GENETIC DIVERSITY OF NIGERIAN INDIGENOUS GOAT BREEDS USING MICROSATELLITE MARKERS

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DEPARTMENT OF ANIMAL SCIENCE,
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NIGERIA

JULY 2014

DECLARATION

I declare that the work in this Dissertation entitled "Genetic Diversity of Nigerian Indigenous goat breeds using Microsatellite Markers" has been carried out by me in the Department of Animal Science, Ahmadu Bello University. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at any other Institution.

Oluwakemi Aderonke OJO	_____	_____
Name of student	Signature	Date

CERTIFICATION

This dissertation entitled "**GENETIC DIVERSITY OF NIGERIAN INDIGENOUS GOAT BREEDS USING MICROSATELLITE MARKERS**" by **Oluwakemi Aderonke OJO** meets the regulations governing the award of the degree of Doctor of Philosophy in Animal Science of the Ahmadu Bello University and is approved for its contribution to knowledge and literary presentation.

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‘Then Samuel took a stone and set it up between Mizpah and Shen, and called its name EBENEZER, saying’’ Thus far the Lord has helped us’’ (I Samuel 7:12). I give thanks and all the glory to the Almighty God for seeing me through years of relentless effort to acquire knowledge, thus far the Lord has brought me! I am grateful!!

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ABSTRACT

A total of 200 goats from three breeds namely Sahel (60), Red Sokoto (60), West-African Dwarf (60) and one strain; Kano Brown (20) were used to determine the phenotypic variation and Genetic diversity of Nigerian Indigenous goats. Morphological measurements were taken using a measuring tape in centimeters, they included; back length, height at withers and chest girth. Studies were also undertaken to determine the genetic diversity of Nigerian indigenous goats. Tissue samples were taken from the ears of 200 goats, 60 each from Sahel, Red Sokoto and West African Dwarf, while 20 were taken for the Kano Brown goat. DNA was extracted from the tissue using the Pure-link DNA kit and was genotyped using 25 microsatellite markers. The results of the morphological measurements indicated an average back length of 65.40 cm, 61.58 cm, 59.90 cm and 52.97 cm in Sahel, Red Sokoto, Kano Brown and West African Dwarf goats respectively, while the average heights at withers were 63.17 cm, 61.25 cm, 59.20 cm and 55.88 cm in Sahel, Red Sokoto, Kano Brown and West African Dwarf goats respectively. An average of 65.42 cm, 63.27 cm, 62.65 cm and 55.87 cm was obtained for chest girth in Sahel, Red Sokoto, Kano Brown and West African Dwarf goats respectively. Phenotypic correlations among the measured traits were positive and highly significant ($P< 0.05$) in most cases, ranging from 0.843 to 0.904, 0.671 to 0.737 and 0.626 to 0.728 for Sahel, Red Sokoto and West African Dwarf goats respectively. For the Kano Brown strain the correlations were not significant ($P > 0.05$) and ranged from 0.237 to 0.383. Out of the 25 micro-satellite markers, 23 were observed to be highly informative and polymorphic with the number of alleles ranging from 3 to 19. The highest observed heterozygosity was found in West African Dwarf and Kano Brown goats and the minimum in Sahel goat. In the entire goat populations, low inbreeding was observed (Mean inbreeding coefficient (F_{is}) was 0.105 and total inbreeding coefficient (F_{it})

was 0.129). The mean genetic differentiation (F_{st}) was 0.030 and demonstrated that about 96% of the total genetic variation was due to the genetic differentiation within individuals in each breed, while 4% was due to differences between breeds. The Red Sokoto and the Kano Brown breeds showed higher genetic similarity as observed in the genetic distance (0.022). High gene flow (Nm) was observed between Red Sokoto and Kano Brown breeds (13.59), similarly a high gene flow of 14.868 was observed across the studied goat populations. Significant deviations from the Hardy-Weinberg equilibrium were observed in all the populations. The phylogenetic tree and population structure displayed a remarkable degree of consistency with the goat's geographical origins in the country while the population structure showed that the Nigerian goats are admixed possibly due to indiscriminate mating and high gene flow. From the results obtained, inbreeding and genetic differentiation among the studied populations was low whereas gene flow was high. Measures to conserve distinctiveness and uniqueness of indigenous breeds such as ex-situ and in-situ conservation should be carried out in Nigeria. Gene flow between populations should be controlled by adopting improved breeding and management practices.

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CHAPTER ONE

1.0. INTRODUCTION

Small ruminants are important genetic resources in the tropics, where they play a predominant role in the sustenance of the livelihoods of rural farmers. Goats constitute the largest group of small ruminant livestock in Nigeria totaling about 53.8 million, goats in Nigeria constitutes 6.25% of the world's goat population (FAO, STAT 2011). Surveys have shown that up to 85% of rural-poor and smallholder farmers of all age groups and sexes keep them (FDLPCS, 2007).

The ability of goats to tolerate harsh climates, the presence of trypanotolerance in some breeds (Salako, 2004), suitability to traditional systems on account of small size, short generation interval (Abdul-Aziz, 2010) and ability to thrive on poor quality diets provided by scarce grazing on marginal lands (Adedeji *et al.*, 2011) all combined to make small ruminants strategic to increasing livestock productivity in rural agricultural systems (Adebambo *et al.*, 2004).

Goats are one of the main economic resources in many developing countries and their economic importance is growing in Western countries (Luikart *et al.*, 2001). Fortunately, the market demand at least in some parts of the world, are changing and the demand for specialty products (niche-products) is growing. This gives breeders of original/rare breeds an opportunity to expand the stock and preserve its genetic diversity. Genetic diversity is defined as the sum of genetic differences in multiple loci among individuals in a population, and is most readily reflected in the phenotypic variation seen in many populations. It is a valuable asset as the adaptability of a population, that is the population's ability to adjust to changes, depends on it (Woolliams *et al.*, 2005). It is well known that

species can face great environmental changes over time, such as in climate, pollution and diseases. Genetic diversity is required for populations to adapt to these changes (Frankham *et al.,* 2002).

Loss of genetic diversity is often associated with inbreeding and reduction in reproductive fitness, Frankham *et al.* (2002); Willi *et al.* (2006) although, there has been some disagreement regarding the importance of genetic factors in population extinctions (Frankham *et al.*, 2002). It has been established that most species do not become extinct before genetic factors negatively affect them (Spielman *et al.,* 2004). This has been demonstrated to apply to species Spielman *et al.* (2004) and there is no reason that it could not apply to individual breeds within species as well. Many plant and animal species around the world are at risk of extinction, largely due to human activities (Lande, 1998). During the past fifteen years, 300 of the 6000 farm animal breeds identified by the Food and Agricultural Organization FAO, (2000) have become extinct and 1350 breeds are at risk of extinction in the near future. During this period, fourteen (14) European goat breeds have become extinct (Taberlet *et al.*, 2008).

One of the primary goals in the management of animal populations is to maintain their genetic diversity at a high level and their inbreeding at a low level (Fernández *et al.*, 2005). To estimate the future breeding potential of a livestock breed, it is necessary to characterize the genetic structure and estimate the level of genetic diversity within the breed. For this purpose, pedigree information (Caballero and Toro 2000, Cervantes *et al.* 2008) and genetic material (Saitbekova *et al.* 1999, Dasmahapatra, *et al.* 2008) are used. From genetic material, microsatellite markers and mitochondrial DNA displacement loop (D-loop) are commonly used to describe the genetic polymorphism, genetic distance and geographical

origin of the domestic goat (Saitbekova *et al.*, 1999; Luikart *et al.*, 2001; Naderi *et al.*, 2007; Agha *et al.*, 2008).

The introduction of molecular genetics in recent decades has made it possible to differentiate the relationship between pairs of individuals, which according to the pedigree have the same relationship. The diversity of gene pool, different climatic conditions within the country, free mating and natural selection have given rise to different local populations of goats. Therefore, one would expect much differential adaptive development to be reflected in the morphology (Hall, 1991).

Since characterization of a breed is the first approach to a sustainable use of its animal genetic resource; studies on diversity and variability between indigenous goat breeds on the basis of morphological variables have been extensively carried out on-station and on-farm in Southern Nigeria (Orheruata *et al.*, 1997; Ozoje and Kadri 2001; Ozoje and Mgbere, 2002) and Northern Nigeria (Akpa *et al*., 1998, Yakubu *et al*., 2010).

Polymorphic DNA markers are very useful in the assessment of genetic diversity within and between breeds. Microsatellites are widely used as genetic markers for the analysis of genetic variability within and between breeds due to their polymorphism distribution throughout the genome and the efficacy of genotyping (Barker *et al*., 1997, Chenyambuga *et al.*, 2004, Okpeku *et al.*, 2011).

There are several studies on genetic diversity of goats, based on microsatellite markers, such as Swiss breeds (Saitbekova *et al.*, 1999), Chinese indigenous populations (Li *et al.*, 2002, Li *et al.*, 2004), Indian domestic goats (Rout *et al.*, 2008), goats from Europe and

Middle East (Canon *et al.*, 2006). Very scanty studies have been carried out on the genetic diversity of Nigerian indigenous goats.

1.1 Problem Statement

The Nigerian indigenous goat breeds have not been effectively characterized and it would be tragic to lose their unique genetic resource resulting from centuries of human and natural selection. Also, the rate of erosion of indigenous animal genetic resource threatens the prospect of improving the livelihood of present and future human generations. This is especially so when one considers that the demand for animal products is expected to double over the next 20 years as a consequence of urbanization, population growth and increased income (FAO, 1999).

Developing countries such as Nigeria hosts a vast majority of indigenous farm animal genetic resources (over 90%), that unfortunately have not being improved to respond to global food security needs (FAO, 1999).

1.2 Justification

A species without enough genetic diversity is thought to be unable to cope with changing environments or evolving competitors and parasites (Schaal *et al*., 1991). Also the ability of a population to respond adaptively to environmental changes depends on the level of genetic variation or diversity it contains (Ayala and Kiger, 1984). Therefore, studies on population, genetic diversity and the structure of population within and between species may not only illustrate the evolutionary process and mechanism but also provide information useful for biological conservation of the livestock species (Notter, 1999). Maintaining the integrity of livestock species as well as their genetic diversity is one of the

paramount interests for agricultural policies and serious steps have to be taken in order to protect the stocks of local animals which are in danger of losing their uniqueness. The preservation of the biodiversity can be achieved through the protection of gene reserves. One major use of DNA techniques in conservation is to reveal genetic diversity within and between populations. Inter-simple sequence repeat (ISSR) which is a DNA marker can be used without knowing the sequence information for genomic DNA (Zietkiewicz *et al*., 1994). The ISSR marker technique involves polymerase chain reaction (PCR), amplification of DNA using a single primer composed of a microsatellite sequence. The ISSR has mild technical difficulty, good reproductive ability and reasonable cost, permitting its use for genetic studies of population (Chen *et al*., 2005; Li and Xia, 2005).

1.3 Hypothesis

The following null hypotheses (Ho) were postulated for the study:

i) There are no relationships between the body parameters of the Nigerian goat populations.

ii) The Nigerian goat populations are not genetically diverse.

1.4 Objectives

The objectives of this study were to:

i) evaluate some phenotypic characteristics of the Nigerian goat breeds.

ii) estimate the allelic frequencies of different gene loci for the different breeds.

iii) estimate the degree of polymorphism in markers from the indigenous goat breeds.

iv) determine the genetic relationships between the Nigerian goat populations.

v) determine the genetic diversity within and between the Nigerian goat breeds.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1. Classification of Goats

Taxonomically the domestic goat (*Capra hircus*) belongs to the order *Artiodactyla*, suborder *Ruminantia*, family *Bovidae* and tribe *Caprini*. The tribe *Caprini* includes different members: - The goat-like sheep or sheep like goat: *Ammotragus lervia* (Barbary sheep) and *Pseudois nayaur* (Blue sheep), the goat like antelope: *Oreamnos americanus* (Mountain goat), and the sheep and goats.

Goats are divided into two genera: *Capra* and *Hemitragus*. The later is the tahr, which has three species (Ellerman and Morrison-Scott, 1966): - *H. jemlahicus,* found in the Himalayan region from Kashmir to Sikkim (northern India); *H. jayakari,* found in Oman; and *H. hylocrius,* found in Nilgiri Hills and adjacent ranges in southern India.

The genus *Capra* is divided into eight species (Alados, 1985; Corbet, 1978).

i) *C. aegagrus* (bezoar or wild goat), found in mountains from Asia Minor to Afghanistan and Pakistan, Oman, Crete, Aegean Islands;

ii*) C. hircus* (domestic goat), found worldwide in association with people, feral in many / some areas;

iii) *C. ibex* (ibex), found in European Alps, Palestine, Sinai, Arabian Peninsula, mountainous region from Afghanistan and northern India to Lake Baikal, Egypt and Sudan east of the Nile, northern Ethiopia;

iv) *C. walie* (Walia ibex), Simien Mountains of north-central Ethiopia;

v) *C. caucasica* (west Caucasian tur), western Caucasus;

vi) *C. cylindricornis* (east Caucasian tur), eastern Caucasus;

vii) *C. pyrenaica* (Spanish ibex), Spain and formerly southern France and Portugal;

viii) *C. falconeri* (markhor), mountains of southern Uzbek and Tadzhik (formerly Soviet Socialist Republic), Afghanistan, northern and central Pakistan, and Kashmir (India).

Epstein (1971) suggested that all these different species or subgenera have contributed to the domesticated stock. According to Mason (1984), the bezoar is the direct ancestor of the domestic goat and the markhor is said to have contributed to some breeds of Central Asia (Mason, 1981). However, for Mason (1984) it is less likely that the markhor has influenced some breeds. The ibexes and turs have not been domesticated. Table 2.1 illustrates the main differences between goat and sheep.

Table 2. 1: The main differences between goat and sheep

Goat	**Sheep**
60 chromosomes	54 chromosomes
Presence of beards in most	Absence of beards
Presence of face and caudal glands	Absence of face and caudal glands
Absence of foot glands	Presence of foot glands
Tail held up	Tail hanging down

(http://capra.iespana.es/capra/ingles/origen/origen.htm).

2.2 Domestication of Goats

Domestic animals have played a key role in human history. Despite their importance, the origin of most domestic species remains poorly understood. The goat is the earliest domestic animal and probably the first ruminant livestock, after the wolf to be domesticated (Hole, 1996; Uerpmann, 1996). There are two reasons for this: Firstly, the wild goat was

reported to be present in the regions of Southwest Asia during the time when agriculture was developing. Secondly, the goat is an extremely hardy animal, hence, could have withstood the rigors of being reduced to the state of domestication better than other ruminants. Goats were domesticated at first for meat (Hole, 1996).

However, as a dairy animal, the goat is regarded as the oldest, even older than cattle (Devendra and Burns, 1983). Domestic goats (*Capra hircus*) might have played a central role in the Neolithic agricultural revolution and the spread of human civilizations around the globe (Porter, 1996). The evidence of time and place for goat domestication is based on the identification of bones from archaeological sites. Domestication of goats is considered to have occurred in the mountainous area of western Asia between the 7^{th} and 9^{th} millennium B.C. (Epstein, 1971; Devendra and Burns, 1983).

The origin of domestic goats remains uncertain and controversial; however, archaeological evidence suggests that they were probably first domesticated in the Fertile Crescent region of the Near East, possibly first in the Zagros Mountains area 10,000 years ago (Zeder and Hesse, 2000). However it has been suggested that the goats could have also been domesticated outside the Zagros Mountains range. Some studies hint that a second domestication area is the Sindhu valley in North-Western part of Pakistan from which the Cashmere breeds could have originated (Porter, 1996). Others suggest that at least two wild species of *Capra* could have contributed to the gene pool of domestic goats (Clutton-Brock, 1981). Takada *et al*. (1997), studied the diversity of the cytochrome b gene of mitochondrial DNA (mtDNA) in Asian goats, and concluded that the strongest candidate for a matriarch ancestor of domestic goats is the bezoar or pasang (*Capra aegagrus*).

Furthermore, phylogeographic analysis of mtDNA revealed three highly divergent lineages (estimated divergence > 200,000 years ago) suggesting three separate maternal origins of domestic goats (Luikart *et al.*, 2001). In the report of Sultana *et al.* (2003) on the mtDNA diversity of Pakistani goats, four distinct mtDNA lineages termed as A, B and C (previously reported) and a new lineage D were identified. They concluded that at least four different strains of wild *Capra* might have been the source of the modern domestic goats with the most likely wild ancestor being the bezoar (Harris, 1961; Clutton-Brock, 1981).

Joshi *et al.* (2004) have undertaken the investigation of 363 goats belonging to 10 different breeds from different geographic regions of India using mtDNA sequence data from the hyper variable region. They found evidence for population structure and new mitochondrial DNA in Indian goats and cannot reconcile the genetic diversity found within the major lineage with domestication starting 10,000 years ago from a single mtDNA ancestor. Thus, they proposed a more complex origin for domestic goats. The Bezoar (*Capra aegagrus*) is thought to be the progenitor of the domestic goat. *Bezoar* means counter poison, as in the savage animals, it is possible to find a concrete in the stomach which is believed to be an antidote.

The main characteristic of the Bezoar is the long saber or scimitar shaped horns. It is believed that goats were probably present at Cayönü in Southeast Anatolia about 7000 BC. They were present at Jarmo in Iraqi Kurdistan where the site is dated from about 6500 BC to the end of the 6th millennium BC and at Sialk on the Iranian plateau as early as the 6th millennium BC. Domestic goats with scimitar horns like the bezoar spread over a large part of the whole World during the Neolithic period. They were represented in vase paintings from ancient Mesopotamia in the 4th millennium BC.

The small short-eared sabre-horned generalized type was occasionally also illustrated in the Mesopotamia tomb painting of the same period. It is believed that by the 5th millennium BC goats had reached Egypt and by about 3500 BC goats with spiral or corkscrew horns entered Egypt from the Middle East, from Egypt the goats moved to the South and West part of the continent.

2.2.1 Indigenous Goat Breeds in Nigeria.

The indigenous goat breeds of Nigeria are West African Dwarf, Sahel goats (Borno white) and Red Sokoto (Maradi), while Kano Brown is a strain of Red Sokoto goat.

2.2.1.1 West African Dwarf goat

The West African Dwarf (WAD) goat (Plate I), which is trypano-tolerant, is widely distributed across the rainforest belt of Southern Nigeria. They are short-legged and small-bodied animals, weighing between 22 to 26kg (Mourad *et al.*, 2000). They also present variable coat colours, ranging from black, brown, gray and white, and sometimes combinations of these in a variety of patterns (Mourad *et al.*, 2000). It has coarser bones and greater body surface area than Red Sokoto, they are prolific and can kid three times in two years. Herd structure consists of 23.9% males and 76.1% females. Age at first kidding is 18-24 months. Mean kidding interval is 11.0 months, peak conception period from August onwards. Kidding can occur all year but the peak is in dry season months of December to April. Litter size 1.4, pre-weaning mortality rate 30.6 % (Mourad *et al*., 2000).



Plate I: West African Dwarf buck.

Some of the goats found in the Northern parts of the country include the Sahel, Red Sokoto and Kano Brown.

2.2.1.2 Sahel goat

The long-legged Sahel goats (Borno White or Kyalla) as seen in Plate II are common in the arid or Sahel region of Nigeria. It is medium to large in size, long legged, and covered with coat of short fine hair. The breed is highly adapted to normadic life and a wide range of vegetation in desert to semi-desert condition. It is tolerant to hot, dry environment but unsuited to humid regions. They are used for meat and skin production, and a few can be described as good milkers. Ears are long and droopy, herd structure consists of 71.5% females and 28.5% males. It has a kidding interval of 11.3 months and a litter size of 1.4. (Akpa *et al.*, 1998).



Plate II: Sahel doe.

