

1 **STUDIES ON THE PRODUCTIVE TRAITS AND RELATIONSHIP BETWEEN**
2 **BREEDS OF CATTLE (FRIESIAN BUNAJI CROSS, BUNAJI AND SOKOTO GUDALI)**
3 **USING BLOOD BIOCHEMICAL POLYMORPHISM**
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6
7 **Abstract**

8 We studied the blood haemoglobin polymorphism and activity in relation to morphology and
9 milk traits in Nigerian breeds of cattle. A total of 150 animals, 50 per breed of the Bunaji,
10 Friesian X Bunaji and Gudali were used to study the relationship between breeds. Variables
11 measured were BW: Body weight (Kg); BL: Body Length (Cm); HW: Height at withers (cm);
12 CW: Chest width (cm); HG: Heart Girth (cm); Rumwi: Rump width (cm); TL: Teat Length (cm);
13 RUH: Rear Udder Height (cm); UC: Udder Circumference (cm); TY: Total Yield (Litres); ADY:
14 Average Daily Yield (Litres/day) and LL: Lactation Length (days) and blood protein
15 polymorphism of the Haemoglobin. Observed results showed overall frequencies of 0.62 (HbA)
16 and 0.48 (HbB). Higher frequencies of the A allele (0.52, 0.54 and 0.68) were obtained
17 respectively for the Bunaji, Friesian X Bunaji and Gudali cattle in the Haemoglobin locus. Study
18 of blood protein polymorphism and productivity indicated significant ($p<0.05$) influence of the
19 haemoglobin. There is a need for a genetic study using other proteins and at DNA level to
20 compliment the results arisen from morphometric differentiation of the two most populous
21 Nigerian breeds of cattle in the NAPRI herd.

22 **Keywords:** Genetic characterization, gene introgression, haemoglobin, polymorphism
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26 **INTRODUCTION**

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

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

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

49 determine blood haemoglobin activity in relation to morphology and milk traits in Nigerian
50 breeds of cattle.

51
52 **Location of the study**

53
54 The research was conducted at the Dairy Breeding Unit of National Animal Production Research
55 Institute (NAPRI), Shika, Zaria, Kaduna State. NAPRI is geographically located between
56 latitude 11° and 12° N and longitude 7° and 8° E at an altitude of 640 m above sea level.

57
58 **Animals and Management**

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

61 **Sampling size and Sampling structure**

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

64 **Age Determination**

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

66 **Quantitative Characters**

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

71 **Metric Variables**

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

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

77 **Udder Measurements**

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

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

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

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

84 **Milk yield characteristics**

85 Milk yield characteristics were measured as follows:

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

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

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

91 **Blood Collection**

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

94 **Sample Preparation**

95 **Blood Haemolysates**

96 Red blood cell was prepared from the erythrocyte fraction of heparinised blood by centrifuging
97 at 2500 –3000 rpm for 10 mins at 4 °C. The RBC were washed in saline (0.155M NaCl) three
98 times and centrifuged at 2500 –3000 rpm for 5mins at 4°C. The RBCs were lysed with a fourfold
99 volume of distilled H₂O to release haemoglobin.

100 **Plasma**

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

103 **Gel Soaking**

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

108 **Sample Loading**

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

111 **Gel Running**

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

116

117 **Gel Staining**

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

122 **Gel Scoring**

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

126 **Electrophoretic Conditions**

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

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 129

130 Table 1: Electrophoretic Conditions

131	BUFFER	TIME (MINS)	p ^H	STAIN
132 Haemoglobin	Tris EDTA borate	40	8.4	Ponceau Stain

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136 **Bands Scoring**

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

140 **Statistical Analysis**

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

145

146 **Model for the Analysis was a factorial anova design as illustrated below:**

147

148 $Y_{ijk} = \mu + B_i + P_j + BP_{ij} + E_{ijk}$

149 Where Y_{ijk} is the record observed
150 μ = population mean
151 B_i = Fixed effect of the i^{th} breed of cattle
152 P_j = Fixed effect of the j^{th} polymorphic types of blood protein
153 BP_{ij} = interaction between breed and blood protein polymorphic class
154 E_{ijk} = random error particular to the ijk^{th} observation assumed to be independently randomly
155 distributed with mean zero and variance σ^2_e , i . e , NID (0, σ^2_e).

