International Journal of Chemical and Pharmaceutical Research Updates

Journal homepage: https://orionjournals.com/ijcpru/

(RESEARCH ARTICLE)

Check for updates

Genomics and Bioinformatics Analyses of *Interleukin-10* gene in three Plumage Colour Variants of Nigerian Local Turkey

Jessie Ezekiel Udoh *, Emmanuel Godwin David and Ekaette Ndifreke Mbaba

Department of Animal Science, Faculty of Agriculture, University of Uyo, Akwa Ibom State, Nigeria.

International Journal of Chemical and Pharmaceutical Research Updates, 2026, 06(02), 009-021

Publication history: Received on 15 April 2026; revised on 26 May 2026; accepted on 29 May 2026

Article DOI: https://doi.org/10.53430/ijcpru.2026.6.2.0037

Abstract

This study evaluated the genomics and bioinformatics analysis of *Interleukin-10(IL-10*) gene in three plumage colour variants of Nigerian local turkeys (White, Black and Lavender) in response to immune system and their evolutionary relationship. A total of 90 Poults (30 per variant) were reared intensively in the T&R Farm of the University of Uyo. Experimental design was CRD. Blood samples were collected for gDNA extraction, PCR-RFLP amplification, Amplicons quality checked through Electrophoresis before sent for sequencing at South Africa. Sequenced results obtained were analyzed using Molecular Software Packages and Bioinformatics Approach were used to determine genetic diversities. The coding regions of *Interleukin-10* genes in the three variants of Nigerian Turkeys were conserved in exon 4. The result showed that White variants turkey recorded 0.58 for Nucleotide diversity (Pi) while Lavender variant came the least with 0.28. Although the White variants had high expression of the gene action due to SNPs in the non-coding region of the gene which produces mRNA for the expression of *IL-10* gene in all tissues. The result showed that both the White and Black variants expressed high level of percent identity. The result showed the evolutionary relationship existing among the 3 turkey variants through phylogenic tree which revealed that the Lavender variant of IL-10 gene arose independently, hence, having more recent ancestor from the Black and White variants. White variant of Nigerian local turkeys was considered the best in this study due to high level of Pi expressed and SNPs in the non-coding region of the gene.

Keywords: Bioinformatics Analysis; Genomic; *Interleukin-10* gene; Plumage colour variants; Turkeys

1 Introduction

Changes in coat or plumage colour is one of the first hallmarks of domestication Wilkins *et al.[1]*, and human interest in coat colours make it more diverse [2]. The coat colour is an important trait in breed recognition for numerous domestic mammalian and avian species. According to Fontanesi *et al.[3]* who reported that a large number of coat colour phenotypes were described in different domestic animals. Traditional genetic selection techniques have resulted in significant improvements in many economically important traits in chickens, turkey and other animals based on pedigree information. Currently, new molecular approach are adopted in turkey and other animals for selection through genomics. One of these is a candidate gene approach which is a powerful method for detecting the QTL responsible for genetic variation of the traits of interest in livestock species [4]. Similarly, other economic traits, like immune response are under complex genetic control. The elucidation of genes and their networks involved in immune response determination, which is key in turkey genetics and breeding. This is because, resolution of infections depends on the host's ability to mount a protective immune response based on the recruitment of immune-competent effectors and the establishment of immune memory. When immune response is impaired, as exemplified by primary and secondary immune-deficiencies, infections are no longer controlled. However, exacerbated responses to infectious diseases induce tissue or systemic lesions, which may be deleterious to the animal. In other words, immune-regulatory mechanisms are necessary to shape the amplitude of immune response and to prevent infection-associated lesions [5].

* Corresponding author: Udoh, Jessie Ezekiel

Interleukin-10 (IL-10) gene is a critical immune-regulatory molecule and is a member of an expanding family consisting of cellular and viral cytokines. Its cellular version is produced by myeloid cells, B cells, and as reported by regulatory cells [6]. IL-10 is an anti-inflammatory cytokine which is important in balancing inflammatory response to pathogens, and is secreted by macrophages, dendritic cells, T cells, B cells, neutrophils, eosinophil and mast cells [7]. Much attention has been focused on the study of *inter-leukin10* gene due to its important role in anti-viral defense. Some reports have focused on variation of the interleukin 10 gene within species of animals but no comparative study of interleukin 10 gene within Nigerian local turkey. A genetic polymorphism study aimed at evaluating genetic variation between and within animals for improvement and conservation purposes should be considered.

Therefore, there is need to study *interleukin10* gene at genomic level among Nigerian local turkey so that novel sequences with previously characterized *interleukin10* gene within the specie can provide important insights into their common attributes and evolutionary origins. Also, probing for similarities between DNA and protein sequences within specie through bioinformatics analysis which would contribute to better understanding of relationship in the three plumage colour variants of Nigerian Local Turkeys. Hence, the research aimed to determine genetic variability of *Interleukin 10 gene* (IL-10) amongst the three plumage colour variants of Nigerian local turkey in response to immune system.

2 Materials and Methods

2.1 Experimental Site

The experiment was conducted at the Poultry Unit of the Teaching and Research Farm of the Department of Animal science, University of Uyo, Nigeria. Uyo is within the tropical rainforest zone in Nigeria. It has an average rainfall range per annum between 2200mm to 3500mm. Temperature of the area ranges from 28^0C to 32^0C year round. Uyo is located between latitude $4^0$31' and $6^0$30' north and longitude $7^0$30' and $8^0$20' East of the equator [8].

2.2 Management of experimental birds

2.2.1 Procurement of experimental birds

A total of 150 day old Poults of turkey (*Meleagris gallopavo*) from three plumage colour variants (50) per variant) were purchased from a reputable farm in Oyo State.

2.2.2 Pen preparation

The brooding pen were washed, disinfected, cleaned and fumigated for one week prior to the arrival of the day old Poults. The feeders and drinkers were washed and disinfected, rinsed and dried prior to usage. The Brooder was provided with beddings made of wood shavings which were changed on weekly basis until termination of the research. 10 Poults per brooder (0.3m^2 width and 0.5m^{2}length). Heat and light sources were; lantern and electrical source which were provided around the feeding areas. On the first day of brooding, the brooding environment was maintained at 35^{o}C in the first day for the Poults. The heat sources and the temperature was monitored with thermometer by regulating 1^{o}C every 3 days until it got to the heat requirement of 21^0C which was maintained till the termination of the research at 8 weeks of age.

