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GENETIC VARIABILITY AMONG CATTLE BREEDS OF NIGERIA USING THYROID HORMONE RESPONSIVE SPOT 14 ALPHA GENE (THRSPα) THROUGH POLYMERASE CHAIN REACTION (PCR)

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Abstract: The genetic variability among three Nigerian indigenous cattle breeds-White Fulani (WF), Red Bororo (RB) and Sokoto Gudali (SG) was carried out using Thyroid Hormone Responsive Spot 14 Alpha Gene (THRSPα) through Polymerase Chain Reaction (PCR). A total of sixty-seven (67) cattle blood samples comprising 30 WF, 25 RB and 12 SG were used. The DNA were extracted from blood samples using Zymobead Genomic DNA extraction kit after which PCR was carried out using 50 ng template DNA, 1.0µM primer (THRSPα), 16µl Nuclease-free water in a BIONEER Accupower premix. Gel electrophoresis was carried out and the gels scored. Statistical analyses were carried out using GENEPOP, PAST, SPSS version 16 and Tools for Population Genetic Analysis (TFPGA) version 1.3. The allelic frequency ranged from 0.3600 to 0.6400 in A and B alleles, average heterozygosity ranged from 0.4608 to 0.4861. Lowest genetic distance of 0.0010 between WF and RB and highest genetic distance of 0.0058 between SG and RB were identified. Lowest genetic identity of 0.9942 between RB and SG and highest genetic identity of 0.9990 between WF and RB was also identified. Two (2) genetic population clusters were identified in the dendogram; WF and RB are in cluster 1 while SG is in cluster 2. Test of Hardy Weinberg equilibrium revealed variation in genotypic frequencies. These results therefore demonstrate variation among these three Nigerian indigenous cattle breeds which is attributed to the response of the breeds to various stimuli which also enhance their survivability and adaptation to their specific environments. Such variation can also be harnessed for conservation and improvement of our indigenous breeds through selection and breeding strategies.

Keywords: Genetic identity, Heterozygosity, Indigenous cattle, Allelic frequency

Introduction

Livestock play a vital role in the agricultural and rural economies of the developing world. Not only do they produce food directly, they also provide key inputs to crop agriculture through animal power (*ILRI, 1997*). In Nigeria, cattle production represents one of the major protein suppliers to the populace. It has been estimated that there are 13.9 million cattle in Nigeria (*Lawal-Adebowale, 2012*). Generally, cattle are raised for meat, milk, leather, dung, and draft purposes. Cattle as an economically important farm animal in Nigeria requires sound management and adequate conservation strategy through characterization at the molecular level for sustainable development. Genetic variability exists between and within individual livestock genotypes in a population. Such genetic variability can be measured through genetic markers, mitochondrial DNA, DNA sequence or specific genes that produce a detectable trait with a known location on a chromosome and that can be used to study family and population characteristics. The genetic variability found in domestic breeds allows farmers to develop new characteristics in response to changes in environment, diseases or market conditions (*Georg and Christina, 2007)*. Variations at DNA level contribute to the genetic characterization of livestock populations and this may help to identify possible hybridization events as well as past evolutionary trends (*Choudhary et al., 2006)*.

Thyroid Hormone Responsive Spot 14 Alpha gene (THRSPα) is primarily a nuclear protein which is important in the regulation of lipid metabolism. It is a gene that encodes a small acidic protein; it is a transcription factor that controls expression of blood and major lipogenic tissue such as liver and fat. The gene is highly correlated with intramuscular fat content (*Wang et al., 2009*). The expression of this was determined in bovine mammary gland and mapped the THRSP gene to bovine chromosome 29 nearest microsatellite marker RM179 on the USDA linkage map. THRSPα gene have been used successfully in estimating genetic relatedness and variability among various breeds and populations of goats and chicken (*Hirwa et al., 2009; Xiaopeng et al., 2012)* respectively. Most of the indigenous livestock populations in developing countries such as Nigeria have not yet been characterized and evaluated at phenotypic and genetic molecular levels (*Hannotte and Jianlin, 2005*), it is therefore necessary to determine genetic variability among cattle breeds indigenous to Nigeria. This research therefore is aimed at determining genetic variability among White Fulani, Red Bororo, and Sokoto Gudali breeds of cattle by Thyroid Hormone Responsive Spot 14 Alpha gene (THRSPα) through Polymerase Chain Reaction (PCR).

