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Genetic diversity at four Nigerian sheep breeds assessed by variation of albumin and carbonic anhydrase in cellulose acetate electrophoretic systems

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Abstract. The aim of the study was to evaluate the occurrence and distribution of variations in blood protein markers in sheep breeds in Nigeria and to evaluate the relationships that exist among them. A total of 100 sheep comprising of twenty-five each of Balami, Uda, Yankassa and West African Dwarf (WAD) breeds were sampled for biochemical studies. Blood was collected to determine variations at the Albumin and Carbonic-Anhydrase (CA) structural protein loci using cellulose acetate electrophoresis. All tested loci were polymorphic yielding four allelic variants (CAA, CAA, Alb, and Alb) at the two protein loci. Genetic variability in the studied population was assessed using heterozygosity (observed — Ho and expected — He), effective number of alleles (Ne), fixation index (F) and gene flow (Nm). Genotypic frequencies ranged from 0.01 to 0.62 for CA and 0.25 to 0.49 for Alb loci. Estimated heterozygosity values ranged from 0.32±0.28 at Balami sheep to 0.70±0.22 in WAD with a global average estimated at 0.43±0.09 for all the breeds studied. The gene flow values for each of the loci studied were 0.82 and 1.24 for Alb and CA, respectively, with an average value of 1.03. The results showed that Yankassa and Balami sheep populations are more genetically (Nei's genetic identity value — 0.99) alike compared to Yankassa and WAD (0.73). Based on the values of heterozygosity (mean He =0.70 and Ho =0.45) assessed by variation of albumin and carbonic anhydrase the most genetic diverse is WAD sheep breed among the studied populations.

Keywords: indigenous sheep, genetic diversity, blood protein polymorphism, cellulose acetate electrophoresis

Introduction

Genetic diversity studies in domestic animals aim at evaluating genetic variation within and between breeds, since the breed is the management unit for which factors such as inbreeding are controlled (Tapio et al., 2005). However, the definition of a breed, as applied by the Food and Agricultural Organisation (FAO), frequently does not reflect the underlying genetic population structure. Therefore, a molecular genetics study of the population diversity and structure improves the understanding of the actual genetic resources.

The domestic sheep (Ovis aries) has, during the last 10000 years attained a relatively significant increase in body size, a decrease in horn size and a change from a hairy, moulty fleece to a white woolly fleece (Ryder, 1983). Hundreds of local breeds and strains have been developed for different production systems throughout the world. Further genetic improvement has occurred in the last 50 years as a result of the application of quantitative genetics and selective breeding methods (Adebambo et al., 2004).

Nigeria has basically four definitive sheep breeds – the West African Dwarf (WAD), the Uda, the Balami and the Yankassa, all of which are well adapted to different ecological niches within the country’s geographical sphere, except for the Yankassa which has a very wide spread across the country. With a relatively unslected sheep population of more than 14 million in Nigeria, widely dispersed throughout the country, yet highly localized in their existence and adaptation, the need to characterize and exploit these diverse genotypes cannot be over-emphasized (Adebambo et al., 2004).

Blood protein polymorphism has been used by several researchers as markers to study the evolutionary relationships in mammals. Manwell and Baker (1977) compared the genetic variation at 30 blood protein loci to determine genetic distance (Nei, 1972) between Australian Merino and Poll Dorset breeds. Most blood protein polymorphisms are genetically controlled by allelic series. If an animal has a gene for a specific substance, this can be detected in the blood by appropriate procedures, such as electrophoresis and the presence or absence of the specific substance is directly related to their genotype (Daly, 1979; Adebambo, 2004).

The aim of this study was to evaluate the occurrence and distribution of variations in blood protein markers in indigenous sheep breeds in Nigeria and of the relationships existing among them.

