create two mutant models. Both the wild-type and mutant architectures were optimised to decrease energy use.

Protein–Protein Interaction Prediction

The study focused on investigating protein-protein interactions in order to reveal and identify all functional connections between proteins within cells. The internet database STRING (STRING;

http://string-db.org/) was utilised for the anticipation of interactions among proteins (Szklarczyk et al., 2015). Protein-protein interactions determine the role of the target protein in a certain pathway, aiding in the mapping of the disease-causing network.

Results and discussion

The SNPs were identified as shown in Figure 1. The Figure 1 showed Adenine changed to cytosine at position 22 of the sequences among some of the chickens.

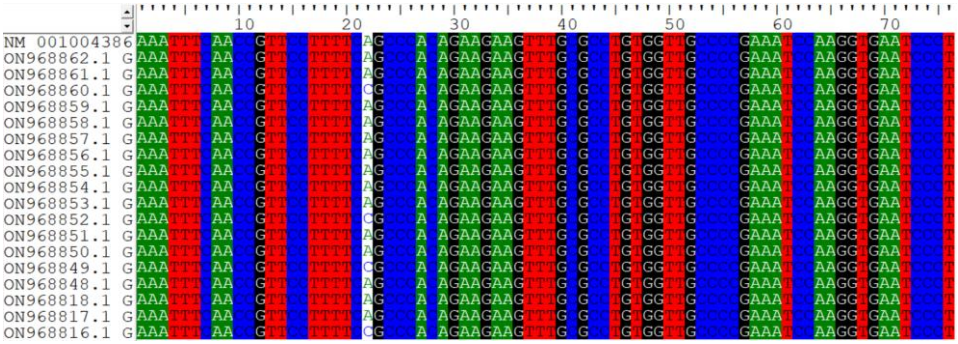

Figure 1. Single nucleotide polymorphism identified at position 22 among Nigerian indigenous chickens.

PhD-SNP and SNAP predicted all the nsSNPs as neutral. PredictSNP, PolyPhen-1, PolyPhen-2 and SIFT predicted R46H has deleterious. All the tools predicted T221P and T221S as neutral except PolyPhen-1 predicted T221P as deleterious. The percent expected accuracy showed ranged between 50 and 98%. The nsSNPAnalyzer and PANTHER were unsuccessfully predicted effects of the variants. *Zyxin* structure was predicted by AlphaFold v2 method with GMQE of .47, percent identify of 71.08.

Table 1 above provides a thorough summary of single nucleotide polymorphisms (SNPs) in the *Zyxin* gene of Nigerian indigenous chickens, showcasing their regions, location on the gene, genotypes, SNP types, and allelic frequencies. It is worth mentioning that there are non-synonymous SNPs (A > C at position 22 and G > A at position 137) present in Exon 1 with all the three genotypes having the A > C SNP at exon 1 whereas only the Normal feather had the G > A SNP type at the same exon. Also, there existed a non-synonymous SNPs in Exon 3 (A > C and A > T both at position 861) of the gene with Synonymous SNPs observed only in exon 3 (A > G at position 812 and 854, T > C at position 863). These SNPs and their allelic frequency highlight the genetic variability observed in various chicken genotypes, including Normal feather (Nm), Nera Black (NB), and Frizzled feather (Nk). The genetic diversity observed in these chicken populations suggests the presence of genetic diversity, wherein Naked neck chickens exhibit a relatively higher allelic frequency for certain SNPs. Furthermore, synonymous SNPs (A > G and A > G) were detected in Exon 3 at different position, indicating that certain genetic variations within this area might not result in alterations to the corresponding amino acids. The existence of both synonymous and non-synonymous SNPs highlights the intricate genetic terrain of the *Zyxin* gene in Nigerian indigenous chickens, potentially impacting variations in coccidiosis resistance and other functional aspects of this gene. The genetic variation observed in this study is consistent with previous findings that have suggested the importance of *Zyxin* in immune response and cell regulation (Kotb et al., 2018). The non-synonymous SNPs detected and observed were further expatiated on Table 2.

Table1. Single nucleotide polymorphisms in Nigerian indigenous chickens.

