# Hemoglobin polymorphism and its association with some morphometric traits of Sheko cattle in southwestern Ethiopia

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## Abstract

The present study investigated the biochemical polymorphism of hemoglobin and its association with some morphometric traits in Sheko cattle. For this, a total of 155 Sheko cattle of both sexes were evaluated, and distinguishing phenotypic characteristics of the breed, horn length, and hump size measurements were taken. To study the polymorphic activities of hemoglobin, blood samples from the same animals were collected by jugular vein puncture, and the samples were subjected to agarose gel electrophoresis (pH range 8.4-8.5). Three Hb genotypes, $Hb^{AA}$, $Hb^{AB}$, and $Hb^{BB}$ were observed. The most frequent genotype was $Hb^{AB}$ (0.62). The frequency of $Hb^{A}$ and $Hb^{B}$ alleles were 0.66 and 0.34, respectively. The chi-square ($\chi 2$) test revealed that the observed genotype frequency of the population is not under Hardy-Weinberg equilibrium. In addition, the typical phenotypic features of Sheko cattle, polled and no or a small hump size, correspond to the $Hb^{AB}$ genotype. The present study identified possible associations between hemoglobin variants and some visible traits, suggesting its potential significance as a marker for selecting Sheko cattle.

**Keywords**: Genotype; Hemoglobin polymorphism; Sheko cattle.

## Introduction

Ethiopia has diverse cattle genetic resources adapted to various local environmental conditions and acquired unique features. Sheko is one of the Ethiopian indigenous cattle maintained in warm and humid parts of southwestern Ethiopia. The breed has been portrayed for a long time as the remnants of Africa's

original *Bos taurus* cattle. However, inconsistent with the previous phylogenetic, genetic distance-based analysis, which inferred the breed is distantly related to the Ethiopian Sanga cattle breeds (Dadi *et al.,* 2008) genome-wide, analyses classified the breed as more of a Sanga type (originated from African taurine and Asian zebu) (Mbole-Kariuki *et al*., 2014). On the other hand, locus-ancestry deviation analysis revealed genomic regions that showed substantial deviation in Asian zebu ancestry and their inclination towards the taurine haplotypes (Bahbahani *et al*., 2018).

In pure or admixed populations, selection and/or migration and the associated gene flow and genetic drift might have shaped the genome. Accordingly, the breed maximizes its performance traits or adaptability to environmental challenges (Bahbahani *et al*., 2018). In this regard, positive selection might contribute to Sheko's resilience characteristics to extreme environments and tolerant to tsetse fly (the vector of trypanosomiasis) transmitted trypanosomiasis, which could prove the breed possesses unique traits that meet the current and the unpredictable future environment (Lemecha *et al.,* 2006; Dadi *et al.,* 2009; Taye *et al*., 2012).

Despite its potential for diversified genetic resources with unique features, the breed is at risk of dilution. Different factors contribute to it, and the chief among them are indiscriminate breeding with non-Sheko and unplanned crossbreeding with exotic breeds (Dadi *et al*., 2009; Taye *et al.,* 2011). The manifestation of the small humps in Sheko cattle can be considered a visible sign of zebu introgression (Taye *et al*., 2011; Woldu and Beyene, 2013).

Characterization of a breed is crucial for monitoring gene flow in populations, conservation of the breed, and determination of inbreeding and crossbreeding levels. This is particularly pertinent for the breed at risk of breed replacement and genetic dilution through crossbreeding (Salzano *et al*., 2015). Genetic variation can be captured using different methods, including morphological, biochemical, and DNA characterization. Blood groups and proteins have also been widely used to characterize animal populations, especially hemoglobin (Ajayi *et al*., 2013). Hemoglobin (Hb) is the simplest of the genetic markers which helps to investigate the possible relationship and variation within and between cattle populations. Combining morphological characteristics with genetic markers can effectively identify pure individuals for conservation, breed improvement, and research (Dadi *et al.,* 2009). Apart from the information available concerning hemoglobin polymorphism in Ogaden cattle found in

Southeastern Ethiopia of the Somali region (Sanjoy and Yesihak, 2014), little is known about the relationship of hemoglobin genetic types of Sheko cattle with morphometric traits. Therefore, this study aimed to determine hemoglobin types and their association with some visible traits in Sheko cattle to establish baseline information for breed conservation.

