A MOLECULAR MARKER FOR FATTY ACID BINDING PROTEIN 4 (FABP4) GROWTH DETERMINANT FUNCTION IN NIGERIAN FULANI ECOTYPE CHICKEN

BY

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INTRODUCTION

- Nigerian local chickens;
- Widely kept across Nigeria, though low in growth rate relative to exotic chicken,
- Favoured with exceptional qualities
- The genetic improvement of Local breeds is desirable to preserve their exceptional qualities while improving their productivity
- Improvement through selection for economic traits using conventional breeding methods is slow and inefficient (slow genetic gain)
- Candidate gene approaches are faster and more efficient
- The approach directly targets genes regulating production traits to identify superior alleles for Marker Assisted Selection
- A growth related gene such as FABP4 gene is also regarded as a candidate gene for intramuscular fat (IMF) deposition, as it can enhance the deposition of triglyceride during adipocyte differentiation in muscle (Gerbens *et al.,* 1998; de Koning *et al.,* 1999; Saez *et al.,* 2009).

AIM AND OBJECTIVES

Aim:

- To identify and describe natural variation within conserved regions of the Adipose fatty acid binding protein gene as a first step towards examining the effect of such variation on adipose related variation in Nigerian Local Chicken (Fulani Ecotype)

Objectives:

- To examine the nucleotide sequence of the gene that encodes chicken FABP4 a growth associated gene, for polymorphism that may determine differential protein function between fast growing broiler chickens (Hubbard breed) and slow growing Nigerian Local Chicken (Fulani Ecotype) as a prelude to marker confirmation and use in improvement of the latter

MATERIALS AND METHODS

CANDIDATE GENE IDENTIFICATION (FABP4)

PRIMER DESIGN; forward and reverse primer sequences (of 18 – 23 base pair long at temperature of 58 ± 5^0c)

BLOOD COLLECTION AND DNA EXTRACTION

PCR reactions were performed in peltier effect thermal cycler, programmed for 2’ at 95°C, 35 cycles of 30” at 95°C, 1’ at 55°C, 58°C or 60°C, 30” at 72°C and a final step of by 3’ at 72°C.

Sequencing**;** Amplicons products were subjected to forward and reverse primer sequencing
Output files were viewed using Finch TV v1.4.0. (Geospiza, USA).

MSA was performed by use of MultAlin (Corpet, 1996).

Sequences were translated *in-silico* using a standard genetic code by means of ExPASy Translate tool

Phylogenetic analysis was carried out using Phylogeny.fr (Dereeper *et al.,* 2008).

