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Preliminary investigation of haemoglobin polymorphism and association with morphometric traits in West African Dwarf goats in north central Nigeria

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Abstract

The aim of this study was to characterize the genetic pool of the West African Dwarf (WAD) goats using haemoglobin (Hb) polymorphism, as well as the association of some morphological traits with the Hb variants. Blood samples were collected from a total of 104 mature goats of both sexes belonging to the WAD breed in north central Nigeria. The red cell lysates were subjected to cellulose acetate electrophoresis and specific staining procedure to reveal the band patterns of haemoglobin. Three co-dominant alleles, causing the presence of three genotypes (AA, AB and AC) were detected among individual goats. The frequencies of the A, B and C alleles were 0.69, 0.30 and 0.01, respectively. The corresponding genotype frequencies for AA, AB and AC in the goat population were 0.37, 0.61 and 0.02, respectively. The discrepancy between the observed and the expected genotype number was significant ($P<0.05$) thereby violating the Hardy-Weinberg frequencies. The expected heterozygosity (H), which is a measure of gene diversity in the population, was 0.57; while the local inbreeding coefficient (F) was -0.055 indicating disassortative mating. Apart from body weight and heart girth which were higher ($P<0.05$) in individuals with Hb AA genotype, other body parameters were not significantly influenced ($P>0.05$) by Hb variants. There is a need for further study encompassing more number of goats covering larger areas, use of DNA markers and their relationship with economic traits as well as performance study for detailed understanding of breed characteristics, conservation and genetic improvement of WAD goats in north central Nigeria.

Key words: haemoglobin, polymorphism, morphometric traits, goats, Nigeria

Introduction

The importance of goat breeding has been increasing in Nigerian economic conditions. Genetic variation has become the objective tool traditionally used for improving animal species (Melus et al., 2009). Recent developments in the different branches of biotechnology, including reproductive technologies, the use of genetic markers and, to some extent, the possibility of genetic modifications may provide the opportunity for new approaches in the characterization, conservation and sustainable utilization of animal genetic resources (AnGR) (Gama and Bressan, 2011; Ivanova et al., 2011). A number of blood protein systems have been found to exhibit heterogeneity in different species. One of the important blood proteins is haemoglobin, an "evergreen" red protein (Bettati et al., 2009) that

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has attracted attention because of its biochemical, biophysical and physiological properties, having relevance to the selection phenomenon of animals (Hrinca, 2008; Yakubu and Aya, 2012).

Vertebrate Haemoglobin, contained in erythrocytes, is a globular protein with a quaternary structure composed of four globin chains (two alpha and two beta) and a prosthetic group named heme bound to each other (De Souza and Bonilla-Rodriguez, 2007). The alpha globin genes are a relevant example of gene families which have arisen from a single-gene by gene duplication events. In mammals, nonallelic loci produce unequal amounts of alpha globin which may give rise to quantitative polymorphism with the upstream gene being the most efficient (Hurles, 2004; Pieragostini et al., 2005). Nafti et al. (2013) reported frequencies of 0.779 and 0.757 (HbAA), 0.206 and 0.226 (HbAB) and 0.013 and 0.017 (HbBB) for Arbi and Serti goats in Tunisian Oases. Bindu and Raghavan (2010) reported the predominance of HbA (0.987) allele compared to the frequency of 0.012 of HbB allele in the pooled population of Malabari goats in India. The frequencies or HbA and HbB alleles were calculated as 0.83+0.022 and 0.17±0.022 in hair goats (Elmaci, 2001). Type A was the only adult Hb observed in adult Batinah and Jebel Akhdar goats. In contrast, only 34 % of the Dhofari goats were homozygous for Hb A, while 66 % were heterozygous for Hb A and Hb B. Dhofari goats with type AB could further be differentiated into those with approximately 67 % type A and 33 % type B and those with approximately 33 % type A and 67 % type B. None of the goats was homozygous for type B (Johnson et al., 2002). Haemoglobin types have been associated with some performance traits in animals (Bezova et al., 2007; Boonprong et al., 2007). Sam (2012) reported that HbAA was superior to other genotypes in body conformation traits of goats in north western Nigeria where Red Sokoto goats predominate; and these are morphologically close to WAD goats in the north central zone of the country. In a related species, Akinyemi and Salako (2010) reported that hair and horn length were longer for HbAA than HbBB phenotypes in Nigerian sheep. The most valuable features, concerning shape and size of hair curls, as well as the quality and lustre of hair fibres are associated with phenotype HbAB and the weakest association of these features occur with the phenotype HbBB (Hrincă and Vicovan, 2011). Chineke et al. (2007) reported that Hb of AB rabbits performed better than HbAA in individual kit weight and average litter weight at weaning and post-weaning ages of 35 and 56 days.

