Sudan University of Science & Technology
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Phenotypic and Genotypic Characterization of Fuga Cattle of Western Sudan Compared to (Kenana and Butana) Dairy Breeds

الصفات المظهرية والوراثية لأبقار فوجا بغرب السودان ومقارنتها مع سلالتي الحليب (كنانة وبطانة)

Submitted in fulfillment of the requirements for the degree of PhD

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2014

Dedication

This thesis is dedicated to my parents, whose constant support and encouragement made this possible, and also dedicated to my Dear wife, and my Daughter (Saga)

Acknowledgements

First and above of all, I would like to express my great thanks to Allah, for helping me to accomplish this work.

Special thanks to my Supervisor Prof. Dr Abdel Aziz Makkawi, Reproductive physiologist, Faculty of Agricultural science, Department of animal production, Sudan University of science & Technology for all the experience and patience endowed to me.

A lot of thanks are to Dr. Salah-Eldein A. Sid-Ahmed, Co. Supervisor , Dr. Animal Genetics, Faculty of Agricultural science , Department of animal production ,for continuous advise, encouragement and help .

Particular thanks to my external supervisor. Prof. Dr. Ahmed S. Abdoon, Department Animal Reproduction Veterinary Research Division, National Research Center Egypt, for all the help he extended to me.

And thanks to Prof. Dr. Mohammed Saber Hassanane, Professor of Animal Genetics, Cell Biology Department, National Research Center in Egypt, A lot of thanks are to Prof. Dr Omima M.Kandil Department Animal Reproduction Veterinary Research Division, National Research Center Egypt and Dr.Sally.A.Allam. Animal Genetics, Cell Biology Department, National Research Center in Egypt. To all of them, I appreciate their kind attitude and help extended to me during my practical work in their lab.

I would like to thanks all the colleagues who helped me in the blood samples collection.

Finally, I am also grateful to all staff members of Atbara research station for Butana cattle and Um.banein research station for Kenana cattle . and all Owners of Fuga cattle .

I gratefully offer my sincerest thanks to my friends and I would like to thank any one else who helped me.

Abstract

This study aims to investigate the phenotypic and genotypic characterization of Fuga cattle in North Kordufan. A set of detailed structured questionnaires were used to collect information from Fuga cattle owners (150) .The questionnaires were pre-tested to check clarity and appropriateness of the questions. Some of the informations collected during interviews were supported by observations on milk yield per day, milk yield per lactations ,lactation length , age at first calving , Calving interval, number of services per conception ,and weight of birth of Fuga cattle breed were presented (5.26±0.29 kg,1100.96±86.18 kg, 208.60 ± 5.37 days, 40.49 , 15.90± 0.44 months, 2.27±0.7 , and 22.06±0.44kg) respectively. For the genotypic characterization of this study nine bovine microsatellites markers SPS115, TGLA122, TGLA126, TGLA227, ETH10, ETH225, ,,INRA023 BM2113 , BM1824 were analyzed in three different breeds of Sudanese cattle raised in Sudan representing three suspected breeds: Fuga form western Kordufan (Fuga area), Butana Breed for Atbara Research Station, and kenana Breed from Um.Banein Research Station. The purpose of the study was to measure the genetic diversity between these three breeds as well as measuring the purity or the heterogeneity of each breed and establish the relationship amongst three Sudanese zebu cattle breeds using 9 bovine specific microsatellite markers recommended by ISAG (2012) for such studies,Blood samples were collected. The study was performed on a total of 90 unrelated cattle individuals from Fuga; Kenana and Butana .The samples were collected from respective habitats. 30 sample from each breeds were loaded on vacutainer EDTA as an anticoagulant matter, DNA was isolated from the white blood cells, in to Central Laboratory of Ministry of Science and Technology, Sudan. Microsatellite analysis was carried out in Madrid - Spain .One PCR multiplex consists of nine fluorescence-labeled. with specific software. The results were statistically analyzed. Out of the nine markers studied all of them proved to be highly polymorphic : ETH10 ,SPS115,BM2113(9 alleles);BM1824(8alleles);ETH225(10alleles);INERA023(11alleles);TGLA227(13 alleles); TGLA122 (19 alleles) and TGLA126(7 alleles). A total of (74) microsatellite alleles were identified with number of alleles at one locus ranging from 5 to 11 alleles while the average number of observed alleles per locus (8.22). Sharing allele analysis

showed total number of alleles shared between breeds study is 34 alleles, and then alleles shared between Fuga and Kenana (43) higher than alleles shared between Fuag and Butana(40) , Kenana and Butana (36). High values for the average heterozygosity were observed all over the loci and the three breeds studied: Fuga (0.778); Butana (0.737) and Kenana (0.692). Moreover, gene diversity were also high for the 9 microsatellite studied in the three breeds, the average gene diversity over all loci were 0.684, while for individual loci ranged between 0.468 (SPS115) in Fuga breed and 0.885 (TGLA122) in Fuga breed also. Fuga breed displaying somewhat higher levels of genetic polymorphisms compared to Butana and Kenana breeds. The values of polymorphism Information Content (PIC) obtained from the present study were 0.664 in Fuga , 0.630 in Butana and 0.596 in Kenana. The result inbreeding between different breeds and within the breeds itself, indicated that there is no any inbreeding between or within the breeds under study. In addition, a high values for gene flow or migration in our breeds for the nine markers studied with an average value of 4.335. Higher levels of gene flow were observed ranged from (0.814) in SPS115 to (8.754) in ETH10. It can be assumed that migration and admixture could have taken place between these breeds. The Nei's dendrogram or phylogenic relationship gave a primary idea about the possible migration of cattle, migration starts from the northern east part of Sudan to the middle part in north kordufan.Drawing phylogeny tree between the breeds proved that Butana and Kenana are within one cluster while Fuga is in another cluster, the three breeds are then coming from one ancestor. Although admix is occurred due to hybridization with Friesian breed, a high genetic diversity in the three breeds studied, which can be used in designing good programs for genetic improvement in Sudanese zebu cattle.

ملخص الدراسة

هدفت هذه الدراسة لتحديد الصفات المظهرية والوراثية لابقار فوجا في شمال كردفان، لتحديد الصفات المظهرية لأبقار فوجا استخدم الاستبيان والملاحظة في جمع المعلومات عن الصفات المظهرية لعدد 150 من مالكي ومربى الأبقار وقد وجد كل من انتاج الحليب الكلى ، وانتاج الحليب اليومي ، وفترة الإدرار، العمر عند اول ولادة، والفترة بين الولادتين، عدد مرات التلقيح والوزن عند الميلاد وكانت النتائج كالأتى(1100.96±86.18 كيلوجرام ، 5.26±0.29 كيلوجرام ،208.60 ± 5.37 يوم،40.49±6.76 شهر ،15.90±0.44 شهر ،2.27±0.7 و22.06±0.44 كيلوجرام) على التوالى.

وللصفات الوراثية تناولت هذه الدراسة استخدام تسع من واسمات التتابعات الوراثية الدقيقة (Microsatellites) الموصي بها الجمعية الدولية لوراثة الحيوان(ISAG-2012) لمثل هذه الدراسات والمستخلصة من قبل من الأبقار وهى:, ETH10, ETH225, TGLA227, TGLA122, TGLA126, SPS115, INRA023, BM2113 , BM1824 وذلك فى تحليل ثلاث من عشائر الأبقار المحلية السودانية والتى تم تجميعها من المناطق التالية: منطقة شمال كردفان (فوجا) محطة ابحاث عطبرة (سلالة بطانة) محطة ابحاث أم بنين (كنانة) .وقياس الاختلافات الوراثية ومقارنتها مع سلالتى ابقار بطانة وكنانة وقياس نسبة نقاء العشائر ومدى تجانس الأفراد (homozygosity) بداخلها وقياس كمية الخلط الأليلى بها (heterozygosity) علاوة على دراسة درجة القرابة بين هذه السلالات وقياس معدل التدفق الجينى أو معدل الهجرة للأبقار بين المناطق تحت الدراسة .

ولإجراء هذا البحث تم تجميع تسعين عينة دم من أبقار ليس بينها درجة قرابة من المناطق المشار إليها سابقا 30 عينة من كل سلالة، ووضعها على مادة مانعة للتجلط وهى EDTA وتم عزل المادة الوراثية (DNA) الموجودة بكرات الدم البيضاء بالمعمل المركزي بوزارة العلوم والتقانة بالسودان، وقد أجريت التجارب لتفاعل إنزيم البلمرة بإستخدام(One PCR multiplex consists of nine) في اسبانيا – مدريد ، حوي واسمات التتابعات الدقيقة المختلفة في وجود مادة وراثية معلومة الطول (DNA size marker) لذلك ثم إدخالها على برنامج كمبيوتر متخصص لتحليلها و تحديد أطوال الاليلات لهذه الحيوانات. وبعد الحصول على قياسات هذه الأطوال تم جدولة النتائج وتحليلها إحصائيا باستخدام برامج كمبيوتر متخصصة فى مجال وراثة العشائر. ومن خلال النتائج تم التعرف على عدد (74) الاليلات الجينية الموجودة بـ(9) من الواسمات الوراثية كما تم التعرف على الإليلات بكل سلالة على حدة وبالسلا لات كلها. كما تم أيضا حساب التكرارات الاليلية لكل واسم فى كل سلالة على حدة علاوة على السلالات ككل.

هذا وقد وجد من خلال تحليل النتائج أن تسع من واسمات التتابعات الدقيقة المستخدمة ، قد أظهرت نتائج عالية فى تعدد الأشكال المظهرية (polymorphism) لها حيث وجد أن كل من SPS115, ETH10, BM2113 تحوى تسع أليل، واظهر BM1824, ثمانى أليلات، و الواسم ETH225 حوي 10 اليل ، و INRA023 حوي 11 اليل والواسم TGLA227 حوي 13 أليل ، والواسم TGLA 122 حوي 19 من الأليلات بينما أظهر الواسم TGLA126 سبع أليلات فقط. وقد وجد أن بعضا من هذه الاليلات موجودة فى بعض من سلالات الأبقار الآسيوية و الأوربية والأفريقية. وعليه فانه يمكن استخدام هذه الواسمات فى اختبار

معرفة الأبوية أو النسب وبالإضافة إلى ذلك يمكن استخدامها فى التعرف على المواقع الجينية المرتبطة بالصفات الإنتاجية الكمية واستخدمها فى برامج التربية الحديثة والتى تسمى بالانتخاب بمساعدة الواسمات.وقد وجد من خلال إحصائية نتائج هذه الدراسةأن متوسط عدد الاليلات المشاهدة هو 8.22 أليلات لكل واسمة. ولم يظهر اختبار المشاركة الاليلية (allele sharing) وجود أى ارتباط لأليل معين مع منطقة معينة. وهذا أول مؤشر يدل على أن عشائر الأبقار السودانية المأخوذة من مناطق مختلفة التى كانت تحت الدراسة تنحدر كلها من سلف واحدة ، كما وجد أن عدد الإليلات المشتركة بين السلالات 34 أليل بينما عدد الإليلات المشتركة بين سلالتي فوجا وكنانة 43 آليل أكبر من عدد الآليلات المشتركة بين فوجا وبطانة 40 أليل وبين بطانة وكنانة 36 أليل.كما وجد من خلال نتائج التحليل الإحصائى ارتفاع ملحوظ لجميع العشائر فى قيم متوسطات التنوع الوراثى الجينى (Average Gene Diversity) لجميع الواسمات المستخدمة فى تحليل عشائر الحيوانات المختلفة و أنه يتراوح من0.536 (ETH210) إلى (TGLA122) 0.810 والمتوسط الكلى لها يساوى 0.684.وقد وجد أن قيمة محتوى معلومات تعدد المظاهر الوراثية أو ما يعرف أيضا بالتنوع الوراثى: {Polymorphism information Content (PIC)}المتحصل عليها تتراوح من (0.483– 0.745) وهى قيم عالية مما يثبت وجود درجة عالية من التنوع الوراثى فى الأبقار تحت الدراسة.وفيما يتعلق بنتائج التربية الداخلية (inbreeding) بين العشائر تحت الدراسة فقد وجد عدم وجود تربية داخلية. وبالإضافة إلى ذلك وجد أن قيمة الهجرة أو التدفق الجينى (gene flow or migration) المرتبطة بعشائر الحيونات المحلية (وهى احد العوامل المؤثرة على إحداث التغيير الجينى) تراوحت مابين (0.814–8.754) ومتوسط القيم يساوى 4.335. وتعتبر هذه القيم عالية بمقارنتها بسلالات أخرى وهذا يدل على زيادة معدل الهجرة بين عشائر الحيوانات تحت الدراسة.

