Carbonic anhydrase polymorphism in West African Dwarf goat populations of western Nigeria

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Carbonic anhydrase polymorphism in West African Dwarf (WAD) goat populations, from Oyo and Ogun States was investigated using the cellulose acetate electrophoresis system. Blood samples of 5 mL were collected from 60 WAD goats (30 from Ibadan (Oyo State) and 30 from Ijebu-Ode (Ogun State)), via the jugular veins and placed into heparinised bottles. The results of the electrophoresis analyses were subjected to statistical analysis using Tools For Population Genetic Analysis (TFPGA) software. The results revealed that two alleles, CA^F and CA^S govern the three genotypes (CA^{FF}, CA^{FS} and CA^{SS}) with CA^{SS} being prevalent. Allele frequencies ranged from 0.24 for CA^F in Ijebu-Ode to 0.76 in CA^S also from Ijebu-Ode while the genotype frequencies ranged from 0.09 in CA^{FF} from Ijebu-Ode to 0.61 in CA^{SS} also from the Ijebu-Ode population. Deviation from the Hardy-Weinberg equilibrium was significant ($P \leq 0.05$) in the population from Ibadan. Average gene diversity was 0.43, while the expected heterozygosity H_E(0.47) was higher than the observed heterozygosity H_O(0.25).Wright analysis showed that the populations sampled were from inbred populations with a positive F_{IT} and F_{IS} value (0.47, and 0.06 respectively) implying deficiency in heterozygote and the F_{ST} value of 0.45 indicating greater genetic differentiations among the populations giving opportunity for improvement.

Keywords: Carbonic anhydrase, West African Dwarf goat, polymorphism, heterozygosities

Goats constitute the largest group of small ruminant livestock in Nigeria totaling about 53.8 million and also constituting 6.2% of the world goat population (FAOSTAT 2011). Surveys have shown that up to 85% of rural households, poor farmers and small business people of all age groups and gender keep goats (FDLPCS 2007). They rank next to cattle in income generation and their meat (chevon) is quite popular and well relished (Ladele et al. 2006). There are three main breeds of goat in Nigeria, the West African Dwarf, the Sokoto Red and the Sahel. The ability of goats to tolerate harsh climates, the presence of trypanosomal tolerance in some breeds (Salako 2004), suitability to traditional systems due to small size, short generation interval (Abdul-Aziz, 2010) and the ability to thrive on poor quality diets, due to poor grazing lands (Adedeji et al. 2011), combine to make small ruminants strategic to increasing livestock productivity in rural agricultural systems (Adebambo et al. 2004).

Goats are one of the main economic resources in many developing countries and their economic importance is growing in developed countries (Luikart et al. 2001). The Nigerian dwarf goat is native to West Africa, its native habitat is the humid and sub-humid zones of Nigeria (Wilson 1991) and they are characterised by small size and variable colours. It is believed that coat colour and various breed characteristics also provide these goats with unique abilities to survive. Detailed genetic characterisation on West African Dwarf goats will establish the following: number of alleles, their frequency and diversity, polymorphic information content, as well as expected and observed heterozygosity (Hanotte and Jianlin 2005). Protein polymorphisms have proved to be a cheap and fast method of analysing single locus variation in breeds (Thairu-Muigai 2002). The main objective of this research was to investigate carbonic anhydrase polymorphism of West African Dwarf goat populations in Oyo and Ogun States.