SNPs	SNPs region	Location on the gene	Genotypes	SNPs type	Allelic frequency
A > C	Exon 1	22	Nm, NB, Nk	Non-synonymous	Nm(.22), NB(.31), Nk(.19)
G > A	Exon 1	137	Nm	Non-synonymous	Nm (.09)
A > G	Exon 3	812	NB, Nk	Synonymous	NB(.08), Nk(.10)
A > G	Exon 3	854	NB, Nk	Synonymous	NB(.08), Nk(.05)
A > C	Exon 3	861	Nm, Nk	Non-synonymous	Nm(.04), Nk(.05)
A > T	Exon 3	861	Nk	Non-synonymous	Nk(.05)
T > C	Exon 3	863	Nm, Nk	Synonymous	Nm(.04), Nk(.05)

Nm: Normal feather; Nk: Naked neck; NB: Frizzle feather.

Table 2. Non-synonymous SNPs and their corresponding amino acids in Nigerian indigenous and Nera black chickens.

nsSNPs	Location on the gene	Location on the protein	Mutated Amino acids
A > C	22	8	Serine > Arginine
G > A	137	46	Arginine > Histridine
A > C	861	221	Threonine > Proline
A > T	861	221	Threonine > Serine

Table 2 provides a concise summary of non-synonymous single nucleotide polymorphisms (nsSNPs) identified in the *Zyxin* gene of Nigerian indigenous and Nera black chickens, indicating the specific locations within the gene, the corresponding locations on the protein, and the amino acid changes resulting from these mutations. Notably, it reveals non-synonymous SNPS (nsSNPs) leading to amino acid alterations at positions 8, 46, and 221 of the *Zyxin* protein. For instance, the A > C mutation at position 22 in the gene results in the replacement of serine with arginine at position 8 in the protein. Similarly, the G > A mutation at position 137 in the gene leads to the substitution of arginine with histidine at position 46 in the protein. Additionally, nsSNPs in Exon 3 at position 861 result in different amino acid changes, with A > C leading to a threonine-to-proline alteration, while A > T results in a threonine-to-serine replacement. These amino acid changes are of significant interest as they may impact the protein's structure and, consequently, its function, which aligns with previous research emphasizing the importance of *Zyxin* in various cellular processes (Siddiqui et al., 2021; Wang et al., 2019). Understanding the implications of these nsSNPs on *Zyxin*'s function is crucial for elucidating their potential roles in coccidiosis resistance and other physiological aspects in Nigerian indigenous and Nera black chickens.

Table 3 summarizes the percentage expected accuracy of deleterious non-synonymous single nucleotide polymorphisms (nsSNPs) in the *Zyxin* gene, as predicted by various computational tools (PredictSNP, PhD-SNP, PolyPhen-1, PolyPhen-2, SIFT and SNAP). These tools provide insights into the potential impact of nsSNPs on protein function and their likelihood of being deleterious as described by Lim et al. (2021). For the R46H mutation (also see the structure of this mutated amino acid in Figure 2), PredictSNP indicates a 55% probability of being deleterious, while PhD-SNP and SIFT offer relatively higher probabilities at 78 and 79%, respectively. Both PolyPhen-1 and PolyPhen-2 suggest a moderate likelihood of 74 and 65%, respectively, for this mutation, and SNAP provides a lower probability of 50%. In contrast, for the T221P mutation, PredictSNP and SNAP offer probabilities of 74 and 77%, respectively, while PhD-SNP predicts a high probability of 98%. PolyPhen-1 and PolyPhen-2 provide more conservative probabilities of 59 and 87%, respectively, and SIFT offers the highest probability at 90%. Similarly, for the T221S mutation, PredictSNP suggests a probability of 83%, while PhD-SNP offers 89%. PolyPhen-1 and PolyPhen-2 present probabilities of 67 and 75%, respectively, while SIFT and SNAP indicate probabilities of 76 and 71%, respectively. These predictions from various computational tools adopted provide valuable insights into the potential impact of specific nsSNPs on protein function and stability. The variations in probability percentages among the tools highlight the complexity of the mutations identified and their possible interactions with other genetic factors with potential probability of being damaging, deleterious and diseased (as explained by Lim et al., 2021).

Table 3. Percentage expected accuracy of deleterious nsSNPs in the *Zyxin* gene using computational tools.