2.2.1.3 Red Sokoto goat

Red Sokoto goat (Maradi) as seen in Plate III) is one of the few well defined breeds of goat in Africa. It is the most numerous goat breed in Nigeria originally confined to the Niger Republic and Sokoto province of Nigeria but has spread throughout the Savannah belt. Ears are short and curved horizontally both sexes are horned, it is uniformly dark red in colour. The legs are shorter than the Sahel breed. The skin of the Red Sokoto goat is among the most valuable of all goat skins, it is used in the leather industry locally and internationally (Akpa *et al.,* 1998). The breed is a good meat animal in Niger, it is a milk yielding breed with about 0.5kg in dry season and up to 1.5kg in wet season per day. Birth weight varies from 1.0 – 2.5kg. It is a relatively small animal; at maturity (23 – 30 kg), (Akpa *et al.,* 1998)



Plate III: Red Sokoto doe.

2.2.1.4 Kano Brown goat

Kano Brown goat (Plate IV) is a strain of Red Sokoto goat; it is light brown in colour.



Plate IV: Kano Brown buck.

2.3 Morphological measurements: An Overview

According to Lanari *et al.* (2003) morphometric measurements have been used to evaluate the characteristics of various breeds of animals and could provide first hand information on the suitability of animals for selection. It is believed that various breed characteristics provide, to some extent reasonable, economic indicators. Body size and shape are important traits in meat animals and meat production can be better estimated than other production properties by taking body measurements (Bene *et al.*, 2007).

Proper measurement of body weight on-farm or on-station is often very difficult, as a result of unavailability of weighing scale especially in the rural areas where most of the animals are located (Adeyinka and Mohammed, 2006). It has been documented that body weight and linear body measurements of meat animals have been relevant in estimating body size and shape (Tsado *et al*., 2009, Cam *et al*., 2010, Sowande *et al*., 2010 and Ojedapo *et al*., 2012). Body linear measurements in addition to body weight are better tools to describe an individual or a population as opposed to the weighing and grading methods (Hill, 1990)

2.3.1 Body weight

Hassan and Ciroma (1992) obtained body weight of Red Sokoto goats at 1-2, 3-4 and 5 years and above as 16.41 ±0.39, 22.94±0.47 and 30.02±0.78 kg respectively. According to Akpa *et al.* (2009), the average body weight in Red Sokoto goats at 3, 6 and 9 months of age were 7.6, 10.6 and 12.8 kg respectively. Okpeku *et al.* (2011b) reported body weight in West African Dwarf and Red Sokoto goats at 2 years to be 15.37±0.38 and18.99±0.36kg, respectively.

2.3.2 Back length

For Red Sokoto goats at 1-2, 3-4 and 5 years and above, Hassan and Ciroma (1992) obtained back length as 79.15±0.57, 87.33±0.64 and 96.57±0.87cm, respectively. However, Yakubu *et al*. (2010) obtained back length of 51.63±0.41cm in Red Sokoto goats, while Akpa *et al.* (2009), reported the average back length in Red Sokoto goats at 3, 6 and 9 months of age as 40.8, 49.0 and 52.1cm respectively.

In West African Dwarf goats, Yakubu *et al*. (2010) reported a back length of 45.10±0.38cm, while Okpeku *et al*. (2011b) obtained a back length of 44.86±0.50 and 57.89±0.53cm at 2 years in West African Dwarf and Red Sokoto goats respectively.

2.3.3 Height at withers

Hassan and Ciroma (1992) obtained height at withers in Red Sokoto goats at 1-2, 3-4 and 5 years and above as 57.49±0.51, 62. 77±0.37 and 67.39±0.63 cm respectively. According to Akpa *et al.* (2009), the average height at withers in Red Sokoto goats at 3, 6 and 9 months of age were 40.2, 45.9 and 57.5cm, respectively. However, Okpeku *et al.* (2011b) observed slightly different values in height at withers of 47.88 ±0.50 and 61.04±0.54 in West African Dwarf and Red Sokoto goats at 2 years of age respectively.

2.3.4 Chest girth

For Red Sokoto goats at 1-2, 3-4 and 5 years and above, Hassan and Ciroma (1992) obtained chest girth to be 61. 78 ±0.73, 67.86±0.51 and 75.77 ±0.86 cm where as Yakubu *et al.* (2010) obtained a chest girth of 69.81±0.56 in Red Sokoto goats of 19 months of age.

However, Akpa *et al.* (2009) obtained average chest girth in Red Sokoto goats at 3, 6 and 9 months of age to be 43.3, 52.3 and 56.0cm, respectively.

In West African Dwarf goats of age 19 months, Yakubu *et al.* (2010) obtained a chest girth of 60.09±0.49, while Okpeku *et al*. (2011b) observed slightly different values in chest girth of 58.21±0.60 and 65.95±0.69 in West African Dwarf and Red Sokoto goats at 2 years of age respectively.

2.3.5 Pearson correlation between linear body measurements in goats

Phenotypic correlation coefficients are used to measure the relationship between two quantitative traits.

Ogah *et al*. (2013) reported correlation coefficients of 0.12 between Chest girth and Body length in adult male West African Dwarf goats, 0.90 between body length and height at withers and 0.41 between height at withers and chest girth for the same breed.

For the does, the researchers obtained correlation coefficients of 0.86 between chest girth and body length, 0.37 between height at withers and body length and 0.61 between height at withers and chest girth.

Okpeku *et al*. (2011b) reported high and positive correlations between body weight, withers height, neck-length, body- length and chest girth in West African Dwarf goats of Nigeria, for both bucks and does. They also obtained high and positive correlations for the linear body measurements in Red Sokoto goats for bucks and does.

In adult West African Dwarf goats Ojedapo *et al.* (2007) obtained correlation coefficients of 0.924 between height at withers and body length, 0.908 between chest girth and body length and 0.915 between height at withers and chest girth.

According to Khan *et al*. (2006) they reported correlation coefficients of 0.893 between height at withers and body length, 0.857 between chest girth and body length and 0.870 between height at withers and chest girth in adult Beetal goats of Pakistan.

For the Ethiopian indigenous goats Hassen *et al*. (2012) obtained high and significant correlation coefficients between body weight and body length (0.729), body length and chest girth (0.627).

2.4 Principal Component Analysis

Principal component analysis is a multivariate technique which could be used with success when morphological variables are independent. Principal components are a weighted linear combination of correlated variables, explaining a maximal amount of variance of the variables (Truxillo, 2003). This aids in data reduction and breaks multicollinearity which may lead to wrong inferences. Many researchers have used the independent factor scores derived from multivariate technique of principal component factor analysis to estimate body weight (Yakubu and Ayoade 2009), functional traits (Karacaoren and Kardamideen 2008) and as a selection criterion for the improvement of body size (Pinto *et al*., 2006). This technique has also been used to reduce the number of independent variables in the prediction of genomic breeding values (Macciotta and Gaspa, 2009).

2.5 Conservation of Domestic Animal Diversity

2.5.1 Meaning and importance of domestic animal diversity

Domestic animal diversity has been defined by Hammond (1993) as the spectrum of genetic variation existing among species, breeds and individuals of all animal species, which have been domesticated, and their immediate wild relatives. This genetic variation has been developed during the millions of years of evolution to form and stabilize each species. Globally, there are about 40 species of domestic livestock (FAO, 1995), which have been domesticated by humans over the last 12,000 years. In the process of domestication, however, separate and genetically unique breeds and strains have developed within each species as a result of human development and occupation of new areas over the planet.

According to FAO (1995), there are about 4,000 to 5,000 breeds and strains of domestic animals in the world, of which 300 are breeds of goats (Devendra and Burns 1983). These breeds and strains are referred to as the global animal genetic resources and the genetic variation both between and within the breeds are described as the diversity within the species of domestic animals. The selection process, both environmental and human-directed, has resulted in much of the diversity existing between the breeds. Differences among the breeds have been created by reproductive isolation, often imposed by human through pursuit of different breeding objectives and physical separation for various length of time.

As a consequence of physical separation, each breed/strain has been adapted to particular ecological condition to suit the local climate and the requirements of the community. Thus,

in the conservation of domesticated species, it is the diversity between breeds, rather than the variation between species, which is of crucial importance (Hammond, 1993; Barker, 1994). Intra-specific diversity has got both within-population and between-population genetic components.

Genetic variation at the population level consists of the differences in the types of alleles present and their frequencies across all members of a population considered together. Genetic variation within population is caused by change of allele frequencies over time due to selection, random genetic drift and gene flow (immigration from or emigration to other population).

Genetic variation within population is important as it is related to heterozygosity, which is known to enhance fitness-related characteristics. Variability between populations arises both from random processes (founder effects, demographic bottlenecks, genetic drift and mutations) and from local selection imposed by environment and humans (Hartl and Clark, 1997). Variability between populations is also the result of adaptation of populations to their local conditions and it is important since locally adapted populations may have particular genes or gene combinations critical for viability in their local environment.

For conservation of animal diversity, it is therefore, important that both within and between populations variation be maintained. This will enable better utilization of animals for production of food and agriculture to meet the current need of human kind while maintaining the potential to meet the range of future possible changes and needs (Hammond, 1993; FAO, 1995). This is because genetic diversity is the basis for a species

evolutionary flexibility and responsiveness to environmental changes. The main reason to advocate conservation of genetic diversity is that the improvement of domestic animals in order to meet human needs is dependent on genetic variation both within and between breeds/strains.

Loss of genetic diversity will likely decrease the ability of animals to respond to environmental change and will result in loss of genetic information potentially useful for breeding improvement (Hunter, 1996). The earth's climate varies widely and is continuously changing. It is the diversity of species and breeds that will allow the environment to be utilized efficiently throughout the years both in temperate areas and in the tropics.

The urge for conservation, particularly in developing countries, is strengthened by the predictions of the scale of human population growth. It has been predicted that, human needs for food will double over the next two generations with the demand for animal products increasing more rapidly than that of plants (Hammond, 1993; Hammond, 1994; Delgado *et al.,* 1999). With increasing population pressure, the quantity of food and other products must increase.

In developed countries consumer emphasis on product quality is increasing. Changing the production level and product quality requires different types and varieties. In addition, changes in food and agriculture production will require new combinations of plant and animal species and lines (e.g. breeds and varieties) and changes of management and production strategies. Sustainability in this new production system will require different genetic types (FAO, 1995).

The second reason to advocate conservation of domestic animal diversity is that, we can reasonably expect that large differences observed today between geographic regions in human needs for food and agriculture and in production capability will persist in the future. According to Hammond (1994) almost three quarters of the world agriculture will remain at the low to medium input levels where animal production environments incorporate combinations of stress such as feed shortage, drought and heat. Breeds in these environments are reservoirs of genetic information responsible for the adaptations necessary for production under adverse environment. Although they have been overlooked, the need for those which are able to produce in hard conditions, is making and will make in future important contribution to feeding humans (Simm, 1998).

A third reason for conservation of domestic animal diversity is that sustainable crossbreeding schemes require at least two viable purebred populations and sometimes more. Trends towards fewer breeds will reduce the opportunities for improvement of productivity through crossbreeding.

The fourth reason is that the wide variety of livestock breeds and strains available today is part of our cultural heritage and it deserves protection. At the breed level, unique genetic differences exist between the breeds of each domestic species, for example in goats, the Nubians have high milk yield, the Small East African are hardy animals and the West African Dwarf goats are trypanotolerant. Red Sokoto goats provide good quality skin. Thus, genetic diversity at the level of species can be exploited to satisfy human needs for varieties.

2.5.2 Loss of genetic diversity in domestic animals

Reduction in genetic diversity has been expressed primarily in terms of loss of breeds and strains. It is postulated that the current rate of extinction of species, breeds and strains is greater now than at any time in the past (Hammond, 1993). It is estimated that at least 30% to 40% of all animal genetic resources are currently at high risk of extinction (Hammond, 1994). However, adequate records do not exist to enable reliable estimates of either loss rates of the breeds or of domestic animal diversity itself. The existing data on the number of endangered breeds are likely to be underestimates of the magnitude of the problem. The loss of genetic diversity is occurring both within populations and among populations.

2.5.3 Loss of diversity within populations.

The factors that diminish genetic diversity within populations are genetic bottlenecks, random genetic drift, inbreeding and human activities. All the four factors are functions of population size (Lacy, 1987; Lande and Barrow-Claugh, 1987). These are described as follows:

2.5.3.1 Genetic Bottlenecks

A demographic bottleneck occurs when a large population experiences a severe, temporary reduction in size due to environmental or demographic events. For example, natural catastrophes, which occur at unpredictable intervals including events, like drought, disease outbreak and war. These events may destroy a certain percentage of a population and therefore reduce the effective population size. The result is that the genetic variability of all subsequent generations is contained in the few individuals that survive the bottleneck and reproduce. Hence, some genetic diversity is lost in the process. The magnitude of the loss

in diversity depends on the size of the bottleneck and the growth rate of the population afterward (Hunter, 1996). Another demographic event which may lead to bottleneck effect is the founder event. A founder event occurs when a few individuals of a population establish a new population.

The genetic constitution of the new population depends on the genetics of the founder animals. The genetic diversity of the original larger populations is reduced because the sample of genes in the few founder animals is not likely to be representative of the original gene pool. Generally passing through a genetic bottleneck can create two problems (Carson, 1983): a loss of certain alleles, especially rare alleles, if no individuals possessing those alleles survive, and a reduction in the amount of variation in genetically determined characteristics due to the presence of fewer alleles and decline in heterozygosity. Baker and Moeed (1987) have shown that the founder populations of several species of introduced birds have fewer alleles and lower heterozygosity than the source populations.

Bottlenecks have been shown to reduce allozyme heterozygosity. Leberg (1992) suggested that extremely low levels of allozyme heterozygosity in broad geographical surveys imply the occurrence of one or more recent severe bottlenecks. The overall effect of bottlenecks is the decline in fitness of the individuals in the population.

2.5.3.2 Random genetic drift

Genetic drift is a random change in gene frequencies in small populations, attributable to sampling error. In small populations each generation retains just a proportion of the gene pool of the previous generation. Subsequently after many generations of random genetic

drift, small populations will usually result in loss of variability as a consequence of fixation of some alleles on loci and loss of other alleles.

2.5.3.3 Inbreeding depression

Inbreeding results from the mating of two closely related individuals. However, the degree of relationship may vary. “Close breeding” refers to the mating of very close relatives such as sibling to sibling or parent to offspring. Its probability of occurrence increases in small populations if mating occurs at random. Inbreeding allows the rare, harmful recessive alleles to become expressed in the homozygous form, with resulting harmful effects on the offspring (Selander, 1983; Ralls *et al.*, 1988; and Charlesworth, 1987; Lomker and Simon, 1994) such as reduction in fertility, fecundity, offspring size, growth and survival, and physical deformities. Since inbreeding depresses reproductive fitness, it is assumed to increase the risk of extinction.

This presumption is supported by correlation between extinction and inbreeding on laboratory and domestic animals (Soule, 1980). However, gene flow from outside populations is beneficial in avoiding inbreeding and the erosion of genetic diversity (Miller and Waits, 2003).

2.5.3.4 Human activities

Extinction of species, breeds and strains/varieties through human activities represents the greatest threat to genetic diversity. The major threats to genetic diversity that result from human activity are habitat destruction and degradation, pollution, introduction of exotic species, and over-exploitation (Frankham, 1994). These threats are all caused by an ever-

increasing use of the world's natural resources due to expanding human population and development of market economy.

The growth of towns, factories and mines in developing countries creates a cash market for livestock products. Consequently, the traditional farmers who formerly kept animals for their own needs begin to supply the cash market. Genetic variation is being lost as farmers in developing countries abandon their local breeds in favour of high-yielding breeds for commercial production. Even if human activities do not directly eliminate a breed or strain, loss of genetic variation is taking place as the number of individuals in populations is reduced. In the long run the population size of a breed or strain may become so small that the breed/strain is no longer viable and may eventually go extinct.

2.5.4 Loss of diversity among populations

Loss of genetic diversity among-populations occurs when historically divergent and isolated populations experience an artificially high rate of gene flow from other populations. In domestic animals this is typically happening, as traditional farmers mix and interbreed their populations by moving from one place to another when they set up new settlements. It also occurs through crossbreeding by increasingly promoting universal use of very few superior breeds per species to upgrade local breeds. The result is that the uniqueness of formerly distinct breeds/strains is diminished or lost.

Moreover, there are concerns that, if there exist co-adapted groups of genes within each subpopulation, merging may result in the break-up of these and the loss of the particular adaptability of each subpopulation to its environment (Templeton, 1986). Lacy (1987) has shown that the effect of gene exchange between subpopulations is to increase the variance within groups, decrease the variance between groups and decrease the total variance.

According to Hammond (1994) about 50 % of the total variance for several quantitative traits in cattle, sheep and pig breeds is at the between-breed level, the remainder being common to all breeds. Hence, a move to one breed would eliminate half of the total variation. Loss of genotypic variance is irreversible as is the loss of alleles from a population. This is because it is impossible to sort out the alleles into groups of genotypes resembling the original subpopulations (Nelson and Soulé 1987).

2.6 Approach to conservation of animal genetic diversity

Conservation of genetic resources has become a major issue of public interest in the last decade or so. This is due to increased awareness that the genetic diversity is the basis for a species evolutionary flexibility and responsiveness to environmental changes and that loss of genetic diversity will restrict the opportunities available to mould domestic animals to meet unpredictable future changes and requirements.

Hence, genetic diversity needs to be preserved. The loss of variation within breeds is continually balanced by the introduction of new variation through new mutations; however, it is unwise to rely on new mutations as they are rare events and generally have deleterious effects on reproductive fitness (Frankham, 1994). Moreover, the genetic variation presented as differences among breeds cannot be regenerated. Each breed or strain is a result of mutation, genetic drift as well as separate adaptation and evolution, often over many centuries following different selection pressures imposed by climate, endemic parasites and diseases, nutrition and human selection. Each breed thus comprises a unique set of genes, which must be conserved.

Conservation of domestic animal diversity has been defined as the sum total of all operations involved in the management of animal genetic resources so that the pool of genetic diversity is maintained over time (Hammond, 1993). It encompasses management of human activities in such a way that animal genetic resources are best utilized and developed to meet immediate and short term human needs for future generations. Most attention in conservation of animal diversity has been directed toward rare breeds. However, in management of animal genetic resources, the fundamental problem is not the distinction between the breeds that are endangered and those that are not, but between those that are perceived to have little or no current utility and those which do have current utility or seem likely to have an immediate future use (Barker, 1994).

The critical issues in conservation of domestic animal diversity according to Notter *et al.* (1994) include:

(i) defining utilization strategies for available breeds to optimize production efficiency and sustainable utilization throughout the global range of environments and production systems;

(ii) livestock improvement strategies that make appropriate use of global domestic animal diversity;

(iii) development of optimum strategies for assessment and preservation of the genetic diversity, which exists in breeds that have currently little or no value in commercial production.

FAO (1995) has recommended the following strategies for effective management of domestic animal diversity at global level and for each species:

• Identifying and listing all breeds.

• Describing and characterizing breeds in order to understand their unique qualities and potential contributions and to understand which breeds have the potential to make the greatest variety of future contributions.

• Monitoring the population statistics for each breed and regularly reporting to the world the populations currently at risk of extinction.

• Sorting adequate samples of as many breeds as possible, generally in the form of frozen semen, ova, and embryos, to enable the future regeneration of lost populations of animals.

An overview of the historical background of animal genetic resources and the reasons for concern about their future, both in the developing and developed world, has been presented by Barker (2002). He concluded that the primary focus in the conservation of domestic animal diversity is on conservation of breeds. In general, two methods are used for conservation of genetic resources to support future livestock improvement: *in-situ* conservation and *ex-situ* conservation.

2.6.1 In-situ conservation

This involves maintaining breeds in their farm environment. *In-situ* conservation is primarily the active breeding of animal populations for food and agriculture, such that diversity is best utilized in the short term and maintained for the longer term (Hammond, 1994). Operations pertaining to *In-situ* conservation include performance recording and development of breeding programmes. It also includes ecosystem management for sustainable production of food and agriculture. It has been suggested that the number of animals required for maintaining a breed in western countries ranges from 150 to 1500

breeding females, depending on the species and reproductive rate. However, in developing countries the number should not decline below 5000 breeding females (Hodges, 1990).

