NSAP 47th Annual Conference (JOS 2022) CONFERENCE PROCEEDINGS
THEME: SECURING ANIMAL AGRICULTURE AMIDST GLOBAL CHALLENGES

EFFICACY OF MICROSATELLITE MARKER FOR GENETIC STUDIES ON GUINEA FOWL IN NIGERIA

Umar, U.A[1]., Kabir, M[2]., Nwagu, B. I[1]., Umar, H. A[3].Yusuf, S.S[4].
[1]National Animal Production Research Institute, Shika-Zaria, Nigeria.
[2]Department of Animal Science, Ahmadu Bello University, Zaria, Nigeria.
[3]Department of Animal Science, Bayero University Kano, Nigeria.
[4]Centre for Biotechnology Research and Training, Ahmadu Bello University, Zaria
Corresponding Author: uualiyu9@gmail.com, 08098621234.

ABSTRACT

A study was carried out to evaluate efficacy of 31 sets of microsatellite marker developed for helmeted guinea fowl (*Numida meleagris galeata* pallas). A total of 200 randomly sampled indigenous guinea fowl strains namely; lavender (ash), gray breasted (white breasted), pearl, black and broiler guinea fowl comprising 40 per strain from north western Nigeria were used for the study. Two milliliters of blood was obtained from each bird and analyzed using multiplex PCR optimized from different conditions and adjustments. The bands on the electropherogramme were analysed and scored using the image lab™ and result was used to compute Polymorphism Information Content (PIC). The Result showed that the markers were lowly (PIC<0.25) polymorphic in Ash, moderate (0.50 > PIC > 0.25) in Pearl while highly (PIC>0.5) polymorphic in Broiler, Black and white breasted guinea fowl. This research shows that the markers can be used for molecular genetic studies and conservation programmes in Nigeria.
Key words: Efficacy, Microsatellite, Helmeted Guinea fowl, Nigeria.

INTRODUCTION

Until 2005 the genotyping of the individual animals for marker assisted conservation scheme was carried out using the amplified fragment length polymorphism (AFLP) technique (Christopher *et al*., 1997). Afterward, microsatellites have been applied because they are well dispersed in the genome and highly polymorphic (Cheng, *et al*., 1998).Their application to characterize chicken has been used in many countries to study the genetic relationships among native breeds of poultry (Hillel *et al.*, 2003; Takahito *et al.*, 2011).

Microsatellites were described by (Tautz, 1989) as simple sequence-stretches with a high degree of hyper-variability, and are abundant and well distributed in eukaryotic genomes (Cheng & Crittenden, 1994). An important index of polymorphism for microsatellite DNA sites is to estimate the polymorphism of gene markers. The value of polymorphism information content (PIC) indicates the degree of polymorphism as reported by Umar *et al*.(2019).Kong *et al. (*2006)also reported PIC to be an important index of polymorphism of microsatellite DNA sites which were first used to estimate the polymorphism of gene markers in linkage analysis. A site is highly polymorphic when PIC>0.5, normally polymorphic when 0.50 > PIC > 0.25, and lowly polymorphic when PIC<0.25. The study was carried out to evaluate efficacy of 31 set of microsatellite marker developed for helmeted guinea fowl in Nigeria.

MATERIALS AND METHODS

A total of 200 randomly sampled indigenous Guinea fowl strains collected from some parts of North Western States of Nigeria. The strains considered were Lavender (Ash), Gray breasted (white breasted), Pearl, Black and Broiler Guinea fowl y. For each strain, a maximum of forty (40) birds were sampled and 2ml blood was collected from the wing vein of each bird and taken to centre for Biotechnology Research and training of ABU Zaria. Thirty-one (31) sets of primers (Table1) included in the lists of recommended primers for guinea fowl suggested by Botchway *et al*. (2013), fowl gDNA was isolated from blood using a Thermo Scientific® kit according to the procedures described by (Sambrook, *et al.*,1998). The purified gDNA was checked by running it on a 1.0 % Agarose gel stained with G-green.

