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POLYMORPHISMS WITHIN THE EXON 3 REGION OF IGF1 GENE OF TWO SAVANNAH MUTURU POPULATIONS IN NIGERIA USING RFLP MARKER

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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 IGF1 gene in two Nigerian Savannah 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 (www.whatman.com). The amplified DNA were 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. The mean number of alleles (Na), mean number of effective alleles (Ne), mean information index (I), mean observed heterozygosity (Ho) and mean expected heterozygosity (He) were 5.000 ±1.000, 3.700 ±0.700, 1.416 ±0.202, 0.045 ±0.045 and 0.720 ±0.053 respectively. This study demonstrates low level of genetic diversity therefore, there is 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. In the past, conventional animal genetic improvement strategies such as crossbreeding and selection methods have been used to produce animals with superior traits. These strategies require large number of animals and are time consuming and laborious. Recently, improved technologies have been developed based on genetic markers that allow for rapid and accurate selection of important and unique traits with low estimates of heritability (Zhang *et al*., 2010). These technologies represent a breakthrough for the animal industry. One of such technologies is the DNA-based molecular techniques.

Molecular genetics techniques are of great benefit or use in the identification of variations in genetic markers which are associated with different production and reproduction traits in farm animals (Jiang *et al.*, 2002; Arora and Bhatia, 2006; Missohou *et al.*, 2006). These genetic variations affect the physiological pathways that consequently lead to quantitative variations in different phenotypic characteristics (Schwerine *et al*., 1995; Lan *et al*., 2007). In quantitative genetics, there are number of single genes associated with muscle growth, development and function which have been studied as excellent candidates for linkage relationships with traits of economic importance (Othman *et al*., 2013). Among traits of economic importance are growth performance traits.

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 somatotrophic axis (GH/IGF-I axis) is involved in the neuro-endocrine pathways and it is considered as the key in postnatal growth and metabolism in different mammals including farm animals (Shoshana *et al.*, 2000; Burkhard *et al.*, 2005). One of the most important members of the somatotrophic axis is insulin-like growth factor I (IGF-I) which has a remarkable variation of its biological effect like protein synthesis and skeletal growth (Froesch *et al.*, 1985; Baxter, 1985; Clemmons *et al.*, 1987).

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. This complex system (often referred to as the IGF "axis") consists of two cell-surface receptors (IGF1R and IGF2R), two ligands (Insulin-like growth factor 1 (IGF-1) and Insulin-like growth factor 2 (IGF-2)), a family of seven high-affinity IGF-binding proteins (IGFBP1 to IGFBP7), as well as associated IGFBP degrading enzymes, referred to collectively as proteases (Scarth, 2006).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.

PCR-RFLP generally refers to the differences in banding patterns obtained from DNA fragments, after sequence-specific cleavage with restriction enzymes. In Nigeria, two types of Muturu cattle have been identified; a larger savannah type and a dwarf forest type. Muturu has ability to maintain good body condition by grazing and browsing throughout the year. Although, their management level is low as Adebambo (2001) has asserted, they have been able to survive due to their sacred functionality. The weight of Muturu is low as compared to many Zebus and Sanga breeds, however, their fertility, tolerance to trypanosomiasis and cultural roles makes them important breed in their traditional breeding areas. Despite these utilities, the Muturu faces threat of extinction, productivity is quite low due to poor production environment and absence of genetic improvement intervention since 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. Benue State lies between latitude $60^0$25'N and $80^0$8'N and longitude $70^0$47'E and 100^0E. It is located in the middle belt area of Nigeria. Ebony lies approximately $5^0$40' and $6^0$45'N, and longitudes $7^0$30' and $8^0$28'E (Ebonyi State House of Assembly, 2006)

Experimental Animals

A total of 20 Muturu cattle were randomly sampled with 11 samples from Tarka in Benue State and 10 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, one per animal.

