ABG -03

β-Lactoglobulin Gene Polymorphism of Indigenous Nigerian Goat Breeds Using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism

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Abstract
An investigation of the genetic polymorphism at the β-Lactoglobulin (β-LG) gene of Nigerian indigenous goat breeds (West African Dwarf, Sahel and Red Sokoto) was carried out using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method. Restriction endonucleases (Rsal) were used in the study to cut the extracted DNA into fragments. The results provided evidence that the β-LG gene locus of West African Dwarf, Red Sokoto and Sahel goats is monomorphic with only one genetic variant (allele A) with a gene frequency of 1.00.The amplified DNA products were observed at 120 bp. The restriction digestion with Rsal revealed just one genotype at the β-LG locus. In conclusion, the results revealed the absence of polymorphism at the β-LG locus of the goats.

Keywords: polymorphism, goats, gene locus, PCR-RFLP, *β*-Lactoglobulin

Introduction

Goats are kept for a wide variety of reasons by farmers but majorly for milk, meat, fibre and pelts.In Nigeria, they are kept mainly for meat production; if they are used for the production of milk, it is at a very low frequency. This might be attributed to inadequate knowledge of the nutritional quality of goat milk. The Nigerian Forum for Agricultural Advisory Services, recommended goat milk consumption for its beneficial health benefits which included: high levels of protein, higher levels of essential amino acids, calcium, potassium, phosphorus and vitamin A (Vanguard, 2017). Because of its low lactose level compared to the milk of other animals, the milk of goat is an important nutrient for human consumption, particularly those who have problem of digesting milk lactose (lactose intolerance); the milk also contains about 25% more vitamin B6, 47% more vitamin A, and 13% more calcium than cow's milk (NAFIS, 2017).

The *β*-lactoglobulin protein coded by *β*-LG gene represents about 50% of the total milk protein. The *β*-LG gene is located on chromosome 3 with 4.9 Kb long transcription units comprising of 7 exons and 6 introns; *β*-LG has the ability to bind a variety of hydrophobic molecules including retinol and small fatty acids making it to play a role in the transport of vitamin A (Basanti *et al.,* 2014).Milk protein polymorphism can serve as a genetic marker and hence, have been considered as a potential genetic tool for carrying out a selection of dairy animals. It is one of the many positional candidate genes which may affect milk production traits in goats. Milk polymorphism is owed to single nucleotide substitution in exonII (T–C), which is expressed, as a change of the 20th amino acid (Tyr to His). This substitution disrupts *Rsa*I site which can be detected by PCR-RFLP. Allelic variants A and B *β*-LG only differ by a Tyr/His substitution, where *β*-LG A has Tyr and *β-LG* B has His (Kolde and Braunitzer, 1983). The rare variant *β*-LG C is a subtype of *β*-LG A with a single exchange of Arg-Glu (Erhardt, 1989). Only two genetic variants of *β*-LG (A and B) have been reported in goats (Kumar *et al*., 2006; El-Hanafy *et al*., 2010).

The distinctiveness of goat milk as stated earlier should ignite extensive research into the genetic structure of milk-related genes in Nigerian indigenous goat populations. Milk production of a doe will definitely have a significant effect on pre-weaning growth and survival of kids especially when born as twins and triplets. Improvement in the milk-producing ability of does and kid survival will not only lead to significant economic returns to most goat farmers, but probably a goat dairy niche that will improve the health status of the populace. It has been established that milk yield is influenced by polymorphisms in genes coding for whey proteins (Kumar *et al*., 2006; El-Hanafy *et al*., 2010). This relationship between genetic variants of *β*-lactoglobulin gene and milk yield, as well as its composition and cheese-making ability have raised interest for the polymorphic identification of *β*-LG in dairy animal populations. *β*-LGAA and AB genotypes are associated withcasein content and curd yield whereas the BB genotype is associated with a significantly higher milk yield (Basanti *et al.,* 2014).Polymorphisms of the milk protein genes of goats can be used therefore as a genetic marker in modern breeding programmes targeted at the animal and other milk-producing ruminants. Considering the importance of this gene, it is important to determine the superior genotypes in Nigerian indigenous goat breeds as this will help in developing breeding programmes and selection strategies. The forms and frequency of *β*-LG gene have not been studied in indigenous Nigerian goats.

This study is aimedat identifying the allelic variants of *β*-LG gene in threeindigenousNigeriangoat breeds of different agro-climatic regions using the PCR-RFLP method.

