



Prediction of Quaternary Protein Structure of HSP90AA1 Gene in Three Nigerian Indigenous Breeds of Sheep

*¹De Campos, J.S., ¹Olatunji, O.I., ²Okunlola, M.A., ⁵Oliyide, O.M., ²Bajomo, E.T., ⁴Aremu, J.O., ¹Bello, T.S., ¹Adegoke, S.J., ⁷Kujero, G.O., ⁶Onasanya, G.O., ¹Adeleye, A.A., ³Olusoji, B. I., ⁸Popoola, A.A., ¹Badmus, M.O., ¹Ajayi, B.J., ¹Abatti, O.S., ¹Adeneye, I.B., ¹Amoo, S.T., ¹Dairo, C.O., ¹Ikeobi, C.O.N.

¹Department of Animal Breeding and Genetics, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.

²Department of Animal Production, University of Ilorin, Ilorin, Nigeria.

³Department of Animal Production Technology, Yaba College of Technology, Yaba, Lagos State, Nigeria.

⁴Animal Production Department, Kwara State University, Malete, Kwara State, Nigeria.

⁵Department of Veterinary Pharmacology and Toxicology, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.

⁶Department of Animal Science, Federal University Dutse, Dutse Nigeria.

⁷Department of Animal Production and Health, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.

⁸Department of Animal Nutrition, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.

*08038296550; decamposjs@funaab.edu.ng

Abstract

This study aimed to predict and compare the quaternary structure and functions of the HSP90AA1 protein in three indigenous sheep breeds in Nigeria: West African Dwarf (WAD), Yankasa, and Uda. Fifteen healthy adult sheep, comprising five animals per breed, were randomly selected for the study. 2 mls of blood was collected from each animal via jugular vein and preserved on FTA cards for genomic DNA extraction. The HSP90AA1 gene was amplified using two primer sets targeting the 5' flanking region (300 bp) and intron 10 (700 bp), followed by bidirectional Sanger sequencing. A total of thirty (30) sequence reads were generated, quality-checked using Phred scores (≥ 30), and aligned using BioEdit software to produce consensus sequences for each breed. The nucleotide sequences were translated into amino acid sequences and analysed using ExPASy. ProtParam software was used for detecting the physicochemical properties and SOPMA software was used for secondary structure prediction. Quaternary structures were predicted using AlphaFold2 Multimer software and visualised using Mol Viewer. Results revealed clear breed-specific variation in amino acid length, with WAD, Yankasa, and Uda having 1540, 1680, and 1890 amino acids, respectively. Physicochemical analysis showed that the Uda breed exhibited a lower instability index (31.25) and higher aliphatic index (40.68), indicating greater structural stability and thermo-tolerance compared to WAD and Yankasa. All predicted proteins were slightly acidic and hydrophilic. Quaternary structure prediction confirmed a conserved homo-dimeric organisation across all breeds; however, structural differences were observed, with WAD and Yankasa displaying compact V-shaped conformations, while Uda exhibited a more elongated open-state structure. The study demonstrates that breed-specific variations in the HSP90AA1 gene result in distinct structural and functional differences in the protein, which may contribute to environmental adaptabilities of the Nigerian indigenous sheep. The findings highlight the potential of HSP90AA1 as a molecular marker for breeding heat-resilient sheep and underscores the importance of computational approaches in livestock genetic improvement.*

Keywords: Gene, Heat Shock Protein, Prediction, Quaternary Structure, Sheep.

Introduction

Ruminant livestock make huge contributions to global agricultural production, food security, and livelihood sustainability,

especially in the developing world (Osei Amponsah *et al.*, 2019). Sheep, specifically, are of immense importance to the socioeconomic lives of people worldwide, and

Nigeria alone has a sheep population of around 50 million. Sheep farming is a significant part of Nigeria's agricultural sector, providing meat and other products to local markets (Agaviezor *et al.*, 2012; FAOSTAT, 2023). The major indigenous Nigerian sheep breeds, namely the West African Dwarf (WAD), Yankasa, Uda, and Balami, are highly valued for their toughness and adaptability in different agro-ecological zones, and as a result, they constitute a valuable genetic resource for sustainable meat production in the local farming systems (Agaviezor *et al.*, 2012). The toughness of these indigenous breeds is a direct result of their biological makeup, which is basically fueled by proteins.

