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# Genetic differences among selected breeds of sheep using blood biochemical polymorphisms

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## ABSTRACT

**Aim:** Purpose of the study was to investigate the genetic distance among three breeds of sheep using blood biochemical Polymorphisms - Yankasa, Balami and Uda.

**Method and materials:** A total of 30 animals, comprising of 10 individuals from each breed were examined to determine genotype distribution, allele frequencies, Hardy–Weinberg equilibrium (HWE), heterozygosity, inbreeding coefficients and genetic distance. About 5ml of Blood were collected via jugular vein and transferred immediately into a well level bottle (EDTA) anti-coagulant, placed to a chill ice pack and sample were analyzed using an automated haematology analyzer.

**Results:** Results revealed three haemoglobin genotypes (AA, AB and BB) with Yankasa exhibiting all variants while Balami and Uda lacked the heterozygous AB genotype. Hb BB was predominant across all breeds particularly in Balami (90%) and Uda (90%) suggesting possible adaptive advantage or directional selection for the B allele. Chi-square analysis indicated significant deviation from HWE in Yankasa and Uda whereas Balami approximated equilibrium conditions. Observed heterozygosity was extremely low in all breeds particularly Yankasa and Uda (Ho = 0.00) indicating high levels of homozygosity and potential inbreeding. F-statistics showed complete heterozygote deficit in Yankasa and Uda (FIS = 1.00) while Balami exhibited a slight heterozygote excess (FIS = –0.0526). Genetic distance estimates revealed close affinity between Balami and Uda (0.0029) and greater divergence between Yankasa and Balami (0.2076).

**Conclusion:** The findings demonstrated varying levels of genetic diversity among the breeds with evidence of genetic differentiation, reduced heterozygosity and restricted gene flow in some populations. It was concluded that the need for structured breeding, conservation strategies and molecular characterization to enhance genetic diversity and sustain the productivity of indigenous sheep breeds in Nigeria.

**Keywords:** Genetic distance, haemoglobin, genotyping and polymorphism

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## Introduction

Sheep production is an emerging sector for employment and income generation for the rural poor especially land less, it is also an effective tool for poverty alleviation for rural poor (Ahmed *et al*., 2010). Sheep plays a significant role in socio-cultural activities practices as they are great used during cultural and religious festivals such as naming ceremony. In Nigeria, sheep production is the best way of enhancing meat supply and also generate income to the small holder farmers. Sheep are found throughout Nigeria but the highest population is found in the northern part where three of the four breeds of sheep which are Balami, Uda and Yankasa. Efficient intensive production of meat, milk and other food require blends and balance diets (FAO, 2010).



The Uda breed of Ram is a specific breed of sheep that originated in Nigeria. Uda Rams are known for their long, spiral-shaped horns and their meat which is highly valuable for its flavors and tenderness. They are primarily raised for meat production. And are well-adapted to local climatic condition in Nigeria. Uda Rams are often utilized in cross breeding programs to improve the meat quality and productivity of other sheep breeds (Ahmed *et al*., 2010). The characterization of animal diversity is essential to meet future needs in Nigeria in particular, in order to cope with unpredictable responding to directional forces imposed by broad spectrum of environment must be maintained. Maintaining genetic diversity is an important insurance package against future adverse condition (Shoyombo *et al*., 2015).

Protein electrophoresis has been widely used in studying genetic diversity in several farm animals (Osailyuwu *et al*., 2017). The genetic diversity found in domestic breed allows breeders to select

and develop new breeds in respond to changes in demand and environment (Fatai *et al*., 2017) polymorphism of blood protein provide the possibility to study genetic differentiation before the advent of molecular markers. Consequently, several livestock breeds had been characterized for variation in blood protein (Akinyemi *et al*., 2012). There are several important functions of blood proteins, studies in sheep have linked these markers to production traits and environmental adaptations (Vicovan *et al*., 2011). Information on blood protein has also been used to study genetic relationship among breeds. (Nwacharo *et al*., 2002 and Ibeagh-Awemu & Brhardt, 2012). The need for the determination of genetic distance among the breeds of sheep is of paramount importance so as to assess the genetic structure and relatedness to other breeds and to be able to predict potential gains from cross breeding among the individual populations there by replacing the previous resource wasting trait and error method. The general objective of the study was to determine the genetic distance among three breeds of sheep using blood biochemical polymorphisms.

