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PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF FUGA CATTLE BREED BASED ON THE ANALYSIS OF MICROSATELLITES MARKERS

Ibrahim H. Ahmed, Abdel Aziz A. Makkawi, Salah-Eldein. Sid-Ahmed, Ahmed S. Abdoon, Mohammed Saber Hassanane, Sally S Alam

Sudan University of Science and Technology, College of Agriculture Studies, Dept. of Animal Production, Sudan.

ABSTRACT

A survey was conducted through questionnaire guided interviews with cattle owners (household survey) in selected districts in Fuga cattle habitats. Five villages were selected according to the clustering of villages within the district and accessibility. A set of detailed structured questionnaires were prepared and used to collect information from a total of 150 cattle owners in fuga cattle area. The questionnaires were pre-tested to check clarity and appropriateness of the questions. Some of the information collected during interviews was supported by observation. Milk yield per lactations , milk yield per day ,lactation length , age at first calving ,Calving interval, number of services per conception ,and weight of birth of Fuga cattle breed were presented (1100.96±86.18 kg, 5.26±0.29 kg, 208.60 ± 5.37 days, 40.49, 15.90± 0.44 months, 2.27±0.7 and 22.06±0.44kg) respectively. The population structure and genetic diversity were analyzed using nine microsatellite markers, These markers showed a high level of polymorphism; 64 alleles were identified. The mean number of alleles per locus was 7.11. The mean expected heterozygosity was (0.725) lower than the mean of observed heterozygosity which was (0.778), Polymorphic information content(PIC),Showed substantial variation in PIC among the markers. The mean PIC was 0.664. FST value in all loci was within the average of 0.149.

Keywords: Phenotypic, Genotypic, microsatellite DNA, Fuga cattle Breed.

INTRODUCTION

Livestock plays an important role in production of food and represent great socio-economic and cultural values in various societies around the world. Indigenous cattle form the backbone of relevant and sustainable livestock production in most Eastern African countries. In Sudan, the rural communities own 80% of the livestock the nomadic tribes own 90% of that rural holdings, with livestock playing a central role in their livelihoods. They are well adapted to the local environmental conditions (e.g. tolerance to heat stress and they are able to survive long periods of feed and water shortage [1], however, they show correspondingly low performance level demonstrated by a low juvenile growth rate, late sexual maturity, low milk yield and long calving interval. There are many types of cattle in the Sudan, and many authors have attempted to classify the local breeds on the basis of their origin and phenotypic characteristics. Bennett *et al*., (1948) classified the Sudanese local cattle into three main groups; namely, Northern or Arab, Southern or Nilotic and the small cattle of the Nuba mountains [2]. According to Joshi *et al*., (1957) and Payne (1970), the Northern Sudan cattle include Kenana, Butana, Western Baggara, White Nile and Northern Province. However, those classifications were based on phenotypic characteristics or geographic origin and are not related to genotype except in as much as the phenotype is in part a reflection of genotype [3-4]. Other types of Northern Sudan Zebu cattle include Aryshi (of eastern Sudan), White Nile cattle, Fuga or Dar El Reeh cattle of the North Kordofan. Cattle population in Sudan is 41.56 millionheads [5]. Thegenetic characterizationof

Corresponding Author:-Ibrahim H. Ahmed Email:- hummeada1977@hotmail.com

populations, breeds and species allows evaluation of genetic variability ,which is a fundamental element in working out breeding strategies and genetic conservation plans. Molecular markers have been comprehensively exploited to assess this variability as they contributeinformation on every region of the genome, regardless of the level of gene expression. Microsatellites (highly polymorphic simple sequence repeats) are presently the most favoured molecular markers, essentially owing to the option of blending their analysis with use of the polymerase chain reaction (PCR). Microsatellites have been effectively exploited to understand bovine domestication and migration pattern [6-8] and to evaluate genetic diversity and relationships among cattle populations [9-13]. The objective of this study to identify the phenotypic and genotypic characterization of Fuga cattle in western Sudan, Based on the analysis of Microsatellites Markers.