Beyond their role in stress tolerance, these breeds also possess distinct production, performance, and disease resistance traits that determine their suitability across Nigeria's different agro-ecological zones. The WAD breed is known for early sexual maturity, high prolificacy, and a well-documented trypanotolerance, which enables it to survive in the humid, tsetse-infested forest zones of southern Nigeria (Daramola and Adelaye, 2009; Obidike *et al.*, 2009). The Yankasa breed, found mainly in the northern Guinea and Sudan savannah zones, has a larger body frame, faster growth rate, and produces more meat and wool than WAD, and as a result, it is preferred for commercial mutton production (Sam *et al.*, 2023; Yakubu *et al.*, 2025). The Uda breed, which is adapted to the semi-arid Sudan savannah and Sahel zones, is known for its large body size, long legs, and pendulous ears, and it also shows good resistance to ecto- and endoparasitic infections that are common in its drier habitat (Agaviezor *et al.*, 2012; Adebambo, 2002). These breed-specific production and disease resistance traits, just like the structural variation reported for *HSP90AA1* in this study, show the wider pattern of adaptive divergence among Nigerian indigenous sheep breeds in response

to their different ecological niches (Muthusamy *et al.*, 2025).

Proteins are essential biomolecules possessing a wide range of cellular functions, from enzymatic catalysis to structural support (Alberts *et al.*, 2015). A protein's function is closely related to its three-dimensional structure, which is organised hierarchically into four orders. Whereas the primary structure is the linear sequence of amino acids, the quaternary structure, involving the assembly of multiple polypeptide subunits into a single functional complex, is significant for many proteins. This complex structure dictates their stability, activity, and how they interact with other molecules (Goodsell and Olson, 2000; Zhang, 2008).

The quaternary structure of a protein refers to the spatial arrangement and assembly of multiple polypeptide subunits (each with its own primary, secondary, and tertiary structure) into a functional multimeric complex. This level of structure is stabilised by non-covalent interactions such as hydrogen bonds, hydrophobic interactions, van der Waals forces, and sometimes disulfide bonds or metal coordination (Goodsell *et al.*, 2000). One family of proteins that has a vital role in the flexibility of ruminants is the heat shock proteins (*HSPs*), which are molecular chaperones. These proteins protect cells by assisting in protein folding, stabilisation, and repair under various kinds of stress (Lindquist and Craig, 1988; Taipale *et al.*, 2010). The *HSP90AA1* gene particularly encodes for a beta isoform of *HSP90* that is important in the maintenance of cellular balance (proteostasis) and cell survival both under normal circumstances and in response to stress (Jackson, 2013). Research proved that genetic variation in *HSP90* genes is linked with thermo-tolerance and production traits in ruminants, reflecting their potential as genetic markers in breeding programs aimed at improving livestock tolerance to tropical

environments (Deb *et al.*, 2014; Hassan *et al.*, 2020). With the help of bioinformatics, it is now possible to investigate the molecular mechanisms of these adaptations. Computation based protein structure prediction has become a reliable and cost effective method for the determination of protein function and diversity. More recent software such as Alpha Fold Multimer is able to predict complex quaternary structures from sequence data and evolutionary information (Sheng *et al.*, 2014). Given the role of *HSP90AA1* in stress tolerance, its predicted quaternary structure in native Nigerian sheep breeds will provide information on the structural adaptations that allow them to survive and will inform livestock breeding programs in the future.