## Materials and Methods

*Study area*

This study was conducted at the Teaching and Research Farm University of Maiduguri, Borno State, Nigeria. The University of Maiduguri is located in the northeastern region of Nigeria with geographical coordinates of approximately 11.8333° N latitude and 13.1500° E longitude. This characterized by a semi-arid climate with an average annual rainfall of 500–1000 mm and a temperature range of 25–40°C (NIMET, 2020). The availability of grazing land and agricultural byproducts in Borno State supports sheep rearing making it an important area for livestock production (Yakubu *et al*., 2010).

*Experimental Animals*

A total of 30 sheep comprising 10 Balami, 10 Uda and 10 Yankasa was used for the study. The animals were randomly selected from the Maiduguri abattior cattle market (Kasuwan Shanu). The animals were brought from different parts of the state for sale and slaughter. The laboratory analysis was conducted at the Department of Animal Science University of Maiduguri.

*Blood collection*

About 5ml of blood sample was collected via jugular vein and transfer immediately into a well level bottle containing ethylene diamine tetra acetic acid (EDTA) anti-coagulant. It was then placed in chill ice box before taken to the laboratory for analysis. The sample was analyze using an automated hematology analyzer. Equipment's and Reagents used includes: Hemoglobin genotyping machine, Cellulose acetate paper, Tris buffer TB (PH 8.6), Filter paper, Genotype applicator, Whatman filter paper, Forceps and Tile.

*Laboratory Analysis*

About 0.1 - 1ml of whole unsedimented blood was drawn into a vacutainer tubes and normal saline was added to washed the red blood cells, the samples were centrifuge at 5°c for 5 minutes at 3000 rpm. The supernatant saline was discarded and distilled water was added to the sedimented cells to release the hemoglobin by hemolysis. A transfer pipette was used to remove the hemolysate, a cellulose acetate paper was prepared and labeled with pencil. The strip was later suck with Tris Buffer (TB, PH 8.6) and blotte light with a filter paper. After that put a drop of sample on the tile of each sample. Prepared genotype machine by pouring Tris Buffer in to the two side of the genotype tank avoiding the bridge at the middle. Used forcep to remove cellulose acetate paper and blood in between two filter paper, apply the drop on the tile on the cellulose acetate paper on a straight line at the base of the Tris buffer. Used the forceps to transfer the cellulose acetate paper with the sample and placed it across the bridge of the genotype tank in a straight position and close the cover then apply light. Observe it to 5-10 minutes and interpret the movement.

AA Single Faster Band Homozygote

BB Slower Single Band Homozygote

AB Double Band Heterozygote

*statistical analysis*

Allele and genotype frequencies for the locus in each sample was computed by direct gene counting. The distribution of the haemoglobin genotypes was tested using chi-square analysis. The genotype frequency for each population was estimated using the following expression (Salako *et al*., 2007).

$$\text{AA} = \frac{Total No.\ of AA}{No.\ of Individual Breed} \times 100$$

$$\text{BB} = \frac{Total No.\ of BB}{No.\ of Individual Breed} \times 100$$

$$\text{AB} = \frac{Total No.\ of AB}{No.\ of Individual Breed} \times 100$$

The frequency for each breed was estimated using the expression as reported by (Salako *et al.,* 2007).

$$\text{Allele A} = \frac{2nAA + nAB}{N/2}$$

$$\text{Allele B} = \frac{2nBB + nAB}{N/2}$$

Where,
N = total number of Individual sampled in each population
nAA = observed genotype number for AA
nBB = observed genotype number for BB
nAB = observed genotype number for AB
The genetic distance between Balami, Uda and Yankasa sheep was estimated according to the method of Bodmer and Cavallisforza (1976) with the following expression
$d^2 = (P1 - P2)^2$
Where,
$d^2$ = genetic distance
P1 = gene frequency of allele A
P2 = gene frequency of allele B
$d^2$ is simply the square of the difference of the two gene frequency from the pair population.