ويعطى الشكل التخطيطى الناتج من اختبار العالم Nei والخاص بتكوين شجرة العائلة وانبثاق السلالات من بعضها أن الهجرة بدأت له ذه السلالات في السودان قد بدأت من الجزء الشمال الشرقي ثم انتقلت الى الوسط (شمال كردفان).

TABLE OF CONTENTS

CHAPTER THREE : MATERIAL AND METHODS

List of Table

CHAPTER ONE

INTRODUCTION

Livestock plays an important role in production of food and represent great socio-economic and cultural values in various societies around the world. Indigenous cattle form the backbone of relevant and sustainable livestock production in most Eastern African countries.

In Sudan, the rural communities own 80% of the livestock and the nomadic tribes own 90% of the rural holdings with livestock playing a central role in their livelihoods. They are well adapted to the local environmental conditions (e.g. tolerance to heat stress and they are able to survive long periods of feed and water shortage (Okomo-Adhiambo, 2002), however, they show correspondingly low performance level demonstrated by a low juvenile growth rate, late sexual maturity, low milk yield and long calving interval.

There are many types of cattle in the Sudan, and many authors have attempted to classify the local breeds on the basis of their origin and phenotypic characteristics. Bennett et al. (1948) classified the Sudanese local cattle into three main groups; namely, Northern or Arab, Southern or Nilotic and the small cattle of the Nuba mountains. According to Joshi et al. (1957) and Payne (1970), the Northern Sudan cattle include Kenana, Butana, Western Baggara, White Nile and Northern Province. However, these classifications are based on phenotypic characteristics or geographic origin and are not related to genotype except in as much as the phenotype is in part a reflection of genotype. Other types of northern Sudan Zebu cattle include Eryshai (of eastern Sudan), White Nile cattle, Fuga or Dar El Reeh cattle of the North Kordofan.

Cattle population in Sudan is 41.56 million heads (MARF, 2009). Among the cattle population Kenana and Butana are promising indigenous milk breeds, which under improved feeding and management in research stations yield more than 1500 kg milk per lactation (Musa et al., 2005). The two breeds constitute about 24% of the total cattle population of the country (Abdel-Aziz et al., 2005). Through experience, many herdsmen have come to understand that the best results are

obtained by crossing the best local cattle (usually Butana and Kenana) with exotic breeds (usually Friesian) (Musa et al., 2005).

This has raised concern over the fate of Butana and Kenana types and efforts for their conservation for both present and future use. This concern is motivated by the fact that the genotypes of the improved indigenous breeds may be required to upgrade or replace low producing cattle in harsh nomadic environments where exotic cattle cannot survive. Recognizing the need to ensure that livestock production become more efficient, sustainable and responding to economic pressures, the FAO initiated the Global Program and Strategy for Animal Genetic Resources. The program aims to overcome the erosion of animal genetic resources through better development and use, together with improving the production levels of adaptive breeds as central to the better management/ conservation of AnGR (FAO 1997). With the advent of molecular technology, a powerful new tool is available for characterization, classification and estimation of distances between breeds and strains. There are tendencies for genetic improvement programs to focus on single market driven traits such as milk or meat production in isolation from environmental constraints and broader livestock system functions which cattle perform in developing countries (Ouma et al., 2004).

In addition, the development of genetic improvement programs for cattle will only be successful when accompanied by a good understanding of the production systems and when simultaneously addressing several constrains e.g.; feeding, health control and management (Baker and Gray 2003). Genetic characterization it is necessary at this point to decide exactly what is meant by the term "breed". The animal-oriented definition recognizes that breeds differ by the totality of average differences observed in many quantitative and qualitative traits.

The differences may overlap, but they have a genetic basis, and these differences taken together provide a unique description (Baker and Manwell, 1991). This definition provides a solid basis for the application of population genetic techniques and is in stark contrast to the arbitrary and often colloquial designation of a particular type to one breed or another based on anthropocentric criteria, it is

instructive to make a distinction between those genetic markers that are used as a tool to identify and locate useful traits and those that are used to describe population structures. The characterization of production traits in cattle using molecular markers is currently a very active area of research. The identification of such markers requires the examination of either large pedigrees or populations that are carefully phenotyped. Markers themselves may be polymorphisms within genes that are suspected to actually confer a particular trait (candidate genes) or, more commonly, they may be anonymous regions of the genome that are examined with the expectation that they will be adjacent, i.e. linked, to genes of importance.

In order to ensure proper conservation and utilization of indigenous breeds, it is necessary to evaluate genetic variations that exist within and among breeds (Kunene *et al.,* 2009).The genetic characterization of domestic animals is the first step in considering the sustainable management or conservation of a particular population. It is important to know how unique or how different it is from other populations (FAO, 2000b). It is also valuable to establish DNA archives that can act as benchmark material against which the success of management programmes aimed at the conservation of genetic variability can be monitored (FAO, 2007).

Throughout the world researchers utilize molecular technology as a standard approach to establish the genetic distances among breeds. Recently, the loss of genetic diversity between breeds of sheep has been a major concern (Kunene *et al.*, 2008). It is has been suggested by Ali (2003) and FAO (2007) that the highest level of genetic diversity in these populations of livestock is found in the developing world, where record keeping is poor but the risk of extinction is high and is increasing.

The investigation of genetic variation is very important for future monitoring of gene flow in populations, conservation of species, determination of the level of inbreeding and crossbreeding within and between breeds (Hetzel and Drinkwater, 1992).Polymorphism in blood proteins first offered the possibility to study genetic variations in livestock including domestic sheep breeds before the advent of molecular markers (Distasio, 1997). Protein markers have also been used in

studying biochemical and molecular characterization of some sheep breeds in Egypt (Mwacharo et al., 2002).Population-level characterization is distinctly different from that of trait-based characterization. A primary aim of the population geneticist is to detail in an unambiguous and often numerical manner parameters that are useful in describing a population.

The knowledge of how genetic variation is partitioned among populations may have important implications not only in evolutionary biology and ecology, but also in conservation biology. In order to understand the amount of genetic variation in a population, methods are required to quantify this information. Although several measures have been used to describe genetic variation at a single locus or a number of loci, heterozygosity has remained the most widely used (Hedrick, 2005).

Up till now there is no available literature on the phenotypic and genotypic characterization of Fuga cattle in Sudan. This study was carried out in Fuga cattle areas in western Sudan with the objectives to understand the conditions of production systems and to identify breeding objectives, husbandry practices and production constrains as a first step toward development of a sustainable breed improvement program. Therefore, the objective of this research is to investigate on the phenotype and genotype characterization of Fuga cattle as one of the promising dairy animal in Sudan. The parameters to be used of researching objective are:

1. Estimation of Some phenotypic characterization (productive and reproductive traits), this include:
   1.1. Age at first calving,(months).
   1.2. Number of service preconception.
   1.3. Pregnancy rat.
   1.4. Calving interval (days).
   1.5. Weight of birth.
   1.6. Milk yield.
   1.7. Length of lactation (days).

2. Estimation of genetic diversity in Fuga compared to Kenana and Butana cattle by using:

2.1. Level of polymorphism according to DNA markers, between and within breeds.

2.2. Unique markers and fingerprints.

2.3. Genetic similarity "genetic distance "between and within breed.

2.4. Genetic relationship "between and within breed.

2.5. Glusters analysis of microsatellite data.

CHAPTER TWO
LITERATURE REVIEW

2.1. Cattle in Africa

With its unique set of genetic resources, Africa is known to be one of the centers of cattle diversity and domestication (Hanotte et al., 2002). However, the site of initial domestication of cattle in Africa is as yet without any consensus. The indigenous Zebu cattle accounts for about 90 % of the total cattle population in most African countries (Teale et al., 1993). The expectation is that there is more genetic diversity among indigenous cattle breeds in Africa than in the rest of the world. One explanation for this diversity is that breeds in the developed world have been highly selected while most cattle in Africa are free-roaming and indigenous and are under little or no artificial selection (Giovambattista et al., 2001).

Cattle in Africa can be classified into four major groups: the humpless *Bos Taurus*; the humped *Bos indicus* (Zebu), distributed widely in Africa; *B. taurus* x *B. indcus* (Sanga), found mainly in eastern and southern Africa; and Sanga x Zebu types also known as Senga (Reisti-Marti et al., 2003).

However, indiscriminate cross breeding of the Zebu with Taurine breeds has been a major contributor to the genetic dilution of this resource in most African counties. This has been accelerated by advances in new reproductive techniques, particularly artificial insemination and increased exchange of breeding stocks between regions (Moazami-Goudarzi et al., 1997).

Mburu and Hannotte (2005) described a study that looked at genetic diversity and population structure in 52 populations of African cattle using 15 microsatellite markers. The study indicated a wide range of genetic diversity among African cattle based on mean number of alleles, effective number of alleles, and expected heterozygosity.

There was high differentiation among African cattle breeds. Diversity was highest in the North African *Bos taurus* and lowest in the western cattle. The

analysis supported isolation by distance in all, but the Southern African region, with extensive gene flow between populations. Within these, there is extensive gene flow between populations. These and other molecular studies have revealed that cattle in Africa are appropriately partitioned by origin into East, West, North, and South. Though both *Bos Taurus* and *Bos indicus* can be found in West Africa, Red Bororo and White Fulani are the primary cattle raised in that region.

Other breeds that can be found in the West African region include the Baoule-Somba and various Zebu strains (Alderson and Bodo,1992; and Belemsaga et al, 2005).

Though there are no recent updates, examples of both were previously reported by Epstein (1974). Most North African cattle were reported to be long or giant horned and humpless types, currently, only the N'Dama and Muturu of West Africa, the Namji of Northern Cameroon and the Kuri of Lake Chad with these characteristics can be found. In the Republic of Sudan, the humpless Nuba short-horned cattle that were previously found in the mountain region of the southern Kardofan, are now shard to find even (Alderson and Bodo, 1992).

In Ethiopia, the Sanga cattle maitained by the Danakil and the Balla Azebo people are also very hard to find. In Uganda, the giant-horned Ankole Sanga are said to have been restricted by the advances of *Glossina morsitans* to a few isolated enclaves.Aderson and Bodo (1992) have also reported that in Zimbabwe, the original Amabowe have become exceedingly rare owing to the disastrous effects of the rinderpest which destroyed a large proportion of African herds. In South Africa, the Sanga types of cattle are still present in the Zululand, Swaziland and a few enclaves of Northern Transvaal. However, in other parts of South Africa, Knowledge of the genetic variation that can be effectively measured within and between populations is important and also required for breed characterization (Hetzel and Drinkwater, 1992). The relationship among most of the African cattle breeds has remained poorly understood. Often, the same breed may be known by different names and equally, two breeds may be known by the same name depending on the Geographical locations of such breeds (Gwakisa et al. 1994).