2.2.3 Feeding

Formulated feed was fed to the poults. The birds were given starter ratio from 0-4 weeks that contained 2832.71 Kcal of Metabolizable energy and 28.44% Crude protein and grower mash (3000 Kcal Metabolizable Energy and 29% Crude protein) from 5 – 8 weeks. Small amounts of feed starter mash were evenly distributed over the floor of the brooders in the first day of brooding. On the third day, feed were presented in small feeders and drinkers for the Poults. The Poults were introduced to water by dipping their beaks in the water immediately they were placed on the floor. Both feed and water were provided *ad libitum*. Birds were vaccinated against Newcastle disease at day old (intraocular). Gumboro vaccine was administered on the 7th, 14th, 21st and 28th day and Lasota vaccine on the 21st day.

2.2.4 Blood collection for DNA extraction

Blood samples were collected from the 90 Poults wing veins at the 8th weeks using 3ml syringe and needle. Blood collected were dropped on Whatman FTA (Flinders Technology Associate) card. The stained FTA cards were air dried and preserved in air-tight identified paper envelope and sealed to prevent contamination. The blood stained FTA cards were taken to Biotechnology Laboratory at Federal University of Agriculture, Abeokuta in Ogun State for genomic DNA extraction and PCR amplification.

2.2.5 DNA extraction

Genomic DNA (gDNA) extraction was carried out with solution-based Jena Bioscience Animal and Fungi DNA Preparation Kit following manufacturer's instructions.

In a *Eppendorf* tube, 300µl of cell lysate solution and 1.5 µL Proteinase K was added to FTA card containing dried blood sample. The tube was incubated at 55^{o}C overnight. 100 µL of protein precipitation solution was added to the cell lysate; mixture was vortexes vigorously and centrifuged at 15,000g for 3minutes to precipitate the protein present. The supernatant was transferred to a sterile 2 µL *Eppendorf* containing 300 µL of Isopropanol and mixed gently by inverting for 1minute to precipitate the DNA. DNA was pelleted by centrifugation at 15,000pprm for 1min, washed with 500 µL washing buffer and allowed to completely air-dry after the washing buffer was discarded. 50 µL hydration solution and 1.5µl R-Nase was added to the air-dried DNA pellet. The samples were subjected to initial incubation at 37 °C for 1 hour followed by a final incubation at 65°C for 1 hour to completely hydrate the dried DNA pellet. The DNA was stored at -20^{o}C for later use. The extracted DNA were quantified for concentration and purity using Thermo Scientific Nanodrop Tm 2005 spectrophometer following the protocol described by [9].

2.2.6 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 also checked by Ultra-Violet illumination.

Primers information: A pair of Published primers for exon 4 of the IL-10 gene used in the amplification of the segment of the DNA through polymerase chain reaction (PCR) in the amplified region of exon 4 which is highly polymorphic table 1.

Table 1 Primer sequence for amplification of Interleukin-10 gene

Gene	Accession Number	Product size	Primer Sequence	References
IL-10	AJ621254	454	F-5^{I}TGGAGCCTTAAATCCCACTG3^{I} R-5^{I}TAATGCAAGCCTCATTGTGC3^{I}	Rothwel *et al.[10]*.

2.3 Gel Electrophoresis and DNA Purification

Electrophoresis with 1.5% agarose gel was carried out to check for the integrity and purity of the genomic DNA. After the electrophoresis ended, the gel was taken out from the electrophoretic machine and placed in gel documentation machine to view the bands.

2.4 Polymerase Chain Reaction (PCR) Amplification

The polymerase chain reaction (PCR) was carried out using IL-10 forward and revere primers to amplify 50bp region covering both 5^{1} UTR, exon 4 and 3^{1} UTR of turkey IL-10 9 (Table 2.3.3.1). The amplification reaction was carried out in a programmable Thermo cycler (Master cycler pro by *Eppendorf)*. A total of 25 µl reaction mixture was prepared comprising of 12.5µl master mix, 1 µl each of forward and that of reversed primer respectively, 2µl of DNA template and 8.5µl of Nuclease free water. The contents were thoroughly mixed by vortex. The PCR conditions included initial denaturation of 94°C for 5minutes, 35 cycles of final denaturation at 94°C for 15seconds, 60°C of annealing for 20 seconds and 72 °C of extension for 30 seconds and final extension for 72°C for 7minutes. The amplicons were purified with magnetic beads carboxylate (MCLab, USA) and sent out to Iqaba Biotechnology Centre in South Africa for sequencing.

2.5 Sequencing, Purification and data analysis

The sequencing of PCR products was done with BigDye Terminator version 3.1 using ABI 3730 XL DNA Analyzer (BioNeer, Daejeon, South Korea) following the supplier's protocol at STAB Vida Genetics Laboratory, Campus FCT UNL Edificio Departamental de Quimica, Laboratorio 009, 2829-516 Caparica, Portugal. All the raw sequences were curated and assembled using CodonCode Aligner (http://www.codoncode.com/aligner) and submitted to GenBank with accession number MT347739.

2.6 Bioinformatics

2.6.1 Analysis of IL-10 gene

All nucleotide sequences obtained were trimmed and edited using Bioedit [11]. The nucleotide sequences were blast using BLAST-N to ascertain the sequences returned from the sequencer. The blast sequence were aligned using CLUSTAL-W. Codon Code Aligner was used for the detection of the Single Nucleotide Polymorphisms in the three plumage colour variant of Nigerian Local Turkeys.

