POLYMORPHISMS WITHIN EXON-3 REGION OF IGF-1 GENE OF TWO MUTURU POPULATIONS IN NIGERIA USING RFLP MARKER

[1]*Antenyi, E.F., [1]Momoh, O.M. and [1]Tor, N.E.T.
[1]Department of Animal breeding and Physiology, College of Animal Science, Joseph Sarwuan Tarka University Makurdi.
*Corresponding author: antenyifaith@gmail.com

ABSTRACT
The IGF1 gene (insulin-like growth factor 1) is a candidate gene for marker-assisted selection strategies. This study was designed to identify and analyze various genetic diversity indices within exon 3 region of the gene in two Nigerian Muturu cattle populations using PCR-RFLP. 20 Muturu cattle were randomly sampled with 11 samples from Tarka in Benue State and 9 samples from Onueke in Ebonyi State. About 0.2ml of blood was collected from the coccygeal vein of the tail-head of the selected cattle with the use of 1-inch, 18-gauge classic needle and syringe. The blood samples obtained from each cattle was used for DNA extraction and amplification following standard procedures. The amplified DNA was digested with the use of SnaB1enzyme and the resultant DNA fragments subjected to Agarose Gel Electrophoresis. Two genotypes (CC and CT) were identified. The genotypic frequencies were 0.95 for CC and 0.05 for CT. A total of 10 Alleles were found with lengths ranging from 300 to 440 base pairs. The allelic frequency ranged from 0.025 to 0.200. This study demonstrates low level of genetic diversity among the studied populations, highlighting the need for genetic improvement and conservation considering the endangered nature of the Muturu cattle breed.
Keywords: Muturu, Genetic diversity, IGF1 Gene, PCR-RFLP

INTRODUCTION

Animal genetic improvement is important for increasing animal productivity. Growth in animals is a complex process in which the interactions between different neuro-endocrine pathways take place and is expressed as growth phenotypically. The genes that regulate or control growth include growth hormone (GH) and insulin-like growth factor-1 (IGF-1) genes (Soller *et al.,* 2000) and they are implicated in the somatotrophic axis. The insulin-like growth factors (IGFs) are proteins with high sequence similarity to insulin. IGFs are part of a complex system that cells use to communicate with their physiologic environment. IGF-1 gene is localized on chromosome 5 and consists of 6 exons in cattle; it is considered a marker for growth rate and meat production because of its role in cell proliferation and growth.

In Nigeria, two types of Muturu cattle have been identified; a larger savannah type and a dwarf forest type. Despite Muturu's ability to maintain good body condition, their fertility, tolerance to trypanosomiasis and cultural roles, Muturu faces threat of extinction since their management level is low as Adebambo (2001) has asserted and low genetic improvement intervention because not much has been reported on the molecular characterization of IGF1 gene which is one of the genes that regulate growth and development, among other genes. The aim of this study is to identify and analyze various genetic diversity indices within exon 3 region of IGF1 gene in the Nigerian Savannah Muturu populations of Benue and Ebonyi states using Restricted Fragment Length Polymorphism (RFLP) as a marker.

MATERIALS AND METHODS

The study was carried out in Tarka local government area of Benue State and Onueke in Ezza South local government area of Ebonyi State.

Experimental Animals

A total of 20 Muturu cattle were randomly sampled with 11 samples from Tarka in Benue State and 9 samples from Onueke in Ebonyi State. Twenty 1-inch of 18 guage classic needle and syringe was used to take about 0.2ml of blood from the coccygeal vein of the tail-head of the selected cattle

DataCollection and Analysis

The blood samples obtained from each cattle was used for DNA extraction which was assessed for quantity and quality using a Spectrophotometer. The set of forward (ATTACAAAGCTGCCTGCCCC) and reverse (ACCTTACCCGTATGAAAGGAATATACGT) primers used by Siadkowska *et al*. (2006) was adopted in the current study for polymerase chain reaction (PCR). Digestion was done with the use of SnaB1 enzyme. Resultant DNA fragments from the Digestion was subjected to 2% Agarose Gel Electrophoresis for visualization and Photography. Genotypes obtained were subjected to statistical analysis to determine AMOVA, Information index,

Heterozygosity, Fixation index, pairwise genetic distance and frequencies of the various alleles using GenAlex software while Gel analyzer was used to detect the base pairs.

RESULTS AND DISCUSSION

Genotypes and Genotype frequencies among the studied Muturu populations

The results of PCR-RFLP of IGF1 gene of the studied Muturu Cattle populations (Table 1) showed the existence of two genotypes (CC and CT), with total frequencies of 95% and 5% respctivly. The presence of only one genotype in the onueke population is similar to the report of Putra *et al.* (2019) who showed only CC genotype in Pasundan cattle. The result of the present study is comparable with the results of Romaz *et al.* (2021) who reported 86% CC and 14% CT in Nyalawi subtype of Baggara cattle. The result of the genotype frequency indicate low genetic diversity among the studied populations which could be the result of gene flow occassioned by geographic proximity.

Table 1: Genotypes and Genotype frequencies among the studied Muturu populations

Genotype	Counts	Genotype Frequencies
CC	19	0.95
CT	1	0.05

Allele Frequencies in the studied Muturu Populations

A total of 10 alleles were observed across the locus for the two populations studied (4 and 6, respectively for Onueke and Tarka) as shown in Table 2. This observed number of alleles difference in the present study might be due to sample size. The number averaged 5 alleles and allele frequency proportion ranging from 0.025 to 0.200 which indicates low allelic frequency. The mean number of alleles obtained was comparable to those obtained in cattle breeds from Mozambique (Besa *et al*., 2009. A higher mean number of alleles were previously reported for different African cattle breeds genetic diversity studies (Ema *et al*., 2014; Cameroon cattle at 10.7, Okomo-Adhiambo, 2002; Kenya cattle at 11.6).

