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

Ismaila Yada Sudi^{1,4*}, Mohammed Shuaibu², Malachi Albert Tizhe³, Augustine Clement²

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 TM kit used for DNA extraction and amplified using forward and reverse primers (CSN1S1F 5'-ACCCCTCAGGTACCCTAAGAAA-3' 5'and CSN1S1R 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.

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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 selfsufficiency 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 α_{s_1} -(CSN1S1), α_{s_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 $^{\circ}$ 05' N/ 10 $^{\circ}$ 30' N and Longitude 13 $^{\circ}10'$ E/ 13 $^{\circ}30'$ 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 °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°C. In August, the average temperature is 23.4 °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 °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 DNATM MicroPrep Kit from Zymo Research according to manufacturer's instruction. 400 μ l of Genomic Lysis Buffer was added to 100 μ l 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- spinTM 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- spinTM ll column was transferred to a new collection tube, 200 μ l of DNA pre - wash buffer added to the spin column and centrifuge at 100,000 × g for one minute. Again, 500 μ l 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 μ l 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 \times 10 cm minigel cast, 1% agaros gel was prepared by dissolving 0.5g of agarose in 50cm of 1 \times 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 \times 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 μ l of the extracted DNA sample with 1 μ l 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 μ l reaction consisting of 4 μ l of 5× Solisbiodyne master mix. 0.6 μ l each of the forward and reverse primers, 12.8 μ l of nuclease free water and 2 μ l 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 α s₂ 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

TGCATTCATTTCAGACATGGCTATTCGCATCACAAGAGATGTTTACTCTGTGAGGAAAACAGAGAAACCAAACTCTTCCCT Fig 1 Nucleotide sequence of Balami sheep breed A

>CSN1S1-Balami-2

TGGTCTTTCTCTCAGCTTTTCAGACATTCTACCAGCTGGACGCCTATCCATCTGGTGCCTGGTATTACCTTCCACTAGGCA CACAATACACTGATGCCCCCTCATTCTCTGACATCCCTAATCCCATTGGCTCTGAGAACAGTGGAAAGACTACTATGCCAC TGTGGTGGTAAGTTCATTTAAATGACTGCCATATTGCTGCCGTATCAAGGGAAATAGAAGAAAAACATAATAAAAATAAA TTTAGAATAAGCATGACACTTAAATGCTTAGTGTCCTATGCTAGAATTTTCTGAAATGGAAAATTGATGATAACTTTCTGA AATAGGAGGAAAAATTTTCTCTCCAAAGTAAAAATTCAACTTTATCCTCCTTGCACTTTTGCTAATCTTTAAATGCCTTTCTT

Fig 2 Nucleotide sequence of Balami sheep breed B

>CSN1S1-Balami-3

 ${\tt GCAAGGGGGGGGGGGGGGGAAAAACAAAGGGAAGAGTTTGGTTTCCTCTGTTTTCCTCACAGAGTAAACATCTCTTGTGATGC}$ GAATAGCCATGTCTGAAATGAATGCAATGATTCATTTTCAGAGATTCAAAACTGATTTCTCATACACTGTTGCTTTTTCAAT GGTCTTTCTCTAGCTTTTCAGACAATTCTACCAGCTGGACGCCTATCCATCTGGTGCCTGGTATTACCTTCCACTAGGCA CACAATACACTGATGCCCCCTCATTCTCTGACATCCCTAATCCCATTGGCTCTGAGAACAGTGGAAAGACTACTATGCCAC TGTGGTGGTAAGTTCATTTAAATGACTGCCATATTGCTGCCGTATCAAGGGAAATAGAAGAAAAACATAATAAAAATAAA TTTAGAATAAGCATGACACTTAAATGCTTAGTGTCCTATGCTAGAATTTTCTGAAATGGAAAATTGATGATAACTTTCTGA AATAGGAGGAAAAATTTCTCTCCAAAGTAAAAATTCAACTTTATCCTCCTTGCACTTTTGCTAATCTTTAAATGCCTTTCTT