2.6.2 Single Nucleotide Polymorphism (SNP) Identification and Genotyping

All novel sequences of three plumage colour variants of Nigerian local Turkey were assembled in BioEdit V7.2.5 [11] and aligned with the Clustal W multiple aligning tools for identification of SNPs and further analysis. The SNPs were also confirmed using DnaSp v5.10.01 [12](Librado and Rozas, 2009). The resultant amino acid variation of each SNP was also predicted using CodonCode Aligner (http://www.codoncode. com/aligner). Genetic diversity indices such as number of haplotypes, haplotype diversity, total number of mutation, nucleotide diversity, synonymous nucleotide diversity, non-synonymous nucleotide diversity, number of polymorphic site, and nucleotide substitution site were estimated for the coding region of turkey *IL-10* gene using DnaSp v5 [12]. The three plumage colour variant of Nigerian local turkey *IL-10 genes* obtained were analyzed and compared to the sample in the Gene Bank using the NCBI Nucleotide Blast-n approach.

2.6.3 Nucleotide Identity and Protein Similarity of Interleukin-10 gene

The percent identity and similarity among all the nucleotide sequences for both homologous and divergent plumage colour variants were determined through pair-wise comparison of the sequences of BLAST N option to create matrix table.

2.7 Phylogenic Tree

MEGA-X V.6 software [13] was used to determine the phylogenetic relationship among the plumage colour variants. The phylogenetic tree was inferred using neighbor joining method. The reliability of the inferred tree was evaluated using bootstrap analysis of 1000 replications.

2.7.1 IL-10 Gene Protein Structure

Finally, the Jpred Secondary Protein Structure online tool was used to predict the secondary structure of the 3 plumage colour variants of Nigerian local Turkey IL-10 protein.

*2.7.2 **Molecular Data Analysis***

The expression data for turkey Interleukin-10 genes obtained was subjected to analysis of variance using molecular software package.

Model 1

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

Where; Y_{ij} = $_i^{th}$ Plumage colour variants of $_j^{th}$ turkey population

μ = population mean

T_i = turkey plumage colour variants ($_i$ = 3)

$\mathcal{E}_{ij}$ = error term

3 Result and Discussion

3.1 Genetic Diversity among the three Plumage Colour Variants of Nigerian Local Turkey

The result of the analysis (Table 2) showed the descriptive statistics of genetic diversity indices of IL-10 gene in three plumage colour variants of Nigerian Local Turkeys (White, Black and Lavender) of 15 per plumage colour variant.

The results of the analysis showed that *IL-10* gene selective regions ranged between 1-448 and 1-718. The selected regions were 1-718, 1-569 and 1-448 for White, Lavender and Black plumage colour variant, respectively. The nucleotide site for White Turkey variant was located at position 718bp, followed by sequence of lavender plumage colour variant at 569bp and 448bp for black turkey variant. It was observed that, there was no parsimony informative site in all the turkey variants measured. The result indicated that, there were 15 haplotype diversities in the 3 plumage colour variant of the Nigerian Local Turkeys. The results of the analysis showed that, White Turkey variants had the highest average number of nucleotide diversity of 0.58, followed by black variants with 0.40 while Lavender variant came least with 0.28 (Table 2) and also (figure 1) graphically presented.

The analysis indicated that, the White turkey variant had the highest number of non-synonymous nucleotide diversity of 0.59, followed by Black turkey variant with 0.39 and 0.28 for Lavender turkey variant as the least.

It was observed that Lavender turkey plumage colour variant sequence had the highest Synonymous Nucleotide Diversity of 0.58, followed by Black Turkey variant sequence with 0.41, and 0.28 for White Turkey Variants least as the .

The result showed that, White Turkey plumage colour variant sequence had highest total number of Mutation of 284, followed by Black Turkey Variant sequence with 255 value, and 180 for Lavender Turkey variant sequence as the least value.

The polymorphic site for the Nigerian Local turkeys were located on the chromosome at position 227bp, 224bp, and 166bp for the White Turkey variant, followed by the Black colour variant and Lavender colour variant, respectively.

The candidate gene approach is a very powerful tool for diversity studies. The results of Nucleotide Diversity within white and black plumage colour variants of Nigerian local turkey for all selected sequences were higher than the lavender colour variant. White Nigerian local turkey's variant had the highest Nucleotide Diversity, which makes it adapt to the harsh environmental condition of Nigeria. The divergence in the immune response of the Plumage could be inferred from high genetic diversity of the IL-10 gene. The result of this study agrees with the report of Pablo [14] who reported that genetic diversity is measured by nucleotide diversity (Pi), and by the number of segregate sites. The highest value for average number of nucleotide diversity was found in white variant in this study, which makes it fit for selection as observed by Cañón, *et al. [15]* who reported that breeds whose genetic diversity is high, are more useful in meeting the changes alteration of human desire.

The graph of Nucleotide diversity showed the frequency of the White turkey variant to vary and adapt to changing environment than the other two turkey colour variants.

The highest value of Non-Synonymous nucleotide diversity in White plumage colour variant of Nigerian local Turkey showed that the genetic substitution of the IL-10 gene caused changes in the amino acid of the protein which resulted in high genetic diversity in the White Plumage colour variant of Nigerian local Turkey. This could be attributed to direct selection in the White plumage colour variant over time. The result of this study is in consistence with the report of Salwa [16] who reported that SNPs in the noncoding region of human and animal genes cause high expression of the gene, indicates that, the individual genome consisted of regulatory elements that controlled high expression of the gene.

The highest value of synonymous nucleotide diversity in Lavender Plumage colour variant of Nigerian local turkey reveals that the substitution (mutation) taking place in their IL-10 gene causes no change in the amino acid of the protein, hence, leading to low expression of the gene action in the Lavender plumage colour variant of Nigerian local Turkey. This study agrees with the explanation of Salwa [16], who observed that synonymous substitution or mutation do not have any effect on the organism and are said to be selectively silent as the substitution cause no change in the amino acid of the protein produced.

Higher mutation was detected in white colour variant of Nigerian Local Turkey followed by black variant when compared within sequences. The higher values obtained in white and black colour variant was due to the presence of high mutation in amino acids of their IL-10 genes. Hence, some favorable prognosis was associated with elevated IL-10 gene expression [16]. White and Black plumage colour variants of Nigerian local Turkey had high pleiotropic activities of IL-10 gene. This result is in agreement with the reports of Mark [17], who reported that phenotypic traits were influenced by a large number of genes (loci), each of which had a high number of alleles.