RESULTS AND DISCUSSIONS

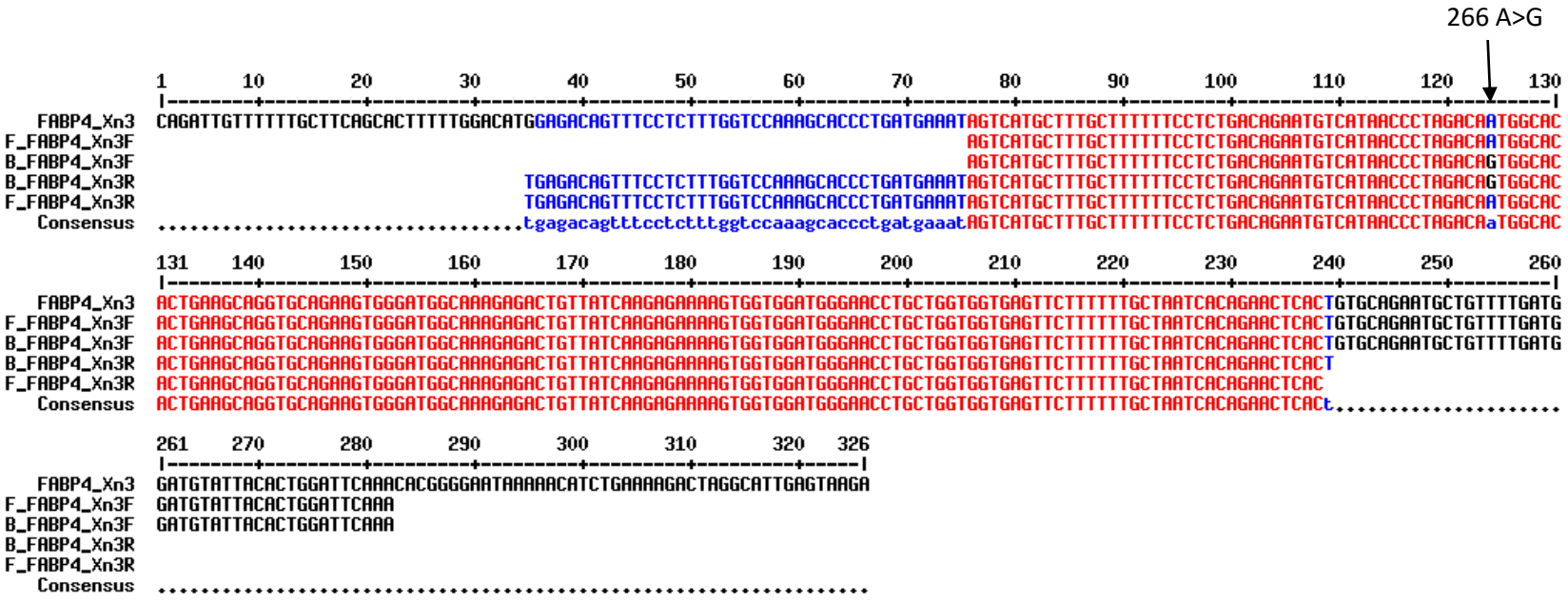

Figure 2: Multiple DNA sequence alignment of FABP4 exon 3 from : Red Jungle Fowl ENSGALT00000025427.3 nt 1741 – 2067, Fulani ecotype using Forward (F_FABP4_Xn3F) and Reverse (F_FABP4_Xn3R) primers in sequencing and; Hubbard broiler using Forward (B_FABP4_Xn3F) and Reverse (B_FABP4_Xn3R) primers in sequencing. Polymorphic base shown (arrow).

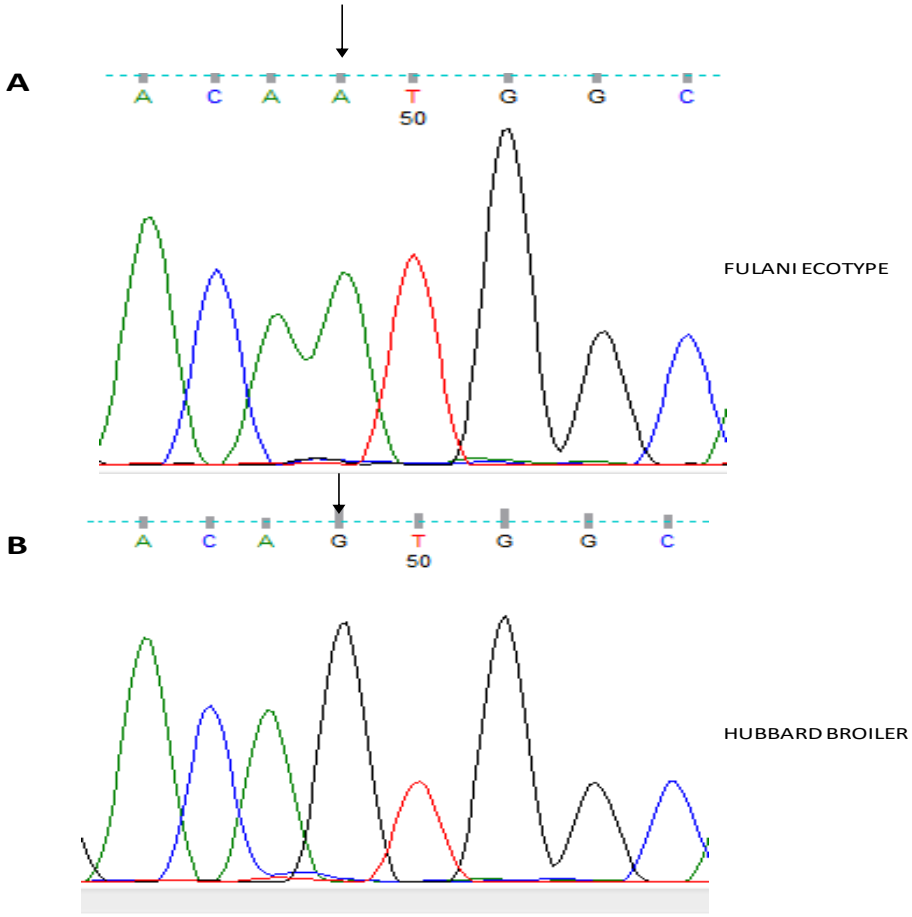

Figure 3: (A) : electropherograms of Fulani ecotype/ Red Jungle Fowl sequence and (B) Hubbard broiler sequence FABP4 exon 3 mutations, polymorphic base is arrowed