Material and methods

Experimental design

One hundred animals, twenty-five each of Yankasa, Balami, Uda and West African Dwarf (WAD) sheep were purposefully sampled from various representative populations across Nigeria to ensure that the animals were not closely related. Blood samples were collected from adult and presumably unrelated animals of both sexes. Whole blood was collected from jugular vein (v. jugularis) into 5ml heparinized vacutainer tubes and separated into plasma and red cells by centrifugation at 4°C for 10min at 3500rpm. Red cells were washed three times in normal saline solution and lysed with cold distilled water. Hemolysates were stored in a refrigerator at -20°C until the electrophoretic studies were carried out.

Two protein coding loci: Albumin (Alb) and Carbonic anhydrase (CA) were typed, using cellulose acetate electrophoresis as described by Bader (1998) and RIKEN (2006) with minor modifications. Typing of CA was done using RBCs in 4 volume dH2O.
Prepared and labelled cellulose acetate strips were soaked in EDTA Sodium acetate buffer at pH 5.6 and blotted lightly with filter paper to remove excess buffer. Hemolysates were applied and electrophoresis carried out with EDTA Sodium acetate buffer at pH 5.6 as electrode buffer at 200V for 35min. The strips were then stained for 2 hours in a dark place using ponceau S while destaining was done using 1% acetic acid solution. Albumin alleles were genotyped using plasma in Tris citrate buffer at pH5.6 as electrode buffer at 150V for 30min. The strips were stained in aniline blue solution and destained using distilled water.

Statistical analysis
Allele and genotype frequencies for each locus in each sample were computed by direct counting and tested for fit to Hardy-Weinberg ratios using chi square (χ²) goodness of fit test. The observed and expected heterozygosity were calculated according to Nei (1973) with the correction for small samples (Levene, 1949). The genetic distances were calculated using the method described by Nei (1978). Genetic distances were used to construct dendrograms by un-weighted pair-group arithmetic averages (UPGMA) method as described by Sneath and Sokal (1973) for single locus and pooled values for both loci. The genetic differentiation among populations and fixation indices were analyzed according to Nei (1987), using Wright's (1978) F-statistics. All computations were performed using Popgen (Yeh et al., 1999) and Tools for population genetic analysis (TFPGA; Miller, 1997).

Results
Allele frequency
The two studied loci were polymorphic in all breeds. Frequencies of observed alleles at the investigated loci are given in Table 1. Four allelic variants with two alleles each were observed at the studied loci. The most frequent alleles were CA in Balami (0.98), Uda (0.84) and Yankassa (0.86) while Al was most frequent in WAD (0.72).

Genotype frequencies
The genotypic frequencies of the two studied loci are presented in Table 2.

Carbonic anhydrase (CA). The CA locus was polymorphic, having three genotypes controlled by two codominant alleles. The CA and CA genotypes occurred in the Balami, Yankassa and WAD population while the Uda alone had the CA genotype occurring with a frequency of 0.04. The frequency of CA was similar in the Balami, Uda and Yankassa, and ranged from 0.72 (Uda and Yankassa) to 0.96 (Balami), whereas the CA was most frequent (0.92) in the WAD.

Albumin (Alb). Three genotypes of albumin (Al, Al and Al) determined by two codominant alleles were observed. The genotype heterozygous Al had the highest value (0.60) among the Balami population while the homozygous Al had the largest frequency (0.52) in the Uda population. The Homozygous Al had the largest occurrence (0.60) in the Yankassa population while the heterozygous Al and homozygous Al had the same level of occurrence (0.48) in the WAD population. All three genotypes were observed in the WAD population while only the homozygous Al and heterozygous Al were present in the Balami and Yankassa populations and the Uda population had only homozygous Al and the heterozygous Al.