$$\text{Chi square } (x^2) = \frac{(observed\ number - expected\ number)^2}{expected\ number}$$

$$\text{Fis} = \frac{Hs - H}{Hs}$$

Where, FIS = Inbreeding Co-efficient
FSt= Genetic differentiation
FIT= Heteroygote deficit across population.
Dendogram (Phylogennetic tree) was constructed from genetic distance of the three population
Inbreeding Co-efficient (FIS), genetic differentiation (FST) at Heterozygosity indices following the procedure of Reynoid's (Reynoid *et al*., 1983) genetic distance.

## Results and Discussion

*Distribution of Haemoglobin Genotypes in Three Sheep Breeds*

The distribution of haemoglobin (Hb) genotypes among Yankasa, Balami and Uda sheep breeds revealed the presence of the three classical haemoglobin variants (AA, AB and BB), although not uniformly across breeds (Table1). Yankasa exhibited all three genotypes with a predominance of Hb BB (60%) followed by Hb AA (40%), Balami had the highest proportion of Hb BB (90%) and (10%) Hb AB with absence of Hb AA. Whereas Uda recorded 90% Hb BB and only 10% Hb AA whereas Yankasa and Uda showed complete absence of the heterozygous genotype (AB).

The dominance of Hb BB in Balami and Uda implies possible adaptive advantage in their production environments consistent with the report of Hrinca (2008) who associated the fixation of Hb B allele with moderate temperature and favorable breeding conditions. Similar observations were made by Salako *et al.* (2007) who found variable distribution of haemoglobin genotypes across Nigerian small ruminants. The absence of AB in both Yankasa and Uda suggests reduced heterozygosity which may result from population fragmentation or non-random mating. According to Alphonsus *et al.* (2012), loss of heterozygotes across generations may signal reduced genetic exchange and possible drift effects. The results therefore, indicate considerable variation in haemoglobin genotype structure among the three sheep breeds suggesting differing levels of genetic diversity and possible environmental or demographic influences affecting genotype frequencies.

*Genotype Frequencies of the Sheep Breeds*

The genotype frequencies of three breeds of sheep, In Yankasa sheep all three genotypes (AA, AB and BB) were present with frequencies of 0.4 (AA), 0.0 (AB) and 0.6 (BB). This distribution indicates moderate heterogeneity but with a clear dominance of the BB genotype. The coexistence of two homozygous genotypes suggests a more variable gene pool compared to the other breeds. In Balami sheep the genotype frequencies were 0.0 (AA), 0.05 (AB) and 0.95 (BB). The nearly complete fixation of the BB genotype and near absence of the AA genotype indicate extremely low genetic diversity at the Hb locus. The rare occurrence of the AB genotype (0.05) reflects minimal heterozygosity implying strong directional selection for the B allele or historical inbreeding effects. In Uda sheep the frequencies also showed a similar trend with AA = 0.1, AB = 0.0 and BB = 0.9. Like Balami and Uda exhibited a high proportion of BB individuals although with a slightly higher presence of AA individuals when compared to Balami (Table 2). The total absence of heterozygotes may suggest genetic drift restricted gene flow or environmental adaptation that favors the B allele. Yankasa with a more balanced AA and BB distribution appears to maintain higher genetic variability which may confer greater adaptive flexibility. Mabruka and Ahmad (2018) documented variable Hb genotype frequencies among Nigerian sheep and goats, emphasizing the influence of environmental adaptation. Their observation of fluctuating AB proportions across regions agrees with the

depressed AB frequencies found in Balami and Uda in this study. Ganiyu et al. (2018) reported that high homozygosity—particularly for Hb B—is common in isolated or intensively selected populations. This is consistent with the high BB frequencies (0.6–0.95) recorded in all breeds examined. Abdulmalik *et al.* (2023) similarly observed dominance of the B allele in Nigerian sheep populations and linked it to adaptation to arid and sub-humid environments. The strong presence of BB in Balami and Uda which are traditionally associated with northern savannah regions supports this explanation. Asadollahpour Nanaei *et al.* (2020) documented low heterozygosity in some sheep breeds as a consequence of reduced gene flow or selective breeding. This parallels the absence or near-absence of AB genotypes in Balami and Uda.

