Research Article

Sudi, I.Y., et al., Alpha S1-Casein Gene Polymorphısm in Nigerian Balami Sheep Breed Indigenous to Mubi. International Journal of Life Sciences and Biotechnology, 2019. 2(3): p. 231-242.

Alpha S1-Casein Gene Polymorphısm in Nigerian Balami Sheep Breed Indigenous to Mubi

Ismaila Yada Sudi[1,4*], Mohammed Shuaibu[2], Malachi Albert Tizhe[3], Augustine Clement[2]

ABSTRACT

The study was conducted on five Balami sheep breed to determine and characterize the alpha S1 casein gene. Five blood samples were collected from each animal intravenously using sterile needle and syringe. The blood samples were placed in labelled tubes containing ethylene diamine tetraacetic acid (EDTA). The blood samples were transported on ice cold chain container to the laboratory for analysis. Quick-DNA Miniprep ™ kit used for DNA extraction and amplified using forward and reverse primers (CSN1S1F 5'-ACCCCTCAGGTACCCTAAGAAA-3' and CSN1S1R 5'-GTTTATCCCCCACACTGCATTC-3'). The gene was sequenced and blasted against the NCBI database. Single nucleotide polymorphism analysis was performed to ascertain the variations. Multiple sequence alignment and phylogenetic analysis within and with the reference sequence was conducted online using bioinformatics tools. Result from the analysis reveals that, the extracted DNA were found on chromosome 6, intron 16 and exon 17. The Balami breeds of sheep showed total number of polymorphic and monomorphic site of 68 and 600 respectively, and percentage of polymorphism of 10.18%. The Balami breed showed one amino acid substitution and genetic variation within breeds. Complete molecular characterization, genotyping and determination of allele frequency of alpha S1 gene in Balami breed of sheep indigenous to Nigeria and its variations is recommended for further research.

ARTICLE HISTORY
Received
10 October 2019
Accepted
28 November 2019

KEYWORDS
Balami breeds, casein gene, genetic variation, ovine, polymorphism

Introduction

The population of sheep in Nigeria is estimated at 33.9 million representing 3.1% of the world's total [1]. Sheep milk production started with the beginning of domestication [2] and production in 2008 represented 4.92%, 0.02%, 1.70%, and 1.44% of milk produced in Africa, America, Asia and Europe, respectively (FAO, 2010). Ovine are better suited than bovine to environmental conditions prevalent in sub-Saharan Africa [4, 5, 6], they are

[1] Department of Biochemistry, Faculty of Science, Adamawa State University, Mubi, Nigeria.
[2] Department of Animal Production, Faculty of Agriculture, Adamawa State University, Mubi, Nigeria.
[3] Department of Animal Physiology, School of Agricultural Technology, Federal Polytechnic Mubi, Nigeria.
[4] North East Zonal Biotechnology Centre, University of Maiduguri, Nigeria.
Corresponding author's Email: yada280@gmail.com H/P: +2348160787132

extensively held as “the cows of the small holder” [7], to provide home supply and self-sufficiency for families, to avoid starving and malnutrition especially in high-quality protein [4, 6, 8]. Ovine milk and meat are widely consumed without inhibition as they thrived well with little supplements on browse and pasture.

Ovine milk product such as cheese and yoghurt can provide a profitable alternative to cow milk products owing to their specific taste, texture, natural and healthy image [9] and ovine milk contains higher level of total solids and major nutrient than goat or cow, especially of average protein and fat [10]. This result in higher cheese yield by approximately 15% in sheep compared to 10% in bovine, as cheese curd constitute mostly fat and casein [7, 11]. Whereas mineral contents of ovine milk are comparable with caprine mostly higher than that in bovine milk [10].

Casein is the main proteins in ovine (MPOM); present in colloidal solution and form 76 - 83% of total milk proteins in ovine [10, 12]. Casein play a nutritive function as a source of amino acids, calcium and phosphorus [13]. There are four casein fractions; namely αs_1-(CSN1S1), αs_2- (CSN1S2), β-(CSN2) and κ- casein CN; CSN3). They are differentiated according to their homology of primary structure [14] and are tightly linked within a 250 kb cluster [15, 16, 17] on ovine chromosome six (OAR6) [18].

The research on determining the relationship between the presence of genetic marker and production traits of animals is being conducted for many years now. In livestock farming the emphasis was put on milk protein genes. With the increasing population in the country the demand for milk proteins through sustainable animal agriculture is increasing. There is vigorous research for efficient production system that will supplement nutrition. Therefore, maintaining genetic variation is very important to avoid the loss of breeds by farmers and consumers. There are numerous breeds of sheep that are extinct and others classified at high risk of loss. There is need for characterization, though studying the genetic polymorphism of milk proteins have raised considerable research interest in caprine and bovine species, there are little description of casein gene polymorphism of ovine milk of native sheep breeds of Nigeria.