Although attempts have been made at the morphological characterization of goats in northern Nigeria (Yakubu et al., 2010; Yakubu et al., 2011), there is dearth of information on their molecular characterization. Therefore, the present investigation aimed at describing the genetic structure from the level of the determinant locus of haemoglobin in West African Dwarf (WAD) goats in north central Nigeria, and the association of the haemoglobin variants with morphometric traits.

Materials and methods

A total of 104 West African Dwarf goats of both sexes and of equal number (52 animals in both cases) were randomly sampled in Nasarawa State, north central Nigeria. The State falls within the guinea savanna agro-ecological zone, and is found between latitudes 07º52'N and 08º56'N and longitudes 07º25'E and 09º37'E, respectively. The mean monthly temperatures in the State range between 20 and 34 ºC, with the hottest months being March/April and the coolest months being December/January (Lyam, 2000). The animals which were reared through the extensive management system originated from different herds sampled in the state. They were approximately three years of age. Age was determined from the available records on goats provided by the livestock keepers; and where information was missing, the age of each goat was estimated using dentition.

Blood samples (5 mL per animal) were obtained from the jugular vein and placed in tubes with EDTA (Ethylene-diamine tetra-acetic acid) as anticoagulant. The red cells were separated, washed in saline solution and lysed with distilled water. Haemoglobin was typed using cellulose acetate electrophoresis as described by Imumorin et al. (1999) with a slight modification (Yakubu and Aya, 2012). The identification of the haemoglobin types in goats was achieved in accordance with the migration speed of the light spots on the electrophoretical substratum, detected from the start line towards the cathodal zone. The direct gene counting method was used to score Hb bands based on the separation of Hb variants. Haemoglobin typing was carried out at the Dalhatu Araf Specialist Hospital, Lafia, Nasarawa State, Nigeria. Body weight (kg) and nine linear body measurements (cm) namely, height at withers (WH), rump height (RH), heart girth (HG), body length (BL), face length (FL), ear length (EL), horn length (HL), rump length (RL) and rump width (RW) of each animal were also measured. The anatomical reference points and procedure were as adopted by Yakubu et al. (2011).

Statistical analysis

Genotype and gene frequencies of Haemoglobin (Hb) alleles were estimated according to Hrinca (2008). Data on Hb alleles and of genotype frequencies were subjected to chi-square analysis to test for goodness-of-fit for observed and expected frequencies under Hardy-Weinberg equilibrium (HWE). Heterozygosity (H) was estimated as the expected proportion of heterozygotes under HWE. Local inbreeding coefficient (F) was also calculated to know the extent of inbreeding or otherwise in the goat population. The t-test was employed to determine the effects of haemoglobin variants on the morphometric traits of WAD goats using SPSS (2010) software.

Results

The haemoglobin polymorphism in the WAD goats stood out by identification in the electrophoretical field of three migration zones: the fast haemoglobin named HbAA type, the haemoglobin with intermediate migration labelled HbAB type and the rare allele (Hb AC) found in two animals. The slow haemoglobin designed HbBB type was not found in the goat population.

The frequencies of the A, B and C alleles were 0.69, 0.30 and 0.01, respectively. The corresponding genotype frequencies for AA, AB and AC in the goat population were 0.37, 0.61 and 0.02, respectively (Table 1). The gene and genotype frequencies of the goats deviated significantly (chi-square=20.84; $P<0.05$) from HWE (Table 2). The estimated heterozygosity (H) was 0.57 while the local inbreeding coefficient (F) was -0.055. While BW and HG were significantly ($P<0.05$) higher in animals belonging to Hb AA genetic type, WH, RH, BL, FL, EL, HL, RL and RW were not significantly influenced ($P>0.05$) (Table 3).