Materials and Methods

This research was carried out at the Animal Science laboratory, Faculty of Agriculture University of Port Harcourt. Blood samples were collected from sixty seven (67) cattle which comprised the following Nigerian indigenous breeds- 25 Red Bororo (RB), 30 White Fulani (WF), and 12 Sokoto Gudali (SG). Three (3) ml of blood was collected from each cattle in a 5ml EDTA bottle and kept at -4^{o}C until the extraction of DNA commenced.

DNA Extraction and Polymerase Chain Reaction (PCR)

DNA from these blood samples of cattle was extracted using ZymoBead Genomic DNA kit protocol. The PCR followed as described by *Hirwa et al. (2009).* The DNA was amplified via PCR in a PCT- 100 Thermal Cycler (Biorad, Hercules, CA) using forward primer (Deletion F: 5'-GCC TCC GTC ACC GAT CAG- 3').The 20 µl amplification reactions contained 50 ng templates DNA, 1.0 µl of each primer. 16 µl Nuclease-free water in a BIONEER AccuPower® TLA PCR Premix. PCR was performed for 33 cycles for 30 sec at 59.5°C, and 1 min at 72°C, after denaturation at 94°C for 2 minute, final extension was carried out for 10 minutes. The forward and reverse primers produce a 127 or 136bp is representative of THRSPα AA genotype and 127bp is representative of THRSPα BB genotype which is indicated by 96bp deletion.

Gel Electrophoresis and scorings of gels

10µl of PCR product was loaded in a 1.5% agarose gel pre-stained with 0.5µg/ml ethidium bromide. Electrophoresis was carried out at room temperature for 1 hour at 100 volts using a Biorad Power Pac^{TM} electrophoresis apparatus (Biorad, Hercules, CA, USA). The resulting amplified bands were visualized with UV light and photographed and were scored using GENE Mate Quanti- Marker 100bp DNA ladder (Bioexpress, UT, USA).

Statistical Analysis

The allelic frequencies and genotype frequencies were estimated by GENEPOP Software package (*Raymond and Rousset, 1995*). Other genetic analyses of data were performed using PAST, SPP version 16 and Tools for Population Genetic Analyses (TFPGA) version 1.3 (*Miller, 1997*).

Results and Discussion

The results of analysis of data are shown in Table 1-3 and Figure 1. Table 1 shows allelic frequency, heterozygosity and percent polymorphic loci in the three

cattle breeds used. Allele frequency is highest in Red Bororo at allele B with the value 0.6400 and lowest at allele A with the value 0.3600. These values are however lower than those reported by *Douglas (2008)* and *Bessa et al. (2009)* who observed allele frequency ranges of 10.16 to 89.84 and 2% to 10% respectively. Variations observed may be attributable to differences in environmental and /or genetic factors. Sokoto Gudali recorded the highest average heterozygosity of 0.5833 at B allele. The result is in consonance with earlier reports on mean observed heterozygosity of 0.574 and 0.5401 for Kherigarh and Kenkatha cattle respectively (*Pandey et al., 2006)*. A 100% polymorphic loci was recorded in this study. This value is at variance with earlier reports of 75% and 98.3% respectively by *Nguyen et al. (2007)* and *Makkawi et al*. *(2007).*

Table 1. Allele, heterozygosity and percentage polymorphic loci for the entire population