Mutation	Predict SNP	PhD-SNP	PolyPhen-1	PolyPhen-2	SIFT	SNAP
R46H	55%	78%	74%	65%	79%	50%
T221P	74%	98%	59%	87%	90%	77%
T221S	83%	89%	67%	75%	76%	71%

nsSNPs: non-synonymous single nucleotide polymorphisms.

Table 4 presents the predictions of changes in amino acid stability resulting from specific mutations in the *Zyxin* protein, as assessed by I-Mutant 2.0 and MUpro software tools. For the R46H mutation, both I-Mutant 2.0 and MUpro suggest a decrease in stability, with ΔΔG values of -1.05 kcal/mol and -0.3124 kcal/mol, respectively. The presence of negative ΔΔG values suggests that the mutation results in a protein structure that is less stable (Figure 3). This finding aligns with the assertion made by Chen et al. (2021) that mutations leading to decreased stability of proteins may contribute to tumorigenesis. Specifically, the reduced stability of normal proteins may decrease their fitness level, while simultaneously conferring fitness advantages to tumorigenic proteins (Chen et al., 2021; Wang & Fersht, 2017). The reliability index for I-Mutant is 7, indicating a relatively confident prediction, while MUpro provides a confidence score of -

0.8407. Conversely, for the T221P mutation, I-Mutant 2.0 predicts a slight increase in stability with a ΔΔG value of -0.10 kcal mol^{-1}, suggesting that this mutation makes the protein slightly more stable. However, MUpro predicts a decrease in stability with a ΔΔG value of -0.8438 kcal mol^{-1}. The reliability index for I-Mutant is 6, indicating a reasonable level of confidence, while MUpro offers a confidence score of -0.9409. Similarly, for the T221S mutation, both I-Mutant 2.0 and MUpro predict a decrease in stability with ΔΔG values of -0.71 kcal mol^{-1} and -0.4609 kcal mol^{-1}, respectively. These negative values suggest that the mutation results in decreased protein stability. The reliability index for I-Mutant is 1, indicating a lower level of confidence, while MUpro provides a confidence score of -1.

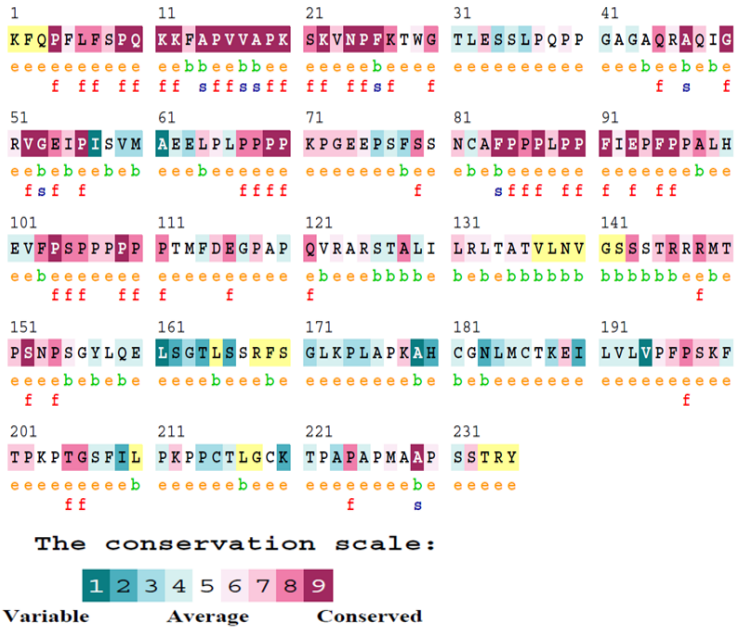

Figure 2. Results from consurf tool. **e** - An exposed residue according to the neural network algorithm. **b** - A buried residue according to the neural network algorithm. **f** - A predicted functional residue (highly conserved and exposed). **s** - A predicted structural residue (highly conserved and buried). **x** - Insufficient data - the calculation for this site was performed on less than 10% of the sequences.

Table 4. Prediction of change in amino acids stability using I-Mutant and Mupro software.