Discussion

This is the first study on blood protein polymorphism in goats from north central Nigeria. A genetic

Table 1. Distribution of haemoglobin genotypes and gene frequencies in WAD goats

		Genotype frequency				Gene frequency		
Sex	No	AA	AB	BB	AC	A	B	C
Male	52	18 (0.35)	34 (0.65)	0 (0.00)	0 (0.00)	0.67	0.33	0.00
Female	52	21 (0.40)	29 (0.56)	0 (0.00)	2 (0.04)	0.70	0.28	0.02
Total	104	39 (0.37)	63 (0.61)	0 (0.00)	2 (0.02)	0.69	0.30	0.01

Table 2. The observed and expected number of the genotypes of haemoglobin in WAD goats

Parameters	Genotype				
	AA	AB	BB	AC	Total
Observed number	39	63	0	2	104
Expected number	49.51	43.05	9.36	2.08	104
Deviation	-10.51	19.95	-9.36	-0.08	-
Chi-square	2.23	9.25	9.36	0.003	20.84*

*Significant at $P<0.05$
Heterozygosity (gene diversity index) = 0.57
Local inbreeding coefficient (F) = -0.055

Table 3. Effects of haemoglobin variants on the morphometric traits (Mean±SE) of WAD goats

Variable	Hb AA	Hb AB
Body weight	22.14 ± 0.33^{a}	21.01 ± 0.32^{b}
Height at withers	47.99 ± 0.24^{a}	48.23 ± 0.27^{a}
Rump height	48.86 ± 0.24^{a}	49.20 ± 0.27^{a}
Heart girth	62.36 ± 0.45^{a}	57.96 ± 0.51^{b}
Body length	46.13 ± 0.18^{a}	45.62 ± 0.21^{a}
Face length	15.56 ± 0.18^{a}	15.59 ± 0.18^{a}
Ear length	10.67 ± 0.13^{a}	10.93 ± 0.14^{a}
Horn length	9.79 ± 0.10^{a}	9.99 ± 0.12^{a}
Rump length	11.34 ± 0.14^{a}	11.01 ± 0.12^{a}
Rump width	9.96 ± 0.10^{a}	9.81 ± 0.11^{a}

SE= standard error
Means within the same row bearing similar superscripts are not significantly different (P>0.05)

character is known to be polymorphic when the rarest phenotype has a frequency greater than one percent (Das and Deb, 2008). Based on electrophoretic separation of proteins in the present study, three different alleles, encoding three different genotypes were found in the locus of haemoglobin with higher Hb A than Hb B frequencies. Similarly, Bindu and Raghavan (2010) reported the predominance of the frequency of HbA over HbB in Malabari goats. Higher genotype frequencies of HbAA were also recorded in goats in Tunisian Oases (Nafti et al., 2013). This is not quite unexpected, because in the biological respect, as in the case of goats too, the allele HbA is characterized by a great natural selection advantage in comparison with the allele HbB as observed in the present study. It is believed that the balance between the adaptive values of different gene types under varying environments would be responsible for its maintenance. Generally, the extreme temperatures (acute cold or sultry heats), the extreme forms of relief (dessert or mountain) or precarious nutrition and breeding conditions favour the fixing of the allele HbA.

The absence of HbBB genotype in the present study is congruous to the findings of Fésüs et al. (1983) in Hungarian Edelziegen "grün" native goat breed, Canatan and Boztepe (2000) in Turkish hair goats, Kuwar et al. (2001) in Nepalese Hill goats and Johnson et al. (2002) in Omani Dhofari goats. However, the relative high number of HbAB and the absence of HbBB could be due to the analytical procedure employed in the present study. According to Pieragostini et al. (2005), who used a more sophisticated procedure, the truly AB goats are only those exhibiting the 67:33 band pattern; 67 being equal to the percent gene efficiencies of both ${}^{II}_{\alpha}$ genes plus that of the single ${}^{I}_{\alpha}{}^{A}$ genes (≈36 % + ≈32 %), while 33 % is equal to the percent gene efficiencies of the single ${}^{I}_{\alpha}{}^{B}$ gene. The goats exhibiting the 33:67 band pattern reasonably seem to be BB where 33 is equal to the percent gene efficiencies of two ${}^{II}_{\alpha}$ genes, while 67 is equal to the percent gene efficiencies of the two ${}^{I}_{\alpha}{}^{B}$ genes. However, the application of this latest procedure is a subject for future investigation in Nigerian goats.

Elucidating the molecular genetic basis of adaptive traits is a central goal of evolutionary genetics. According to Hrinca (2009), the genetic types of haemoglobin can be used to elucidate if a breed is indigenous in its area or it derives from different crossing systems among other domestic breeds. Altitudinal patterns of nucleotide diversity and linkage disequilibrium are indicative of local adaptation of a- and b-globin genes to different altitudinal zones, and Hb genotypes differ in oxygen affinity and aerobic performance (measured by VO2 max) (Storz, 2010; Cheviron and Brumfield, 2011). It is possible that, sometimes, the profile of haemoglobin system in some goat breeds could be due to the reproduction isolation phenomenon (Hrinca, 2008). The observation of low HbC frequency in the present study is similar to the reports of Deza et al. (2000). This might be attributed to the anaemic conditions of the animals (not confirmed through appropriate diagnosis) or environmental perturbations. Goats and sheep under conditions of erythropoietic stress (anaemia) or hypoxia, synthesize a juvenile haemoglobin type, HbC (Alloggio et al., 2009). According to Pieragostini et al. (2005), haemoglobin switching to HbC in adults during anaemic episodes is known to conceal polymorphism at the beta globin level because of the substitution of all beta variants by the beta C globin chain encoded by the dormant gene C_β.