2.2. Sudan Cattle Breed:

Sudanese cattle were broadly classified into three main types; *the Arab* or northern Sudan short horned Zebu cattle, the Nilotic and Nuba mountains cattle breeds (Bennet et al., 1954). The best two defined indigenous type for milk production are the kenana and Buttana cattle. These breeds will be subjected to substantial pressers from outside genetic sources in the coming decades for purposes of the cross breeding or up-grading to the foreign breeds (Cumminghan;1987).

2.2.1.The Northern Sudan short horn Zebu cattle:

This type mainly found in northern and central Sudan, heard by nomadic tribes between the 10 th and 14 th parallels of latitude (Khalil. 1961) The Arab cattle include Kenana, Butana, and western Baggara.

2.2.2. Origin and distribution of Kenana cattle:

The Kenana cattle breed is among the Large East African Zebu and has several strains, namely Rufáai El Hoi, Rufáai El Sherik, Fung, Gezira and White Nile. The breed is believed to have descended from the first zebu introduction into Africa from West Asia. This breed remained largely with semi-nomadic pastoralists in the northern Sudan. They are distributed east of the confluence of the Blue and White Niles, south-east to the Ethiopian border, and the western banks of the Blue Nile to southern Khartoum.

2.2.2.1. Physical characteristics of Kenana cattle:

They are medium to large in size. Coat colour is white or steel grey with darker shading on the neck and shoulders and a black switch to the tail; blue-grey to white with black shadings on the head, neck hump, hindquarters and legs; horns are black. Calves are reddish-brown at birth turning grey by about six months of age (Maule 1990). The hump is prominent in males and in most cases cervico-thoracic in position; large and has tendency to hang over at the rear (Mason and Maule 1960). They have well-developed dewlap commencing under the chin often as a double fold, and runs well back along the floor of the chest, frequently joining the large

umbilical fold(Epstein 1971).They also possess sheath.The udder is well developed. Horns are often loose; females have shorter horns than males (Maule 1990).

2.2.2.2 Breed status:

The Kenana breed is not at risk of extinction, population estimate in 1994 being 1500 000 (DAD-IS, 2005) and 1 670 000 (Rege, 1999). However, the breed has been extensively crossed with other breeds during the past 2-3 decades. Increasingly these animals are being kept by sedentary agro-pastoralists at the expense of the nomadic system but still mainly owned by nomadic and semi-nomadic breeders.

The average weight of a bull is 500 kg whilst that of a cow is 400 kg. The average milk yield is 1860 kg in 222 days lactation (Osman 1981). In this breed, the peak milk yield is during the 4th lactation and the average length of productive herd life of a cow is 5.42 lactations (Alim 1960). The age at first calving is 45.2 months but with good management; this could be reduced to 32 months (Khalafalla 1977). It is to be noted however that around 61 percent of heifers in a herd calve at 37-48 months. The calves weigh 24.8 kg and 23.5 kg at birth respectively for male and female. With good management considerable improvement could be achieved in the reproductive performance of the breed.

2.2.3. Origin and distribution of Butana cattle:

Butana cattle breed have descended from the first zebu introduction into Africa from West Asia. This breed remained largely with nomadic pastoralists (Batahin, Shukria), but the Dongola and Shendi strains are kept by settled farmers. They inhabit the Butana plain in central Sudan, an acacia scrub and desert area lying between the Blue Nile and Atbara rivers. Being originated in the Butana plain, east of the Blue Nile, have spread to Gizera in central Sudan and along the river Nile in the northern region. They are referred to as Dar El Reih cattle across the White Nile in the northern part of Darfur and Kordofan.

2.2.3.1. Physical characteristics of Butana cattle:

As one of the Large East African Zebu breeds of cattle such as the present-day Boran of Ethiopia, Kenya and Somalia, the Butana breed of Sudan are very similar morphologically to the zebu breeds of Asia. The predominant coat colours is red or dark red, the tongue, muzzle eyelids and around the coronet are black; light red with white may also exist (Maule 1990). Dewlap is well developed and runs down in front of the forelegs forming few folds (Mason and Maule 1960) and is prominent in both sexes. Most of them possess small and black horns although some animals are polled. They have well developed thoracic or cervico-thoracic humps, which are large, hanging over backwards. The humps are relatively large in males but small in the cows (Mason and Maule 1960; Epstein 1971).

2.2.3.2. Breed status:

The Butana breed is not at risk of extinction. Their population size was 258 000 in 1991 (Rege 1999) and 1 000 000 in 1994 (DAD-IS, 2005). According to this FAO database, this breed has been subjected to extensive cross-breeding with European cattle (since 1956) and has also been exposed to extensive droughts in 1972/73, 1983/84, 1989/90. Consequently the breed is declining in numbers. The population size is much smaller than that reported in R-1992 as that report used the figure from the 1976 population census without taking subsequent droughts, wars and famines into consideration. The breed is still bred pure at the Atbara Research Station but not at NDRI, Shukaba. The age at first calving is 43 months with calving intervals of 373 days.The birth weights for male are 25.64 kg and for female 24.29 kg respectively (Khalifa 1966). The average milk yield is 2253 kg in 240 days lactation (Osman 1981).

2.2.4 Dar El Reeh cattle (Fuga cattle)

Bos indicus (Zebu) cattle. Two thoughts for their origin; One thought to be originating from Butana cattle in Butana area brought to Kordofan by El Mahdi followers. Another, reported by the intervied producers at Foja, said that they are

crosses of Kenana bulls with Baggara cattle stabilized as a subtype or breed in Foja area. The Kenana Bulls were said to have been brought from Sennar area in the Blue Nile State to Kordofan on their way to Darfur Sultanate during Sultan Ali Dinnar time. But, following the death of Ali Dinnar in 1916, these bulls were restrained in Kordofan at Fuga area close to Darfur border. There they were crossed to Baggara cattle and the Foja cattle emerged. Both thoughts of origin seem to hold since there are variabilities in color of cows, some are red and light red resembling Butana cattle while others are white like Kenana cattle of the Blue Nile State. However, the latter thought seems to be sound in that Foja bulls are a close resemblance of Kenana ones and the red color in some cows might have been from the crossing with Baggara cattle.

This type of cattle used spread all over north Kordofan, but now confined to Fuga area in west Kordofan. They are reputed for their high milk yield; 5.0-8.0 litres/day at the Peace University Research Farm at El Fula. Calf weight at birth is ranging from 20.0-23.0 kg for female calves to 28.0 kg for males.(WSRMP Livestock Breed Characterizations Study 2011).

2.2.5 Husbandary systems:

The interviewed livestock owners stated that they used to keep their cattle close to their villages and feed them on natural range, crop residues and oilseed byproducts. However, due to drought and deteriorated rangelands they said that they only keep milking cows and send dry and that at early pregnancy south east as far as Iyal Bakheit area during the dry season and bring them back during the wet season.(WSRMP Livestock Breed Characterizations Study 2011).

2.3. Reproductive Performance:

Reproduction is one of most important physiological traits that affect animal performance and is of primary concern to the breeder. It is economically important because it affects milk yield per cow and number of replacements produce per cow and culling practices (Berger et ,al 1981).Reproductive performance is a composite characters made up of several components that interplay together to produce and

overall effect , which is the number of calves produced per cow over specified period of time to fulfill this requirement ,the following traits among others are to be considered; The reproductive efficiency of a dairy herd can be measured in several ways, such as by measuring pregnancy rate, percentage of cows calving each year, average calving interval, average number of days dry, and number of live calves born each year. Although each of these measures affects the profitability of the dairy business in a slightly different way, the calving interval affects both the total milk production of the dairy herd and the number of calves born. In most modern dairies, the general practice is to breed cows early, with the aim of establishing a calving interval of 12 to 13 months, which is considered optimum; hence, calving interval is considered an important index of reproductive performance (Roberts, 1986; Arbel et al., 2001).

2.3.1. Age at first calving:

This term defines Age as the period from the date of birth to the date of first calving .age at first calving was found to be and important factor that influences milk yield, lactation length, and first calving interval.It was shown that very early calvers had proved to be poor total yielders with short lactation length but with correspondingly shorter first calving interval .these cows can be expected to have more calving, thus longer productive life (Venkaya adAnantakrishnan.1956).

First calving marks the beginning of a cow's productive life. Age at first calving is closely related to generation interval and, therefore, influences response to selection. Under controlled breeding, heifers are usually mated when they are mature enough to withstand the stress of parturition and lactation. This increases the likelihood of early conception after parturition. In traditional production systems, however, breeding is often uncontrolled and heifers are bred at the first opportunity.This frequently results in longer subsequent calving intervals. The average age at first calving in *Bos indicus* cattle is about 44 months ,compared with about 34 months in *Bos taurus* and *Bos indicus* x *Bos taurus* crosses in the tropics . Heritabilities of age at puberty, at first conception and at first calving are generally

low , indicating that these traits are highly influenced by environmental factors. Jochle (1972) studied the effect of season on the reproductive performance of Brahman heifers that first conceived at between 15 and 37 months old in the Mexican Gulf coast. Of 111 heifers that first conceived at 15 to 24 months old, significantly more ($P<0.001$) did so during the dry season than during the wet season. However, among heifers that first conceived at more than 24 months old, most conceived during the rainy season and overall there was no significant difference between the percentages of heifers conceiving first during the rainy or dry season .Oliveira (1974) observed that Nellore cows in Brazil that calved first in the dry season were younger than those that calved first in the rainy season. Miranda et al (1982a) found that age at first calving in Brazilian Nellore heifers was significantly affected by year and month of birth: calves born from January to May tended to be younger at first calving than those born between June and December. Sabino et al (1981) also found a year-of-birth effect among Haryana, Gir and another unspecified zebu type cattle in Venezuela, as did Sharma (1983).

In Nagauri cattle in India. However, Sabino et al (1981) found that neither month of birth nor breed significantly affected age at first calving. Gregory (1981) found no significant difference in age at first calving ($P>0.05$) between Boran and Sahiwal heifers on a ranch in the Kenya Rift Valley, but Chhikara et al (1979) found that breed differences had a significant effect on age at first calving in Haryana, selected Haryana, Tharparkar and Sahiwal heifers in India. In an analysis of production data covering 14 years, Aroeria et al (1977) found that Gir heifers tended to be older than Nellore or InduBrazil heifers at first calving. Breed differences probably reflect differences in management conditions. The time taken by an animal to attain puberty and sexual maturity depends on the quality and quantity of feed available, which affects growth rate.

Wagenaar et al (1986) found a mean age at first calving of 50.2 ±9.1 months in 146 Fulani-type dams in Niger. None of the factors tested for in the least squares analysis (herd, season and year of birth of dam, sex of the calf) significantly

affected this parameter. However, Saeed et al (1987) found that year of birth significantly ($P<0.001$) affected age at first calving in Kenana cattle in Sudan but that month of birth did not. Wagenaar et al (1986) observed a significant correlation ($P<0.001$, $r = -0.52$) between age at first calving and body weight at 3 years; heifers that weighed 10 kg more than average at 3 years old first calved 2 months earlier than average-weight heifers. There was no significant correlation between age at first calving and weight at 1, 2 or 4 years.Dennis and Thiongane (1978) found that Gobra (Senegal Fulani) heifers kept on pasture and fed a balanced concentrate supplement calved first at 31 months old, compared with 40 months for unsupplemented heifers. El-Khidir et al (1979), working in Sudan, also found that improved nutrition significantly decreased age at first oestrus ($P<0.001$), which in turn reduced age at sexual maturity, first conception, calving and total rearing costs.