Heterozygosity. Heterozygosity values were calculated to determine the level of genetic variation within the populations (Table 3). The observed heterozygosity (H) values were between 0.32 (32.0%) for Balami and Yankassa populations, and 0.70 (70.0%) for

Table 1. Allele frequency for two polymorphic loci in four indigenous sheep populations

<table>
<thead>
<tr>
<th>Locus</th>
<th>n</th>
<th>Allele</th>
<th>Observed no of alleles</th>
<th>Balami</th>
<th>Uda</th>
<th>Yankassa</th>
<th>WAD</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA</td>
<td>25</td>
<td>F</td>
<td>0.02</td>
<td>0.16</td>
<td>0.14</td>
<td>0.46</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>S</td>
<td>0.98</td>
<td>0.84</td>
<td>0.86</td>
<td>0.54</td>
<td>0.81</td>
<td></td>
</tr>
<tr>
<td>AL</td>
<td>25</td>
<td>A</td>
<td>0.70</td>
<td>0.24</td>
<td>0.80</td>
<td>0.28</td>
<td>0.51</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>0.30</td>
<td>0.76</td>
<td>0.20</td>
<td>0.72</td>
<td>0.50</td>
<td></td>
</tr>
</tbody>
</table>

*WAD= West African Dwarf; CA = Carbonic Anhydrase; AL = Albumin

Table 2. Genotype frequencies at 2 allozyme locus in 4 indigenous sheep breeds

<table>
<thead>
<tr>
<th>Locus</th>
<th>Genotypes</th>
<th>Balami</th>
<th>Uda</th>
<th>Yankassa</th>
<th>WAD</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbonic</td>
<td>FF</td>
<td>0.00</td>
<td>0.04</td>
<td>0.00</td>
<td>0.00</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>FS</td>
<td>0.04</td>
<td>0.24</td>
<td>0.28</td>
<td>0.92</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td>SS</td>
<td>0.96</td>
<td>0.72</td>
<td>0.72</td>
<td>0.08</td>
<td>0.62</td>
</tr>
<tr>
<td>Albumin</td>
<td>AA</td>
<td>0.40</td>
<td>0.00</td>
<td>0.60</td>
<td>0.04</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>AB</td>
<td>0.60</td>
<td>0.48</td>
<td>0.40</td>
<td>0.48</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td>BB</td>
<td>0.00</td>
<td>0.52</td>
<td>0.00</td>
<td>0.48</td>
<td>0.25</td>
</tr>
</tbody>
</table>

*WAD= West African Dwarf
WAD population. The average observed heterozygosity for all populations was 0.43 (43%). The value for expected heterozygosity ($H_e$) was observed in the Balami population to be the lowest (0.23) while the highest value was recorded for the WAD population (0.45). The mean value of $H_e$ for all the populations was 0.32 and this was higher than the values recorded for each of the population in the current study except WAD. The most diverse population recorded is the WAD with expected heterozygosity value of 45.0%.

Hardy-Weinberg equilibrium. Hardy-Weinberg equilibrium test for single locus was conducted for the populations within the two allozyme markers. Results shown in Table 4 revealed that there were some genotypes with loci that deviated significantly from HWE (P<0.05). The following loci were found not to be in HWE (P<0.05) for a particular population: for Balami - Alb with P-Values of 0.032 had deviations from HWE. The locus CA was found to be in disequilibrium (P<0.05) with P-Value of 0.000 for WAD population. However, Yankassa and Uda sheep populations were in equilibrium for the two loci studied.

Genetic identity and Genetic distance. Genetic distance estimates of Nei (1972) were calculated using allele frequencies and recorded at 1.03. for Alb and CA, respectively. The mean gene flow over all loci was 0.82 and 1.24 for CA and Alb, respectively, with a mean of -0.34 for all loci. Global breed differentiation evaluated by Fst, was estimated at 0.03. and -25 for CA and Alb, respectively, with a mean of -0.34 for all loci. The gene flow values for each of the loci studied were 0.82 and 1.24 for CA and Alb, respectively. The mean gene flow over all loci was recorded at 1.03.

The results of genetic identity were as presented in Table 6. The distance between populations ranged from 0.01 to 0.32. The smallest genetic distance was observed between Yankassa and Balami populations while the farthest distance was observed between WAD and Yankassa populations. The results of genetic identity were as presented in Table 6. The results indicate that the Yankassa and Balami populations are more genetically alike (0.99) while the Yankassa and WAD populations were the least genetically identical (0.73).

