

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

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ABSTRACT

A candidate gene for growth determination (Adipocyte Fatty Acid Binding Protein (A-FABP/FABP4) was examined for polymorphisms which can serve as markers of its differential functional status and role in growth performance between genetically slow growing indigenous chickens (Fulani Ecotype, and Yoruba Ecotype Nigeria Local Chicken, FE-NLC and Y-NLC) and fast growing exotic commercial chicken (Hubbard broiler) breeds. Primers for amplification of each exon of the predicted primary transcript of A-FABP of Red Jungle Fowl and the 50 base sequences immediately preceding and following each exon were designed, synthesised and used to amplify their target region in Hubbard and FE-NLC genomic DNA by use of the Polymerase Chain Reaction (PCR). PCR products were sequenced using ABI 3500XL Genetic Analyzer (Applied Biosystems). Exon sequences were subjected to multiple sequence alignment (MultAlin software). Phylogeny trees were constructed using Phylogeny.fr. Multiple sequence alignment (MSA) of Hubbard broiler and FE-NLC FABP4 exon 3 DNA sequences against FABP4 exon 3 of the Red Jungle Fowl primary transcript (ENSGALT00000025427.3 nt 1746 -1847) and flanking intron DNA revealed the existence of a purine to purine Single Nucleotide Polymorphism (SNP) in position 1765 (position 266 of the mature transcript predicted from NM_204290; position 20 of exon 3) of the primary transcript. The Red Jungle Fowl and Fulani Ecotype chicken carried an Adenine (A) base and Hubbard broiler carried Guanine (G) which led to an Asparagine to Serine change at an evolutionarily conserved region of codon 7 of exon 3 of the gene. Phylogenetic analysis of the predicted peptide sequence of exons using the order aves (within superorder Palaeognathae) as in-group and eutherian and non eutherian mammals as out-group revealed that exon 3 of Hubbard broiler was the most recently emerged in the examined group of bird. The location of the FABP4 polymorphism within the coding portion of the gene (exon) and specifically its location within an evolutionarily conserved codon, coupled with its non-synonymous nature suggest that the broiler variant may affect function of the encoded protein and the mutation might contribute to differential fat deposition and early growth rate in Hubbard Broilers relative to NLC chickens carrying the consensus/ancestral variant.

Keyword: Fatty Acid Binding Protein, molecular marker, phylogeny, Single nucleotide polymorphism, Fulani ecotype chicken.

INTRODUCTION

Nigerian local chicken (NLC) is widely kept across Nigeria constituting 80% of the National poultry flock (60% of 120 million chickens). Though low in growth rate relative to exotic chicken, NLC are favoured with exceptional qualities (including hardiness, biotic and abiotic stress tolerance, gustatory quality of meat, high brooding efficiency, high fertility and hatchability) and preserved for the same reason (RIM, 1992). Marker assisted selection offers a

highly attractive, efficient and effective tool for improving growth performance of the NLC while preserving its existing merits. To use Marker Assisted selection, polymorphisms within genes known or presumed to determine variation in the trait of interest (or to be closely linked) must first be identified. Subsequently, linkage or association studies in populations in which the trait of interest and known polymorphisms in the gene are segregating are used to test whether and to what extent the gene

of interest co-segregates with the trait of interest and by implication is causally linked or associated. Linked/associated markers are then used to select birds that carry the allele associated with superior trait performance in the straight-bred population under improvement. Equally, where the superior allele is absent from a breed under improvement interest, crosses may be made between a breed carrying the allele of interest and the breed under improvement, followed by iterative crossing and selection of birds carrying the superior allele in each generation to introgress the marker's linked superior trait performance with speed and efficiency. This will improve supply and contribute to animal protein sufficiency in Africa.

Several candidate gene determinants of growth may be amenable to selection but such an approach is currently not possible because polymorphisms that may contribute to candidate gene function in determining growth in the NLC have not yet been identified to fill the existing gap in knowledge.