The result of the 15 aligned sequence revealed that, white and black plumage colour variants of Nigerian local turkey showed higher polymorphism than Lavender variant counterpart. This could be as a result of the presence of Single

Nucleotide Polymorphisms (SNPs) in the variants. The polymorphism of each gene was in the intron region of the gene, suggesting that the associations were due to pleiotropic effect of the SNP to other functional polymorphisms in the same gene or nearby genes. The result of this study is in line with the observation of Fadhil and Zulkadir [18] who reported that SNP was maintained in Hardy-Weinberg equilibrium (HWE) and was not associated with coat coloration.

The highest Nucleotide Substitution Site of 415 was observed in White Turkey plumage colour variant, followed by the sequence of the Lavender turkey plumage colour variant with 26 while 16 was recorded for Black Turkey plumage colour variant sequence as the least value.

The larger the average number of nucleotide substitutions per site, the smaller the genetic distance between plumage colour variants of Nigerian local turkey. The smallest average number of nucleotide substitutions observed per site showed the farthest relationship between black and white colours variant. The largest average number of nucleotide substitutions per site displayed differentiation between white and black plumage colour variants of Nigerian local Turkey of both belong to the same family. The lower average number of nucleotide substitutions per site between lavender and white implied that there was higher comparability existing between the two variants. The closeness in relationship between black and white plumage colour variants of Nigerian local turkey was in accordance with the observation of Fadhil and Zulkadir [18] who reported the relatively low degree of genetic variation for animal breeds and colour variants which can be attributed to emergence process of genetically affected factors that interacted and might be restricted to geographical distribution.

The high value in the non-synonymous protein coding site of White plumage colour variant of Nigerian local turkey showed the positive changed in the structure and the function of the protein coded by IL-10 gene and this is in line with the report of Donger *et al.[19]* who stated that polymorphisms in the non-coding regions (site) of IL-10 gene was associated with high expression of the gene function. The high non-synonymous protein coding site of white plumage colour variants revealed its high IL-10 gene potentials controlling point for self-regulatory functions. This resulted in both transcription and translation processes of anti-inflammatory response for immune regulation which is good and quick medium in which targeted cells process it mRNA transcripts and form a newly synthesized IL-10 protein. The result obtained in this study is in line with the report of Trifunović *et al*. [20] who explained that breed showing good production in the cytokine secretion is due to certain allelic variants of mediator gene. Stop codons observed in all the 3 plumage colour variants of Nigerian local turkey coding regions means that they could code for rare amino acid.

The higher result of lavender plumage colour turkey variant in synonymous protein coding site over Black and White colour variants revealed its slowness in speed during translation and post-transcription of IL-10 gene for expression of the gene actions anti-inflammatory response as a result of pathogens invasion, indicating its IL-10 gene level as low, hence, the turkey plumage colour variant has difficulties in withstanding the harsh environmental condition of Nigeria.

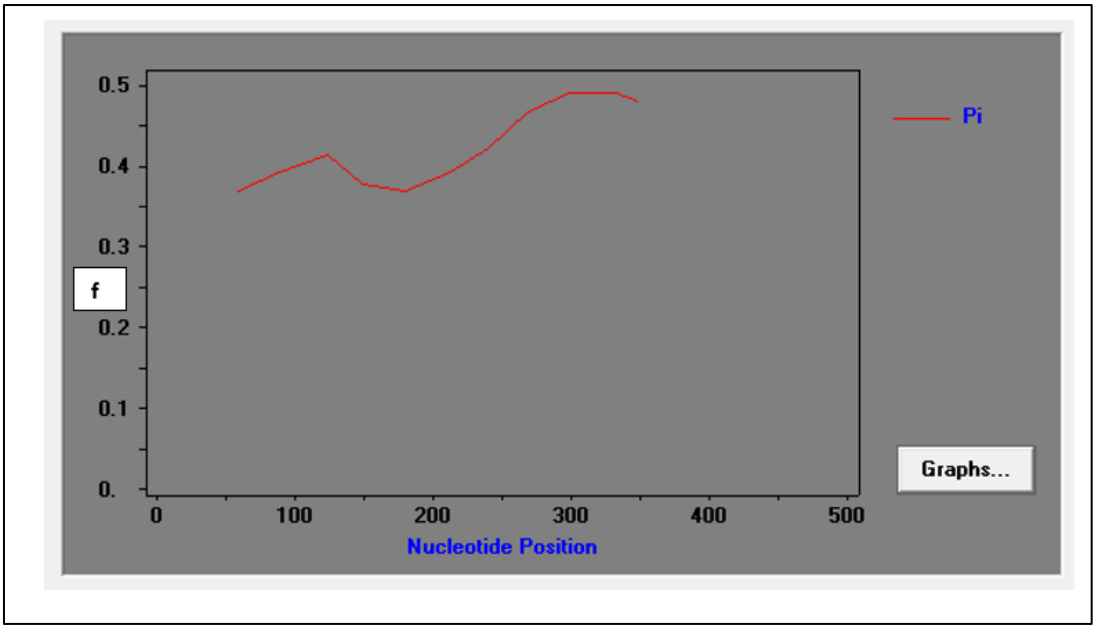
3.2 Graphical presentation of genetic diversity among the three plumage colour variants of Nigerian local turkey

A graphical representation of the Nucleotide Diversity of the 3 plumage colour variants of Nigerian local turkey as white Plumage colour variant of Nigerian local turkeys had a high expression of Nucleotide Diversity (pi) of 0.58 at 0.5 frequency with 360 wavelength, while black colour turkey colour variant had 0.40 Pi at 0.45 frequency with 260 wavelength, and the least value was observed in Lavender turkey colour variants which had 0.25 pi at 0.35 frequency with 180 wavelength, Figure 1

Table 2 Genetic Diversity among the three plumage colour variants of Nigerian local Turkeys

Data file	hap	Hd	Region	Site	Pi	NSND	SND	Eta	NPs	NS
Black	15	15	1-448	448	0.40	0.39	0.41	225	224	16
White	15	15	1-718	718	0.58	0.59	0.28	284	227	415
Lavender	15	15	1-569	569	0.28	0.28	0.58	180	166	16

Eta = total number of mutation, hap = number of haplotype, hd = haplotype (gene) Diversity, Pi = Nucleotide Diversity, SND = synonymous Nucleotide Diversity, NSND = Non-synonymous Nucleotide Diversity, nPS = Number of Polymorphic Sites, NS = Nucleotide Substitution site



Pi= Nucleotide diversity, f= frequency

Figure 1 Graph of genetic diversity of the three plumage colour variants of Nigerian local turkey

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

The result of protein coding region in each plumage colour variants showed that Lavender turkey plumage colour variant sequence had the highest synonymous sites of 72.17bp, while the Black turkey plumage Colour variants sequence recoded 70.33bp and White turkey plumage colour variants sequence recoded the lowest as 69.67bp.

