

GENETIC COMPARISON OF *MELANOCORTIN-1 RECEPTOR (MC1R)* GENE NUCLEOTIDE SEQUENCES AND PROTEIN MODELLING IN THREE PLUMAGE COLOUR VARIANTS OF NIGERIAN LOCAL TURKEY

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ABSTRACT

This study was carried out to compare the nucleotide and protein sequence similarity and identity of three plumage colour variants (white, black and lavender) of Nigerian local turkey. A total of 120 poults (40 per variant) were raised for this study. 30 Poults (10 poults per variant) were randomly selected before Blood collection for DNA extraction and PCR Amplification. Electrophoresis was done to check for extracted DNA purity and concentration before PCR amplification. Out of 30 samples 23 samples were fit for RFLP digestion process in which the PCR-RFLP products were sequenced then edited through Bioinformatics approach and analyzed using molecular software packages to compare per cent identity and per cent similarity of their nucleotide and protein sequences, evolutionary relationships and protein modelling in the MC1R gene of Nigerian Local turkey. The result showed that there was a high percentage similarity of 99.95% between white, lavender and black plumage colour variants of Nigerian local turkey. It also indicated that there was a high per cent identity between white and lavender (94.85%), black and white (93.93%), then black and lavender (88.78%). The results indicated that there was an evolutionary relationship among the three plumage colour variants in which the white and lavender variants indicated the existence of high genetic polymorphism derived from a common ancestral form, while the black plumage colour variant had a divergent ancestor. The result indicated a mutation in white plumage colour variants due to the presence of SNPs in the gene's protein. It is concluded that the variants were similar and originated from a common ancestor.

Key words: Evolutionary relationship, Nucleotide sequences, Similarity, Protein structure, Turkey variant

J. Agric. Prod. & Tech. 2024; 13:37-50

INTRODUCTION

Turkeys provide a number of values to the ecosystem and to humans. Ecologically, they are integral to food chains, foraging a wide variety of plants and animals' food, including acorns and nuts, seeds, berries, roots, insects, and even small

vertebrates, like frogs and salamanders. In turn, they provide food for animals such as foxes, bobcats, and coyotes. For humans, turkeys provide a popular and nutritious in protein, niacin, and B vitamins (Bender & Bender, 2005). Animal plumage colour has been the subject of studies for some years.

While most phenotypic features of animals result from the interaction of genetic and environmental factors, plumage colour is considered to be exclusively genetically determined (Kasprzak- Fillipek *et al.*, 2020).

There are many genes responsible for the appearance of different plumage colours, among which the melanocortin 1 receptor gene plays an important role in controlling the production of black and red pigments that determine basic colours. Other interacting genes also influence colour formation. The MC1R gene polymorphisms have impacts on the plumage colours and skin traits in domestic animals (Vidal *et al.*, 2010). Studies have found a significant association between the MC1R genetic variation and plumage colours of chicken and geese (Huang *et al.*, 2014). Animals display a wide variety of coat or skin colours, which depend on the black-brown eumelanin to yellow reddish pheomelanin in the skin (Wang and Hebert, 2006). However, the melanocortin-1 receptor (MC1R) gene, with its ligands melanocortins and adrenocorticotrophic hormone (ACTH), is the main positive regulator (Slominski *et al.*, 2004). The cytogenetic location of the melanocortin-1 receptor is the long arm (q) of chromosome 16 at position 24.3 and mainly controls which types of melanin are produced by melanocytes (Garcia-Borrón *et al.*, 2005; Wang *et al.*, 2009). When the *MC1R* gene is activated by external factors, a series of chemical reactions are triggered inside melanocytes, stimulating production of eumelanin (Ha *et al.*, 2003). MC1R signalling stimulates antioxidant and DNA repair pathways, as reviewed (Maresca & Flori, 2015).

Pheomelanin is responsible for the reddish-brown pigments and predominates in human red hair, red and yellow fur of

mammals as well as in chestnut and rufous feathers. Melanins are not derived from the diet as carotenoids but are produced endogenously in peripheral tissue like skin, more specifically in the specialised cells called melanocytes present in the epidermis of birds and mammals (Mason & Mason, 2000; Wang *et al.*, 2019). Melanin-based pigmentation provides the ultimate base for a colourful breeding plumage observed in many bird species, and may serve as a potential honesty-reinforcing mechanism, as it is maintained by hormonal effects with potential consequences on metabolism and immune function (Folstad & Karter, 1992). The great variation with respect to pigment production and the molecular processes underlying pigment distribution. The impressive diversity of pigments identified in bird feathers to date is predicted to serve numerous functions such as protection from various destructive sources, aiding in thermoregulation, facilitating camouflage and communication between and within species.

Sequences of biological macromolecules in various breeds of animal species share a common evolutionary ancestry, especially those with conserved catalytic activity, presumably fold into the same structure and are used for optimisation of their similarity based on observed sequence variation. It can also be used to obtain a single optimal alignment, which provides an accurate measure of similarity and identity, structure, function and evolutionary history. DNA and protein sequence evolution are highly complex and need to be researched for proper documentation. Hence, the topic is the comparison of the MC1R gene's Nucleotide and protein sequences in three plumage colour variants of Nigerian local turkey and their evolutionary relationships.