MATERIAL AND METHOD

Study area :North Kordofan State is located between latitudes 11.15 and 16.45$^{o}_{N}$ and longitudes 27.05 to 32^{o}E. It covers an area of about 245,000 km^{2}, representing two third of the region In North Kordofan, the rainy season does not last for more than three months. Rains occur between May-October with the peak in August. Northern part of Kordofan region characterized by extremely high rainfall variability from year to year as well as from one place to another. Within and between seasons variation in rainfall amount and distribution is common.

Questionnaires survey method: Structured questionnaire was prepared and used to collect information from cow owners under field condition in one visit interview and productive and reproductive performance of their cows were studied. The questionnaires were checked for clarity of the questions prior the interview respondents were briefed to the objective of the study. Following that, the actual questions and questionnaires were presented. Accordingly, information about the .milk yield per lactations , milk yield per day ,lactation length , age at first calving , Calving interval, number of services per conception ,and weight of birth were collected.

For blood sample collection , thirty blood samples were collected from different regions representing the breed under study.Randomly selected pure adult individuals of Dar el Reel cattle (Fuga cattle breed) collected sample were from fuga area. 5 ml from peripheral blood was collected from a vein in the neck of each animal on a vacationer tube containing EDTA as Anti.coagulant matter. The samples were transferred to the laboratory in a shadow and kept away from the direct sunlight. The samples were kept at 4 °C and processed for DNA extraction in a period not exceeds 7 days from its arrival to the laboratory.

As for DNA it was extracted in the Ministry of science and Technology, central Lab . Sudan. The method described by Sambrook*et al.,* (1989), was used for DNA isolation proteinase K digestion phenol/chloroform extraction and ethanol precipitation [14].

For DNA quantification; the DNA concentration was measured in National Research Center; Dokki Cairo, Egypt using the U.V spectrophotometer at wavelength 260 nm. The concentration was adjusted to 50 ng (nano-gram) by adding sterile double distilled water. Concerning Microsatellite analysis, it was carried out in Facultad de veterinaria 28040 Madrid - Spain. One PCR multiplex consists of nine fluorescence-labeled microsatellite primers(ETH 10, ETH 225, BM2113, BM1824, SPS115, TGLA122, TGLA126, TGLA227 and INRA23) were used for the analysis. The multiplex is under the recommendation of*ISAG (2012).* For amplification, 100 ng of genomic DNA was added to a reaction mixture containing 50 pMol of fluorescence-labeled forward and reverse primers; 200 µM of every dNTPs; 1.5 mM of MgCl2 and 0.5U of Taq polymerase in a final volume of 25 µl. The amplification procedure was: initial denaturation step of 1 min at 95°C, 35 cycles of 1 min at 95°C, annealing 1 min at 57°C and 1 min at 72°C and a final extension of 5 min at 72°C.

STATISTICAL ANALYSIS

POPGENE Version 1.31.software package [15] was used to calculate allele frequencies, observed number of alleles, effective number of alleles [16], observed (Ho) and expected (He) heterozygosity at each locus in the breed under study. Polymorphism information content (PIC) value for each locus was calculated by using the method described by Bostein *et al.,* (1980) [17]. Using the variance-base method of *Weir and* Cockerham (1984) [18], population differentiation by F-statistics was computed using FSTAT version 2.9.3.2 computer program [19]. Mean and standard deviations of the F-statistics program, F .f, that are analogue to Wright's (1951, 1978) [20-21] FST and FST, were obtained across breeds by the Jackknifing procedure over loci [22]. .The extent of global inbreeding was further studied with the same software by estimated FST value.