The present study was designed to examine the nucleotide sequence of the gene that encodes chicken Fatty Acid Binding Protein 4 (FABP4) also known as Adipocyte Fatty Acid Binding Protein (AFABP), 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. Earlier work by others did implicate FABP4 in cell growth and differentiation, cellular signaling, gene transcription, and protection of enzymes from the toxic effects of free fatty acids (Glatz and Veerkamp, 1985; Zimmerman and Veerkamp, 2002), which are all component of growth. Fatty acid-binding proteins (FABPs) such as A-FABP/FABP4 exhibit a high affinity for long-chain fatty acids and appear to function in the metabolism and intracellular transportation of lipids in poultry birds. According to Fisher *et al.* (2001), FABP4 is expressed in many tissues, especially in adipose tissue, and is associated with lipid metabolic enzymes. The 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). Intramuscular fat which contribute to overall tissue weight has been described to have a positive influence on meat quality, with respect to tenderness and flavor of the meat (Wood *et al.*, 1999; Mourot and Hermier, 2001). Because polymorphisms that disrupt gene function are more likely to be found in the conserved portion of the genome, including regulatory and coding portions of genes (promoters, splice sites, and exons), the study targeted the complete collection of exons constituting the coding portion of the gene (i.e, the FABP4 exon) and the 50 base pair non-coding region immediately preceding (5') and following (3') each exon, in which splice donor and acceptor sites reside and in which other regulatory sequences may also reside.

The present study was predicated on three linked hypotheses; that differences in FABP4 gene coding and near-adjacent DNA sequences exist between slow (Fulani NLC) and fast growing (Hubbard broiler) chickens; that through *in silico* translation of DNA variant/ polymorphic forms distinguishing between the examined groups, consequences of polymorphism at the level of primary protein structure (peptide sequence can be ascertained), and; through comparison of the region of polymorphism in chickens against genetically diverse extant species, clues of the evolutionary constraints on variation of the polymorphic region (which point to functional importance of such a region) can be gleaned.

MATERIALS AND METHODS

Primer Design

The sequence of the primary transcript of FABP4 gene (ENSGALT00000025427.3) was retrieved from Ensembl (<http://www.ensembl.org>). forward and reverse primer sequences (of 18 – 23 base pair long at temperature of $58 \pm 5^{\circ}\text{C}$) for each exon of the predicted primary transcript of FABP4 of Red Jungle Fowl and the 50 base sequences immediately preceding and following each exon were designed, synthesised and used to amplify their target region in Hubbard Broiler and FE-

NLC genomic DNA by use of the Polymerase Chain Reaction (PCR).

Blood Collection and DNA Extraction

Blood samples of 1ml each were taken from birds via the brachial vein to aspirate blood from each bird using a sterilized disposable 2mls syringe and needle into an ethylene diamine-tetra acetate (EDTA) sample bottle to prevent coagulation of the blood samples. This was then carried to the laboratory for DNA extraction.

Extraction of DNA from the blood samples collected was carried out in line with the procedure described by Wang *et al.* (2006). The DNA was then subjected to Purity, Quantity and Quality tests using Nano-drop (NANODROP 2000) spectrophotometer.

Polymerase Chain Reaction (PCR)

A PCR cocktail was prepared 12.5µl New England Biolabs 2X Master mix (20mM of Tris-HCl, 1.8mM of MgCl₂, 22mM of NH₄Cl, 22mM of KCl, 0.2mM of dNTPs, 5% glycerol, 0.06% IGEPAL, 0.05% OF Tween, Xylene Cyanol FF, Tartrazine and 5 units of Taq DNA polymerase), 4µl of 25ng/ µl chicken DNA template, 2.5 µl each of forward and reverse primer (10 mM) and distilled deionized H₂O to a final volume of 25µl. PCR reactions were performed in an MG96G MyGene (LongGene Scientific Instrument Co.,Ltd.) 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. Amplicons were resolved by gel electrophoresis (1% agarose, 0.5 µg /ml ethidium bromide 10V/cm, 45"). Stained gels were view under a UV trans-illuminator.