Amino acids change	I-Mutant 2.0			MUpro		
	Stability	ΔΔ(kcal mol^{-1})	ReIiability index	Stability	ΔΔ(kcal mol^{-1})	Confidence score
R46H	Decrease	-1.05	7	Decrease	-.3124	-.8407
T221P	Increase	-.10	6	Decrease	-.8438	-.9409
T221S	Increase	-.71	1	Decrease	-.4609	-1

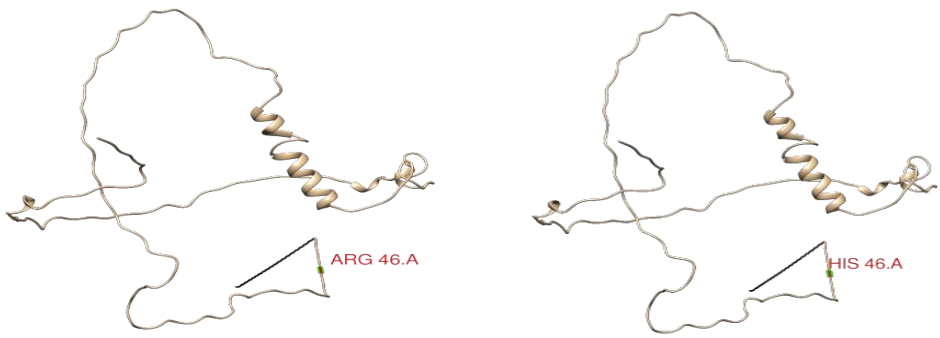

Figure 3. a. Normal *Zyxin* with Arginine at position 46 b. Arginine mutated to Histidine (R46H).

These stability predictions are essential in understanding how specific mutations may impact the structural integrity of the *Zyxin* protein. The variation in predictions between the two tools highlights the complexity of protein stability changes resulting from these mutations (Lim et al., 2021). It emphasizes the importance of considering multiple computational methods to assess stability and the need for

experimental validation to ascertain the functional consequences of these mutations in the context of coccidiosis resistance and other physiological processes in chickens (Ahmad et al., 2023).

Table 5 provides information on post-translational modification sites in the *Zyxin* protein and their conservation analysis, specifically for the R46H, T221P, and T221S mutations. For the R46H mutation, it is associated with proteolytic cleavage and is assigned a score of 0.56. This score indicates the likelihood of this site being modified, with a higher score suggesting a greater probability of modification and also the mutation (R46H) is capable of affecting post-translational modifications, potentially influencing the functional role of *Zyxin* at position 46 (see Figure 2, 3a and 3b). Additionally, the confidence interval color is marked as 8, indicating a relatively high level of confidence in this prediction. The designation "E" in the "B/E" column indicates that this residue is exposed, making it accessible for modifications. Furthermore, the "Function" column designates this residue as functional (f), and it is noted to be highly conserved and exposed. This suggests that the R46H mutation may play a functional role and could potentially influence *Zyxin*'s involvement in various cellular processes (Alberts et al., 2015).

Table 5. Post-translational modification sites in *Zyxin* protein and their conservation analysis.

Mutation	Modification	Score	Confidence interval colours	B/E	Function
R46H	Proteolytic cleavage	0.56	8	E	f
T221P			5	E	
T221S			5	E	

E – exposed residue, functional (f), f - highly conserved and exposed.

On the other hand, the T221P and T221S mutations do not have specific post-translational modifications associated with them, as indicated by the empty cells in the "Modification" column. The scores for these mutations are both 5, suggesting a moderate likelihood of modification. The designation "E" in the "B/E" column again indicates that these residues are exposed and accessible for modifications. While no specific function is assigned, their exposure implies the potential for functional significance. Additionally, they are marked as highly conserved and exposed, suggesting their importance in the protein's structure and function.

These results indicate that the R46H mutation is linked to proteolytic cleavage and is predicted to have functional significance, potentially affecting *Zyxin*'s role in cellular processes. In contrast, the T221P and T221S mutations are also exposed and may have functional roles, although specific modifications are not identified (See Figure 4a, 4b and 4c). This analysis emphasizes the potential impact of these mutations on *Zyxin*'s functional properties and highlights the significance of this study in their roles in coccidiosis resistance and other physiological aspects in chickens (Xue et al., 2020).

Figure 4. a. Normal *Zyxin* with Threonine at position 221 b. Threonine mutated to Serine c. Threonine mutated to Proline.