Sample Collection 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 DNA was extracted following standard procedures (www.whatman.com). 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 SnaB1enzyme. 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

Results

The agarose gel electrophoresis result of the PCR-RFLP analysis of amplified IGF1 gene of savannah Muturu is presented in Figure 1, 2 and 3. The banding pattern revealed two RFLP variants which were assigned as CC and CT genotypes. The CC and CT genotypes were detected by the presence of one band and two bands, respectively. Out of the 20 Muturu cattle sampled, 19 had CC genotype showing the single banding pattern while 1 (sample 14) had CT genotype showing the double banding pattern.

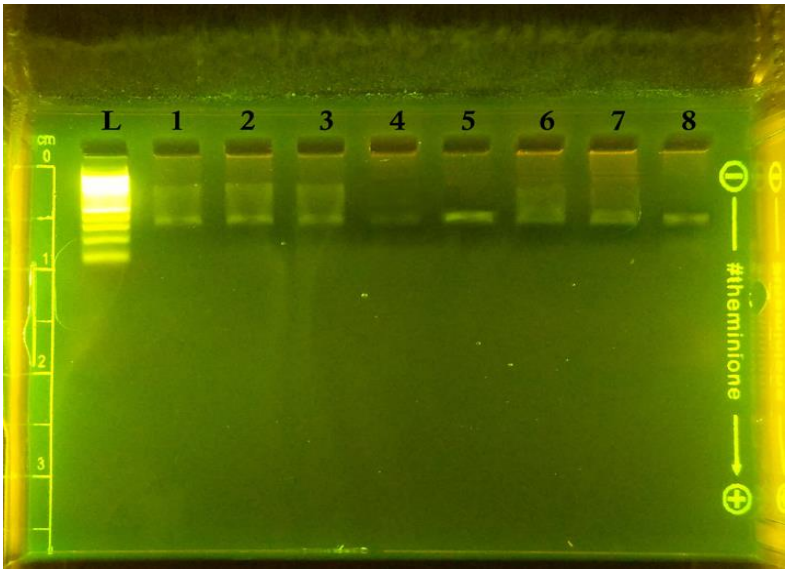

Figure 1: Agarose gel electrophoresis result of the PCR-RFLP analysis of amplified IGF1 gene (Sample 1-8)

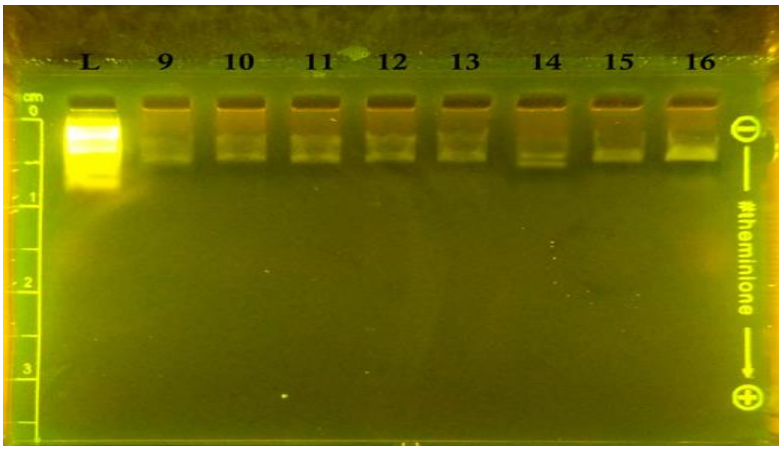

Figure 2: Agarose gel electrophoresis result of the PCR-RFLP analysis of amplified IGF1 gene (sample 9-16)