MATERIALS AND METHODS

Experimental site

The experiment was carried out at the Poultry Unit of the Teaching and Research Farm of the Department of Animal Science, Faculty of Agriculture, University of Uyo, Annex campus, Akwa Ibom State, Nigeria and genomic DNA extraction was done in the Biotechnology Laboratory of Federal University of Agriculture, Abeokuta, Ogun State. Uyo falls within the Tropical rainforest zone in Nigeria. The area has two distinct seasons, rainy and dry seasons. It has an annual rainfall range of 2200 mm to 3500 mm. Temperature of the area ranges from 28 °C to 32 °C year-round. Uyo is located between latitude 4°31' and 6°30' North and longitude 7°30' and 8°20' east of the equator. The mean altitude is 38.1m above sea level. Sunshine is between 1400 and 1500 hours per year; Relative humidity is from 71% to 88% annually (University of Uyo Meteorological Station, 2019).

Procurement and Management of Experimental Birds

A total of 120-day-old Poults (turkey) with 40 Poults each of the three plumage colour variants (white, lavender, and black) were procured from a turkey supplier in Uyo, Akwa Ibom State. The Poults were reared intensively in using standard management practices.

The brooding pen was washed, cleaned, disinfected, and fumigated for a week prior to the arrival of the day old Poults. The feeders and drinkers were washed, rinsed and dried before use. The Brooder house was bedded with wood shavings, and the bedding was changed on weekly basis to prevent disease outbreaks to the birds. The dimensions of the brooders constructed were large enough to accommodate 5 Poults per brooder (0.3m2

width and 0.5m2 length) throughout the experimental period of 8 weeks of termination. Heat and light sources were: a lantern and an electrical source, which was provided around the feeding area on the first day of brooding and the brooding environment was maintained at 35°C on the first day for the Poults. The heat source and the temperature were monitored with a thermometer by regulating 1°C every 3 days until it got to the heat requirement of 21°C. Feed ingredients were purchased and compounded at the experimental site. The birds were given starter mash from 0-4 weeks that contained 2832.71 Kcal metabolizable energy and 28.44% Crude protein, and grower mash (3000 Kcal Metabolizable Energy and 29.00% crude protein) from 5-8 weeks. Small amounts of feed starter mash were evenly distributed over the floor of the brooders on the first day of brooding. On the third day, feed was presented in small feeders and drinkers for the Poults. The Poults were introduced to water by dipping their beaks after they were placed on the floor. Both feed and water were provided *ad libitum*.

Vaccination: Birds were vaccinated against Newcastle disease at day old (intraocular). Gumboro vaccine was administered on the 7th, 14th, 21st and 28th days and Lasota vaccine on the 21st day.

Data collection

Blood collection for DNA extraction

Blood samples were collected from the turkeys' wing veins on the 8th week using a 3 mL syringe and needle and dropped onto the circular section of the Whatman FTA (Flinders Technology Associate) cards. The stained FTA cards were dried, placed in a well-identified paper envelope, and sealed and kept in a cool, dry place to prevent contamination. The blood-stained FTA cards were taken to the

Biotechnology Laboratory at Federal University of Agriculture, Abeokuta, in Ogun State, for genomic DNA extraction and PCR amplification.

DNA Extraction

Genomic DNA (gDNA) extraction was carried out with a solution-based Jena Bioscience Animal and Fungi DNA Preparation Kit following the manufacturer's instructions. In a clean Eppendorf tube, 300 µl of cell lysis solution and 1.5 µl of Proteinase K were added to the FTA card containing the dried blood sample. The tube was incubated at 55°C overnight. 100 µl of protein precipitation solution was added to the cell lysate the mixture was vortexed vigorously and centrifuged at 15,000 rpm for 3 min to precipitate the protein present. The supernatant was transferred to a sterile 2 µl Eppendorf tube containing 300 µl of Isopropanol and gently mixed by inverting for 1 min to precipitate the DNA. DNA was pelleted by centrifugation at 15,000 rpm for 1 min, then washed with 500 µl of washing buffer and all were completely air-dry after the washing buffer was discarded.

50 µl hydration solution and 1.5 µl RNase inhibitor were added to the air-dried DNA pellet. The samples were subjected to inhibitor were added to the air-dried DNA pellet. The samples were subjected to an initial incubation at 37 °C for 1 hour, followed by a final incubation at 65 °C for 1 hour, to fully hydrate the dried DNA pellet.

The extraction of genomic DNA was performed strictly according to the FTA purification kit protocol. Steps for extraction were as follows:

Using a Harris cutting/punching mat and a Harris micro punch (1mm). Sample discs were punched from each stained Flinders Technology Associates (FTA) card

and dropped into a labelled 0.2 µl Eppendorf tube. 0.15 µl 100 µl of distilled water and Tris 0.1% SDS of FTA purification Reagent (200 µl for each disk) were added and rotated up and down for 30 minutes, then tipped off the spent solution carefully. 200 µl of distilled water was added and rotated up and down for 10 minutes, then decant the solution carefully, the process was repeated. Then repeated the process without shaking and tipped of the spent solution carefully. 50 µl water was added to the tube. Then heated in the 50 µl water at 90°C for 15 minute using water bath. DNA was ready for PCR reaction.