## Materials and methods

### Study area description

The study was conducted in the Bench Sheko and Kaffa zones of southwestern Ethiopia. Geographically, Sheko cattle are available in these two zones of the country. The mean annual temperature and rainfall of the Bench Sheko zone range from 15.1 to 27.3°C and 400 to 2000 mm, respectively. The mean annual temperature and rainfall of the Kaffa zone varies from 10.1 to 27.5°C and 1001 to 2200 mm, respectively.

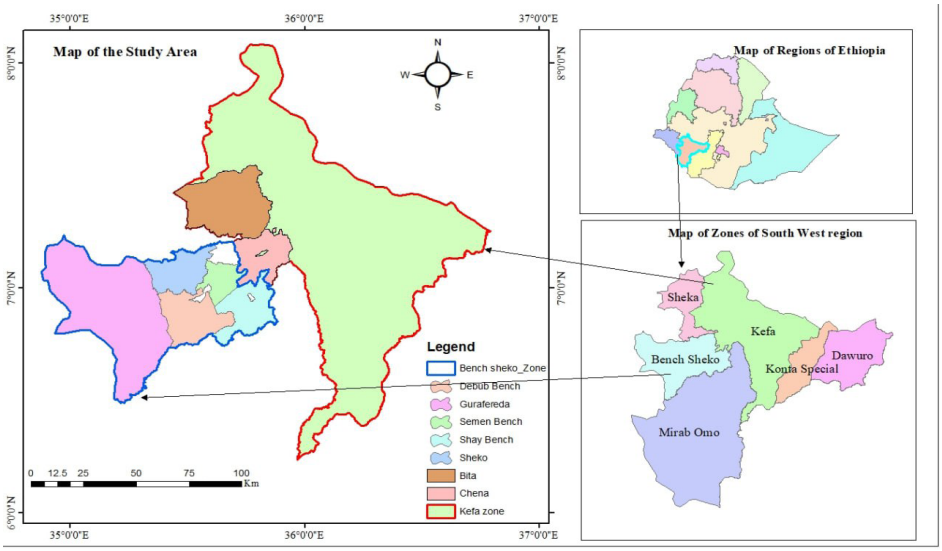


**Figure 1. Map of the study area.**

### Study animals

Sheko cattle were identified based on morphological characteristics, herd owners, and expert consultation. Accordingly, 155 Sheko cattle of both sexes (89 female and 66 male) were sampled from two zones (144 from Bench Sheko and

11 from Kafa) and seven districts. Based on the availability of Sheko cattle, Debub Bench, Gura Ferda, Semen Bench, Shay Bench, and Sheko districts were selected from the Bench Sheko zone. Additionally, Chena and Bita were included from the Kaffa zone. The visible trait of the breed, such as the coat color, was recorded. In addition, horn length and hump size measurements were taken from the sampled animals using a measuring tape in centimeters, following the phenotypic descriptor list of FAO (2012), before the blood sample collection.

## Hemoglobin genotyping

From each of the 155 animals, 10ml blood was collected from the jugular vein using a sterile vacutainer containing ethylene-diamine-tetra-acetic acid (EDTA) and then kept at -20°C until processing. The red blood cells were washed by the addition of an equal volume of normal saline (9.0 g of NaCl in 1 liter of deionized water) and allowed to stand for 30 minutes. Then, the washing solution was decanted. The separated RBC was lysed by adding two volumes of deionized water (Chuku and Uwakwe, 2012).

Hemoglobin genotypes were determined by agarose gel (1%) (Weigh 1 g agarose and dissolve it in 100 mL of 0.5x TBE Buffer) electrophoresis. Carefully transfer the gel to the electrophoresis tank and filled with 0.5x TBE buffer of pH 8.4 - 8.5. A sample size of ten microliters was loaded into the wells, and a voltage of 200 volts was applied constantly and allowed to run for about 2 hrs. Then, the haemoglobin pattern was read directly from the gel without staining according to their electrophoretic mobility. $Hb^{AA}$ had a slower band or electrophoretic mobility than the ($Hb^{BB}$) and $Hb^{AB}$ (heterozygous) had an intermediate speed in between the two bands (Naik *et al*., 1969; Essien *et al.,* 2011; Chukka and Uwakwe, 2012).

## Statistical Analysis

Descriptive statistics was used to estimate the gene and genotype frequencies. Genotype frequencies were subjected to Chi-square analysis to test for goodness of observed and expected frequencies under Hardy-Weinberg equilibrium (HWE) (Chuku and Uwakwe, 2012). Continuous variables with or without normal distribution were expressed as mean ±SE. Categorical variables were shown as numbers and percentages.