Sequencing

Amplicons products were subjected to forward and reverse primer sequencing on an ABI 3500XL Genetic Analyzer (Applied Biosystem Inc.) through a commercial service (Inqaba Biotechnology Laboratories, South Africa). Output files were viewed using Finch TV v1.4.0. (Geospiza, PerkinElmer, USA).

Multiple Sequence Alignment

Multiple Sequence Alignment was performed by use of MultAlin (Corpet, 1996). Each sequence was first aligned against the predicted transcript from the chicken genome sequence derived from Red Jungle Fowl (ENSGALT00000025427.3) to

verify that the intended portion of the target gene was correctly amplified and sequenced.

In silico Translation

Sequences were translated *in-silico* using a standard genetic code by means of ExPASy Translate tool (Swiss Institute of Bioinformatics, <http://web.expasy.org/translate>).

Phylogenetic Analysis

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

RESULTS

Three out of four primer pairs corresponding to FABP4 exon 1 to 4 successfully amplified a fragment of the predicted size in at least one of the three breeds examined (Hubbard broiler, Fulani Ecotype and Yoruba Ecotype NLC) although non amplified fragments in all three breeds as shown in Table 1 and Figure 1

DNA Primary Structure – Nucleotide Sequence

Multiple sequence alignment of the Fulani ecotype, Yoruba ecotype and Hubbard Broiler FABP4 exon 3 sequences and flanking intron DNA produced in the present study against exon 3 of the Red Jungle Fowl primary transcript (ENSGALT00000025427.3 nt 1741 – 2067) revealed the existence of a purine to purine Single Nucleotide Polymorphism (SNP) in position 1765 (i.e. position 20 of exon 3; position 266 of the CDS) as shown in Figure 2. The Red Jungle Fowl and Fulani Ecotype chicken carried an Adenine (A) base and broiler carried Guanine (G) (c. 266A>G) as shown in Figures 2 and 3.

Predicted peptide sequence

Multiple Sequence Alignment of Peptide sequences from the translation of Jungle Fowl (ENSGALT00000025427.3 nt 1741 – 2067), Fulani and Hubbard broiler sequences (Figure 4) showed that the c. 266A>G mutation resulted in a missense variation at codon 7 of exon 3 (codon 89 of the full peptide sequence), in which codon 7 of exon 3 in Red Jungle fowl encoded the polar amino acid Asparagine (Asp, N), and the corresponding codon in broiler encoded Serine (Ser, S) (also a polar amino acid), as shown in Figure 4A.

Multiple sequence alignment of the predicted peptide sequence of FABP4 exon 3 from three randomly chosen species representing three

genera within the taxonomic order Aves (Turkey, Duck and Zebra finch) along with predicted peptide sequence of exon 3 of Broiler and Red Jungle Fowl/Fulani chicken (Figure 4B), revealed that the use of asparagine at codon 7 of exon 3 in the Jungle Fowl/Fulani chicken is conserved in avian evolution

Multiple peptide sequence alignment of FABP4, exon 3 for Aves, Eutherian and non-eutherian mammals showed that FABP4 exon 3 codon 7 (numbering relative to the chicken exon) 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).

Protein Prototype Assisted Modelling

Comparative Protein prototype of assisted modelling of the folded/tertiary/3-dimensional structure of FABP4 containing the peptide Serine at position 89 (Hubbard broiler) and the alternative protein structure containing Asparagine at position 89 (Fulani/ Red Jungle Fowl) of the complete FABP4 CDS revealed no profound effect of the N89S missense mutation on protein structure (Figure 6).

Phylogenetic Analysis of FABP4 Exon 3

A phylogram constructed from FABP4 exon 3 peptide sequence of chicken exon 3 of FABP4 of Hubbard Broiler, Red Jungle Fowl and Fulani Ecotype chicken, and other Aves (duck, turkey, zebra finch) in the Palaeognathae super order, using Eutherian and non-eutherian mammals as out-group, showed that the Red Jungle Fowl and Fulani Ecotype allele N89 (c. 266A) is ancestral to the broiler allele S89 (c. 266G) and the broiler allele is the more recently evolved form, the conventions c. 266A>G and N89S are therefore most appropriate in describing the mutation at the level of DNA and peptide sequence as shown in Figure 2 and 3 and Figure 4B and 5 respectively.

