FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 6 No. 2, April, 2022, pp 156 - 159
DOI: https://doi.org/10.33003/fjs-2022-0602-917

SINGLE NUCLEOTIDE POLYMORPHISM DETECTION AND SEQUENCE CHARACTERIZATION OF BETA CASEIN GENE IN BUNAJI AND FRIESIAN-BUNAJI CROSSBRED COW

***[1]Dubagari N. G., [2]Nwagu B. I., [3]Ojo O. A.**

[1]Department of Animal Science, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.
[2]National animal production research institute, Zaria, Kaduna State, Nigeria.
[3]Department of Livestock and Fisheries, National Agricultural Extension and Research Liason Services, Zaria, Kaduna State, Nigeria.

*Corresponding authors' email: **maxwellgambo2@gmail.com** Phone: +2347038741089

ABSTRACT

Milk is an important food constituent in diet. This study was undertaken to detect the presence of SNP in βcasein gene of Bunaji and Friesian X Bunaji crossbred cows using sequencing. 60 animal comprising of 30 Bunaji and 30 friesian X Bunaji cows were understudy. DNA was extracted from 200 µl of the blood using high salt method. The electrophoresis revealed molecular DNA with a single band. The exon 7 of βcasein gene was amplified by PCR using published primer. 20 amplicon were sent for sequencing, 10 each for Bunaji and Friesian X Bunaji crossbred. The DNA sequence of exon 7 of beta casein gene were aligned using the MAFFT online sequence alignment tools. The results revealed, 6 polymorphic sites at exon 7 beta casein gene. The SNP were at positions; 221, 350, 381, 387, 510, 387 and 516. Four of the SNPs were nonsynonymous in nature while 2 were synonymous. A nucleotide substitution from C→A was observed in Bunaji and its crossbred counterpart resulted in substitution of amino acid proline → histidine; and C→G substitution resulting in a Serine → Arginine substitution implying that Bunaji and Friesian X Bunaji had the preponderance of A2, A1 and B variant, resulting in potentials release of bioactive peptide upon digestion of A1 and B variant. SNPs discovered in this study can be used as molecular genetic markers for marker assisted selection (MAS) to increase the rate of genetic improvement of milk production traits in Bunaji and Friesian Bunaji crossbred cows.

Keywords: Beta, Bunaji, casein gene, Exon IIV, SNP and Milk

INTRODUCTION

Milk is a common source of food in our diet. The major constituents of milk are water, lactose, fat, protein, organic acids and minerals. The quality of milk is mainly determined by its protein and fat contents. Milk is made up of two major protein groups viz., casein and whey protein. About 3.3 per cent of bovine milk is made up of protein (Walstra et al., 2006). Bovine milk protein constitutes 80 percent of casein and 20 percent of whey protein (Roginsky, 2003; Hoffman and Falvo, 2004; Sohdi *et al.*, 2017). The four casein types in milk are alpha S1, alpha S2, Beta and kappa casein encoded as just many genes (CSN1S1-CSN1S2-CSN2-CSN3) respectively located on chromosome 6 (Roginsky, 2003). Gamma- casein derives from degradation of beta casein (Kaminski *et al*., 2007). Beta casein is one of the major proteins present in cow milk.

Beta-casein (*CSN2*) is relevant in relation to milk production parameters and milk protein quality. Such genes that are correlated with production Parameters explain a part of the genetic variance and can improve the estimation of breeding value, therefore they can be used as a suitable supplement to conventional breeding procedures. The polymorphism related to the differences in animal performance can be taken into account in the selection process. There are 12 genetic variants of beta-casein viz., A1, A2, A3, B, C, D, E, F, H1, H2, I and G (Roginsky, 2003). Among them, A1 and A2 variants are most common in Holstein-Friesian dairy cattle worldwide (Kaminski *et al*., 2007). The A1 and A2 types differ in one amino acid at the position 67 of the peptide chain, where A1 variant has histidine and A2 variant has proline. It is thought that beta-casein variant A1 play some role in the development of some human diseases like artheriosclerosis and type I diabetes as it yields the bioactive peptide beta casomorphin-7 (Kaminski *et al*., 2007). In some countries like New Zealand and Australia, the A2 milk is preferred over A1 milk, this because of the structural difference between this two beta casein types results to differential digestion in the guts by the action of digestive enzymes resulting in the release of beta casomorphin which is a threads to human health (Tailford *et al.,* 2003). Several research works have been carried out for determining the casein genotype of exotic cattle breeds. However, such works on indigenous cattle breeds in Nigeria are scanty. Furthermore, very little work had been carried out on Bunaji cattle with regard to molecular genetic characterization using single nucleotide polymorphism. Therefore, this study seeks to identify the variant types of beta casein gene in Bunaji and Friesian-Bunaji crossbred cattle.