The genetic distance estimates were used to construct dendrogram based on individual locus and the pooled distances for the two loci studied. The dendrograms are presented in Figures 1 to 3. The tree topology of the genetic distances obtained at the Carbonic anhydrase locus can be used to differentiate two sub clusters of Uda – Yankassa at node 1 (with a distance of 0.0021) and Balami – Yankassa at node 2 (with a distance of 0.0128) and the distance at node 3=0.4949 (including all populations) (Figure 1). The tree topology of the genetic distance obtained at the Albumin locus divided the four breeds into two distinct clusters of Uda – WAD at node 1 (genetic distance of 0.0004) and Balami at node 2 (with a distance of 0.0121 including Balami, Uda and Yankassa). The WAD was totally separated from the above two sub clusters at node 3 with a distance of 0.1840 including all four populations (Figure 2). The Phylogenetic tree of the genetic distances pooled for the two loci studied supports the genetic distance estimates where the Yankassa population is the most genetically distant from the WAD population. The Balami and Yankassa populations formed a different cluster at node 1, indicating a closer relationship between the two populations, whereas the Uda and WAD clustered at node 2 (Figure 3).

<table>
<thead>
<tr>
<th>Populations</th>
<th>Locus</th>
<th>N</th>
<th>Na</th>
<th>Ne</th>
<th>I</th>
<th>$H_e$</th>
<th>$H_s$</th>
<th>uHe</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Balami</td>
<td>Alb</td>
<td>25</td>
<td>2.00</td>
<td>1.72</td>
<td>0.61</td>
<td>0.60</td>
<td>0.42</td>
<td>0.43</td>
<td>-0.43</td>
</tr>
<tr>
<td></td>
<td>CA</td>
<td>25</td>
<td>2.00</td>
<td>1.04</td>
<td>0.10</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>-0.02</td>
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<tr>
<td></td>
<td>Mean</td>
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<td>2.00</td>
<td>1.38</td>
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<td>0.23</td>
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<td>-0.22</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>0.34</td>
<td>0.26</td>
<td>0.28</td>
<td>0.19</td>
<td>0.19</td>
<td>0.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uda</td>
<td>Alb</td>
<td>25</td>
<td>2.00</td>
<td>1.57</td>
<td>0.55</td>
<td>0.48</td>
<td>0.37</td>
<td>0.37</td>
<td>-0.32</td>
</tr>
<tr>
<td></td>
<td>CA</td>
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<td>2.00</td>
<td>1.37</td>
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<td>0.27</td>
<td>0.27</td>
<td>0.11</td>
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<tr>
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<td>Mean</td>
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<td>1.47</td>
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<td>0.32</td>
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<td>0.05</td>
<td>0.05</td>
<td>0.21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yankassa</td>
<td>Alb</td>
<td>25</td>
<td>2.00</td>
<td>1.52</td>
<td>0.53</td>
<td>0.36</td>
<td>0.34</td>
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<td>0.24</td>
<td>-0.16</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
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<td>1.42</td>
<td>0.47</td>
<td>0.32</td>
<td>0.29</td>
<td>0.30</td>
<td>-0.11</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>0.10</td>
<td>0.06</td>
<td>0.04</td>
<td>0.05</td>
<td>0.05</td>
<td>0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WAD</td>
<td>Alb</td>
<td>25</td>
<td>2.00</td>
<td>1.68</td>
<td>0.59</td>
<td>0.48</td>
<td>0.40</td>
<td>0.41</td>
<td>-0.19</td>
</tr>
<tr>
<td></td>
<td>CA</td>
<td>25</td>
<td>2.00</td>
<td>1.99</td>
<td>0.69</td>
<td>0.92</td>
<td>0.50</td>
<td>0.51</td>
<td>-0.85</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>25</td>
<td>2.00</td>
<td>1.83</td>
<td>0.64</td>
<td>0.70</td>
<td>0.45</td>
<td>0.46</td>
<td>-0.52</td>
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<tr>
<td></td>
<td>SE</td>
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<td>0.05</td>
<td>0.22</td>
<td>0.05</td>
<td>0.05</td>
<td>0.33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grand Mean</td>
<td>Mean</td>
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<td>2.00</td>
<td>1.53</td>
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<td>-0.239</td>
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<tr>
<td></td>
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<td>0.09</td>
<td>0.05</td>
<td>0.051</td>
<td>0.106</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Ab = Albumin locus; CA = Carbonic anhydrase locus; SE = Standard error; Na = No. of Alleles; Ne = No. of Effective Alleles = 1 / (Sum pi^2); I = Shannon’s Information Index = -1 * Sum (pi * Ln (pi)); Ho = Observed Heterozygosity = No. of Hets / N; He = Expected Heterozygosity = 1 - Sum pi^2; uHe = Unbiased Expected Heterozygosity = (2N / (2N-1)) * He; F = Fixation Index = (He - Ho) / He = 1 - (Ho / He); Where pi is the frequency of the ith allele for the population and Sum pi^2 is the sum of the squared population allele frequencies.
Table 4. Summary of Chi-Square Tests for Hardy-Weinberg equilibrium