RESULTS AND DISCUSSIONS

- DNA Primary Structure – Nucleotide Sequence
- ➢ Multiple Sequence Alignment (MSA) of FE, HB FABP4 exon 3 sequence against RJF primary transcript
- ➢ Purine to purine change SNP
- ➢ FABP4 was more expressed in fast growing birds.
- ➢ Fat breakdown related enzyme thiolesterase B is expressed higher in slow growing birds, Zheng *et al.* (2009)
- ➢ This suggested that different metabolic regulatory network are indispensable for differential growth rate between fast growing and slow growing bird.

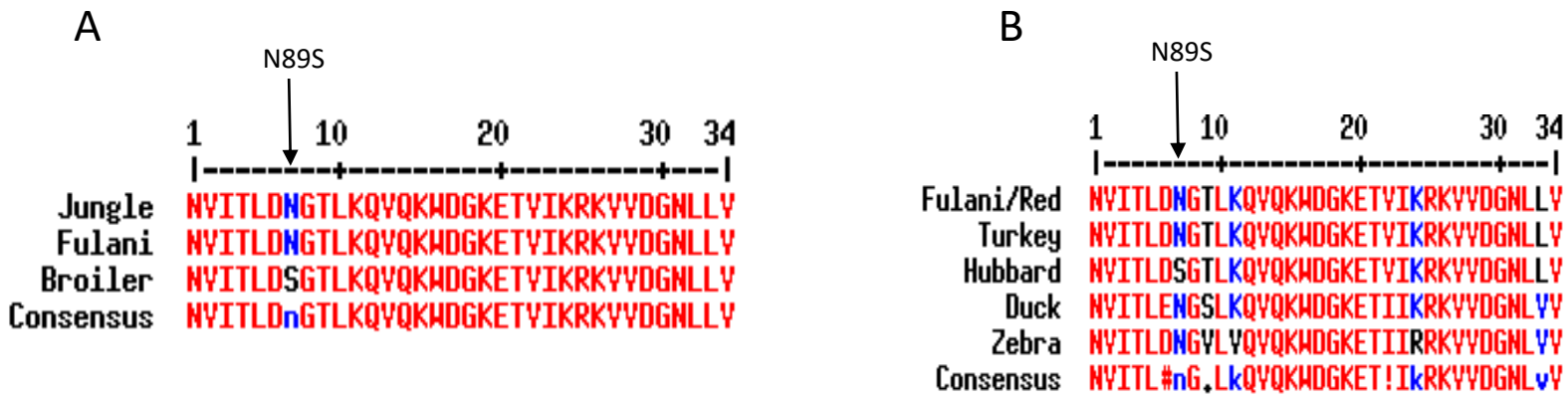

Figure 4: (A) Alignment of the FABP4 exon 3 peptide sequences of Red Jungle Fowl (Jungle ENSGALP00000025381 CODON 83 - 116), Fulani ecotype (Fulani) and Hubbard Broiler (Broiler). Mutated base shown (arrow), showing a polymorphism at codon 7 of exon 3 corresponding to codon 89 of the FABP4 CDS. (B) Alignment of the FABP4 exon 3 peptide sequences of Fulani ecotype/Red Jungle Fowl (Fulani/Red), Turkey, Hubbard Broiler (Hubbard), Duck and Zebra Finch (Zebra), showing polymorphic peptide at codon 7 (codon 89 of the FABP4 CDS) Mutated base shown (arrow).

