HAEMOGLOBIN AND MORPHOMETRIC POLYMORPHISM OF FOUR STRAINS OF DONKEY (*Equus asinus L.*) IN GAMAWA LOCAL GOVERNMENT AREA, BAUCHI STATE, NIGERIA.

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ABSTRACT
The study was conducted in 2018 (March to July). Blood samples and some morphometric characteristic of donkeys were taken in Gamawa Local Government Area (LGA) of Bauchi State, Nigeria. The aim was to describe the haemoglobin polymorphism and to characterize the genetic pool of four commonly kept strains (*Auraki*, *Duni*, *Fari* and *Idabari*) of donkeys in the study area. Eighty (80) matured donkeys were used for this research, comprising both sexes (39 males and 41 females), with 16 donkeys from each of five agro-ecological zones (Gamawa East and West, Udubo East and West and donkey market) of Gamawa. The red lysates were subjected to cellulose acetate electrophoresis and specific staining procedure to reveal the band patterns of the haemoglobin. The results revealed the presence of three genotypes (AA, AB and BB) with BB found only in *Fari* strain. The frequencies of A and B alleles were 0.925 and 0.075, respectively. The implication is that donkeys with AA genotype are more resistant to infections. Analysis of the morphometric measurements revealed that height at withers and neck circumference were significantly ($P<0.01$) correlated (0.514). Negatively lower correlation (-0.224) was significantly ($P<0.05$) found between lengths of ear and tail. Body length and neck measurements were significantly ($P<0.01$) Correlated (0.324 to 0.497). It could be concluded that, donkeys in Gamawa LGA of Bauchi State with A gene (*Auraki*, *Duni* and *Idabari*) can be selected for resistance and performance. Further study could be undertaken to evaluate the effects of genotypes on the productivity.
Keywords: Donkey, Haemoglobin, Polymorphism and Morphometric

INTRODUCTION
Donkeys are valued for their ability to survive under harsh conditions (Blench, 1990), yet they are often regarded as animals of low social status and neglected by research (Starkey, 1995). Nigeria is estimated to have about 800,000 heads of donkey (Mabayoje and Ademiluyu, 2014). Mainlyconcentrated in the North where they are used as pack animals (RIM, 1992). In south eastern Nigeria, donkeys are used as meat animals and about 16,000 donkeys are transported annually from the northern states for this purpose and these has become a major threat to donkey population (Blench, 2014). However, there are needs to conserve the existing population to avoid exilement. To this, haemoglobin is an important erythrocyte protein inherited by co-dominance in Mendelian fashion and has been reported to be a useful marker through which many economic traits (Akinyemi and Salako, 2010).

The genetically determined system of haemoglobin polymorphous in donkey provides an opportunities for using them as genetic markers that make it possible to conduct studies related to the genetic structure (Slavov *et al.*, 2004). Although these genetic markers and their mode of inheritance were clarified in most animals, information on donkeys are rather limited. This this, the study aimed to determine the haemoglobin and morphometric polymorphism of four Nigeria indigenous strains of donkeys in Gamawa Local Government Area of Bauchi State.

METHODOLOGY
Study Area
This research study was conducted in Gamawa Local government Area of Bauchi State, Nigeria (North Eastern Nigeria) bordering Yobe State to the East and Jigawa State to the west. It lies between latitude 12.13^0N, longitude 10.54^0E of the equator and 397 meters elevation above the sea level (Maps view, 2018). It has an area of 2,925km^2 and has a population of 286,388 as at 2006 census. The annual rainfall ranges between 700mm to 1600mm which start in May and ends in October. The rainy season months are from July to September and the dry season commence fully in November and end in April. The relative humidity ranges between 20- 30 % and the mean monthly temperature ranges between 26^0C to 39^0C (BSADP, 2017).

Research Animals

The animals used for this study were obtained from several small donkey holder flocks especially four strains predominant to the dry climates of the north. A total of eighty (80) animals, twenty (20) per strain – *Auraki*, *Duni*, *Fari* and *Idabari* were used. Sampled animals from these four strain comprised both sexeswithin breeding age group. Donkey management in the study area is dominated by semi extensive production system, in which donkeys are allowed to graze freely during the day time and kept on open enclosure during the night.

Data Collection

Blood collection

5ml of blood was collected from each of the sampled animals by jugular vein puncture using needle and syringe and put into sample bottles containing Ethylene Diamine Tetra Acetic acid (EDTA) as anticoagulant, properly labelled with respect to the different strains and sex and preserved in a cooler containing ice blocks from where they were transported to immaculate medical laboratory in Minna, Niger State for analyses.