<table>
<thead>
<tr>
<th>Populations</th>
<th>Locus</th>
<th>ChiSq</th>
<th>Prob</th>
<th>Signif</th>
</tr>
</thead>
<tbody>
<tr>
<td>Balami</td>
<td>Alb</td>
<td>4.59</td>
<td>0.03</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>CA</td>
<td>0.01</td>
<td>0.92</td>
<td>ns</td>
</tr>
<tr>
<td>Uda</td>
<td>Alb</td>
<td>2.49</td>
<td>0.11</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>CA</td>
<td>0.29</td>
<td>0.59</td>
<td>ns</td>
</tr>
<tr>
<td>Yankassa</td>
<td>Alb</td>
<td>0.06</td>
<td>0.81</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>CA</td>
<td>0.66</td>
<td>0.42</td>
<td>ns</td>
</tr>
<tr>
<td>WAD</td>
<td>Alb</td>
<td>0.91</td>
<td>0.34</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>CA</td>
<td>18.14</td>
<td>0.00</td>
<td>***</td>
</tr>
</tbody>
</table>

ns = not significant; * P<0.05; *** P<0.001

Table 5. F-Statistics and estimates of gene flow over all populations for each locus

<table>
<thead>
<tr>
<th>Locus</th>
<th>Fis</th>
<th>Fit</th>
<th>Fst</th>
<th>Nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alb</td>
<td>-0.25</td>
<td>0.04</td>
<td>0.23</td>
<td>0.82</td>
</tr>
<tr>
<td>CA</td>
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<td>0.17</td>
<td>1.24</td>
</tr>
<tr>
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<td>-0.07</td>
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<td>1.03</td>
</tr>
<tr>
<td>SE</td>
<td>0.08</td>
<td>0.11</td>
<td>0.03</td>
<td>0.21</td>
</tr>
</tbody>
</table>

Fis = (Mean He - Mean Ho) / Mean He; Fit = (Ht - Mean Ho) / Ht; Fst = (Ht - Mean He) / Ht;
Mean He = Average He across the populations. Mean Ho = Average Ho across the populations;
He = Expected Heterozygosity; Ho = Observed Heterozygosity;
Ht = Total Expected Heterozygosity = 1 - Sum tpi^2, where tpi is the frequency of the ith allele for the total and Sum tpi^2 is the sum of the squared total allele frequencies.

Table 6. Nei’s genetic identity and genetic distance between Nigerian sheep populations

<table>
<thead>
<tr>
<th>Population</th>
<th>Balami</th>
<th>Uda</th>
<th>Yankassa</th>
<th>WAD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Balami</td>
<td>***</td>
<td>0.84</td>
<td>0.99</td>
<td>0.73</td>
</tr>
<tr>
<td>Uda</td>
<td>0.17</td>
<td>***</td>
<td>0.79</td>
<td>0.93</td>
</tr>
<tr>
<td>Yankassa</td>
<td>0.01</td>
<td>0.24</td>
<td>***</td>
<td>0.73</td>
</tr>
<tr>
<td>WAD</td>
<td>0.32</td>
<td>0.07</td>
<td>0.32</td>
<td>***</td>
</tr>
</tbody>
</table>