The *HSP90AA1* gene is a key molecular chaperone with a function in stress tolerance. While *HSP90* polymorphisms have been associated with thermotolerance based on research in cattle and goats (Deb *et al.*, 2014; Hassan *et al.*, 2020), not much such work has been conducted on native Nigerian sheep, which are a crucial part of rural livelihood. By predicting the quaternary structure of the *HSP90AA1* protein, this study will shed light on how specific structural differences may be conferring an enhanced degree of environmental adaptation and stress tolerance in these breeds. The objective of this study was to predict and compare the quaternary structure of the *HSP90AA1* protein in three Nigerian indigenous sheep breeds in order to determine the differences in structures associated with stress tolerance.

Materials and Methods

Experimental site

The blood samples were collected at the Akinyele market, located in Akinyele, Ibadan, Nigeria. This area lies within the Southern Guinea Savannah ecological zone, characterised by a typical tropical climate with

a distinct wet season (April and October) and dry season (November and March). Average annual rainfall ranged between 1000 and 1200 mm, with mean daily temperatures of 25°C and 35°C and relative humidity of 40 and 70% (NIMET, 2018). The vegetation was dominated by grasses interspersed with shrubs and scattered trees, providing suitable forage for small ruminants. This location was selected due to its significance as a sheep and producing region in Nigeria, where the three indigenous breeds; Yankasa, Uda, and West African Dwarf (WAD) sheep were readily available and commonly reared. Conducting the study in this environment ensured that the findings are relevant to local farmers and livestock managers.

Experimental Animals and Sample Collection

A total of 15 healthy adult sheep (5 per breed) were randomly selected for this experiment. The animals were observed to be free from visible signs of disease at the time of collection. Sampling occurred during the dry season to minimise environmental variability. Blood samples (2ml each) were collected from the jugular vein using sterile syringes. A portion of the blood was immediately dropped into the FTA cards and allowed to air-dry thoroughly at room temperature. The dried FTA cards were then stored in individual moisture barrier envelopes to ensure long-term DNA stability and transported to the laboratory.

DNA Extraction

Genomic DNA was extracted from blood samples dropped on FTA cards using a Quick-DNATM Miniprep Kit, strictly following the manufacturer's recommended protocol. To begin, 200 µl of each blood sample was transferred into a 1.5 ml micro-centrifuge tube. Lysis of the blood cells was achieved by adding 20 µl of Proteinase K alongside 200 µl of Buffer AL directly to the sample. The

mixture was then thoroughly vortexed to ensure uniform mixing before being incubated at 56°C for 10 minutes. This step was crucial for complete cell disruption and the release of genomic DNA from the blood matrix. Following lysis, 200 µl of absolute ethanol (96–100%) was pipetted into the lysate and mixed immediately by pulse-vortexing for 15 seconds. This alcohol precipitation step aids in binding the DNA to the silica membrane of the spin column in the subsequent stages. The entire mixture, including any precipitate that may have formed, was carefully transferred onto a DNeasy Mini spin column placed inside a 2 ml collection tube and centrifuged at $6,000 \times g$ (8,000 rpm) for 1 minute. The flow-through and collection tube were discarded after centrifugation.

The spin column was then subjected to a two-step washing process to remove residual contaminants. First, 500 µl of Buffer AW1 was added to the column and centrifuged at $6,000 \times g$ for 1 minute, after which the flow-through was discarded. A second wash was performed by adding 500 µl of Buffer AW2, followed by centrifugation at $20,000 \times g$ (14,000 rpm) for 3 minutes to completely dry the silica membrane. This step was important to eliminate any carry-over of ethanol, which could inhibit downstream enzymatic reactions. Finally, the purified DNA was eluted by placing the spin column in a clean

1.5 ml micro-centrifuge tube, adding 100 µl of pre-warmed Buffer AE (70°C) directly onto the silica membrane, and incubating at room temperature for 1 minute before centrifugation at $6,000 \times g$ for 1 minute. The eluted DNA was collected and stored at –20°C until further use. To assess the quality and quantity of the extracted DNA, all samples were measured using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific). Only samples with an A260/A280 absorbance ratio falling within the acceptable range of 1.8 to 2.0 — indicating sufficient purity free from protein or solvent contamination and a DNA concentration greater than 50 ng/µl were retained. Samples that failed to meet these quality thresholds were subjected to re-extraction to ensure the reliability and reproducibility of subsequent PCR amplification.