The advantages of this approach are that the animals are still being utilized, the performance characteristics can be properly recorded and evaluated, and the breeds have the opportunity to evolve. The disadvantages are that selection and genetic drift may result in unfavorable genetic changes if the population is small. There is a risk of increasing inbreeding and homozygosity, which is associated with reduced fitness. The animals are at risk from disease and other natural disasters. Also, they are likely to be less productive and so more costly to maintain.

2.6.2 Ex-situ conservation

In the context of conservation of domestic animal diversity, ex-situ conservation means storage. Generally three approaches are used for Ex-situ conservation.

2.6.2.1 Maintaining breeds in farm parks or other animal collections

It involves breeding animals of a sample of a breed outside its normal production environment or habitat. Many of the pros and cons of this approach are similar to the *in-situ* conservation method. However there is potentially more control over management of the population. It has been suggested that 50 individuals should be the minimum number necessary to maintain genetic variability (Franklin, 1980). With such population only 1 % of the genetic variability will be lost per generation. This figure is on the safe side. Indeed it has been suggested that animal stocks can be maintained with a loss of 2–3% of the variability per generation. According to Notter *et al.* (1994) populations can be maintained at an inbreeding rate of 1% per generation corresponding to an effective population size (*N*e) of 50%, which can be achieved with 14 males and 100 females under random mating.

2.6.2.2 Creating a conservation herd (gene pool)

This involves crossing several rare breeds together, then breeding them to maintain genetic variability. It is an effective way of conserving genetic variation for two or three breeds. Maintenance of genetic diversity is almost better served by pooling five breeds in a conservation herd with *Ne* of 250 (Notter *et al.*, 1994).

However there is a greater risk of losing useful genes when more populations are combined. The disadvantage of this approach is that, although useful genes may be conserved, the identity of individual breeds is lost.

2.6.2.3 Cryopreservation

This involves frozen storage of rare breeds in the form of living semen, ova, embryos or tissues, which can be used to regenerate animals. Cryopreservation of semen and embryos is a powerful tool for preservation of genetic diversity (Moore, 1985). In the situation of a critical threat with high probability that a breed will become extinct, preservation of genetic material of individual animals in the form of germ cells and embryos is necessary to ensure that an adequate genetic pool is retained for future improvement programs.

Breeding technologies as artificial insemination (AI) and embryo transfer (ET) may provide support for this approach. The use of frozen semen in conservation program is particularly feasible where a tradition of use of AI is already strong. Collection of semen of endangered local breeds should take place as part of the AI program. According to Smith (1984), semen from 25 unrelated sires would provide an exceptionally good sample of the genetic diversity of a breed. Also Notter *et al.* (1994) recommended cryopreservation of semen

from 5 breeds with 10 sires from each breed and argued that it will provide greater preserved diversity than frozen semen of sires of each of two breeds.

For loci with multiple alleles, the probability of retaining all alleles from the rare breed is higher for the conservation herd supported with frozen semen than for a conservation herd maintained by random mating alone (Lomker and Simon, 1994), however the ability to rapidly recreate living purebreds is a key issue in germ plasm utilization. If frozen semen is used as the only method of conservation, then several generations of backcrossing are needed to reinstate the breed concerned. In contrast, breeds can be reinstated rapidly from frozen embryos. Embryo preservation is particularly important when the reproductive rate is low or the generation interval is long.

Furthermore, maternal cytoplasmic genes (e.g. mtDNA) cannot be preserved from semen, but can be effectively conserved by maintenance of even a modest number of female lineages. However, for all species, the likely cost per haploid genotype maintained or per animal sampled is much less for semen than for embryos (Notter *et al*., 1994). In general, embryo preservation should be attempted only when Embryo transfer is already in use for livestock improvement or when a breed has both clear potential value and a high likelihood of extinction.

Frozen storage of semen and embryos are relatively expensive, but it has the advantage that after the initial investment, semen and embryos provide much greater flexibility for finding ways of enhancing the reproduction of individuals, which are refractory to natural breeding conditions (Moore, 1985). In addition, the breed being conserved is free from unintended genetic changes (Simm, 1998). During storage frozen genetic material is at less risk from

disease and natural disasters than live animals, but obviously at risk from technological failures. Splitting the material collected from a particular breed or population and storing it in/at different locations can minimize these risks.

The disadvantages are that reproductive technologies are not uniformly successful or presently available for all individuals or all species and the expertise is not always available in the places where it is needed most. Also the breeds conserved in this way are not able to adapt to changes in the production environment or new disease challenges. Semen or embryos frozen now may as well be unable to meet future requirements for improvement.

2.7 Molecular Techniques for the Assessment of Animal Genetic Diversity

2.7.1 Rationale for molecular genetic approach

Biological variation is of wide occurrence in nature. Morphological variations in type, traits and production characteristics have been known and characterized within and between species for many centuries. Phenotypic traits have traditionally been used in the description of breeds. The characters vary from external features (horns, ears, coat colour), physical body measurements (heart girth, height, body length), production traits (body weight, milk yield, wool production, carcass traits), reproductive traits (fertility, age at first kidding, kidding interval), to survival traits (mortality, resistance to diseases, tolerance to heat and poor management and feeding) (Aboagye, 1992; Aboagye *et al.*, 1994).

The description of breeds with respect to coat colour, horns, physical body measurements and productive traits are based upon observation of the phenotype. An organism's phenotype is principally a manifestation of its genotype and environment. Phenotypic characters have the advantages of being easily seen and measured and usually much lower costs are incurred during phenotypic characterization compared to genetic characterization

(Minelli, 1993). For these reasons, phenotypic characters have been used extensively for characterization and identification of breeds.

One of the problems associated with phenotypic characterization is the difficulty in combining different measures in order to provide a useful tool for the description of a breed. Most phenotypic characters are poly-genetically inherited and many of them are influenced by environmental effects and sometimes with strong genotype-environment interaction. Furthermore, phenotypic characters are affected by natural selection. Therefore, due to the influence of different environmental conditions and different selection pressure on different kinds of characters, interbreed phenotypic comparison is unlikely to give meaningful results.

Earlier work to study genetic variability of individual animals and populations employed screening of protein variants by gel electrophoresis. Polymorphism in gene products such as enzymes, blood group systems and leucocytes antigens have been used for investigating genetic diversity at the molecular level. Using the above protein markers, numerous studies have estimated genetic variability, gene flow and phylogenetic relationships among populations (Barbancho *et al.*, 1984; Nguyen, 1990; Pepin and Nguyen, 1994). Protein polymorphism has proven to be a cheap and fast method of analyzing single locus variation in breeds (Thairu- Muigai, 2002). However, these techniques lack the power to resolve the differences between closely related breeds since a great deal of genetic variation remains undetectable by using protein markers (Meghen *et al.*, 1994; Dowling *et al.*, 1996).

Moreover, the genotypic frequencies estimated from protein markers may be influenced by natural selection among alleles making it difficult to interpret inter-population comparisons

(Alexandrino *et al.*, 1983; Pemberton *et al.*, 1988; Mopper *et al.*, 1991)The goal of breed characterization is to determine the genetic diversity within and between breeds. The near ultimate description of an animal should be a description of the sequence of nucleotides that comprise its genome. Describing differences and similarities in the DNA of two or more populations can provide the measure of relative genetic distances of such populations from each other.

The analysis of DNA has several significant advantages over protein markers for the study of molecular population genetics and systematics (Dowling *et al*., 1996): (i) the genotype rather than the phenotype is assayed; (ii) the expression of DNA markers is not influenced by development or by environmental factors; (iii) one or more sequences appropriate to a problem can be selected on the basis of evolutionary rate or mode of inheritance; (iv) the methods are for most part general to any type of DNA and, (v) DNA can be prepared from small amounts of tissues and is relatively stable, even in non cryogenetically stored tissues.

The last attribute according to Taberlet *et al.* (1997) means that genetic information on rare or endangered species can be obtained without destructive sampling and it is possible to analyze DNA from extinct populations or species (Taylor *et al.*, 1994). More recently, molecular data from DNA markers have received particular attention in the study of population variability because of their possible use to determine the chronology of evolutionary events using neutral DNA markers.

2.7.2 Molecular techniques for studying animal genetic diversity

DNA markers (molecular markers) are the most important tools added to the armory of the scientists to characterize, distinguish, or relate individuals within or between breeds,

strains, or species. The advances of the last two decades in techniques on DNA polymorphism and subsequent data analysis have greatly increased the ability to understand the genetic relationship among species at the molecular level. DNA genetic markers have now been used for the molecular genetics characterization and genetic diversity studies in numerous species (Schlotterer and Pemberton 1994).

Restriction fragment length polymorphism (RFLP) has been used for genome characterization and analysis (Botstein *et al*., 1980). According to Grodzicker *et al.* (1974) large amount of data have been generated since the first demonstration of RFLP and RFLP have been used as markers in human genetics (Botstein *et al.*, 1980), in genetic improvement of plant (Tanksley *et al.*, 1989) and animals (Soller and Beckmann, 1982). The reports of Saiki *et al*. (1988) revealed that the introduction of PCR in conjunction with the constantly increasing availability of DNA sequence data represents a milestone in this endeavor. A variety of different molecular techniques are now being used in various laboratories for the study of inter-and intra-specific genetic variation at the DNA level.

The most widely used molecular techniques of genetic variation are restriction fragment length polymorphism (Botstein *et al*., 1980) of nuclear DNA (Jeffreys and Morton, 1987) and mitochondrial DNA (Loftus *et al.*, 1994), minisatellites probing (Jeffreys *et al*., 1985; Jeffreys and Morton, 1987), microsatellites PCR amplification (Litt and Luty, 1989; Weber and May, 1989), random amplified polymorphic DNA (RAPD) (Welsh and McClelland, 1990; Williams *et al*., 1990), amplified fragment length polymorphism (AFLP) (Vos *et al*., 1995) and sequencing of mitochondrial DNA fragments (Cunningham *et al*., 1994). These techniques differ in the type of data they generate, in the way that they resolve genetic variations and in the taxonomic levels at which they may be most appropriate.

2.7.3 Application of Microsatellite markers for population studies

2.7.3.1 Microsatellite as genetic markers for population studies

Microsatellites usually called Simple Tandem Repeats (STRs), are a highly polymorphic class of genetic markers (Weber and May 1989), they consist of short sequence repeat motifs of tandemly repeated di, tri, tetra or penta nucleotide sequences that occur at large number of loci throughout the eukaryotic genome (Hamada *et al*., 1984; Tauz, 1989). The dinucleotide repeats: cytosine – adenine (CA) or Guanine –thymine (GT) on the other strand is the most common in mammalian genomes (Meghen *et al*., 1994). Polymorphism of microsatellite markers takes the form of variation in the number of repeats at any given locus. Microsatellites with more than ten dinucleotide repeats tend to be highly informative (Weber, 1990). For instance, in haploid human genome there are at least 35,000 CA repeats and they are located every 100,000 base pairs (Weber, 1990). Tri- and tetra-nucleotides have been shown to occur at a frequency of 1 every 300 – 500 kb on X-chromosome (Edwards *et al*., 1991). Due to exceptionally high rate of mutation, the majority of microsatellite loci are highly polymorphic in most mammalian species (Jeffreys *et al*., 1988; Weber, 1990). Alleles at microsatellite loci conform generally to Hardy-Weinberg equilibrium and segregate in a Mendelian fashion (Weber, 1990).

Most microsatellite loci are selectively neutral. This makes them compatible with the assumption of the neutral theory of population genetics (Kimura and Crow, 1964). Microsatellite polymorphism has advantages over other techniques in that it is a very reliable, highly accurate and repeatable method and has the potential of being standardized all over the world. The other potentially very large advantage of microsatellite in

population genetics is the fact that primers developed for a particular species can be sometimes applicable across a wide range of related taxa. For example, some primers developed for cattle can be used for sheep, goats, yak, etc.

Microsatellite markers are used for a wide range of molecular genetic studies such as establishing genetic linkage maps (Kappes *et al*., 1997), analysis of mating system and population structures (Bruford and Wayne, 1993; Queller *et al*., 1993; Schlötterer and Pemberton, 1994) and reconstruction of phylogenetic relationships among populations (Bowcock *et al*., 1994; Roy *et al.*, 1994, Forbes *et al*., 1995; Takezaki and Nei, 1996; MacHugh *et al.*, 1997). Their desirable characteristics are that: microsatellite loci are found in large numbers and are relatively evenly distributed throughout mammalian genomes, Mendelian co-dominant inheritance and relative ease of scoring through polymerase chain reaction.

Microsatellites are conserved across related species. Moreover microsatellite variation is independent of age, sex and environmental changes and hence can be detected at the early stage of development. The majority of microsatellites are found in non-coding regions of genome. However Morin *et al.* (1994) also reported the presence of microsatellite in protein coding region exhibiting regulatory role in gene expression and trinucleotide repeats have been used for linkage analysis in association with disease susceptibility genes in human (Richards and Sutherland, 1994).

Microsatellites have been found to be conserved between closely related species (Stallings *et al.*, 1991) allowing primer sets to be used across species (Moore *et al*., 1991). They have been extensively used for linkage mapping in diverse organisms from human to mosquitoes

(Weissenbach *et al.*, 1992; Crawford *et al.*, 1995; Vaiman *et al.*, 1996; Barendse *et al.*, 1997) and their use has enabled the identification of quantitative trait loci in major livestock species (Georges *et al.*, 1993). Microsatellites have proven useful in the analysis of paternity and kinship (Queller *et al*., 1993) and statistical calculation of probability at the individual (Edward *et al.*, 1992) and the population levels (Paetkau *et al.*, 1995). Microsatellite variation has been used to study the amount of hybridization between closely related species (Gottelli *et al.*, 1994; Roy *et al*., 1994).

Spatial distribution of alleles has been used to study local gene flow and population substructure (Gottelli *et al*., 1994; Allen *et al.*, 1995). Effective population sizes and inbreeding have been estimated from microsatellite data (Edwards *et al*., 1992). Microsatellites have been increasingly used for the study of genetic variation between and within animal populations. They have been successfully applied in the study of genetic variation in vertebrates (Kashi *et al.*, 1990a and b) and more particularly in livestock species such as poultry, sheep, goats, buffaloes and bovines (Haberfeld *et al.*, 1991; Ganai and Yadav, 2001, Arora *et al.*, 2004, Li and Valentini 2004).

Microsatellite DNA variation has been employed to assess genetic distances between strains of poultry (Kuhnlein *et al.*, 1989) and among European and African cattle (MacHugh *et al.*, 1994; MacHugh *et al*., 1997; Okomo *et al.*, 1998) and sheep breeds (Buchanan *et al.*, 1994; Arranz *et al.*, 1998). Microsatellite markers are well suited for forensic applications; population genetics studies etc. and consequently, have become the basis of genomic analysis in rodents, pigs, dogs, cattle and goats (Barendse *et al.*, 1994; Ellegren, 1993; Roy *et al*., 1994; Serikawa *et al.*, 1992; Ganai and Yadav, 2001).

2.7.3.2 Microsatellite genetic distances in livestock

Moazami-Goudarzi *et al*. (1997) used 17 microsatellite loci and 13 biochemical markers for studying the genetic variability between 10 cattle breeds. They used Nei's (1972) genetic distance and chord distance (*D*C) (Cavalli-Sforza and Edwards, 1967) for studying the genetic relationships between breeds. Yadav *et al*. (1998) reported the use of 22 bovine microsatellite primer pairs in 36 animals of Murrah breed of buffalo. All the 22 primer pairs amplified a product, and 14 of which were polymorphic. Pandey (1999) also reported the conservation of cattle microsatellites in two breeds of Indian buffalo.

Loftus *et al.* (1999) carried out a microsatellite survey on 8 hump-less cattle breeds from the Near East, 3 from Europe, 1 from West Africa and 2 zebu breeds from India with 20 microsatellite loci. They reported a large genetic divergence between the non-hybrid taurine and zebu groups. Mommens *et al*. (1999) studied variation between an African and five European taurine using data on 31 microsatellites loci. They calculated genetic distance and constructed Phylogenetic tree using neighbor joining (NJ) method. They reported American bison as an out-group. Wilson and Strobeck (1999) have shown genetic relatedness among wood and plains bison populations by using 11 microsatellite loci. Yang *et al.* (1999) studied the genetic relationship among five indigenous Chinese goat breeds with six microsatellite markers. A neighbor joining tree was constructed using Nei's standard genetic distance.

In this tree, Neimonggol and Liaoning were grouped together, then with Taihang; while Tibetan and Matou each had their own branch. Hanslik *et al.* (2000) studied the introgression of new world Holstein-Friesian into European Holstein-Friesian populations. A tree of individuals based on the proportion of shared alleles was produced; a clear

distinction between old world and new world Holstein-Friesian populations was found. Ritz *et al*. (2000) studied the phylogenetic analysis of the tribe bovine using 20 microsatellite loci. The authors inferred that the genetic distances used for the study: (δμ) 2 and *D*C are both considered being appropriate for phylogenetic studies among species and subgenus.

The authors concluded that *Bos*, *Poephagus* and *Bibos* each emerged as a subgenus within the genus *Bos*. On the other hand, *Bison*, *Bubalus* and *Syncerus* each formed a separate discrete genus. A suitable large panel of polymorphic markers for each species, especially those that are less common and less studied. Cross-species utilization of microsatellite loci not only save time and efforts in the laboratory, but also enable the construction of comparative maps between related species.

MacHugh *et al*. (1998) studied genetic demarcation among the breed of seven European cattle using 20 microsatellite markers. They reported correct breed designation with accuracy approaching 100% using data from a panel of 10 microsatellite loci. Martin *et al*. (1999) studied genetic diversity in six Spanish native cattle breeds using 30 microsatellites markers. Martin *et al*. (1999) used allele frequencies and genetic distance for the construction of dendrogram. The closest populations were those representing Austrian breeds, the most divergent being Menorquina and fighting bull.

2.8 Hardy-Weinberg Equilibrium (HWE)

Hardy-Weinberg Equilibrium law states that in a large random mated population with no selection, mutation or migration, the allelic or gene frequencies and the genotype frequencies remain constant from one generation to the next. Thus a population with constant gene and genotype frequencies is said to be in Hardy-Weinberg equilibrium

(HWE), Chi-square test is useful for determining whether the allelic frequencies are in HWE (Falconer and Mackay, 1996). Hardy-Weinberg assumption is under the following consideration: diploid, sexual reproduction, random mating, no selection, no mutation and no migration (Mariette *et al*., 1999).

2.8.1 Factors that affect HWE

2.8.1.1 Non-random mating

Non-random mating can occur in cases where related individuals have a greater probability of mating with each other than with other members of the population and where individuals that are geographically close are more likely to mate with each other than those that are not geographically close (Falconer and Mackay, 1996).

2.8.1.2 Mutation

This is the process that produces a gene or chromosome that is different from the wild type. A mutation occurs when a DNA gene is damaged or changed in such a way as to alter the genetic message carried by that gene. Mutation brings about genetic variation in a population by producing novel variants of genes (Cavalli-Sforza *et al*., 1994).

2.8.1.3 Migration

The movements of genes caused by individuals moving, including new individuals entering (immigration) or leaving (emigration) a population, introducing or removing genetic material and thereby changing allele frequencies. Migration of genes into a population results in an increase in a population's genetic variation and the migration of genes out of a population may result in a reduction of the genetic variation (Falconer and Mackay, 1996).

2.8.1.4 Selection

A natural process resulting in the evolution of organisms' best adapted to the environment. Only the individuals that are better adapted to the environment or able to mate successfully could pass their genes on to the next generation. Selection generally results in a reduction of genetic variation in a population (Cavalli-Sforza *et al.*, 1994)

2.8.1.5 Random genetic drift

Random genetic drift is the change in gene frequencies due to chance or sampling effects, it is the random sampling of alleles at every generation and it is very dependent upon population size. Genetic drift is fundamentally the result of a finite population size. The effect of genetic drift is infinitely proportional to the population size. Thus when the population size is small, e.g. due to strong bottleneck effects in the past, there are greater changes in gene frequency under genetic drift at every generation (Cavalli-Sforza *et al.*, 1994). Therefore, the smaller the population size, the greater the chances of sampling errors occurring.