Weitze (1984) found that supplemental feeding during the dry season reduced the average age at first calving from 45.0 to 37.5 months in Nellore, Gir and InduBrazil cattle in Brazil. Calving interval was also shortened by 52 days to 492 days (16.4 months), with a calving rate of 83%. Singh et al (1982) found age at first calving to be positively correlated with lactation milk yield and lactation length in Gangatiri cattle, as did El-Khidir et al (1979) and Singh et al (1981) working with Kenana and Indian-type cattle, respectively. In general, earlier first calving increases lifetime productivity of cows. For example, Meaker et al (1980) showed that, despite lower first conception rates, Africander heifers calving first at 2 years old produced 0.6 more calves over their productive lifetime than those calving first at 3 years old, while Pinney et al (1962) estimated the increase to be 0.8 of a calf.

Meaker et al (1980) recorded heart girth, wither height, rump height, chest depth, body length, height and width, and hip width of beef cows until 6 years of age and found that only bodyweight at 2 years and wither height at 3 years were affected by early calving. They concluded that animal growth up to 6 years is not significantly depressed by early calving. Ahmad and Ahmad (1974) found that late first calving was associated with longer first dry periods ($r = 0.29$) and longer

calving intervals (r = 0.36). Basu et al (1979) observed also that the number of services per conception increased with increasing age at first calving. Most data thus suggest that it is advantageous to breed heifers as early as is physiologically possible.

2.3.2. Number of services per conception:

This is one of the breeding efficiency measures. It was defined by (Esslemont et al., 1985) as the total number of services given to a group of cows over a defined period divided by the number cows served. Which resulted in a diagnosed pregnancy not less than 42 days after services including services to culled cows hould.(Ageeb and Hayes, 2000) working in the pure bred Holstein kept near Khartoum is Sudan reported number of services per conception, as (4.2) , Ahmed and Warf . 1999), in Pakistan reported that the number of service per conception in cross-bred cows averages (1.6±0.1) and ranged from (1to 12) and was positively correlated with daily milk yield and negatively correlated with lactation length.

The number of services per conception (NSC) depends largely on the breeding system used. It is higher under uncontrolled natural breeding and low where hand-mating or artificial insemination is used. NSC values greater than 2.0 should be regarded as poor, and some of the factors contributing to high NSC values are elaborated below. Choudhuri et al (1984) estimated the repeatability of NSC to be 19% from 2152 records for Haryana cattle.

The NSC was 2.81 ±0.03 and was significantly affected by herd, season, placenta expulsion time, lactation length and milk yield. Since heritability can be broadly estimated from repeatability, this study indicates that heritability of NSC is low and most of the variation in NSC is attributable to environmental factors.

Sharma and Bhatnagar (1975) found a significant effect of parity on NSC in Sahiwal, Red Sindhi and Tharparkar cattle. The NSC was highest at the fourth lactation for F1 crosses with Brown Swiss. Kumar and Bhat (1979) noted that Haryana heifers needed more services per conception than cows. Azage Tegegn et

al (1981), using 3 local Ethiopian breeds, the Barca, Horro and Boran, found that NSC was lower for animals from wet areas than for those from drier areas (1.74 ±0.6 vs 1.98 ±0.07). Crossbred cows required 0.12 and 0.14 fewer services per conception than local zebu cows in wet and dry areas, respectively.

El-Amin et al (1981) concluded that NSC did not differ significantly between Red Butana and Red Butana crosses (average 2.6) but was influenced by month of calving. NSC increased over the study period, probably due to changes in management. This is partly supported by an analysis by Busch and Furstenberg (1984) of 483 600 inseminations performed by 379 technicians on 623 farms in the USA, which showed that the 90- and 120-day non-return rate differed significantly among inseminators and the inseminator effect was greater than the farm effect. However, non-return rate did not differ among bulls.

2.3.3. Calving interval:

Defined as the number of elapsed days between two consecutive calving or calving interval is the period between two consecutive calving . Regular calving of dairy cows every 12-13 month is economically desirable for profitable production(Bath et al 1985) and it is an important measure of the productive efficiency.Significant ($p<0.01$)correlation exisisted between calving interval and lactation milk yield and lactation length and peak yield. In indication that animals with optimum calving intervals will have high yield in all aspects.However, there was a negative and significant ($p<0.01$) correlation between calving interval and season of calving (Banerjee and Banerjee. 2002).

Calving interval can be divided into three periods: gestation, postpartum anoestrus (from calving to first oestrus) and the service period (first postpartum oestrus to conception) .The following section therefore relates to factors that influence the length of the postpartum anoestrous and service periods. This is sometimes also called the "days open", period and is the part of the calving interval that can be shortened by improved herd management. The "days open" period should not exceed 80-85 days if a calving interval of 12 months is to be achieved

(Peters, 1984).This requires re-establishment of ovarian activity soon after calving and high conception rates. The duration of this period is influenced by nutrition (Wiltbank et al, 1962), season, milk yield, parity (Buck et al, 1975), suckling and uterine involution. At any time, the effects of one or more of those factors may be confounded. Calving interval has been extensively analyzed and reported. It is probably the best index of a cattle herd's reproductive efficiency. Resumption of ovarian activity in the postpartum period does not necessarily lead to conception and methods of stimulating oestrus must be considered in relation to their effect on conception (Holness et al, 1980) and, indirectly, calving intervals.

2.4. Productive performance:

2.4.1. Lactation milk yield:

Milk yield is the most important economic trait that determines productivity and profitability in dairy cattle herds. Milk yield produced during the first 305-days of lactation is used as a standard measure for milk yield per lactation .Milk yield measures the worth of daily cow's from and economic point of view milk yield is a basic and most important trait in dairy animals. Milk yield is a complex character because it is result of connection between phenotypic, genotypic characters and environmental interactions. There are obvious differences in milk yield between breeds. Holstein cattle performance is negatively affected by heat stress.

Cooling system based on spraying and fans are effective alternative to increases productive and reproductive efficiency in lactating Holstein cows under hot environmental condition. (Armstrong et al, 2002). Studies in milk production of Friesian cows in Kenya, shows lactation milk yield was (2495) kg.(wilkins,1979) average milk yield in Holstein cows in Californiawas (8127.77±361.11)kg and was influenced by the number of lactation and season . Daily milk production of Holstein Friesian cows in Maharashtra India ranged from (4.53-4.96) kg, milk yield with total first lactation yield as (2857.0±60.2)kg .

Performance of the indigenous dairy cattle in Sudan was investigated by many researches (Bayoumi and Dinasoury., 1962) revealed that the average milk production for Sudanese dairy cows was (442.5) gallons with C.V of 32% while (Fangaly .,1980) reported that the 305 days milk yield for Kenana cows at Umbenein and Nesheshiba station. Were (1872.5) and (1367.13)kg respectively Butana cows at Atabra station showed milk yield of (2254.2)kg per lactations .selection and cross breeding are the most important tools of cross breeding are the most enhance milk yield production potentiality of our indigenous breeds .(Ali et al.,1988) reported that the lactation yield for cross-breed(Friesian × Kenana or butane)cows having 50% , 62.5% and 75% Friesian blood were 4306 , 5733 , and 4136 Ib, respectively. Bahatanger et al., 1986) revealed that the total lactation yield for brown Swiss × Sahiwal was (3259) kg and was higher than yield of Sahiwal which recorded (1836)kg .(Mackintosjh.1925) found that a gradual increase in yield up to the 5^{th} and 6^{th}lactations and decreases after the7^{th}or 8^{th}.

The estimated lactation milk yield for Butana cattle (538.26 kg) and for Kenana cattle (598.73 kg) showed that both breeds had a comparable milk performance under field condition which was much lower than their yield under station condition. However, Butana cattle in Atbara Livestock Research Station yielded 1662.57 ± 108.96 kg/ lactation (Musa et al 2005) and Kenana cattle in Um-Benein Livestock Research Station yielded 1423.58 ± 551.70/ lactation (El-Habeeb 1991). For both breeds the lactation period was also much shorter under field condition.

2.4.2. Lactation length:

Lactation length is period from calving to drying up of a cow and is and important factor for influence the milk yield. The standard length of lactation has taken as (305) days which corresponds to the reproductive cycle of cows. Lactation length difference between breeds and even between individual in the same breed according to management policies adopted (Mahadevan.et al., 1958). The length of first lactation was (244± 6.9) day for Friesian cows in India (Kulkarni et al., 2001). A lactation length of (322) days was reported for Frisian cows in Kenya by

(Wilkins.1979.Bhattacharya et al., 2002) working on Indian-born Holstein Friesian reported and average lactation length of (295±8.28) days. He pointed out that the effect of season on lactation length was significant in Holstein Friesian breed. (Osman and Elaminm.1971)reported lactation length of (294) days with coefficient variation of (31%) for northern zebu Sudanese cattle for butana cattle and Atbara .(Alim.,1962) reported lactation length of (253±103)days (Alim.,1960)reported length 224±85)days for kenana at the Gazira Agriculture Research station herd.

The lactation performance of dairy cattle is usually measured by determining the total milk yield per lactation or per year, average daily milk yield, lactation length, lactation persistency, and milk composition. Generally, the reproductive performance and lactation performance of dairy cattle are closely associated with each other. Breeding failure has a clear negative influence on milk production and farm income and determines the future sustainability of a dairy farming operation. Milk production level and lactation persistency are crucial factors determining the appropriate calving interval (Arbel *et al*., 2001). On the other hand, the costs of fertility depend on the stage of lactation and the shape of the lactation curve.

Cows normally have a lactation curve that loses 8 to 10% per month after the peak, but those rare animals whose production declines by only 4% or so may make a longer calving interval justified (Esslemont, 2003).In most modern dairy farms, a lactation length of 305 days is commonly accepted as a standard. However, such a standard lactation length might not work for smallholder dairy cows in which the lactation length is extended considerably in most cases (Teodoro and Madalena, 2003; Masama *et al*., 2003; Msangi, Bryant & Thorne, 2005).

Furthermore, such an extended lactation period has practical significance for the smallholder dairy farmer as it provides compensation for the usually extended calving interval (Tanner, McCarthy & Omore, 1998). The profitability of short or extended lactation length depends on various factors, including the lactation length persistency. Numerous studies have documented that additional days in which cows

are not pregnant beyond the optimal time post calving are costly (Groenendaal, Galligan &Mulder, 2004; Meadows, Rajala- Schultz &Frazer, 2005).

However, Borman, Macmillan & Fahey (2004) demonstrated that extended lactations are suitable for some dairy enterprises and that the suitability depends particularly on cow milk potential, the ability to grow pasture or feed supplements economically, management expertise, environmental constraints, herd size and labour availability. Moreover, Osterman and Bertilsson (2003) suggested that by combining a longer calving interval with increased milking frequency, daily milk production from one calving to another could be increased, making an increased calving interval an interesting option for dairy farmers. In addition, an economic advantage in extending lactations (by 60 days) was found even in the case of high-yielding cows.

This advantage was greater for primiparous cows, because of the high persistency of their milk production and the increase in the fat and protein contents of their milk as lactation progresses (Arbel *et al*., 2001). Thus, it is of interest to properly evaluate the economic benefits and subsequently optimize both the lactation length and calving interval under the given production level and prevailing management conditions.