The result showed that White turkey plumage colour variant sequence had the highest Non-synonymous site of 224.33, followed by the Black turkey plumage colour variants sequence with the value of 223.67 then Lavender with 221.83 though they were not significantly different.

It was observed that there was an existence of stop codon in the coding region from the result obtained in the analysis (Table 3).

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

The result of percent identity among the plumage colour variants of Nigerian local turkey indicated that White and Black plumage colour variant of Nigerian Local Turkeys had 77.60%, while black and lavender recorded 75.60% and between white and lavender was 75.60% which showed no significant differences (Table 4)

The percent identity between black and white nucleotide sequences was high 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 black and white suggests that nucleotide sequences from the two plumage colour turkey variants had comparatively a short evolutionary distance, meaning, common ancestor and their enzymatic functions would be the same than with White/Lavender or Black/Lavender. This is in line with Rost [21] who stated that when two sequences shared more than 50% sequence identity, their enzymatic function will be the same.

Black and lavender as well as white and lavender colour variants have the same percent identity lower than that observed in White and Black plumage colour variants. This implied that, their IL-10 sequences have long evolutionary distance, hence, having different ancestors. However, this is in line with Rost [21] who observed that, 70% of pair fragments above 50% sequence identity might not have a completely identical function.

Generally, it was observed that percentage identity between the three plumage colour variants of Nigerian local turkey were relatively high, hence, one plumage colour variant has a more recent mutant ancestor.

Table 3 Protein coding region of Synonymous and Non-synonymous site of the three plumage colour variants of Nigerian local turkey

Turkey Variant	SS (bp)	NSS (bp)
Black	70.33	223.67
White	69.67	224.33
Lavender	72.17	221.83

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

Table 4 Percentage identity between nucleotide sequences of IL-10 gene among the plumage colour variants of the Nigerian local turkey

	White	Black	Lavender
White	1		
Black	77.60	1	
Lavender	75.60	75.60	1

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

The result of the analysis shows that percent similarities among the three turkey variants observed was that white and black turkey plumage variants were highly similar with 72.92%, followed by 49.77% for Black and Lavender variants, then 47.50% for white and lavender variants relationship in similarity, Table 5.

The percent similarity between black and white was relatively high. This revealed that their sequence share a common evolutionary ancestor. Hence, the two sequences did not arise independently, they arose from a common ancestor. The low percent similarity between black and lavender as well as White and Lavender implied that their *IL-10* sequences arose independently. Hence, having different ancestors. Therefore, their *Interleukin-10* nucleotides might be poorly conserved and might not have a complete identical function in comparison. The result of this study is in line with George [22], who reported that amino acids were conserved through evolution at specific locations in a protein sequence because they were regained to stabilize the protein structure to a specific binding site or act as a catalyst.

Both the relative percentage identity and similarity between the turkey plumage colour variants were relatively high and this could confirm the IL-10 nucleotide and protein sequences that they were homologous proteins [23]. The percent similarity between black and white were relatively high. This means that, *IL-10* protein from them might be functionally different and divergence between them was more ancient.

Table 5 Matrix table showing percent similarities between protein sequences of IL-10 gene among the 3 plumage colour variants of Nigerian local turkey

	White	Black	Lavender
White	1		
Black	72.92	1	
Lavender	49. 77	47.50	1

3.6 Evolutionary divergences of IL-10 gene among the 3 plumage colour variants of Nigerian local turkey.

The Phylogenic tree shows evolutionary relationship of the IL-10 gene sequences among the 3 plumage colour variants of Nigerian local turkey (figure 2). The tree composed of outer branches representing relationship among the 3 plumage colour variants of Nigerian Local Turkey. Plumage colour variant joint together in the tree are likely from one polymorphic ancestor. Plumage colour variant with a single node is a descendant that showed most recent common polymorphic ancestor from the original ancestor(s). All plumage colour variants retained their positions and relationships in the phylogenic tree of IL-10 gene sequences as predicted.

The phylogenetic findings demonstrated a clear distinction between *IL-10* genes of the 3 three plumage colour variant of Nigerian local turkeys. Sequences from White and Black Plumage colour variants confirmed the existence of high genetic polymorphism derived from a common ancestral form. This result agrees with the findings of Donger *et al.[19]* 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 dendograms in this study showed that the Lavender plumage colour variant was clearly separated from White and Black colour variants ancestor. This showed that, Lavender plumage colour variant of Nigerian local Turkey has a different divergent ancestor from the ancestor shared with White and Black plumage colour variants of Nigerian local Turkey on the phylogeny trees. In the phylogenetic trees, the internal nodes of White and Black represented the common ancestors that existed before divergence. The terminal nodes represent the species *IL-10* sequences (gene and protein) for which the phylogenies were constructed. Branches were the evolutionary connections between plumage colour variants. This is in line with Donger *et al. [19]* who stated that the distance of one group from the other groups indicates the degree of relationship; *i.e.,* closely related groups are located on branches close to one another.