In the study of many populations, it is important to determine whether the loci and the populations genotyped were in HWE and whether there were any significant deviations from the HWE. Deviations in Hardy Weinberg equilibrium at microsatellite level has been reported in various studies (Barker *et al.*, 2001; Hassan *et al.*, 2003; Laval *et al.*, 2000; Luikart *et al.*, 1999). It is known that a population is considered to be within HWE only when it is able to maintain its relative allele frequencies. Heterozygote deficiency is one of the parameters underlying departure from HWE.

2.9 Genetic Relationship Between Populations

2.9.1 Allelic frequencies and heterozygosities in population studies

The Mean Number of Alleles (MNA) detected in each population and the expected heterozygosities are good indicators of the genetic polymorphism within populations. The MNA is the average number of alleles observed in a population, while the expected heterozygosities are the proportion of heterozygote expected in a population. Numbers of alleles per locus per population are obtained by direct counting. Generally, the MNA is dependent on the sample size because of the presence of unique alleles that occur in low frequencies in populations and also because the number of observed alleles tend to increase with increases with population size (Nei, 1987).

Therefore the comparison of the MNA between samples of different sizes may not be meaningful unless the sample sizes are more or less the same (Nei, 1987; Hart and Clark, 1989). Among the Sub-Saharan African breeds, the MNA ranged from 5.26±0.464 to 7.05±0.516 in Djallonke and Mubende, respectively. For all these breeds the lowest mean number of alleles (4.05±0.442) was observed in Toggenburg while the highest (7.47±0.646) was found in Bandipur. Observed average heterozygosity ranged from 0.342±0.038 in Ndebele to 0.547±0.048 in Mongolian Cashmere. The expected heterozygosity was lowest (0.542±0.036) in Pafuri and highest in Ndebele (0.672±0.031). In all cases, average observed heterozygosities were lower than the heterozygosities expected under Hardy-Weinberg Equilibrium (HWE) and all populations showed significant deviation ($p< 0.001$) from HWE expectations.

Mahmoudi *et al*. (2011) studied the Iranian goat population using 13 micro-satellite markers and reported that the number of observed alleles for each locus ranged from 3 to

13.The authors observed that the highest and lowest number of effective allele were 8.7 and 2.6 for oarJMP23 locus in Raeini and oarAE133 locus in Kori Jonob Khorasan (K.J.K), respectively.

The average number of allele objective and effective was 7.67 and 5.14 respectively for the entire Iranian goat population. Highest and lowest polymorphism information content (PIC) value was 0.778 and 0.725 for Raeini and Lori respectively; polymorphism information content and expected hetrozygosity over all populations were 5.14, 0.797 and 0.755, the average expected heterozygosity overall loci in Raeini, Lori and K.J.K. were 0.805, 0.778 and 0.809, respectively. Among the three populations, K.J.K. populations displayed the highest values for mean Heterozygosity and Raeini PIC, while the Lori populations showed lower variability levels. However, Yang *et al*. (1999) reported that the mean effective number of alleles ranged between 0.746 to 0.8 in Chinese goats.

2.9.2 Genetic distance between populations

Genetic distance is a measurement of genetic relatedness of samples of populations (whereas genetic diversity represents diversity within a population). The estimate is based on the number of allelic substitutions per locus that have occurred during the separate evolution of two populations.

This difference measured between two populations provides a good estimate of how divergent they are genetically (Avise, 1994). Thus when the genetic distance is large, the genetic similarity is low and the time they diverged from each other is greater, while when genetic distance is small the genetic similarity is high and the time they diverged from each other is smaller (Avise, 1994). Cavalli-Sforza and Edwards (1964; 1967) were the first to

use gene frequency data to classify populations. They used genetic distance data to construct a phylogenetic tree to infer the history of various human populations.

One of the common measures of genetic distance in use today is Nei's standard genetic distance (DS) (Nei, 1972, 1978). In the reports of Okpeku *et al*., (2011a) on the Nigerian goat breeds, genetic distance between Sahel and West African Dwarf was (0.662), Sahel and Red Sokoto (0.444), Red Sokoto and West African Dwarf (0.268). Adebambo *et al*. (2011) reported that the genetic distance between Western African Dwarf goat and Red Sokoto (Maradi) was 0.39.

According to reports of Chenyambuga *et al*. (2004) who studied 16 Sub-Saharan African goats and Ethopian goats, they reported that the lowest distance (0.087) was observed between Arsi-Bale and North West Highland (i.e. between Ethiopian breeds), followed by that between Pafuri and Ndebele (0.125). Among the Sub-Saharan African goat breeds, the largest distance was found between Pafuri and Djallonke (0.273), followed by that between WAD and Pafuri (0.256). For all the 16 breeds analysed, the largest distance was found between Mongolian Cashmere and Djallonke (0.382) and between Toggenburg and Arab (0.382).

Mahmoudi *et al*. (2011) studied the Iranian goat populations and the standard genetic distances were calculated for these populations. The closest distance was observed between Raeini and Kori Jonob Khorasan (K.J.K.) (D = 0.4891) and the largest between Raeini and Lori (D = 0.6298).

Populations with many similar genes have small genetic distances. This indicates that they are closely related and have a recent common ancestor. Genetic distance is useful for

reconstructing the history of populations. For example, evidence from genetic distance suggests that African and Eurasian people diverged about 100,000 years ago (Nei and Roychoudhury 1974). Genetic distance is also used for understanding the origin of biodiversity. For example, the genetic distances between different breeds of domesticated animals are often investigated in order to determine which breeds should be protected to maintain genetic diversity (Raune, 1999)

2.9.3 F-statistics

This refers to fixation indices F_{is}, F_{st} and F_{it} which are used to partition genetic variation. Fixation indices are measures of standardized variances in allele frequencies that detect departure from HWE caused by inbreeding, biased out-breeding or population subdivision and drift (Weir and Cockerham, 1984). The subscript i, s and t refer to individual, subpopulation and total population respectively. The F statistics is a measure of the difference between the mean heterozygosity among subdivisions in a population and potential frequency of heterozygote, if all members of a population mixed freely and more assertively.

F_{is} detects inbreeding in individuals relative to subpopulation (within individuals within populations). This parameter can range from -1 to 1 indicating maximal inbreeding and out-breeding respectively. A positive F_{is} value indicates inbreeding as the observed heterozygosity is lower than the expected heterozygosity (Weir and Cockerham, 1984).

F_{st} detects inbreeding in subpopulations relative to the total population, when F_{st} equals zero, it means that subpopulations have identical allele frequency and fixed for different

allele. It is always positive and F_{st} is better for population estimates in cases where high level of gene flow is present. If F_{st} is between 0-0.05, it is considered small, 0.05-0.15, moderate and 0.15 and above large (Weir and Cockerham, 1984).

F_{it} can range from -1 to 1, indicating maximal inbreeding and out-breeding respectively (Weir and Cockerham, 1984).

2.9.3.1 Genetic differentiation of populations (F_{st})

The populations of most, if not all species show some levels of genetic structure, which may be due to a variety of non-mutually exclusive agents. Environmental barriers, historical processes and life histories (e.g. mating system) may all, to some extent, shape the genetic structure of populations (e.g. Donnelly and Townson, 2000; Gerlach and Musolf, 2000; Palsson, 2000; Tiedemann *et al.*, 2000). The understanding of the genetic differentiation between populations is of interest to population geneticists because it reflects the number of alleles exchanged between populations which influence the genetic composition of individuals within these populations (Balloux and Lugon-Moulin, 2002).

F_{st} measures the effect of population subdivision, which is the reduction in heterozygosity in a subpopulation due to genetic drift. F_{st} is the most inclusive measure of population substructure and is most useful for examining the overall genetic divergence among subpopulations. It is also called co-ancestry coefficient (θ) (Weir and Cockerham, 1984) or 'fixation index' and is defined as correlation of gametes within subpopulations relative to gametes drawn at random from the entire population (Subpopulation within the Total population).

F_{st} is always positive; it ranges between 0 = panmixis (no subdivision, random mating occurring, no genetic divergence within the population) and 1 = complete isolation (extreme subdivision). F_{st} values between 0-0.05 indicate negligible (small) genetic differentiation whereas, 0.05-0.15 moderate differentiation, greater than 0.15 means very great genetic differentiation within the population analysed. F_{st} is usually calculated for different genes, and then averaged across all loci, and all populations. This highly versatile parameter is even used as a genetic distance measure between two populations instead of a fixation index among many populations (Weir, Genetic Data Analysis II, 1996, and Kalinowski, 2002).

2.9.4 Gene flow between populations (Nm)

Gene flow is the transfer of alleles from one population to another population through immigration of individuals. In population genetics, gene flow is also known as gene migration. Migration into or out of a population may be responsible for a marked change in allele frequencies, immigration may also result in the addition of new genetic variants to the established gene pool of a particular species or population (Nassiry *et al*., 2009).

Gene flow between populations determines the effects of selection and genetic drift; generates new polymorphisms and increases the local effective population size (Balloux and Lugon-Moulin, 2002).

2.9.5 Genetic diversity

Genetic diversity refers to the variation at the level of individual genes (polymorphism), and provides a mechanism for populations to adapt to their ever-changing environment.

The more variation the better the chance that at least some of the individuals will have an allelic variant that is suited for the new environment and will produce offspring with the variant that will in turn reproduce and continue the population into subsequent generations. The level of genetic diversity in Swiss goat breeds according to Saitbekova *et al*. (1999) ranged from (51 to 58%) for 20 microsatellite loci while for Chinese goat breeds (77.7 to 82.3%) for 6 microsatellite loci (Yang *et al*., 1999). In general, the levels of gene diversity observed in the goats of Sub-Saharan Africa as reported by Chenyambuga *et al*. (2004) ranged from 54.2% in Pafuri to 67.2% in Ndebele.

2.9.5.1 Shannon index

Shannon Index is a commonly used diversity index that takes into account both abundance and evenness of species present in the community. A diversity index is a mathematical measure of species diversity in a community. Diversity indices provide more information about community composition than simply species richness (i.e., the number of species present); they provide important information about rarity and commonness of species in a community. The ability to quantify diversity in this way is an important tool for biologists trying to understand community structure. (Magurran, 1988).

Shannon index is explained by the formula:

$$H = -\sum_{i=1}^{s} (P_i * \ln P_i)$$

Where,
H = the Shannon diversity index
P_i = fraction of the entire population made up of species i (proportion of a species i relative to total number of species present, not encountered)
S = numbers of species encountered

Here, a high value of H (3.5) would be a representative of a diverse and equally distributed community and lower values represent less diverse community. A value of 0 would represent a community with just one species (Krebs, 1989). Shannon index can be compared when calculated for two loci, if one primer has higher amount of Shannon index than other primers, it means that primer is more suitable for genetic diversity studies in that breed or population (Nassiry *et al*., 2009).

2.9.6 Principal component analysis (PCA)

The construction of phylogeny and calculation of genetic distances have the disadvantage of not being able to detect the effects of past bottleneck effects on populations and any admixture that has taken place between populations (Cavalli-Sforza *et al*., 1994).

An alternative method taking into account the admixture is multivariate analysis, for example, principal component analysis. PCA can be used to reduce a large number of variables to a smaller number of variables without losing too much information (Cavalli-Sforza *et al*., 1994). PCA involves a mathematical procedure that transforms a number of (possibly) correlated variables into a (smaller) number of uncorrelated variables called principal components.

The first principal component accounts for as much of the variability in the data as possible, and each succeeding component accounts for as much of the remaining variability as possible.

PCA simply describes the variation of the multivariate data in terms of some linear combinations of the variables, in this case the observed microsatellite allele frequencies, which are resolved into smaller reference vectors. The new variables are uncorrelated with

each other. The coefficients defining the linear transformation are chosen so as to minimize the variation of the transformed data measure along each new co-ordinate axis (Cavalli-Sforza *et al*., 1994).

2.9.7 Construction of phylogeny

The neighbor-joining (NJ) methodology (Saitou and Nei, 1987) is used to construct the phylogenetic tree of population relationships. The NJ is known to be more efficient than many other methods in obtaining the correct tree (Rzhetsky and Nei, 1992). The neighbor-joining method does not assume an equal rate of evolution between lineages unlike the unweighted pair group-method with arithmetic mean (UPGMA), another method commonly used to construct phylogenetic trees (Nei *et al*., 1983).

2.9.8 Assignment of individuals to populations

In population genetics, individuals in a sample have to be classified into specific populations or breeds using a set of phenotypic criteria. Population assignment methods assign individuals to populations using a clustering method. Here individuals that are genetically similar are assigned to the same cluster. There are broadly two types of clustering methods that could be used; distance based and model based method, with the most commonly used method being the distance method. Distance based method first calculates a pair-wise distance matrix (for example, Nei's distance or Cavalli-Sforza and Edwards Chord distance), then uses them to assign the individual to the closest population. These methods are very easy to apply but have the disadvantage of being too dependent on

the distance measure and it is difficult to assess the confidence level that should be assigned to clusters obtained (Pritchard *et al.*, 2000).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study location and Source of Animals

This study was carried out using goat populations in three states of Nigeria namely, Jigawa, Kaduna and Oyo state. A total of 200 randomly sampled indigenous goats from three (3) breeds namely: Sahel goats, Red-Sokoto and West-African Dwarf and one strain namely; Kano- Brown were used for the study. For each breed, a maximum of sixty (60) animals were sampled and twenty (20) for the Kano Brown.

The Sahel goats were sourced from Maigatari International market in Maigatari district of Jigawa state. It has an average elevation of 352 meters above sea level. The area is mildly densely populated with 148 people per km^2, with a total population of 7,799 people. Its coordinates are 12^o 48 26 N and 9^o 25 5 E, the average annual temperature is 27.3^oC with an average rainfall of about 418mm per annum. Maigatari is semi arid in nature and the soil is very sand (Ibrahim *et al*., 2012).

The Red Sokoto and Kano Brown goats were sourced from the Teaching and Research Farm of the Department of Animal Science, Ahmadu Bello University Zaria. The University is located in the Northern Guinea Savannah zone of Nigeria. Being in the Northern Guinea Savannah zone, it receives a mean precipitation of 1,107mm per annum, stretching over 120-170 days from late April to early September (Akpa *et al*., 2002, Issa *et al* 2011). Seasonal distribution of rainfall is approximately 0.1%, late dry (January-March), 25.8% early wet (April-June) 69.6% late wet (July-September) and 4.5%, early dry (October-December) season. Average maximum temperature is 38.89^oC while the average minimum ambient temperature is 8.89^oC and the yearly average for the past 7 years was

25.55^{o}C (Issa *et al*., 2011). The mean relative humidity during dry and wet seasons is 21 and 72% respectively. Good quality forage is more abundant in the late wet and early dry seasons while forages are generally scarce and very low in quality during late dry and early wet seasons (Akpa *et al*., 2002).

The West African Dwarf goats were sourced from the Bodija Abattoir at Ibadan Oyo state. Bodija has a latitude of 7.436^{o} and longitude of 3.919^{o}, its temperature ranges from 21.15^{o} to 34.8^{o}C with a mean of 26.6^{o}C.The peak rainfall is in the month of September while the least is in January and December. The mean annual rainfall is 1258.9mm with a relative humidity of 94 % (Cadmus *et al*., 2008).

3.2 Data Collection

3.2.1 Linear Body Measurements.

Height at withers (HW): The height at withers was measured as the distance from the ground to the withers using a tape rule measured in centimeters.

Back Length (BL): Body length was measured using a tape rule as the distance from the occipital protuberance to the base of the tail.

Chest girth (CG): The chest girth was measured by taking the circumference of the chest using a tape measure in centimeters.

The linear body measurements taken are shown in Plate V.

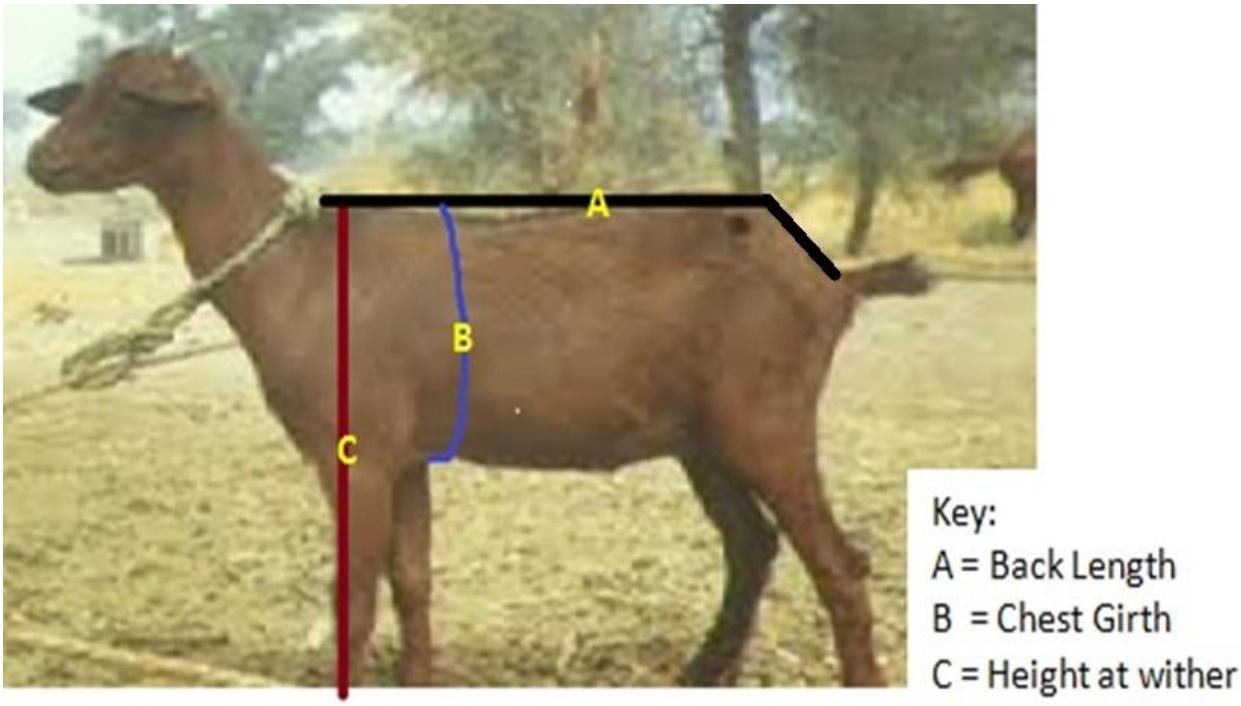


Plate V: Linear body measurements in goats

3.2.2 Sampling

Six goats each from ten different herds of Sahel, Red Sokoto and West-African Dwarf breeds were sampled, while for Kano-Brown goat, two goats were sampled from ten different herds. The number of sampled breeds and their sources are shown in Table 3.1 and Figure 3.1.

Tissue samples were taken from the ears of these goats using an Allflex ear punch tissue sample collector (Plate VI) and aliquoted into plastic tubes (Plates VII and VIII) containing the Allflex tissue preservative.