156

157 **RESULTS**

158 **Performance of Breeds in morphometric and selected milk Production traits**

159 The result of mean comparison of morphometric and selected milk Production traits of the three
160 breeds is presented in Table 2. Body Weight (BW) and all other measured traits differed
161 significantly ($p < 0.05$) between the breeds; the highest BW and BL was obtained with the
162 Friesian–Bunaji and this differed from the Gudali and the Gudali differed from the Bunaji with
163 the least BW and BL. The Gudali had the highest HW, CW, HG, Rumwi and TY (178.42, 31.69,
164 127.78, 50.18 and 1388.52 cm); the Friesian-Bunaji (174.99, 25.13, 124.09, 43.53 cm and
165 1097.59 L). TL was significantly higher in the hybrid (5.10) and differed from the Bunaji (4.67)
166 which also differed from the Gudali (4.47 cm) being the least. The same trend was observed with
167 RUH and UC with the exception that UC were similar in magnitude between the Bunaji and
168 Gudali. TY in this study was significantly higher with the Gudali (1203.52), while the Bunaji and
169 its hybrid were statistically ($p > 0.05$) similar; ADY indicated a reversed trend with the Friesian–
170 Bunaji having the highest Production while the Bunaji and Gudali were similar. LL was higher
171 and similar between the Bunaji and Friesian–Bunaji compared to the Gudali which had the least
172 LL value.

Table 2: Performance of Breeds in morphometric and selected milk Production traits

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

^{abc}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).

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.

Table 3: Effect of Haemoglobin Types on Morphology and Milk Production

Haemoglobin	AA	AB	BB	SEM
BW	383.83 ^b	394.15 ^a	387.92 ^b	3.35
BL	176.99 ^b	180.95 ^a	178.59 ^b	1.01
HW	174.17	174.87	173.88	0.78
CW	24.98 ^c	28.58 ^a	25.75 ^b	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

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201 ^{abc}: Means with different superscript across rows differ significantly (p<0.05)

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205 **Effect of Breed and Haemoglobin forms on Morphology and Milk traits**

206 Table 4 shows the result of the interaction between breed and haemoglobin forms. All measured
207 characteristics varied significantly ($p < 0.05$) within and between breed due to the effect of
208 haemoglobin polymorphs. The highest BW (398.81 Kg) was observed in the AB phenotype of
209 Friesian -Bunaji, followed by the BB (392.32 Kg) which ranked next and was similar to the AA
210 in this breed and all the other polymorphs in the Gudali but differed from the Bunaji were all the
211 Haemoglobin polymorphs were similar and had the least BW. BL were highest and similar
212 among forms in the Friesian-Bunaji (180.40 – 180.70 cm) and differed from the Gudali where
213 the AB and BB ranked next (178.86 and 178.95) but differed from the AA with the least length
214 in this breed (176.83) that was statistically similar to the records obtained among the polymorphs
215 in the Bunaji breed (175.12 – 175.99 cm). Generally for HW, CW, HG and Rumwi, the
216 interaction between polymorphic forms of Haemoglobin and the different breeds indicates lower
217 measures in the Bunaji irrespective of forms and medium to higher means for the Bunaji-Friesian
218 and the best performance for these traits in the Gudali. TL, RUH, UC, TYA, ADY and LL
219 means indicated a reversal of these trends with the best mean profile existing with the Friesian-
220 Bunaji followed by the Bunaji and finally the Gudali. Also it was observed that for most studied
221 characteristics, the highest mean were noted for the AB phenotype among haemoglobin forms
222 with the exception of HW, CW and HG were the AA ranked highest and in Rumwi were the BB
223 had the best mean value.

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

228 abcd: Means with different superscript across rows differ significantly (p<0.05).
 229 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
 230 Circumference (cm); TY: Total Yield (Litres); ADY: Average Daily Yield (Litres/day) and LL: Lactation Length
 (days).