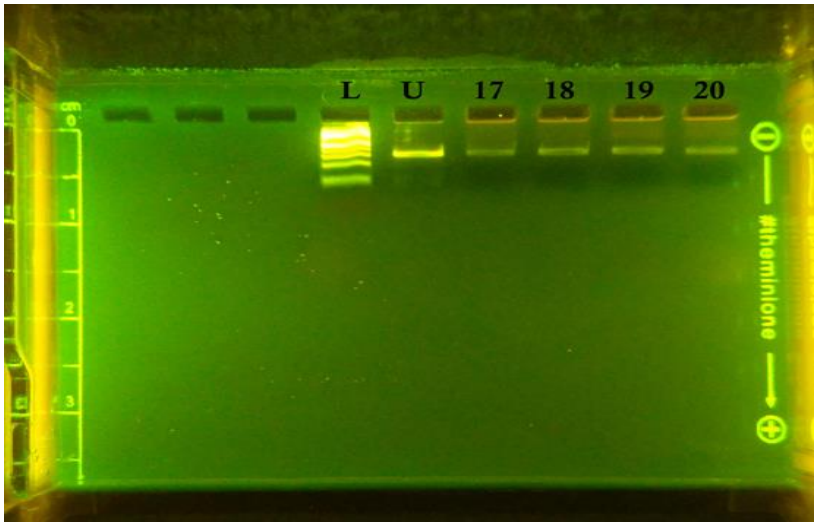

Figure 3: Agarose gel electrophoresis result of the PCR-RFLP analysis of amplified IGF1 gene (sample 17-20)

The genotype and genotypic frequencies of the studied Muturu population is Presented in Table 1. From the result only two genotypes were obtained (CC and CT). The CC genotypic frequency (0.95) was higher than the CT genotypic frequency (0.05) as only one sampled animal out of the 20 has the heterozygous CT genotype.

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

Location	Number of samples	Genotype	counts	Genotype Frequencies	Percentage (%)
Onueke	9	CC	9	1.00	100
		CT	0	0.00	0
Tarka	11	CC	10	0.91	91
		CT	1	0.09	9
Total	20	CC	19	0.95	95
		CT	1	0.05	5

Table 2 shows the identified alleles (the different RFLP fragments), their counts and frequencies in the two studied Muturu populations. A total of 10 Alleles were found with lengths ranging from 300 to 440 base pairs. One common allele (310) was observed. By population the allele 300 was observed to have the highest count (8) and frequency (0.444) under Onueke while allele 400 had the highest count (8) and frequency (0.364) under Tarka. These Two alleles (300 and 400) however had the highest frequency (0.200) across the populations.

Table 2: Allele counts and Frequencies by Locus and Population

Locus	Allele	Allele counts (Onueke)	Allele counts (Tarka)	Allele frequency (Onueke)	Allele frequency (Tarka)	Allele frequency across populations
IGF	300	8	0	0.444	0.000	0.200
	310	2	2	0.111	0.091	0.100
	340	0	4	0.000	0.182	0.100
	350	6	0	0.333	0.000	0.150
	370	0	3	0.000	0.136	0.075
	380	2	0	0.111	0.000	0.050
	400	0	8	0.000	0.364	0.200
	430	0	4	0.000	0.182	0.100
	440	0	1	0.000	0.045	0.025

The genetic diversity at exon 3 of IGF1 gene of the studied Muturu population is presented in Table 3. The table shows the mean number of alleles (Na), mean number of effective alleles (Ne), mean information index (I), mean observed heterozygosity (Ho), mean expected heterozygosity (He), mean unbiased expected heterozygosity (uHe), mean fixation Index(F) to be 5.000 ±1.000, 3.700 ±0.700, 1.416 ±0.202, 0.045 ±0.045, 0.720 ±0.053, 0.758 ±0.052, 0.941 ±0.059 respectively. It was observed that Onueke Muturu had lower Na (4.000), Ne (3.000), I (1.215), Ho (0.000), He (0.667) and uHe (0.706) but has the highest F (0.583). The entire gene locus was found to be polymorphic (100%).