*Nei’s (1972) original measure of genetic identity and genetic distance;
Genetic Identity (above Diagonal) and Genetic Distance (below Diagonal)

Figure 1. Dendrogram of genetic distance between four Nigerian indigenous sheep populations at the Albumin locus

Figure 2. Dendrogram of genetic distance between four Nigerian indigenous sheep populations at the Aarobic anhydrase locus
Starch gel electrophoresis. Mwacharo et al. while in the experiment of Savic et al. (2000) blood protein in this current study may be due to the electroperentic membrane often found in native Norwegian sheep. The observed polymorphism sheep breeds. However, the authors mentioned that the Alb is most identified as Alb phenotypes, which commonly occurs in other sheep breeds. However, the authors established that the Alb allele to be the same as the Alb, while Alb is most likely the same as the Alb allele established by Mwacharo et al (2002) who reported that Albumin was monomorphic for Alb allele in Yugoslavia Tsigai sheep. Sargent et al. (1999) reported the predominance of CA allele (0.98) in Yugoslav Tsigai sheep. Sargent et al. (1999) reported the presence of CA allele in all of the breeds studied and speculated that the latter allele may be the same as the CA allele reported by other authors. Bis and Tucker (1983) and Zanotti et al. (1990) established that CA is generally the most frequently occurring allele in sheep breeds. However, Ibeagha-Awemu and Erhardt (2004) found a fixation of CA in Yankassa, Uda, Mbororo and West African Dwarf sheep breeds and in Merino, East Friesian Milk and German Grey Heath sheep breeds. The fixation of CA was contradicted by the report of CA and CA in similar breeds (Missouhou et al., 1999). Akinyemi and Salako (2012) also reported CA as the most frequent allele in Balami, Uda and Yankassa sheep breeds while adding that data on the polymorphism of this blood enzyme and the physiological advantages it confers, is however scarce.

Albumin. All samples tested were polymorphic at the Alb locus. However, this was not the case in the report of Mwacharo et al. (2002) who reported that Albumin was monomorphic for Alb allele in all the fat tailed sheep studied. The authors, however, recorded polymorphism in the Merino sheep with the frequency of Alb being very low (0.0063). The allele Alb in this current study is speculated to be the same as the Alb, while Alb is most likely the same as the Alb allele established by Mwacharo et al. (2002).

Savic et al. (2000) found that no albumin polymorphism in Tsigai sheep has been observed so far and so all samples tested were identified as Alb phenotypes, which commonly occurs in other sheep breeds. However, the authors mentioned that the Alb is most often found in native Norwegian sheep. The observed polymorphism in this current study may be due to the electroperentic membrane used. In this study, cellulose acetate electrophoresis was used, while in the experiment of Savic et al. (2000) blood protein separation was carried out by Horizontal electrophoresis and at Mwacharo et al. (2002) blood typing was done using horizontal Starch gel electrophoresis.

In a study of 19 sheep breeds from Southern Africa, Sargent et al. (1999) reported that all breeds, except the South African meat Merino and the Van Rooy breeds, were monomorphic at the Alb system with Alb being fixed for all other breeds studied. The authors also noted that the frequencies of the Alb alleles were low (0.019 and 0.006, respectively) in the two breeds that were reported to be polymorphic at the Alb system. The result from this present study however, does not agree with the report of previous authors as the four breeds in the study were polymorphic and had frequencies ranging from 0.24 to 0.80 for Alb and 0.20 to 0.76 for Alb. The overall allele frequency at the Alb locus was 0.5050 for Alb and 0.4950 for Alb. It will be difficult to compare the results of this present study with other reports, as data on cellulose acetate electrophoresis for this locus is scarce.