Casein genetic polymorphism of milk proteins are of importance as association to quantitative and qualitative parameter in milk proteins and used in breeding strategies and

in the dairy industry. They have effects on quantitative traits and technology properties of milk, it has been shown that ovine genetic polymorphisms affect the physicochemical properties of milk hence, there is need for in-depth knowledge of the genetic polymorphism of indigenous ovine milk proteins for the improvement of the quality of ovine milk for its contribution to the Nigerian dairy industry. It is in the light of this that present work is prompted to study alpha casein gene polymorphism in Nigerian indigenous Balami breed of sheep in Mubi area of Adamawa State, Nigeria.

Materials and Methods

Location and Size

The study was conducted in Mubi which is the second largest town in Adamawa State of Nigeria and covers an area of about 600 Square Km. The town lies about 260 Km north of Yola, the state capital. Mubi metropolitan area situated between Latitude 10 º 05' N/ 10 º 30' N and Longitude 13 º10' E/ 13 º30'E. The town is centrally located on the border line between Mubi-North L.G.A and Mubi-South L.G.A [19].

Metrological data

Mubi has a tropical climate. In winter, there is much less rainfall than in summer. According to Koppen and Gieger, this climate is classified as Aw. In Mubi, the average annual temperature is 25 ^{o}C. About 935 mm of precipitation falls annually. The driest month is January, with 0 mm of rainfall. Most precipitation falls in August, with an average of 258 mm. The warmest month of the year is April, with an asverage temperature of 29.3^{o}C. In August, the average temperature is 23.4 ^{o}C, which is the lowest for the whole year. The difference in precipitation between the dust month and the wettest month is 258 mm. the average temperatures vary during the year by 5.9 ^{o}C [20].

Experimental Animals and Blood Collection

Blood samples were randomly collected from five adult female ovine (Balami breed of Sheep) in Mubi, Adamawa State. Blood samples were collected through the jugular vein, using a needle and syringe (5 ml) and preserved in EDTA in a tube. All the samples were conveyed to the laboratory in an ice park.

Casein Gene Extraction

The casein gene (DNA) extraction was carried out using Quick DNA^{TM} MicroPrep Kit from Zymo Research according to manufacturer's instruction. 400 µl of Genomic Lysis Buffer was added to 100 µl of blood to make 4:1 volume, and mixed completely by vortexing for about 4-6 seconds and was left to stand for about 5-10 minutes at room temperature. The mixture was transferred to a Zymo- spin^{TM} ll column in a collection tube and was centrifuged at 10,000 × g for one minute, the collection tube was discarded with the flow through.

The Zymo- spin^{TM} ll column was transferred to a new collection tube, 200 µl of DNA pre - wash buffer added to the spin column and centrifuge at 100,000 × g for one minute. Again, 500 µl of g-DNA wash buffer was added to the spin column and centrifuged at 10, 00 × g for one minute.

The spin column was transferred to a clean microcentrifuge tube and 50 µl DNA for elution. Elution buffer was added to the spin column and incubated at room temperature for about 2-5 minute and thereafter, centrifuged at top speed for 30 seconds to elute the DNA. The eluted DNA was immediately used for molecular based application.

All genomic DNA was checked on 1% agarose gel electrophoresis and all amplicons on 1.5% agarose gel electrophoresis. The gel was stained with ethidium bromide and visualized under UV light transilluminator. For a 10 cm × 10 cm minigel cast, 1% agaros gel was prepared by dissolving 0.5g of agarose in 50cm of 1 × TAE (Tris Acetate EDTA) buffer, while 1.5% agarose gel for the amplicons was prepared by dissolving 0.75 g of agarose in 5 ml of 1×TAE. The mixture of agarose and buffer was swilled gently to ensure complete dissolution. The colloidal solution formed was heated in the microwave oven for 1-3 minute or till a clear solution was obtained.

The gel was allowed to cool to about 50°C (gel should not solidify) under running tap. Precaution was taken to prevent water from the running tap from splashing into gel. Ethidium bromide was mixed with DNA samples and loaded in the wells created by the comb in addition to loading dye. Genomic DNA samples was prepared for loading into the well by mixing 4 µl of the extracted DNA sample with 1 µl of the 5× loading dye. 5'-ACCCCTCAGGTACCCTAAGAAA-3' and CSN1S1R 5'-

GTTTATCCCCCACACTGCATTC-3') were designed from reference genomic sequence NC-019463.2. The primers spans intron 16 – exon 17 – intron 17 of casein alpha S1 gene on oar_v17 genomic sequence assembly.