DNA Quality and purification checked (Electrophoresis)

Quality and purity of DNA were checked by agarose gel electrophoresis at 1.5% in (0.5x TE buffer stained with ethidium bromide) at 100 V for 1 hour. Quality and concentration of DNA were viewed via Ultra-Violet illumination.

Primers: A synthesized Primers (MC1R – Forward 5' CTG GCT GAG GCC GGG GCC 3' Reverse 5' GAG GTC GGG CAG CCC AAC 3') manufactured by Macrogen Europe, Netherlands was used. 10 picomol concentration of primers was used. 10 µl of the stock solution of the original primer was added to 90 µl of molecular grade water (ddH₂O). Primer came in form of a pellet it was reconstituted to form the stock primer solution which was preserved.

Polymerase Chain Reaction (PCR) for DNA Amplification

The reaction was carried out with Jena Bioscience High Fidelity Core kit. The extracted DNA was amplified using the polymerase chain reaction method. The amplification reaction was carried out in a programmable applied biosystem 2720 thermocycler (Mastercycler pro by

Eppendorf). For each constituent or reagent of one polymerase chain reaction (PCR) tube, 25 µl mixture was prepared which comprised of 2 µl of DNA template, 0.5 µl of dNTP (Deoxynucleotide Triphosphates), 2.5 µl PCR buffer, 0.25 µl DNA polymerase, 2 µl of primer (forward and reverse) (10 pmol/ µl) and 17.75 µl of molecular water. The contents were thoroughly mixed. The amplification went through phases of cycles as follows: 94 °C of initial denaturation for 5 minutes, 94 °C of denealing for 30 seconds, 64 °C of annealing for 40 seconds and 72 °C of extension for 30 seconds (the denealing, annealing and extension phase went through 35 cycles) and 72 °C of final extension for 5 minutes. The amplicons were obtained when all the cycles completed. The polymerase chain reaction (PCR) amplification was confirmed by agarose gel electrophoresis (1%). The amplified products were visualized of the bands of the expected size of MC1R under UV trans-illumination.

The products of PCR were digested with HinfI restriction endonuclease. The digestion process was carried out in 15 µl mixtures comprising of 5 µl of PCR product, 1U HinfI endonuclease, 1.5 HinfI buffer and 7.5 µl of molecular water. The mixture were incubated for 2 hour at 37°C. The 23 digested DNA were then run on a 2% agarose gel and imaged by gel documentation system (Syngene, Gene Geenius Bio- Imaging, USA).

Sequencing, Purification, and data analysis

Cycle total sequencing of 23 samples was carried out in a total reaction volume of 20 µL using BigDye^R. Terminator v3.1. Cycle Sequencing Ready Reaction- 100 mix (Thermo Fishers Scientific, Applied Biosystem),

forward/reverse primers, and 50-70 ng/µL purified Polymerease Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) products on 2720 Thermal cycler (Applied Biosystems). Cycle sequencing conditions consisted of 95°C for 5 mins, followed by 32 cycles of 95 °C for 20 s, at 55 °C for 15 s and at 60 °C for 4 min. The 23 sequenced reactions were purified using Zymo Research DNA Sequencing Clean-upTM Kit (The Epigenetics Company, USA) and sequenced by capillar Melanin Cortin 1-Receptor y electrophoresis on an automated DNA sequencer (ABI PRISM 3500 Genetic Analyzer). 23 raw sequences were curated and assembled using bioinformatics tools.

Bioinformatics

Analysis of Melanin Cortin 1- Receptor (MC1R) gene

The 23 nucleotide sequences were trimmed and edited using Bioedit (Hall, 1999). The 23 nucleotide sequences obtained were analyzed using BLAST-N to ascertain the returned sequences. The gene sequence obtained were BLAST using BLAST-N and aligned using CLUSTAL-W (Thompson, 1994) and save in Bioedit. Codon Code Aligner was used for the detection of the Single Nucleotide Polymorphisms in the three plumage colour variant of Nigerian Locally adapted Turkeys.

Data/Statistical Analysis

Single Nucleotide Polymorphism (SNP) Identification and Genotyping

SNPs were confirmed using DnaSp v5.10.01 (Librado and Rozas, 2009). The resultant amino acid variation of each SNPs were predicted using CodonCode Aligner (<http://www.codoncode.com/aligner>). The 23 Sequences of the three turkey variants through the (National Center for

Biotechnology Information) NCBI Nucleotide Blast-N approach were carried out in Gene Bank of the NCBI Nucleotide replacement export data from the MC1R (Melanin Cortin 1- Receptor) gene were carried out to detect polymorphisms using MEGA software version 4.0.2 (Kumar *et al.*, 2008). Correlation analysis, the Polymorphic Informative Content (PIC) and Principal Component Analysis was computed using PASW/SPSS version 20. Also haplotype frequencies were investigated using the algorithm from the Haploview program (Haploview, USA).