Fig 3 Nucleotide sequence of Balami sheep breed C

>CSN1S1-Balami-4

ATCCTTACTGTGATTTACCATAGGGAAGAGTTTGGTTTCCTCGTTTTCCTCACAGAGTAAACATCTCTTGTGATGCGAATAG ${\tt CCATGTCTGAAATGAATGCAATGATTCATTTTCAGAGATTCAAAACTGATTTCTCATACACTGTTGCTTTTTCAATGGTCTT$ TCTCTCTAGCTTTTCAGACAATTCTACCAGCTGGACGCCTATCCATCTGGTGCCTGGTATTACCTTCCACTAGGCACAAAT ACACTGATGCCCCCTCATTCTCTGACATCCCTAATCCCATTGGCTCTGAGAACAGTGGAAAGATTACTATGCCACTGTGGT ATAAGCATGACACTTAAATGCTTAGTGTCCTATGCTAGAATTTTCTGAAATGGAAAATTGATGATAACTTTCTGATATATG

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.

	1	10	20	30	40	50	60	70	80	90	100	110	120	130
3A 3B 3D 3C Consensus	AAGAAA	ITCCCGGTGAA	CF G	ATTTCCCTTA Atcctta Craggggggg	CTGTGATTTA Ctgtgattta Ggggagcaar	ICCATAGGGAA ICCATAGGGAA IACAAAGGGAA	GAGTTTA GAGTTT- GAGTTT-	AAGTTGAAT-T GGTTTCTCTAG GGTTTCTCT-G GGTTTCTCT-G ggtTTctcT-g	TTTTCCTCA TTTTCCTCA TTTTCCTCA	CAGAGTAAA Cagagtaaa Cagagtaaa	CATCTC CATCTC CATCTC	TTGTGATGCGA TTGTGATGCGA TTGTGATGCGA	ATAGCCATGI Atagccatgi Atagccatgi	TCT
	131	140	150	160	170	180	190	200	210	220	230	240	250	260
3A 3B 3D 3C Consensus	GAAA GAAA GAAA	ITGAATGCAAT ITGAATGCAAT ITGAATGCAAT	GATTCATTTI Gattcattti Gattcattti	CAGAGATTCA Cagagattca Cagagattca	AAACTGATTT AAACTGATTT AAACTGATTT	CTCATACACT CTCATACACT CTCATACACT	GTTGCTTTT GTTGCTTTT GTTGCTTTT	AAAGTTATCAT TCAATGGTCTT TCAATGGTCTT TCAATGGTCTT LcAaTggTCLT	TCTCTCTAGO TCTCTCTAGO TCTCTCTAGO	CTTTTCAGA <mark>C</mark> CTTTTCAGA <mark>C</mark> CTTTTCAGAC	AATTCTA <mark>CC</mark> A AATTCTA <mark>C</mark> CA AATTCTA <mark>C</mark> CA	GCTGGACGCCT GCTGGACGCCT GCTGGACGCCT	ATCCATCTGO ATCCATCTGO ATCCATCTGO	GTGCC GTGCC GTGCC
	261	270	280	290	300	310	320	330	340	350	360	370	380	390
3A 3B 3D 3C Consensus	TGGTAT TGGTAT TGGTAT	TACCTTCCAC TACCTTCCAC TACCTTCCAC	TAGGCACACA Taggcacaca Taggcacaca	ATACACTGAT Atacactgat Atacactgat	GCCCCCTCAT GCCCCCTCAT GCCCCCTCAT	TCTCTGACAT TCTCTGACAT TCTCTGACAT	CCCTAATCC CCCTAATCC CCCTAATCC	AATATGCAGTC Cattggctctg Cattggctctg Cattggctctg Cattggctctg cattggctctg	agaacagtg(agaacagtg(agaacagtg(SAAAGACTAC Saaagattac Saaagactac	T <mark>atgccactg</mark> Tatgccactg Tatgccactg	TGGTGGTAAGT TGGTGGTAAGT TGGTGGTAAGT	TCATTTAAA1 TCATTTAAA1 TCATTTAAA1	TGACT Tgact Tgact