MATERIALS AND METHODS

Experimental Animals

This study was carried out on 60 animals consisting of 30 Friesian-Bunaji crossbred from NAPRI and 30 Bunaji cattle from small-holder cattle herds in Guga, Giwa Local Government Area of Kaduna Sate, Nigeria.

DNA Extraction and Sequencing: A total of 60 blood sample were collected from Bunaji and Friesian-Bunaji crossbred. The blood sample were collected in EDTA Vacutainer tubes by jugular vein puncture. The samples were transported in an ice container to the laboratory and were accordingly stored at -20^{0}c till DNA extraction at African Bioscience Lab (Ibadan, Nigeria). Genomic DNA was extracted using high salt DNA extraction procedure (Miller et *al*., 1988). The quality of isolated DNA was checked on 0.6 percent agarose. The quantity of DNA was also checked at 260/280nm suing Ultra violet spectrophotometer. The sample having the OD ratio between 1.8-1.9 were good and used for polymerase chain reaction (PCR).

PCR Amplification

Amplification of 965bp fragment spanning exon VII of beta casein gene were performed using the already established primer by Melisa et al. (2017) with the following sequence;

Table 1: Sequence and position of Primers used for the Beta Casein gene

Gene	Location	Length	Primer Sequence (5-3)	Denature	Annealing
Leptin	Exon 7	965bp	F; AGGCAACTCAGGAAGAGGTG R; ATCTCCACGGGTAAGCCTAGA	72.0^{o}C	60.0^{0}C

The polymerase chain reaction was carried out in the final volume of 25µl containing 12.5 µl of master mix, 2µl of DNA template 1 µl of each primer (10pmol/ µl) and buffer up to 25 µl. Amplification was performed with thermal cycler with an initial denaturation at 95^{o}C for 3 minutes followed by 30 cycles of 95^{o}C for 30sec, 60^{o}C for 40sec, 72^{o}C for 40sec with the final extension at 72^{0}C for 10 minutes.

DNA Sequencing

PCR product were purified and sequenced using both the forward and reverse primer of PCR amplification. The obtained sequence were edited manually using BioEdit and aligned with MAFFT online sequence alignment tools.

RESULTS AND DISCUSSION

The quality and quantity of DNA from 20 Bunaji and Friesian x Bunaji crossbred cow were assessed by spectrophotometer readings and electrophoresis which revealed high molecular weight DNA with a single band. The exon 7 of CSN2 gene was amplified (965 bp) by Polymerase Chain Reaction (PCR) is presented in Plate 1

SNP Detection and Sequence Characterisation of Beta Casein Gene in Bunaji and Friesian- Bunaji

The sequence data generated for 20 animals, 10 for each of Bunaji and Friesian x Bunaji crossbred cattle revealed only 6 polymorphic sites in the coding region (-350 C>A, -221 C>A, -516C>G, 387 C>G, -510 T>C and -381 T>C). The C>A variation at -350 position showed an allelic frequency of 0.278, amino acid substitution of His67Pro and A2 variant type. The C>A variation at -221 position showed an allelic frequency of 0.728, amino acid substitution of Pro67His and A1 variant type. The C>G variation at -516 and -387position both showed an allelic frequency of 0.111, an amino acid substitution of Ser122Arg and B variant type. While the T>C variation at 510 and -381 locations both showed an allelic frequency of 0.944, an amino acid substitution of Thre170%3D. The amino acid substitutions were Thre170%3D and Thre127%3D at -510 and 381 positions respectively (table 2).

The β-casein variants discovered in this work had a look at and corresponding amino acid variation presumed from the sequence analysis confirmed β-casein has 209 amino acids and there are at least 13 variants of this protein that vary at difference amino acid location (Gallinat *et al*., 2013; Haq *et al*., 2014; Singh *et al*., 2015). For instance, the A2 allele differs from A1 and A3 alleles by only one amino acid change at positions 67 (His/Pro) and 106 (Gln/His), respectively (Barroso *et al*. 1999; Kaminski *et al*., 2007; Cieslinska *et al*., 2012; Farrell *et al.,* 2004). The distiction between A1 and B variants involves only one amino acid substitution (Serine for Arginine) at location 122 of the β-casein. B variant differs from A2 in having a proline in position 67 and an arginine in place of a serine at location 122 (Farrell *et al.,* 2004; Clemens, 2011; Vallas *et al*., 2012).