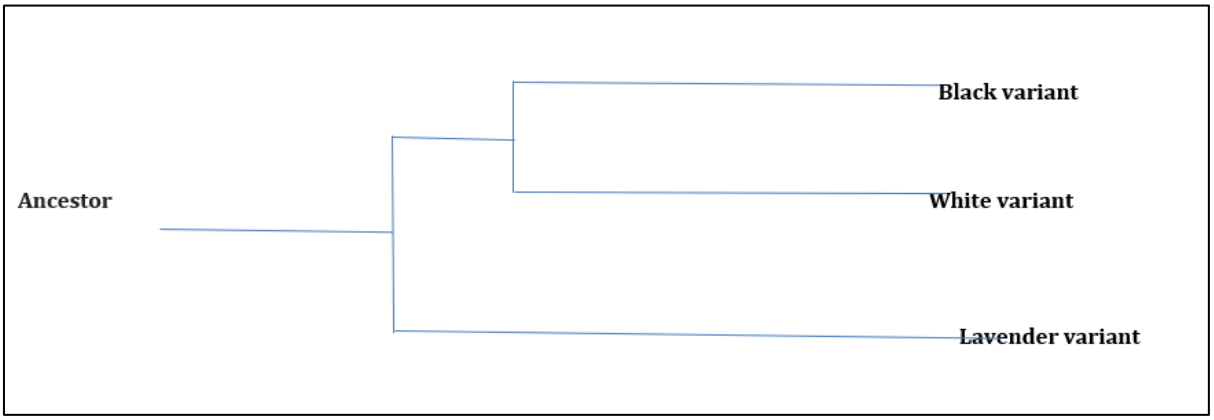


Figure 2 Phylogenic Tree of 3 plumage colour variants of Nigerian Local Turkey

3.7 Protein homology modelling of the 3 plumage colour variants of Nigerian local turkey.

The result shows that on using Jalview, the Jpred Secondary Protein Structure of the IL-10 gene of the three plumage colour variants of Nigerian local turkey were predicted as presented in Figures 3 and 5 The original multiple alignments returned using multiple alignment sequences annoted with prediction.

It showed that in the predicted structures, Lupas_21, Lupas_14, Lupa_28 there is coiled-ciol predictions for the sequences. It indicated that there were binary predictions for each location.

The bJnet Burial was a prediction of solvent accessibility of which were of levels:

0 – exposed,

3 – 25% or more S.A accessible,

5 – 6% or more S.A accessible,

9 – buried (<5% exposed).

The JnetPred which is for the consensus prediction had the helices marked as red tubes, and sheets as dark green arrows (figures 3, 4 and 5).

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

JNETALIGN aligned 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.

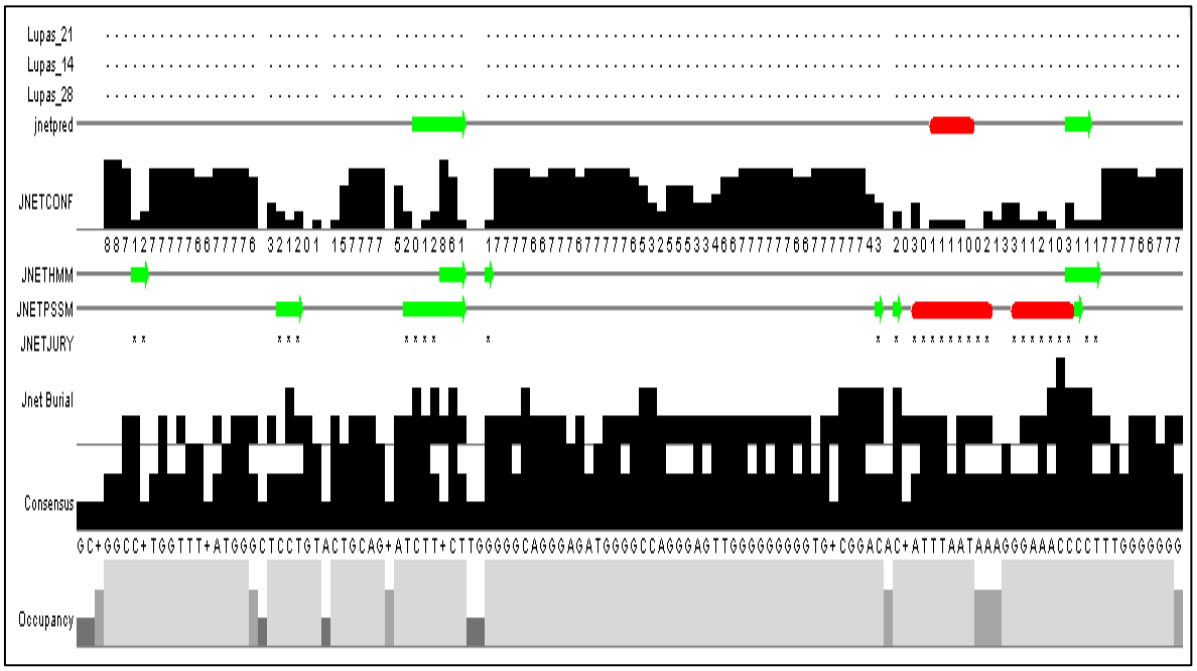
The result of the analysis shows that the structure of the White turkey variant of Nigerian Local turkeys indicated a single nucleotide polymorphism of the alpha helixes in the Lupas 14 line of its structure (figure 4). It also showed that White Turkey variant had more beta sheets than the Black and Lavender turkey variants in this study (Tables 3 to 5).

The protein structure of the 3 plumage colour variants of Nigerian local turkey were joined by hydrogen bonds between carbonyl and amino groups that make 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 turkey structures, the helix and beta sheets form 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. This agrees with the report of Sneha and George [24] (2016) who stated that secondary structure of protein is determined by the pattern and stabilization of hydrogen bonding.

The 3 plumage colour variants 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 or 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 deoxynucleotidyl residue. Guanine base paired via three hydrogen bonds with the pyrimidine cytosine. Guanine bonds to cytosine because they both share three hydrogen bonds.