Nucleotide identity and protein similarity of Melanin Cortin 1- Receptor (MC1R) gene were determined through per cent identity and per cent similarity among 23 nucleotide sequences for both homologous and divergent plumage colour turkey variants through pair-wise comparison of the sequences of BLAST N option to create matrix table. Phylogenetic tree was constructed using MEGA-X V.6 software (Tamura *et al.*, 2013) to determine the phylogenetic relationship among the three plumage colour turkey variants. The phylogenetic tree was inferred using neighbor joining method. The reliability of the inferred tree was evaluated using bootstrap analysis of 1000 replications. The MC1R gene protein structure was predicted using the Jpred secondary protein structure online tool to predict the secondary structure of the three plumage colour variants of Nigerian local turkey in MC1R protein. Gene Expression in MC1R gene of the three plumage colour variant of local Turkey was determined through NCBI Geo. The expression data for Turkey MC1R gene were subjected to analysis of variance.

Molecular Data Analysis

The expression data for turkey MC1R gene obtained was subjected to

analysis of variance using molecular software package DnaSp v5.10.01 (Librado & Rozas, 2009).. The model is given as:

$$Y_{ij} = \mu + T_i + \epsilon_{ij}$$

Where, Y_{ij} represents log ratio of sample target source2/sample target source1; μ represents population mean; T_i represents turkey variants (1, 2, 3); ϵ_{ij} represents error term.

RESULTS AND DISCUSSION

The Gel Image of the Three Plumage Colour Variants of Nigerian Locally Adapted Turkey

The gel image of RFLP patterns in MC1R gene of exon 4 cutting at 500bp and 1000bp of the three Plumage colour Variants of Nigerian locally adapted turkeys presented in Figures 1 and 2 depicts RFLP patterns generated by HinfI restriction enzyme (*Haemophilus influenza*) cutting and electrophoresed on 2% agarose gel. L is the molecular marker (50bp).

Comparison of protein coding region of the three plumage colour variants of Nigerian local turkey

The result in Table 1 showed protein coding region in each plumage colour variants showed that black turkey plumage colour variant sequence had the highest synonymous sites of 141.17 bp, while the lavender turkey plumage colour variants sequence recoded 139.67 bp and white turkey plumage colour variants sequence recoded the lowest as 188 bp. The high value of synonymous protein coding site of black plumage colour variant indicated that substitution of one base for another in the exon of the gene coding for a protein, produces an amino acid sequence that is not modified. This is possible because the genetic code is "degenerate", meaning that some amino acids are coded for by more

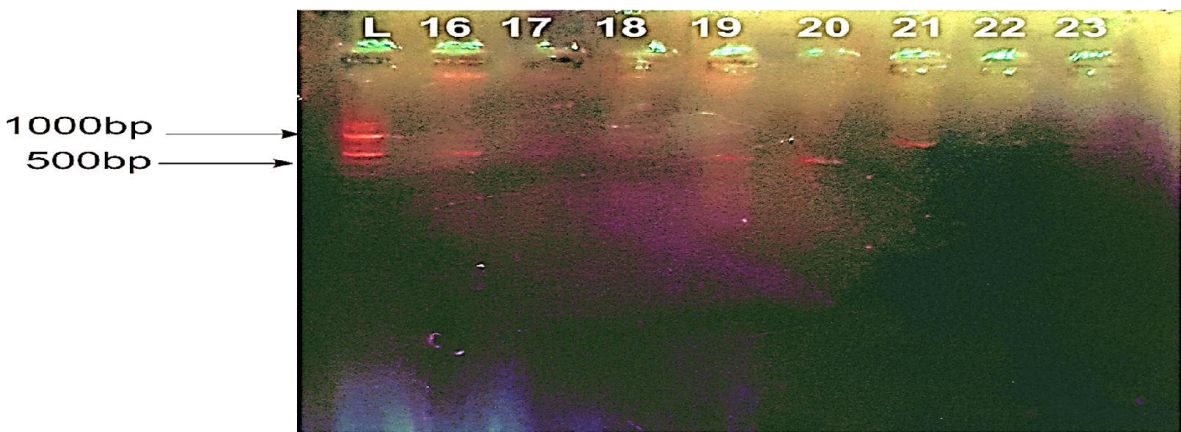
than one three-base-pair codon; since some of the codons for a given amino acid differ by just one base pair from others coding for the same amino acid, a mutation that replaces the "normal" base by one of the alternatives will result in incorporation of the same amino acid into the growing polypeptide chain when the gene is

translated. Hence, slowness in speed during translation and post-transcription of the gene in thriving the harsh environmental condition of economy. Therefore, the change is often assumed to be neutral, meaning that it does not affect the fitness of the individual carrying the new gene to survive.



mid-range ladder

Figure 1: MC1R gene RFLP Products of the three Nigerian locally adapted Turkey Variants Population



mid-range ladder

Figure 2: RFLP patterns generated by HinfI enzyme cutting and electrophoresed on 2% agarose gel.

The result showed that white plumage colour variant turkey sequences had the highest non-synonymous site of 381, followed by the lavender turkey plumage colour variants sequence with the value of 379 then black with 377 though they were not significantly different. The high value of non-synonymous site of the protein coding in the white plumage colour

Table 1: Protein coding region of synonymous and non-synonymous site of three plumage colour variants of Nigerian local turkey.