A multiplex PCR was done by a PX2 Thermo hybaid thermal cycler with 5ng/µL of gDNA as a template in 25µL PCR reaction performed using 9.0µL of Taq 2x Master Mix (Tqman of Thermo fisher scientific™) which includes 0.4mM dNTPs, 50U/ml Taq polymerase, 1.5mM $MgCl_2$, 1.5µL Standard Taq Reaction Buffer and 8µL of primers. The PCR amplification was done using an initial Denaturation at 96°C (4 min), Annealing at 53°C (45 sec), and Extension at 72°C (1.5 min) for 35 cycles according to procedure of (Sanger *et al.*, 1977). PCR products were subjected to electrophoresis on Bio-Rad gel electrophoresis unit on 1.5% Agarose gel prepared in 0.5 × Tris borate EDTA (TBE) buffer. Ethidium bromide (2 µL / 200 ml gel) was added as an illusion buffer and 100 bp (10 µL) ladder by Gei Nei, Meark was used as a size standard. The running buffer comprised of 0.5 X TBE. PCR product (25 µL) was loaded in each well, while the Gel was run at 70 mA Current for 45m to 1 h depending on the voltage for minimum 10 cm distance (3/4 gel slab).

After electrophoresis the product was visualized and scored by Molecular Imager® Gel Doc™ XR+ system with image Lab™ Software of BIO-RAD (Jean-Louis, 2000). The data generated was used to estimate PIC determined using excel macros.

RESULTS AND DISCUSSION

Table 2 showed the Microsatellite markers and their Polymorphism Information Content (PIC) from the five (5) Guinea fowl population studied. The value of Polymorphism Information Content (PIC) ranged from 0.650 to 0.960 in Pearl guinea fowl with mean of 0.269 while Black showed 0.785 to 1.000 with a mean of 0.642. Broiler guinea fowl presented a PIC range of 0.985 to 1.000 with mean of 0.642 as well, and then Ash guinea fowl expressed 0.857 to 0.980 with mean value of 0.207, finally white breasted guinea fowl revealed a value of rage of 0.929 to 0.993 with a mean PIC value of 0.591.

An important index of polymorphism of microsatellite DNA sites ware first used to estimate the polymorphism of gene markers in linkage analysis.

From the result in this study the markers were lowly (PIC<0.25) polymorphic in Ash, moderate (0.50 > PIC > 0.25) in Pearl while highly (PIC>0.5) polymorphic in Broiler, Black and white breasted guinea fowl, indicated a moderate to high gene diversity among the studied markers which is a good indication of high polymorphism(Chang *et al.*, 2007) and they can be used for molecular genetic studies, similar result was also reported by (Liu, *et al.*, 2006) while Kong *et al. (*2006) assessed the genetic variation and established the relationship amongst breeds and strains using 15 chicken specific microsatellite markers, and founded that PIC ranged from 0.562 to 0.872, likewise Chang *et al. (*2007) studied genetic diversity of quail breeds in China based on microsatellite markers, and indicated the highest PIC was 0.573

CONCLUSION

. It can be concluded that the result revealed that the markers were lowly (PIC<0.25) polymorphic in Ash, moderate (0.50 > PIC > 0.25) in Pearl while highly (PIC>0.5) polymorphic in Broiler, Black and white breasted guinea fowl, indicated a moderate to high gene diversity among the studied markers which is a good indication of high polymorphism.

REFERENCES

Chang, G. B., Chang, H., Liu, X. P., Zhao, W. M., Ji, D. J., Mao, Y. J., … Shi, X. K. (2007). Genetic Diversity of Wild Quail in China Ascertained with Microsatellite DNA Markers, *20*(12), 1783–1790.

Cheng, H. H., Burt, D. W., & Dodgson, J. B. (1998). Recent advances in poultry genome mapping. In *Proceeding Symposium Series 1 of the 8th World Conference on AnimalProduction* (pp. 168–178). Seoul, Korea: Seoul National University.

Cheng, H. H., & Crittenden, L. B. (1994). Microsatellite markers for genetic mapping in the chicken. *Poultry Science*, *73*, 539–546.

Christopher, D., Pierre, C. and Claude, C. (1997). Impact of livestock and fisheries on food availability

and demand in 2020. *American Journal of Agricultural Economics*, *9*(5), 147–1475.

Hillel, J., Groenen, M. A. ., Tixier Boichard, M., Korol, A. B., David, L., Kirzhner, V. M., … Weigend, S. (2003). Biodiversity of 52 chicken populations assessed by microsatellite typing of DNA pools. *Genetics Selection Evolution*,*35*, 533–557.

Jean-Louis, V. (2000). Electrophoresis of DNA and other polyelectrolytes: Physical mechanisms. *Reviews of Modern Physics*, *72*, 813–872. https://doi.org/doi:10.1103/RevModPhys.72.813.