The Single Nucleotide Polymorphism (SNP) in the white plumage colour variants mask the high expression of the gene over the other two plumage colour variants of Nigerian Local Turkeys. Hence, there is an interaction with other amino acid of the gene in the environment and there was higher level of conservation of the chemical properties of the amino acid of interleukin-10 gene in the white plumage colour variant. This agrees with Donger *et al*. [19] who stated that Single Nucleotide Polymorphisms may change the structure or the function of the protein coded by a gene, especially the polymorphisms located in the promoter region

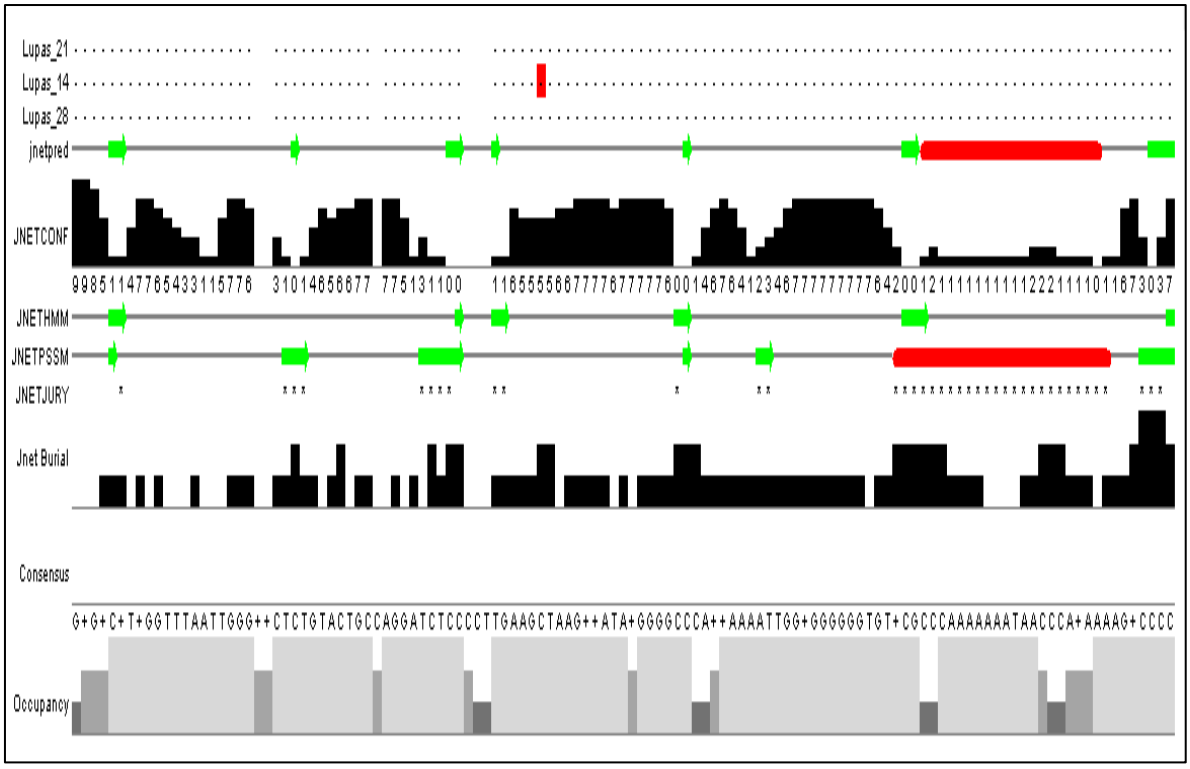
The abundance of beta sheets in White plumage colour variants makes their IL-10 gene have high pleitrophic effect in response to inflammation cause by foreign bodies like bacteria, virus, fungi and algae.



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

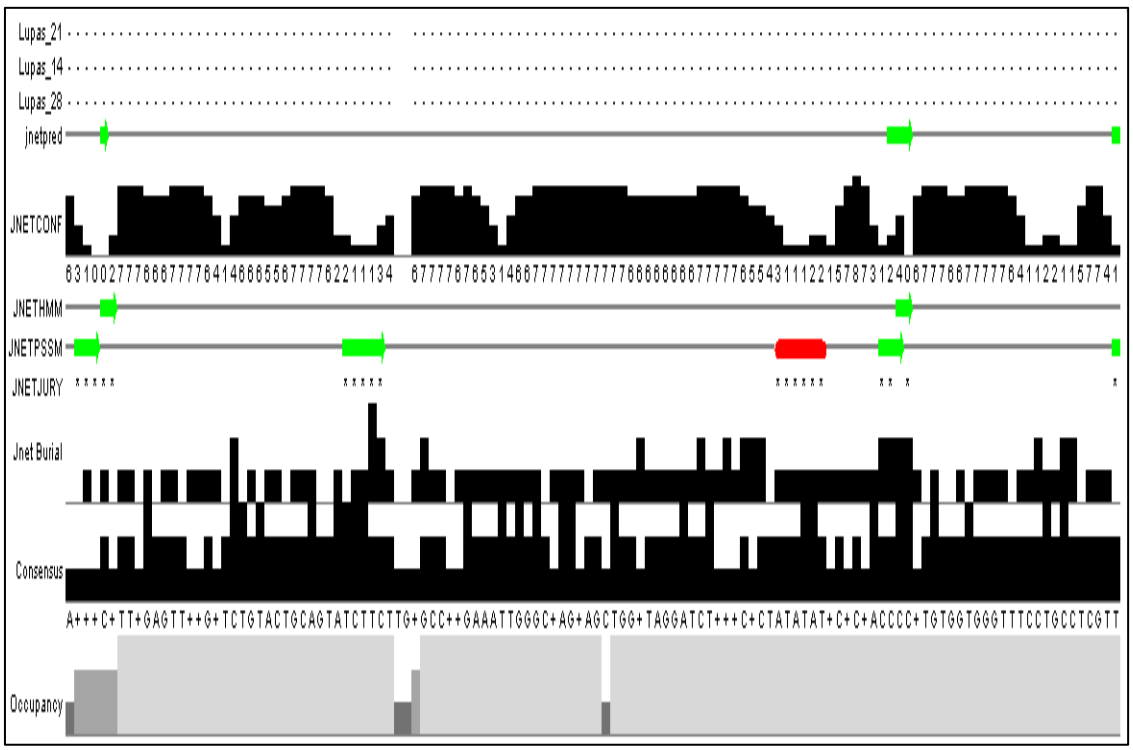
Source: Researcher's field work

Figure 3 Secondary protein structure of black plumage colour variant of Nigerian local turkey



KEYS: RED = alpha helices; Dark green = beta sheets; Black = structure as a result of Nucleotide combination; **Source:** Researcher's field work

Figure 4 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; **Source:** Researcher's field work

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

4 Conclusion

White plumage colour variant of Nigerian local turkey was considered as the best variant that could be adaptive to the harsh environmental condition of Nigeria, due to high genetic diversity of their IL-10 gene which is good for immune regulation and inflammation as a result of their high nucleotide diversity (Pi) and polymorphic site which indicate high mutation rate existing in this variant.