2.5. Measures of genetic variation and differentiation in cattle

It is generally accepted that genetic variation is the raw material of evolution, without which populations cannot evolve in response to changing environments. The knowledge of how genetic variation is partitioned among populations may have important implications not only in evolutionary biology and ecology, but also in conservation biology. In order to understand the amount of genetic variation in a population, methods are required to quantify this information.

Although several measures have been used to describe genetic variation at a single locus or a number of loci, heterozygosity has remained the most widely used (Hedrick, 2005). Heterozygosity refers to the state of being heterozygous and it is

believed to be a good predicator of chances for long-term survival of a population. This is because it reflects the number of genetic options available within a population. Another measure that has sometimes been used to measure genetic variation is the number of allelesobserved at a given locus in a population. But again, according to Hedrick (2005), this measure is strongly influenced by the population size so that comparisons across populations with different sample sizes should be made cautiously. The effective number of alleles, ne, which is the inverse of the expected homozygosity, is also sometimes usedto measure the amount of genetic variation in a population. In a recent study in cattle, Zhou et al (2005) used all these three measurements to analyze genetic diversity in breeds native to China. Based on polymorphisms of 10 microsatellite loci, they examined thegenetic diversity among five native Chinese cattle breeds and also estimated the genetic differentiation and relationship within and between breeds. From their results, the number of alleles, n, heterozygosity, and effective number of alleles, ranged from 4-12,0.51-0.86 and 2.48-5.41 per locus, respectively.

Genetic distance: based methods have been widely used to measure genetic differentiation among populations. These methods involve calculating pair wise distance matrices, whose entries give an estimate of the distance between pairs of individuals. Amongst distance methods, Nei's genetic distance (Nei 1972) has been the preferred approach. Recently, for example, MacNeil et al (2006) used Nei's genetic distance metric to calculate the relationships among cattle in Chirik Island, off the coast of Alaska, and commercial breeds in North America.

However, for closely related breeds within which drift is important, particularly in the developing world the modified Cavalli-sforza distance is recommended (Nei and Yatano, 1983). Another measure which partitions the geneticvariation and provides a description of differentiation was developed by Wright (1951, 1965). This approach consists of varying types of differentiation measurements called F coefficients. One commonly reported F coefficient is Fst, which measures the degree of genetic differentiation based on standardized

variances in allele frequencies among populations (Balloux and Goudet, 2002). Metta et al. (2004), for example, genetically characterized two Indian cattle breeds, Ongole and Deoni, using coefficients Fst and Fis, the levels of differentiation and inbreeding, respectively.

Phylogenetic trees often are constructed to accompany distance measurements. For example, Edwards et al. (2000) constructed two Neighbor-joining trees, one in which they used the genetic distances to visualize the relationships between the endangered pustertaler-Sprinzer and three European cattle breeds and another in which they used the allele sharing statistic suggested by Bowcock et al. (1994) with individual animals acting as operational taxonomical units (OTUs).

2. 6. Tools to understand livestock diversity–molecular characterization

Genetic marker types that provide different estimates of genetic diversity information have been described. The choice of the marker to use for genetic diversity is quite often dictated by the power of the method used to generate a reproducible polymorphism that can either be tracked in a Mendelian fashion or can show segregation of a phenotypic trait in a predictable manner (Hannotte and Jianlin, 2005). The choice can also be influenced by the availability of specialized equipment, operating of equipment and assays, and technical competence.

The first biomarkers to be widely used in livestock characterization were protein polymorphisms known as allozymes (Queller et al., 1993). Several livestock breeds have been characterized for variations in different proteins (Di Stasio, 1997).

Using published data on protein polymorphisms from approximately 1000 papers in about 216 breeds of cattle, Manwell and Baker (1980) analyzed differences among animals from different continents. Protein polymorphisms, although still used in population studies, are of limited value in the assessment of genetic variation at the level of cattle breeds. This is largely because of the relatively low levels of polymorphism found in protein loci, resulting in a lower taxonomic limit for the resolving power of protein electrophoresis.

Molecular DNA polymorphisms are now the tools of choice for the assessment of genetic diversity among livestock breeds. They have great potential for discovery of fundamental parameters or characteristics important in conservation, including past effective population size (Garrigan et al., 2002), past bottlenecks (Luikart and Cornuet, 1998), population origin (Cornuet et al., 1999), inbreeding status (Ellegren, 1999; Lynch and Ritland, 1999), and sex-specific gene flow. According to Hannotte and Jianlin (2005), important assumptions that are needed for the use of genetic markers include: (i) neutrality of the polymorphisms and (ii) the use of a relatively small number of independently segregating marker loci are a good predictor of the overall genomic diversity of a population.

One of the oldest molecular DNA markers used for assessing diversity is restriction fragment length polymorphisms (RFLPs). This method involves comparing the number and size of deoxyribonucleic acid (DNA) fragments produced by the digestion of DNA with various restriction enzymes. The banding pattern is generated by the presence or absence of restriction cutting sites and these in turn are produced by mutation. The fragments are blotted to membranes and probed with cloned radio-labeled DNA that binds to a single locus. This technique can be applied to nuclear DNA or to mitochondrial DNA (also to chloroplast DNA in the case of plants). It has applications in the study of genetic distance, population variation, gene flow, effective population size, patterns of historical biogeography and analyses of parentage and relatedness. For example, Thiemann et al. (1989), used nine RFLPs in six breeds of cattle to show that the Brahman (Bos indicus) differed significantly from the Bos taurus. Amongst the most widely used molecular markers for assessing relatedness in livestock are microsatellites. In recent years, in addition to being the markers of choice for gene mapping, they have been used in population studies to assess diversity (Wall et al., 1993).

Microsatellites are highly polymorphic, densely distributed in the genome, highly variable, and relatively easy to detect using the polymerase chain reaction. As hypervariability is highly significant for detecting differences in a population and between individuals, microsatellite typing can reveal degrees of polymorphism

that are easy to interpret. Microsatellites have been isolated in large numbers from most livestock species and FAO has recommended a list of microsatellite markers for genetic diversity studies that are now publicly available (http://www.fao.org/dad-is).

The primary disadvantage of this technique is that a prior knowledge of the DNA sequence is required to allow the design of PCR primers.

2.7. Molecular Markers in Animal Genome Analysis

The discovery of the genetic code, first published by Watson and Crick in 1953, has showed how the genetic information was passed from generation to the next. In the past years the developments in understanding the genetic code of animal species have opened the possibility to evolve the genetic evaluation of all species of livestock. Traditionally, the genetic improvement of livestock breeds has been based on phenotypic selection. The past century was characterized by the development of quantitative theory and methodology towards the accurate selection and prediction of genetic response *(Walsh, 2000)*.This resulted in the selection of a number of economically important genetic traits in cattle, sheep, pigs and poultry. The first work on detection of genome variation in animal livestock's is based on morphological, chromosomal and biochemical markers. Most of morphological markers are sex limited, age dependent, and are significantly influenced by the environment. Biochemical markers show low degree of polymorphism. The various genotypic classes are indistinguishable at the phenotypic level because of the dominance effect of the marker and low genome coverage (*Montaldo and Meza-Herrera, 1998*). The development of molecular biology during the past three decades created new means for studying livestock genetics and animal breeding. Selection according to genotype has become an important tool in the breeding of farm animals. The molecular markers, capable of detecting the genetic variation at the DNA sequence level, have removed the above mentioned limitations of morphological, chromosome and protein markers but also possess unique genetic properties that make them more useful than other genetic markers. They are numerous and distributed ubiquitously throughout the genome. These markers follow a typical Mendelian inheritance which usually expresses in a co-dominant

fashion, and are often multiallelic giving mean heterozygosity of more than 70 per cent. They are non influenced by the environmental factors, and generally do not have pleiotropic affect on quantitative trait loci (QTL).

Molecular markers, revealing the polymorphism at the DNA level are now key players in animal genetics. Molecular markers serve as an useful tool for animal identification and genetic distance estimation. Parentage testing using molecular markers yields much higher exclusion probability (> 90%) than the testing with blood groups (70–90%) or other biochemical markers (40–60%). These polymorphisms referred to as molecular markers can be used to build up genetic maps and to evaluate differences between markers in the expression of particular traits that might indicate a direct effect of these differences in terms of genetic determination on the trait (Vignal et al., 2002). Extensive genetic maps were prepared in the last few decades in a variety of animal species such as cattle, sheep, and swine, used for marker assisted selection (MAS), QTL segregating analysis and for detection of major genes (Kinghorn et al., 1993; Roher et al., 1994; Kinghorn et al., 1997; Kinghorn 1997; Vignal et al., 2002). The present review is a brief account of molecular markers and techniques and its various applications in animal genetic improvement.

2.8. Importance of genetic variation:

The loss of genetic variation within and between breeds is detrimental not only from the perspectives of culture, conservation and investigation but also for utility since lost genes may be of future economic interest (Hetzel and Drinkwater, 1992). Within breeds, high rates of loss of genetic variation lead to reduced chances of breed survival due to decreased fitness through inbreeding depression. These breeds become subject to faster changes in gene frequencies, greater rate of loss of genes and genetic constitutions (haplotypes). These are all due to small population sizes or high rates of inbreeding (Meuwissen, 1991). Regrettably, once animal genetic diversity has been lost it cannot be replaced (FAO, 2000b).

Inbreeding can lead to loss of important characteristics of a breed, and can result in the misinterpretation and incorrect classification and identification of breeds (Hetzel and Drinkwater, 1992). Before the advent of genetic studies, the classification of breeds was based on historical and anthropological evidence and morphological characteristics that were and are still not satisfactory or significant for the purpose and objective of conservation, parentage and future monitoring of breeds (Mwacharo *et al.,* 2006).

In order to ensure proper conservation and utilization of indigenous breeds it is important to evaluate genetic variations that exist within and among breeds (Kunene *et al.,* 2008, 2009). Most of the indigenous livestock populations in developing countries have not yet been characterised and evaluated at phenotypic and genetic levels (Hannotte and Jianlin, 2005). Genetic classification of African livestock is essential. Breed classification requires knowledge of genetics that can be effectively measured within and between populations (Nei *et al.,* 1983). The importance of maintaining domestic animal diversity has been emphasised in several reports (Kantanen *et al*., 1995; FAO, 2007; Hassen *et al.,* 2007).

Domestic animal diversity is not only important for food security but also to meet unpredictable future requirements.

2.9. The impact of Molecular Biology:

In the past few years, molecular biology has given access to the entire genome by using the DNA techniques (Ali, 2003). It has been reported that the studies of protein and DNA markers are providing useful and practical information at different levels: populations‟ structure; levels of gene flow; phylogenetic relationships; patterns of historical biogeography; the analysis of parentage and relatedness (Feral, 2002).The study of the structure and function of genes at the molecular level in a breeding population can help to determine the similarities of the genetic material carried by populations and the genetic variation they possess (Buduram, 2004).

Several techniques have been developed to estimate the genetic variation or polymorphism in populations and hence the genetic relationship amongst populations. Such techniques include DNA markers: [DNA hybridization, restriction fragments length polymorphism (RFLP), mitochondrial DNA (mtDNA), microsatellites and random amplified polymorphism DNA (RAPD)] and protein markers: [starch gel electrophoresis (STAGE) and polyacrylamide gel electrophoresis (PAGE)] (Buduram, 2004).