A 20 µl reaction consisting of 4 µl of 5× Solisbiodyne master mix. 0.6 µl each of the forward and reverse primers, 12.8µl of nuclease free water and 2µl DNA template was made. The cycling condition were as follows: initial denaturation at 95°C for 3 minute, denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 40 seconds and final extension at 72 °C for 5 minutes. PCR products was run on 1% agarose, viewed and photographed using UV light according to manufacturer's instruction.

DNA Sequence Analysis

The sequencing was carried out at Xcelri Genomics, India, according to Sanger [21] using F 5'-ACCCCTCAGGTACCCTAAGAAA-3' and R 5'-GTTTATCCCCCACACTGCATTC-3 primers. Sequence was blasted against the database in NCBI and multiple sequence alignment using Multalin [22], Clustal W and trimming of sequence on BioEdit [23]. Phylogeny construction in MEGA X using Nei's genetic distance was used generated in Genalex 6.503 [24, 25], and Muscle phylogenetic Neighbour-joining tree 3.8 [26].

Results and Discussion

Sequence Analysis

The DNA isolated sequences of were presented (Fig. 1-4). The highest number of nucleotide sequence (714) with Balami A sheep breed (Fig. 1), and lowest (651) in Balami D (Fig. 4). This result is not consistent with the findings of Rumunno et al. [27] who observed the gene CSN1S1 encoding αs_2 to have the length of 18438 nucleotide and divided in to 19 exons ranging from 24 to 266 nucleotide, the observed differences may be due to differences of class of casein, exon, breed and geographical location.

>CSN1S1- Balami-1
AAGAAATCCCGGTGAATACTCCAGAGTAGAGGCATTTAAAGATTAGCAAAAGTGCAAGGAGGATAAAGTTGAATTTTTAC
TTTGGAGAGAAAATTTTCCTCCTATTTTCATCAGAATGATTATCACTTATCAAATAGATAGCACTGCTCCACATGTTCCTGA
GTAATGGATTAACATTAGCCATATATCAGAAAGTTATCATCAATTTTCCATTTCAGAAAATTCTAGCATAGGACACTAAGC
ATTTAAGTGTCATGCTTATTCTAAATTTATTTTTATATTATGTTTTCTTCTATTTCCCTTGATACGGCAGCAATATGCAGTCA
TTTAAATGAACTTACCACCACAGTGGCATAGTAATCTTTCCACTGTTCTCAGAGCCAATGGGATTAGGGATGTCAGAGAAT
GAGGGGGCATCAGTGTATTGTGTGCCTAGTGGAAGGTAATACCAGGCACCAGATGGATAGGCGTCCAGCTGGTAGAATTG
TCTGAAAAGCTAGAGAGAAAGACCATTGAAAAAGCGACAGTGTATGAGAAATCAGTTTTGAATCTCTGAAAATGAATCAT

TGCATTCATTTCAGACATGGCTATTCGCATCACAAGAGATGTTTACTCTGTGAGGAAAACAGAGAAACCAAACTCTTCCCT
ATGGTAAATCACAGCAAGGAAATTTTGGAAAGGATTTTTCTTAGGGGACCTGAGGGGGGGGAAAAC

Fig 1 Nucleotide sequence of Balami sheep breed A

>CSN1S1-Balami-2
CAATTTCCCTTACTGTGATTTACCATAGGGAAGAGTTTAGGTTTCTCTAGTTTTCCTCACAGAGTAAACATCTCTTGTGATG
CGAATAGCCATGTCTGAAATGAATGCAATGATTCATTTTCAGAGATTCAAAACTGATTTCTCATACACTGTTGCTTTTTCAA
TGGTCTTTCTCTCTAGCTTTTCAGACATTCTACCAGCTGGACGCCTATCCATCTGGTGCCTGGTATTACCTTCCACTAGGCA
CACAATACACTGATGCCCCCTCATTCTCTGACATCCCTAATCCCATTGGCTCTGAGAACAGTGGAAAGACTACTATGCCAC
TGTGGTGGTAAGTTCATTTAAATGACTGCATATTGCTGCCGTATCAAGGGAAATAGAAGAAAACATAATATAAAAATAAA
TTTAGAATAAGCATGACACTTAAATGCTTAGTGTCCTATGCTAGAATTTTCTGAAATGGAAAATTGATGATAACTTTCTGA
TATATGGCTAATGTTAATCCATTACTCAGGAACATGTGGAGCAGTGCTATCTATTTGATAAGTGATAATCATTCTGATGAA
AATAGGAGGAAAATTTTCTCTCCAAAGTAAAAATTCAACTTTATCCTCCTTGCACTTTTGCTAATCTTTAAATGCCTTTCTT
TGGATTATACCCATGATATACATTAGAATGCATGAGGGGGGAAAAAAAAACCAAAA