White and Black turkey variants of Nigerian local turkey are closely related as they share common ancestor and as such they are identical.

It was observed that all the 3 plumage colour variants of Nigerian local turkey exhibited approximately 80% of alpha helices and beta sheets in their secondary protein structure.

White plumage colour variant of Nigerian local turkey showed high expression of Single Nucleotide Polymorphism (SNP) in the non-coding region of the gene resulting in a change of amino acid nucleotide (mutation).

It was observed that all the 3 plumage colour variants of Nigerian local turkey exhibited a stop codon which showed the presence of regulatory element that can code for the production of rare amino acid. The White plumage colour variant of Nigerian local Turkey should be used to improve variants of Nigerian local turkey because of its high variability expression.

Compliance with ethical standards

Acknowledgments

Author is grateful to students who assisted during sampling and carcass measurement and the Institution that allowed this study to be carried out there.

Disclosure of conflict of interest

The authors declare that they have no competing interests.

Statement of ethical approval

This research was carried out according to the ethical guidelines of the Institutional Animal Care and Use Committee (IACUC) of the Department of Animal Science University of Uyo as per the ethics application approval numbers 2014.15 and 2014.16.

References

[1] Wilkins AS, Wrangham RW and Fitch WT. The "Domestication Syndrome" in Mammals: a Unified Explanation Based on Neural Crest Cell Behavior and Genetics. *Genetics*; 2014; 197:795-808.

[2] Cieslak M, Reissmann M and Hofreiter M, Ludwig A. Colours of Domestication. *Biological reviews of the Cambridge Philosophical Society 2011;* 86:885-99.

[3] Fontanesi L, Beretti F and Riggio V. Missense and nonsense mutations in melanocortin 1 receptor (*MC1R*) gene of different goat breeds: association with red and black coat color phenotypes but with unexpected evidences. *BMC Genetics* 2009; 47:102-111.

[4] Bai JY, Zhang Q and Jia XP. Comparison of different foreground and background selection methods in marker marker-assisted introgression. *Journal of Genetics and Genomics*, 2006; 33(12):1073-1080.

[5] Dalloul RA, Long JA, Zimin AV, Aslam L, Beal K and Blomberg LA. *Multi-platform next-generation sequencing of the domestic turkey (Meleagris gallopavo):* genome assembly and analysis 2010. PLoS Biology 8, e1000475.g008.

[6] Walter MR. Structural analysis of IL-10 and type I interferon family members and their complexes with receptor. *Advance Protein Chemokines,* 2004; 68: 171–223

[7] Moore, KW, R de Waal Malefyt, RL Coffman and AO Garra (2001). Interleukin-10 and the interleukin-10 receptor. *Annual Review of Immunology* 19:683–765.

[8] University of Uyo Meteorology Station. Atmospheric condition of University of Uyo Camposes, 2021; 23-30.

[9] Desjardins P, Conklin D. Nanodrop microvolume quantification of nucleic acid. *Journal of Visualized Experiment*, 2010; 22 (45): 2565-2570

[10] Rothwel L, Young JR, Zoorob R, Whiltaker CA, Hesketh P, Archer A, Smith A L and Kaiser P. Cloning and Characterization of Chicken *IL-10* and its role in the Immune Response to Eimeria Maxima. *Journal of immunology, 2004;* 173 (4), 2675 – 2682.

[11] Hall TA. BioEdit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT. *Nucleic acids symposium series, 1999* 41: 95–98

[12] Librado P and Rozas J. DnaSP V5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics, 200;*5(11): 1451-1452

[13] Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution, 2013;* 30: 2725–2729.

[14] Pablo GG. Neiker-Tecnalia Basque Institute for Agricultural Research and Development, 2017: 22-25

[15] Cañón J, Alexandrino P, Bessa I, Carleos C, Carretero Y, Dunner S, Ferran N, Garcia D, Jordana J, Laloë D, Pereira A, Sanchez A and Moazami-Goudarzi K. Genetic diversity measures of local European beef cattle breeds for conservation purposes. *Genetics Selection in Evolution*, 2001; 311–332.

[16] Salwa T. DNA Polymorphisms: DNA-Based Molecular Markers and Their Application in Medicine. Genetivc diversity and disease susceptibility, 2018; 26

[17] Mark O. Johnston. Mutations and New Variation: Overview. Dalhousie University, Halifax, Nova Scotia, Canada. *Animal Genetics , 2006; 90:* 20–27.

[18] Fadhil M and Zülkadir U. Molecular Characterization of MSTN Gene in Holstein Friesians and Brown Swiss Cattle Breeds. *Journal of Agricultural Food Science*, 2017; 31 (3), 151-153.

[19] Donger C. Georges JL, Nicaud V, Morrison C, Evans A and Kee F. New polymorphisms in the interleukin-10 gene–relationships to myocardial interaction. *European Journal of Clinical Investigation, 2001;* 31:9–14.

[20] Trifunović J, Miller L, Debeljak Ž, and Horvat V. Pathologic patterns of interleukin 10 expression--a review. *Biochemical Medicine, 2015* 25(1): 36-48.

[21] Rost B. Enzyme function is less conserved than anticipated. *Journal of Molecular Biology, 2002;* 318: 595–608.

[22] George R. (2002). The nascent polypeptide-associated complex (NAC) promotes interaction of ribosomes with mitochondrial surface in vivo. *Journal of biology and evolution, 2002;* 5: 213-216

[23] Shuto Y, Shibasaki T, Otagiri A, Kuriyama H, Ohata H, Tamura H, Kamegai J, Sugihara H, Oikawa S and Wakabayashi I. Hypothalamic Growth Hormone Secretagogue Receptor Regulates Growth Hormone Secretion, Feeding, and Adiposity. *Journal of Clinical Investigation, 2002;* 109:1429–1436

[24] Sneha PC and George Priya Doss (2016). Advances in protein chemistry and structural Biology, 201:12-14.