*Hardy–Weinberg Equilibrium and Chi-Square Analysis*

The observed and expected genotype counts along with Chi-square results (Table 3) provide insight into population equilibrium states. For Yankasa, the Chi-square components (3.6 + 4.8 + 1.6 ≈ 10.0) exceeded the table value of 5.99 at 2 df, indicating significant deviation from Hardy–Weinberg equilibrium (HWE). The genotype $\chi^2$ total values to give the overall chi-square statistic for each population Yankasa 10, Balami 0.02770 and Uda 10. The deviation between observed and expected frequencies is statistically significant in both Yankasa and Uda breeds whereas the Balami has no significant difference between observed and the expected values, if the observed value and expected value are equal there is no significant difference (McHugh *et al.,* 2013). The same was observed in Uda though the deviation was marginal. In Balami, the Chi-square value was extremely small (0.00263 + 0.000069) suggesting near conformity to HWE.

Deviation from HWE in Yankasa and Uda may be attributed to random genetic drift, small population size or non-random mating. Similar deviations were reported by Abdulraheem et al. (2024) in Nigerian chicken populations and by Gwaza *et al.* (2019) in WAD goats. In contrast, Balami's approximate conformity to equilibrium implies a stable mating structure, large population size or absence of strong selective pressures. The predominance of Hb BB and absence of AB in Balami and Uda also explain the deviations as equilibrium requires the presence of all genotypes in frequencies proportional to allele distributions (Reynolds *et al.*, 1983).

*Heterozygosity, Inbreeding Coefficients and F-Statistics*

Results of heterozygosity indices and F-statistics (Table 4) provide deeper genetic diversity insights. Observed heterozygosity (Ho) was zero in both Yankasa and Uda reflecting complete absence of heterozygous individuals. Balami had a slightly higher Ho (0.1) consistent with one heterozygote recorded. Expected heterozygosity (He) values however, were moderately higher Yankasa (0.4), Balami (0.095) and Uda (0.18). High He relative to Ho indicates excess homozygosity and suggests inbreeding or selective breeding. This was confirmed by FIS values: Yankasa (1.0) and Uda (1.0) indicating complete heterozygote deficit. Negative FIS in Balami (–0.0526) reflects heterozygote excess, implying outbreeding or mixing, consistent with reports by Awobajo *et al.* (2016) on WAD goats. Negative Fst values in Balami (–0.0526) indicate disassortative mating or immigration of genetically distinct individuals. High Fit and Fst in Yankasa and Uda show both within- and across-population genetic deficits, aligning with findings by Diyaware *et al.,* (2018) who associated high F-statistics with fragmented or isolated populations.

The heterozygosity and inbreeding patterns indicate:

Yankasa and Uda = high inbreeding, low heterozygosity

Balami = slightly outbred, genetically more stable.

*Genetic Distance and Breed Relationships*

Genetic distance values (Table 5) indicated measurable differentiation among the breeds. The highest distance was observed between Yankasa and Balami (0.2076) followed by Yankasa and Uda (0.1552). Balami and Uda exhibited very low genetic distance (0.0029) indicating close genetic similarity. High genetic distance suggests reduced gene flow and possible long-term separation. Similar observations were reported by Yakubu *et al.* (2010) who found higher distances between Sahel and WAD than between Sokoto red and WAD. The low distance between Balami and Uda may reflect shared geographical distribution, similar management systems or historical crossbreeding. Dafur *et al.* (2018) low genetic distance is a strong indicator of phylogenetic relatedness, while high distance reflects differentiation that may be useful for breed classification or selection programmes.