Morphometric measurements

Flexible measuring tape was used to take the body measurement. During body measurement, animals were made to stand upright and restrained by assistants in such a way that their necks, heads and ears were stretched almost in a straight line. Each measurement was taken for at least two times and recorded to the nearest centimetre. Reference marks for body measurement according to the method of Salako (2006a) were adapted.

Head Length (HL), Ear length (EL), Neck length (NL), Neck circumference (NC), Height at Wither (HW), Body length (BL) and Tail length (TL) as defined by Salako (2006a)

Laboratory Analysis of Blood Collected

Blood Preparation

The blood samples were then washed with normal saline and haemolysed with distilled water to release the haemoglobin. The supernatant was removed after centrifuging at 3000 rpm for 5 min and the sample haemoglobin stored at refrigeration temperature pending electrophoresis.

Electrophoresis

The red cell lysate was impregnated on a cellulose acetate paper with a control, placed on the electrophoresis tank using forceps and subjected to electrophoresis according to the standard. The strips were run for 5 minutes at a constant voltage of 250v until a clear separation was observed. Interpretations were made based on the relative mobility of the haemoglobin bands towards the anode. The resulting electrophoregram showed haemoglobin bands distinct movement toward anodic end of the electrophoretogram (Boyers and Hinner, 1963).The genotype migrated was labelled as described by Boyers and Hinner (1963).

Statistical Analysis

Since only two alleles (A and B) were detected; then, haemoglobin genotype and gene frequencies were estimated as follows:

$$\text{Genotype frequency AA} = \frac{\text{No of individuals with AA}}{\text{No of idividuals sampled}} \text{ X } 100$$

$$\text{Genotype frequency AB} = \frac{\text{No of individuals with AB}}{\text{No of idividuals sampled}} \text{ X } 100$$

$$\text{Genotype frequency BB} = \frac{\text{No of individuals with BB}}{\text{No of idividuals sampled}} \text{ X } 100$$

Genotype frequency was estimated with respect to breeds and sex sampled. For the estimation of Gene frequency, the equation was adopted as thus;

P = (2NAA + NAB) / 2N and

Q = (2NBB + NAB) / 2N

Data collected for morphometric traits were subjected to Special Package for Social Science (SPSS 21.0), according to the method of Steel and Torrie (1980). The difference between the means were separated using Duncan Multipe range test (DMRT).

RESULTS AND DISCUSSION

Table 1: Distribution of haemoglobin types on strains and between sex of donkeys in Gamawa

Strain	N	Sex	N	AA	AB	BB
Auraki	20	Male	15	15	0	0
		Female	5	4	1	0
		Total	20	19	1	0
Duni	20	Male	8	7	1	0
		Female	12	10	2	0
		Total	20	17	3	0
Fari	20	Male	6	5	0	1
		Female	14	12	1	1
		Total	20	17	1	2
Idabari	20	Male	10	9	1	0
		Female	10	8	2	0
		Total	20	17	3	0

Table 2: Distribution of genotype frequency on strain and sex of donkey in Gamawa

Strains	**N**	**AA%**	**AB%**	**BB%**	**Total**
Auraki	20	95.00	5.00	0.00	100.00
Duni	20	85.00	15.00	0.00	100.00
Fari	20	85.00	5.00	10.00	100.00
Idabari	20	85.00	15.00	0.00	100.00
Polled (All)	80	87.50	10.00	2.50	100.00
Sex					
Male	39	92.31	5.13	2.56	100.00
Female	41	82.93	14.63	2.44	100.00

Table 3: Gene frequency according to strain and sex of donkey in Gamawa.

Strains	N	A	B	Total
Auraki	20	0.975	0.025	1
Duni	20	0.925	0.075	1
Fari	20	0.875	0.125	1
Idabari	20	0.925	0.075	1
Polled (All)	80	0.925	0.075	1
Sex				
Male	39	0.949	0.051	1
Female	41	0.902	0.098	1

Table 4: Effect of strains on morphometric parameters of donkey in Gamawa.

	Strains					
Trait (cm)	**Auraki**	**Duni**	**Fari**	**Idabari**	**SEM**	**P- value**
HL	46.57^{b}	45.54^{c}	48.41^{a}	47.19^{b}	0.16	0.00
EL	25.36	26.15	25.10	25.01	0.28	0.49
NL	46.90^{a}	44.61^{b}	46.49^{a}	46.38^{a}	0.22	0.03
NC	64.40	63.39	62.92	62.74	0.36	0.16
HW	105.32	104.93	104.42	104.92	0.17	0.31
HG	109.93^{c}	114.03^{a}	111.42^{bc}	112.47^{ab}	0.41	0.003
BL	107.39^{ab}	108.27^{a}	106.74^{b}	106.01^{b}	0.25	0.04
TL	57.77	55.46	57.58	57.34	0.45	0.43

HL = Head Length EL = Ear Length, NL = Neck Length, NC= Neck Circumference,
HW =Height at Wither, BL = Body Length, HG = Heart Girth and TL = Tail Length
SEM: Standard error of means
abc: means with different superscript in row are significantly different (P<0.01; 0.05)
P-value: Probability level of significant.