Primer Design and PCR Amplification

Previously published primers by Marcos-Carcavilla *et al.*, 2008 targeting the *HSP90AA1* gene were used based on conserved sequence regions of the *Ovis aries* genome. Two sets of primers were employed in this study, targeting the 5' flanking region and Intron 10 of the *HSP90AA1* gene respectively, with amplicon sizes of 300 bp and 700 bp. The primer sequences used for amplification and sequencing are presented in Table 1.

Table 1. List of primers used to amplify and/or sequence the *HSP90AA1* gene.

	5' Flanking region	Intron 10	Amplicon size
Forward (5'-3')	ACTTGTTGCCCGAATTCCT	ACAGGATCTACAGGATGAT	300bp
Reverse (5'-3')	AAGCGTGTCCCCAGATAGTG	ACTAGCTCTGCTTTAGTGC	700bp
Sequencing (5'-3')	GCTAGGTTTCGAGCCTTGAGG	ACAGGACCTACAGGATGAT	

Marcos-Carcavilla *et al.* (2008)

PCR amplification was carried out in a total reaction volume of 25 µl. Each reaction mixture consisted of 50 ng of template DNA, 0.5 µM of each primer (forward and reverse), 12.5 µl of 2× PCR Master Mix (Thermo Fisher Scientific), and nuclease-free water added to bring the final volume to 25 µl. All reactions were assembled on ice to minimise non-specific amplification and premature enzymatic activity. Amplification was performed using a thermal cycler programmed with the following cycling conditions: an initial denaturation step at 95°C for 5 minutes to fully denature the double-stranded DNA template; followed by 35 cycles of denaturation at 95°C for 30 seconds, primer annealing at temperatures between 55°C and 60°C for 30 seconds (optimized per primer pair), and extension at 72°C for 45 seconds; and a final extension step at 72°C for 10 minutes to ensure complete synthesis of all amplified products. To confirm successful amplification and verify the expected product sizes, PCR products were resolved by electrophoresis on a 1.5% agarose gel stained with ethidium bromide (0.5 µg/ml), and visualized under UV light using a gel documentation system. A 100 bp DNA ladder was included alongside the samples to serve as a molecular size reference. Only samples yielding clear, distinct bands of the expected fragment sizes of 300 bp and 700 bp were considered for subsequent purification and Sanger sequencing.

Sequencing

Amplified PCR products were purified using a QIA quick PCR Purification Kit and sequenced bidirectionally via Sanger sequencing (outsourced to a certified facility). Sequences were obtained in FASTA format and quality and checked using Phred scores (Greater than 30 for reliable bases).

Sequence Analysis and SNP Identification:

Raw Sanger sequencing data in FASTA

format from HSP90AA1 amplicons were processed and analysed using BioEdit. The sequences were manually quality-trimmed to remove low confidence bases at the terminal ends. These sequences were then aligned against the *Ovis aries* HSP90AA1 reference sequence within BioEdit to resolve any variation. Multiple sequence alignment was subsequently performed across the three breeds (Yankasa, Uda, and WAD) to identify polymorphisms, including Single Nucleotide Polymorphisms (SNPs) and insertions/deletions (indels).

Protein Sequence and Physicochemical Property Prediction

Nucleotide sequences were translated into amino acid sequences using the ExPASy Translate tool, accounting for breed-specific variations. The reference protein sequence (724 amino acids) served as a baseline. The primary physicochemical properties, including molecular weight, isoelectric point (pI), and instability index, were determined using ExPASy ProtParam. Secondary structure features, such as alpha helices and beta turns, were predicted using SOPMA to provide a structural foundation for higher-order modeling.