Fig 2 Nucleotide sequence of Balami sheep breed B

>CSN1S1-Balami-3
GCAAGGGGGGGGGGGAGCAAAACAAAGGGAAGAGTTTGGTTTCTCTGTTTTCCTCACAGAGTAAACATCTCTTGTGATGC
GAATAGCCATGTCTGAAATGAATGCAATGATTCATTTTCAGAGATTCAAAACTGATTTCTCATACACTGTTGCTTTTTCAAT
GGTCTTTCTCTCTAGCTTTTCAGACAATTCTACCAGCTGGACGCCTATCCATCTGGTGCCTGGTATTACCTTCCACTAGGCA
CACAATACACTGATGCCCCCTCATTCTCTGACATCCCTAATCCCATTGGCTCTGAGAACAGTGGAAAGACTACTATGCCAC
TGTGGTGGTAAGTTCATTTAAATGACTGCATATTGCTGCCGTATCAAGGGAAATAGAAGAAAACATAATATAAAAATAAA
TTTAGAATAAGCATGACACTTAAATGCTTAGTGTCCTATGCTAGAATTTTCTGAAATGGAAAATTGATGATAACTTTCTGA
TATATGGCTAATGTTAATCCATTACTCAGGAACATGTGGAGCAGTGCTATCTATTTGATAAGTGATAATCATTCTGATGAA
AATAGGAGGAAAATTTTCTCTCCAAAGTAAAAATTCAACTTTATCCTCCTTGCACTTTTGCTAATCTTTAAATGCCTTTCTT
TGGATTATACCCATGATATACATTAGAATGCAGTAGGGGGGGAGAAAAAAACAAAAT

Fig 3 Nucleotide sequence of Balami sheep breed C

>CSN1S1-Balami-4
ATCCTTACTGTGATTTACCATAGGGAAGAGTTTGGTTTCTCTGTTTTCCTCACAGAGTAAACATCTCTTGTGATGCGAATAG
CCATGTCTGAAATGAATGCAATGATTCATTTTCAGAGATTCAAAACTGATTTCTCATACACTGTTGCTTTTTCAATGGTCTT
TCTCTCTAGCTTTTCAGACAATTCTACCAGCTGGACGCCTATCCATCTGGTGCCTGGTATTACCTTCCACTAGGCACACAAT
ACACTGATGCCCCCTCATTCTCTGACATCCCTAATCCCATTGGCTCTGAGAACAGTGGAAAGATTACTATGCCACTGTGGT
GGTAAGTTCATTTAAATGACTGCATATTGCTGCCGTATCAAGGGAAATAGAAGAAAACATAATATAAAAATAAATTTAGA
ATAAGCATGACACTTAAATGCTTAGTGTCCTATGCTAGAATTTTCTGAAATGGAAAATTGATGATAACTTTCTGATATATG
GCTAATGTTAATCCATTACTCAGGAACATGTGGAGCAGTGCTATCTATTTGATAAGTGATAATCATTCTGATGAAAATAGG
AGGAAAATTTTCTCTCCAAAGTAAAAATTCAACTTTATCCTCCTTGCACTTTTGCTAATCTTTAAATGCCTTTCTTTGGATTA
TACCCATGATATACATTAGAATGCATGGGGGGGAAAAAAAAAAAAAAAA

Fig 4 Nucleotide sequence of Balami sheep breed D

However, consensus was found at various positions, at position 75-78 (TTT), 217-220(TTT), 515- 518 (GAA), among many other positions within the alignment (Fig. 5). Many consensus were found with respect to the reference, at position 1477-1480 (CTC), 15037-150170(TCT), 15187-15190 (ATT) as observed (Fig. 6). Phylogenetic tree within Balami sheep breed shows that 3A and 3C is genetically distance away from 3B and 3D (7), while phylogenetic tree of Balami sheep with reference showed 3A are genetically closer to the reference, 3C and 3B at the same distance to reference and 3A, 3D distantly away from reference, 3A, 3B, 3D are at the same distance respectively. Multiple sequence alignment within all breeds, showed consensus at position 79-83 (TTT), 97 -100 (AAA), 501-503 (TTT) among many consensus observed through multiple sequence alignment. It was observed that, the nucleotide sequence variation among Balami sheep within 154 bp of

intron 16 and exon 17, the highest number of nucleotide sequence (714) in Balami A and lowest (651) in Balami D nucleotide positions when compared to reference gene NC_040257.1, while the variation in G, A, C, and T, was confirmed by using multiple sequence alignment. The polymorphic sites and frequency of polymorphism confirmed the variation and similarity in the multiple sequence alignment, where amino acids substitution and polymorphism were identified within the open reading frame of the CSN1S1 gene as compared with the reference sequence (DNA sequence: 94714744- 94715051). This result is similar to the finding of Calvo et al., [28] who observed 61 polymorphism in Assaf sheep breed on exon 17.