There were more heterozygotes than homozygotes in the present study. The deviation from HWE might not be unconnected with management systems and mating pattern since the goats have not been artificially selected. Population size might equally be a contributing factor as the WAD goats are not native to the northern but the southern part of the country. The current H estimate falls within the recommended average heterozygosity between 0.3 and 0.8 in a population (Takezaki and Nei, 1996), for markers to be useful for measuring genetic variation. The negative F value obtained is in tandem with the H value indicating that the goat population might currently have disassortative mating or be experiencing a Wahlund effect (more heterozygotes than expected). Hence the need to design appropriate breeding and conservation schemes to prevent the erosion of the valuable adaptive traits of WAD goats.

The higher BW and HG of animals with Hb AA type is an indication of favourable natural selective advantage. This is consistent with the findings of Sam (2012). However, there were dearth of literature to compare our present observation with as regards the relationship between Hb types and morphometric traits in goats. Generally, the present findings may be useful because an apparently inverse trend seems to be taking place with regard to the annotation of protein mutations with their relevance for phenotypic expression which has been long neglected. Recently, growing attention has been focused on functional genetics, an area that is recurrently included amongst animal breeding research priorities (Pieragostini et al., 2010).

Conclusion

The study revealed the preponderance of Hb A allele over B and C alleles, respectively. There were also more heterozygotes (Hb AB) than homozygotes (AA). Individuals with Hb AA type had higher BW and HG compared to their Hb AB counterparts. The average H and F values are indications of the extent of uncontrolled breeding in the goat population. However, as this is a preliminary study, further work for detailed understanding of breed characteristics should be carried out with a large data set employing more sophisticated Hb screening method, neutral DNA markers (microsatellites) and single nucleotide polymorphisms (SNPs); and correlate them with productive and reproductive traits of WAD goats in north central Nigeria.

Preliminarna istraživanja polimorfizma hemoglobina i povezanosti s morfometrijskim svojstvima zapadnoafričkih patuljastih koza u sjevernom dijelu središnje Nigerije

Sažetak

Cilj ovog istraživanja bila je genetska karakterizacija zapadnoafričkih patuljastih koza (WAD) pomoću polimorfizma hemoglobina (Hb), kao i povezanost nekih morfoloških obilježja s HB varijantama. Uzorci krvi spolno zrelih koza WAD pasmine (n=104) prikupljeni su u sjevernom dijelu središnje Nigerije. Uzorci su uzeti od oba spola. Za utvrđivanje profila hemoglobina provedena je elektroforeza (celuloza acetat) i specifična tehnika bojanja gela. Tri kodominantna alela koja su uvjetovala prisutnost tri genotipa (AA, AB i AC) utvrđena su između pojedinih koza. Frekvencije alela A, B i C bile su 0,69, 0,30 i 0,01. Frekvencija genotipova AA bila je 0,37, AB 0,61 i AC 0,02. Nesukladnost između promatranog i očekivanog broja genotipova bila je značajna ($P<0,05$), što narušava Hardy-Weinberg frekvencije. Očekivana heterozigotnost (H), koja predstavlja raznolikost gena u populaciji je 0,57; dok lokalni koeficijent inbreedinga (F) iznosi -0,055 i ukazuje na disasortativno parenje. Osim tjelesne težine i srčanog obujma, čije su vrijednosti

bile veće (P<0,05) u individua Hb AA genotipa, Hb varijante nisu značajno utjecale (P>0,05) na ostale parametre tijela. Postoji potreba za daljnje istraživanje koje će obuhvatiti veći broj koza koje pokrivaju veća područja, korištenjem DNA markera i njihova odnosa s gospodarskim svojstvima, kao i performans studije za razumijevanje svojstava pasmine, te očuvanje i genetsko unapređenje WAD pasmine koza u sjevernom dijelu središnje Nigerije.

Ključne riječi: hemoglobin, polimorfizam, morfometrijska svojstva, koze, Nigerija

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