Quaternary Structure Prediction: The quaternary structures of HSP90AA1, specifically the multimeric assembly's characteristic of this protein, were predicted using AlphaFold2. Input sequences from the identified variants across the three sheep breeds were processed through the AlphaFold2 multimer workflow to model the interaction between subunits. Structural refinement was achieved through multiple recycling iterations to ensure high-confidence residue placement and interface accuracy. The resulting models were visualised and analysed to assess the structural stability of the protein complex.

Results

Comparison of *HSP90AA1* Gene Protein Sequences Across Three Nigerian Indigenous Sheep Breeds

The *HSP90AA1* protein sequences obtained directly from laboratory sequencing were aligned and consensus sequences were generated for each breed using BioEdit software. The consensus protein sequences of WAD, Yankasa, and Uda were then compared. The results revealed breed-specific differences in the total number of amino acids. The WAD breed had 1,540 amino acids, Yankasa had 1,680 amino acids, and Uda had 1,890 amino acids. These differences indicate the presence

of breed-specific variation across the *HSP90AA1* protein sequences of these three Nigerian indigenous sheep breeds.

Physicochemical Properties of the Predicted *HSP90AA1* Protein

The physicochemical properties of the *HSP90AA1* protein in the three breeds were analysed using ExPASy ProtParam, which provided the molecular weight, theoretical pI, instability index, aliphatic index, and GRAVY score. The alpha helix, beta sheet, and random coil content were obtained separately using SOPMA. The results are presented in Tables 2, 3 and 4.

Table 2. Physicochemical properties of the predicted *HSP90AA1* protein in WAD sheep

PROPERTY	VALUE	INTERPRETATION
Molecular Weight	192.40 kDa	Large dimeric protein
Theoretical (pI)	6.71	Slightly acidic
Instability Index	41.33	Unstable protein
Aliphatic Index	23.90	Low thermo-stability
GRAVY score	-1.536	Hydrophilic protein
Beta sheet	27.99%	Functional dynamics
Alpha Helix Content	28.83%	Moderate structure rigidity
Coil Content	43.18%	Flexibility for binding

Table 3. Physicochemical properties of the predicted *HSP90AA1* protein in YANKASA sheep

PROPERTY	VALUE	INTERPRETATION
Molecular Weight	215.86 kDa	Large dimeric protein
Theoretical (pI)	6.54	Slightly acidic
Instability Index	42.18	Unstable protein
Aliphatic Index	27.88	Low thermo-stability
GRAVY score	-1.865	Hydrophilic protein
Beta sheet	28.10%	Moderate structural rigidity
Alpha Helix Content	25.30%	Functional dynamics
Coil Content	46.61%	Flexibility for binding

Table 4 Physicochemical properties of the predicted *HSP90AA1* protein in Uda sheep

PROPERTY	VALUE	INTERPRETATION
Molecular Weight	220.42 kDa	Large dimeric protein
Theoretical (pI)	6.35	Slightly acidic
Instability Index	31.25	Stable protein
Aliphatic Index	40.68	Moderate thermo-stability
GRAVY score	-1.65	Hydrophilic protein
Beta sheet	32.28%	Functional dynamics
Alpha Helix Content	12.28%	Moderate structural rigidity
Coil Content	55.45%	Flexibility for binding

Predicted Quaternary Structures of *HSP90AA1* Protein in Three Nigerian Indigenous Sheep Breeds

The quaternary structures of the *HSP90AA1* protein in the three breeds were predicted using AlphaFold and visualised using Mol* Viewer. The predicted structures for WAD, Yankasa, and Uda are presented in Figures 1, 2, and 3, respectively.