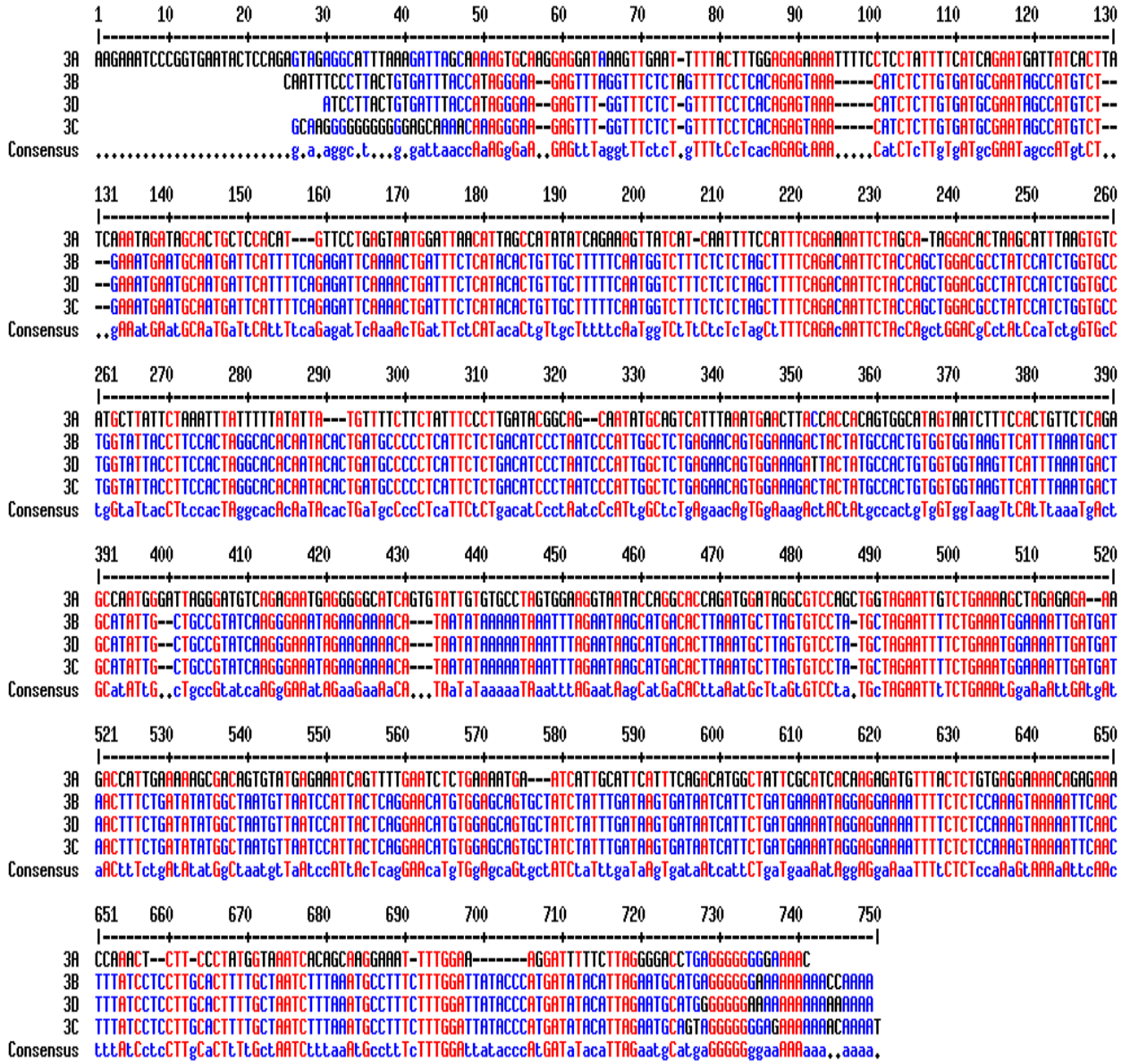

Fig 5 Multiple sequence alignment of nucleotide sequences within Balami sheep breed. Note: 3A =Nucleotide sequence for Balami sheep 1, 3B =Nucleotide sequence for Balami sheep 2, 3D= Nucleotide sequence for Balami sheep 4, and 3C= Nucleotide sequence for Balami sheep 3