Discussion

Allele frequency

Carbonic anhydrase. The four breeds in this current study were polymorphic at the CA locus. The CA was the most common in all of the breeds. A study by Ordas and Primitivo (1986) on Churra sheep breeds was compared with the current investigation. They discovered a new allele (CA) at the red cell carbonic anhydrase locus in Churra sheep. They found the CA allele to be present in all of the breeds studied (Churra, Lacha and Manchega), and this allele was fixed (monotype) in most of the breeds.

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Heterozygosity. Genetic diversity measured as the amount of actual or potential heterozygosity in this study had values which were moderate to high (0.32-0.70) for all the breeds with an overall H of 0.43±0.09 and were within the range of values (0.3 and 0.8) in a population (Takezaki and Nei, 1996) recommended for markers to be useful for genetic variation. These values indicate that there is sufficient genetic variability within the populations studied. The values observed in this study were lower than those published by Kowalska and Zaton-Dobrowolska (2008), who found heterozygosity coefficients in the range of 0.591 and 0.703 with an overall value of 0.655. This result was 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). However, the present values are higher than the range of 0.229-0.259 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 has higher level of polymorphism (Ibeagha-Awemu and Erhardt, 2004).

Hardy-Weinberg equilibrium. Tests of departure from Hardy-Weinberg proportions are frequently made to check on random mating in populations, and the deviations from expectation are used to estimate inbreeding coefficients. The significant deviations observed in Balami population at the Alb locus (P<0.05) and in the WAD population at the CA locus (P<0.001) are in line with the report of Sargent et al. (1999) who established deviations from HWE at protein loci in Southern Africa Sheep. Significant deviations from HWE could be as a result of any one or a combination of unobserved null alleles, excess of heterozygote, migration, high mutation rate and artificial selection (Aminafshar et al., 2008), crossing and linking, inbreeding, sample error, population bottenecks and random genetic drift. Ideal Hardy-Weinberg populations do not actually occur in nature owing to various factors, which can shift the equilibrium and disrupt the stability of a population, giving rise to change in the genetic structure.

Gene flow and F-statistics. Wright’s (1978) fixation index 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 estimate of Fis in the current study were negative at both loci examined. The average Fis was -0.335±0.081, indicating excess of heterozygotes among the sheep population. The global Fit value (-0.069±0.109) also indicated a deficiency of homozygotes in the population indicating that mates were less closely related. The Mean Fis was -0.335±0.081, indicating excess of heterozygotes among the sheep population. The global Fit value (-0.069±0.109) also indicated a deficiency of homozygotes in the population indicating that mates were less closely related. The Mean Fis was -0.335±0.081, indicating excess of heterozygotes among the sheep population. The global Fit value (-0.069±0.109) also indicated a deficiency of homozygotes in the population indicating that mates were less closely related.
Hartle (1980), Fst values up to 0.05 indicate negligible genetic variations while values greater than 0.25 indicate large genetic differentiation amongst populations. The current study reported moderate level of genetic differentiation among the four population of sheep studied. The mean Fst value obtained was higher than values reported for other sheep breeds in different studies. Zhong et al. (2010) reported 0.048 in ten Chinese indigenous sheep breeds, Dixit et al. (2009) reported 13% among different goat populations in India and El Nahas et al. (2008), reported 3.7% in different goat breeds. The average genetic differentiation (Fst) between populations in the current study interval indicates that 20.1% of the genetic diversity can be explained by differentiation among the populations while the remaining 79.9% can be explained by differences among individuals within the populations. The overall global excess of heterozygotes (FIt) across populations has a 6.9% increase in the amount of heterozygote individuals within the populations indicating a situation of non-random mating.