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Materials and methods

Blood samples of 5 mL were collected from 60 (30 from Ibadan and 30 from Ijubu-Ode) West African Dwarf goats by jugular venipuncture, using needle and syringe and placed into heparinised vacutainer tubes. However only 23 of the blood samples from Ijubu-Ode were able to be analysed. The blood samples were centrifuged for about 10 minutes at 4^0C. The red blood samples were washed in saline three times by repeating centrifugation for 5 minutes at 4^0C. Centrifugation helps in the separation of the plasma and the erythrocyte fraction of heparinised blood. For transferring protein, the plasma was used to conduct the test using an electrophoresis apparatus. A buffer was prepared with tris glycerine with a pH of 8.5; 12.10 g of Tris was poured into a conical flask and dissolved with 600 mL of distilled water and was brought up to 1000 mL with distilled water. The buffer was then poured into the Titan Gel Chamber and the corrected plasma, after the red blood cells centrifugation, was placed in the chamber for about 50 minutes at 4^0C at 350V and a migration from cathode [-] and anode [+]. The plasma was collected after 40 minutes and was stained with Ponceau S for about 15 minutes and was destained in 5% acetic acid. Tools for Population Genetic Analyses (TFPGA) (Miller 1997) software was used to generate the genetic distance according to Nei (1972), the allele frequency, observed and expected heterozygosity, Hardy-Weinberg equilibrium, the inbreeding coefficients i.e. Wright's F_{IS} and F_{IT}, F_{ST} estimates. Bands produced were scored visually according to the method described by RIKEN (2006) and direct counting was used for calculating gene frequencies.

Results

Table 1 shows the total numbers of allele and the mean numbers of allele (2 and 2.00 respectively) across the locus in the two populations are the same. The observed heterozygosity across the population is lower (0.20–0.30) than the expected heterozygosity which ranges from (0.37–0.51). In all cases, average observed heterozygosities were lower than the heterozygosities expected and the Hardy-Weinberg equilibrium (HWE) in each of the populations showed significant deviation ($P \leq 0.001$) from HWE expectations**.** (Nei 1979) in the Ibadan population and no significant difference ($P > 0.05$) in the Ijebu-Ode population.

Table 1: Total number of alleles, mean number of alleles, observed and expected heterozygosity and deviation from the Hardy-Weinberg equilibrium

Population	N	TNA	MNA	H_O	H_E	DHWE
Ibadan	60	2	2.0000	0.2000	0.5062	***
Ijebu-Ode	46	2	2.0000	0.3043	0.3720	NS

N = Sample size there were 30 animals selected from each population with two allele per locus; however only 23 of the samples for Ijebu-Ode were useful TNA = Total no of allele, MNA = Alleles/locus, Ho and He = observed and expected heterozygosity, DHWE = deviation from Hardy-Weinberg equilibrium, ***: $P \leq 0.001$, NS: not significant

The frequency of carbonic anhydrase (Table 2) ranged from 0.24 – 0.47 for CA^F while the frequency ranged from 0.53 – 0.76 for CA^S.

Table 2: Allele frequencies.

Allele	Ibadan	Ijebu-Ode	Total	Average
CA^F	0.4667	0.2391	0.3679	0.3529
CA^S	0.5333	0.7609	0.6321	0.6471

The deviation from the Hardy-Weinberg equilibrium value for the population from Ibadan showed very a highly significant difference ($P \leq 0.001$) while no significant difference ($P > 0.05$) was revealed for the population from Ijebu-Ode. The value for the overall population of both samples showed a significant difference ($P \leq 0.05$) (Table 3).

The observed heterozygosity (Table 4) in the two populations ranged between 0.20 – 0.30. They do not match because there is not random mating in the combined population. Our sample includes two populations that do not mix. This is an example of what's known as the Wahlund effect. It is lower than the value of expected heterozygosity which ranged from 0.37 – 0.51 with an average of 0.44. Table 4 shows that the alleles were detected at the carbonic anhydrase locus in both populations sampled. Carbonic anhydrase polymorphism was highly informative (Shannon Information Index <0.60: Li et al. 2002; Yang et al.1999). The observed number of alleles across both populations are more than the effective number of alleles expected; the effective number of alleles was 1.99 (Ibadan) and 1.57 (Ijebu-Ode) with an overall mean of 0.94.

All $f(F_{IS})$ estimates across the locus were positive (significant heterozygote deficit) indicating heterozygote deficiency based on table wide randomisations ($P \leq 0.05$) Wright (1978). (Table 5).