Turkey Variant	SS (bp)	NSS (bp)
Black	141.17	377
White	188	381
Lavender	139.67	379

SS-synonymous site, NSS; Non-Synonymous site, bp- base pairs

Comparison of percentage identity among the three plumage colour variants of Nigerian local turkey

The result in Table 2 showed percent identity among the plumage colour variants of Nigerian local turkey indicated that white and black plumage colour variants had 93.93%, while black and lavender recorded 88.78% and between white and lavender was 94.85% which showed no significant differences. The percentage identity between lavender and white variants nucleotide sequences was higher compared to white and lavender or black and lavender sequences. This implied that, highly related plumage colour variants of Nigerian local turkeys showed high level of identity. The high sequence identities between lavender and white suggested that nucleotide sequences from the two plumage colour turkey variants had comparatively a short evolutionary distance, meaning, they have common ancestor and their enzymatic

variant indicated the presence of single nucleotide polymorphism (SNP) that lead to changes in the amino acid of the gene's proteins. Hence, masking their high expression of their nucleotide diversity (Pi). This is in line with the report of (Hepp *et al.*, 2016) who observed that *MC1R* gene could be involved in color polymorphism through epistasis interaction with other loci.

functions would be the same than with black/Lavender. This is in line with Rost (2002) who stated that when two sequences shared more than 50% sequence identity, their enzymatic function will be the same. Generally, it was observed that percentage identity between the three plumage colour variants of Nigerian local turkey were relatively high than 50% thus proof to emerge from one ancestor.

Table 2: Percentage identity of MC1R nucleotide sequences among the three plumage colour variants of Nigerian local turkey

	White	Black	Lavender
White	1		
Black	93.93	1	
Lavender	94.85	88.78	1

Comparison of percentage similarities among the three plumage colour variants of Nigerian local turkey

The result of the analysis indicated that in percentage similarities, white, lavender and black turkey plumage colour variants were highly similar with 99.95 in comparison, as presented in table 3. The percentage similarity between black, lavender and white was relatively high. This revealed that their sequence share a common evolutionary ancestor, hence, the three sequences differences. The percentage identity between lavender and white

variants nucleotide sequences was higher compared to white and lavender or black and lavender sequences. This implied that, highly related plumage colour variants of Nigerian local turkeys showed high level of identity. The high sequence identities between lavender and white suggested that nucleotide sequences from the two plumage colour turkey variants had comparatively a short evolutionary distance, meaning, they have common ancestor and their enzymatic functions would be the same than with black/Lavender. This is in line with Rost (2002) who stated that when two sequences shared more than 50% sequence identity, their enzymatic function will be the same. Generally, it was observed that percentage identity between the three plumage colour variants of Nigerian local turkey were relatively high than 50% thus proof to emerge from one ancestor.

Table 3: Percentage identity of MC1R nucleotide sequences among the three plumage colour variants of Nigerian local turkey

	White	Black	Lavender
White	1		
Black	93.93	1	
Lavender	94.85	88.78	1

Comparison of percentage similarities among the three plumage colour variants of Nigerian local turkey

The result of the analysis indicated that in percentage similarities, white, lavender and black turkey plumage colour variants were highly similar with 99.95 in comparison, as presented in Table 4. The percentage similarity between black, lavender and white was relatively high. This revealed that their sequence share a common evolutionary ancestor, hence, the three sequences did not arise independently, they arose from a common ancestor.

Table 4: Percentage similarities of MC1R nucleotide sequences among the three plumage colour variants of Nigerian local turkey

	White	Black	Lavender
White	1		
Black	99.5	1	
Lavender	99.5	99.95	1

Evolutionary divergences in the three plumage colour variants of Nigerian local turkey.

The phylogenic tree showed evolutionary relationship of the *MC1R* gene sequences among the three plumage colour variants of Nigerian local turkey as presented in the dendrogram (Figure 3). The tree composed of outer branches representing relationship among the three plumage colour variants of Nigerian local turkey. Plumage colour variant joined together in the tree likely come from one polymorphic ancestor. Plumage colour with a single node is a descendant that showed most recent common polymorphic ancestor from the original ancestor(s).

The phylogenetic findings demonstrated a clear distinction between *MC1R* genes of the three plumage colour variant of Nigerian local turkeys. Sequences from white and lavender plumage colour variants confirmed the existence of high genetic polymorphism derived from a common ancestral form. There was a high per cent similarity between white, lavender and black plumage colour variants of Nigeria local turkey recording (99.95%). There was also a high per cent identity between white and lavender with both recording (94.85%), black and white (93.93%), black and lavender (88.78%). This result agrees with the findings of Donger *et al.*, (2001) who reported that Polymorphism of gene was

due to the genetic variation and its variants were transmitted by simple Mendelian inheritance with no dominance. The dendrogram in this study showed that the black plumage colour variant was clearly separated from white and lavender plumage colour variants ancestor. This showed that, black plumage colour variant of Nigerian local turkey has a divergent ancestor than the ancestor shared with white and lavender plumage colour variants of Nigerian local turkey on the phylogenetic trees. In the phylogenetic trees, the internal nodes of White and lavender represented the common ancestors that existed before

divergence. The terminal nodes represent the species MC1R sequences (gene and protein) for which the phylogenies were constructed. Branches were the evolutionary connections between plumage colours. This is in line with Donger *et al.* (2001) who stated that the distance of one group from the other groups indicates the degree of relationship; that is closely related groups are located on branches close to one another. All plumage colour variants retained their positions and relationships in the phylogenetic tree of MC1R gene sequences as predicted.