The natural mutations that give rise to this change are as a result of a single nucleotide polymorphism of the β-casein gene. In this study β-casein A1 allele varies from A2 allele by an A→C substitution at location 350 and 221 of the cow β-casein reference sequence (GenBank, NC_037333.1). The B allele varies by 2 non synonymous mutations from the A2 allele. The variant is characterized by an A→C transition at nucleotide location 350 and 221, ensuing in the amino acid exchange Pro→His, and a C→G transition at location 516 and 387, leading in a Ser→Arg substitution. This variance in the amino acid sequence suggests the secondary structural difference of the expressed protein (Elliott *et al*., 1999; McLachlan, 2001).

Selection for improvement must take this under consideration as selection for improved milk production may upturn the frequency of harmful A1 allele in our bovine population. Efforts should be made to increase the A2 allele with a view of transforming Nigeria cattle population capable of producing A2 variant of beta casein which has a world demand.

CONCLUSION

This study showed the preponderances of A2, A1 and B variants over other 12 Variant reported in the literature. SNPs discovered in this study can be used as molecular genetic markers for marker assisted selection (MAS) to increase and quicken the rate of genetic improvement of milk production traits in Bunaji and Friesian Bunaji crossbred cattle.

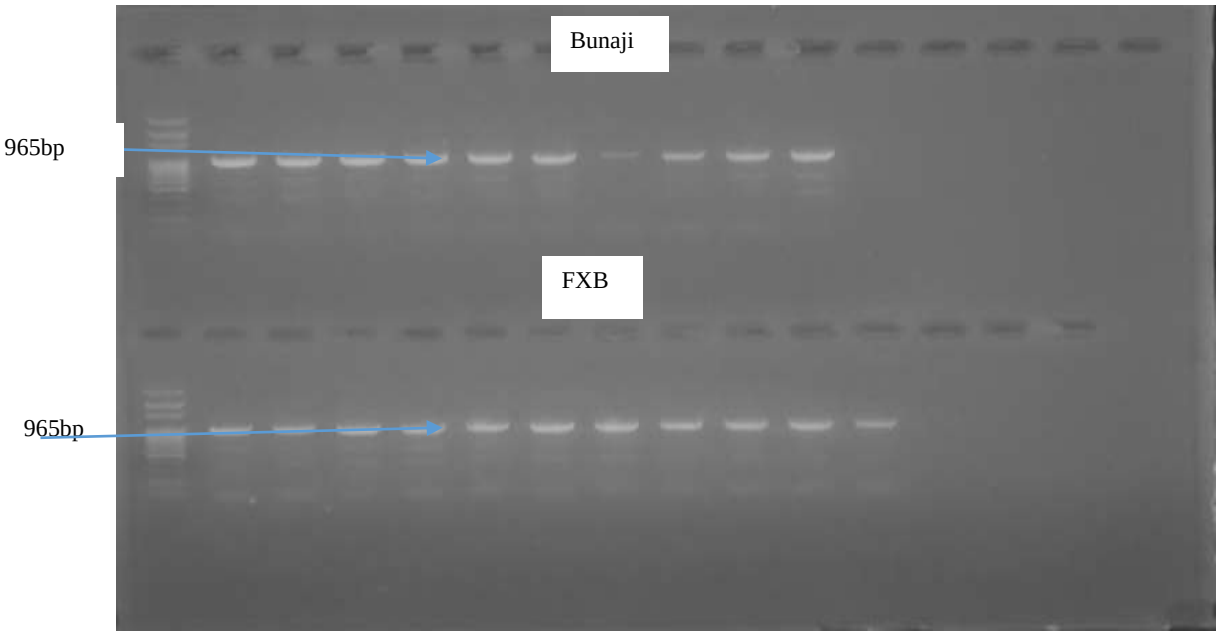

Plate 1: PCR patterns of 965bp fragment of CSN2 exon 7 of Bunaji and Friesian x Bunaji crossbred

Table 2. SNP Detection and sequence characterization of Beta Casein Gene

Number	Location of SNP	Consequence	Position on Bautarus	CDS position	Allele Substitution	Frequencies	Amino Acid Substitution	Variant type
1	Exon 7	Missense Variant	6_85451298	350	C>A	0.278	His67Pro	A2
2	Exon 7	Missense Variant		221	C>A	0.278	Pro67His	A1
3	Exon7	Missense Variant	6_85451132	516	G>C	0.111	Ser122Arg	B
4	Exon7	Missense Variant		387	G>C	0.111	Ser122Arg	B
5	Exon7	Synonymous	6_85451138	510	T>C	0.944	Thre170%3D	-
		Synonymous		381	T>C	0.944	Thre127%3D	-