DISCUSSION

The Adenine (A) to Guanine (G) substitution (G 266 variant) at the DNA level at position 20 of the FABP4 gene, exon 3 transcript identified in the present study had previously been described (SNP Identification number rs431850290) by Luo *et al.* (2006) and Wang *et al.* (2009) in Arbor Acres broiler chickens and that the Red

Jungle Fowl A266 variant was carried by the Baier egg laying Chinese Local Chicken breed. It was suggested that the N89S mutation allele might be associated with higher fat deposition (Wang *et al.*, 2006). Zheng *et al.* (2009) found that FABP4 was more expressed in fast growing birds than in slow growing birds whereas a fat breakdown related enzyme thiolesterase B is expressed higher in slow growing birds, suggested that different metabolic regulatory network are indispensable for differential growth rate between fast growing and slow growing bird.

A Cytosine (C) to Thymine (T) substitution which is a silent (synonymous) mutation was also previously detected by Wang *et al.* (2006) in a separate study in exon 1 of chicken FABP4 using China Agricultural University Resource Population (CAURP) and Northeast Agricultural University Resource Population (NEAURP) which was obtained by crossing broiler sires, derived from lines at NEAU divergently selected for abdominal fat, with Baier layer dams, a Chinese local breed. The C variant is ascribed to Baier (local Chinese egg layer) grandsire and the T is ascribed to Broiler grandsire (Arbor acre). A study of the same location in the present work revealed no variation was found at the revealed base position for Hubbard broiler and Yoruba ecotype.

In the study of Wang *et al.* (2006), this C to T mutation at the DNA level did not change the function of the amino acid as both still encoded phenylalanine but statistic analysis indicated that the substitution was correlated with chicken abdominal fat content in the F2 recourse populations. This may be due to linkage disequilibrium between the polymorphic base and a proximal or distal causal variant and/or linkage disequilibrium between other loci acting in Trans. Li *et al.* (2008) recently reported that the expression of *FABP4* significantly correlated with abdominal fat, breast intramuscular fat and thigh intramuscular fat and was highly expressed in the Beijing-YOU chicken compared to the Jing Xing chicken.

This C/T substitution mutation in exon 1 (*FABP4* gene) was later separately reported by Luo *et al.* (2006) to also have significant correlation with abdominal fat, subcutaneous fat

and intramuscular fat content of pectoralis major muscle in Beijing-YOU chickens. Although it was unclear to the author whether the exon 3 A to G substitution in the chicken *FABP4* gene provides a molecular explanation for the previously reported linkage between a silent mutation in exon 1 of the gene and chicken abdominal fat, subcutaneous fat and intramuscular fat contents earlier reported by (Luo *et al.*, 2006; Wang *et al.*, 2006), it seems the most parsimonious explanation for their results.

In the present study, multiple sequence alignment using Aves (Broiler, Jungle Fowl/Fulani, Turkey, Duck and Zebra finch) as in group and with peptide sequences which showed that the use of asparagine at codon 7 of exon 3 (amino acid position 89 of the *FABP4* coding sequence) is conserved in avian evolution, suggested that, while at the nucleotide level the SNP represents a purine to purine change, its consequence at the amino acid level is a substitution of a polar for another polar amino acid, its (the SNP) localization within an exon (coding region of the gene), its non-synonymous consequence at the level of amino acid/peptide sequence, and the difference in the properties of the alternative variant amino acids (asparagine and serine), coupled with the conserved location of the non-synonymous substitution, it has consequence(s) for function of the encoded protein (*FABP4*). Indeed, a search through the literature confirmed that others have shown previously that the asparagine to serine mutation alters the functional properties of the protein (Wang *et al.*, 2009). The phylogeny of exon 3 for Palaeognathae was consistent with published literature on the molecular classification and phylogeny of mammals (Tarver *et al.*, 2016). In the out-group however, although the molecular phylogeny of exon 3 was largely consistent with the overall phylogeny of mammals (Tarver *et al.*, 2016), the super order Laurasiatheria was split with a suggestion that perissodactyla (a member of the super-order Laurasiatheria) split from Laurasiatheria prior to the emergence of super-orders Laurasiatheria, Xenarthra, Afrotheria and Eumarchontoglires from a common ancestor. Relationships among orders of the eutherian and

the non-eutherian mammals according to literature are largely unresolved partly because of their extreme rapid diversification in the late Cretaceous and early tertiary period and partly because of extremes in convergent and divergent adaptation to a wide range of habitats (Tarver *et al.*, 2016).