Table 3: P values calculated from the Hardy-Weinberg equilibrium

Population	Population size	P value	S.E
Ibadan	60	0.0010***	
Ijebu-Ode	46	0.5609NS	
Overall	106	0.0290*	0.0046

*: $P \leq 0.05$; ***: $P \leq 0.001$; NS: not significant

Table 4: Measure of genetic variation

Population	N	Na	Ne	I	H_O	H_E	Nei
Ibadan	60	2.0000	1.9912	0.6909	0.2000	0.5062	0.4978
Ijebu-Ode	46	2.0000	1.5721	0.5501	0.3043	0.3720	0.3639
Total	106	2.0000	1.8696	1.2410	0.2453	0.4695	0.4651
Mean	53	2.0000	0.9348	0.6205	0.1226	0.2347	0.4309

N = sample size; Na = observed number of alleles; Ne = effective number of alleles; I = Shannon Information Index; HO= observed heterozygosity**;** HE = Expected Heterozygosity; Nei = gene diversity

Table 5: Wright's F-statistic analysis

Population	$f(F_{IS})$	$ø(F_{ST})$	$F(F_{IT})$	Nm
Ibadan	0.5982			
Ijebu-Ode	0.1636			
Mean	0.4726	0.4479	0.0567 NS	4.1608

f(FIS) = within population inbreeding estimate, ø(FST) = estimate of population differentiation, F(FIT) = overall global heterozygote deficit across population, Nm = gene flow

Table 6 shows the genotype frequency for both populations and the overall frequency. The frequency ranges from genotype CA^{FF} with 0.09 (Ijebu-Ode) to CA^{SS} with 0.61 (Ijebu-Ode). In the overall value, the frequency of CA^{SS} was the highest 0.51 while CA^{FF} and CA^{FS} have same frequency of 0.25.

Table 6: Genotype frequency

Genotypes	Ibadan	Ijebu-Ode	Overall
CA^{FF}	0.3667	0.0869	0.2452
CA^{FS}	0.2000	0.3043	0.2452
CA^{SS}	0.4333	0.6086	0.5094

Discussion

The allelic richness values of 2 (Table 1) in this study fall within the range obtained for Nigerian sheep (2 - 7) using the same protein maker (Akinyemi and Salako 2012). Similar values were reported by Awobajo et al. (2020), Awobajo et al. (2016) and Salako et al. (2007) for haemoglobin in West African Dwarf goats while a higher value (3) was reported by Jaayid 2012 for Iraqi goats. The H_O across both populations is lower (ranging from 0.20 – 0.30) than the H_E which range from (0.37 – 0.51) (Table 1), implying departure from random mating in the populations and suggesting an on-going selection or inbreeding (Dixit et al. 2008, Bruno-de-Sousa et al. 2011). The Hardy-Weinberg equilibrium for the Ijebu Ode population was not significant ($P > 0.05$), while that of the Ibadan population showed significant deviation ($P \leq 0.001$) from expectations, confirming deviation from random mating as a result of mating between relatives and consequent genetic drift, similar to what has been observed in many other goat populations (Agha et al. 2008, Rout et al. 2008, Dixit et al. 2009). The most frequent allele is CA^S in both Ibadan and Ijebu-Ode populations with 0.53 and 0.76 respectively, allele CA^F is the least frequent in both populations with 0.47 and 0.24 in Ibadan and Ijebu-Ode populations respectively (Table 2). Savic et al. (2000) reported the predominance of C^{AS} allele (0.98) in Yugoslavia Tsigai sheep, Akinyemi and Salako (2012) also reported C^{AS} as the most frequent allele in Balami, Uda and Yankassa sheep breeds. The Hardy-Weinberg Equilibrium for the overall population was significant ($P \leq 0.05$) and this is due to the effect of very high significant deviation from the Hardy-Weinberg Equilibrium in the Ibadan population (Table 3). The value for H_O was lower than the value of H_E (Table 4) in both populations. This implies a departure from random mating, which suggests that the populations were homozygous in nature and may also indicate on-going selection (Dixit et al. 2008, Bruno-de-Sousa et al. 2011), or result from mating between relative and consequent genetic drift, similar to what has been observed in many other goat populations (Agha et al. 2008; Rout et al. 2008; Dixit et al. 2009). The Shannon Information Index values ranged from 0.55 – 0.69 with a mean of 0.62 (Table 4); high Shannon Information Index values suggest that these markers are informative for genetic diversity in the West African Dwarf goats sampled and that Nigerian goats possess a wide genetic base that allows for adaptation to a wide variety of ecological environments. The Shannon Information Index values obtained in this study are similar to 0.63 as reported by Awobajo et al. 2016. The gene diversity ranged between 0.36 – 0.50, with a mean of 0.43 (Table 4) which is within the range of 0.3 – 0.8 in a population recommended for markers to be useful for genetic variation (Takezaki and Nei 1996). These values indicate that there is sufficient genetic variability within the populations studied. The gene diversity values are lower than 0.54 reported by Muema et al. (2009),