Table 6 provides an overview of the physiochemical properties of the wild-type and mutated *Zyxin* proteins in Nigerian indigenous and Nera black chickens, including molecular weight, isoelectric point, extinction coefficients, instability index, aliphatic index, and the Grand average of hydropathicity (GRAVY). The molecular weight of the wild-type *Zyxin* protein is approximately 25061.32 Da, while the mutated (HS) version has a slightly lower molecular weight of 25028.24 Da, and the mutated (hp) variant has a molecular weight of 25038.28 Da. This indicates that the mutations at positions R46H and T221P have a minimal impact on the overall molecular weight of the protein which is as suggested by Ozturk and Carter (2021). Meanwhile, the isoelectric point (pI) represents the pH at which the protein carries no net electrical charge (Novák & Havlíček, 2016). So in this case, the wild-type and both mutated variants have very similar pI

values, with the wild-type at 9.84 and both mutated proteins at 9.75. This suggests that the mutations do not significantly alter the protein's charge characteristics. Also, the extinction coefficients are the measure of how strongly a protein absorbs light at a specific wavelength, often used for protein quantification (Anthis & Clore, 2013). In this context, all three variants have the same extinction coefficient value of 8730, indicating no substantial change in their light-absorbing properties.

Table 6. Physiochemical properties of wild-type and mutated *Zyxin* protein in Nigerian indigenous and Nera black chickens.

nsSNPs	Molecular weight	Isoelectric point	Extinction coefficients	Instability index	Aliphatic index	GRAVY
Wild-type	25061.32	9.84	8730	77.50 (unstable)	67.70	-0.217
Mutated(HS)	25028.24	9.75	8730	79.39	67.70	-0.211
Mutated (hp)	25038.28	9.75	8730	78.00	67.70	-0.215

GRAVY: Grand average of hydropathicity.

The instability index is an indicator of protein stability, with higher values suggesting greater instability (Gamage et al., 2019). The wild-type *Zyxin* protein has an instability index of 77.50 (unstable), while both mutated proteins (HS and hp) have slightly higher values of 79.39 and 78.00, respectively. This suggests that the mutations may lead to slightly increased protein instability. The aliphatic index measures the relative volume occupied by aliphatic side chains, which are typically hydrophobic. All three variants have the same aliphatic index value of 67.70, indicating that the mutations do not significantly affect the protein's hydrophobic properties. The GRAVY score reflects the protein's hydropathicity, with negative values indicating hydrophilic characteristics (Wang et al., 2021). In this case, the wild-type *Zyxin* has a GRAVY score of -0.217, while both mutated variants have similar scores, with -0.211 for HS and -0.215 for hp. This suggests that the mutations do not induce substantial changes in the protein's hydrophilic nature (see Figure 5 for the diagrammatic representation and interpretation of *Zyxin* protein-protein interactions as well as its structure).

The total energies obtained after 100 iterations were 2843.774 KJ mol^{-1} and 2065.011KJ mol^{-1} for native and R46H, respectively. For mutation at the position 221, total energies obtained after 100 iterations were 2850.277 KJ mol^{-1}, 2938.116 KJ mol^{-1} and 3035.955 KJ mol^{-1} for native, T221S and T221P, respectively. The distance and structural similarities between the modeled native and mutant structures were determined using the root mean square deviation (RMSD). The RMSD values obtained for amino acid residue at position 46 were 0.00507Å and 0.00505Å for native and R46H, respectively. RMSD values obtained for amino acid residue at position 221 were 0.00506Å, 0.00507Å and 0.00506Å for native T221S and T221P, respectively. Increases in RMSD indicate a greater deviation from the native structure, which may alter the protein's function.