2.10. DNA as a hereditary material:

Genetics is the science of that which is heritable, a quality that is bound up essentially in the deoxyribonucleic acid (DNA) of organisms. Cattle, like humans, possess approximately three billion units of information, or base pairs, of DNA and this information is transmitted faithfully through the generations, unaltered except by rare mutations and the mixing of parental contributions through sexual reproduction. The double helical DNA molecule, the library of the cell, encodes in its structure the instructions that program all of a cell's activities. It consists of sugar-phosphate backbones linked together by hydrogen bonds between the corresponding nitrogenous bases, the sequences of which code for proteins. Unique among molecules, it is able to self-replicate. The strands separate, exposing the bases for bonding with the complementary nucleotides. Genes are the units of functionality written in the four-letter DNA code (Adenine, Guanine, Thymine, and Cytosine). They consist of a discrete segment of deoxyribonucleic acid (DNA); a linear molecule composed of sequences of four different nucleotide bases. Each of the hundred thousand or so genes in mammalian genomes codes for a protein (or RNA) structure, the function of which is enabled by its own, DNA-coded, macromolecular sequence. Proteins and the biochemical partners and substrates with which they interact make up the basic constituents of cellular and hence, organism, life (Bradley et al., 1998).

Genes are arranged linearly along the DNA and in most eukaryote organisms there is something like 50,000 of them. The actual quantity of DNA in each cell of

different species of eukaryotes varies over three orders of magnitude. The DNA of a cell is divided among a number of chromosomes. These chromosomes generally exist in two copies within each cell of the body and the organism is then said to be diploid. For the majority of organisms, which have sexual reproduction, one of these copies comes from the mother and the other from the father. Sex in genetic terms is the coming together of genetic information from separate individuals. In this way genetic differences from different individuals may be combined in their offspring to produce new combinations upon which evolutionary processes can work. A sexually reproducing organism must wait for the occurrence of different mutations in the same lineage to achieve these new combinations of genes (Groombridge, 1992).

Mutations are changes in the DNA, which occur in many ways and produce variation. "Variation is the raw material on which the breeder works" (Lush, 1945). The same gene can exist in a number of variants and these variants are called alleles. Normal individuals have two copies of each gene at a given locus one from the father and one from the mother.

Consequently, at each locus examined by DNA tests, a person typically has two alleles, one maternal and one paternal. This pair of alleles is a genotype. If the two copies of a particular gene possessed by an individual are different alleles, the individual is said to be heterozygous at that gene. If not, the individual is homozygous at that gene. If there are two alleles for a gene, there are two possible homozygotes and one heterozygote. If there are three alleles, there are three homozygotes and three heterozygotes. For four alleles there are four homozygotes and six heterozygotes, and so on.

A population of a species that has more than one allelic form of a particular gene is said to be polymorphic for that gene and the difference is known as polymorphism. The set of genotypes possessed by a person at two or more loci is a multi-locus genotype or DNA profile. Even though DNA from different individuals has more similarities than differences, there are areas on chromosomes where an

individual's DNA is highly distinguishable. Some of these areas, usually in the 90% of the genome that does not code for protein, are known as Variable Number of Tandem Repeats (VNTR). VNTR's involve a large number of alleles, have high heterozygozity, and occur frequently in the genome. The highly polymorphic characteristics of the VNTR's make them useful in (human) gene mapping, identification of individuals, and determining how closely individuals are related. Additional repeats of the base sequence will make the marker region longer, resulting in a length difference between the allelic forms.

One feature of heritable characteristics in animals is their variability. At the basic level of the DNA sequence, cattle differ from each other by one base pair in every several hundred. This variation is also manifest at the level of protein sequences and, as a result, in their biochemistry (the different blood groups are the most familiar example). Because of the accumulation of such variation, there are differences between animals in the peaks and troughs of their cellular physiological landscape. For the same reason, genetic variation is evident at the most gross level of measurement, that of the phenotype (Bradley et al., 1998).

Nowadays, the genetic analysis of organisms has been revolutionized by recombination DNA technology, allowing the direct identification and manipulation of DNA sequences, and the application of a marker assisted selection (MAS) to complement traditional selection methods that accelerate genetic improvement of economically important traits.

2.11. Types of DNA:

The DNA can be classified in two broad categories: nuclear and organellar. Nuclear genomes are much larger than organellar, ranging from [less than][10.sup.6] nucleotide bases (= 1000 kb [kilobase]) in some bacteria to [greater than][10.sup.11] in some plants (Cavalier-Smith, 1985, Li et al., 1985). Diploid organisms have two copies of each genetic region (locus) on homologous pairs of chromosomes. These two copies are called alleles, regardless of whether they represent coding or noncoding regions of the genome. Coding regions (exons) are

often interspersed with more variable noncoding regions (introns or intergenic regions).Nuclear DNA (nDNA) contains both unique single-copy and nonunique, duplicated or repetitive regions. Single-copy regions generally code for a particular gene product. Repetitive DNA consists of core sequences that are repeated in varying degrees. They may be made up of coding segments such as the ribosomal RNA (rRNA) genes, or noncoding tandemly repeated units. The latter include some of the most variable markers identified in the genome of eukaryotes: minisatellite and microsatellite sequences. The repeated units are linked together as consecutive tandem repeats and these repetitive sites may be found at one locus or at many regions scattered throughout the genome. Variation in the number of repeat units is common (Lewin, 1990). These variable number tandem repeats (VNTRs) are also called satellite DNA, in reference to the distinct "satellite" peaks seen in a CsC1 density gradient centrifugation. Large repetitive units of satellite DNA are often associated with heterochromatin near the centromere. Smaller regions (made up of repeat units [less than] 65 bp [base pairs]) are known as minisatellite DNA and can occur throughout the genome (Jeffreys et al., 1985a). Smaller still is microsatellite DNA, which has consecutive repeat units of only 2-6 bp (Tautz, 1989).

Another class of repetitive nuclear DNA is ribosomal DNA (rDNA). In eukaryotes, ribosomal DNA occurs in tandem repeats and codes for ribosomal RNA. Much of the sequence of rDNA is conserved across taxa, while other regions are quite variable. Therefore, these molecules are extremely useful in determining phylogenetic relationships, whether ancient or more recently derived (Hillis and Dixon, 1991). Within taxa, however, rDNA sequences are highly conserved so these regions are often less appropriate for most within-population studies (Flavell et al., 1986; Learn and Schaal, 1987 and Capossela et al., 1992).

Organellar DNA: the second major class of DNA is found in chloroplasts and mitochondria. Organellar DNA is inherited in a non-Mendelian, cytoplasmic fashion, often with uniparental (usually maternal) transmission. This unique feature of organellar DNA can be used to trace matrilines and is useful in studies of founder

effects, hybridization, and introgression (e.g., Avise et al., 1987, Moritz et al., 1987 and Cruzan et al., 1993).

Chloroplast and mitochondrial DNA molecules are small, [approximately] 120-220 kb and 15-17 kb, respectively, and circular (Brown et al. 1979, Palmer, 1985, 1987). Neither chloroplast DNA (cpDNA) nor plant mitochondrial DNA (mtDNA) are commonly used by population biologists because the former are highly conserved and the latter undergo frequent structural mutations, making it difficult to identify homologous regions (Dowling et al., 1990). In contrast, regions of animal mtDNA may exhibit considerable variation within and among populations. Various studies have shown that the rate of nucleotide substitution is greater in mtDNA than in coding regions of nuclear DNA (Brown et al., 1979). The mitochondrial genome also includes a small noncoding region known as the Displacement Loop (D-Loop), which serves as the origin of replication for the mitochondrial genome. In most animals, the D-Loop is much more variable than the rest of the mitochondrial genome and is therefore a very useful marker for the study of very recently divergent populations or species.

2.12. Genetic mutations; their types, places and importance:

Based on a book of Raven and Johnson, (1995), mutations are any permanent heritable change in the genetic material which is passed on to daughter cells. Mutations can occur in the genetic material of both germinal and somatic cells (nuclear DNA) and also in mitochondrial DNA (mtDNA). Mutations that may result in abnormal phenotypes include chromosome abnormalities (aneuploidy and structural changes), microdeletions (of genes), trinucleotide repeats (in 5' or 3' sequences, introns, exons), point mutations (one or a few bases). Mutations can be caused by defects in the DNA replication system, DNA repair system, or environmental mutagens.

Mutations are the **source of variety** upon which evolution acts. Natural selection allows the most "fit" genotypes to survive to reproduce. In prokaryotes which are haploid, mutation is the primary source of variation since sexual

recombination is rare. An example of selection which has profound effects for our health is antibiotic resistance of bacteria. The resistance is due to the selection of bacteria which have mutations that allow them to bypass the effects of the antibiotic. The antibiotic resistant bacteria survive to reproduce in that medium. Their DNA can be taken up by unrelated bacteria through a process known as transfection. Thus the transfected bacteria are also antibiotic resistant. Diploid organisms can harbor mutations without expressing them immediately since they can have a normal allele to cover for them (in the heterozygous state). Gene duplication (often in tandem) and diploidy (two copies of each gene) allow us to acquire and retain mutations which are bad, neutral or good. The neutral or bad (at the time) could prove useful for the species in the future.

2.13. Types of Mutations:

Mutations could be classified into two types according to the cell type which occurred as:

2.13.1. Somatic mutations (somatic cells): Somatic cells are the non-reproductive cells that make up our phenotype. Mutations in these cells are not passed on to offspring and therefore do not contribute to the genetic variation present in the next generation. They therefore do not directly affect the allelic composition of the population. However, note they can affect the allelic combination indirectly if they have an adverse effect on an individual. Cancer is an excellent example of this. Many types of cancer result in a mutation in the genes responsible for preventing the over-reproduction of a cell. Such mutations may result in runaway cell growth that eventually kills an individual.

2.13.2. Gametic mutations (cells destined to become gametes):

Gametic mutations are those mutations that occur in cells destined to become gametes. These are the important ones for evolution since it means these genes may be passed on to the next generation. It is gametic mutations that are the original source of new allelic variation in a population. Point mutations were originally defined as those involving a chromosomal region that was too small for the change to be detected cytologically.

2.14. Types and effects of point mutations:

2.14.1. Molecular nature of point mutations:

Point mutations can occur in a variety of ways (including frameshift mutations). Any change in a single base pair that alters a codon and causes an amino acid substitution in the coded protein is called a *missense* mutation. If one purine is replaced by another purine or if one pryimidine is replaced by another pyrimidine in the sense strand base sequence (with complementary changes in the antisense strand), the substitution is called a transition. If the substitution involves replacement of a purine with a pyrimidine or a pyrimidine with a purine, it is called a transversion.

2.14.2. Missense mutations:

Most base pair substitutions change the amino acid specified by the codon in which they occur. Such mutations are described as *missense* mutations because they cause an amino acid substitution in the coded protein. Depending on the nature of the amino acid substitution and its location within the protein, missense mutations may have a variety of effects, ranging from complete loss of biological activity to reduced activity or temperature-sensitive activity or no functional effect at all.

2.14.3. Nonsense mutations:

Base pair mutations that generate a translation stop codon (TAA, TAG or TGA in the DNA sense strand, transcribed as UAA, UAG or UGA in the mRNA) cause premature termination of translation of the coded protein and are referred to as *nonsense* mutations. In some cases, the effects of nonsense mutations can be suppressed by modified tRNA molecules that insert an amino acid with a low efficiency when a stop codon is encountered. Bacterial strains that contain such tRNAs are referred to as suppressor strains.