Table 3: Genetic diversity in the studied Muturu populations

Population	N	Na	Ne	I	Ho	He	uHe	F	%P
Onueke	9	4.000	3.000	1.215	0.000	0.667	0.706	1.000	100
Tarka	11	6.000	4.400	1.618	0.091	0.773	0.810	0.882	100
Total Mean	10.000	5.000	3.700	1.416	0.045	0.720	0.758	0.941	100
SE	1.000	1.000	0.700	0.202	0.045	0.053	0.052	0.059	0.00

N= Sample Size, Na= Number of different Alleles, Ne= Number of Effective Alleles, I= Shannon's Information Index, Ho= Observed Heterozygosity, He= Expected Heterozygosity uHe= Unbiased Expected Heterozygosity, F= Fixation Index, %P= Percentage of Polymorphic Loci

Pairwise Population Nei Genetic Distance between Onueke and Tarka was observed to be 0.305 (Table 4) indicating that genetic relationship exists among the two studied populations.

Table 4: Pairwise population differentiation using Nei genetic distance

Population	Onueke	Tarka
Onueke	0.00	
Tarka	0.305	0.00

Variation within and among populations of the two Muturu cattle populations estimated using AMOVA (Table 5) revealed that a large proportion (76%) of the observed variance occurred among individuals within populations, 19% of the variance was contributed due to differences among populations and variation within individual was 5%. Values of FIS, FIT, and FST across the locus were 0.938, 0.950 and 0.193 respectively, while the value of gene flow between the two populations was 1.047.

Table 5: 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	

Discussion

Genotypes and Genotype frequencies among the studied Muturu populations

The results of PCR-RFLP of IGF1 gene of the studied Muturu Cattle populations showed the existence of two genotypes (CC and CT). In the total population the genotype frequencies were 95% (CC) and 5% (CT). 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.

Allele counts and Frequencies by Locus and Population

A total of 10 alleles were observed across the locus for the two populations studied (Onueke 4, Tarka 6). 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 were comparable to those obtained in cattle breeds from Mozambique (Besa *et al*., 2009; 5.9). 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, Ndumu *et al*., 2008; African Great Lakes Region Ankole longhorn cattle at 13.8, Kugonza *et al*., 2011; Ankole cattle of Uganda at 10.5, Ndiaye *et al*., 2015; Senegal cattle at 7.5, Grema *et al*., 2017; Niger at 7.86).

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. The mean observed heterozygosity in this study was lower than expected heterozygosity. This report is similar to the report of Nwachukwu *et al.* (2022), Sharma *et al.* (2015), Demir and Balcioglu (2019) and Suh *et al*. (2014). Conversely, is the report of Adido *et al*. (2019) who reported higher values of mean observed heterozygosity for three cattle breeds in Benin. 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 Adido *et al*. (2019) 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. A heterozygosity of less than 0.5 indicated low variation for this gene in the studied populations. It is suggested that strategies such as migration, introduction of new diversity and crossbreeding for increasing gene diversity and its conservation besides exploration of this potential genetic diversity should be adapted. The positive fixation index (F is an inbreeding index) suggests inbreeding is occurring in the studied Muturu cattle populations.

Genetic Distance between the studied Muturu populations
Pairwise Population Nei's genetic distance estimate (0.305) established fairly high genetic relationships among the studied Muturu cattle populations. This value is similar to the values reported by Madilindi *et al.* (2019) who reported 0.383 genetic distance between Landim and Angone cattle breeds of Mozambique. Similarly, Shelema *et al.* (2023) also reported values ranging between 0.407-0.460 genetic distance between three Ethiopian Indigenous Cattle. This genetic relationship between Onueke Muturu cattle and Tarka Muturu cattle could be due to possible gene flow between the two cattle populations owing to the proximity between the two geographical locations and also the animals being of the same breed type.