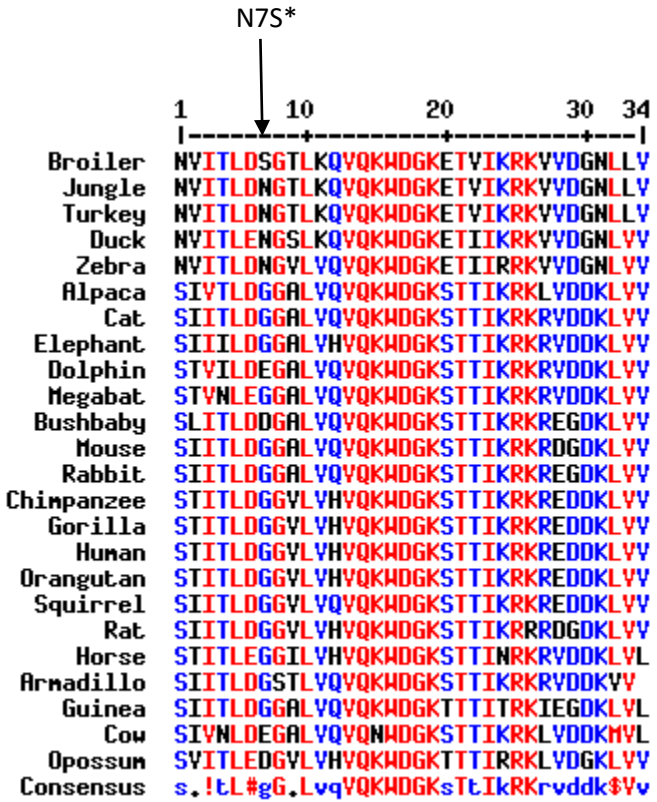

Figure 5; Multiple peptide sequence alignment (MultAlin Corpet, 1996) of FABP4, exon 3 for Aves, Eutherian and non-eutherian mammals. Alignment was produced by Multalin software. Location of Polymorphic codon/peptides shown as N7S, * numbering relative to reference gene transcript Chicken – (ENSGALP00000025381), Alpaca (ENSVPAP00000001054), Duck (ENSAPLP00000005715), Armadillo (ENSDNOP00000033224), Bushbaby (ENSOGAP00000011712), Cat (ENSFCAP00000017433), Chimpanzee (ENSPTRP00000034857), Cow (ENSBTAP00000000079), Dolphin (ENSTTRP00000006583), Elephant (ENSLAFP00000000507), Gorilla (ENSGGOP00000007182), Guinea Pig (ENSCPOP00000006483), Horse (ENSECAP00000021250), Human (ENSP00000256104), Megabat (ENSPVAP00000013471), Mouse (ENSMUSP00000029041), Opossum (ENSMODP00000008038), (ENSPPYP00000020978), Rabbit (ENSOCUP00000013299), Rat (ENSRNOP00000073333), Squirrel (ENSSTOP00000007662), Turkey (ENSMGAP00000012217) and Zebra Finch (ENSTGUP00000012023).

- **Predicted peptide sequence**
- Multiple Sequence Alignment of Peptide sequences
- Missense variation at codon 7 of exon
- Use of asparagine at codon 7 of exon 3 in the Jungle Fowl/Fulani chicken is conserved in avian evolution.
- FABP4 was less well conserved over a broad evolutionary history spanning over 300 million years captured in the set of exon 3 peptide sequences examined (Figure 5).