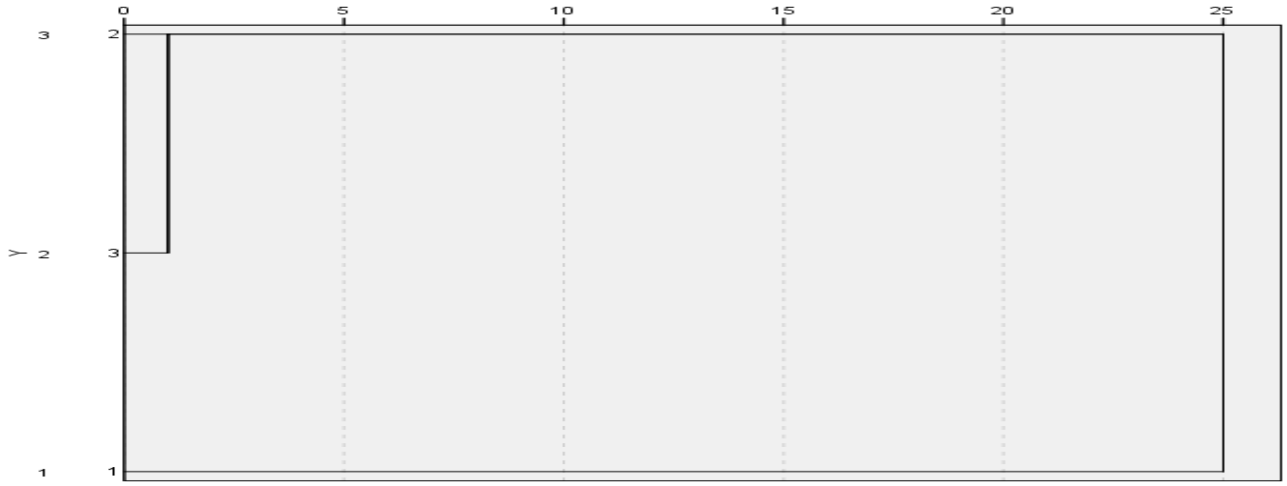


Fig. 1. Dendrogram using average linkage (between groups) rescaled distance combine

Table 1. Distribution and frequency of haemoglobin genotyping of three breeds of sheep

| Population | Genotype Frequency | | | | Gene Frequency | | |
|---|---|---|---|---|---|---|---|
| | AA | AB | BB | Total | AA | AB | BB |
| Yankasa | 4 | 0 | 6 | 10 | 40 | 0 | 60 |
| Balami | 0 | 1 | 9 | 10 | 0 | 10 | 90 |
| Uda | 1 | 0 | 9 | 10 | 10 | 0 | 90 |

Table 2. Genotype Frequency of three Breeds of Sheep

| Population Genotype | Frequency | | | |
|---|---|---|---|---|
| | AA | AB | BB | Total |
| Yankasa | 0.4 | 0.0 | 0.6 | 1.00 |
| Balami | 0.0 | 0.05 | 0.95 | 1.00 |
| Uda | 0.1 | 0.0 | 0.9 | 1.00 |

Table 3. Observed and expected genotype count and chi-square of three breeds of sheep

| | Population | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Yankasa | | | Balami | | | Uda | | |
| Genotype | AA | AB | BB | AA | AB | BB | AA | AB | BB |
| Observed | 4.0 | 0.0 | 6.0 | 0.0 | 1.0 | 9.6 | 1.0 | 0.0 | 9.0 |
| Expected 1.6 | 4.8 | 3.6 | 0.025 | 0.095 | 9.025 | 0.1 | 1.8 | 8.1 | |
| Deviation | 2.4 | -4.8 | 2.4 | -0.025 | 0.05 | -0.025 | 0.9 | -1.8 | 0.9 |
| Exp-Obser | -2.4 | 4.8 | -2.4 | 0.025 | -0.05 | 0.025 | -0.9 | 1.8 | -0.9 |
| $X^2$ | 3.6 | 4.8 | 1.6 | 0.025 | 0.00263 | 0.000069 | 8.1 | 1.8 | 0.1 |
| $X^2$ total | | 10 | | | 0.02770 | | 10 | | |
| Tab value | 5.99 | at2df | | | | | | | |
| Significance | | P<0.05 | | P>0.05 | | P<0.05 | | | |