Liu, G. Q., Jiang, X. P., Wang, J. Y., & Wang, Z. Y. (2006). Correlations between heterozygosity at microsatellite loci, mean d2 and body weight in a Chinese native chicken. *Asian- Australian Journal of Animal Science*, *19*(12), 1671–1678.

Raymond, M., & Rousset, F. (1955). Testing of heterozygote in a population. *Genetics*, *200*(9), 14–19.

Sanger, F., Niclen, S., & Coulson, A. R. (1977). DNA Sequencing with chain terminator inhibitors. *Proceeding of National Academy of Science USA*, *74*, 5463–5467.

Takahito, O. K., Matsubara, M. T., Masayuki, Furuhashi Masateru, T., & Kawai, T. (2011). *Single-Molecule Electrical Random Resequencing of DNA and RNA*. *Nature*. 8-1 Mihogaoka, Ibaraki, Osaka 567-0047, Japan. https://doi.org/2 : 501 | DOI: 10.1038/srep00501

Tautz, D. (1989). Hypervariability of simple sequences as a general source for polymoorphic DNA markers. *Nucleic Acids Research*, *17*, 6463–6471.

Umar, U. A., Kabir, M., Nwagu, B. I., Umar, H. A., Sulaiman, A., & Abdulrahman, M. (2019). Genetic finger print of helmeted guinea fowl (Numida Meleagris Galeata Pallas) Using Microsatellite Marker In Nigeria. *Journal of Animal Production Research*, *31*(2), 01–14. Retrieved from journals.napri.gov.ng

Table 1. Guinea fowl primers (directly labeled) for genetic diversity assessment

LOCUS	ACCESSION NUMBER	PRIMER SEQUENCE	NUMBER OF BASES	SIZE RANGE (bp)
Nmg01_F	AB819290	CATCCAATACCCTGAACCTA	20	192-196
Nmg01_R		TTAAACCACAAGACCATTCC	20	
Nmg02_F	AB819291	TGAACACGGGCTTAGATAGT	20	123-213
Nmg02_R		GCTTTAGATGGCAATAGTGG	20	
Nmg03_F	AB819292	GTCTTCTCTGACTTTTGGAAAT	22	164-168
Nmg03_R		TACCCACACTGGTACTCTCC	20	
Nmg04_F	AB819293	GCACTAATAGTAGAGTACGCAGAA	24	94-110
Nmg04_R		TGCTAACTCCAAATGACACA	20	
Nmg05_F	AB819294	TGTACATGGTGCGTGTTTAT	20	116-152
Nmg05_R		CGTTTTTGTCCGTACTCAAC	20	
Nmg06_F	AB819295	TGCAAATCATCTTTTTCCTT	20	164-172
Nmg06_R		TCCTCTGACTTATACCAGTTGA	22	
Nmg07_F	AB819296	TGAGAGTGAAATACCTGCAA	20	169-172
Nmg07_R		GATCTGTTAGGGCTGCTAGA	20	
Nmg08_F	AB819297	GATGGCTATTGGGAAATACA	20	206-214
Nmg08_R		CTGGCTTACATATCCTTCCA	20	
Nmg09_F	AB819298	GTCTCCGAGATGTTGGTTT	19	142-147
Nmg09_R		AATCTTTCGCCTCTTACACA	19	
Nmg10_F	AB819299	TCTTGTTCCAGTTGTCATCA	19	108-116
Nmg10_R		ATGCCTCTGCAAATTAGTGT	20	
Nmg11_F	AB819300	AAGTTTTCAGCAAAATCCAG	20	242-246
Nmg11_R		CACATACAGATCATGGGACA	20	
Nmg12_F	AB819301	CAACTAAGTTCCTTGATTTCTCA	23	145-210
Nmg12_R		TGCAGAGTTTCTCTCTTTGAC	21	
Nmg13_F	AB819302	AACAAAGGATGTTTTGTGCT	21	214-233
Nmg13_R		TAAACCAATTTCCAGCATTT	20	
Nmg14_F	AB819303	GGCTGTGTGAAAGGAGAGTA	22	149-163
Nmg14_R		GCCAACATGCCTAACTGTAT	20	
Nmg15_F	AB819304	TCTTGTTCTCCATCAGCTCT	20	118-120
Nmg15_R		CACAAAACACCTGCTCACTA	20	
Nmg16_F	AB819306	CAACATTTGTCTGGTGTCAG	20	252-257
Nmg16_R		AGTCAAATGGTTGTGGATGT	20	
Nmg17_F	AB819307	TCTTCCTTCAGAGGTACCAA	20	223-232
Nmg17_R		TGAAGACCATAGAAGCCTGT	20	
Nmg18_F	AB819308	ACTTCATGAGGTTCAAATGG	20	264-266
Nmg18_R		TGGAATCTAGCTTGTTGGTT	20	