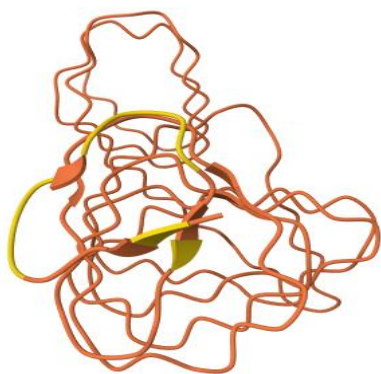


Figure 1: Predicted quaternary structure of *HSP90AA1* protein in WAD sheep



Figure 2: Predicted quaternary structure of *HSP90AA1* protein in Yankasa sheep



Figure 3: Predicted quaternary structure of *HSP90AA1* protein in Uda sheep

The AlphaFold-predicted quaternary structures revealed that all three breeds formed dimeric assemblies consisting of two polypeptide chains. The WAD and Yankasa breeds displayed a compact, V-shaped dimeric conformation with two prominent alpha-helical coiled regions connected by a central flexible loop region. The Uda breed, by contrast, displayed a more elongated and extended dimeric conformation with the two helical chains arranged in a more linear and vertically stacked organisation. These structural differences are consistent with the variation observed in the physicochemical and secondary structure properties across the three breeds.

Comparison of Predicted Quaternary Structures Across the Three Breeds

A comparison of the predicted quaternary structures across the three breeds revealed both similarities and differences. All three breeds produced dimeric *HSP90AA1* structures, confirming the conserved quaternary organisation of the *HSP90AA1* protein. However, the WAD and Yankasa breeds shared a structurally similar compact dimeric conformation, while the Uda breed displayed a distinctly different elongated open-state dimeric conformation. In terms of physicochemical properties, Uda recorded the highest molecular weight (220.42 kDa), the most stable instability index (31.25), the highest aliphatic index (40.68), and the highest random coil content (55.45%), distinguishing it clearly from WAD and Yankasa. WAD and Yankasa had comparable instability index values (41.33 and 42.18 respectively) and similar random coil contents of 43.18% and 46.61% respectively. All three breeds shared similar slightly acidic pI values and negative GRAVY scores, indicating a conserved hydrophilic and acidic character of the *HSP90AA1* protein across Nigerian indigenous sheep breeds.

Discussion

The comparison of *HSP90AA1* protein sequences across the three breeds revealed breed-specific differences in amino acid length: WAD (1,540 aa), Yankasa (1,680 aa), and Uda (1,890 aa). These differences in sequence length suggest the presence of breed-specific variation in the *HSP90AA1* protein across the three breeds. This finding is consistent with reports by Deb *et al.* (2014), who observed significant sequence variation in HSP90 proteins across tropical livestock breeds and attributed such variation to selective pressure imposed by different ecological environments. Similarly, Hassan *et al.* (2020) reported that HSP90 protein variation in ruminants reflects adaptive evolution in response to climatic conditions. The longer amino acid sequence observed in the Uda breed (1,890 aa) compared to WAD and Yankasa may indicate the presence of additional functional motifs or repeat regions that enhance the chaperone capacity of the *HSP90AA1* protein in this breed. This difference could be attributed to the fact that the Uda breed is predominantly adapted to the semi-arid Sudan savannah zone of Nigeria, where exposure to high ambient temperatures may have driven the evolution of a more elaborate stress-response protein.

The molecular weights of the *HSP90AA1* protein across the three breeds (192.40 kDa for WAD, 215.86 kDa for Yankasa, and 220.42 kDa for Uda) are consistent with the expected size of dimeric HSP90 complexes, where each monomer contributes approximately 90 kDa to the functional dimer. These values are in agreement with findings reported by Pearl and Prodromou (2006), who characterised the molecular weight of HSP90 dimers in mammalian cells to be in the range of 170 to 240 kDa. The slightly higher molecular weight observed in the Uda breed compared to WAD and Yankasa is directly attributable to its longer amino acid sequence and may indicate

a more complex multi-domain architecture of the protein in this breed. All three breeds recorded theoretical pI values below 7.0 (WAD: 6.71, Yankasa: 6.54, Uda: 6.35), confirming that the *HSP90AA1* protein is slightly acidic across all breeds. This is consistent with the known biochemical characteristics of HSP90 proteins reported in the literature. Pearl and Prodromou (2006) and Taipale *et al.* (2010) both noted that the slightly acidic nature of HSP90 proteins is essential for their cytoplasmic function and interaction with co-chaperones under physiological pH conditions. The slightly more acidic pI of Uda (6.35) compared to WAD (6.71) could reflect differences in the charged amino acid composition of the protein, which may influence its interaction with client proteins and co-chaperones under heat stress.