Analysis of Molecular Variance
The AMOVA in the present study revealed 76% among individual variation, 19% among population variation and 5% within individual variation. 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), 2.6% by Grema *et al.* (2017) in Niger cattle, 6.1% by Ema *et al*. (2014) in Cameroonian cattle and 4.35% by Tu *et al*. (2014) in yellow cattle in Taiwan. This implies some genetic differentiation, how be it weak among the studied populations (supported by FST = 0.193). In this study, genetic variation within the population was higher than among populations, this suggests interpopulation gene flow, sexual recombination, and mutations.

CONCLUSION
The results of this study revealed that a large proportion of individuals sampled were homozygous at the studied locus of the IGF1 gene. Low genetic diversity exists between the studied Muturu cattle populations. There was Low heterozygosity, high level of inbreeding in the studied Muturu populations and the marker was highly informative in showing genetic diversity. High genetic relationships exist among the studied Muturu cattle populations. Genetic variation within the population was higher than among populations, indicative of lack of population genetic structure and there was High gene flow between the populations. There is therefore need for possible improvement through breeding and maintenance of diversity by applying appropriate conservation strategies such as cross breeding, introgression of alleles and recombinant DNA technologies

REFERENCES
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.

Arora, R., Bhatia, S. (2006). Genetic diversity of magra sheep from India using microsatellite analysis. *Asian-Australasian Journal of Animal Sciences*. 19(7): 938-942.

Baxter, R. C. (1985). The somatomedins: insulin-like growth factors. Advances in Clinical Chemistry, 25: 94-115.

Bessa, I., Pinheiro, I., Matola, M., Dzama, K., Rocha, A. and Alexandrino, P. (2009) Genetic Diversity and Relationships among Indigenous Mozambican Cattle Breeds. *South African Journal of Animal Science*, 39: 61-72.

Burkhard, T., Daniela, K. & Sonia, C. (2005). Growth hormone/insulin-like growth factor-I system in children with chronic renal failure. Pediatric Nephrology, 20:279-289.

Clemmons, D. R., Dehoff, M., McCusker, R., Elgin, R. & Busby, W. (1987). The role of insulin-like growth factor I in the regulation of growth. Journal of Animal Science 65(2): 168-179.

Demir, E. & Balcioglu, M. S. (2019). Genetic diversity and population structure of four cattle breeds raised in Turkey using microsatellite markers. *Czech Journal of Animal Science*, 64: 411– 419.

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

Froesch, E. R., Schmid, C., Schwander, J., Zapf, J. (1985). Actions of insulin like growth factors. Annual Review of Physiology, 47:443-467.

Grema, M., Traoré, A., Issa, M., Hamani, M. and Abdou, M. (2017). Short Tandem Repeat (STR) based genetic diversity and relationship of indigenous Niger cattle. *Archives Animal Breeding*, 60: 399-408.

Jiang, Y. L., Fan, X. Z., Xiao, L. R., Xiang, R. L., Hu, X. X., Du, L. X., Wu, C. X. (2002). Association of T-A mutation in the promoter region of myostatin gene with birth weight in Yorkshire pigs. *Asian-Australasian Journal of Animal Science*, 15:1543-1545

Kugonza, D. R., Jianlin, H., Nabasirye, M., Mpairwe, D., Kiwuwa, G. H., Okeyo, A. M. & Hanotte, O. (2011) Genetic Diversity and Differentiation of Ankole Cattle Populations in Uganda Inferred from Microsatellite Data. *Livestock Science*, 135: 140-147.

Lan, X. Y., Pan, C. Y., Chen, H., Lei, C. Z., Hua, L. S., Yang, X. B., Qiu, G. Y., Zhang, R. F., Lun, Y. Z. (2007). Ddel polymorphism in coding region of goat POU1F1 gene and its association with production traits. Asian Australasian Journal of Animal Science, 20(9):1342-1348.

Madilindi, M. A., Banga, C. B., Bhebhe, E., Sanarana, Y. P., Nxumalo, K. S., Taela, M. G. and Mapholi, M. O. (2019). Genetic differentiation and population structure of four Mozambican indigenous cattle populations. *Livestock Research for Rural Development*, 31(4).

