



## **Studies on the Productive Traits and Relationship between Breeds of Cattle (Friesian Bunaji Cross, Bunaji and Sokoto Gudali) Using Blood Biochemical Polymorphism**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Author DSBU designed the study. Author UAU performed the statistical analysis. Author BIN wrote the protocol. Author OOR managed the literature searches. Author IS managed the analyses of the study. All authors read and approved the final manuscript.*

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### **ABSTRACT**

We studied the blood haemoglobin polymorphism and activity in relation to body morphology and milk traits in Nigerian indigenous breeds of cattle. A total of 150 animals, 50 per breed of the Bunaji, Friesian X Bunaji and Gudali were used to study the relationship between breeds. Variables measured were BW: Body weight (Kg); BL: Body Length (Cm); HW: Height at withers (cm); CW: Chest width (cm); HG: Heart Girth (cm); Rumwi: Rump width (cm); TL: Teat Length (cm); RUH: Rear Udder Height (cm); UC: Udder Circumference (cm); TY: Total Yield (Litres); ADY: Average Daily Yield (Litres/day) and LL: Lactation Length (days) and blood protein polymorphism of the Haemoglobin. Observed results showed overall frequencies of 0.62 (HbA) and 0.48 (HbB). Higher

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frequencies of the A allele (0.52, 0.54 and 0.68) were obtained respectively for the Bunaji, Friesian X Bunaji and Gudali cattle in the Haemoglobin locus. Study of blood protein polymorphism and productivity indicated significant ( $p < 0.05$ ) influence of the haemoglobin. There is a need for a genetic study using other proteins and at DNA level to compliment the results arisen from morphometric differentiation of the two most populous Nigerian breeds of cattle in the NAPRI herd.

*Keywords: Genetic characterization; gene introgression; haemoglobin; polymorphism.*

## 1. INTRODUCTION

Much genetic research is now directed towards the investigation of the relationship between physiological, biochemical and metabolic products/markers to the productive efficiency of farm animals. Biochemical traits, including blood groups, blood proteins and enzymes have been studied to explain the physiological basis of performance traits and the effect of heterosis. Polymorphism of blood protein first offered the possibility to study genetic differentiation before the advent of molecular markers. Consequently, several livestock breeds including the domestic cattle, sheep and goat have been characterized for variations in major blood proteins [1]. In addition to several important functions of blood proteins, several studies in cattle and sheep have already linked these markers to production traits and environmental adaptation [2,3].

Blood polymorphism studies have been conducted extensively to identify biodiversity among livestock. Biochemical particles of blood can be determined easily at the post-natal period of young animals, and these components are merely or not affected by the environmental factors. Several works have been conducted to detect the different types of blood components such as Haemoglobin, Albumin, Glutathione, Transferrin and Potassium [3,4].

Haemoglobin variants have been extensively studied in Zebu cattle and at least eight variants have been identified. Four migration bands were found, Hba, Hbl, Hbc and HbB, but the last band (HbB) may be possibly broken into two, named; HbB1 and HbB2. The respective gene frequencies were  $0.563 \pm 0.012$ ,  $0.007 \pm 0.01$ ,  $0.021 \pm 0.002$ ,  $0.188 \pm 0.007$  and  $0.221 \pm 0.007$ . The genetic frequencies were in equilibrium [5]. The existence of two types of haemoglobin; Hb and HB has been established [6]. They are expressed as homozygous  $Hb^{AA}$  and  $Hb^{BB}$  and phenotypes with  $Hb^{AC}$  being a pre-adult form of Hb [7]. However, this study was designed to determine blood haemoglobin activity about body morphology and milk traits in Nigerian breeds of cattle.

## 2. MATERIALS AND METHODS

### 2.1 Location of the Study

The research was conducted at the Dairy Breeding Unit of National Animal Production Research Institute (NAPRI), Shika, Zaria, Kaduna State, Nigeria. NAPRI is geographically located between latitude  $11^\circ$  and  $12^\circ N$  and longitude  $7^\circ$  and  $8^\circ E$  at an altitude of 640 m above sea level.

### 2.2 Animals and Management

Animals used for this research were sourced in the National Animal Production Research Institute (NAPRI). They were raised under semi-intensive system of management.

### 2.3 Sampling Size and Sampling Structure

A total of 150 cattle comprising of an equal number of Friesian X Bunaji cross, Sokoto Gudali and White Fulani were used for the study.