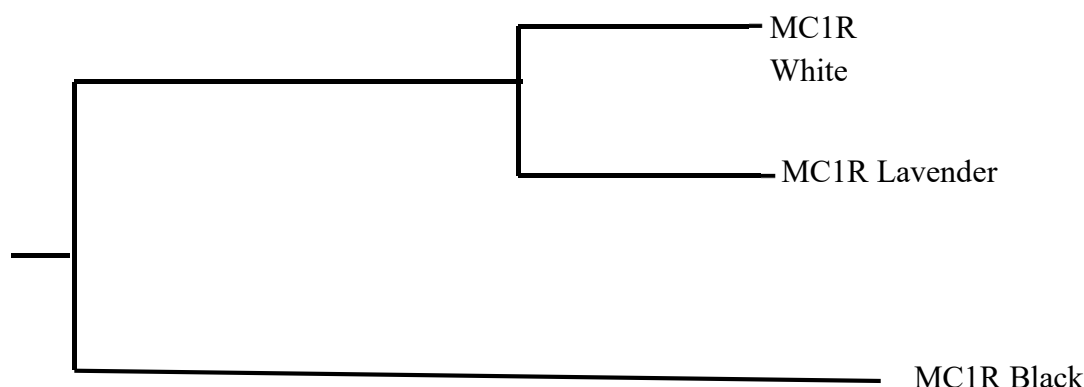


Figure 3: Phylogenetic tree relationship of three plumage colour variants

Protein homology modelling of the three plumage colour variants of Nigerian local turkey.

The result showed that on using Jalview, the Jpred secondary protein structure of the *MC1R* gene of the three plumage colour variants of Nigerian local turkey, were predicted as presented in figures 4, 5 and 6. The original multiple alignments returned using multiple alignment sequences are noted with prediction.

It showed that in the predicted structures, Lupas_21, Lupas_14, Lupa_28 is coiled-coil predictions for the sequences.

It indicated that there were binary predictions for each location.

The bJnet burial was a prediction of solvent accessibility (S.A) of which were in levels: 0 = exposed; 3 – 25% or more SA; 5 – 6% or more SA; 9 = buried (<5% exposed).

The JnetPred analyzed consensus prediction which showed the helices marked as red tubes, and sheets as dark green arrows as seen in the figures 4, 5 and 6.

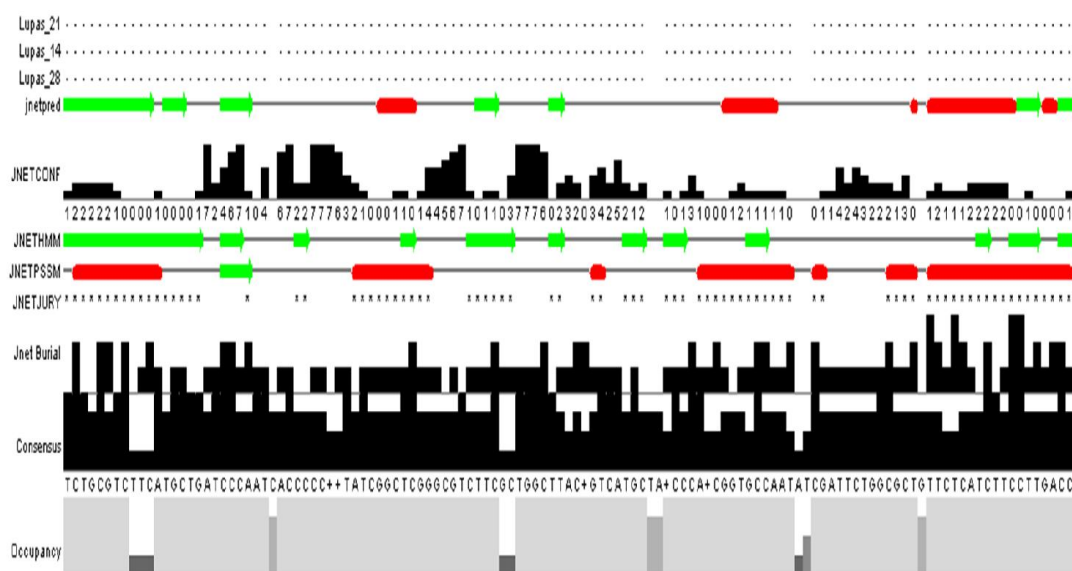
The JNETCONF analyzed confidence estimated for the prediction. High values obtained implied high confidence.

JNETALIGN analyzed the alignment based prediction. JNETHMM aligned Hidden

Markov Model (HMM) profile based prediction. JNETPSSM aligned position specific scoring matrices (PSSM) based prediction. JNETJURY had to do with a ‘*’ and annotation indicated the JNETJURY was invoked to rationalize significantly different primary predictions.