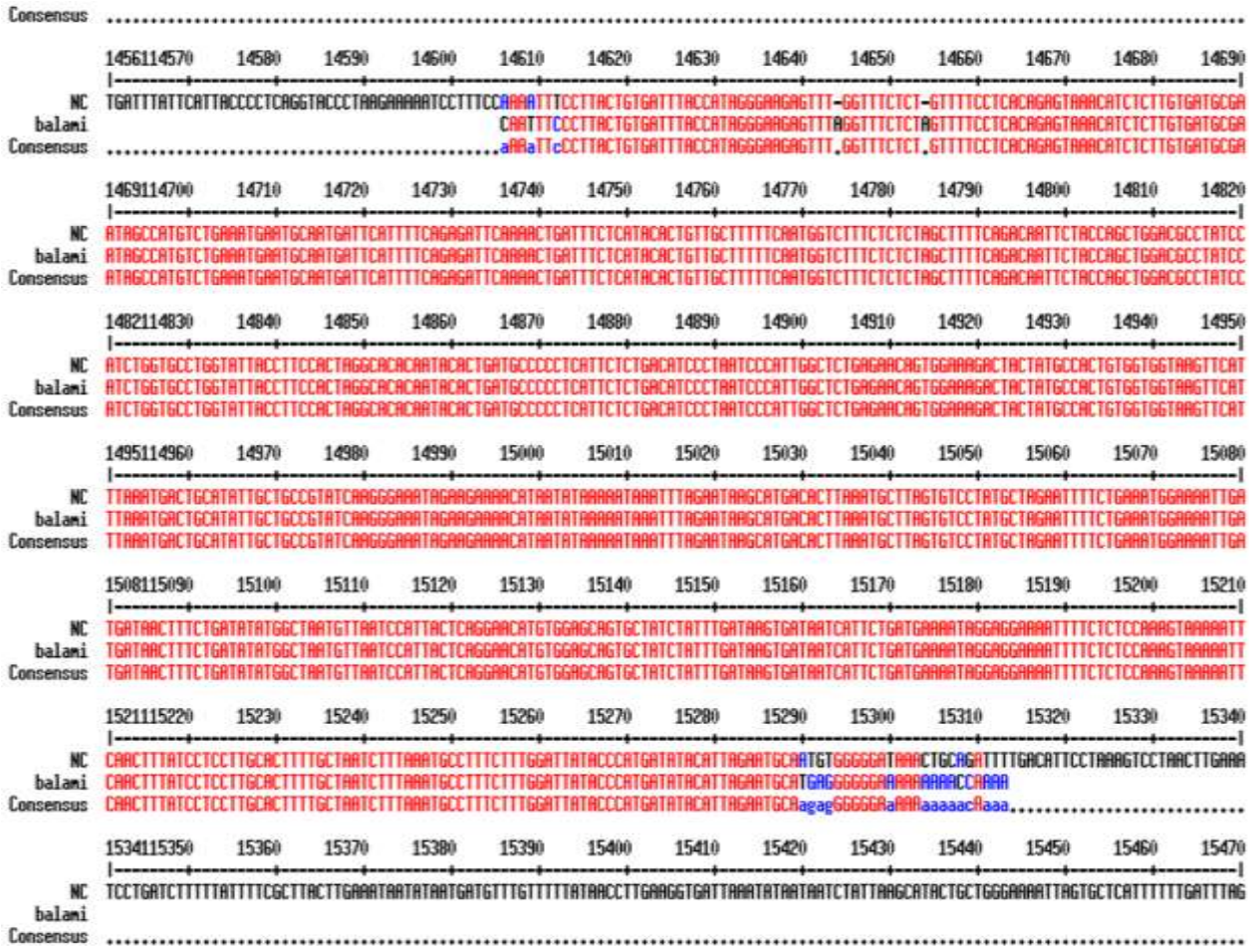

Fig 6 Multiple sequence alignment of nucleotide sequence of Balami sheep with reference sequence. Note: NC= Nucleotide sequence for reference NC-040257.1, balami= Nucleotide sequence for Balami sheep

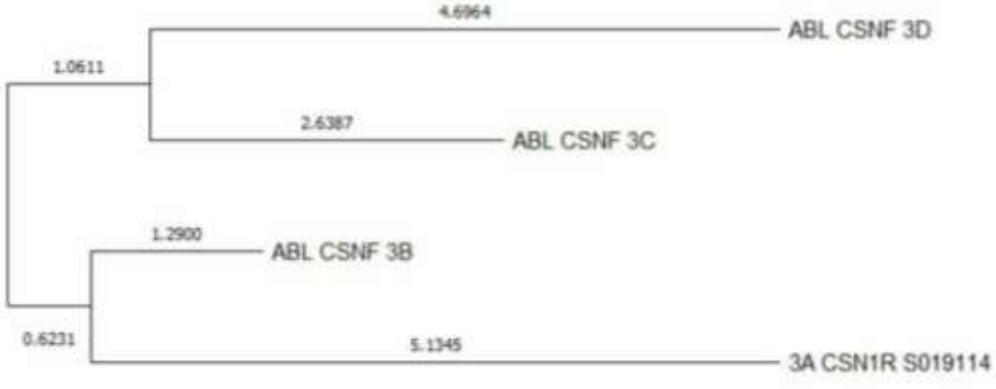

Fig 7 Phylogenetic tree within Balami sheep breeds. Note: 3A CSNIR S019114 = Balami sheep 1, ABL CSNF 3B = Balami sheep 2, ABL CSNF 3C = Balami sheep 3, and ABL CSNF 3D = Balami sheep 4.