REFERENCES

Cieślinska A, Kostyra E, Kostyra H, Oleński K, Fiedorowicz E & Kamiński S (2012). Milk from cows of differentβ-casein genotypes as a source of β-casomorphin-7. *International Journal of Food Sciences and Nutrition* 63426–430

Clemens RA, (2011). Milk A1 and A2 peptides and diabetes.Nestle NutritionInstitute Workshop Series: *Pediatric Program* 67187–195

EFSA. 2009. Scientific Report of EFSA prepared by a DATEXWorking Group on the Potential Health Impact ofβ-Casomorphins and Related Peptides. European Food SafetyAuthority Science Report, pp 1–107

Melisa, M., Silvia, P., Gaetano, L., Angelo, V. Z. and Andrea, S. (2017). Evaluation of bovine betacasein polymorphism in two dairy farms located in northern Italy. *Italian Journal of Food Safety*, 6(6904):131-133.

Elliot, R.B., Harris, D.P., Hill, J.P., Bibby, N.J. and Wasmuth, H.E., (1999). Type I (insulin dependent) diabetes mellitus and cow milk: casein variant consumption. *Diabetologia*, 42: 292-296

Farrell, H. M., Jr, Jimenez-Flores, R., Bleck, G.T., Brown, E.M., Butler, J.E. and Creamer, L.K. (2004). Nomenclature of the proteins of cow's milk-sixth revision. *Journal of Dairy Science*, 87: 1641–1674

Tailford KA, Berry CL, Thomas AC, Campbell JH. A casein variant in cow's milk is atherogenic. Atherosclerosis. 2003;170:13–19. [PubMed] [Google Scholar]

Gallinat JL, Qanbari S, Drögemüller C, Pimentel ECG, Thaller G & Tetens J(2013). DNA-based identification of novel bovine casein gene variants. *Journal of Dairy Science* 96699–709

Haq U, Kapila R, Shandilya UK & Kapila S, (2014). Impact of milk derivedβ-casomorphins on physiological functions and trends in research: *a review.International Journal of Food Properties* 171726–1741

Hoffman, J. R. Falvo, M. J. (2004). Protein which is best? *Journal sport science medicine* 3(1): p 18-30.

Kaminski, S., Cieslinska, A. and Kostyra, E. (2007). Polymorphism of bovine beta-casein and its potential effect on human health. *Journal of Applied Genet*ics, 48(3): 189-198.

Legates J.E and E.J Warwick. (1990). Breeding and improvement of farm animals. 8th Edition. Mc Graw Hill Publishing Company. Pp 241-273.

Mclachlan, C.N.S., (2001). Beta-casein ischemic heart diseases mortality and other illnesses. Med. Hypotheses., 56: 262-272

Miller, S.A., Dykes, D.D. and Polesky, H.F., (1988). A simple salting out procedure for extracting DNA from human nucleated cells. *Nuclear Acids Resource*, 16: 1215

Niki, R., Kim, G.Y., Kimura, T., Takahashi, K., Koyama, K. and Nishinari, K. (1994). Physical properties and microstructure of rennet gels from casein micelles of different sizes. *Milchwissenschaft*, 49: 325–329.

Roginsky, H. (2003). Encyclopedia of Dairy Sciences. Academic Press, London.

Singh, L. V., Jayakumar, S., Sharma, A., Gupta, S. K., Dixit, S. P., Gupta, N. and Gupta, S. C. (2015) Comparative screening of single nucleotide polymorphisms in β-casein and κ-casein gene in different livestock breeds of India. *Meta Gene,* 485–91

Sodhi, M., Mukesh, M., Mishra, B.P., Amit Kishore, Prakash, B., Kapil, R., Keviletsu Khate., Kataria, S. and Joshi, B. K., (2012). Screening of taurine and crossbred breeding bulls for A1/A2 variants of β-casein gene. *Ind. J. Anim. Sci.*, 82(2): 183-186

Vallas, M., Kaart, T., Varv, S., Parna, K., Joudu, I., Viinalass, H.and Parna, E. (2012). Compositeβ-κ-casein genotypes and their effect on composition and coagulation of milk from Estonian Holstein cows. *Journal of Dairy Science*, 95: 6760–6769

Walstra, P., Wouters, J.T.M. and Geurts, T.J. (2006). Dairy Science and Technology. *Taylor & Francis*, New York.