Distribution of haemoglobin types on strains and between sex of donkey found in Gamawa Area of Bauchi State are shown in Table 1. Three haemoglobin genotypes were detected; two homozygotes (AA and BB) and one heterozygous (AB) which corresponds to the general observation of A and B alleles with their corresponding genotypes AA, AB and BB in different species (Tunon *et al*., 1989). Out of 20 from each strains, *Auraki* sampled had 19 AA Hb (15 males and 4 females), 1 AB Hb (1 female) and no BB Hb type was present. *Duni* had 17 AA Hb (7 males and 10 females), 3 AB Hb (1 male and 2 females) and no BB Hb type. *Fari* strain had 17 AA Hb (5 males and 12 females), 1 AB Hb (I female) and 2 BB Hb (1 male and I female) and *Idabari* strain had 17 AA Hb (9 males and 8 females), 3 AB Hb (2 males and 3 females) and no BB Hb type was present.

The presence of homozygote Hb BB genotypes of donkey disagreed with Salvaggi *et al.* (2015) who observed one haemoglobin types in Martina Franca donkeys and concluded that, it might not be readily apparent or is not widely distributed. Pearson and Ouassat (1996) believed that, the specific conditions of the African continent enabled donkeys to develop a typical habit thereby causing a change in its genotypes to enable them play key roles in order to survive in dry locations. Therefore, the predominant of Hb AA over Hb AB and Hb BB types among the four strains of donkey could be attributed to donkey adaptive feature for survival and genetic resistance to diseases as reported by (Wold *et al*., 2004). Donkeys appear to be less susceptible to a number of pathological conditions and diseases affecting horses and cattle. Similar report was documented by (Meutchieye *et al*., 2014).

Auraki strain had the highest genotypes frequency for AA with 95 %. *Duni*, *Fari* and *Idabari* had the same genotype frequencies of 85 % for AA. AB genotypes for the four strains showed that, *Duni* and *Idabari* had the highest genotypic frequency of 15 %, followed by *Auraki* and *Fari* with 5 %. BB genotypic frequency was not visible in *Auraki*, *Duni*, and *Idabari* but visible only on *Fari* strain with genotypic frequency of 10 %. The genotypic frequencies polled (all together) of the four strains of donkey were 87.5, 10 and 2.5 % for AA, AB and BB genotypes, respectively.

The gene frequencies A and B alleles among the four strains of donkey showed that, *Auraki* had the highest frequency (0.975) while Fari had the lowest (0.875) frequency of the A Allele. However, *Fari* had the highest gene frequency (0.125) while *Auraki* has the lowest (0.025) gene frequency of the B Allele. More so, they all obeyed Hardy Weinberg's equilibrium (P + Q = 1). Gene frequency of A allele on sex were higher on males (0.949) compared to females (0.902). More so, the B allele were higher in females frequencies (0.098) compared to males (0.051). There is not enough reason however, to conclude that sex has an effect on the disparity in B types.

Differences in the on strains among the qualitative traits of donkeys such as HL, NL, HG and BL could be attributed to genetic variations among the donkey strains. The study agreed with Ellis *et al*. (1994) who reported that, other body dimensions of the Zimbabwean donkeys as well as live weight indicated no major difference between male and female donkeys of similar maturity. However, there were no significant differences between EL, NC, SW, HW and TL in adult donkeys; this implied that, those donkeys were somewhat similar to Nigeria donkeys.

CONCLUSIONS

Gamawa donkeys haemoglobin polymorphism is defined by expression of three genotypes: two homozygotes (Hb AA and Hb BB) and one heterozygote (Hb AB). The phenotypization of the haemoglobin variants is determined by two co-dominant alleles, Hb A and Hb B. The allele Hb A registers a higher frequency than Hb B allele. Consequently, in the haemoglobin tables, the homozygotes Hb AA reaches a high incidence, the heterozygotes Hb AB have a middling frequency and the homozygotes Hb BB were less present. Finally, it could be suggested that the Hb AA type evident in the *Auraki* donkeys having the highest altitude, Gamawa local Government Area of Bauchi State, donkeys were favoured by natural selection, possibly for resistance against certain parasitic (internal and external) and some Haemo-parasitic infections.