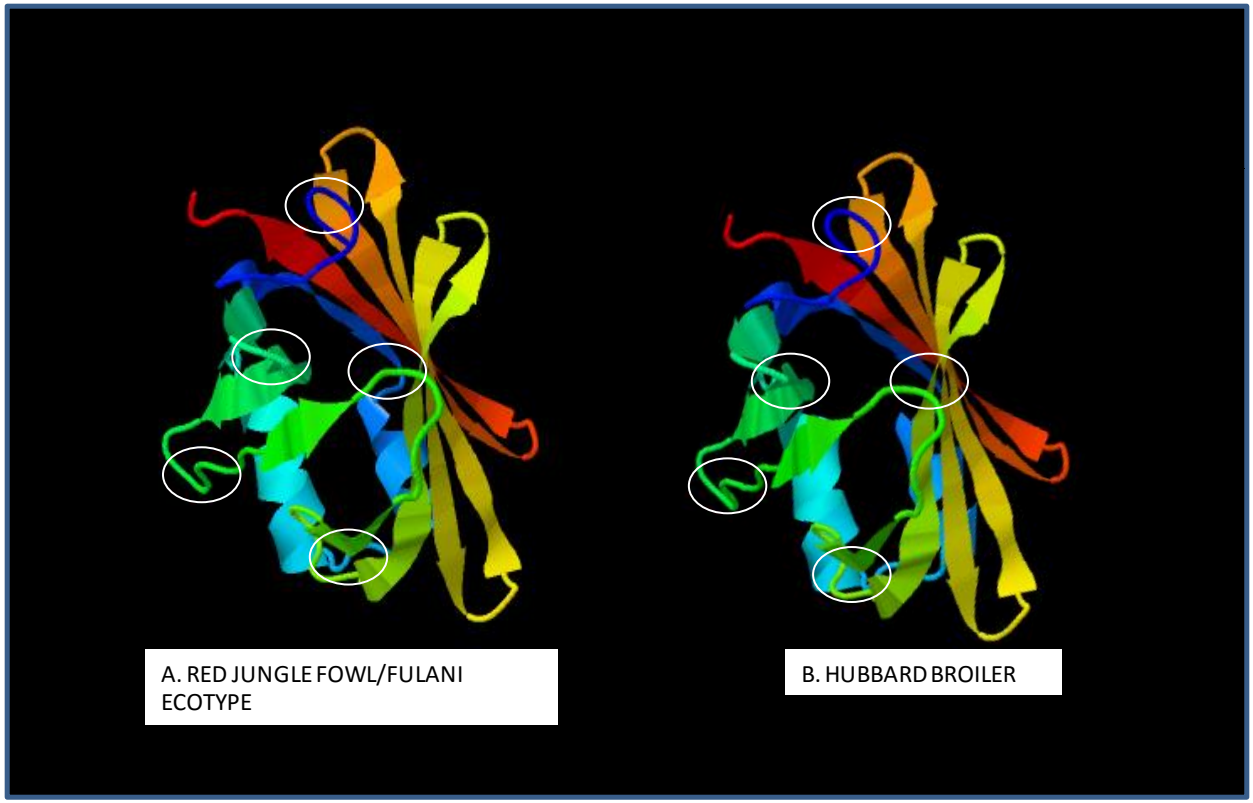

Figure 6: Predicted structure of FABP4 protein encoded by Red Jungle Fowl/Fulani genome (A) and Hubbard Broiler genome (B) 3D structure when produced by phyre 2. White rings in figures A and B highlight location of structural/conformational differences observed between Red Jungle Fowl/Fulani ecotype and Hubbard broiler FABP4 protein structure

- **Protein Prototype Assisted Modelling**
- Comparative Protein prototype of assisted modelling of the 3-dimensional structure of FABP4 revealed no profound effect of the N89S missense mutation on protein structure (Figure 6).