Materials and Methods

Total DNA was isolated using ZymoBeadTM Genomic DNA Kit. Gel monitoring was used to determine DNA quality.*β*-LG genotypes were identified as described by Anton *et al.* (1999). The *β*-LG genotypes were identified in 2 steps; in the 1st step,

the 120 bp fragments were amplified using forward primer 5-CAACTCAAGGTCCCTCTCCA-3 and reverse primer 5-CTTCAGCTCC TCCAGGTACA-3, while in the 2nd step, the 105 bp fragments were amplified using forward primer 5-TCAGGACCCCGGAGGTGGACAAC-3 and reverse primer 5-CCTCCAGCTGGGTCGGGTTGAAG-3. The restriction fragments were analyzed by electrophoresis and visualized using ultraviolet light. The genotypes were recognized using the restriction fragments observed in the gel.Direct counting was used to estimate phenotypic and allele frequencies of *β*-LG genetic variants. Chi-square test (*χ2*) was used to check for whether the populations were in Hardy-Weinberg equilibrium.

Chi-square test was carried out using Genalex software v. 6.502 (Peakall and Smouse, 2008).

Results and Discussion

The DNA extracted was of high quality without any sheared fragments (FiguresI andII) meaning that the Zymoresearch kit was appropriate in extracting genomic DNA from the blood samples as has been reported also in coral reef (Santos *et al.,* 2012). It is also said to be more time saving and cost effective than other extraction methods for forensic samples (Yunjie and Oluseyi, 2013).

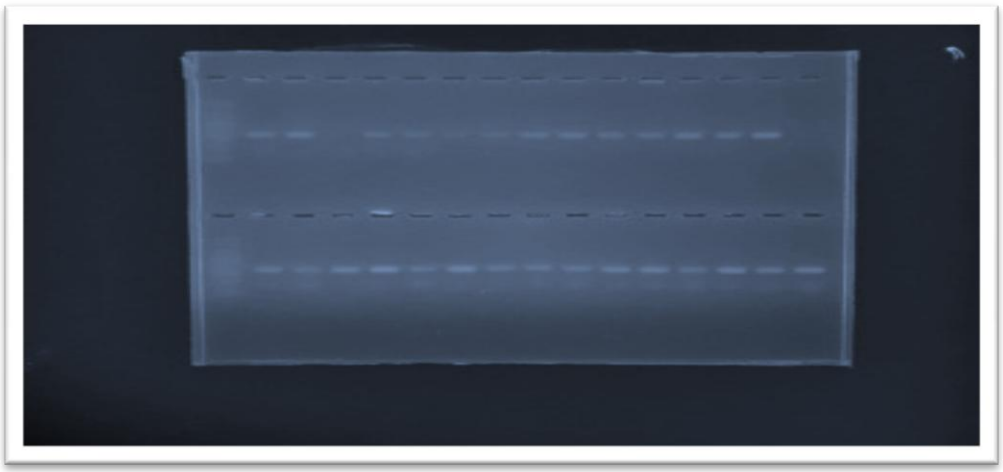


Fig. I:Gel electrophoresis of genomic DNA extracted from Nigerian indigenous goat breeds

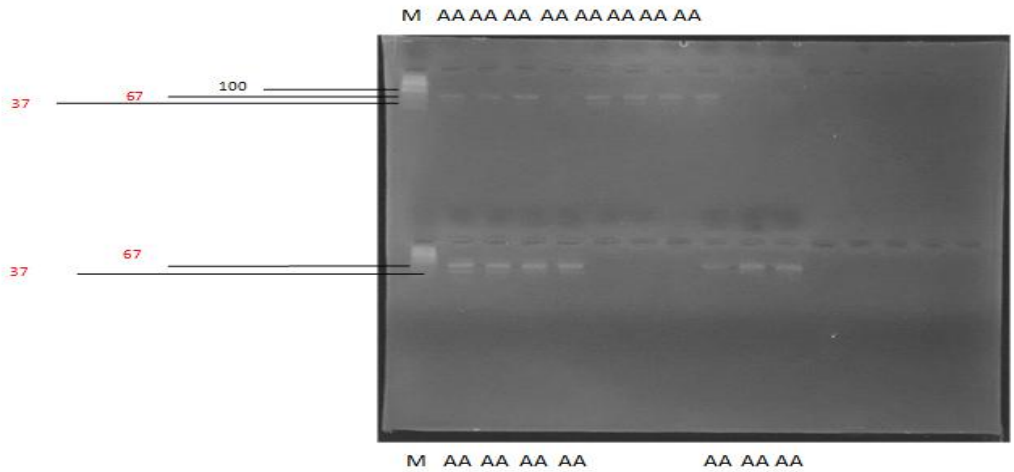


Fig. II: Gel electrophoresis of 120 bp*β*-LG genotyping by PCR-RFLP with Rsal enzyme in indigenous Nigerian goats. Lane M = DNA ladder; Lane 1-12 = AA genotype; Lane 15-18 = AA genotype

The genotype and gene frequency of the PCR products (Table 1) revealed the presence of only one allele (A) at the *β*-LG locus of the goats sampled. This is in agreement with the reports of El-Shazly *et al*. (2012) in Saudi goat breeds. This is however at variance with the reports of El- Hanafy *et al*. (2010) and Lekerpes *et al.* (2014) who all reported the presence of two genetic variants of *β*-LG (A and B) using SacII restriction enzyme in Egyptian and Kenyan goats, respectively.The difference observed may be due to the different restriction enzymes used in their study. Hardy-Weinberg equilibrium could not be tested in the goat populations sampled due to the monomorphic nature of the *β*-LG gene locus.