Genotype and allele frequencies were computed following the principle of Hardy-Weinberg Equilibrium as follows:
Genotype frequency of $Hb^{AA}$(P) = (Number of individuals with $Hb^{AA}$ )/(Total number of individuals samped)×100
Genotype frequency of $Hb^{AB}$(Q) = (Number of individuals with $Hb^{AB}$)/(Total number of individuals sampled)×100
Genotype frequency of $Hb^{BB}$(H) = (Number of individuals with $Hb^{BB}$)/(Total number of individuals samped)×100

The gene frequencies were also calculated according to Hardy-Weinberg equation as;

p = P+ ½ H  Where  P= Genotypic frequency of allele AA
q= Q+ ½ HQ  Where  = Genotypic frequency of allele BB
p = frequency of allele A
q = frequency of allele B
H = Genotypic frequency of allele AB

The genetic variability in the studied cattle population was measured (Nei, 1978) by using the following formula.

Heterozygosity (H) = 1- $\sum_{i=1}^{n} x^2 i$ *Where*, X = the gene frequency of the $i^{th}$ allele in a locus, i = the number of tested loci.

The effect of hemoglobin variants on morphometric traits was analyzed by using the following model:

$$Y_{ij} = \mu + \alpha_i + e_{ij}$$

Where: $Y_{ij}$ = the observation belonging to $i^{th}$ Hb type,

μ = overall mean,
$\alpha_i$ = effect of $i^{th}$ hemoglobin type (i = $Hb^{AA}$, $Hb^{AB}$, $Hb^{BB}$),
$e_{ij}$ = random residual error.

The results were reported as the least square mean ± (SE) standard error. All the analyses were performed using the Statistical Package for Social Sciences (SPSS) version 21.0 and the significance level was set at $p < 0.05$.

# Results

## Phenotypic characteristics of the breed

The qualitative characteristic and morphometric measurements of the Sheko cattle varied among the tested animals. The dominant coat color of the breed was brown (50.3%), followed by multi-colored (44.5 %). About 62.6 % of Shekos' were polled, and 96.1 % of the breed was humped (Table 1).

**Table 1. Description of some morphometric traits in Sheko cattle.**

| Traits | Description | N | Percentage |
|---|---|---|---|
| Coat color | Black | 8 | 5.2 |
| | Brown | 78 | 50.3 |
| | Multi-colored | 69 | 44.5 |
| Horn | Horned | 58 | 37.4 |
| | Hornless (polled) | 97 | 62.6 |
| Hump | Humped | 149 | 96.1 |
| | Humpless | 6 | 3.9 |

# Genotype

Based on the electrophoretic motility, three hemoglobin genotypes (AA, AB, and BB) were identified across the sexes of the tested population (Figure 2). Among these genotypes, $Hb^{AB}$ (0.62) was the most prevalent and its incidence in Sheko cattle was statistically higher ($p < 0.05$) than the other two variants. Allelic frequencies of $Hb^{A}$ and $Hb^{B}$ were 0.66 and 0.34, respectively (Table 2).

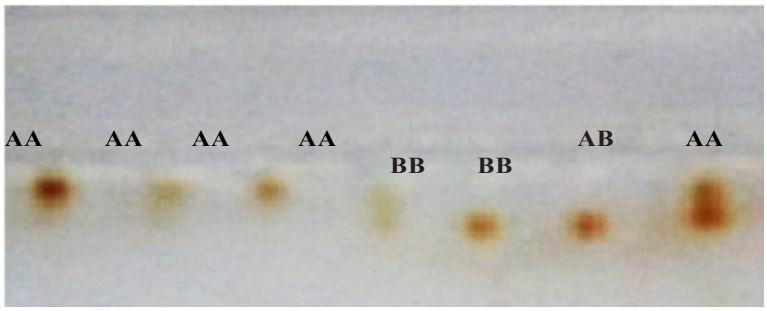


**Figure 2. The observed hemoglobin polymorphism in Sheko cattle.**

**Table 2. Allele and genotype frequencies of hemoglobin in tested population of Sheko cattle.**

| Allele frequency | Genotype | Genotype Frequency | Genotype observed | Genotype expected | $\chi^2$ df=4 |
|---|---|---|---|---|---|
| A=0.66 | $Hb^{AA}$ | 0.35 | 55 | 53.6 | 310 |
| | $Hb^{AB}$ | 0.62 | 96 | 93.6 | |
| B =0.34 | $Hb^{BB}$ | 0.03 | 4 | 2.6 | |

A relatively higher prevalence of $Hb^{AB}$ (0.36) in female Sheko cattle was observed (Table 3). Despite differences in genotype and allele frequencies within the sex of the studied animals, no statistically significant association between the Hb genotype and the sex of the animals was evident.