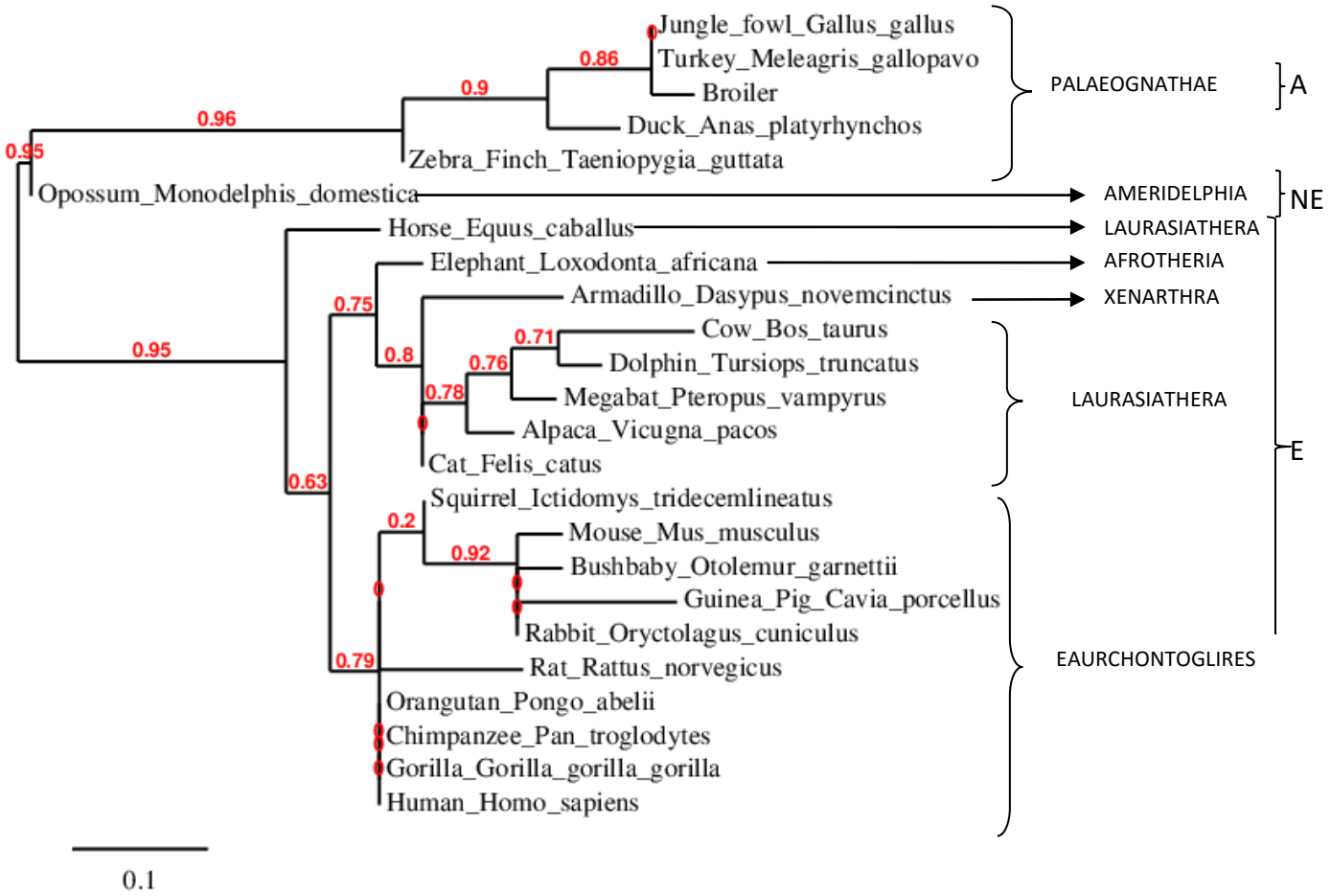

Figure 7; Phylogram of Aves (A), Eutherian (E) and non-eutherian (NE) mammals based on FABP4 exon 3 peptide sequence. Phylogram was produced by the use of Phylogeny.fr program.

- **Phylogenetic Analysis of FABP4 Exon 3**
- Extremes in convergent and divergent adaptation to a wide range of habitats (Tarver *et al.,* 2016).
- Horse split from Laurasiatheria prior to the emergence of super-orders Laurasiatheria, Xenarthra, Afrotheria and Eaurcongtoglires from a common ancestor.
- Horse has retained the ancestral form of exon 3 of FABP4

CONCLUSION

- It can be concluded from this experiment that;
- Natural variation exists in the coding protein of FABP4 gene distinguishing exotic Hubbard Broiler from indigenous local chicken (FE-NLC).
- Natural variation in FABP4 between exotic Hubbard Broiler and indigenous local chicken (FE-NLC) results in change in the peptide sequence (primary structure at the level of sequence) of the encoded protein.
- The FABP4 exon 3 gene can as serve as a marker for function of the gene and its region of linkage disequilibrum between Hubbard Broiler and Nigerian local chicken
- The FABP4 exon 3 mutation (266A > G, N89S) may determine variation in growth rate, fat deposition and meat quality between exotic Hubbard Broiler and Nigerian Local chicken.

RECOMMENDATION

- **RECOMMENDATIONS**

- This Single Nucleotide Polymorphism (SNP) c 266 A >G should be used as a marker for studying effect of FABP4 on growth and performance in chickens
- Association studies should be conducted in a cohort (Hubbard broiler, Fulani ecotype, unimproved Yoruba ecotype and improved Yoruba ecotype) in which alleles are segregating.
- This study should be repeated to ensure sequencing of all exons of both (FABP4) gene for Hubbard broiler, Fulani ecotype and Yoruba ecotype so as to examine the consequence of variation in as-yet-unsequenced portions of the gene.

THANKS
FOR
LISTENING.