	Allele	Number of observed allele	Allele frequency	Number of heterozygosity	Heterozygosity frequency	Average heterozygosity	Average heterozygosity (unbiased)	Average heterozygosity (direct count)	% polymorphic loci
Entire population	1	51	0.3806	41.0000	0.6119	0.4715	0.4750	0.6119	100.000
	2	83	0.6194	41.0000	0.6119				
White Fulani	1	23	0.3833	19.0000	0.6333	0.4728	0.4808	0.6333	100.000
	2	37	0.6167	19.0000	0.6333				
Red Bororo	1	18	0.3600	14.0000	0.5600	0.4608	0.4702	0.5600	100.000
	2	32	0.6400	14.0000	0.5600				
Sokoto Gudali	1	10	0.4167	8.0000	0.6667	0.4861	0.5072	0.6667	100.000
	2	14	0.5833	8.0000	0.6667				

The genetic distance and Nei's identities for similarities and relatedness in the breeds are shown in Table 2. Genetic distance is lowest between White Fulani and Red Bororo with the value of 0.0010 and highest between Red Bororo and Sokoto Gudali with the value of 0.0058. A probable reason for the low genetic distance observed between White Fulani and Red Bororo could be attributed to homogeneity in the environmental and management factors prevalent in similar agro-ecological zones where these breeds are found in Nigeria. The two breeds – White Fulani and Red Bororo also recorded highest genetic similarities/ identities (0.9990).This result could be an indication of gene flow due to hybridization between populations (*Oliveira et al., 2005*).

Table 2. Nei's Identities/distances

Populations compared	Distances	Identities	Unbiased distances	Unbiased identities
White Fulani vs Red Bororo	0.0010	0.9990	-.0155	1.0156
White Fulani Vs Sokoto Gudali	0.0021	0.9979	-.0266	1.0270
Red Bororo Vs Sokoto Gudali	0.0058	0.9942	-.0240	1.0243

The population structures of breeds of cattle used in this study are presented in Figure 1 by the dendogram. Two clusters were identified. White Fulani and Red Bororo are in Cluster 1 with pair wise distances of 0.010. This shows that the two breeds are closely related. Sokoto Gudali is in Cluster 2 with a pair wise distance of 0.020 from Cluster 1 which implies it is not closely related to the other two breeds. Test of Hardy Weinberg's Equilibrium is as indicated in Table 3. Variations in allelic and genotypic frequencies were observed for the AA, AB and BB genotypes across breeds. The gel electrophoresis band shown in Figure 2 also confirm further that polymorphism exist across the cattle breeds being studied. Such variations could be attributed to the response of the breeds to various stimuli which also enhance their survivability and adaptation to the environment. This information could be relevant in breeding planning and strategy for genetic improvement of the Nigerian indigenous cattle breeds.

Table 3: Test of Hardy-Weinberg's Equilibruim

	White Fulani			Red Bororo			Sokoto Gudali		
	Genotype	Observed number of genotype	Expected number of genotype	Genotype	Observed number of genotype	Expected Number of genotype	Genotype	Observed number of genotype	Expected number of genotype
	AA	9	11.4083	AA	9	10.2400	AA	3	4.0833
	AB	19	14.1833	AB	14	11.5200	AB	8	5.8333
	BB	2	4.4083	BB	2	3.2400	BB	1	2.0833
Exact probability			**0.1219**			**0.4095**			**0.5477**

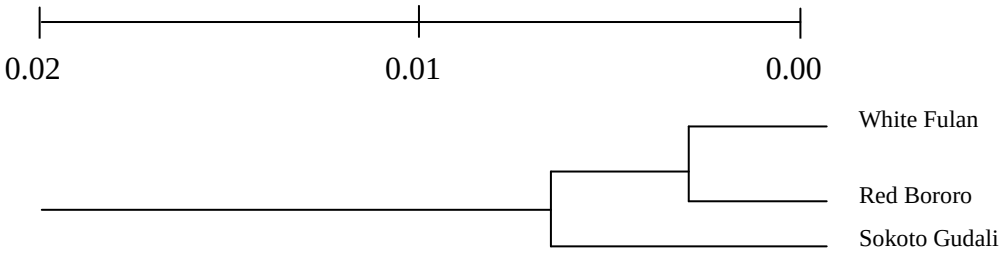

Figure 1. Dendogram showing genetic variability among White Fulani, Red Bororo and Sokoto Gudali indigenous cattle breeds of Nigeria.