Table 2: Allele Frequencies

Allele	300	310	340	350	370	380	400	430	440
Allele Frequency	0.200	0.100	0.100	0.150	0.075	0.050	0.200	0.100	0.025

Genetic diversity in the studied Muturu populations

The mean estimates of observed and expected heterozygosity obtained in this study were 0.045±0.045 and 0.720±0.053 respectively (Table 3). The mean observed heterozygosity in this study was lower than expected heterozygosity. This report is similar to the report of Nwachukwu *et al.* (2022). Conversely, is the report of Adido *et al*. (2019) who reported higher values of mean observed heterozygosity for three cattle breeds in Benin.

Table 3: Heterozygosity in the studied Muturu populations

Population	Ho	He
Onueke	0.000	0.667
Tarka	0.091	0.773
Total Mean	0.045	0.720
SE	0.045	0.053

Ho= Observed Heterozygosity, He= Expected Heterozygosity, SE= Standard Error

The lower mean observed heterozygosity suggests that a large proportion of individuals sampled were homozygous at the locus. Small effective population, positive assortative mating (mating of likes), inadvertent selection against heterozygotes, long years of inbreeding, and limited contact with other cattle types could be responsible for the low levels of heterozygosity observed across the sampled populations of Muturu, since the animals were sampled from small herds kept by small holder farmers (Adido *et al*. 2019). Pairwise Population Nei's genetic distance estimate (0.305) indicates fairly high genetic relationships among the studied Muturu cattle populations**,** suggestive of possible gene flow between the two cattle populations owing to geographic proximity and breed.

Analysis of Molecular Variance

The AMOVA in the present study revealed 76% among individual variation, 19% among population variation and 5% within individual variation (Table 4). The among population variation of 19% observed in the present study is

similar to the report of Nwachukwu *et al.* (2022) who revealed that 19% of total genetic variation exist among the four Nigerian indigenous cattle populations. This value is higher compared to 1.14% reported by Adido *et al*. (2019). This implies weak or absence of genetic differentiation.

Table 4: Results of Analysis of Molecular Variance

Source of variation	Degree of freedom	Sum of Squares	Mean Square	Estimate of variance component	Variation (%)	F statistics	P value
Among Populations	1	2.675	2.675	0.096	19	FST= 0.193	0.001
Among Individuals	18	14.000	0.778	0.376	76	FIS= 0.938	0.001
Within Individual	20	0.500	0.025	0.025	5	FIT= 0.950	0.001
Total	39	17.175		0.497	100		
Gene flow (Nm)						1.047	

CONCLUSION

The results of this study revealed low genetic diversity among the studied Muturu populations. There is, therefore, a need for possible improvement through breeding and maintenance of diversity by applying appropriate conservation strategies such as introgression of alleles and recombinant DNA technologies

REFERENCES

Adebambo, O. A. (2001). The muturu: a rare sacred breed of cattle in Nigeria. *Animal Genetic Resources,* 31:27–36

Adido, M. S., Gicheha, M. G., Adoligbe, M. C. and Atchou, K. (2019). Genetic diversity of three indigenous cattle breeds reared in Benin. *American Journal of Animal and Veterinary Science*, 14(1): 40 – 29.

Ema, P. J. N., Manjeli, Y., Meutchieyié, F., Keambou, C., Wanjala, B., Desta, A. F., Ommeh, S., Skilton, R. and jikeng, A. (2014). Genetic diversity of four Cameroonian indigenous cattle using microsatellite markers. *Journal of Livestock Science,* 5: 9-17.

Nwachukwu, E. N., Kalla, D. J. U., Ukwu, H. O., Ogbu, C. C., Ezea, J., Udoh, U. H. and Ekumankama, O. O. (2022). Genetic diversity and population structure of four Nigerian indigenous cattle breeds. *Tropical Animal Health and Production,* 54: 132

Okomo-Adhiambo, M. (2002). Characterization of genetic diversity in indigenous cattle of East Africa: Use of microsatellite DNA techniques: AGTR Case Study. Nairobi, Kenya: ILRI.

Putra, W. P. B., Anwar, S., Said, S., Indarto, S. A. A. and Wulandari, P. (2019). Genetic characterization of thyroglobulin and leptin genes in Pasundan cattle at West Java. *Buletin Peternakan*, 43 (1): 1-7.

Romaz, M. A. O., Ikhlas, A. N., Ahmed, D. A. B., Lutfi, M. A. M., Mitsuru, T., Faisal, M. E., Khaleel, I. Z. J., Mai, A. M., Yasunori, K. and Mohammed-Khair, A. A. (2021). "Molecular Genetics and Phenotypic Characterization of Some Beef Quality Traits in Sudanese Baggara Cattle". *Asian Journal of Science and Technology*, 12 (07): 11757-11761.

Soller, M., Rubin, B. K., van Eijk, A. M. A., Moorman, A. F. M. and Liu, J. (2000). "Growth hormone regulates the expression of the insulin-like growth factor-1 (IGF-1) gene in the liver of juvenile chickens." *Journal of Endocrinology,* 166 (2): 249-256.

Siadkowska, E., Zwierzchowski, L., Oprzadek, J. and Strzalkowska, N. (2006). Effect of polymorphism in IGF-1 gene on production traits in Polish Holstein-Friesian cattle. *Animal Science Papers and Reports,* 24 (3): 225-237.