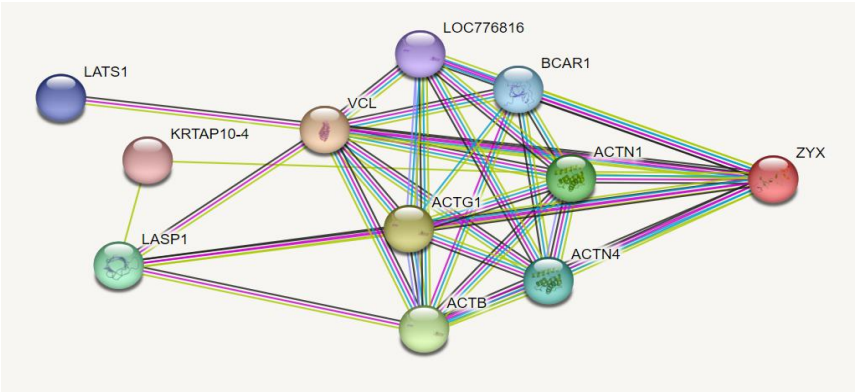

Figure 5. *Zyxin* protein-protein interactions with 10 partners.

All the proteins except KRTAP10-4 were observed for interaction with *Zyxin* by experimental basis. From text mining data, *Zyxin* interactions are detected for VCL, ACTG1, ACTB, ACTN1, LASP1, ACTN4, BCAR1, LATS1, LOC776816 and KRTAP10-4 with 0.989, 0.968, 0.966, 0.965, 0.964, 0.959, 0.959, 0.9912, 0.905 and 0.902 scores, respectively. Strong association pattern of *Zyxin* is predicted for, VCL, ACTG1, ACTB, ACTN1, LASP1, ACTN4, BCAR1, LATS1, LOC776816 and KRTAP10-4 partners with high confidence. Vinculin (VCL) and Actin, cytoplasmic type 5 (ACTG1) are highly coexpressed with *Zyxin*. Actin filament –binding protein (VCL) involved in cell-matrix adhesion and cell-cell adhesion and regulates cell surface. ACTG1 are highly conserved proteins that are involved in various types of cell motility and are ubiquitous.

Conclusion

This work aimed to use molecular screening to identify functional single nucleotide polymorphisms (SNPs) in the *Zyxin* gene that are linked with coccidiosis in chickens. Multiple servers were integrated to enhance the predictive efficiency. Two non-synonymous single nucleotide polymorphisms (nsSNPs) were forecasted to exert the greatest influence on the structure, stability, and activities of *Zyxin*, thereby heightening the vulnerability to coccidiosis. The two anticipated non-synonymous single nucleotide polymorphisms (nsSNPs) in the chicken *Zyxin* gene can be subjected to additional experimental validation using chickens infected with coccidiosis. This validation process aims to confirm the presence and impact of these nsSNPs, which in turn can guide the development of targeted disease diagnostics and treatment strategies by focusing on functionally significant SNPs.

References

Adenaike, A. S., Mabunmi, A. O., Takeet, M. I., Adenaike, O. D., & Ikeobi, C. O. (2016). Genetic differences in the body weight and haematological traits of Nigerian indigenous chickens infected with Eimeria tenella. *Tropical Animal Health and Production*, *48*(7), 1443–1447. https://doi.org/10.1007/s11250-016-1114-6

Adenaike, A. S., Peters, S. O., Adeleke, M. A., Fafiolu, A. O., Takeet, M. I., & Ikeobi, C. O. N. (2018). Use of discriminant analysis for the evaluation of coccidiosis resistance parameters in chickens raised in hot humid tropical environment. *Tropical Animal Health and Production*, *50*(5), 1161–1166. https://doi.org/10.1007/s11250-018-1547-1

Adenaike, A.S.,; Peters, S.O.,; Fafiolu, A.O.,; Adeleke, M.A.,; Takeet, M.I.,; Wheto, M.,; Adebambo, O.A., &; Ikeobi, C.O.N. (2019). Genetic Diversity of zyxin and TNFRSF1A genes in Nigerian Local Chickens and Nera Black Chickens. *Agriculturae Conspectus Scientificus*, *84*(3), 305-311.

Ahmad, R., Yu, Y. H., Hua, K. F., Chen, W. J., Zaborski, D., Dybus, A., Hsiao, F. S., & Cheng, Y. H. (2023). Management and control of coccidiosis in poultry: A review. *Animal Bioscience, 37*, 1–15. https://doi.org/10.5713/ab.23.0189

Ajala, A. O., Ogunjimi, S. I., Famuwagun, O. S., & Adebimpe, A. T. (2021). Poultry production in Nigeria: exploiting its potentials for rural youth empowerment and entrepreneurship. *Nigerian Journal of Animal Production*, *48*, 114–123. https://doi.org/10.51791/njap.v48i1.2890

Alberts, B., Johnson, A., ; Lewis, J.,; Raff, M.,; Roberts, K., &; Walter, P. (2015). *Studying Gene Expression and Function*. Nih.gov; Garland Science, 2015.