Nmg19_F	AB819309	TCTGAAGTATCTGCCCTGAG	20	135-139
Nmg19_R		TTATCAAGTGAGCGATCAGA	20	
Nmg20_F	AB819310	ATTCTCTGGAATGGGAATTT	20	200-204
Nmg20_R		ATAGTGGCATGGTTCTCTTC	20	
Nmg21_F	AB819311	TGCTAAATTATGTGCAGCAG	20	164-173
Nmg21_R		CCAACCTAAGCCATACTAGG	20	
Nmg22_F	AB819312	TTCCAGCTTGAAAACTGACT	20	203-234
Nmg22_R		CACAGACACAGACCATTGAG	20	
Nmg23_F	AB819313	AACCTGCAGAAACACATTTT	20	230-236
Nmg23_R		CTGCAATACTTCATTTGTGG	20	
Nmg24_F	AB819314	TCTCTCCTGACTTCCAAAAA	20	226-236
Nmg24_R		AGGCTTGAACTCATGGACTA	20	
Nmg25_F	AB819315	CACAAGTGTGAAGCAATGTC	20	207-211
Nmg25_R		ACATCTATGGCCTCAGACAC	20	
Nmg26_F	AB819316	AAACAGAAGGTGAATGCTGT	20	261-286
Nmg26_R		GTAGCTGTGCACCTCACC	18	
Nmg27_F	AB819317	GGCCCTATCCTCAAATAGTCTCC	23	236-252
Nmg27_R		TCAAAGCCTGTAAGAAGTGCTC	22	
Nmg28_F	AB819318	GAATCCAGTGACACACCTTT	20	209-235
Nmg28_R		TATTTAATGAGGGCTGCTTT	20	
Nmg29_F	AB819319	CCTCTTCCTCCTCGTTTAAT	20	241-278
Nmg29_R		TCCTTTTGCTAGAACACACA	20	
Nmg30_F	AB819320	TGAGATCAGGTGGAAAAACT	20	203-209
Nmg30_R		TACATTTCCTGCTCTCCAGT	20	
Nmg31_F	AB819321	CCTTGTTCCAGAGCTGTAGT	20	175-181
Nmg31_R		GGAAGGACAAATAAAGCAAA	20	

F: forward sequence **(5'→3')**, **R:** reverse sequence **(3'→5'), bp**: Base pair

Table 3. Microsatellite markers, polymorphism information content (PIC) Pearl guinea fowl

	PIC				
LOCUS	**Pearl**	**Black**	**Broiler**	**Ash**	**White Breasted**
AB819290	0.96	0.785	0.985	-	-
AB819291	0.96	0.785	0.985	0.857	0.929
AB819292	-	-	-	-	-
AB819293	0.96	0.806	0.993	0.878	0.943
AB819294	-	0.857	0.994	-	0.953
AB819295	-	0.857	-	-	-
AB819296	-	0.857	-	-	-
AB819297	0.96	0.857	-	0.898	0.955
AB819298	-	0.860	0.994	-	0.955
AB819299	-	0.860	-	0.918	0.956
AB819300	-	0.860	0.995	-	0.958
AB819301	0.96	0.860	0.995	0.939	0.959
AB819302	-	0.992	0.996	-	0.959
AB819303	-	0.992	0.997	-	0.966
AB819304	-	-	-	-	-
AB819306	-	0.992	0.997	-	0.967
AB819307	-	0.992	0.997	-	0.968
AB819308	-	-	-	-	-
AB819309	-	-	-	-	0.970
AB819310	-	-	0.998	-	0.969
AB819311	-	0.992	-	-	-
AB819312	0.65	0.992	0.998	-	0.974
AB819313	-	0.994	0.998	-	-
AB819314	-	0.994	0.998	-	0.982
AB819315	0.96	0.994	-	0.959	-
AB819316	0.96	0.996	-	-	-
AB819317	-	-	0.999	-	0.984

AB819318	0.96	0.998	0.999	0.980	0.986
AB819319	-	1	0.999	-	0.993
AB819320	-	-	1.000	-	-
AB819321	-	1	1.000	-	-
Mean PIC	**0.269**	**0.715**	**0.642**	**0.207**	**0.591**

Polymorphism Information Content: (PIC)