### 2.4 Age Determination

Bio-information of birth date of all the animals used in this study was collected from NAPRI.

### 2.5 Quantitative Characters

Nine metric characters including body weight and ten linear measurements were taken on each sampled animal. They include: BW: Body weight (kg), BL: Body length (cm), HW= Height at withers (cm), CW: Chest width (cm), HG: Heart girth (cm), RW: Rump width (cm), TL: Teat Length (cm), RUH: Rear udder height (cm), UC: Udder circumference (cm).

### 2.6 Metric Variables

Weights of the animals were taken using a spring balance and walk-in weighing bridge (kg). Flexible tape rule was used to take the body measurement. During body measurement

animals were made to stand upright and restrained by two assistants in such a way that their heads, necks, and chest were stretched almost in a straight line, each measurement was taken at least three times and the mean recorded to the nearest cm.

## 2.7 Udder Measurements

The Udder and teat measurements were done using flexible tape measures (cm) as follows:

**Udder Circumference (cm):** Measured at the widest point of the Udder round it.

**Udder height (cm):** Measured from the rear attachment of the Udder to the front of it where it blends with the body.

**Teat length (cm):** measured as the distance from the upper part of the teat, where it hangs perpendicularly from the Udder to the tip of the teat.

## 2.8 Milk Yield Characteristics

Milk yield characteristics were measured as follows:

**Average Daily Yield (ADY):** - As an average of all test day yields within the milking period.

**Total Yield (TY):** - As milk production during the lactation period up to the point where the production of the cow dropped below 100 ml.

**Lactation Length (LL):** - As the period from calving to the point when the milk yield of the cow falls below 100 ml.

## 2.9 Blood Collection

Blood was collected from each animal by jugular vein puncture and placed in heparinized tubes to prevent coagulation. It was refrigerated prior to analyses.

## 2.10 Sample Preparation

### 2.10.1 Blood haemolysates

Red blood cell was prepared from the erythrocyte fraction of heparinised blood by centrifuging at 2500–3000 rpm for 10 mins at 4°C. The RBC were washed in saline (0.155M NaCl) three times and centrifuged at 2500–3000 rpm for 5mins at 4°C. The RBCs were lysed with a fourfold volume of distilled H<sub>2</sub>O to release haemoglobin.

### 2.10.2 Plasma

The plasma fraction is separated from the erythrocyte fraction of heparinised blood by centrifuging at 2500 – 3000 rpm.

### 2.10.3 Gel soaking

Cellulose acetate plates were soaked in the same buffer as the electrode buffer. This is often referred to as a continuous buffer system. Multiple gels were simultaneously soaked in an 800 ml beaker with individual gel plates separated by glass rods to ensure complete soaking of every plate.

### 2.10.4 Sample loading

Prepared blood samples were added to the wells of the sample plate. Cellulose acetate paper was blotted dry between sheets of filter paper to remove excess moisture.

### 2.10.5 Gel running

The side bearing the acetate was placed on the wick. Since the current runs from the cathode to the anode electrode (negative to positive), the loaded zones on the plate were positioned at the cathodal end of the tank. For the majority of the enzyme systems which migrate anodally e.g. haemoglobin, the loading zone was placed on the anodal end.

### 2.10.6 Gel staining

When the gel run is complete, the plates were removed from the tank and placed in an empty petri dish. Again, care was taken to ensure that the cellulose acetate plate lies horizontally. Once plates have been removed from the tank, they were stained with ponceau stain before they dry out.

### 2.10.7 Gel scoring

After 20 minutes the plates were sufficiently stained, they were destained several times with trichloroacetic acid until clear and sharp bands appear. The bands were scored visually based on their migratory pattern as described by Riken [8].

### 2.10.8 Electrophoretic conditions

The method to be used is as described by Riken [8] are shown in Table 1.

**Table 1. Electrophoretic conditions of haemoglobin polymorphism**

	<b>Buffer</b>	<b>Time (mins)</b>	<b>p<sup>H</sup></b>	<b>Stain</b>
Haemoglobin	Tris EDTA borate	40	8.4	Ponceau Stain

**2.10.9 Bands scoring**

Bands were scored visually as described by Riken [8] according to the migration of the bands. Direct counting was used for calculating gene frequencies. Frequencies generated were used to compute genotypic frequencies.