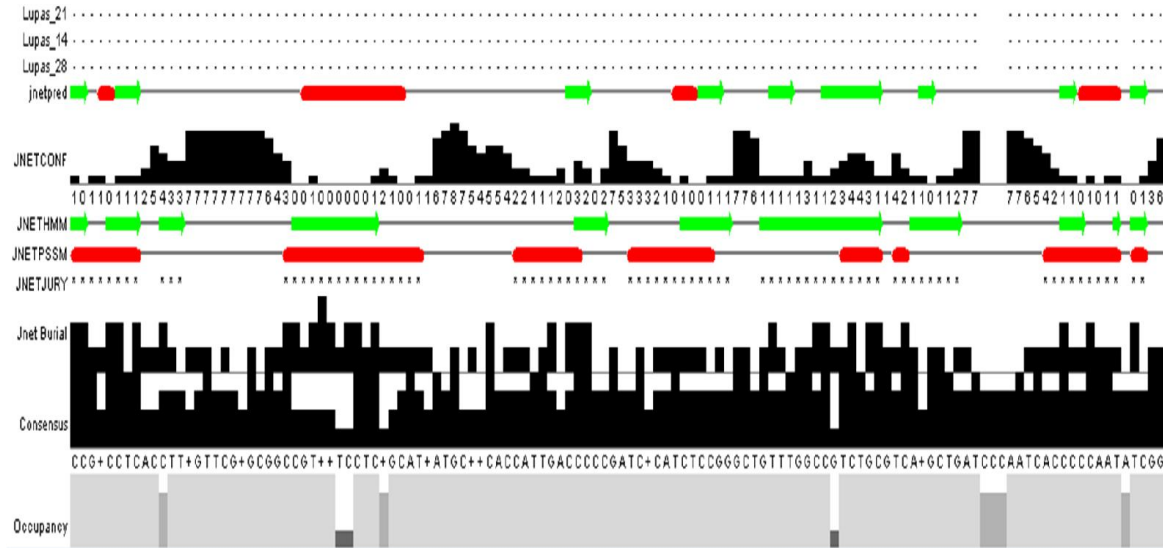
The protein structure of the MC1R gene in three plumage colour variants of Nigerian local turkey were joined by hydrogen bonds between carbonyl and amino groups that makes up the polypeptide backbone and causes the molecule to either bend and fold (beta pleated sheet) or spiral around (helices). In the plumage colour variant of Nigerian local turkey structures, the helix and beta sheets formed when polypeptide backbone folds periodically as a result of formation of stabilizing hydrogen bond. Alpha-helix was a corkscrew type structure with the main chain forming the backbone and the side chains of the amino acids projecting outward from the helix.

The three plumage colour variant structures were coded by the formation of two or more nucleotide bases of Adenine (A) and Thymine (T), Guanine (G), and Cytosine (C), being the basis of any DNA sequences and the building block of protein/nucleic acid. Adenine was one of the two purine nucleotide bases (the other being guanine) used in forming nucleotides of the nucleic acids. Adenine bonded to thymine via two hydrogen bonds stabilized the nucleic acid structures. Guanine was a purine (molecular formula $C_5H_5N_5O$) found in the form of a de-oxynucleotidyl residue. Guanine base paired via three hydrogen bonds with the pyrimidine cytosine. Guanine bonds to cytosine because they both share three hydrogen bonds. The abundance of beta sheets in protein structure made the gene of interest to have high pleiotropic effect in response to inflammation caused by foreign bodies like bacteria, virus, fungi, algae etc.



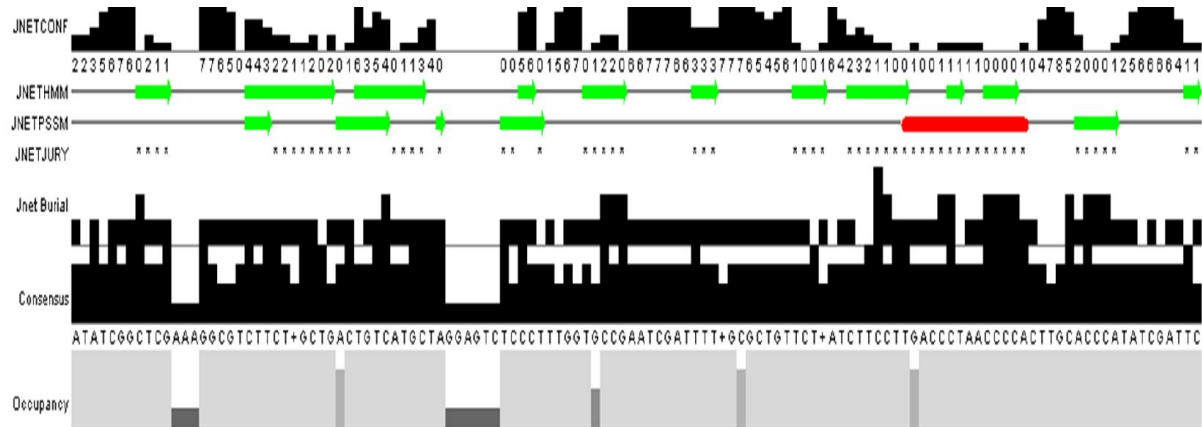
KEYS: RED = alpha helices; Dark green = beta sheets; Black = structure as a result of nucleotide combination

Figure 4: Secondary protein structure of lavender plumage colour variant of Nigerian local turkey



KEYS: RED = alpha helices; Dark green = beta sheets; Black = structure as a result of nucleotide combination

Figure 5: Secondary protein structure of white plumage colour variant of Nigerian local turkey



KEYS: RED = alpha helices; Dark green = beta sheets; Black = structure as a result of nucleotide combination

Figure 6: Secondary protein structure of black plumage colour variant of Nigerian local turkey

CONCLUSIONS AND RECOMMENDATION

- There was an existence of evolutionary relationship (88.78 – 99.95%) amongst the three

plumage colour variants in which white and lavender plumage colour variants indicated the existence of high genetic polymorphism derived from a

common ancestral form while the black plumage colour variant has a divergent ancestor.