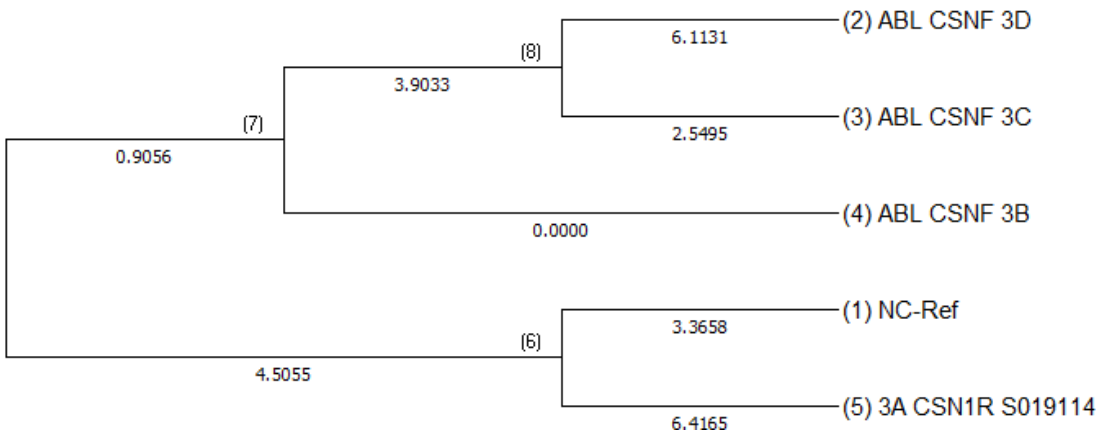

Fig 8 Phylogenetic tree of Balami sheep with reference gene. Note: 3A CSNIR S019114 = Balami sheep 1, ABL CSNF 3B = Balami sheep 2, ABL CSNF 3C = Balami sheep 3, ABL CSNF 3D = Balami sheep 4, and NC-Ref. = Reference sequence.

However, Balami sheep breed showed percentage polymorphism of 5.09%, and the number of polymorphic and monomorphic site of 24 and 644 respectively compared with NC-040257.1 and showed only one amino acid substitution compared with reference.

Although, Chessa et. al., [29] found and reported that amino exchanged at position 200 of αs_2 Asn>lys was observed in bovine, amino acid exchange in Balami was observed at position 201 Thr> Ile with high frequency of 0.733. This difference could be as a result of species different as well as difference of class of casein gene one in Balami sheep breeds indicated high genetic variation within breeds which is very important for breeds adaptability, production and long term survival. All the amino acid exchanged were caused by single nucleotide polymorphism, this is not consistent with the work of Giambra et al. [30]. Balami sheep is genetically distant in repect to the refrence gene (NC_040257.1) by 0.031, and was also in consonance with the phylogenetic analysis and multiple sequenced alignment.

Conclusion

All the balami sheep breeds showed total number of polymorphic and monomorphic site of 68 and 600 respectively, and percentage of polymorphism of 10.18% and the same number of site. Amino acid substitution in Ouda sheep breed was higher (7) than Yankasa sheep breed (4), and Balami sheep breed shown the lowest amino acid substitution of 1, it showed

variation exist within and between breeds these is very important for species long term survival. High frequency of 0.733 was observed at position 201 in all the breeds, showed amino acid exchanged on exon 17 position 183Met>Val with frequency of 0.12 to 0.26 this difference could be as a result of differences in targeted segments on the exon as well as the position of the exchanged. Balami sheep breeds were genetically closer compared to Ouda sheep. Ouda and Balami sheep were therefore genetically related.

There is need for complete characterization, genotyping and finding the allele frequencies of casein gene of indigenous sheep breeds, this will offer the possibility to get a complete picture about milk protein gene and to consider milk protein variation in specific breeding programmed in improving consumer preference. In conclusion, casein CSN1S1 was isolated in Balami sheep within 154 bp of chromosome 6, intron 16 and exon 17. It was characterized, shown polymorphism, genetic variation within and between breeds. These sequence obtained from all breeds will be deposited on NCBI data base for further research. This will assists in conserving the genes of the native animals for breeding purposes.