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236 **DISCUSSION**

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238 The observed significant ($p < 0.05$) differences in all body and milk production traits
239 measurements of the three studied population indicates clear breed distinction. Observed
240 value for the Bunaji BW (Kg) (379.95) was higher than the value of 249 reported for the
241 NAPRI cow herd [10] but fell between the range 250 – 380 as reported in Tawah and Rege
242 (1994), while the value 395.40 obtained was lower than the value 491 recorded by these
243 authors for the Friesian-Bunaji. This may be attributed to differences in age of sampled herds,
244 season of study amongst other things. The value of 388.42 kg observed in the Gudali was
245 higher than the range of 241-353 kg, 335-336 kg and 360 – 363 kg reported for the Sokoto
246 Gudali, Adamawa Banyo and Yola Gudali but within the range 330-408 kg for the Nguadere
247 Gudali of Adamawa [11]. Since it has been noted that the Gudali is more of a beef cattle
248 compared to the Bunaji, the observed superiority of the Gudali to the Bunaji in BW appears
249 tenable. The Superiority of the Gudali over the Bunaji in HW, CW, HG and Rumwi were
250 consistent with the findings [12]. They stated that generally, the linear body measurements of
251 Sokoto Gudali were significantly ($P < 0.05$) higher than those of the Bunaji cattle with the
252 exception of body length and face length respectively, this study however showed differences
253 in BL between the two breeds.

254 Comparative measurements of morphometric traits can provide evidence of breed
255 relationships and size. The considerable variation in body dimensions of the two cattle breeds
256 might not be unconnected with individual breed's potential and peculiarities. While the
257 Bunaji cattle is noted for milk production, their Sokoto Gudali counterparts which is often
258 ranked second in milk production produce more meat and appear to have more draught power
259 than the former [12]. The superiority of cross bred animals to local breeds in this study needs
260 not be emphasised as it is a generally accepted trend in animal improvement work. The
261 estimates for BL (175.48 – 180.63) were comparable to 175.29 – 179.02 reported by [12].

262 HW estimates of 170.02 -178.42 were higher than 110-148.40 reported by various authors for
263 different cattle breed [13, 14]. Observed measures of HG range of 124.09-127.78 cm
264 obtained were lower than the values 141-151cm reported for Bunaji (Kanai *et al.*, 2013).
265 Average daily yield of 3.37 to 4.43 l observed were comparable to 4.8l reported for Bunajii
266 and Friesian X Bunaji [14] But the value 7.40 observed for the hybrid was higher than this
267 estimate. LL (days) of 245.33 were within the range of 173 – 249.5 reported by [14]. While
268 the LL of 218.99 days obtained for the Gudali were comparable to the values of 216 – 225 for
269 Yola Gudali in Kafare station [14]. The significant superiority of the Gudali in milk
270 production compared to the Bunaji was contrary to the claim by [14] that generally the
271 Gudali is a relatively poor milker compared to the White Fulani and the other important zebu
272 breeds in this region. This could be explained by the claim that the range in milk yield and
273 lactation length of the Gudali indicates substantial variation in these traits unlike the Bunaji
274 and their crosses for whose selection efforts have been intensified. These figures point to the
275 opportunity for genetic improvement of milk traits through stringent selection.

276 It was observed that BW, BL and CW were observed to be significantly ($p < 0.05$) influenced
277 by Hb types. There exists a deficit of literature reports on the impact of Hb types on growth
278 and production traits. However, [15] reported that haemoglobin types had no significant
279 influence on LL. No direct evidence exists of differences among the three Hb phenotypes
280 (AA, AB and BB) for fitness in cattle [16]. [17] reported that Hb type did not significantly
281 affect milk yield and butterfat percentage in dairy cattle. The influence of Hb variants on BL
282 and CW were comparable to the observation of [18, 19] in goats. There exist variations in
283 literature reports on the effect of polymorphic forms of Hb on body traits; [20] had reported
284 that haemoglobin type had influence on performance of sheep and goats. It should be noted
285 that goats exhibit a very complex Hb polymorphism due to the presence of a number of
286 allelic and non-allelic chains both in the alpha and beta globin systems [21]. This may be

287 responsible for the lack of clear pattern and accord in obtained results and literature reports
288 on their impact on morphological traits. However, the significant effect of the interaction
289 between breed and Hb types on growth and milk production traits in this study may point to
290 the impact of Hb on fitness which may in turn influence growth and productivity. However,
291 the lack of preceding literatures makes it difficult to compare and contrast. It may be posited
292 that breed and Hb interaction may be a good source of variation in adaptability and
293 productivity.

294 **Conclusions**

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

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