- Evolutionary relationship showed that White and Lavender turkey variants shared a common ancestor while Black turkey variant had a divergent ancestor from the other two variants studied.
- White plumage colour variants showed a high value in non-synonymous site which possibly indicated the presence of mutation (SNPs) that changed structure and function of the amino acid.
- It is therefore recommended in this study that since MC1R gene has variations, it could be used in developing breeding program for the breeders who are interested in maintaining or developing plumage colour varieties in their farms.

REFERENCES

- Bender, D. A., and A. E. Bender. 2005. *A Dictionary of Food and Nutrition*. New York: Oxford University Press. ISBN 0198609612.
- Donger, C., Georges, J. L. Nicaud, V., Morrison C., Evans, A., Kee, F. 2001. New Polymorphisms in the Melanocortin 1 receptor gene-relationships to myocardial infarction. *European Journal of Clinical Investigations*, 31:9-14.
- Folstad, I. and Karter, A. J. 1992. Parasites, bright males, and the immune competence handicap. *The American Naturalist*, 139:603-622.
- Garcia-Bórron J. C. Sánchez-Laorden B. L., Jiménez-Cervantes C. 2005. Melanocortin-1 receptor structure and functional regulation. *Pigment Cell and Melanoma Research* 18(6):393-410.
- Ha, T. Naysmith L. Waterson K., Oh, C., Weller, R. Rees J. L. 2003. Defining the quantitative contribution of the melanocortin 1 receptor (MC1R) to variation in pigimentary phenotype *Annals of the New York Academic of Sciences*, 994(1): 339-347.
- Hall, T. A. (1999). Bioedit: a friendly biological sequence alignment editor and analysis program for windows 95/98/NT. *Nucleic acids symposium series*, 41:95-98.
- Hepp, D., Gonçalves, G.L., Moreira G. R. and De-freitas T.R. (2016). Epistatic interaction of the Melanocortin 1 Receptor and Agouti Signaling Protein genes modulates wool color in the Brazilian Creole Sheep. *J Hered* 107: 544-553.
- Huang, W., Wang, W., Zhou, M. and Zhang, X (2014). association of single nucleotide polymorphism. *Clinical and experimental ophthalmology* 42(6); 515-521.
- Kasprzak-Filipek K, Sawicka-Zugaji, W., Litwininczuk, Z., Chabuz, W. Sveistiene R., Bulla J. 2020. Polymorphism of the Melanocortin 1 Receptor (MC1R) Gene and its Role in Determining the Coat Colour of Central European Cattle Breeds. *Animals*, 10 (10):1878. <https://doi.org/10.3390/ani10101878>.
- Kumar, S., Nei, M., Dudley, J. and Tamura, K. 2008. MEGA: A biologist-centric software for evolutionary analysis of DNA and protein sequences. *Briefings in bioinformatics*, 9(4), 299-306. <https://doi.org/10.1093/bib/bbn017>.
- Librado, P and Rozas, J. (2009). DnaSP v5: A software for comprehensive analysis of DNA Polymorphism data. *Bioinformatics* 25:1451-1452.
- Maresca V, Flori E. and Picardo, M (July 2015). "Skin phototype: a new perspective". *Pigment Cell & Melanoma Research*. 28 (4):378-89. doi:10.1111/pcmr.12365. PMID 25786343. S2CID 12806815.

- Mason, K. A. and Mason, S. K. 2000. Evolution and development of pigment cells: at the crossroads of the discipline. *Pigment Cell Res*, 13 Suppl 8, 150-5.ress, London.
- Rost B. 2002. Enzyme function is less conserved than anticipated. *Journal of Molecular Biology* 318: 595–608.
- Slominski A., Tobin D. J. Shibahara, S. Wortsman J. 2004. Melanin pigmentation in mammalian skin and its hormonal regulation. *Physiological Reviews* 84 (4): 1155-1228.
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. and Kumar, S. 2011. MEGA 5: Molecular Evolutionary Genetic Analysis using maxium likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol Evol.* 28: pp 2731-9.
- University of Uyo Meterological Station, (2019).
- Vidal, O. J., Viñas, J. and Pla, C. (2010). “Variability of the Melanocortin 1 Receptor (MC1R) Gene Explains the Segregation of the Bronze Locus in Turkey (Meleagris Gallopavo).” *Poultry Science* 89 (8):1599–1602.
- Wang, N., Hebert D. N. (2006). Tyrosinase maturation through the mammalian secretory pathway: bringing color to life. *Pigment Cell & Melanoma Research* 19(1): 3-18.
- Wang, Wei; Guo, Dong-Ui; Lin, Yue-Jun; Tao, Ya-Xiong (2019). "Melanocortin Regulation of Inflammation". *Frontiers in Endocrinology*. 10:683. doi:10.3389/fendo.2019.00683. PMID 31649620. (<http://www.codoncode.com/aligner>).