The instability index values showed a clear distinction between the breeds: WAD (41.33) and Yankasa (42.18) were classified as unstable proteins, while Uda (31.25) was classified as stable. This finding differs from studies on HSP90 in cattle, where Kadri *et al.* (2014) generally reported stable HSP90 proteins across all breeds studied. The instability observed in WAD and Yankasa *HSP90AA1* proteins in the present study may be attributed to the unique amino acid composition of these indigenous Nigerian breeds, which have not been widely characterised in previous studies. Notably, *HSP90AA1* is the inducible isoform of HSP90, and it has been suggested that a degree of conformational flexibility or instability in the in vitro-predicted sense may actually be advantageous for a stress-inducible protein, as it allows rapid conformational changes in response to stress signals (Jackson, 2013). The stable profile of the Uda protein, however, suggests it may maintain structural integrity under basal conditions, which combined with its inducible nature, could represent an

efficient molecular strategy for stress response in hot environments.

The aliphatic index values of WAD (23.90), Yankasa (27.88), and Uda (40.68) indicate varying degrees of thermo-stability across the breeds, with Uda showing the highest thermo-stability. This is consistent with the findings of Collier *et al.* (2008), who reported that breeds adapted to hot climates tend to express HSP variants with higher aliphatic indices, reflecting greater thermal stability of the protein. The significantly lower aliphatic index values for WAD and Yankasa suggest that their *HSP90AA1* proteins may be less thermostable, though this does not necessarily imply reduced stress tolerance since the rapid induction and dynamic activity of *HSP90AA1* may compensate for lower thermo-stability. All three breeds recorded negative GRAVY scores (WAD: -1.536, Yankasa: -1.865, Uda: -1.65), confirming the hydrophilic character of the *HSP90AA1* protein. This is in agreement with Taipale *et al.* (2010), who reported that HSP90 proteins are inherently hydrophilic due to their abundance of charged and polar amino acids, which is essential for their solubility and interaction with client proteins in the aqueous cytoplasmic environment.

The alpha helix, beta sheet, and random coil content obtained from SOPMA revealed that all three breeds had high random coil content (WAD: 43.18%, Yankasa: 46.61%, Uda: 55.45%), consistent with the known structural flexibility of HSP90 proteins. Hartl *et al.* (2011) reported that the high loop and coil content of molecular chaperones is essential for enabling the conformational changes required during the chaperone cycle, particularly the open-to-closed transition that drives client protein binding and release. The exceptionally high coil content observed in the Uda breed (55.45%) compared to WAD and Yankasa suggests a higher degree of structural flexibility in the Uda *HSP90AA1* protein,

which may enhance its ability to accommodate a wider range of misfolded client proteins under heat stress conditions. This is a finding unique to the present study, as most previous investigations of HSP90 in livestock have focused on cattle and have not reported such high coil content in tropical breeds (Kadri *et al.*, 2014; Hassan *et al.*, 2020). The differences observed may therefore reflect genuine breed-specific structural adaptations of the *HSP90AA1* protein in Nigerian indigenous sheep that have not been previously documented.