**2.11 Statistical Analysis**

The effect of breed and blood enzyme polymorphism on measured traits and their interaction were determined using the PROC GLM of [9]. Significant (p<0.05) differences in means were separated using Duncan Multiple Range Test (DMRT).

Model for the Analysis was a factorial ANOVA design as illustrated below:

$$Y_{ijk} = \mu + B_i + P_j + BP_{ij} + E_{ijk}$$

Where

- Y<sub>ijk</sub> is the record observed
- μ = population mean
- B<sub>i</sub> = Fixed effect of the i<sup>th</sup> breed of cattle
- P<sub>j</sub> = Fixed effect of the j<sup>th</sup> polymorphic types of blood protein
- BP<sub>ij</sub> = interaction between breed and blood protein polymorphic class
- E<sub>ijk</sub> = random error particular to the ijk<sup>th</sup> observation assumed to be independently randomly distributed with mean zero and variance σ<sup>2</sup><sub>e</sub>, i.e., NID (0, σ<sup>2</sup><sub>e</sub>).

**3. RESULTS**

**3.1 Performance of Breeds in Morphometric and Selected Milk Production Traits**

The result of mean comparison of body morphology and selected milk Production traits of the three breeds is presented in Table 2. Body Weight (BW) and all other measured traits differed significantly (p<0.05) between the breeds; the highest BW and BL was obtained with the Friesian–Bunaji and this differed from the Gudali and the Gudali differed from the Bunaji with the least BW and BL. The Gudali had the highest HW, CW, HG, Rumwi and TY

(178.42, 31.69, 127.78, 50.18 and 1388.52 cm); the Friesian-Bunaji (174.99, 25.13, 124.09, 43.53 cm and 1097.59 L). TL was significantly higher in the hybrid (5.10) and differed from the Bunaji (4.67) which also differed from the Gudali (4.47 cm) being the least. The same trend was observed with RUH and UC with the exception that UC were similar in magnitude between the Bunaji and Gudali. TY in this study was significantly higher with the Gudali (1203.52), while the Bunaji and its hybrid were statistically (p>0.05) similar; ADY indicated a reversed trend with the Friesian–Bunaji having the highest Production while the Bunaji and Gudali were similar. LL was higher and similar between the Bunaji and Friesian–Bunaji compared to the Gudali which had the least LL value.

**3.2 Effect of Haemoglobin Types on Morphology and Milk Production**

The effect of the haemoglobin polymorphism on growth and milk traits of the breeds of cattle are presented in Table 3. Polymorphic forms of haemoglobin only significantly (p<0.05) influenced BW, BL and CW. While the other traits indicated non-significant variations due to haemoglobin types. The highest BW and BL was observed with the AB genotype (391.15 and 178.95) while the BB and AA homozygote individuals exhibited statistically similar (p>0.05) magnitude for these traits. CW was also larger with the homozygote individual and differed from the BB genotype, while the BB genotype differed from the AA genotype. It was also noted that for most traits studied which did not show significant variation, the heterozygote had higher mean values for most of these.

**3.3 Effect of Breed and Haemoglobin forms on Morphology and Milk Traits**

Table 4 shows the result of the interaction between breed and haemoglobin forms. All measured characteristics varied significantly (p<0.05) within and between breed due to the effect of haemoglobin polymorphs. The highest BW (398.81 Kg) was observed in the AB phenotype of Friesian -Bunaji, followed by the BB (392.32 Kg) which ranked next and was similar to the AA in this breed and all the other polymorphs

in the Gudali but differed from the Bunaji were all the Haemoglobin polymorphs were similar and had the least BW. BL were highest and similar among forms in the Friesian-Bunaji (180.40–180.70 cm) and differed from the Gudali where the AB and BB ranked next (178.86 and 178.95) but differed from the AA with the least length in this breed (176.83) that was statistically similar to the records obtained among the polymorphs in the Bunaji breed (175.12–175.99 cm). Generally for HW, CW, HG and Rumwi, the interaction between polymorphic forms of Haemoglobin and the different breeds indicates lower measures in the Bunaji irrespective of forms and medium to higher means for the Bunaji-Friesian and the best performance for these traits in the Gudali. TL, RUH, UC, TYA, ADY and LL means indicated a reversal of these trends with the best

mean profile existing with the Friesian- Bunaji followed by the Bunaji and finally the Gudali. Also it was observed that for most studied characteristics, the highest mean were noted for the AB phenotype among haemoglobin forms with the exception of HW, CW and HG were the AA ranked highest and in Rumwi were the BB had the best mean value.