**Table 3. Allele and genotype frequencies of hemoglobin across the sex in Sheko cattle.**

| Sex | Allele frequency | Genotype | Genotype frequency | Genotype observed | Genotype expected | *p* value |
|---|---|---|---|---|---|---|
| Female | A=0.39 | $Hb^{AA}$ | 0.21 | 31 | 31.6 | 0.929 |
| | | $Hb^{AB}$ | 0.36 | 56 | 55.1 | |
| | B=0.19 | $Hb^{BB}$ | 0.01 | 2 | 2.3 | |
| Male | A=0.28 | $Hb^{AA}$ | 0.15 | 24 | 23.4 | |
| | | $Hb^{AB}$ | 0.26 | 40 | 40.9 | |
| | B=0.14 | $Hb^{BB}$ | 0.01 | 2 | 1.7 | |

Hemoglobin genotypes were found polymorphic across the hump size of Sheko cattle. The most abundant genotype, $Hb^{AB}$ (0.6), corresponded with a mean hump size of 6.42±0.31 cm, followed by $Hb^{AA}$ (0.4) with a mean hump size of 6.16±0.40 cm. The smallest hump size (4.50±0.65 cm) was observed in the least frequent $Hb^{BB}$ (0.03) genotype. Despite variations in the mean hump size among the observed Hb genotypes, there was no statistically significant association ($p > 0.05$) between Hb types with hump size in the studied cattle population (Table 4). In contrast, horn length varied significantly ($p < 0.05$) with hemoglobin genetic variants. Animals with the $Hb^{AA}$ genotype had the longest horn, with a corresponding mean horn length of 4.83±0.62 cm (Table 4).

**Table 4. Effects of hemoglobin types on some morphometric traits in Sheko cattle.**

| Traits | Hemoglobin types | | | *p* value |
|---|---|---|---|---|
| | $Hb^{AA}$ | $Hb^{AB}$ | $Hb^{BB}$ | |
| Hump size (cm) | 6.16±0.40 | 6.42±0. 31 | 4.50±0.65 | 0.425 |
| Horn length (cm) | 4.83±0.62 | 2.23±0.42 | 3.25±2.13 | 0.002 |

Hemoglobin genotypes were found polymorphic across the horn size of Sheko cattle. Among the 155 animals tested, 97 of them were phenotypically polled. The highest proportion of hornless Sheko had a heterozygous $Hb^{AB}$ genotype, while the smallest proportion of polled Sheko carried a homozygous $Hb^{BB}$ genotype with the genotype frequencies of 0.46 and 0.01, respectively (Table 5).

**Table 5. Allele and genotype frequencies of hemoglobin across the hump size and horn length in Sheko cattle.**

| Genotype | Hump | N | Genotype frequency | Allele frequency |
|---|---|---|---|---|
| $Hb^{AA}$ | Humped | 55 | 0.35 | A=0.66 |
| $Hb^{AB}$ | Humped | 90 | 0.58 | |
| | Humpless | 6 | 0.04 | |
| $Hb^{BB}$ | Humped | 4 | 0.03 | B=0.34 |
| Total | | 155 | 1.00 | 1.00 |
| | Horn | | | |
| $Hb^{AA}$ | Horned | 32 | 0.206 | A=0.34 |
| | Polled | 23 | 0.148 | A=0.38 |
| $Hb^{AB}$ | Horned | 24 | 0.154 | |
| | Polled | 72 | 0.464 | |
| $Hb^{BB}$ | Horned | 2 | 0.013 | B=0.09 |
| | Polled | 2 | 0.013 | B=0.25 |
| Total | | 155 | 1.00 | 1.00 |

The level of genetic variability within a studied cattle population is presented in Table 6. The estimated heterozygosity at Hb loci was 0.45.