Plate VI: Allflex Tissue Ear Punch

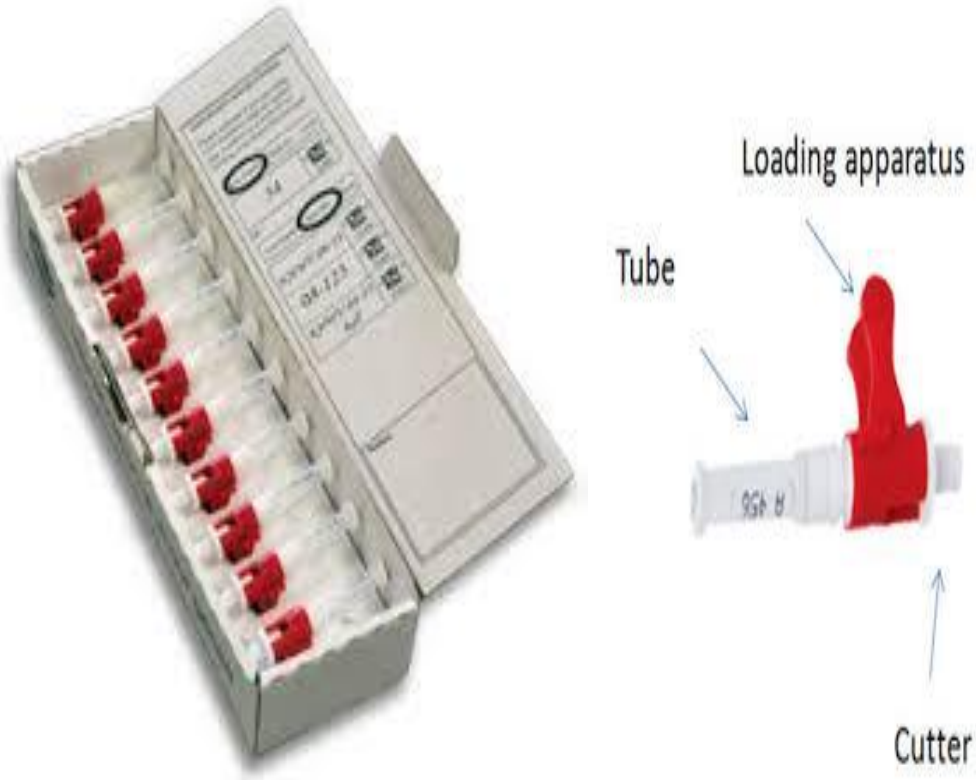


Plate VII: All-flex Tissue sample collection tubes

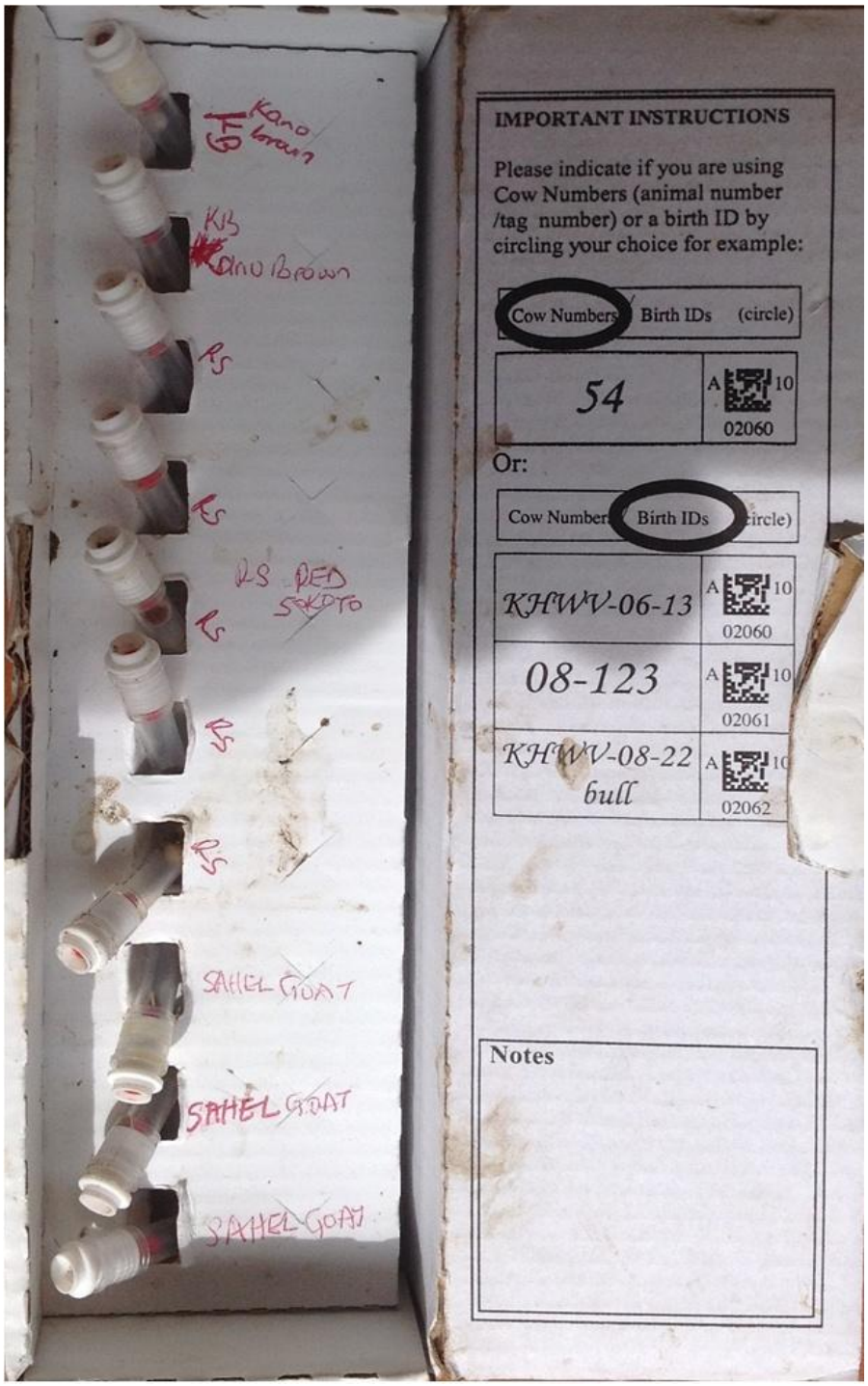


Plate VIII: Plastic tubes containing tissue samples



Figure 3. 1: Map of Nigeria showing distribution of sampled goats

Table 3. 1:Nigerian Goat Populations, Number genotyped and Location.

Goat breeds	Number genotyped	Location(State and Town)
Sahel goats	60	Maigatari market (Jigawa)
Red Sokoto	60	Animal Science Departmental farm Zaria (Kaduna)
Kano Brown	20	Animal Science Departmental farm Zaria (Kaduna)
West-African Dwarf	60	Bodija abbatoir (Oyo)

3.2.3 Genomic DNA extraction

DNA extraction, amplification and sequencing were carried out at the International Livestock Research Institute, (ILRI) Nairobi, Kenya.

Table 3. 2:Components of Purelink Genomic DNA kits.

Component	**K1820-01** 50 preps	**K1820-02** 250 preps
PureLink Genomic Lysis/Binding Buffer	10ml	50ml
PureLink Genomic Digestion Buffer	9ml	45ml
PureLink Genomic Wash Buffer 1	10ml	50ml
PureLink Genomic Wash Buffer 2	7.5ml	37.5ml
PureLink Genomic Elution Buffer(10mM Tris-HCL,pH 9.0,0.1Mm EDTA)	10ml	50ml
RNase A (20mg/ml) in 50Mm Tris-HCL, Ph 8.0, 10Mm EDTA	1ml	5ml
Proteinase K(20 mg/ml) in storage buffer (proprietary)	1ml	5ml
PureLink Spin Columns with Collection Tubes	50	250
PureLink Spin Columns with Collection Tubes (2.0ml)	100	500



Plate IX: PureLink Genomic DNA Kit

3.2.4 Protocol for DNA extraction

DNA was extracted from the tissue cells using the PureLink™ Genomic Kits (Plate IX) for purification of genomic DNA with Catalog nos. K1820-01 and K1820-02 according to the manufacturer's specifications and protocol. Table 3.2 shows the components of the kit.

The tissue samples were chopped into pieces of 2 mm, using a sterile blade and 25mg of the sample was placed into a sterile micro-centrifuge tube.

One hundred and eighty (180 µl) PureLink™ Genomic Digestion Buffer and 20 µl Proteinase K (supplied with the kit) were added to the tube. The mixture was incubated at 55°C with occasional vortexing until lysis was complete (1-4 hours). To remove any particulate materials, the lysate was centrifuged at maximum speed for 3 minutes at room temperature and the supernatant was transferred to a new, sterile micro-centrifuge tube.

Twenty (20) µl RNase A (supplied in the kit) was added to the lysate mixed well by brief vortexing, and incubated at room temperature for 2 minutes. 200 µl PureLink™ Genomic Lysis/Binding Buffer was added and mixed well by vortexing. 200 µl (96-100%) ethanol was added to the lysate and mixed well by vortexing for 5 seconds.

Ethanol was added to PureLink™ Genomic Wash Buffer 1 and PureLink™ Genomic Wash Buffer 2 according to instructions on each label and mixed well and labeled.

3.2.5 Binding DNA

The lysate (~640 µl) prepared with PureLink™ Genomic Lysis/Binding Buffer was added and ethanol to the PureLink™ Spin Column and centrifuged at 10,000 × g for 1 minute at room temperature. The collection tube was discarded and the spin column was placed into a clean PureLink™ collection tube supplied with the kit.

3.2.6 Washing

500 µl of Wash Buffer 1 prepared with ethanol was added to the column and centrifuged at room temperature at 10,000 × g for 1 minute. The collection tube was discarded and the spin column was placed into a clean PureLink™ collection tube supplied with the kit. 500 µl of Wash Buffer 2 prepared with ethanol was added to the column and centrifuged at maximum speed for 3 minutes at room temperature and the collection tube was discarded.

3.2.7 Elution

The spin column was placed in a sterile 1.5-ml micro-centrifuge tube and 25-200µl of PureLink™ Genomic Elution Buffer was added to the column and incubated at room temperature for 1 minute. The column was centrifuged at maximum speed for 1 minute at room temperature and this step was repeated to recover more DNA. The column was centrifuged at maximum speed for 1.5 minutes at room temperature with the tube

containing purified DNA.

3.2.8 DNA amplification and DNA sequencing

A master mix was prepared containing water, buffer, dNTP's, primers and Taq DNA Polymerase in a single tube and latter aliquoted into individual tubes. Magnesium Chloride ($MgCl_{2)}$ and template DNA solutions were latter added. The sample was gently vortexed and briefly centrifuged to collect all drops from the walls of the tube and the samples were placed in a thermocycler using the following setup:

An initial denaturation at 95°C (4 minutes), denaturation (1minute) annealing at 53°C (45 seconds), and extension at 72°C (15 minutes) for 35 cycles.

The PCR products were multiplexed and genotyped using an Applied Biosystems 3700 sequencer and bands were scored using Peakscanner$^{(R)}$ software (Applied Biosystems Foster City, CA, USA). The primers used for PCR are shown in Table 3.3 below.

3.3 Data Analysis

3.3.1 Phenotypic variation

Quantitative data were analyzed using analysis of variance (ANOVA) procedures of SAS (2004) and means were separated using Duncan's multiple range test.

Pearson correlation coefficient for the four goat populations was computed using the generalized linear model of SAS (2004).

Table 3. 3:Characteristics of Microsatellite markers (Primers) used for the study (FAO/ISAG, 2011).

#	Marker	Sequences (F /R: 5' →3')	Annealing T°C	Range (bp)		Dye	GeneBank Access #
1	SRCRSP03	CGGGGATCTGTTCTATGAAC TGATTAGCTGGCTGAATGTCC	55	98	122	NED	L22195
2	ILSTS005	GGAAGCAATTGAAATCTATAGCC TGTTCTGTGAGTTTGTAAGC	55	172	218	VIC	L23481
3	SPS113	CCTCCACACAGGCTTCTCTGACTT CCTAACTTGCTTGAGTTATTGCCC	58	134	158	6FAM	-
4	CSRD247	GGACTTGCCAGAACTCTGCAAT CACTGTGGTTTGTATTAGTCAGG	58	220	247	PET	-
5	MAF209	GATCACAAAAAGTTGGATACAACCGTG TCATGCACTTAAGTATGTAGGATGCTG	55	100	104	VIC	M80358
6	McM527	GTCCATTGCCTCAAATCAATTC AAACCACTTGACTACTCCCCAA	58	165	187	VIC	L34277
7	SRCRSP5	GGACTCTACCAACTGAGCTACAAG TGAAATGAAGCTAAAGCAATGC	55	156	178	PET	L22197
8	ILSTS087	AGCAGACATGATGACTCAGC CTGCCTCTTTTCTTGAGAG	58	135	178	NED	L37279
9	SRCRSP9	AGAGGATCTGGAAATGGAATC GCACTCTTTTCAGCCCTAATG	58	99	135	PET	L22200
10	OarFCB 304	CCCTAGGAGCTTTCAATAAAGAATCG GCGCTGCTGTCAACTGGGTCAGGG	56	150	188	-	L01535
11	ILSTS11	GCTTGCTACATGGAAAGTGC CTAAAATGCAGAGCCCTACC	58	250	300	6FAM	L23481
12	ETH10	GTTCAGGACTGGCCCTGCTAACA CCTCCAGCCCACTTTCTCTTCTC	55	200	210	PET	Z22739
13	MAF065	AAAGGCCAGAGTATGCAATTAGGAG CCACTCCTCCTGAGAATATAACATG	58	116	158	VIC	M67437
14	OarCP34	GCTGAACAATGTGATATGTTCAGG GGGACAATACTGTCTTAGATGCTGC	50	112	130	-	U15699
15	ILSTS029	TGTTTTGATGGAACACAG	55	148	170	NED	L37252

		TGGATTTAGACCAGGGTTGG					
16	INRA023	GAGTAGAGCTACAAGATAAACTTC TAACTACAGGGTGTTAGATGAACT	58	196	215	NED	X80215
17	MAF70	CACGGAGTCACAAAGAGTCAGACC GCAGGACTCTACGGGGCCTTTGC	65	134	168	6FAM	M77199
18	INRA063	GACCACAAAGGGATTTGCACAAGC AAACCACAGAAATGCTTGGAAG	58	164	186	VIC	X71507
19	BM6444	CTCTGGGTACAACACTGAGTCC TAGAGAGTTTCCCTGTCCATCC	65	118	200	6FAM	G18444
20	OarFCB48	GAGTTAGTACAAGGATGACAAGAGGCAC GACTCTAGAGGATCGCAAAGAACCAG	58	149	173	PET	M82875
21	INRABERN172	CCACTTCCCTGTATCCTCCT GGTGCTCCCATTGTGTAGAC	58	234	256	PET	-
22	INRABERN185	CAATCTTGCTCCCACTATGC CTCCTAAAACACTCCCACACTA	55	261	289	PET	X73937
23	TCRVB6	GAGTCCTCAGCAAGCAGGTC CCAGGAATTGGATCACACCT	55	217	255	PET	L18953
24	SRYM18	GGCATCACAAACAGGATCAGCAAT GTGATGGCAGTTCTCACAATCTCCT	58	80	170	6Fam	Y chromosome
25	OarFCB20	GGAAAACCCCCATATATACCTATAC AAATGTGTTTAAGATTCCATACATGTG	58	93	112	VIC	L20004

3.3.2 Genetic variation

Gen AlEX 6.5 genetic analysis statistical package of Peakall and Smouse (2012) was used for computing the number of alleles, number of effective alleles, the heterozygosities, Shanon index, gene flow, genetic distance, genetic diversity and Polymorphic information content (PIC) .

3.3.2.1 F-Statistics

The genetic differentiation among sub-populations (F_{st}), inbreeding coefficient (F_{is}), and total inbreeding (F_{it}) were estimated using GenAlEX 6.5 genetic analysis statistical package of Peakall and Smouse (2012).

3.3.2.2 Phylogenetic tree

Phylogenetic trees were constructed from Nei's standard genetic distance (DS) generated using the Neighbour Joining (NJ) and the Unpaired Group with Arithmetic Mean (UPGMA) methods in the TREEVIEW program, GENEPOP version 3.4 (Raymond and Rousset 1995).

3.3.2.3 Tests for deviation from Hardy-Weinberg Equilibrium

In this study, deviations of the locus/population combinations from Hardy-Weinberg Equilibrium (HWE) were determined using the statistical program of GenAlex 6.5 (Peak and Smouse 2012).

3.3.2.4 AMOVA

Analysis of molecular variance (AMOVA) was performed to quantify further the extent of population differentiation and the distribution of genetic variation in the sampled populations. It was analyzed using GenAlEX 6.5 genetic analysis statistical package (Peakall and Smouse 2012).

3.3.2.5 Evano-graph and Population structure

The Evano-graph and population structure were estimated using the program STRUCTURE 2.0 (Pritchard *et al*., 2000).

CHAPTER FOUR

4.0 RESULTS

4.1 Phenotypic traits of the Nigerian Goat Breeds.

4.1.1 Descriptive statistics of the phenotypic traits

The results of descriptive statistics of the phenotypic traits of the Nigerian indigenous goat breeds are revealed in Table 4.1. The analysis of variance (ANOVA) showed significant ($P< 0.01$) differences in all the measured traits. The Sahelian breed showed higher means and variances in all the traits considered compared to all other breeds. There were no significant differences ($P>0.05$) between Red Sokoto and Kano Brown breeds for all the measured traits, however the West African Dwarf goats were observed to have significantly ($P<0.01$) the lowest means.

Table 4. 1: Descriptive statistics of the phenotypic traits of the Nigerian Indigenous goat breeds.

Trait		Sahel	Red Sokoto	Kano Brown	West African Dwarf
BL	Mean±s.e	65.40±0.90[a]	61.58±0.60[b]	59.90±0.56[b]	52.97±0.84[c]
	S^2	48.72	21.64	6.20	42.71
	CV	10.67	7.55	4.16	12.34
HW	Mean±s.e	63.17±0.70[a]	61.25±0.61[ab]	59.20±0.75[b]	55.88±0.84[c]
	S^2	29.16	22.43	11.12	41.87
	CV	8.55	7.73	5.63	11.58
CG	Mean±s.e	65.42±0.75[a]	63.27±0.98[ab]	62.65±0.66[b]	55.87±0.94[c]
	S^2	33.91	57.62	8.66	52.49
	CV	8.90	12.00	4.70	12.97

BL- body length, HW- height at withers, CG- chest girth.

s.e –standard error; S^2- variance; CV- coefficient of variation.[a, b,c]

[abc]Means in the same row with different superscripts are significantly different at P<0.01.

4.1.2 Phenotypic Correlations of the measured traits.

The Pearson correlation coefficients amongst the linear measurements for Sahel, Red Sokoto, Kano Brown and West African Dwarf are shown in Table 4.2. For the Sahel goats, the highest correlation was observed between body length and chest girth (r = 0.90), although all the correlations were positive and significant (P<0.05).

In the Red Sokoto goats, correlations between body length and height at withers was the highest, (r = 0.74). In all cases, the correlations were significant (P <0.05) and positive while for the Kano Brown goats, correlations between body length and chest girth was highest (r = 0.38) although all the correlations were positive, they were low and not significant (P > 0.05).

For the West African Dwarf goats, the highest correlation was recorded between body length and chest girth (r = 0.73), similarly all the correlations were positive and significant.

Table 4. 2: Phenotypic Correlations among the linear body measurements of the Nigerian indigenous goats.

		BL	CG	HW
CG	Sahel	0.90*		
	Red Sokoto	0.67*		
	Kano Brown	0.38^{NS}		
	West-African Dwarf	0.73*		
HW	Sahel	0.84*	0.89*	
	Red Sokoto	0.74*	0.68*	
	Kano Brown	0.24^{NS}	0.35^{NS}	
	West-African Dwarf	0.72*	0.63*	

BL- body length, HW- height at withers, CG- chest girth * = significant at P<0.05 for all correlations, NS = Not significant at 5% for all correlations.

4.2 Allelic Frequencies of different gene loci for the Nigerian goat breeds

4.2.1 Allelic frequencies, observed and expected heterozygosity and fixation-index (F_{is}) for Sahel goat population.

Table 4.3 shows the number of effective alleles, observed and expected heterozygosity, Shannon index and Fixation index for Sahel goat population. Fixation index (F_{is}) is a measure of standardized variance in allele frequencies that detect departure from Hardy Weinberg Equilibrium caused by inbreeding.

All the markers used in this population were highly informative, with allele numbers ranging from 3 to 19. The number of effective alleles for this population ranged from 1.24 (ILSTS 005) to 8.295 (ILSTS 087). The observed heterozygosity (Ho) ranged from 0.140 (ILSTS 005) to 0.967 (ILSTS 087), while the expected heterozygosity (He) ranged from 0.194 (ILSTS 005) to 0.879 (SRYM 18).The Shannon index (I) ranged from 0.408 to 2.455 and in most cases was above 1.0. Negative F_{is} values were obtained at five (5) loci (ILSTS029, ILSTS087, SPS113, SRCRSP 3, and MAF209).