#### 4. DISCUSSION

The observed significant ( $p < 0.05$ ) differences in all body and milk production traits measurements of the three studied population indicates clear breed distinction. Observed value for the Bunaji BW (Kg) (379.95) was higher than the value of 249 reported for the NAPRI cow herd [10] but fell between the range 250 – 380 as reported in

**Table 2. Performance of breeds in morphometric and selected milk production traits**

Breed	Bunaji	Friesian X Bunaji	Gudali	SEM
BW	379.95 <sup>c</sup>	395.40 <sup>a</sup>	388.42 <sup>b</sup>	3.35
BL	175.48 <sup>c</sup>	180.63 <sup>a</sup>	178.35 <sup>b</sup>	1.01
HW	170.02 <sup>c</sup>	174.99 <sup>b</sup>	178.42 <sup>a</sup>	0.78
CW	22.22 <sup>c</sup>	25.13 <sup>b</sup>	31.69 <sup>a</sup>	1.13
HG	124.94 <sup>b</sup>	124.09 <sup>b</sup>	127.78 <sup>a</sup>	1.20
Rumwi	44.00 <sup>b</sup>	43.53 <sup>b</sup>	50.18 <sup>a</sup>	3.08
TL	4.67 <sup>b</sup>	5.10 <sup>a</sup>	4.47 <sup>c</sup>	0.36
RUH	19.69 <sup>b</sup>	24.43 <sup>a</sup>	17.45 <sup>c</sup>	1.95
UC	41.35 <sup>b</sup>	44.08 <sup>a</sup>	40.08 <sup>b</sup>	1.56
TY	1042.87 <sup>b</sup>	1097.59 <sup>b</sup>	1203.52 <sup>a</sup>	47.71
ADY	4.37 <sup>b</sup>	7.40 <sup>a</sup>	5.43 <sup>b</sup>	0.76
LL	245.33 <sup>a</sup>	255.68 <sup>a</sup>	218.99 <sup>b</sup>	9.61

<sup>abc</sup>: Means with different superscript across rows differ significantly ( $p < 0.05$ )

Keys: BW: Body weight (Kg); BL: Body Length (cm); HW: Height at withers (cm); CW: Chest width (cm); HG: Heart Girth (cm); Rumwi: Rump width (cm); TL: Teat Length (cm); RUH: Rear Udder Height (cm); UC: Udder Circumference (cm); TY: Total Yield (Litres); ADY: Average Daily Yield (Litres/day) and LL: Lactation Length (days)

**Table 3. Effect of haemoglobin types on morphology and milk production**

Haemoglobin	AA	AB	BB	SEM
BW	383.83 <sup>b</sup>	394.15 <sup>a</sup>	387.92 <sup>b</sup>	3.35
BL	176.99 <sup>b</sup>	180.95 <sup>a</sup>	178.59 <sup>b</sup>	1.01
HW	174.17	174.87	173.88	0.78
CW	24.98 <sup>c</sup>	28.58 <sup>a</sup>	25.75 <sup>b</sup>	1.13
HG	126.5	125.63	125.26	1.20
Rumwidth	45.29	46.43	44.80	3.08
TL	4.66	4.83	4.72	0.36
RUH	20.42	20.59	20.72	1.95
UC	41.52	42.04	41.99	1.56
TY	1187.81	1187.81	1172.24	47.71
ADY	4.49	5.45	5.37	0.76
LL	233.34	244.82	241.64	9.61

<sup>abc</sup>: Means with different superscript across rows differ significantly ( $p < 0.05$ )