**Table 6. Heterozygosity at hemoglobin locus in the Sheko cattle.**

| Sheko cattle | Heterozygosity (He) |
|---|---|
| Bull | 0.9 |
| Cow | 0.8 |
| Entire population | 0.45 |

He: expected heterozygosity

## Discussion

### Phenotype

The most commonly observed coat color in Sheko cattle was brown, followed by multi-colored, with brown being the most abundant coat color of the breed, as noted by Alberro and Haile-Mariam (1982). Most Sheko cattle were polled, with only a few individuals exhibiting small, floating horns, which aligns with the findings of Alberro and Haile-Mariam (1982). Moreover, almost all Sheko cattle had a small hump size (96.1%), consistent with the report of Workneh (2001), who observed reduced cervicothoracic hump of the breed. However, the present observation contradicts the humpless phenotypic description of the breed documented by Alberro and Haile-Mariam (1982). The acquisition of the small hump size of the breed might be a signal that the trait has introgressed from zebu cattle (Taye *et al*., 2011; Woldu and Beyene, 2013).

### Genotype

In this study, hemoglobin was found polymorphic in Sheko cattle with three distinct genotypes of $Hb^{AA}$, $Hb^{AB}$, and $Hb^{BB}$ controlled by two alleles with genotype frequencies of 0.35, 0.62, and 0.03, respectively. The present findings are consistent with the observation of Sanjoy and Yesihak (2014) in Ogaden cattle, Ethiopia.

The present study revealed the preponderance of heterozygotes ($Hb^{AB}$) against homozygotes of both types ($Hb^{AA}$and $Hb^{BB}$). Heterozygote genotypes had a superior advantage over the homozygotes in responding to tissue energy needs and output through tissue respiration, the oxygen- carbonic acid gradient in circulating blood, and gaseous exchange between the animal and their environment (Gwaza *et al*., 2019). The higher proportion of $Hb^{AB}$ in the Sheko cattle might favored by natural selection, possibly for the breed's survival, fitness, and adaptation to environmental challenges (hot tropical weather and

tolerance to tick and helminthic infections) (Aygun and Mert, 2007; Essien *et al.,* 2011).

The observed Heterozygosity (0.45) in the present study is in line with the reported heterozygosis of native Sharabi cattle (0.45) in Mosul, north of Iraq Al-Samarrae et al., (2010), Bunaji cattle (0.47) Makurdi, Nigeria Ukwu *et al.* (2018) and Ogaden cattle (0.42) in Ethiopia (Sanjoy and Yesihak, 2014). The average heterozygosity value observed in this study is an index of moderate genetic diversity at the hemoglobin locus in Sheko cattle. This genetic diversity at the hemoglobin locus is likely a result of environmental pressures for adaptability and natural mutation processes. Heterozygosis in the Sheko cattle is beneficial since genetic diversity within a population enables breed adaptation to dynamic environments (Egena and Alao, 2014).

Hemoglobin allele type $Hb^A$ has been speculated to be associated with increased hemoglobin concentration and packed cell volume values (Pieragostini *et al*., 2006). The higher oxygen affinity of the allele $Hb^A$ is due to its biophysical, biochemical, and physiological peculiarities, such as saturation capacity with oxygen, dissociation curve of oxyhemoglobin, erythrocyte load with hemoglobin, and metabolic profile of the erythrocyte (Raushenbach and Kamenek, 1978). These properties of the allele $Hb^A$ with its effects on some blood parameters, such as hemoglobin and packed cell volume, have been suggested to positively influence the resistance of animals carrying this type of hemoglobin against diseases such as helminth infestation (Di Stasio, 1997). The central and West African cattle breeds, Namchi and N'Dama, are resistant to a devastating trypanosomiasis disease believed to be associated with the $Hb^A$ allele (Achukwi *et al*., 1997).

Environmental factors such as temperature are another factor that determines hemoglobin polymorphism (Hrinca, 2008). Extreme hot temperature conditions favor the fixing of allele $Hb^A$, and a moderate temperature favors the fixing of allele $Hb^B$. It can be suggested that the hot and humid environmental condition in the Sheko natural breeding area favors the highest frequency of the $Hb^A$ allele.

The Chi-square (**$\chi$2)** test revealed that the population deviated from Hardy-Weinberg expectations. Natural selection on the Hb locus might contribute to a violation of the assumptions of the theorem.

## Conclusions

In conclusion, the hemoglobin locus was polymorphic across the studied quantitative traits, hump size, and horn length of Sheko cattle. The majority of $Hb^{AB}$ carrier Sheko cattle were hornless or had no or small hump size (6.42±0.31), which is the typical phenotypic feature of the breed, suggesting its potential use as a marker for selecting the breed for appropriate interventions.

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## Conflict of interest

The authors declare no conflict of interest.

## Ethical considerations

This study was conducted with the highest animal welfare considerations, while ethical clearance was not required, as the study did not involve highly invasive procedures.

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