Genetic distance, Genetic identity and Dendrogram

The genetic relationship between populations can be measured by determining the genetic distance and identity between the populations. The distance between two populations provides a good estimate of how divergent they are genetically (Snyman et al., 2013). The distance estimate in this current study revealed a closer relationship between Balami and Yankassa. The genetic distance values obtained in this study ranged from 0.013 to 0.321 and this was similar to 0.01-0.22 reported by Nigussie et al. (2016) for eastern Ethiopia sheep breeds, and higher than 0.001-0.055 reported for Spanish sheep breeds (Ordas and Primitivo, 1986). Buiss and Tucker (1983) found genetic distance values of 0.181-0.308 between different sheep breeds. Zanotti et al. (1990) also reported values in the range of 0.012 to 0.060 in five Italian sheep breeds. The values obtained in the present study where within the prescribed range of genetic distance values (0.000-0.058) for local breeds (Nei, 1976). Ordas and Primitivo (1986) established that genetic distance between flocks of the same breed can be greater than the distance between flocks of different breeds. This they attributed to the effects of selection which have not been parallel or convergent for proteic character and those which participate in the definition of a breed standard. Nei and Roychoudhury (1974) also confirmed that the genetic variations observed between the three major races of man is small when compared to the within race variation. Suggesting that the genes controlling morphological characters have been subjected to natural selection stronger than ‘average genes’ in the racial differentiation process.

The tree topology supported the measure of relationship observed based on estimates of genetic distance and measure of genetic diversity among populations. The tree construct for the Albumin locus generated two clusters with Uda and WAD populations clustered at node 1 and Balami and Yankassa clustering at node 2, whereas, at the Carbonic anhydrase locus the WAD was distinct branching off completely from the other three breeds which formed two sub branches with Uda and Yankassa clustering together, while the Balami formed a second sub group. However, the tree topology for the examined loci combined generated a similar dendogram as was in the albumin locus with two distinct clusters formed. Balami and Yankassa formed the first cluster at node 1 while Uda and WAD formed the second cluster at node 2. The close relationship observed in this study at the loci examined may be due to management system, nomadic nature of most farmers from the northern part of the country or incidents of cross boarder livestock migration and the subsequent interbreeding between such livestock (Mwacharo et al., 2002) or the indiscriminate crossbreeding of livestock with the hope to improve the breed performance.

Conclusion

The two loci studied were polymorphic and informative, yielding four allelic variants. All allele S was found to be more abundant at the Carbonic anhydrase locus, while allele A was predominant at the Albumin locus in Balami and Yankassa populations. The average heterozygosity showed the WAD sheep to be the most diverse of the four populations studied. The Yankassa and Uda populations were found to conform to the Hardy-Weinberg’s equilibrium for all the loci studied, while Balami and WAD populations had significant deviations from Hardy-Weinberg’s equilibrium at the Albumin and Carbonic anhydrase locus, respectively. The tree topology showed that there were two main clusters; the Balami-Yankassa cluster and the Uda-WAD cluster. The average heterozygosity, F-statistics and dendogram are indicators of the extent of controlled breeding or diversity of the sheep populations. However, studies using DNA markers would generate data that will enhance the understanding of the breed characteristics of the population of sheep in Nigeria

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Papers shall be submitted at the editorial office typed on standard typing pages (A4, 30 lines per page, 62 characters per line). The editors recommend up to 15 pages for full research paper (including abstract references, tables, figures and other appendices).

The manuscript should be structured as follows: Title, Names of authors and affiliation address, Abstract, List of keywords, Introduction, Material and methods, Results, Discussion, Conclusion, Acknowledgements (if any), References, Tables, Figures.

The title needs to be as concise and informative about the nature of research. It should be written with small letter /bold, 14/ without any abbreviations.

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The names of the authors should be presented from the initials of first names followed by the family names. The complete address and name of the institution should be stated next. The affiliation of authors are designated by different signs. For the author who is going to be corresponding by the editorial board and readers, an E-mail address and telephone number should be presented as footnote on the first page. Corresponding author is indicated with *.

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Keywords: Up to maximum of 5 keywords should be selected not repeating the title but giving the essence of study.

The introduction must answer the following questions: What is known and what is new on the studied issue? What necessitated the research problem, described in the paper? What is your hypothesis and goal?

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Results are presented in understandable tables and figures, accompanied by the statistical parameters needed for the evaluation. Data from tables and figures should not be repeated in the text. Tables should be as simple and as few as possible. Each table should have its own explanatory title and to be typed on a separate page. They should be outside the main body of the text and an indication should be given where it should be inserted.

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