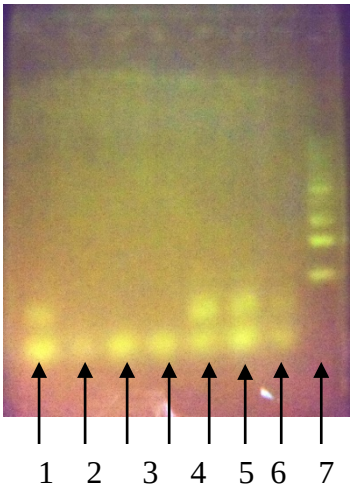

Figure 2. Gel electrophoresis of Thyroid Hormones Responsive spot 14 Alpha Gene (THRSPα) showing both for 7 individuals – 1 is AB, 2 is BB, 3 is BB, 4 is BB, 5 is AB, 6 is AB, 7 is AB and 8 is 100pb Molecular marker

Conclusion

This study revealed that genetic variation exists in White Fulani, Red Bororo and Sokoto Gudali Nigerian indigenous cattle breeds. This study contributes to the knowledge of the genetic variability among the three cattle breeds studied. It also shows that THRSP 14 alpha gene can be used to construct an appropriate measure of variability function through the genetic relationships between these breeds.

The existence of genetic variability allows for the organization of rational conservation and improvement programmes of these breeds based on greater knowledge of their genetic structuring and the relationships between these populations. It was recommended that the number of cattle used be increased in further studies in other to unravel the genetic potentials of Nigerian indigenous cattle breeds for conservation and improvement.

THRSPα u ispitivanju genetske varijabilnosti rasa goveda u Nigeriji metodom lančane reakcije polimeraze (PCR)

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Rezime

Genetska varijabilnost tri nigerijske autohtone rase goveda - White Fulani (WF), Red Bororo (RB) i Sokoto Gudali (SG), ispitana je pomoću hormona štitne žlezde responsivne tačke 14 Alfa gena (THRSPα), korišćenjem lančane reakcije polimeraze (PCR). Ukupno šezdeset sedam (67) uzoraka krvi goveda su korišćeni u istraživanju, od kojih je 30 WF, 25 RB i 12 SG. DNA je ekstrahovana iz uzoraka krvi korišćenjem Zymobead Genomic DNA extraction kit-a nakon čega je izveden PCR korišćenjem 50 ng template DNA, 1.0μM primer (THRSPα), 16μl nukleaze – bez vode u BIONEER Accupower premix-u. Gel elektroforeza je izvedena i gelovi ocenjeni. Statističke analize su izvedene korišćenjem GENEPOP, PAST, SPSS verzija 16 i Tools for Population Genetic Analysis (TFPGA) verzija 1.3. Učestalost alela je bila u rasponu od 0.3600 do 0.6400 u A i B alela, prosečna heterozigotnost u rasponu od 0.4608 do 0.4861. Najmanja genetska distanca od 0.0010 između WF i RB i najveća genetska distanca od 0.0058 između SG i RB su identifikovane. Najniži genetski identitet 0.9942 između RB i SG i najviši genetski identitet 0.9990 između WF i RB je takođe identifikovan. Dva (2) klastera genetske populacije su identifikovana u dendrogramu; WFi i RB su u klasteru 1, dok je SG u klasteru 2. Test Hardy Weinberg equilibrium-a otkriva varijacije u frekvencijama genotipova. Ovi rezultati pokazuju varijaciju imeđu ove tri nigerijske autohtone rase goveda koja se pripisuje odgovoru/reakciji rasa na razne stimuluse čime unapređuju svoje preživljavanje i prilagođavanje njihovim specifičnim sredinama. Takva promena može se iskoristiti za očuvanje i unapređenje naših autohtonih rasa kroz selekciju i strategiju odgoja.

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