	391	400	410	420	430	440	450	460	470	480	490	500	510	520
3A 3B 3D 3C Consensus	GCATAT GCATAT GCATAT	TGCTGCCG TGCTGCCG TGCTGCCG	ITATCAAGGGA Itatcaaggga Itatcaaggga	IAATAGAAGAA IAATAGAAGAA IAATAGAAGAA	AACATAA AACATAA AACATAA	ITATAAAAAATA Itataaaaaata Itataaaaaata	AATTTAGAA AATTTAGAA AATTTAGAA	AATACCAGGCA TAAGCATGACA TAAGCATGACA TAAGCATGACA TAAGCATGACA tAagCatGaCA	ICTTAAATGC1 ICTTAAATGC1 ICTTAAATGC1	ITAGTGTCCT Itagtgtcct Itagtgtcct	<mark>a-tgctagaa</mark> A-tgctagaa A-tgctagaa	TTTTCTGAAAT TTTTCTGAAAT TTTTCTGAAAT	GGAAAATTGA Ggaaaattga Ggaaaattga	ATGAT Atgat Atgat
	521	530	540	550	560	570	580	590	600	610	620	630	640	650
3A 3B 3D 3C Consensus	AACTTT AACTTT AACTTT	ICTGATATATG ICTGATATATG ICTGATATATG	IGCTAATGTTA Igctaatgtta Igctaatgtta	ATCCATTACT ATCCATTACT ATCCATTACT	CAGGAACATO Caggaacato Caggaacato	itggagcagtg itggagcagtg itggagcagtg	CTATCTATT Ctatctatt Ctatctatt	CATTCATTTCA TGATAAGTGAT TGATAAGTGAT TGATAAGTGAT tgaTaAgTgat	TAATCATTCT TAATCATTCT TAATCATTCT	SATGAAAATA Satgaaaata Satgaaaata	ggaggaaaat" ggaggaaaat" ggaggaaaat"	TTTCTCT <mark>CCA</mark> A TTTCTCT <mark>CCA</mark> A TTTCTCT <mark>CCA</mark> A	AGTAAAAAATT Agtaaaaatt Agtaaaaatt	TCAAC TCAAC TCAAC
	651	660	670	680	690	700	710	720	730	740	750			
3A 3B 3D 3C Consensus	TTTATO TTTATO TTTATO	CTCCTTGCAC CTCCTTGCAC CTCCTTGCAC	TTTT <mark>GCT</mark> AAT TTTT <mark>GCT</mark> AAT TTTT <mark>GCT</mark> AAT	CTTTAAATGC CTTTAAATGC CTTTAAATGC	CTTTCTTTGG CTTTCTTTGG CTTTCTTTGG	ATTATACCCA Attataccca Attataccca	TGATATACA Tgatataca Tgatataca	TTAGGGGGACCT TTAGAATGCAT TTAGAATGCAT TTAGAATGCAC TTAGAATGCAC TTAGaatgCat	GAGGGGGGGAA GGGGGGGGGAA TAGGGGGGGGG	<mark>IAAAAAAAACC</mark> IAAAAAAAAAAAAAAAAAAAAAAAAAA	aaaa Aaaat			