Table 4. 3: Allelic Frequencies, Heterozygosity and F-statistics in Sahel goats.

Locus	N	Na	Ne	I	Ho	He	F_{is}
OarFCB20	39	8.000	3.422	1.585	0.282	0.708	0.601
BM6444	58	12.000	4.778	1.858	0.665	0.791	0.171
CSRD247	48	8.000	5.505	1.848	0.729	0.818	0.109
ETH10	60	16.000	5.492	2.216	0.650	0.818	0.205
ILSTS005	57	3.000	1.240	0.408	0.140	0.194	0.276
ILSTS11	47	11.000	3.398	1.666	0.596	0.706	0.156
ILSTS029	60	8.000	1.980	0.974	0.583	0.495	-0.179
ILSTS087	60	17.000	7.895	2.302	0.967	0.873	-0.107
INRA023	48	12.000	5.878	2.046	0.708	0.830	0.146
INRABERNI185	42	4.000	1.477	0.627	0.310	0.323	0.042
INRABERN172	32	9.000	4.983	1.851	0.313	0.799	0.609
MAF65	45	10.000	1.590	0.949	0.200	0.371	0.461
MAF70	59	11.000	4.841	1.866	0.637	0.793	0.210
MCM527	59	6.000	1.902	0.994	0.203	0.473	0.571
OarFCB48	56	8.000	3.158	1.549	0.571	0.683	0.164
OarFCB304	53	15.000	5.739	2.133	0.811	0.826	0.017
SPS113	59	13.000	3.971	1.759	0.898	0.748	-0.201
SRCRSP3	52	5.000	3.414	1.349	0.712	0.707	-0.006
SRCSP9	59	11.000	6.662	2.103	0.778	0.850	0.083
SRYM18	60	19.000	8.295	2.455	0.850	0.879	0.033
TCRVB6	45	13.000	4.776	1.981	0.778	0.791	0.016
OarPC34	33	10.000	7.026	2.079	0.729	0.858	0.081
SRCSP5	55	12.000	5.566	1.975	0.727	0.820	0.113
MAF209	60	4.000	3.259	1.271	0.750	0.693	-0.082
INRA063	52	5.000	2.914	1.210	0.596	0.657	0.092

N=sample size, Na = no. of alleles, Ne= no. of effective alleles, I=Shannon index, Ho = observed heterozygosity, He= expected heterozygosity and F_{is} = fixation index/inbreeding coefficient.

4.2.2 Allelic frequencies, observed and expected heterozygosity and fixation-index (F_{is}) for Red Sokoto goat population.

Table 4.4 shows the number effective alleles (Ne), observed (Ho) and expected heterozygosity (He), Shannon (I) and fixation index (F_{is})using the 25 microsatellite markers for the Red Sokoto goat population. All the primers/markers used for this population were informative, displaying sufficient polymorphism with the number of alleles ranging from 3-17.

The highest value of effective alleles obtained for this population was 9.29 (ILSTS 087) while the least effective allele was 1.336 (INRABERN 185).The observed heterozygosity (Ho) values ranged from 0.299 (INRABERN 185) to 0.983 (SRYM 18), while the expected heterozygosity (He) ranged from 0.251 (INRABERN 185) to 0.892 (ILSTS 087). The Shannon index for this population ranged from 0.482 to 2.392, in most cases, they were higher than 1. The inbreeding index (F_{is}) for this population ranged from -0.018 to 0.666, negative F values were obtained for seven loci (ILSTS 11, ILSTS029, OarFCB 304, SPS 113, SRCRSP 3, SRCSP 9, SRYM 18).

Table 4. 4: Allelic Frequencies, Heterozygosity and F-statistics (F_{is}) in Red Sokot goats

Locus	N	Na	Ne	I	Ho	He	F_{is}
OarFCB20	43	8.000	4.007	1.625	0.488	0.750	0.349
BM6444	57	16.000	5.891	2.178	0.807	0.830	0.028
CSRD247	56	8.000	6.131	1.907	0.750	0.837	0.104
ETH10	60	14.000	4.511	1.933	0.533	0.778	0.315
ILSTS005	60	5.000	1.343	0.557	0.250	0.255	0.021
ILSTS11	57	10.000	2.897	1.511	0.667	0.655	-0.018
ILSTS029	60	4.000	2.035	0.898	0.583	0.509	-0.147
ILSTS087	60	14.000	9.290	2.392	0.867	0.892	0.029
INRA023	56	11.000	5.412	1.955	0.750	0.815	0.080
INRABERNI185	35	3.000	1.336	0.482	0.229	0.251	0.091
INRABERN172	54	12.000	4.780	1.933	0.426	0.791	0.461
MAF65	31	17.000	4.3888	2.140	0.258	0.772	0.666
MAF70	56	10.000	3.831	1.639	0.714	0.739	0.033
MCM527	60	6.000	2.446	1.179	0.367	0.591	0.380
OarFCB48	60	12.000	4.330	1.753	0.733	0.769	0.046
OarFCB304	60	13.000	5.586	1.973	0.850	0.821	-0.046
SPS113	60	13.000	3.329	1.536	0.950	0.700	-0.035
SRCRSP3	59	6.000	2.596	1.175	0.627	0.615	-0.020
SRCSP9	57	11.000	4.570	1.770	0.912	0.781	-0.168
SRYM18	60	14.000	6.040	2.124	0.983	0.834	-0.178
TCRVB6	53	11.000	6.242	2.046	0.736	0.840	0.124
OarPC34	58	13.000	6.451	2.082	0.810	0.845	0.041
SRCSP5	60	9.000	5.169	1.809	0.767	0.807	0.049
MAF209	60	5.000	2.915	1.221	0.533	0.657	0.188
INRA063	60	4.000	2.266	1.059	0.500	0.559	0.105

N=sample size, Na = no. of alleles, Ne = no. of effective alleles, I=Shannon index, Ho = observed heterozygosity, He = expected heterozygosity and F_{is} = fixation index/inbreeding coefficient.

4.2.3 Allelic Frequencies observed and expected heterozygosity and fixation-index (F_{is}) for Kano Brown goat population.

The number of effective alleles (Ne), observed (Ho) and expected heterozygosity (He) as well as fixation index (F_{is}) for Kano Brown goat population are revealed in Table 4.5. The number of available alleles ranged from 2-14. The highest value obtained for the effective allele was 8.989 (ILSTS 087) while the least was 1.133 (ILSTS 005). The observed heterozygosity (Ho) ranged from 0.125 (ILST 005) to 1.000 (SPS 113 and SRYM 18), while the expected heterozygosity (He) ranged from 0.117 (ILSTS 005) to 0.889 (ILST 087 and MAF 65). Most of the primer/markers used for this population were informative and displayed sufficient polymorphism in markers with the exception of ILSTS 005 and INRABERN 185 which had allele numbers of 2 and 3 respectively.

The Shannon index (I) for this population ranged from 0.234 to 2.395. The ILSTS005 marker had low Shannon index (0.234) and number of alleles, where as the inbreeding index (F_{is}) ranged between (-0.012 to 0.444) and were negative at eleven loci for this population (BM6444, ILSTS005, ILSTS11, ILSTS029, ILSTS087, INRABERN185, OarFCB304, SPS113, SRYM18, SRCSP9, TCRVB6).

Table 4. 5: Allelic Frequencies, Heterozygosity and F-statistics (F_{is}) in Kano Brown goats.

Locus	N	Na	Ne	I	Ho	He	F_{is}
OarFCB20	14	5.000	4.170	1.509	0.429	0.760	0.436
BM6444	20	8.000	2.676	1.331	0.750	0.626	-0.198
CSRD247	19	9.000	5.870	1.933	0.789	0.830	0.048
ETH10	20	13.000	7.143	2.235	0.650	0.860	0.244
ILSTS005	16	2.000	1.133	0.234	0.125	0.117	-0.067
ILSTS11	18	8.000	2.521	1.307	0.722	0.603	-0.197
ILSTS029	20	4.000	1.891	0.899	0.500	0.471	-0.061
ILSTS087	20	14.000	8.989	2.394	0.950	0.889	-0.069
INRA023	16	8.000	4.876	1.793	0.750	0.795	0.057
INRABERNI185	13	3.000	1.807	0.733	0.615	0.447	-0.377
INRABERN172	17	11.000	6.494	2.104	0.471	0.846	0.444
MAF65	8	9.000	8.000	2.133	0.750	0.889	0.143
MAF70	18	6.000	3.447	1.448	0.611	0.710	0.143
MCM527	20	4.000	2.432	1.074	0.500	0.589	0.151
OarFCB48	20	9.000	6.612	2.005	0.650	0.849	0.234
OarFCB304	20	10.000	6.250	2.048	0.850	0.840	-0.012
SPS113	20	8.000	3.774	1.586	1.000	0.735	-0.361
SRCRSP3	17	5.000	3.524	1.352	0.647	0.716	0.097
SRCSP9	20	7.000	4.348	1.622	0.800	0.770	-0.039
SRYM18	20	12.000	7.767	2.220	1.000	0.871	-0.148
TCRVB6	12	7.000	5.143	1.772	0.833	0.806	-0.039
OarPC34	14	7.000	4.840	1.729	0.643	0.793	0.190
SRCSP5	19	10.000	6.067	1.972	0.737	0.835	0.118
MAF209	20	6.000	3.941	1.530	0.600	0.746	0.196
INRA063	17	5.000	2.861	1.209	0.588	0.651	0.096

N=sample size, Na= no. of alleles, Ne = no. of effective alleles, I = Shannon index, Ho = observed heterozygosity, He = expected heterozygosity and F_{is} = fixation index/inbreeding coefficient.

4.2.4 Allelic frequencies, observed (Ho) and expected heterozygosities (He) and fixation-index (F_{is}) for West African Dwarf goat population.

The number of effective alleles, observed and expected heterozygosity and fixation index for the West African Dwarf goat population are shown in Table 4.6. The markers used in this population were highly informative with allelic numbers ranging from 3 for ILSTS005 to 19 for BM6444. The highest value obtained for the effective alleles in this population was 8.571 (SRCSP 9) while the least was 1.116 (ILSTS 005). The observed heterozygosities (Ho) ranged from 0.027 (MAF 65) to 1.000 (SPS 113), while the expected heterozygosity (He) ranged from 0.104 (ILSTS 005) to 0.883 (SRCSP 9).The Shannon index (I) ranged from 0.236 to 2.429 and in most cases were higher than 1.0.

The inbreeding index (F_{is}) was negative at six (6) loci (ILSTS029, ILSTS087, SPS113, SRYM18, OarFCB34 and MAF209) displaying some level of out-breeding at such loci and was positive (inbred) for the remaining loci ranging from 0.033 (OarFCB48) to 0.854 (INRABERN172). In all the goat populations the marker ILSTS005 was observed to have low allelic numbers (below 4).

Table 4. 6: Allelic Frequencies, Heterozygosity and F-statistics (F_{is}) in West African Dwarf goats.

Locus	N	Na	Ne	I	Ho	He	F_{is}
OarFCB20	32	7.000	4.188	1.657	0.594	0.761	0.220
BM6444	60	19.000	8.036	2.429	0.783	0.876	0.105
CSRD247	56	8.000	5.733	1.876	0.732	0.826	0.113
ETH10	60	15.000	4.417	1.948	0.533	0.774	0.311
ILSTS005	55	3.000	1.116	0.236	0.073	0.104	0.300
ILSTS11	43	12.000	3.365	1.705	0.651	0.703	0.073
ILSTS029	60	11.000	2.601	1.380	0.683	0.616	-0.110
ILSTS087	60	14.000	8.072	2.227	0.900	0.876	-0.027
INRA023	41	10.000	5.086	1.837	0.634	0.803	0.211
INRABERNI185	36	5.000	1.468	0.701	0.250	0.319	0.215
INRABERN172	25	9.000	5.580	1.950	0.120	0.821	0.854
MAF65	37	4.000	1.214	0.403	0.027	0.176	0.847
MAF70	59	9.000	4.284	1.711	0.746	0.767	0.027
MCM527	60	5.000	2.374	1.171	0.267	0.579	0.539
OarFCB48	60	11.000	3.411	1.598	0.683	0.707	0.033
OarFCB304	59	13.000	3.920	1.756	0.644	0.745	0.135
SPS113	60	10.000	2.684	1.264	1.000	0.627	-0.594
SRCRSP3	60	5.000	3.216	1.294	0.550	0.689	0.202
SRCSP9	58	10.000	8.571	2.207	0.707	0.883	0.200
SRYM18	60	16.000	4.672	2.021	0.967	0.786	-0.230
TCRVB6	44	9.000	2.076	1.235	0.386	0.518	0.255
OarPC34	48	9.000	5.075	1.841	0.813	0.803	-0.012
SRCSP5	59	12.000	6.574	2.046	0.678	0.848	0.200
MAF209	60	6.000	4.162	1.480	0.767	0.760	-0.009
INRA063	58	6.000	2.530	1.176	0.414	0.605	0.316

N=sample size, Na= no. of alleles, Ne= no. of effective alleles, I= Shannon index, Ho = observed heterozygosity, He= expected heterozygosity and F_{is} = fixation index/inbreeding coefficient.

4.3 Polymorphism in Microsatellite Markers from the Nigerian Goat Breeds.

4.3.1 Means and standard errors of the allelic frequencies, Shannon index and heterozygosities for each population.

The means and standard errors of the different alleles, effective alleles, observed and expected heterozygosities for each population are shown in Table 4.7.For the Sahel goat population, the values obtained for the mean number of different alleles, effective alleles, Shannon index, observed and expected heterozygosities as well as fixation index (F_{is}) were 10.00±0.85, 4.37±0.40, 1.64±0.11, 0.61±0.05, 0.70±0.04, and 0.14±0.04, respectively.

For the Red Sokoto population, the mean number of different alleles, effective alleles, Shannon index, observed and expected heterozygosities were 9.96 ± 0.80, 4.31±0.38, 1.635±0.10, 0.64±0.04 and 0.71±0.03, respectively. The fixation index (F_{is}) for this population was 0.09±0.044. In Kano Brown population, the mean number of different alleles was 7.60±0.61, while that of the effective alleles was 4.66 ±0.43. The mean observed and expected heterozygosities for this population were 0.68 ± 0.04 and 0.72 ± 0.04 respectively and the fixation index was 0.04±0.04.

For the West African Dwarf goat, the mean number of different alleles was 9.52±0.80, while the mean effective allele was 4.18 ± 0.42. The values obtained for the observed and expected heterozygosities were 0.58±0.05 and 0.68±0.04 and the fixation index (F_{is}) was 0.17±0.06. The overall means for the entire goat populations are shown in Table 4.8. The means of the different alleles and effective alleles were 9.27±0.39 and 4.38±0.20 respectively. The overall means for the observed and expected heterozygosities were

0.61±0.02 and 0.70±0.02 respectively while the fixation index was 0.11±0.024. In all cases, the average observed heterozygosities were lower than the expected heterozygosities under Hardy-Weinberg Equilibrium (HWE).

4.3.2 Mean observed (Ho) and expected (He) heterozygosity for the entire goat populations.

Table 4.8 shows the Mean Observed (Ho) and Expected (He) Heterozygosity for the entire goat population using the 25 microsatellites. The mean maximum observed heterozygosity was (0.962) at locus SPS 113 while the mean minimum observed heterozygosity was (0.147) at locus ILSTS 005. The maximum expected heterozygosity was 0.883 for locus (ILSTS 087), while the minimum expected heterozygosity was 0.168 recorded at locus ILSTS 005.The mean observed heterozygosity was lower than the expected heterozygosity in twenty (20) loci.

Table 4. 7: Grand Mean and Standard Error over Loci and Populations

Population		N	Na	Ne	I	Ho	He	F_{it}
Sahel	**Mean**	51.920	10.000	4.366	1.642	0.609	0.700	0.143
	SE	1.726	0.845	0.397	0.108	0.047	0.037	0.044
Red Sokoto	**Mean**	55.680	9.960	4.312	1.635	0.644	0.708	0.087
	SE	1.556	0.799	0.375	0.102	0.044	0.034	0.044
Kano Brown	**Mean**	17.520	7.600	4.663	1.607	0.678	0.721	0.041
	SE	0.638	0.611	0.425	0.104	0.038	0.035	0.041
West African Dwarf	**Mean**	52.400	9.520	4.177	1.568	0.584	0.679	0.167
	SE	2.149	0.796	0.417	0.110	0.054	0.041	0.059
Total	**Mean**	44.380	9.270	4.380	1.613	0.613	0.702	0.110
	SE	1.756	0.391	0.200	0.052	0.023	0.018	0.024

N=sample size, Na= no. of alleles, Ne= no. of effective alleles, I= Shannon index, Ho=observed heterozygosity, He= expected heterozygosity and F_{it} = fixation index.

Table 4. 8: Heterozygosity Estimates across the goat populations.

Locus	Ht	Mean He	Mean Ho
OarFCB20	0.797	0.745	0.448
BM6444	0.819	0.781	0.749
CSRD247	0.835	0.828	0.750
ETH10	0.821	0.807	0.592
ILSTS005	0.170	0.168	0.147
ILSTS11	0.674	0.667	0.659
ILSTS029	0.529	0.523	0.588
ILSTS087	0.890	0.883	0.921
INRA023	0.817	0.811	0.711
INRABERN185	0.345	0.335	0.351
INRABERN172	0.833	0.814	0.332
MAF65	0.661	0.549	0.309
MAF70	0.761	0.752	0.675
MCM527	0.566	0.558	0.334
OarFCB48	0.786	0.752	0.660
OarFCB304	0.820	0.808	0.789
SPS113	0.711	0.703	0.962
SRCRSP3	0.693	0.682	0.634
SRCSP9	0.847	0.821	0.800
SRYM18	0.861	0.843	0.950
TCRVB6	0.780	0.739	0.683
OarPC34	0.838	0.825	0.763
SRCSP5	0.839	0.827	0.727
MAF209	0.727	0.714	0.663
INRA063	0.665	0.618	0.525

Ht = total heterozygosity, He= mean expected heterozygosity. Ho= mean observed heterozygosity,

4.4.0 Genetic diversity Within and Between the Nigerian goat breeds.

4.4.1 F- Statistics (F_{is}, F_{it} F_{st}), and estimates of gene flow (Nm) across the goat population.

The F-statistics (F_{is}) and estimates of gene-flow across the goat populations are revealed in Table 4.9. The F_{is} value for the entire population using the 25 markers ranged from -0.043 to 0.592. The F_{is} values were negative for five (5) loci (ILSTS029, ILSTS087, INRABERN 185, SPS 113 and SRYM 18) while the remaining loci revealed positive F_{is} values ranging from 0.012 at ILSTS11 to 0.592 at INRABERN172. The mean F_{is} value was 0.105 ± 0.040. The F_{it} value ranged from -0.016 to 0.601 and a mean of 0.129 ± 0.041, whereas the F_{st} value across the population ranged from 0.007 at INRA023 to 0.169 at MAF65 with a mean value of 0.03 ± 0.07. Gene flow (Nm) ranged from 1.226 for MAF65 to 35.598 for INRA023 with a mean value of 14.868 ± 1.799.

4.4.2 Gene-diversity and Polymorphic Information Content (PIC).

Table 4.10 shows the gene diversity and polymorphic information content for the entire goat population. The gene diversity for the entire goat population ranged from 0.216 at ILSTS005 to 0.878 for CSRD 247 with a mean of 0.73 while the Polymorphic information values, ranged from 0.208 (ILSTS005) to 0.866 (CSRD 247) with a mean of 0.71.

4.4.3 Hardy -Weinberg Equilibrium (HWE) for the Nigerian goat populations.

Table 4.11 reveals the average observed and expected heterozygosities of the different goat populations as well as their Hardy-Weinberg equilibrium values. The average expected heterozygosity was lowest in West African Dwarf goats (0.679), while the Kano Brown goats had the highest expected heterozygosity (0.721). Similarly Kano Brown goats had the

highest observed heterozygosity than all the other three goat breeds. In all cases, the average observed heterozygosities were lower than the expected heterozygosities and all populations showed significant deviations ($P<0.01$) from Hardy-Weinberg expectations.