2.14.4. Silent mutations:

In some cases, base pair substitutions generate a different codon for the same amino acid, with no biological effect whatsoever. This is most likely to happen in

the third position (wobble base) of redundant codons for the same amino acid. Such changes are considered to be mutations because they alter the genetic code. However, because they have no phenotypic effect, even at the level of protein amino acid sequence, they are called silentmutations.

2.14.5. Frameshift mutations:

The genetic code is translated three nucleotide bases (one codon) at a time, with no punctuation between the codons. Addition or deletion of a single base pair in the middle of a coding sequence will result in out-of-frame translation of all of the downstream codons, and thus result in a completely different amino acid sequence, which is often prematurely truncated by stop codons (UAG, UAA, UGA) generated by reading the coding sequence out-of-frame. Such mutations, which are a special subclass of point mutations, are referred to as frameshiftmutations. Deletion of a single base pair results in moving ahead one base in all of the codons, and is often referred to as a positive frameshift. Addition of one base pair (or loss of two base pairs) shifts the reading frame behind by one base, and is often referred to as a negativeframeshift. Note that deletion or addition of three base pairs (or multiples of threes) does not cause a frameshift, but instead results in deletion or addition of one or more amino acids in the coded protein.

2.14.6. Conditional mutations:

Some types of mutations exert their phenotypic effects only under certain environmental conditions. Such mutations are called conditional mutations.

2.15. Genetic polymorphism; identification, types and importance:

In a formal sense, there are only two kinds of DNA polymorphism –those due to replacement of DNA bases and those due to insertion and deletion of bases –point mutation – usually show two alleles at each locus. Those that are due to insertion /deletion events often show more alleles; those that are due to changes in the number of a particular DNA sequence, such as the nucleotides GT repeated many

times, may be hypervariable, with more than five allele at a locus (Fries et al., 1990; Moore et al., 1992; Bishop et al., 1994 and Vaiman et al., 1994b). In some cases, the DNA sequence that is repeated is not exactly the same every time but there is sufficient similarity to discern the fundamental motif. Depending upon the size of the motif, the element is a microsatellite (repeat unit of 1-5 base pairs), a minisatellite (repeat unit of base pairs) or a satellite. The hypervariable loci tend to be either microsatellite or minisatellite. However, the hypervariable loci are less common than point mutations, with point mutations being approximately two orders of magnitude more common than microsatellites (Georges et al., 1987; Steele and Georges, 1991; Steffen et al., 1993; Moore et al., 1994 and Vaiman et al., 1994b).

The detection of these polymorphisms uses essentially the same technology and it is a rare technique indeed that can be used for only one or the other of these kinds of polymorphism. The techniques either recognize the identity of the sequence or recognize the effect of a change in sequence, and some techniques use a combination of the two. For example, a restriction endonuclease will cut DNA at a specific sequence, and mutations at the sequence will result in DNA of different lengths, corresponding to the presence or absence of the mutation. The lengths can be discrimination once the DNA is separated by size on a gel (Southern, 1975). However, the same system can be used to recognize a polymorphism if there is variation in the length of the fragment, due to repetitive elements, even if there has been no mutation at the restriction endonuclease site (Jeffreys et al., 1985b). Then the restriction endonuclease acts merely to cut the DNA to a manageable size for analysis.

Polymorphism can also be divided into those that are in or extremely near a sequence that is transcribed into RNA. The first kind, often called type I polymorphisms (O'Brien, 1991), are in sequences there are conserved across species and so are useful for studies of evolutionary history and for the practical matter of interpolating the expected locations of genes between species. The second kind, often called type II polymorphisms (O'Brien, 1991), are in sequences that are

usually unique to a species or occur in a narrow range of taxonomic groups. Despite their limitation for some studies, they form the backbone of studies to localize genetic factors to linkage maps, primarily due to their large number. They are often hypervariable.

The methods of detection DNA polymorphisms revolve around three major technologies, not all of which are used in every method. These are: (i) gel electrophoresis to separate DNA molecules by either length or volume; (ii) DNA oligonucleotide hybridization, in which oligonucleotides are hybridized to the target DNA; and (iii) DNA visualization, in which the DNA is either stained by silver, intercalated with ethidium bromide, or labeled with radioactive or fluorescent components with detection by various combinations of photography, ultraviolet (UV) fluorescence, autoradiography and laser excitation, with computerized capture of image. These methods have been revolutionized by polymerase chain reactions (Mullis et al., 1986), which result in the amplification of specific DNA sequences to quantities of any predetermined amount. Using genetic polymorphism to study evolution and variation in populations: Genes specify proteins, and proteins form the basis of physiology, development, appearance, and behavior of organisms. Genetic variation can be examined at any of these functional levels.Genetic diversity is measurable at any functional level from blueprint to phenotype.Thus, genetic variability within a population can be measured as:

1. The number (and percentage) of genes in the population that are polymorphic.

2. The number of alleles for each polymorphic gene.

3. The number (and percentage)of genes per individual that are polymorphic(Primack, 1993).

2.16. Factors affecting the amount and kind of genetic variation

All natural populations are exposed to a number of genetic factors or forces affecting the amount and kind of genetic variation. Such factors are mutation, chance events like genetic drift and founder events, selection and migration

(Hedrick, 2000a). Of the four, mutation is the only force that produces new genetic variation (in the form of new alleles). The others forces may alter the genetic composition or structure of a population.

2.16.1. Mutation

Mutation is the first factor, which always increases genetic variation, although most of the times with neutral and sometimes with negative effects. Mutation refers to a structural change in a gene, and it is thought to be a random occurrence. The probability of improving a gene function as the result of a mutation is extremely small. The genetic variation that has accumulated over many thousands of generations, however, reflects not only the environmental conditions to which that population was exposed but also the mutation that have been retained in the gene pool. An important consideration in conserving genetic diversity relates to the extent to which mutation is a factor in maintaining and creating genetic variation over time. Selection responses in livestock populations may continue in part because of genetic variation contributed by mutation (Enfield, 1998; Hill, 1982; Hill and Keightley, 1988). Testing the value of new or newly recognized mutations, however, can be time consuming. In contrast, previously existing mutations in the population presumably have already been studied and may be of greater value as a genetic resource.

2.16.2. Selection:

Selection can be either natural or artificial (imposed by humans); both are strong forces that alter the genetic composition of many livestock population. Natural selection is a continuous process reflecting environmental pressures, and occurs when a certain genetic type confers a reproductive advantage on an individual relative to other genotypes in that population. Artificial selection is superimposed on natural selection by animal breeders in an effort to change various traits in preferred stocks. The rate of response to selection of either kind depends on the variation in the population. More variable populations are expected to produce

more extreme types that can be selection or identified and propagated to produce more rapid genetic changes (BOA, 1993).

In livestock improvement, selection has operated historically at the level of the phenotype. In recent decades, however, methods such as progeny testing have been developed to estimate the genetic merit of an individual based on the performance of its offspring. Now, molecular methods for discerning the genotype are opening up new possibility for artificial selection based on the actual genes carried by an individual.

Natural selection applies selection pressure on a population for certain characteristics, and it may or may not be antagonistic to artificial selection. Likewise, environments vary widely, and a genotype that is at a selective advantage in one environment may lose that advantage or actually be at a disadvantage under different conditions. For example, the small goats living in the tsetse fly zones of East and West Africa are much more resistant to trypanosomiasis than imported European breeds. This phenomenon eventually leads to the development of genetically distinct populations adapted to specific environments. Genotype-by-environment interactions maintain allelic diversity if subpopulation of a species are raised under a wide range of production systems (BOA, 1993).

2.16.3. Migration

Migration or gene flow occurs as a result of the movement of individuals between populations In livestock production, crossbreeding (mating among two or more different breeds) and backcrossing (mating the crossbred progeny back to one of the parental breeds) are used to move favorable alleles from one population into a second population and can result in extremely rapid change in gene frequencies. Crossbreeding, however, can break up favorable combinations of alleles that can only be reconstituted through further breeding and selection (BOA, 1993).

2.16.4. Genetic drift

Genetic drift refers to the random changes in allele frequency. When population size is small, random genetic drift can lead to loss of alleles owing simply to change, particularly if they are present at low frequencies. A practical consequence is that the number of breeding individuals maintained in a preserved population must be sufficiently large to avoid the potential for decreased genetic variation resulting from genetic drift.

Genetic drift, on the other hand, in the end always reduces a population's genetic variation due to random loss of alleles. The other factors may in different situations either increase or reduce genetic variation. The relative importance and strength of each force differ among different species and populations (Barret and Kohn, 1991). In general, even though all factors are responsible for evolution in large as well as small populations, the conditions for mutation, migration and selection are more favorable in large populations at the same time as chance effects, like genetic drift, are small. In contrast, chance effects predominate in small populations where the impact of selection on allele frequencies is reduced, resulting in a loss of alleles and thereby reducing genetic variation (Hedrick, 2000b).

2.17. Identification and evaluation of population variability:

Markers for population identification: Tools to identify genetically different populations are markers. A marker can be described as a phenotypic trait or a genotypic entity easy to detect and having a simple genetic control. One can distinguish between three types of markers: phenotypic, biochemical and DNA markers. Morphological markers (mono or oligogenic) are usually dominant with a low level of polymorphism and are often influenced by environmental variations.

The low levels of variability of allozymes, their tissue specific expression and stringent sampling requirements make their usage restricted. With the isolation of DNA loci, including those with a large number of alleles, similar in all tissues, many novel applications have become possible (Cross et al., 1998). The phenotypic

markers includes: skeletal traits (craniometric parameters, skull shape, size, hump, and thorax depth), color and pattern [hair (morphological difference pelage, length, cuticular pattern), skin thickness; horn and pooling (size, length, color, shape: curvature, rotation, number of segments, distance between horn tips)].

The biochemical markers includes: blood groups; blood proteins [serum, albumin, alkaline phosphatase (ATP), amylase (Amy), transferring (TF)]; red cells [catalase (Cat), haemoglobin (hb), carbonic anhydrase (ca), purine nucleoside phosphorylase, esteras-D (Es-D), X protein, k-transporter (Ke)], lactoproteins: β-casein, κ- casein, α- lactoalbumin, β- lactoglobulin.

Prerequisites to make a genetic marker suitable are (Vienne, 1998): polymorphism: suitable level of variation codominance: no interaction between alleles non-epistatic: no interaction between loci non-pleiotropic :neutrality on other traits neutrality or non-neutrality: the choice will depend on the objectives in sensitive to the environment, single locus, stable, mendelian inheritance unambiguous, and easy scoring, low cost. There has been considerable technological progress in the eighties and early nineties in the development of markers, giving rise to a high proliferation.

Recently developments in molecular biology, such as quantitative genetic analysis, RFLPs, RAPDs, AFLPs, minisatellite, microsatellite and DNA sequencing have been applied to assay genetic diversity in animal population.

2.18. Molecular DNA markers

2.18.1. Types of DNA based markers:

O'Brien (1991) groups genetic markers into two types: Type I markers are associated with a gene of known function, and Type II markers are associated with anonymous gene segments of one sort or another. For now, RFLP remain the most common Type I marker presently mapped in the chicken (43 of 101 Type I markers in the EL map), although they will soon be eclipsed by efforts to add sequence-tagged site (STS: Olson et al., 1989) polymorphisms and microsatellite markers that exist within cloned cDNA [expressed sequence tags (EST)], as described by Smith

et al., 1996. A variety of Type II markers are now available and will be discussed in more detail below.