References

1. FAO, Food and Agriculture Organization. FAO Statistical Yearbook 2009. Agricultural production, (2011). http://www.fao.org/docrep/014/am079m/am079m00.html Retrieved 04.09.2010
2. Haenlein G.F.W., About the evolution of goat and sheep milk production. Small Ruminant Research, 2007. 68: p. 03-06.
3. FAO, Food and Agriculture Organization of the United Nations. Database on Agriculture.15.03, 2010. http://www.fao.org/.15.03
4. Haenlein, G.F.W., Past, present, and future perspectives of small ruminant dairy research. Journal Dairy Science, 2001. 84: p. 2097-2115.
5. Park, Y.W., and Haenlein, G.F.W., Overview of Milk of non-bovine mammals, in Handbook of milk of non-bovine mammals, Y. W. Park, and G. F. W. Haenlein, Editors. 2006, Blackwell Publishing, Ames, Iowa, USA, p. 03-10.
6. Degen, A.A., Sheep and goat milk in pastoral societies. Small Ruminant Research, 2007. 68: p. 07-19.
7. Haenlein, G.F.W., W. L. Wendorff, and Y. W. Park, Sheep milk. Handbook of milk of non-bovine mammal, 2006. Blackwell Publishing, Ames, Iowa, USA, p. 137-194.
8. Morand-Fehr, P., and J. Boyazoglu, Present state and future outlook of the small ruminant sector. Small Ruminant Research, 1999. 34: p. 175-188.
9. Raynal-Ljutovac, K., et al., Composition of goat and sheep milk products: An update. Small Ruminant Research, 2008. 79 (1): p. 57-72.
10. Park, Y.W., et al., Physico-chemical Characteristics of goat and sheep milk. Small Ruminant Research, 2007. 68: p. 88-113.

11. Storry, J.E., et al., Chemical composition and coagulation properties of renneted milks from different breeds and species of ruminants. Journal of Dairy Research, 1983. 50: p. 215-229.
12. Hinrichs, J., et al., Mediterranean milk and milk products. European Journal of Nutrition, 2004. 43 (1): p. 12-17.
13. Holt, C., and L. Sawyer, Primary and predicted secondary structures of the caseins in relation to their biological functions. Protein Engineering, 1988. 2 (4): p. 251-259.
14. Eigel, W.N., et al., Nomenclature of proteins of cow's milk: Fifth Revision. Journal of Dairy Science, 1984. 67: p. 1599-1631.
15. Threadgill, D.W., and J. E. Womack, Genomic analysis of the major bovine milk production. Protein genes. Nucleic Acids Research, 1990. 18: p. 6935-6942.
16. Lévéziel, H., et al., Restriction fragment length polymorphism of ovine casein genes: close linkage between the αs1 -, αs2 -, β- and κ-casein loci. Animal Genetics, 1991. 22: p. 01-10.
17. Bevilacqua, C., et al., Translational efficiency of casein transcripts in the mammary tissue of lactating ruminants. Reproduction Nutrition Development, 2006. 46: p. 567-578.
18. DeGortari, M.J., et al., A second generation linkage map of the sheep genome. Mammal Genome, 1998. 9: p. 204-209.
19. Sudi, I. Y., et al., Alpha S1 casein gene polymorphism in Ouda sheep breed of Mubi, Nigeria. Nigerian Journal of Genetics, 2019. 33 (1): p. 110-116.
20. Degerforsertr, A. M., Climate Mubi. Climate-Data.Org, 2019. http://en.climate-data.org/info/sources Retrieved on 30.09.2019.
21. Sanger, F., and A. R. Coulson, A rapid method for determining sequence in DNA by primed synthesis with DNA polymerase. Journal of Molecular Biology, 1975. 94 (3): p. 441-448.
22. Corpet, F., Multiple sequence alignment with hierarchical clustering. Nucleic Acid Research, 1988. 16 (22): p. 10881-10890.
23. Hall, T. A., BioEdit: A user- friendly biological sequence alignment editor and analysis programmed windows 95/98nt. Nucleic Acids Symposium Series, 1999. 41: p. 95- 98.
24. Kumar, S., et al., Mega X: Molecular Evolutionary Genetics Analysis across computing platforms. Molecular Biology and Evolution, 2018. 35: p.1547-1549.
25. Peakall, R., and P. E. Smouse, GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research – an update. Bioinformatics, 2012. 28 (19): p. 2537-2539.
26. Edgar, R.C., Muscle: Muscle multiple sequence alignment with high accuracy and throughput. Nucleic acid Research, 2004. 32 (5): p. 1792-1797.
27. Ramunno, L., G., et al., Characterization of two new alleles at the goat CSN1S2 locus. Animal Genetics, 2001. 32 (5): p. 264-268.
28. Calvo, J. H., et al., Structural and functional characterization of the αs1- casein gene and association studies with milk traits in Assaf sheep breed. Livestock Science, 2013. p. 01-08.
29. Chessa, S., et al., New genetic polymorphisms within ovine β- and αs_2-caseins. Small Ruminant Research, 2010. 88: p. 84-88.
30. Giambra, I. J., S. Jäger, and G. Erhardt, Isoelectric focusing reveals additional casein variants in German sheep breeds. Small Ruminant Research, 2010. 90 (13): p. 11-17.