Nevertheless, most parsimonious explanation for this anomaly is that Perissodactyla is exceptionally conserved through evolution, and, that the horse has retained the ancestral form of exon 3 of *FABP4* that predates the divergence of Laurasiatheria (to which it belongs) from the Afrotheria and Xenarthra.

The evolutionary and functional basis for differential (extreme) conservation of exon 3 of horse relative to other Laurasiatheria is unclear but may have implications for a differential function of horse *FABP4* protein relative to orthologs for other Laurasiatheria. Exon 3 is more conserved across the primate (relative to the phylogram derived from alignment of the entire gene) suggesting functional conservation of exon 3 in this order (Primates), this was consistent with the findings of Tarver *et al.* (2016).

Previous studies (Hedges and Maxson 1996; Nishihara, *et al.*, 2009; Wildman, *et al.*, 2007) have suggested a clear pattern of biogeographic diversification for placentals into four principle lineages (Afrotheria, Xenarthra, Laurasiatheria and Eumarchontoglires) caused by drift-vicariance. It can be concluded from this experiment that: Natural variation exist in the coding protein of *FABP4* gene distinguishing exotic Hubbard Broiler from indigenous local chicken (YE-NLC and 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 disequilibrium 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.

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..

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Table 1 Primer sequence designed for amplification of Adipose Fatty Acid-Binding Protein genes

GENE	PRIMER	PRIMER SEQUENCE 5' – 3'	Annealing Temperature (°C)	PRIMER LENGTH (bases)	GC%	PRODUCT SIZE (bp)
FABP4	Xn1-F	GGCCAAGGCTCCTTTTA	57.89	18	50.00	268
	Xn1-R		57.92	23	39.13	
	Xn2-F	CAGCATAGACTGAAAAAGCAAGT	58.12	20	55.00	371
	Xn2-R	GGGATGGGAGAGTCTCTTA	58.03	20	45.00	
	Xn3-F	GTTTACCTTTGAAGGCAGCA	56.38	20	50.00	247
	Xn3-R	GGAGACAGTTTCCTCTTTGG	57.06	23	34.78	
	Xn4-F	TTGAATCCAGTGTAATACATCCA	57.45	20	55.00	297
	Xn4-R	GGCTCCCAGTGCATAAGTAG	57.06	22	36.36	
		GGCTAGTTGCATGTAATTTGA				

FABP4 – Fatty Acid-Binding Protein 4, Xn – Exon F – Forward R – Reverse bp – base pair

TABLE 2. Primer sequence used in the amplification of Fatty Acid Binding Protein 4 (FABP4) genes

PRIMER NAME	PRIMER SEQUENCE 5' – 3'	PRIMER LENGTH (bases)	*EXON POSITION (nt)	PRODUCT SIZE (bp)	TA (°C)	ACTUAL PRIMER ANNEALING TEMPERATURE (°C)			SUCCESSFULLY SEQUENCED AMPLICONS		
						HB	FE	YE	HB	FE	YE
FABP4_XN1_F	GGCCAAGGCTCCTTTTA	18	1 - 107	268	57.89	55	55	55	S	F	F
FABP4_XN1_R	CAGCATAGACTGAAAAAGCAAGT	23	1 - 107	268	57.92	55	55	55			
FABP4_XN2F	GGGATGGGAGAGTCTCTTA	20	1349 – 1521	371	58.12	NA	NA	NA	F	F	F
FABP4_XN2R	GTTTACCTTTGAAGGCAGCA	20	1349 – 1521	371	58.03	NA	NA	NA			
FABP4_XN3F	GGAGACAGTTTCCTCTTTGG	20	1746 - 1847	247	56.38	60	60	60	S	S	F
FABP4_XN3R	TTGAATCCAGTGTAATACATCCA	23	1746 - 1847	247	57.06	60	60	60			
FABP4_XN4F	GGCTCCCAGTGCATAAGTAG	20	3132 - 3222	297	57.45	55	55	55	S	F	S
FABP4_XN4R	GGCTAGTTGCATGTAATTTGA	22	3132 - 3222	297	57.06	55	55	55			