0.51 reported by Adebambo et al. (2011) both in Nigerian goats, and 0.61 – 0.78 in Indian goats (Rout et al. 2008, Dixit et al. 2010). They were also lower than those published by Kowalska and Zaton-Dobrowolska (2008), who reported a range of 0.59 – 0.70 with an overall value of 0.66. However the values observed are consistent with the values reported by other authors (Mwacharo et al. 2002; Ibeagha-Awemu and Erhardt 2004; Shahrbabak et al. 2010; Akinyemi and Salako 2012). They are, however, higher than the range of 0.23 – 0.26 reported for Iranian fat-tailed sheep breeds (Shahrbabak et al. 2010). The estimates of heterozygosity obtained with blood protein markers are generally lower than those of microsatellite markers, since the latter have a higher level of polymorphism (Ibeagha-Awemu and Erhardt 2004). Wright's (1978) fixation index (Table 5) is a measure to describe the level of differentiation between populations (i.e. a test whether or not they are from the same gene pool). The average estimate of F_{IS} in the current study was 0.47, indicating a deficiency in heterozygote among the West African Dwarf goat populations. The overall F_{IT} value (0.057) also indicated a deficiency of heterozygote in the populations indicating inbreeding populations. According to Weir and Cockerham (2014), F_{ST} values up to 0.05 indicate negligible genetic variations, while values greater than 0.25 indicate large genetic differentiation among populations. The F_{ST} value of 0.45 obtained in this study indicates large genetic differentiation among the populations studied (Weir and Cockerham 2014). The mean estimates of F-statistics; F_{IS} (0.02 ± 0.02, F_{ST} 0.11 ± 0.01 and F_{IT} (0.12 ± 0.03) reported by Snyman et al. (2013) were different from the values in the current study. The mean F_{ST} value obtained was higher than values reported for sheep breeds in different studies. Zhong et al. (2010) reported 0.05 in ten Chinese indigenous sheep breeds, Dixit et al. (2009) reported 0.13 among different goat populations in India and El Nahas et al. (2008) reported 0.04 in different goat breeds. Gene flow estimates in this study suggest mobility and considerable exchange of genetic material among these West African Dwarf goat populations. Table 6 shows the genotype frequency for each of the populations and the overall frequency. The lowest frequency ranges from genotype CA^{FS} with 0.20 (Ibadan) to CA^{SS} with 0.61 (Ijebu-Ode). The polymorphism is defined by the expression of three genotypes: two heterozygotes, CA^{FF} and CA^{FS} and one homozygote CA^{SS} determined by two co-dominant alleles, F and S. The allele F had lower frequency than the allele S. As a result the homozygotes (SS) had the highest presentation (0.51), the heterozygote CA^{FF} and CA^{FS} reached a low incidence and hence, have a similar occurrence (0.25).

Conclusion

The West African Dwarf goat in this current study is out of the Hardy-Weinberg genetic equilibrium at the carbonic anhydrase locus. The present study reveals that the West African Dwarf goat has substantial genetic variation but there is a high degree of inbreeding as indicated by a positive F_{IS} value (0.47), implying heterozygosity deficiency. According to the locus analysed, there is sufficient genetic variation in the populations of West African Dwarf goat with distinct genetic differentiation between the sample populations. The result of this study will be an addition to the baseline information about the West African Dwarf goat population in the south western part of Nigeria (Oyo and Ogun) which will be useful for the improvement programmes of the breed and serve as reference for large scale diversity studies. Heterozygosity's and allelic richness estimates for the protein locus of this study indicated that the goat populations sampled are a reservoir of West African Dwarf goat diversity. The genetic diversity of the goat populations was high as indicated by the mean number of alleles and gene diversity observed.

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