**Table 4. Effect of breed and haemoglobin forms on morphology and milk traits**

Breed	Bunaji			Friesian X Bunaji			Gudali			SEM
HB	AA	AB	BB	AA	AB	BB	AA	AB	BB	
BW	379.75 <sup>c</sup>	379.67 <sup>c</sup>	381.48 <sup>c</sup>	389.89 <sup>b</sup>	398.81 <sup>a</sup>	392.32 <sup>b</sup>	387.68 <sup>b</sup>	388.63 <sup>b</sup>	388.97 <sup>b</sup>	2.82
BL	175.51 <sup>c</sup>	175.12 <sup>c</sup>	175.99 <sup>c</sup>	180.70 <sup>a</sup>	180.66 <sup>a</sup>	180.40 <sup>a</sup>	176.83 <sup>c</sup>	178.86 <sup>b</sup>	178.95 <sup>b</sup>	0.99
HW	170.22 <sup>c</sup>	170.06 <sup>c</sup>	168.92 <sup>d</sup>	174.72 <sup>b</sup>	174.77 <sup>b</sup>	176.22 <sup>ab</sup>	178.27 <sup>a</sup>	177.23 <sup>a</sup>	170.32 <sup>c</sup>	0.94
CW	22.12 <sup>c</sup>	22.42 <sup>c</sup>	22.38 <sup>c</sup>	25.03 <sup>b</sup>	25.16 <sup>b</sup>	25.19 <sup>b</sup>	31.98 <sup>a</sup>	31.63 <sup>a</sup>	31.30 <sup>a</sup>	0.37
HG	174.35 <sup>c</sup>	175.53 <sup>b</sup>	176.76 <sup>b</sup>	174.53 <sup>c</sup>	173.88 <sup>c</sup>	174.11 <sup>c</sup>	183.3 <sup>4a</sup>	176.74 <sup>b</sup>	176.54 <sup>b</sup>	1.44
Rumwi	43.82 <sup>d</sup>	43.95 <sup>d</sup>	45.03 <sup>c</sup>	43.62 <sup>d</sup>	43.62 <sup>d</sup>	43.07 <sup>d</sup>	50.66 <sup>b</sup>	49.82 <sup>b</sup>	51.38 <sup>a</sup>	0.59
TL	4.63 <sup>b</sup>	4.82 <sup>ab</sup>	4.59 <sup>b</sup>	4.83 <sup>ab</sup>	5.23 <sup>a</sup>	5.04 <sup>a</sup>	4.53 <sup>b</sup>	4.48 <sup>b</sup>	4.35 <sup>c</sup>	0.11
RUH	19.90 <sup>c</sup>	19.29 <sup>c</sup>	19.39 <sup>c</sup>	25.38 <sup>a</sup>	24.23 <sup>b</sup>	23.58 <sup>b</sup>	17.36 <sup>d</sup>	17.48 <sup>d</sup>	17.51 <sup>d</sup>	0.38
UC	41.68 <sup>ab</sup>	40.89 <sup>b</sup>	40.57 <sup>b</sup>	43.50 <sup>a</sup>	44.37 <sup>a</sup>	44.00 <sup>a</sup>	39.12 <sup>b</sup>	40.41 <sup>b</sup>	40.40 <sup>b</sup>	1.26
TYA	942.04 <sup>c</sup>	942.78 <sup>c</sup>	947.19 <sup>c</sup>	1388.32 <sup>a</sup>	1388.39 <sup>a</sup>	1389.33 <sup>a</sup>	1087.99 <sup>b</sup>	1101.36 <sup>b</sup>	1097.75 <sup>b</sup>	9.18
ADY	3.38 <sup>d</sup>	3.36 <sup>d</sup>	3.36 <sup>d</sup>	7.30 <sup>a</sup>	7.52 <sup>a</sup>	7.17 <sup>ab</sup>	4.36 <sup>c</sup>	4.39 <sup>c</sup>	4.96 <sup>b</sup>	0.15
LL	219.67 <sup>d</sup>	217.93 <sup>d</sup>	217.60 <sup>d</sup>	254.11 <sup>a</sup>	256.25 <sup>a</sup>	256.2 <sup>0a</sup>	245.76 <sup>b</sup>	244.66 <sup>b</sup>	248.79 <sup>b</sup>	1.85

<sup>abcd</sup>: Means with different superscript across rows differ significantly (p<0.05)

Keys: BW: Body weight (Kg); BL: Body Length (Cm); HW: Height at withers (cm); CW: Chest width (cm); HG: Heart Girth (cm); Rumwi: Rump width (cm); TL: Teat Length (cm); RUH: Rear Udder Height (cm); UC: Udder Circumference (cm); TY: Total Yield (Litres); ADY: Average Daily Yield (Litres/day) and LL: Lactation Length (days)