The AlphaFold-predicted quaternary structures of the *HSP90AA1* protein confirmed dimeric assembly in all three breeds, consistent with the well-established quaternary organisation of HSP90 proteins reported by Pearl and Prodromou (2006) and Evans *et al.* (2022). The compact V-shaped dimeric conformation observed in WAD and Yankasa is characteristic of the intermediate or semi-closed state of HSP90 dimers, which is associated with ATPase activity and active client protein remodeling (Sheng *et al.*, 2014). The elongated open-state conformation observed in the Uda breed, by contrast, resembles the open apo-state of HSP90 dimers reported by Krukenberg *et al.* (2009), which is associated with increased flexibility and a higher capacity for client protein capture. This structural difference between Uda and the other two breeds is a novel finding that has not been previously reported for sheep *HSP90AA1* and may reflect the unique evolutionary pressures experienced by the Uda breed in its semi-arid habitat. The fact that AlphaFold predicted such a distinct conformation for the Uda breed, despite all three breeds sharing the same gene, strongly suggests that the breed-specific amino acid differences identified in this study have a direct impact on the three-dimensional folding and quaternary organisation of the *HSP90AA1* protein. The structural and physicochemical differences

identified in the *HSP90AA1* protein across the three breeds have important functional implications for thermo-tolerance and environmental adaptability. The combination of a stable instability index, high aliphatic index, elevated molecular weight, and open-state quaternary conformation observed in the Uda breed collectively suggest that its *HSP90AA1* protein is structurally optimised for function under thermal stress conditions. This is consistent with the known ecology of the Uda breed, which is well adapted to the hot, semi-arid Sudan savannah zone of Nigeria (Agaviezor *et al.*, 2012). These findings support the hypothesis that breed-specific structural variation in the *HSP90AA1* protein contributes to differences in environmental adaptability among Nigerian indigenous sheep breeds, a relationship that has been previously proposed but not structurally demonstrated for these specific breeds (Deb *et al.*, 2014; Hassan *et al.*, 2020). The results of the present study therefore provide the first structural evidence for this relationship in Nigerian sheep, contributing a novel and significant finding to the field of livestock molecular biology and computational genomics.

In contrast, the unstable instability index values of WAD and Yankasa, combined with their compact dimeric conformations, suggest that their *HSP90AA1* proteins may be more dynamically responsive to rapid stress signals, which is advantageous in the humid forest and Guinea savannah zones where these breeds are found. The high degree of hydrophilicity observed across all three breeds (negative GRAVY scores) is consistent with the cytoplasmic localisation and water-dependent chaperone activity of *HSP90AA1*, as reported by Taipale *et al.* (2010). Overall, the results of this study suggest that while all three Nigerian indigenous sheep breeds express functional *HSP90AA1* proteins capable of stress response, each breed has evolved a distinct structural strategy for coping with the

environmental challenges of its respective ecological zone, and this structural diversity represents a valuable genetic resource for livestock breeding programs targeting thermo-tolerance under climate change.

Conclusion and Recommendation

The quaternary structures predicted using AlphaFold showed that all three breeds formed dimers, meaning the protein is made up of two chains working together. However, the shape of the dimer was not the same across breeds. WAD and Yankasa both showed a compact, V-shaped dimeric structure, while Uda showed a longer and more extended structure. This tells us that even though all three breeds have the same type of protein, the way it folds and arranges itself is different. When the predicted structures were compared across the three breeds, Uda stood out the most. It had the highest molecular weight (220.42 kDa), the most stable instability index (31.25), the highest aliphatic index (40.68), and the highest coil content (55.45%). WAD and Yankasa were more similar to each other in terms of structure and physicochemical properties, with both showing unstable instability index values and compact dimeric shapes. With respect to these structural differences in line with heat tolerance and adaptability traits, the Uda breed appears to have a more stable and flexible *HSP90AA1* protein that may help it cope better with heat stress, given that Uda sheep are found in the hotter, drier parts of Nigeria. WAD and Yankasa, on the other hand, have more dynamic proteins that respond quickly to stress, which suits the environments they live in.

These findings are important because they help us understand how these breeds survive in their different environments, and they can guide future breeding programs aimed at producing sheep that can handle heat stress and climate change better.

Recommendations

Based on the findings of this study, the following recommendations are made:

1. The Uda breed should be prioritized in livestock breeding programs targeting heat stress tolerance, given the superior thermo-stability and structural integrity of its *HSP90AA1* protein demonstrated in this study.

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