Table 4. 9:F-Statistics and Estimates of Gene flow (Nm) over All Populations

Locus	F_{is}	F_{it}	F_{st}	Nm
OarFCB20	0.398	0.438	0.066	3.562
BM6444	0.041	0.085	0.047	5.115
CSRD247	0.094	0.102	0.009	26.605
ETH10	0.267	0.279	0.016	15.229
ILSTS005	0.123	0.135	0.014	17.639
ILSTS11	0.012	0.022	0.010	24.477
ILSTS029	-0.124	-0.111	0.012	21.108
ILSTS087	-0.043	-0.035	0.008	31.383
ILSTS023	0.124	0.130	0.007	35.598
INRABERN185	-0.047	-0.016	0.030	8.036
INRABERN 172	0.592	0.601	0.023	10.823
MAF65	0.437	0.533	0.169	1.226
MAF70	0.103	0.113	0.011	21.863
MCM527	0.401	0.410	0.014	17.220
OarFCB48	0.123	0.161	0.043	5.518
OarFCB304	0.024	0.038	0.015	16.862
SPS113	-0.369	-0.353	0.012	20.424
SRCRSP3	0.070	0.086	0.017	14.688
SRCSP9	0.026	0.056	0.031	7.875
SRYM18	-0.127	-0.104	0.021	11.709
TCRVB6	0.075	0.124	0.053	4.479
OarPC34	0.074	0.089	0.016	15.249
SRCSP5	0.121	0.133	0.014	17.938
MAF209	0.072	0.089	0.018	13.848
INRA063	0.151	0.212	0.072	3.230
Means	0.105	0.129	0.030	14.868
SE	0.040	0.041	0.07	1.799

F_{is} = within population inbreeding, F_{it} = total inbreeding estimate, F_{st} = measure of population differentiation, Nm = gene flo

Table 4. 10: Gene-diversity and Polymorphic Information Content (PIC).

Marker	Gene Diversity	PIC
OarFCB20	0.7980	0.7723
BM6444	0.7424	0.7215
CSRD247	0.8781	0.8662
ETH10	0.8726	0.8616
ILSTS005	0.2159	0.2083
ILSTS11	0.7615	0.7264
ILSTS029	0.6503	0.6355
ILSTS087	0.8698	0.8570
INRA023	0.8199	0.7967
INRABERN185	0.5246	0.5032
INRABERN172	0.7192	0.6928
MAF65	0.5924	0.5524
MAF70	0.7749	0.7418
MCM527	0.7842	0.7587
OarFCB48	0.7349	0.6932
OarFCB304	0.5742	0.5518
SPS113	0.8176	0.7989
SRCRSP3	0.7024	0.6623
SRCSP9	0.8719	0.8592
SRYM18	0.8072	0.7853
TCRVB6	0.7324	0.7074
OarPC34	0.8185	0.7965
SRCSP5	0.7410	0.7056
MAF209	0.7793	0.7452
Mean	0.7327	0.7083

Table 4. 11: Hardy -Weinberg Equilibrium for the Nigerian goat population.

Populations	**Ho**	**He**	**HWE**	**α (0.01)**
Red Sokoto	0.644	0.708	0.0058	**
Kano Brown	0.678	0.721	0.0026	**
Sahel	0.609	0.700	0.0118	**
West African Dwarf	0.568	0.679	0.0133	**

** = highly significant at (P<0.01),

4.4.4 Population genetic differentiation (F_{st})

The pair-wise genetic differentiation values (F_{st}) are revealed in Table 4.12. The values ranged from 0.011 to 0.037. The least genetically different population was between Kano Brown and Red Sokoto (0.011), while the highest genetically different population was between West African Dwarf and Red Sokoto (0.037).

4.4.5 Gene flow (Nm) between populations

Table 4.13 shows the gene flow (Nm) between the Nigerian goat populations. The gene flow (Nm) represents the number of migrants exchanged per generation. It ranged from 0.31 to 13.59. The highest gene flow was observed between Red Sokoto and Kano Brown population (13.59) while the least was between Sahel and West African Dwarf (0.31).

4.4.6 Genetic distance

Table 4.14 shows the pair-wise population matrix of Nei genetic distance between the goat populations. The shortest distance (0.02) was observed between Red Sokoto and Kano Brown followed by that between Red Sokoto and Sahel (0.04). However, the farthest distance was obtained between West-African dwarf and Kano Brown (0.121).

4.4.7 Analysis of Molecular Variance (AMOVA).

In order to understand the partitioning of the level of genetic diversity of the Nigerian goats, an analysis of variance was conducted as shown in Table 4.15. The results revealed that 96% of the total genetic variability occurred within individuals in the population, while 4% of them occurred amongst the populations.

Table 4. 12: Gene differentiation (F_{st}) Values.

Populations	RS	KB	SH	WD
RS				
KB	0.011			
SH	0.026	0.019		
WD	0.037	0.036	0.017	

RS= Red Sokoto, KB = Kano Brown, SH = Sahel, WD = West-African Dwarf.

Table 4. 13: Gene Flow between Populations.

Population 1	Population 2	Gene Flow (Nm)
Red Sokoto	Kano Brown	13.59
Red Sokoto	Sahel	5.78
Kano Brown	Sahel	8.26
Red Sokoto	West African Dwarf	3.96.
Kano Brown	West African Dwarf	4.21
Sahel	West African Dwarf	0.31

Table 4. 14: Genetic Distance

Population	Red-Sokoto (RS)	Kano-Brown (KB)	Sahel (SH)	West-African Dwarf (WD)
RS				
KB	0.02			
SH	0.04	0.06		
WD	0.09	0.12	0.05	

Table 4. 15: AMOVA of Populations.

Source	Df	SS	MS	Est. Var.	%
Among populations	3	205.09	68.36	0.95	4
Within populations	196	4437.47	22.64	22.64	96
Total	199	4642.56		23.64	100

4.5.0 Genetic Relationships between the Nigerian Goat Populations

4.5.1 Principal Component Analysis (PCA).

The results of the PCA of the allele frequency data of the Nigerian indigenous goat populations using 25 microsatellite markers are shown in Figure 4.1. The variation accounted for by PC1 and PC2 was 31.04% and 19.06% respectively which adds up to 50.1%. This result shows that the Nigerian indigenous breeds are admixed and there are no distinct clusters on the basis of geographical regions where the breeds were sampled. However, a closer observation revealed that Red Sokoto and Kano Brown were more clustered while Sahel and West African Dwarf had closer clustering.

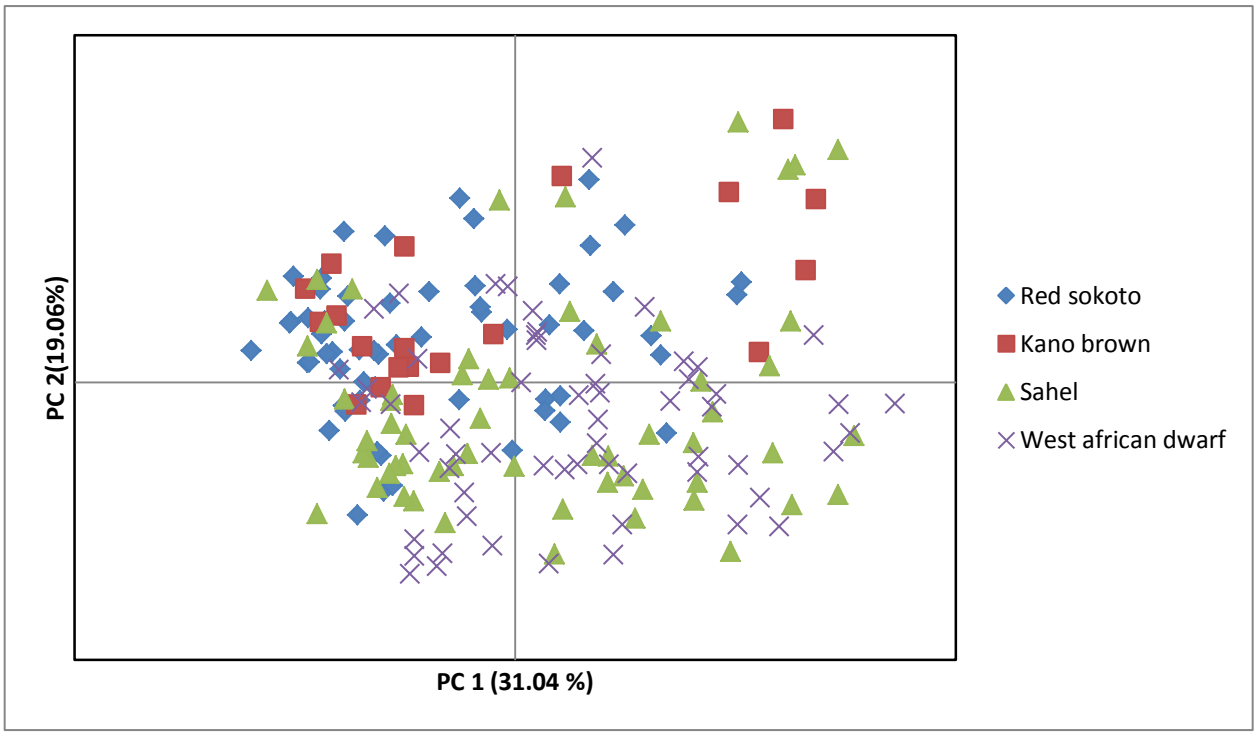


Figure 4.1:Two-dimensional graph representing the relationship between PC1 and PC2 using allele frequency data from 25 micro-satellite loci typed in 4 Nigerian goat populations.

4.5.2 Dendogram of relationships between the four goat populations.

The dendogram or un-weighted pair group mean analysis (UPGMA) tree of the Nigerian goat microsatellite DNA sequences is shown in Figure 4.2. The tree shows that all the breeds originated from a common source/ancestry. However, West- African Dwarf diverged first and is more genetically distinct. This was followed by Sahel and lastly Red Sokoto and Kano Brown goats, although separated are more genetically closely related.

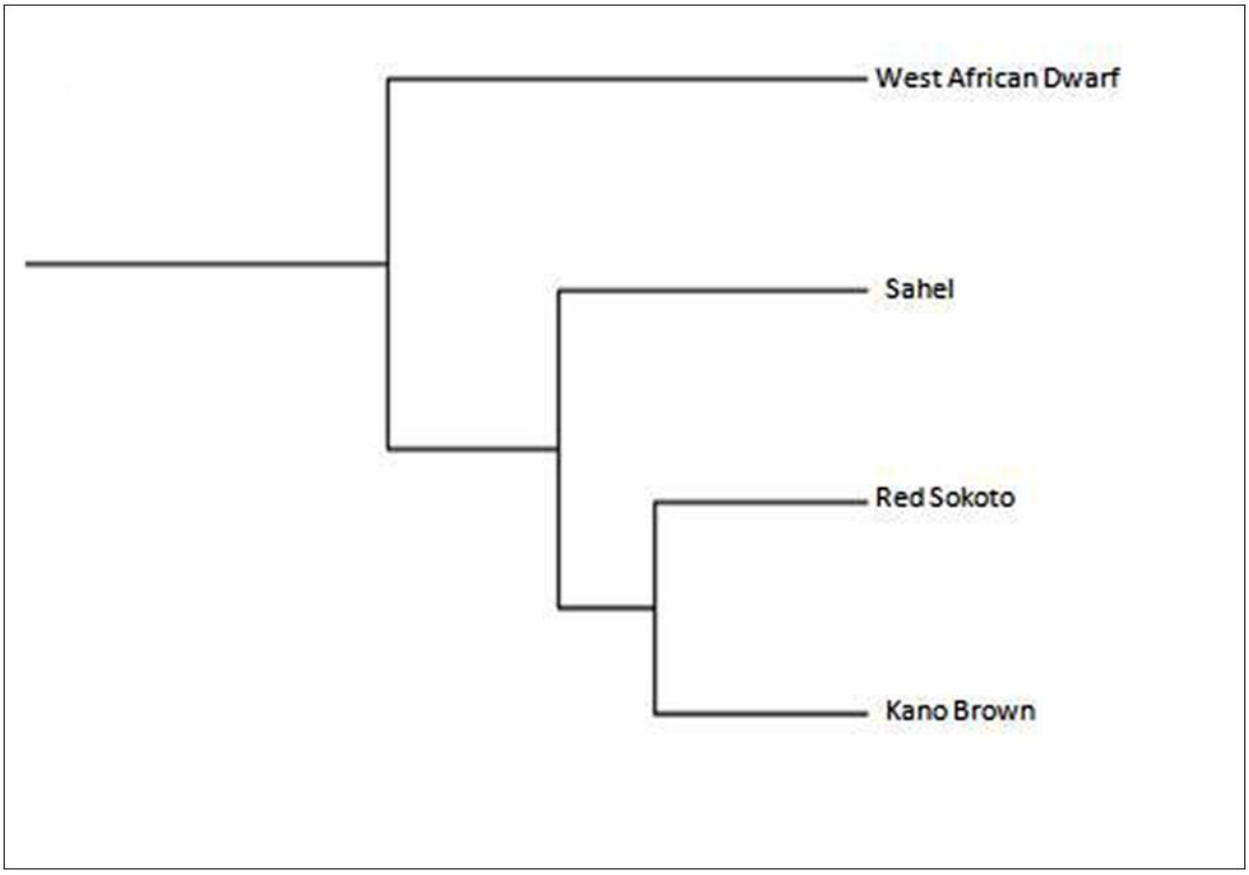


Figure 4. 2: Dendogram representing the genetic relationship among the four Nigerian goat populations.

4.5.3 Evano graph for Nigerian goat population structure.

An evano graph in Figure 4.3 was plotted to detect the genetic structure of the Nigerian indigenous goat populations, as well as to determine the true number of clusters (k) for this population, the value with highest mode (delta k) is usually an indication of the true number of clusters. From the graph below, the variability among the population varied from k = 2 to k =5. The highest likelihood was observed at k = 2, implying that the Nigerian indigenous breeds have two major locations in the country.

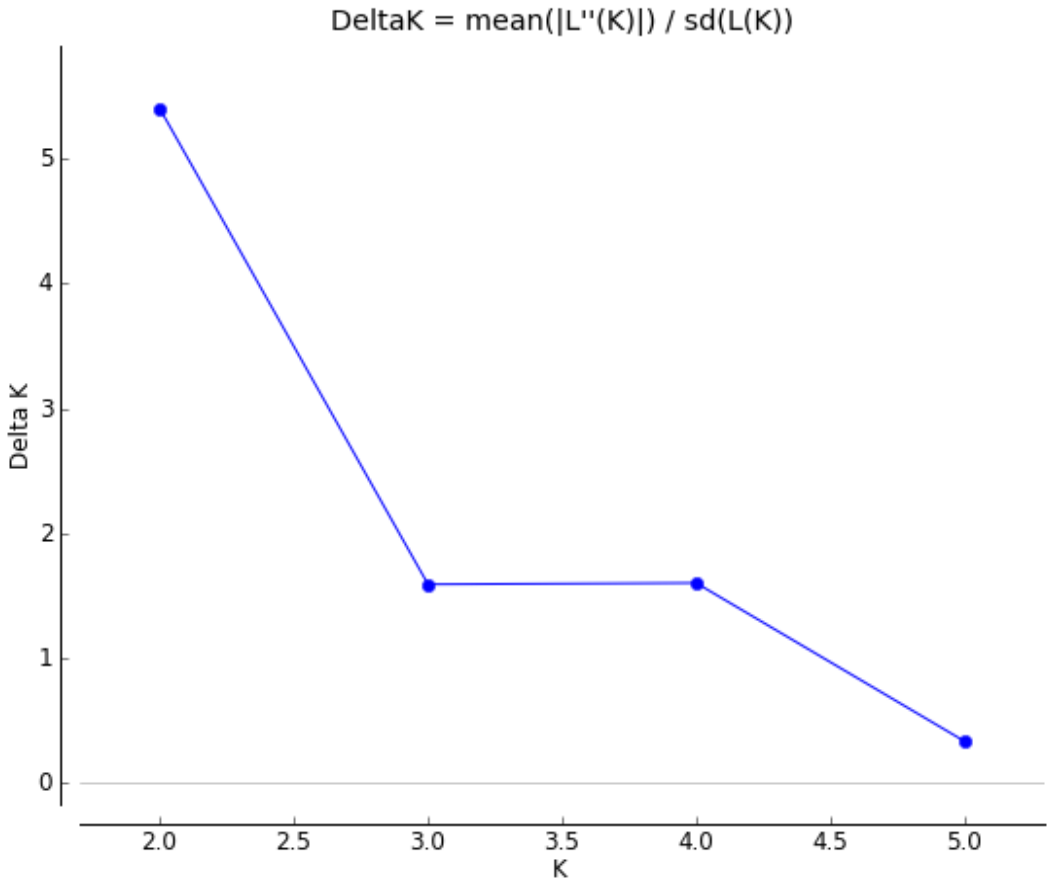


Figure 4. 3: Evano graph of Nigerian goat population.

4.5.4 Estimated population structure among the Nigerian Goat Breeds.

The estimated population structure among individual breeds is revealed in Figure 4.4. To examine relatedness among breeds, allelic frequencies were analyzed using Structure 2.0. Each individual was represented by a vertical line, often partitioned into coloured segments with the length of each segment representing the proportion of the individual's genome from K=2, to K= 6. The first level of clustering (K=2) reflected the primary locations of the Nigerian indigenous goats namely Northern and Southern Nigeria. There was a distinct cluster at this level, which is consistent with the known locations of the goat breeds in Nigeria. Subsequent levels (K=3 to K=6) clearly showed signatures of admixture of the goats used in this study.

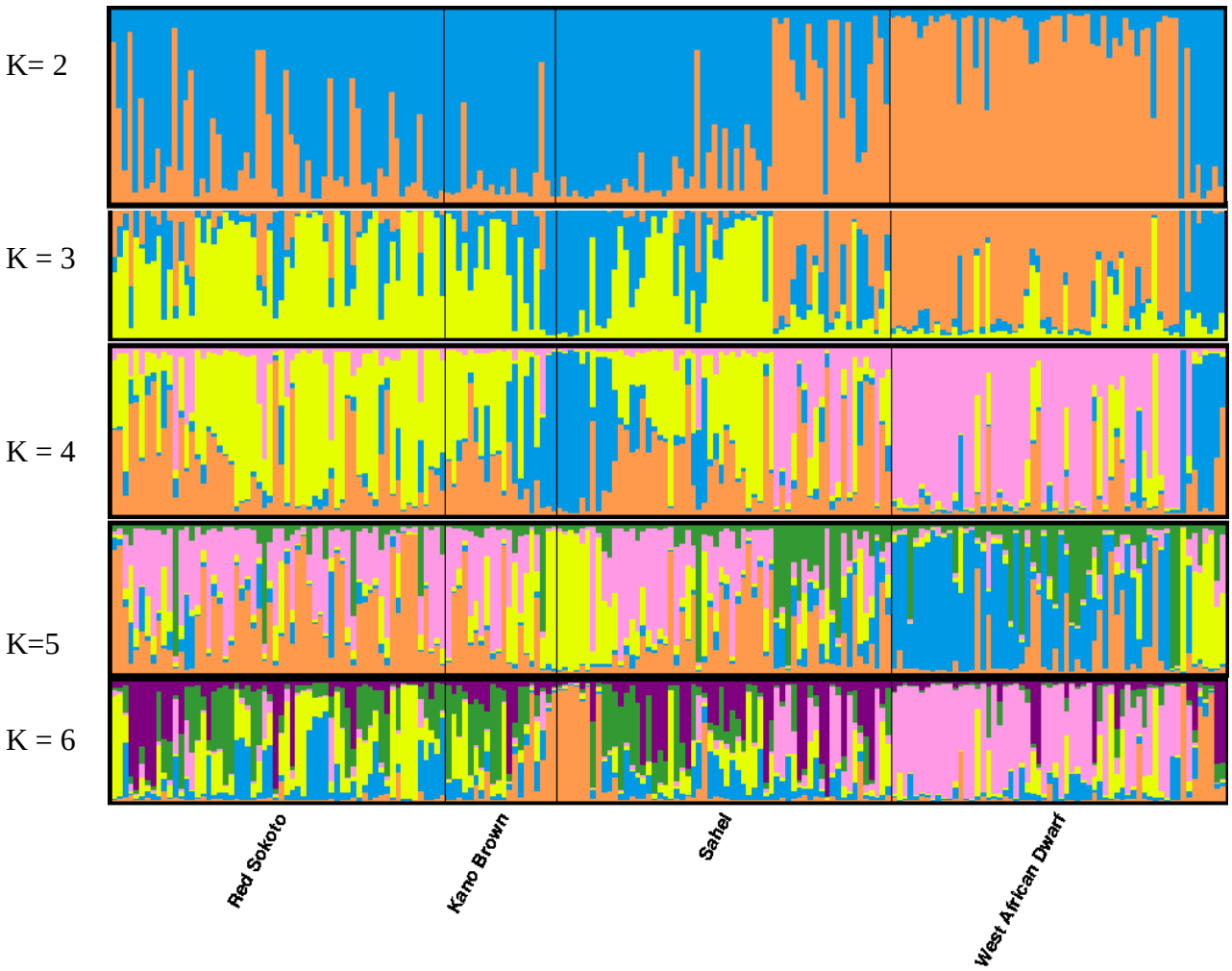


Figure 4. 4 : Estimated population structure of the Nigerian goat population.