The adult body size measurements of donkeys in Gamawa, Bauchi state Nigeria were head length (46.92cm), ear length (25.40cm), neck length (44.09cm), neck circumference (63,36cm), height at withers (104.89cm), heart girth (111.90cm), body length (107.21cm), and tail length (57.04cm).

There were some variations in the morphometric traits of the donkeys due to strain effects of which *Duni* donkey strain having the highest body length and heart girth compared with other strains.

The results of the study is however, subjected to some limitations being that the sample size for the analysis was rather too small and this is due to financial, constraints of the farmer not allow one to get access to the animals due to traditional believes, time as well as animal resources encountered during the course of this study.

RECOMMENDATIONS

The Hb type AA was predominant on the four breeds of donkey which then mean adaption in favour for hot tropical weather and tolerance to ticks and helminthic infections; in other words, hot tropical regions, farmers are therefore encouraged to take advantage of donkey production, since they highly resistance to various diseases compared to cattle and horse. More so, head length, neck length, heart girth and body length could be used in classifying donkeys into strains. Further studies could be carried out to evaluate the effect of genotype on their productivity.

REFERENCES

Akinyemi, M. O. and Salako, A. E. (2010). Haemoglobin polymorphism and morphometric correlates in the West African Dwarf sheep of Nigeria. *International Journal of Morphology,***28**(1): 205-208.

Blench, R. M. (1990). **DonkeysinNigeria**: History, Distribution and Productivity.

Blench, R.M. (2014). A history of donkeys, wild asses, and mules in Africa. In Blench and MacDonald (Eds.) The Origins and Development of African Livestock.

Boyer, S.H. and Hiner, R. (1963). Modified apparatus for starch gel electrophoresis. *Journal of Laboratory Clinical Medicine.***61**:879-81.

BSADP (2017). Bauchi state Agriculture development program metrological record for the year 2017

RIM. (1992). Nigerian national livestock Resource Survey. Report by Resource Inventory and Management Limited (RIM) to Federal Department of livestock pest control services (FDL and PCS). Abuja, Nigeria.

Ellis-Jones J, Muvirimi F, Nengomasha E. M., and Msara P, (1994). Improving the productivity of draft animals in sub-saharan Africa: A rapid rural appraisal of Semukwe., Chikwanda and Sebungwe communal farming areas (with emphasis on the use of draft animals). Project Working Document. OD/94/20.39P.

Steel, R.D.G. and Torrie, J.H. (1980). Principles and procedures of statistics, 2nd edition, McGraw-hill book co Inc, New York.

Mabayoje, A. L., Ademiluyi, Y. S. (2014). A note on animal power and donkey utilization in Nigeria. Animal Traction Network for Eastern and Southern Africa (ATNESA) p. 2.

Map view (2018). Copyright© 2019 maps-streetview.com

Meutchieye, F., Nfor, N. K., Armelle, R. and Nyock, F. (2014). Donkey Husbandry and Production Systems :https://www.researchgate.net/publication/271212674

Pearson, R. A. and Ouassat, M. (1996). Estimation of weight and a body condition scoring system for working donkeys in Morocco. *Veterinary Record*, **138**: 229-233.

Salako, A. E (2006a). Application of morphological indices in the Assessment of Type and Function in Sheep. *International morphology*, **24**: 13-18.

Selvaggi, M., Alessandro**,** A.G. and Dario**,** C. (2015). Comparative characteristics of DNA polymorphisms of κ-casein gene in the horse and donkey. *GeneticMolecular Resource*, **14**(4): 14567-14575.

Slavov, R., Slavova, P. and Laleva S. (2004). Genetic structure of Ile de France sheep breed in Bulgaria according to the transferrin and haemoglobin polymorphous genetic system. *Trakia Journal of Sciences*, **2**: 38–40.

Starkey, P. (1995). Local transport solutions: people, paradoxes and progress-Lessons arising from the spread of intermediate means of transport. Report for Sub-Saharan Africa Transport Policy Program (SSATP) and Rural Travel and Transport Program (RTTP). The World Bank. 71p.

Tunon, M.J., Gonzales, P., VallejO, M. (1989). Blood biochemical polymorphism in Spanish goat breeds. *Comp. Biochemistry and Physiology*, **2**: 513-517.

Wold, A.G., Tegegne, A. and Yami, A. (2004). Research needs of donkey utilisation in Ethiopia in Fielding & Starkey eds Donkeys, people and development: A resource book for the Animal Traction Network for Eastern and Southern Africa. *CTA, Wageningen, Netherlands*, Pp: 77-81.