Anthis, N. J., & Clore, G. M. (2013). Sequence-specific determination of protein and peptide concentrations by absorbance at 205 nm. *Protein Science, 22*(6), 851–858. https://doi.org/10.1002/pro.2253

Chen, S., Wu, J. L., Liang, Y., Tang, Y. G., Song, H. X., Wu, L. L., Xing, Y. F., Yan, N., Li, Y. T., Wang, Z. Y., Xiao, S. J., Lu, X., Chen, S. J., & Lu, M. (2021). Arsenic Trioxide Rescues Structural p53 Mutations through a Cryptic Allosteric Site. *Cancer Cell*, *39*(2), 225–239. https://doi.org/10.1016/j.ccell.2020.11.013

Dai, G., ; Sun, D., ; Sun, M., ; Lin, Y., ; Wang, X., Zhang, G., ; Xie, K., ; Shi, M., ; Olowofeso, O., ; Wang, J. (2014). Study on the relationship between single nucleotide polymorphisms of the coccidiosis-resistance candidate zyxin gene exon 1 and carcass traits in the Jinghai yellow chicken. *Turkish Journal of Veterinary & Animal Sciences*, *38*(2), 121-125.

Gamage, D. G., Gunaratne, A., Periyannan, G. R., & Russell, T. G. (2019). Applicability of Instability Index for In vitro Protein Stability Prediction. *Protein and Peptide Letters*, *26*(5), 339–347. https://doi.org/10.2174/0929866526666190228144219

Heise, H., Crisan, A., & Theuvsen, L. (2015). The poultry market in Nigeria: Market structures and potential for investment in the market. *International Food and Agribusiness Management Review*, *18*, 197-222.https://doi.org/10.22004/ag.econ.207011

Kamani, J., Bwala, F. H., & Weka, P. R. (2021). Coccidiosis: A threat to the poultry industry in Plateau State, Nigeria. *Nigerian Journal of Animal Science*, *23*(1), 80-85.

Kotb, A., Hyndman, M. E., & Patel, T. R. (2018). The role of zyxin in regulation of malignancies. *Heliyon*, *4*(7), e00695. https://doi.org/10.1016/j.heliyon.2018.e00695

Lawal, J. R., Jajere, S. M., Ibrahim, U. I., Geidam, Y. A., Gulani, I. A., Musa, G., & Ibekwe, B. U. (2016). Prevalence of coccidiosis among village and exotic breed of chickens in Maiduguri, Nigeria. *Veterinary World*, *9*(6), 653–659. https://doi.org/10.14202/vetworld.2016.653-659

Li, B., Yang, Y. T., Capra, J. A., & Gerstein, M. B. (2020). Predicting changes in protein thermodynamic stability upon point mutation with deep 3D convolutional neural networks. *PLoS Computational Biology*, *16*(11), e1008291. https://doi.org/10.1371/journal.pcbi.1008291

Lim, S. W., Tan, K. J., Azuraidi, O. M., Sathiya, M., Lim, E. C., Lai, K. S., Yap, W. S., & Afizan, N. A. R. N. M. (2021). Functional and structural analysis of non-synonymous single nucleotide polymorphisms (nsSNPs) in the MYB oncoproteins associated with human cancer. *Scientific Reports*, *11*. https://doi.org/10.1038/s41598-021-03624-x

Mesa-Pineda, C., Navarro-Ruíz, J. L., López-Osorio, S., Chaparro-Gutiérrez, J. J., & Gómez-Osorio, L. M. (2021). Chicken Coccidiosis: From the Parasite Lifecycle to Control of the Disease. *Frontiers in Veterinary Science*, *8*. https://doi.org/10.3389/fvets.2021.787653

Novák, P., & Havlíček, V. (2016). Protein extraction and precipitation. In *Proteomic profiling and analytical chemistry* (pp. 51–62). Elsevier. https://doi.org/10.1016/B978-0-444-63688-1.00004-5