CHAPTER FIVE

5.0 DICUSSION

5.1 Phenotypic Characterization of the Nigerian Goat Breeds.

5.1.1 Descriptive statistics of the phenotypic traits of the Nigerian indigenous goat breeds.

The significant differences observed among the Nigerian goat breeds suggest a direct relationship between the extent of morphometric divergence and geographical separation. This is in accordance with the results of Brown *et al*. (2006) that temporal and spatial variation in weather, plant productivity and subsequent nutrition could have major impacts on physical growth of animals.

The estimates obtained for back length in Red Sokoto goats was higher than those obtained by Okpeku *et al*. (2011b) for the same breed, however, for height at withers in Red Sokoto goats, similar results were obtained by Hassan and Ciroma (1992) and Okpeku *et al.* (2011b) but lower than those of Akpa *et al.* (2009), this may have resulted from differences in sampled animals. For the chest girth in Red Sokoto and Sahel, lower estimates were obtained as compared to those of Yakubu *et al*. (2010). The varying coefficients of variation as well as variance could be attributed to breed differences, influence of environmental factors on the measured parameters especially the width measurements.

5.1.2 Phenotypic Correlations for the linear body measurements for the Nigerian indigenous goat breeds.

The significant, high and positive relationships between the measured traits in Red Sokoto, Sahel and West African Dwarf obtained in the present study indicates that an increase in any one of these traits would result to a corresponding increase in others, as such resulting to in an increase in body size.

Okpeku *et al*. (2011b) also reported high, positive and significant relationships between body weight, withers height, neck-length, body- length and heart girth in West African Dwarf and Red Sokoto goats. Similarly, the correlation results obtained in this study are comparable to the range of values obtained by earlier reports of Adeyinka and Mohammed (2006) for Red Sokoto and Sahel goats, Ojedapo *et al*. (2007), Yakubu (2009), Sowande *et al*. (2010) and Ogah *et al*. (2013) for West African Dwarf goats.

For the Kano Brown goats, the low to moderate, positive but significant correlations between the measured traits may have resulted from the small data size (n= 20) used for its computation.

5.2 Allelic frequencies of different Gene Loci in the Nigerian indigenous goat breeds.

5.2.1 F-statistics and allelic frequencies in Sahel goats.

The different measures of genetic variation such as Na, Ne, and I, showed that all of the studied loci were highly informative, indicating high polymorphism across the loci.

The F-statistics in this population was low (0.14) .The negative F_{is} values located at five (5) loci indicated the presences of more heterozygotes at those loci, while the remaining twenty

(20) loci with positive values suggests an heterozygote deficit, and points towards out-breeding at such loci implying an inbred status of the population.

5.2.2 F-statistic (F_{is}) and allelic frequencies in Red Sokoto goats.

The different measures of genetic variation such as Na, Ne, and I, showed that all of the studied loci are highly informative, indicating high polymorphism across the loci.

The F-statistic (F_{is}) was applied to know the extent of inbreeding and out-breeding in the population. The negative F_{is} values obtained at the seven loci suggests the presence of more heterozygotes at the loci and points towards out-breeding (mating of individuals who are less related than the average relationship of the population). However, there were more positive F_{is} values at the remaining eighteen loci indicating inbreeding/homozygous status of the population.

5.2.3 F-statistics and allelic frequencies in Kano Brown goats.

The F-statistics were applied to know the extent of inbreeding and out-breeding in the population. The negative F values located at eleven (11) loci indicates the presence of more heterozygotes at those loci, while the remaining fourteen (14) loci with positive values suggests an heterozygote deficit, implying an inbred status of the population and this may have resulted from the small population size.

5.2.4 F-statistics and allelic frequencies in West African Dwarf goats.

All loci studied were polymorphic having more than four alleles in most cases, implying that these markers are informative for genetic diversity studies in the West African Dwarf.

The negative F_{is} values observed at six (6) loci indicated the presences of more heterozygotes at those loci, while the remaining nineteen (19) loci with positive values suggests an heterozygote deficit, implying an inbred status of the population.

The presence of negative F_{is} values at six loci suggests that homozygous deficiencies may have arisen from population sub-division owing to genetic drift, null alleles and selection against heterozygotes or inbreeding. These negative values have also been reported in other studies on goats (Barker *et al.,* 1997; Luikart *et al.,* 1999; Agha *et al.,* 2008; Rout *et al.,* 2008; Dixit *et al.,* 2009, Rout *et al.* (2012).

5.3 Polymorphism in Microsatellite Markers for the Indigenous goat breeds.

5.3.1 Mean and standard errors for Shannon index, observed and expected heterozygosities and fixation index for the Nigerian goat population.

The different measures of genetic variation such as Na, Ne, and I, showed that all of the studied loci were highly informative, indicating high polymorphism across the loci.

The mean allelic frequency in Nigerian goats of 9.27 is higher than those of Iranian goats (7.3, Mahmoudi *et al*., 2010), and Canary Island goats (5.9) (Martinez *et al.*, 2006), but lower than Spanish Guadarrama goats with 9-36 (Serrano *et al.,* 2009). The mean observed heterozygosity was lower than those of the expected as such implying a deficit in heterozygosity as observed in the F values as well. This observed inbreeding may have resulted from minimal reproductive isolation and larger effects of migration and genetic drift across the entire Nigerian goat population. The Shannon index of the Nigerian goat breeds in this study revealed low species richness and evenness since all the indices were below 3.5, the mark set for high species evenness and richness (Krebs, 1989). This might

not be unconnected with the level of heterozygote deficiency observed among this population, possibly due to the management system (Mukesh *et al*., 2006)

5.4 Genetic diversity within and between the Nigerian goat breeds.

5.4.1 Within population inbreeding estimate (F_{is})

The presence of negative F_{is} values at five (5) loci (ILSTS029, ILSTS087, INRABERN 185, SPS 113 and SRYM 18) suggests heterozygote deficiencies which have also been reported in other studies on goats (Barker *et al*., 1997; Luikart *et al*., 1999; Agha *et al*., 2008; Rout *et al*., 2008; Dixit *et al*., 2009). This heterozygote deficiency may have arisen due to population sub-structure from pooling together different populations (admixture) in the analysis (Cerda-Flores *et al*., 2002; Muema *et al*., 2009). Additional factors include population subdivision owing to genetic drift, null alleles and inbreeding (Hoarau *et al*., 2005).

The average F_{is} inbreeding coefficient for the entire goat population (0.11±0.04) was higher (0.04±0.13) than that obtained by Okpeku *et al*., (2011a) and could be a consequence of management systems (like tethering of the animals in the case of small flock size during crop season, herding of the flock by children and /or women, where the flock size is larger and some localized transhumance during the dry season). Tethering minimizes the number of different bucks that any particular female (doe) can mate with, leading to service by only males preferred by the owner. While women and children herders usually avoid the mixing of different flocks or exchange of bucks from different areas even at the watering points. Localized transhumance also leads to minimal encounters between different flocks. All these systems and events tend to increase the rate of inbreeding.

The relatively low total inbreeding (F_{it} = 0.129) observed for the entire goat population may merely be an indication of gene flow and migration between the Nigerian goat populations. This implies a gradual erosion of desirable traits within the Nigerian goat population and if not controlled, deleterious genes would begin to express themselves with time.

5.4.2 Genetic differentiation (F_{st})

The F_{st} estimate which is commonly called the coefficient of gene differentiation obtained across all the loci implies that genotypic frequencies among the populations were not randomly distributed, meaning that the Nigerian goat populations have some genetic similarity due to gene flow between sampled populations. The mean Fst value across all loci was low (0.03) compared to previous results of Nigerian goats (F_{st}=0.11, Okpeku *et al*., 2011a), Asian goats (F_{st} = 0.14, Barker *et al.,* 2001) and Chinese goats (F_{st} = 0.105, Li *et al*., 2002). However, Mujibi (2005) reported a moderate F_{st} of 0.058 for West African Dwarf goats in Kenya. These variation may have resulted from population and breed differences. According to Laval *et al.* (2000), migration, interbreeding and genetic drift may exert a greater effect on the reduction in genetic differentiation between populations.

5.4.3 Gene Flow (Nm) between populations

The high mean gene flow (14.868) across the breeds suggests mobility and considerable exchange of genetic material among these goats which may have resulted from minimal reproductive isolation and larger effects of migration and genetic drift. These could be attributed to the fact that most of these animals originated from Northern Nigeria where normadic pastoralism is the dominant livestock management system and to the extensive system of management, allowing the animals to roam freely and fend for themselves in

most rural households and communities in the South. This enables and reinforces the ability of related animals to meet on pasture to breed or for neighbours to exchange related animals for upkeep or breeding.

The high gene flow between Red Sokoto and Kano Brown (13.59) may have resulted from minimal reproductive isolation and further supports proximal interbreeding leading to weakly identified phenotypic features and genetic similarities between the neighbouring breeds. Muhammed *et al*. (2010).

However, the low gene flow (3.96) between Red Sokoto and West African Dwarf may have resulted from minimal effects of migration and genetic drift as such, implying a larger reproductive isolation exists between the Red Sokoto and West African Dwarf goats. Distant geographic regions between these breeds may have contributed to this low gene flow (Okpeku *et al*., 2011a).

5.4.4 Gene-diversity and Polymorphic information content (PIC).

The high average gene diversity among the markers is a function of its high number of alleles or allelic polymorphism.

The results obtained for the PIC in the study (0.21 to 0.87) is an indication that all the markers were highly polymorphic and informative for genetic diversity in Nigerian goats with the exception of ILSTS005 which had low PIC value of 0.2083. It also shows that the Nigerian goats possess a wide genetic base that allows for adaptation to a wide variety of ecological environments. These values are in a similar range compared to other studies on goats, which ranged from 0.11 to 0.78 for Marwari goats (Kumar *et al*., 2005), 0.25 to 0.83

in Canary goats (Martinez *et al.*, 2006) and 0.59 to 0.92 in White and Brown short Haired goats (Jandurova *et al*., 2003).

5.4.5 Hardy -Weinberg Equilibrium (HWE) for the Nigerian Goat Populations

The significant deviations from the Hardy -Weinberg Equilibrium for the Nigerian goat populations, implies that there was a deficit of heterozygosity resulting from the non-random union of gametes in the population i.e. the mating among individuals in the population which are more related than the average relationship. Similarly, migration, mutation and selection might have caused these deviations. Deviations from Hardy-Weinberg equilibrium at microsatellite level has been reported in various studies (Barker *et al*., 2001; Hassan *et al*., 2003; Laval *et al*., 2000; Luikart *et al*., 1999).

5.4.6 Between population Genetic differentiations (F_{st})

Per pair F_{st} value of 0.05 indicates moderate differentiation and those lower than 0.05 indicates low differentiation between populations/breeds (Hartl, 1980).The value of genetic differentiation between populations ranged between 0.011-0.037 this may have resulted from gene flow, migration and genetic drift among sampled populations other than mutations among populations, as such, suggesting that the Nigerian goat populations are not genetically distinct but are related to one another. According to Balloux and Lugon-Moulin 2002, the low F_{st} value as obtained in the Nigerian goat populations reflects large number of alleles exchanged between populations which influence the genetic composition of individuals within these populations. Okpeku *et al*. (2011a) also obtained low F_{st} values (0.05-0.19) for Sahel, Red Sokoto and West African Dwarf goats of Nigeria.

5.4.7 Genetic distance.

The close relationship observed between Red Sokoto and Kano Brown may have resulted from gene flow as these animals are in the same geographical location. The genetic distance between Red Sokoto and West African Dwarf goats (0.09) was lower than those of Adebambo (2003) and Okpeku *et al.* (2011a) who obtained 0.39 and 0.27 respectively for the same breeds. This may have resulted from wider geographical locations from which these animals were sampled.

5.4.8 Analysis of Molecular Variance (AMOVA) of the Nigerian goat populations.

Molecular genetic variation was observed to be higher within populations (96%) than among populations (4%). It showed higher heterozygosity within population samples. These results were quite higher than those reported by Okpeku *et al*. (2011a) who obtained (71%) variation within populations and (29%) variation amongst populations of West African Dwarf, Sahel and Red Sokoto goats of Nigeria. According to Toro and Maki-Tanila (2007) the higher genetic diversity observed within population than between groups may have arisen from overlapping generations with natural selection favouring heterozygosity or subdivision accompanied by genetic drift. Agha *et al*. (2008) reported that the effect of these factors is more pronounced when the effective population size is very large, which is supported by the poor infrastructure on ground presently for livestock improvement and lack of proper breeding policy in Nigeria.

5.5 Genetic relationships between the Nigerian goat breeds.

5.5.1 Principal Component Analysis (PCA)

From the results of the principal component analysis there were no clear distinctions/clusters between the Nigerian goat populations implying that many of the sampled animals were not pure breeds but cross-bred. These might have resulted from migration and interbreeding amongst the breeds.

5.5.2 Dendogram of relationships between the four goat populations.

The dendogram separated the four Nigerian goat breeds according to their geographical location in the country. Evidence from the phylogenetic tree supported first divergence of West African Dwarf, followed by Sahel and latter Red Sokoto and Kano Brown. From the dendogram, geographically adjacent populations were observed to be more genetically related as observed in Red Sokoto, Kano Brown and Sahel which are Northern breeds, though Sahel is located at the extreme north. However, West African Dwarf is a Southern breed and was observed to be more genetically distinct.

5.5.3 Evano graph for the Nigerian goat population structure.

The statistic Delta K peaked at K = 2 indicating support for two groups. This implies that the Nigerian indigenous goat breeds originated primarily from two major locations namely; North and Southern Nigeria.

5.5.4 Estimated population structure of the Nigerian goat breeds.

The results of structure analysis indicated that the Nigerian breeds were not genetically differentiated and there were signatures of admixture within the Nigerian goat populations which might have resulted from indiscriminate mating, cross-breeding as well as migration between breeds. Environmental barriers, historical processes and life histories (mating

system) may all, to some extent, shape the genetic structure of populations (Donnelly and Townson, 2000; Gerlach and Musolf, 2000; Palsson, 2000; Tiedemann *et al.*, 2000).

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 Summary

The knowledge of Phenotypic and Genetic variation is essential for the conservation and utilization of farm animal genetic resources, especially indigenous types that are often disregarded due to low genetic production potential compared to commercial breeds. A total number of two hundred (200) individuals from three breeds namely Sahel (60), Red Sokoto (60), West-African Dwarf (60) and one strain; Kano Brown (20) were used for the study. The data analyzed consisted of body measurements; body length, height at withers and chest girth from adult goats as well as DNA samples obtained from tissues of the ears of these animals. The results revealed an average body length of 65.40, 61.58, 52.97 and 59.90 cm in Sahel, Red Sokoto, West African Dwarf and Kano Brown, respectively. The mean height at withers were 63.17, 61.25, 55.88 and 59.20cm in Sahel, Red Sokoto, West African Dwarf and Kano Brown, respectively, while average Chest girth were 65.42, 63.27, 55.87 and 62.65 in Sahel, Red Sokoto, West African Dwarf and Kano Brown, respectively. Sahel goats had higher body measurements than all other breeds, there were no significant differences between the Red Sokoto and Kano Brown breeds, however the West-African Dwarf goats had the lowest values for body length, height at withers and chest girth.

The genetic characterization of the Nigerian indigenous goat populations involved the sampling of two hundred (200) goats, twenty five microsatellites were used to genotype them. The markers were highly polymorphic and proved to be useful in biodiversity studies as twenty three of the markers had four or more alleles in most cases, with the exception of

ILSTS005 in Kano Brown, Sahel and West-African Dwarf goats and INRABERN185 in Kano Brown and Red Sokoto which had alleleic numbers lower than 4.

The mean expected and observed heterozygosity for the goat populations ranged from 0.68 to 0.72 and 0.58 to 0.68 respectively. About 96% of the total genetic variation was due to the genetic differentiation within individuals in each breed, while 4% was due to differences between breeds. The mean F_{is}, F_{it}, F_{st} and Nm values across the population were found to be 0.11, 0.13, 0.03 and 14.87 respectively. Genetic differentiation between the populations was low (0.03), while gene flow (Nm) across the entire population was high (14.87) and might have resulted from non-random mating and migration across populations.

The West African Dwarf breed was distinct, while the Red Sokoto and the Kano Brown breeds showed higher genetic uniformity or similarity as seen in the genetic distance (0.02). Gene flow played an important role in the genetic uniformity in populations of narrow geographical vicinity as seen between Red Sokoto and Kano Brown breeds (13.59). Significant deviations from the Hardy-Weinberg equilibrium were observed in all the populations suggesting that indiscriminate mating might have occurred. The phylogenetic tree displayed a remarkable degree of consistency with their different geographical origins. From the results obtained, it can be suggested that indiscriminate mating was the most important factor contributing to the low genetic differentiation among the analyzed populations.

6.2 Conclusions

- There was a distinct phenotypic difference among the goat populations except for a close relationship that existed between Red Sokoto and Kano Brown goats.
- There was a high, positive and significant correlation between the measured traits (body length, height at withers and chest girth) for all the breeds (r = 0.63 to 0.90) with the exception of Kano Brown goat whose correlations were low (r = 0.24 to 0.38) and not significant.
- Most of the markers used for the genetic characterization proved to be highly polymorphic (4 to 19) with the exception of ILST005 which had allele numbers lower than 4 (2 to 3).
- The inbreeding coefficient was low (F_{is}=0.11) for the entire goat populations.
- High gene flow (14.87) was observed across the breeds.
- Close genetic relationships were observed between the breeds as seen in the genetic distance between Red Sokoto and Kano Brown (0.02), Red Sokoto and Sahel (0.04), Red Sokoto and West African Dwarf (0.09), West-African Dwarf and Sahel (0.05), West African Dwarf and Kano Brown (0.12), Kano Brown and Sahel goat (0.05).
- From the dendogram, a common source of ancestry was observed for the Nigerian goat population
- It was observed that the Nigerian indigenous goats existed predominantly in two major locations; Northern and Sothern Nigeria.
- The genetic differentiation (F_{st}) between populations was low (0.03) and high level of admixture was observed (k = 3 to k = 6) across the goat populations.

6.3 Recommendations

- The results on phenotypic characterization (high, positive and significant correlations among the linear body measurements) could be used for field assessment and management of the Nigerian goat populations, for future selection and breeding improvement strategies.
- Open nucleus breeding for selective advantage of each of the breeds of goats in Nigeria should be carried out.
- Measures to conserve uniqueness/distinctness of the Nigerian goat breeds such as in-situ and ex-situ conservation should be sought.
- Gene flow between breeds should be controlled by adopting improved management (semi-intensive) and breeding practices.

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