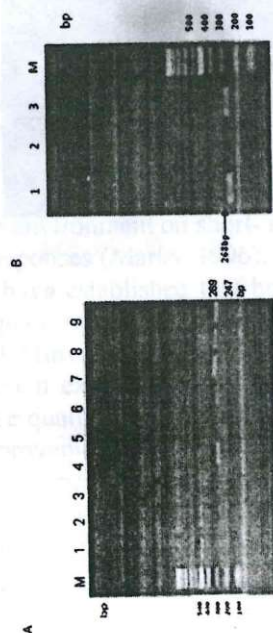


Figure 1 (A) Gel electrophoresis image of amplified PCR FABP4 product. Lane M = marker (100bp DNA ladder), lanes 4, 5, and 6 represent Fulani ecotype (FE), Yoruba ecotype (YE), Hubbard Broiler breed (HB) FABP4 exon 3 respectively and lane 7, 8 and 9 represent FE, YE, HB FABP4 exon 1 respectively, fragment sizes of the marker are listed on the right side, (B): Gel electrophoresis image

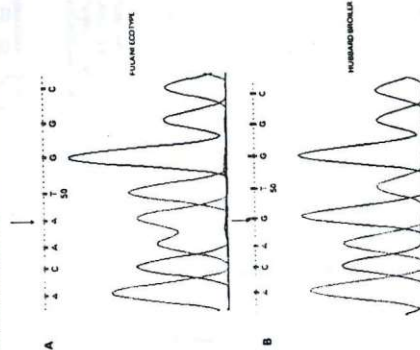


Figure 1 (A) chromatograms of Fulani ecotype/ Red Jungle Fowl sequence and (B) Hubbard broiler sequence FABP4 exon 3 mutations, polymorphic base is arrowed

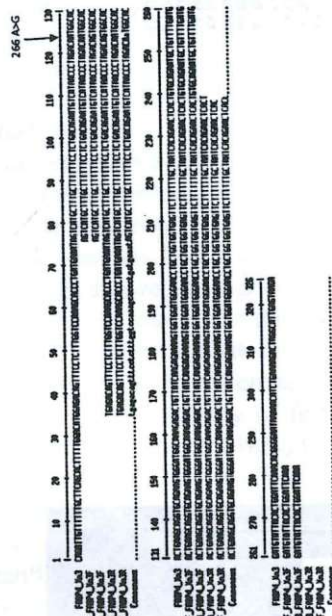


Figure 2: Multiple DNA sequence alignment of FABP4 exon 3 from: Red Jungle Fowl (ENSALP0000002537.3 nt 1741 – 2067 (FABP4_Xn3)), Fulani, using forward (F_FABP4_Xn3F) and reverse (F_FABP4_Xn3R) primers in sequencing and; Hubbard broiler, using forward (F_FABP4_Xn3F) and reverse (B_FABP4_Xn3R) primers in sequencing. Polymorphic base shown (arrow).



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, Turkey, Hubbard Broiler, Duck and Zebra Finch, showing polymorphic peptide at codon 7 (codon 89 of the FABP4 CDS). Mutated base shown (arrow).