Tawah and Rege (1994), while the value 395.40 obtained was lower than the value 491 recorded by these authors for the Friesian-Bunaji. This may be attributed to differences in age of sampled herds, the season of study amongst other things. The value of 388.42 kg observed in the Gudali was higher than the range of 241-353 kg, 335-336 kg and 360-363 kg reported for the Sokoto Gudali, Adamawa Banyo and Yola Gudali but within the range 330-408 kg for the Nguadere Gudali of Adamawa [11]. Since it has been noted that the Gudali is more of a beef cattle compared to the Bunaji, the observed superiority of the Gudali to the Bunaji in BW appears tenable. The Superiority of the Gudali over the Bunaji in HW, CW, HG and Rumwi were consistent with the findings [12]. They stated that generally, the linear body measurements of Sokoto Gudali were significantly ( $P < 0.05$ ) higher than those of the Bunaji cattle except body length and face length respectively, this study, however, showed differences in BL between the two breeds.

Comparative measurements of morphometric traits can provide evidence of breed relationships and size. The considerable variation in body dimensions of the two cattle breeds might not be unconnected with individual breed's potential and peculiarities. While the Bunaji cattle are noted for milk production, their Sokoto Gudali counterparts which are often ranked second in milk production produce more meat and appear to have more draught power than the former [12]. The superiority of crossbred animals to local breeds in this study needs not to be emphasised as it is a generally accepted trend in animal improvement work. The estimates for BL (175.48 -180.63) were comparable to 175.29-179.02 reported by Espinoza, et al. [12]. HW estimates were higher (170.02-178.42) than 110-148.40 reported by various authors for different cattle breed [13,14]. Observed measures of HG range of 124.09-127.78 cm obtained were lower than the values 141-151 cm reported for Bunaji (Kanai, et al. 2013). Average daily yield of 3.37 to 4.43 l observed were comparable to 4.8l reported for Bunajii and Friesian X Bunaji [14]. But the value 7.40 observed for the hybrid was higher than this estimate. LL (days) of 245.33 were within the range of 173-249.5 reported by Lemos, et al. [14]. While the LL of 218.99 days obtained for the Gudali were comparable to the values of 216-225 for Yola Gudali in Kafare station [14]. The significant superiority of the Gudali in milk production compared to the Bunaji was contrary to the claim by Lemos, et al. [14]

that generally, the Gudali is a relatively poor milker compared to the White Fulani and the other important zebu breeds in this region. This could be explained by the claim that the range in milk yield and lactation length of the Gudali indicates substantial variation in these traits unlike the Bunaji and their crosses for whose selection efforts have been intensified. These figures point to the opportunity for genetic improvement of milk traits through stringent selection.

It was observed that BW, BL and CW were observed to be significantly ( $p < 0.05$ ) influenced by Hb types. There exists a deficit of literature reports on the impact of Hb types on growth and production traits. However, [15] reported that haemoglobin types had no significant influence on LL. No direct evidence exists of differences among the three Hb phenotypes (AA, AB and BB) for fitness in cattle [16,17] reported that Hb type did not significantly affect milk yield and butterfat percentage in dairy cattle. The influence of Hb variants on BL and CW were comparable to the observation of [18,19] in goats. There exist variations in literature reports on the effect of polymorphic forms of Hb on body traits; [20] had reported that haemoglobin type influenced the performance of sheep and goats. It should be noted that goats exhibit a very complex Hb polymorphism due to the presence of a number of allelic and non-allelic chains both in the alpha and beta-globin systems [21]. This may be responsible for the lack of clear pattern and accord in obtained results and literature reports on their impact on morphological traits. However, the significant effect of the interaction between breed and Hb types on growth and milk production traits in this study may point to the impact of Hb on fitness which may in turn influence growth and productivity. However, the lack of preceding literature makes it difficult to compare and contrast. It may be posited that breed and Hb interaction may be a good source of variation in adaptability and productivity.

## 5. CONCLUSION

The blood haemoglobin polymorphic studies were found not to be sufficient basis for determining the relationship among the three breeds as evidenced by their frequencies and impact on economic variables; though indicating significant differences among the breeds, it still had many murky and unresolved observations. Also, the low correlated estimates, unclear principal components measures were not

sufficient to delineate the relationship and differences among these breeds in NAPRI.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

As per international standard written ethical approval has been collected and preserved by the author(s).

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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