Exp: Expected Value Obser: Observed Value

Table 4. Local observed expected and inbreeding co-efficient, heteroygosity indices and f statistic for breeds of sheep

| | Population | | |
|---|---|---|---|
| Parameter | Yankasa | Balami | Uda |
| $H_o$ | 0 | 0.1 | 0 |
| $H_e$ | 0.4 | 0.095 | 0.18 |
| $F_s$ | #DIV/0! | -0.0526316 | 1 |
| $H_I$ | 0 | 0.1 | 0 |
| $H_s$ | 0.4 | 0.095 | 0.18 |
| $H_T$ | 0.48 | 0.095 | 0.18 |
| $F_{IS}$ | 1 | -0.0526316 | 1 |
| $F_{st}$ | 0.16666667 | 0 | 0 |
| $F_{it}$ | 1 | -0.0526316 | 1 |

Keys: $H_o$ =Observed Heteroygosity, $H_e$=Expected Heterogosity, $F_s$=Local Inbreeding Co-efficient, $H_i$ $H_s$, $H_T$=Heterozygosity indices, $F_{is}$=Heteroygote deficit within population (Inbreeding Co-efficient), $F_{st}$=Genetic differentiation and $F_{it}$=Hetetoygote deficit across population

Table 5. Genetic Distance between three breeds of sheep

| Genetype | Yankasa | Balami | Uda |
|---|---|---|---|
| Yankasa | 0.000 | 0.207627 | 0.155172 |
| Balami | 0.00916 | 0.000 | 0.002907 |
| Uda | 0.002907 | 0.004725 | 0.000 |

A dendrogram presented that illustrates the results of a hierarchical cluster analysis using the Average Linkage (Between Groups) rescaled distance combine (Fig 1). It visually represents the grouping of three breeds of sheep Balami, Uda and Yankasa. The dendrogram shows similarities among Yankasa, Balami and Uda breeds of sheep hierarchical clustering pattern based on their measured characteristics, the height of the vertical lines reflects the distance or dissimilarity at which the breeds are merged. Balami and Uda cluster at a lower height—represented by shorter vertical lines—indicating a greater degree of similarity between them. This close association suggests that the two breeds share more comparable phenotypic or genetic attributes, shared common ancestors. A finding consistent with earlier reports that classified both Balami and Uda as large-sized desert-adapted breeds with similar morphological and physiological traits share common ancestors (Yakubu *et al.,* 2020 and Okpeku *et al.,* 2021). Yankasa however merges with the Balami–Uda cluster at a higher vertical distance reflected by a longer linkage line. This indicates that Yankasa is more distinct in the evaluated parameters which aligns with previous studies describing Yankasa as a separate, more widely distributed savannah breed with different adaptive and body conformation characteristics (Agaviezor *et al.,* 2012 and Musa *et al.*, 2020). Overall, the dendrogram demonstrates that Balami and Uda are more closely related to each other than to Yankasa reinforcing their shared desert-adaptation profile.

## Conclusion

It was concluded that three Nigerian sheep breeds differ considerably in their haemoglobin genotype composition and genetic structure. The predominance of Hb BB across the populations suggests a breed-level fixation that may be influenced by environmental adaptation or historical breeding practices. Yankasa demonstrated relatively higher genetic variation compared to Balami and Uda. However, significant deviation from Hardy–Weinberg equilibrium and high inbreeding coefficients indicate that genetic drift and restricted gene flow may be influencing its population structure. Balami maintained the most stable population genetics conforming closely to equilibrium expectations. The extremely low heterozygosity recorded among the breeds particularly Yankasa and Uda indicates reduced genetic diversity which may limit the adaptive potential of these populations. Genetic distance patterns further suggest that Yankasa is genetically distinct from Balami and Uda while the latter two share recent common ancestry or exchange of genetic material.

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