RESULT AND DISCUSSION

The demonstrated reproductive performance and milk performance of Fuga cattle under field conditions are shown. Table (1).Milk yield per lactations was 1100.96±86.18 kg/lactation of Fuga cattle under field conditions was much higher than the yield under field condition of Butana (538.26 kg) and Kenana (598.73 kg) cattle . However, Butana cattle in Atbara Livestock Research Station yielded 1662.57 ± 108.96 kg/ lactation (*Musa et al 2005*) and Kenana cattle in Um-Benein Livestock Research Station yielded 1423.58 ± 551.70/ lactation [23]. The milk yield under research stations of

those two breeds was much higher than their yield under field condition and yield of present study.Milk yield per day (kg) in table (1) obtained in this study was 5.26±0.29 kg less than that obtained by El-Habeeb (1991) and Muas *et al.,* (2005) [23-24] for Knana and Butana(5.60 ± 1.77 , 6.10 ± 0.41 kg) respectively. In most modern dairy farms, a lactation length of 305 days is commonly accepted as a standard. The lactation period of fuga cattle in table (1) was 208.60 ± 5.37 days, lower than that reported by Alim., 1960 [25], 224±85) days for kenana at the Gazira Agriculture Research station herd. Compared with present study in field condition. The age at first calving offuga cattle was 40.49 ± 6.76 month, table (1) lower thanButana breed (43 months) , and kenana (45.2 months) but with good management; this could be reduced to 32 months [26]. While El-Habeeb (1991) reported the age at first calving of Kenana Breed is (47.01 ± 12.91 month). Calving interval in the present study was (15.90±0.44) months table (1) higher than, regular calving of dairy cows (12-13 month).this result is comparable with those reported by *El-Habeeb (1991*) for Sudanese Kenanacattle calving interval which was 446.10±2600 days . Table (1),also show the Number of service per conception was 2.27±0.7 in fuga breed, while El-Amin *et al*., (1981) concluded that NSC did not differ significantly between Red Butana and Red Butana crosses (average 2.6) but was influenced by month of calving. Such differences might, probably due to changes in management. The number of services per conception (NSC) depends largely on the breeding system used. It is higher under uncontrolled natural breeding and low where artificial insemination is used. NSC values greater than 2.0 should be regarded as poor. The average birth weight of fuga cattle breed is 22.06±0.44 kg table(1) while The birth weights for Butana breed in male are 25.64 kg and for female 24.29 kg respectively and the calves weight of kenana cattle was 24.8 kg and 23.5 kg at birth respectively for male and female [27]. All were higher when compared with the result in present study. Birth weight is a result of maternal environment and genetic potential-for birth weight of the parents. From the above mentioned results it's clear that Fuga cattle under field condition is doing well without any interference compared to Kenana and Butana cattle, so it conclusion best results for Fuga cattle under controlled environment, can be predicted.

The result in table (2) indicated that all the microsatellite loci analyzed in this study have demonstrated high genetic variation, which indicates rich gene pool in Fugacattle.In the present study of these cattle breed based on this criterion, the 9 microsatellites loci that were used are suitable for population genetic analyses. The total number of alleles found in this study was 64, with the mean of 7.11 per locus was higher than those found in European breeds and South eastern European breeds [28] was (MNA 6.5), and lower than those found in Chinese yellow *cattle(MNA16.55*) [29]. The mean number of alleles per locus 7.11 lies within the range of 6-9 alleles, which was reported in many cattle breeds from Europe, West Africa and Brazil [30-32]. This is an indication for the high ratio of heterozygosity which arises from the absence or weak selection or organized breeding programs for Fuga cattle. The average observed heterozygosity (Ho) in table (2) was 0.778 ranged from(0.500 to1.00), this level in breed under study is higher than obtained in the Mbarara population (Ho 0.737*)* [33] and Bhutan cattle breeds (Ho 0.67). The value of expected heterozygosity (He) was ranged between (0.468- **0**.849) with a mean value of 0.725 which indicated highe level of gene diversity . This result is in agreement with studies of indigenous populations of Chinese yellow cattle [33], Vietnamese cattle population (Shavea)and Ethiopian cattle [34], which was (He 0.73) indicating that high level of gene diversity. The He (0.651 - 0.725) for the present study was much higher than 0.662±0.030 which was observed within the Spanish Alberes cattle breed [35] and 0.45–0.69 [36] reported among African and European cattle breeds respectively. The Creole breed Casanare in Colombia (He 0.82) is higher than present study. The value of genetic diversity or He in breed under study was higher than that found in other breed such as Simmental (He 0.58), Nelore (He 0.51) [37], the Highland breed (He 0.57),Hereford (He 0.60), and Shorthorn (He 0.58) [38].