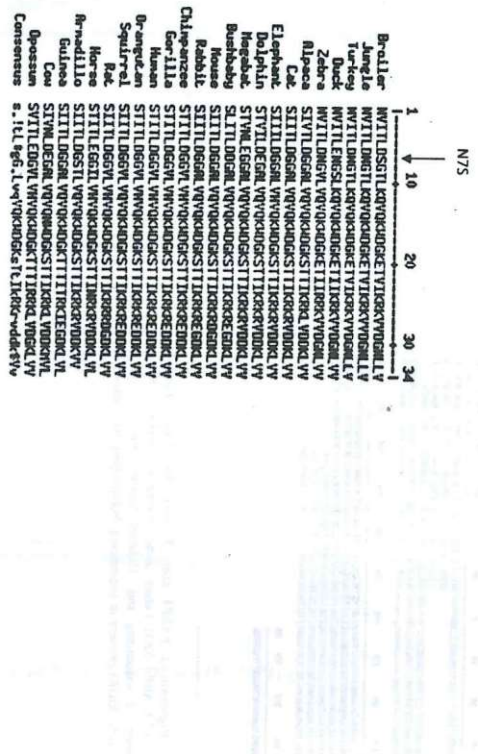


Figure 5. Multiple peptide sequence alignment (Multalin, Corpet, 1996) of FABP4, exon 3 for Aves, Eutherian and non-eutherian mammals. Location of Polymorphic codon/peptides shown as N75. * numbering relative to reference gene transcript Chicken – (ENS GALP00000023381), Alpaca (ENSVPAP0000001054), Duck (ENSAPLP00000005715), Armadillo (ENSNDOP00000033224), Bushbaby (ENSOGAP00000011712), Cat (ENSFCAP00000017433), Chimpanzee (ENSPTRP00000034857), Cow (ENSBTAP00000000079), Dolphin (ENSTTRP00000006583), Elephant (ENSAPF00000000507), Gorilla (ENSOGOP00000007182), Guinea Pig (ENSOP00000000483), Horse (ENSECAP00000021250), Human (ENSP000000256104), Megabat (ENSPVAP00000013471), Mouse (ENSMUSP00000029041), Opossum (ENSMODP00000008038), Orangutan (ENSPPYP00000020978), Rabbit (ENSOCUP00000013299), Rat (ENSRNOP0000007333), Squidrel (ENSTROP00000007662), Turkey (ENSMGAP00000012217) and Zebra Finch (ENSTGUP00000012023).

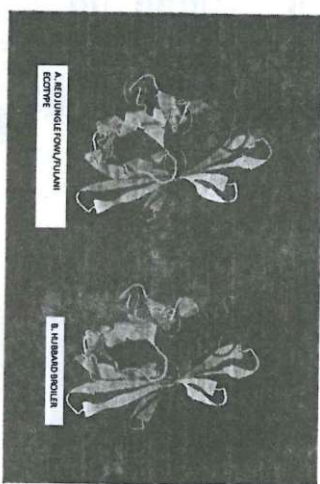


Figure 6. Predicted structure of FABP4 protein encoded by Red Jungle Fowl/Indian genome (A) and Zebra genome (B). 3D structure when produced by Phyre 2. White rings in figures A and B highlight the structural differences observed between Red Jungle Fowl/Indian genome and Zebra Fowl/Indian genome.

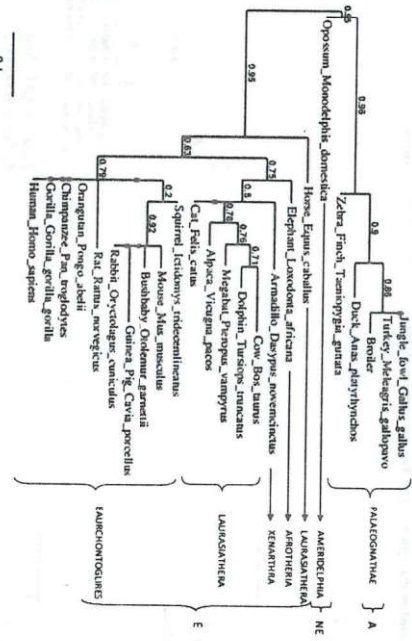


Figure 7. Phylogenetic tree of Aves (A) and non-eutherian (NE) mammals based on FABP4 exon 3 peptide sequence. Phylogenetic tree was produced by the use of Phylogeny.fr program.