Table 1: Genotype and gene frequency of β-LG gene locus of Nigerian indigenous goats

		β-LG genotypes			Gene frequency		
Goat breed	No.	AA	AB	BB	A	B	(χ^2)
Sahel	20	20 (1.00)	0 (0.00)	0 (0.00)	1.00	0.00	Monomorphic
Red Sokoto	20	20 (1.00)	0 (0.00)	0 (0.00)	1.00	0.00	Monomorphic
WAD	20	20 (1.00)	0 (0.00)	0 (0.00)	1.00	0.00	Monomorphic
Total	60	60 (1.00)	0 (0.00)	0 (0.00)	1.00	0.00	Monomorphic

No. = number; WAD = West African Dwarf; AA = β-LG AA; AB = β-LG AB; BB = β-LG BB; (χ^2) = test of Hardy-Weinberg equilibrium

Conclusion

This research provides evidence that the β-LG gene locus of West African Dwarf, Red Sokoto and Sahel goats is monomorphic.

References

Anton, I., Zsolnai, A., andFesus, L. (1999). Identification of the variant C of β-lactoglobulin in sheep using aPolymerase Chain Reaction-Restriction Fragment Length Polymorphism method. *Journal of Animal Breeding and Genetics,* 116: 525.

Basanti, J., Rajiv, K., RajniKumari., A.S., Meena, L.L.L., Prince, V.P. and Satish, K. 2014). β-Lactoglobulin gene polymorphism in Indian sheep breeds of different agro-climatic regions. *Indian Journal of Animal Sciences,*84 (10):1133–1136.

El-Shazly, S.A., Mahfouz, M.E., Al-Otaibi, S.A., and Ahmed, M.M. (2012). Genetic polymorphism in β-lactoglobulin gene of some sheep breeds in the Kingdom of Saudi Arabia (KSA) and its influence on milk composition. *African Journal of Biotechnology,*11(19): 4330-4337.

El-Hanafy, A.A.M., El-Saadani, M.A., Eissa, M., Maharem, G.M. and Khalifa, Z.A. (2010). Polymorphism of β-lactoglobulin gene in Barki and Damascus and their cross bred goats in relation tomilk yield. *Biotechnology in Animal Husbandry,* 26(1-2): 1-12.

Erhardt, G. (1989). Evidence for a third allele at the β-lactoglobulin (β-Lg) locus of sheep and its occurrence in different breeds. *Animal Genetics,*21: 197.

Kolde, H.J. and Braunitzer, G. (1983). The primary structure of ovine beta-lactoglobulin, 2: Discussion and genetic aspects. *Milchwissenschaft,*38: 70.

Kumar, S., Gupta, J., Kumar, N., Dikshit, K., Navani, N., Jain, P. and Nagarajan, M. (2006). Genetic variation and relationships among eight Indian riverine Buffalo breeds. *Molecular Ecology* 15: 593-600.

Lekerpes, S.S., Jung'aa, J.O., Badamana, M.S. and Rubenstein, D.I. (2014). Genetic polymorphism ofbeta-lactoglobulin in Kenyan small east African goat breed using PCR RFLP and sequencing.*Scientific Journal of Animal Science,* 3(8): 233-239.

NAFIS (2017). National Farmers Information Service of Kenya. Nutritional value of goat milk. Available online at http://www.nafis.go.ke/livestock/dairy-goat-production/nutritive-value-of-goat-milk/

Peakall, R. and Smouse, P.E. (2008). Genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics,* 28(19)**:** 2537-2539.

Santos, H.F., Carmo, F.L., Leite, D.C.A., Jesus, H.E., De Carvalho Maalouf, P., Almeida, C., Soriano, A.U., Altomari, D., Suhett, L., Vólaro, V., Valoni, E., Francisco, M., Vieira, J., Rocha, R., Sardinha, B.L., Mendes, L.B., João, R.R., Lacava, B., Jesus, R.F., Sebastian, G.V., Pessoa, A., van Elsas, J.D., Rezende, R.P., Pires, D.O., Duarte, G., Castro, C.B., Rosado, A.S. and Peixoto, R.S. (2012). Comparison of different protocols for the extraction of microbial DNA from reef corals. *Brazilian Journal of Microbiology,*43(2): 819-826.

Vanguard Newspaper (2017). Goat milk best for healthy growth-report. Available online at http://www.vanguardngr.com/2017/10/goat-milk-best-healthy-growth-report/

Yunjie, M. and Oluseyi, V. (2013). Comparison of different DNA extraction methods forforensic samples. *Journal of Natural Sciences Research,* 3(11): 32-38.