When a process of genetic isolation persists for several generations, the main consequence, from the point of view of population genetics, is inbreeding and genetic drift [40]. PIC values indicate the informativeness of the microsatellite loci studied. In the present study the PIC values for all the 9 microsatellite loci ranged from 0.444 to 0.793 in table (2) with the mean value of 0.664, PIC values were observed for BM2113, eight out of nine locus included the present study was highly in formative (PIC > 0.5) and thus will be useful to evaluate the genetic diversity in the breed under study. In general the observed high values of average gene diversity and PIC values could be attributed to the absence of homozygozity and the large number of alleles or heterozygosity observed [40]. In other words, the high value of observed heterozygosity could be due to the absence of selection and the animals were subjected to random mating [41], and this is actually the situation in our cattle population. were in general higher than reported in some Italian cattle breeds (0.55-0.63), which reported by *Del Bo et al. (2001),* and PIC 0.642 in Slovakian Pied cattle [42] is lower than PIC in present study.In Simmental breed mean PIC value was 0.757 in Czech Pied cattle, is higher than PIC in present study. The result in table (2) illustrated Inbreeding estimates (FST) within breed of Fugacattle and showed that Fst value in all loci was not significantly different from zero ($p>0.05$) and the extension between 0.032 (TGLA122) and 0.190 (TGLA126) with average is 0.149 demonstrating absence in the heterozygosis deficit and, consequently, a small probability of inbreeding occurrence in Fuga cattle. In

general it is noticed that most in breeding value were below the zero. This value according to animal breeders meaning the absence of inbreeding within breed study.So it is concluded that there is high allelic diversity in this breed, even though a low effective population size has been maintained and the level of inbreeding has not been monitored.

Table 1. Production and reproduction performance of Fuga cattle under field conditions

Parameters	No.	Mean ± S.E
milk yield per lactation (kg)	150	1100.96±86.18
Milk yield per day (kg)	150	5.26±0.29
Lactation period(days)	150	208.60± 5.37
Age at first calving(months)	150	40.49 ± 6.76
Calving interval(months)	150	15.90 ± 0.44
Number of service per conception	150	2.27±0.7
Weight of birth per (kg)	150	22.06±0.44

Table 2. Number of alleles (No observed, Ne effective), Heterozygosity (Ho observed, He expected), polymorphism information content (PIC), size-range and within population inbreeding estimates (*F*IS) at microsatellite loci in Fuga cattle

Loci	Alleles Size	No	Ne	Ho	He	PIC	FST
BM 1824	178-192	5.00	2.638	0.727	0.635	0.586	0.079
BM 2113	129-145	7.00	5.500	0.773	0.837	0.793	-0.039
ETH10	209-225	7.00	2.495	0.636	0.613	0.562	-0.061
ETH225	140-158	7.00	3.482	0.773	0.729	0.683	0.148
INRA23	196-216	8.00	5.867	0.682	0.849	0.791	-0.069
SPS115	244-260	7.00	1.844	0.500	0.468	0.444	0.076
TGLA122	137-173	9.00	4.156	1.000	0.777	0.783	0.032
TGLA126	115-127	6.00	5.348	0.955	0.832	0.668	0.190
TGLA227	77-101	8.00	4.341	0.955	0.788	0.667	0.035
Mean		7.11	3.963	0.778	0.725	0.664	0.149

No: observed Number of alleles, Ne: effective Number of alleles, Ho: observed Heterozygosity, He: expected Heterozygosity , PIC: Polymorphism Information Content.

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