Missohou, A., Talaki, E., Mamam, L. I. (2006). Diversity and genetic relationships among seven West African goat breeds. *Asian Australasian Journal of Animal Science*, 19(9):1245-1251.

Ndiaye, N. P., Sow, A., Dayo, G. K., Ndiaye, S., Sawadogo, G. J. and Sembène, M. (2015). Genetic diversity and phylogenetic relationships in local cattle breeds of Senegal based on autosomal microsatellite markers. *Veterinary World*, 8: 994-1005.

Ndumu, D. B., et al. (2008) Genetic and Morphological Characterisation of the Ankole Longhorn Cattle in the African

Great Lakes Region. *Genetics Selection Evolution*, 40: 467-490.

Nwachukwu, E. N., Kalla, D. J. U., Ukwu, H. O., Ogbu, C. C., Ezea, J., Udoh, U. H. & 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). Characterisation of genetic diversity in indigenous cattle of East Africa: Use of microsatellite DNA techniques: AGTR Case Study. Nairobi, Kenya: ILRI.

Othman, E., Mohamed, F., Nadia, A. and Karima, M. (2013). Genetic Characterization of Insulin Growth Factor-1 and Its Receptor Genes in Egyptian Buffalo (Bubalus bubalis L.). British Biotechnology Journal, 3(4): 592-604.

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.

Scarth, J. P. (2006). "Modulation of the growth hormone-insulin-like growth factor (GH-IGF) axis by pharmaceutical, nutraceutical and environmental xenobiotics: an emerging role for xenobiotic-metabolizing enzymes and the transcription factors regulating their expression. A review". Xenobiotica; the Fate of Foreign Compounds in Biological Systems. 36 (2–3): 119–218.

Sharma, R., Kishore, A., Mukesh, M., Ahlawat, S., Maitra, A., Pandey, A. K. & Tantia, M. S. (2015) Genetic Diversity and Relationship of Indian Cattle Inferred from Microsatellite and Mitochondrial DNA Markers. *BMC Genetics*, 16: 73.

Shelema, K. B., Tesfaye, S. T. & Gebrerufael, G. (2023). Genetic Diversity and Population Structure of Selected Ethiopian Indigenous Cattle Breeds Using Microsatellite Markers. *Genetic Research* (Camb). 2023; 2023: 1106755. Published online 2023 Jan 14.

Shoshana, Y., Liu, J. L. & Derek, L. R. (2000). The growth hormone/insulin-like growth factor-I system: implications for organ growth and development. Pediatric Nephrology, 14:544-549.

Schwerine, M. T., Brockman, G., Vanselow, J., Seyfert, H. M. (1995). Perspectives of molecular genome analysis in livestock improvement an overview. Animal Research and Development, 42:14-26.

Soller, M., B. K. Rubin, A. M. A. van Eijk, A. F. M. Moorman, and J. Liu. (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.

Suh, S., Kim, Y. S., Cho, C. Y., Byun, M. J., Choi, S. B., Ko, Y. G., Lee, C. W., Jung, K. S., Bae, K. H. & Kim, J. H. (2014). Assessment of Genetic Diversity, Relationships and Structure among Korean Native Cattle Breeds Using Microsatellite Markers. *Asian-Australasian Journal of Animal Science*, 27:1548 – 1553

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.

Tu P., Lin D., Li G., Huang J., Wang D. & Wang P. (2014). Characterization of the genetic diversity and population structure for the yellow cattle in Taiwan based on microsatellite markers. *Animal Biotechnology*, 25: 234–249.

Zhang, F., Huang, J., Li, Q., Ju, Z., Li, J., Shi, F., Zhong, J. and Wang, C. (2010). Novel single nucleotide polymorphism (SNP) of the bovine STAT4 gene and their associations with production traits in chinese Holstein Cattle. *African Journal of Biotechnology*, 9(26): 4003-4008.