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

GATTTATTCATT	BULLET											
TGRTTTATTCATTRCCCCTCRGGTRCCCTARGRARARTCCTTTCCARARATTCCTTACTGTGRTTTACCATRGGGRRGAGTTT_GGTTTCCTC_GTTTCCTCAGRGTRARCATCCTTTGTGRTGGCGA CRRTTTCCCTTACTGTGRTTTACCATRGGGRRGAGTTTAGGTTTCCTAGGTTTCCTCAGRGGGRRAGATCCTTTGTGRTGGGA ARA_TC_CCTTACTGTGRTTTACCATRGGGRRGAGTTT_GGTTTCCT_GTTTTCCTCAGRGGARARCATCTCTTGTGRTGCGA												
469114700	14710	14720	14730	14740	14750	14760	14770	14780	14790	14800	14810	1482
TRECCATETICTE	AAATGAATG	RATGATICAT	TTTCAGAGA	TTCARAACTG	ATTICTCATA	CACTETTECT	TTTTCRATGG	ICTITICICIC	TAGCTITICA	GACABITCTA	CCAGCTGGAC	GCCTATCO
482114830	14840	14850	14860	14870	14880	14890	14900	14910	14920	14930	14940	14950
TCTGGTGCCTGG	TATTACCTTO	CACTAGECRO	CACARTREAC	TGATGCCCCC	TCATTCTCTG	RCATCCCTRA	TCCCATTGGC	TCTGAGAACA	GTEGARAGAC	TACTATECCA	CTGTGGTGGT	ARGITCAT
495114960	14970	14980	14990	15000	15010	15020	15030	15040	15050	15060	15070	1508
TRAATGACTGCR	TATTECTEC	GTATCAAGG	Grantagaag	AAAACATAATI AAAACATAATI	ataaaaataaf Ataaaaataaf	ATTIAGAATA	AGCATGACAC RGCATGACAC	TTARATGCTT TTARATGCTT	AGTGTCCTATI Agtgtcctati	GCTAGAATTT GCTAGAATTT	TCTGARATGG	RAAATTGA
508115090	15100	15110	15120	15130	15140	15150	15160	15170	15180	15190	15200	15210
GRTAACTITCTG	ATATATGEC	ANTGITANTO	CATTACTCA	GGAACATGTG	GAGCAGTECT	RTCTRTTTGR	TAAGTGATAA	TCATTCTGAT	GARARTAGGA	GGRAAATTTT	CTCTCCAAAG CTCTCCAAAG CTCTCCAAAG	TARARATT Taraartt Taraartt
521115220	15230	15240	15250	15260	15270	15280	15290	15300	15310	15320	15330	15340
AACTITATCCTC	CTTGCACTT	TGCTAATCTT	THAATSCCT	TTCTTTGGAT	TATACCCATG	ATATACATTA	GAATGCATGA	GEGEGERARA	ARRACCARA	A	1940003544	ACTTGAAA
534115350	15360	15370	15380	15390	15400	15410	15420	15430	15440	15450	15460	15470
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GETTTCCT, GETTTCCT, GETTTCCTACAGEGAAGAGETT, GETTTCCT, GETTTCCTACAGEGAAGAGETT 469114700 14710 14720 14730 14740 14750 14760 14770 14780 14790 14800 1460114700 14710 14720 14730 14740 14750 14760 14770 14780 14790 14800 1460CATISCTGAAATGATTCATTTCCAGAGETTCATATACATGETTCCTTTCATAGETGTTTCCTTAGETCTTTCCTGAGETTTCCTTAGECTGTTTCCTTAGECTGTTTCCTGAGACAGETTCAGAGETCAGAAGETCAGAGETCAGAGETCAGAGETCCTAGETCCCTAGETCCCTAGETCCCTAGETCCCTAGETCCCTAGETCCCTAGETCCCTAGETCCCTAGETCCCTAGETCCCTAGETCCCTAGETCCCAGEGCCCGGAGEGCCTAGETTCCGAGAGEGCCTCCTAGETCCCAGTGGCCCGGTTGGGCCGGGAGGGCTTCTGGCGAGGGCCTAGETGCCGAGGGCCGGTGGGGGCCGGGAGGGCTGCTGGGGGGGG	and trecttrector control of the second sec

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

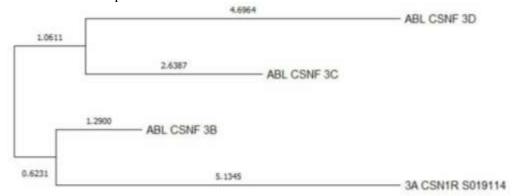


Fig 7 Phylogenetic tree within Balami sheep breeds. Note: 3A CSNIR S019114 = Balami sheep 1, ABL CSNF 3B = Balami sheep 2, ABL CSNF 3C = Balami sheep 3, and ABL CSNF 3D = Balami sheep 4.

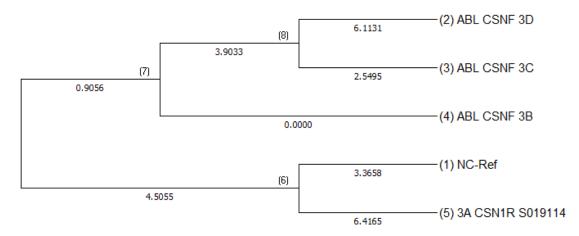


Fig 8 Phylogenetic tree of Balami sheep with reference gene. Note: 3A CSNIR S019114 = Balami sheep 1, ABL CSNF 3B = Balami sheep 2, ABL CSNF 3C = Balami sheep 3, ABL CSNF 3D = Balami sheep 4, and NC-Ref. = Reference sequence.

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.

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