Olutumise, A. I., Oladayo, T. O., Oparinde, L. O., Ajibefun, I. A., Amos, T. T., Hosu, Y. S., & Alimi, I. (2023). Determinants of health management practices' utilization and its effect on poultry farmers' income in Ondo State, Nigeria. *Sustainability*, *15*(3), https://doi.org/10.3390/su15032298

Ozturk, K., & Carter, H. (2022). Predicting functional consequences of mutations using molecular interaction network features. *Human Genetics, 141*(6), 1195–1210. https://doi.org/10.1007/s00439-021-02329-5

Padjasek, M., Kocyła, A., Kluska, K., Kerber, O., Tran, J. B., & Krężel, A. (2020). Structural zinc binding sites shaped for greater works: Structure-function relations in classical zinc finger, hook and clasp domains. *Journal of Inorganic Biochemistry*, *204*. https://doi.org/10.1016/j.jinorgbio.2019.110955

Rashid, M., Akbar, H., Bakhsh, A., Rashid, M. I., Hassan, M. A., Ullah, R., Hussain, T., Manzoor, S., & Yin, H. (2019). Assessing the prevalence and economic significance of coccidiosis individually and in combination with concurrent infections in Pakistani commercial poultry farms. *Poultry Science*, *98*(3), 1167–1175. https://doi.org/10.3382/ps/pey522

Siddiqui, M. Q., Badmalia, M. D., & Patel, T. R. (2021). Bioinformatic Analysis of Structure and Function of LIM Domains of Human Zyxin Family Proteins. *International Journal of Molecular Sciences*, *22*(5). https://doi.org/10.3390/ijms22052647

Svitkina T. (2018). The Actin Cytoskeleton and Actin-Based Motility. *Cold Spring Harbor Perspectives in Biology*, *10*(1), a018267. https://doi.org/10.1101/cshperspect.a018267

Szklarczyk, D., Franceschini, A., Wyder, S., Forslund, K., Heller, D., Huerta-Cepas, J., Simonovic, M., Roth, A., Santos, A., Tsafou, K. P., Kuhn, M., Bork, P., Jensen, L. J., & von Mering, C. (2015). STRING v10: Protein–protein interaction networks, integrated over the tree of life. *Nucleic Acids Research, 43*(D1), D447–D452. https://doi.org/10.1093/nar/gku1003

Wang, G., & Fersht, A. R. (2017). Multisite aggregation of p53 and implications for drug rescue. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(13), E2634–E2643. https://doi.org/10.1073/pnas.1700308114

Wang, H., Zhong, H., Gao, C., Zang, J., & Yang, D. (2021). The Distinct Properties of the Consecutive Disordered Regions Inside or Outside Protein Domains and Their Functional Significance. *International Journal of Molecular Sciences*, *22*(19). https://doi.org/10.3390/ijms221910677

Wang, Y. X., Wang, D. Y., Guo, Y. C., & Guo, J. (2019). Zyxin: a mechanotransductor to regulate gene expression. *European Review for Medical and Pharmacological Sciences*, *23*(1), 413–425. https://doi.org/10.26355/eurrev_201901_16790

Xue, V. W., Chung, J. Y., Córdoba, C. A. G., Cheung, A. H., Kang, W., Lam, E. W., Leung, K. T., To, K. F., Lan, H. Y., & Tang, P. M. (2020). Transforming Growth Factor-β: A Multifunctional Regulator of Cancer Immunity. *Cancers*, *12*(11). https://doi.org/10.3390/cancers12113099

Zampiga, M., Flees, J., Meluzzi, A., Dridi, S., & Sirri, F. (2018). Application of omics technologies for a deeper insight into quali-quantitative production traits in broiler chickens: A review. *Journal of Animal Science and Biotechnology*, *9*. https://doi.org/10.1186/s40104-018-0278-5

Zhang, S., Chong, L. H., Woon, J. Y. X., Chua, T. X., Cheruba, E., Yip, A. K., Li, H. Y., Chiam, K.-H., & Koh, C.-G. (2023). Zyxin regulates embryonic stem cell fate by modulating mechanical and biochemical signaling interface. *Communications Biology, 6*. https://doi.org/10.1038/s42003-023-04421-0