DNA-based markers could alternatively be grouped into clone/sequence-based (CSB) markers and fingerprint (FP) markers. The first category requires the isolation of a cloned DNA fragment and often determination of some, if not all, of its DNA sequence. The CSB markers include microsatellites (Weber and May, 1989), RFLP, STS and EST, among others. The FP markers require no a priori knowledge of the sequence of the polymorphic region or isolation of a cloned DNA fragment, and they include random amplified polymorphic DNA (RAPD, Williams et al., 1990); and their synonyms: DNA amplification fingerprinting (Caetano-Anolles et al., 1991); arbitrarily primed polymerase chain reaction (Welsh and McClelland, 1990), minisatellites [sometimes called variable number of tandem repeats (VNTR), Jeffreys et al., 1985a], amplified fragment length polymorphisms (AFLP) (Vos et al., 1995), and restriction landmark genome scanning (RLGS) (Hirot-sune et al., 1994), among others. The FP markers are inherently Type II markers, whereas CSB markers include both Type I and Type II markers.

In recent years the molecular biological approach has been revolutionized by the application of new technologies. In this regard one of the major role players has been the conceptually simple technique of polymerase chain reaction (PCR), which can be used to dramatically increase any target DNA in a logarithmic and controlled fashion (Nicholas, 1996). Polymerase chain reaction was conceived in 1983 although the first related publications only started to appear in 1985. In 1989, Science magazine selected PCR as the “major scientific development”, Taq polymerase (the enzyme essential to the success of PCR) as “molecule of the year” and in 1993, Kary Mullis received the Nobel Prize (Chemistry) for his role in developing PCR. Although its impact has been extraordinary, the concept of DNA amplification by PCR is simple. The chemistry involved in PCR depends on the complementarily (matching) of the nucleotide bases in the double stranded DNA helix. When a molecule of DNA is sufficiently heated, the hydrogen bonds holding

together the double helix are disrupted and the molecule separates or denatures into single strands. If the DNA solution is allowed to cool, the complementary base pairs are able to restore the original double helix. In order to use classical PCR, the exact sequence of nucleotides that flank (are situated on either side of) the area of interest (the target area to be amplified), must be known. This is the absolute minimum data necessary before a classical PCR reaction can be used. The researcher must determine or use previous data (known sequence data) to synthesize complementary 5´-3´ oligonucleotides (primers) of about 20 nucleotides. However, in using PCR for genotyping purposes, several random priming strategies are commonly employed, as will be discussed during the course of this review. After priming, the key to the amplification of the DNA is a heat stable polymerase. This enzyme reads the original template in the 3´-5´ direction and synthesizes a new complementary template in the 5´-3´ direction, using free deoxynucleotide triphosphates (dNTP's) as building blocks. A PCR thus consists of a number of cycles of denaturation, annealing and extension. The product synthesized in one cycle serves as a template in the next, so that the original DNA is doubled in every cycle and the target DNA molecules are typically increased by several millionfold. The amplicon or PCR-product can then be visualized on an agarose or polyacrylamide gel (Erlich, 1991; Nicholas, 1996; Turner et al., 1998).

PCR has the advantage of being a relatively fast, sensitive and reliable method. Not only can it amplify very small amounts of DNA, but can also amplify degraded or poor sources of DNA (Erlich, 1991; Strachen and Read, 1999). PCR has become an essential tool in molecular biology and plays a leading role in virtually all techniques that are presently applicable to the analysis and characterization of genomes. PCR is applied to amplify known sequences of a sample of DNA or for arbitrary priming of variable regions of the genome; the technique is suitable for use with various DNA markers such as Variable Number Tandem Repeats (VNTR's), Randomly Amplified Polymorphic DNA (RAPD), Amplified Fragment Length

Polymorphism (AFLP) and Restricted Fragment Length Polymorphism (RFLP) (Erlich, 1991).

2.18.2. Polymerase Chain Reaction (PCR):

The process known as polymerase chain reaction or PCR is developed by Kary Mullis in 1983 *(Mullis et al., 1986)*. The PCR is a biochemistry and molecular technique for exponentially amplifying a fragment of DNA, via enzymatic replication, without using a living organism *(Pavlov et al., 2004)*. PCR can be used for amplification of a single or few copies of a piece of DNA – single gene, part of a gene, or a non-coding sequence. As PCR is an *in vitro* technique, it can be performed without restrictions on the form of DNA, and it can be extensively modified to perform a wide array of genetic manipulation *(Altshuler, 2006; Deden et al., 2006)*. PCR is closely patterned after the natural DNA replication. Two oligonucleotide primers flank and define the target sequence to be amplified. These primers hybridize to opposite strands of the DNA to serve as an initiation points for the synthesis of new DNA strands. A thermostable DNA polymerase, such as a Taq DNA Polymerase catalyzes this synthesis. This enzyme reads the original template in the 3´-5´ direction and synthesizes a new complementary template in the 5´-3´ direction, using free deoxynucleotide triphosphates (dNTP's) as building blocks. A PCR thus consists of a number of cycles of denaturation, annealing and extension.The product synthesized in one cycle serves as a template in the next, so that the original DNA is doubled in every cycle and the target DNA molecules are typically increased by several million fold. This is only in 20 cycles PCR provides about million (220) copies of the target *(Mullis and Faloona, 1987; Buratowski, 1994; Koleske and Young, 1995; Stein et al., 1996; Aoyagi, 2001)*.

The amplicon or PCR-product can then be visualized on an agarose or Polyacrylamide gel *(Erlich, 1991; Nicholas, 1996; Turner et al., 1998)*. PCR has the advantage of being a relatively fast, sensitive and reliable method. Not only can it amplify very small amounts of DNA, but can also amplify degraded or poor sources of DNA *(Erlich, 1991; Strachen and Read, 1999)*. PCR has become an

essential tool in molecular biology and plays a leading role in all techniques that are presently applicable to the analysis and characterization of genomes. PCR is applied to amplify known sequences of a sample of DNA or for arbitrary priming of variable regions of the genome. The technique is suitable for use with various DNA markers such as Variable Number Tandem Repeats (VNTR's), Randomly Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP) and Restricted Fragment Length Polymorphism (RFLP) *(Erlich, 1991; Sunnucks, 2001; Freeman et al., 2006).*

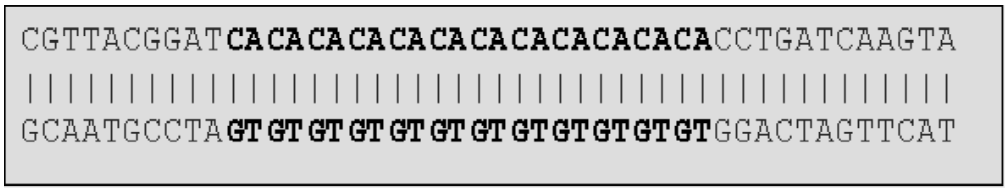
2.18.3. Restriction Fragment Length Polymorphism (RFLP):

This technique relies on the amplification of variable regions of the target genome, with the amplicon then being digested with one or more sequence-specific restriction enzymes. The DNA fragments of different lengths are then subjected to electrophoresis and fragments migrate according to their weights, the smaller fragments faster and the larger fragments more slowly (Nicholas, 1996). Thus, RFLP generally refers to the differences in banding patterns obtained from DNA fragments, after sequence-specific cleavage with restriction enzymes. Before the advent of PCR, RFLP analyses typically entailed restriction enzyme (RE) digestion of chromosomal DNA, followed by electrophoretic separation, membrane blotting and hybridization with a labeled probe, usually radioactive. Although reliable, this process is time-consuming, cumbersome and expensive. However, the ability to amplify a selected target a millionfold through PCR has abolished the need for the problematic blotting and hybridization analyses. Although many enzymes still need to be tested in the initial phase to be able to identify the polymorphism, it is today an easy, reliable and relatively cheap marker to use (Dodgson et al., 1997) and PCR-RFLP is, therefore, probably the simplest PCR procedure for comparisons of sequence polymorphism. Restriction enzymes (BamHI, EcoRI SacI and TaqI) have often been shown to be useful in obtaining RFLP patterns for haplotype identification in individuals (Smith et al., 1996; Spike et al., 1996). Potential disadvantages of the RFLP technique include the dimorphic nature, since a RFLP

only indicates the presence or absence of a cleavage site and, therefore, does not provide a great deal of genotypic information.

2.18.4. Microsatellites:

Microsatellites or Simple tandem repeats (STRs) are sequences made up of a single sequence motif (1-6 bp) which is repeated many times side-by-side. Historically, the term microsatellite has been used to describe only repeats of the dinucleotide motif CA/GT (Litt and Luty, 1989, Weber and May 1989). Microsatellites are generally assumed to be evenly distributed over genomes (e.g. Dietrich et al., 1996) but rare within coding regions (Hancock, 1995). There are, however, some human diseases caused by expansions of polymorphic trinucleotide repeats in genes such as fragile X and myotonic dystrophy, (e.g. Fu et al., 1991, Aslandis et al., 1992 and Rubinsztein, 1999). The figure below is showing the structure of a typical (dC-dA)n microsatellite.



*The structure of a (dC-dA)n microsatellite and the surrounding unique sequence. The (CA)n motif is shown in bold typeface and the unique flanking sequence is shown in plain typeface.*Microsatellites are almost ideal genetic markers. They are abundant, codominant, highly polymorphic and, very importantly, they are spread across the entire euchromatic part of the genome.

The existence of simple repetitive elements (the building blocks of microsatellites) in eukaryotic genomes has been documented since the 1970's, though the large number and almost ubiquitous distribution of these sequences throughout the genetic material of eukaryotes was first highlighted by Hamada et al., (1982), who found hundreds of copies of poly-(dC-dA)n sequences in yeast and tens of thousands in vertebrates. Three different reports appeared in the literature in that year detailing similar PCR based protocols for amplifying microsatellite regions from eukaryotic genomes (Litt and Lutty, 1989; Tautz, 1989; Weber and May, 1989). The microsatellite regions were amplified using two PCR primers

homologous to the unique sequence flanking the microsatellite region, and the resultant PCR products were discriminated on the basis of length using denaturing polyacrylamide gel electrophoresis. It was clearly evident from these initial reports that microsatellites would become vitally important tools for future studies of eukaryotic genomes.

Microsatellites were taken up quickly by the genome mapping community as the primary genetic marker for generating linkage maps of various mammalian genomes. Within a number of years, hundreds of these markers had been characterised from several species and by 1992 the first high density linkage maps were produced for humans and the mouse (Weissenbach et al., 1992; Dietrich et al., 1992). These markers have proved invaluable for the localisation of many human genetic disorders and have greatly expedited the identification of numerous disease lesions in the human genome.

Livestock geneticists were quick to realize the potential that microsatellites offered for studies of genomic variation in important domesticated animal species. It became evident that these loci could provide highly informative markers for the construction of genetic linkage maps which could be used in the search for quantitative trait loci associated with economically important traits (Georges et al., 1995; Womack and Kata, 1995).

Hundreds of microsatellites were characterised from the bovine, ovine and porcine genomes and these eventually led to the production of high density genetic linkage maps (Barendse et al., 1994; Bishop et al., 1994; Eggen and Fries, 1995). The availability of a wide range of microsatellites from livestock species has also generated interest in studies of variation and evolutionary relationships among livestock populations and a number of such studies have appeared in the literature (Buchanan et al., 1994; Machugh et al., 1994 and Forbes et al., 1995).

2.18.4.1 Microsatellite Evolution:

There are two potential mechanisms, which can explain the high mutation rates of microsatellites. The first is recombination between DNA molecules by unequal crossing-over or by gene conversion (Smith 1976; Jeffreys et al. 1994a).

The second mechanism involves a process that has been multiply referred to as "DNA slippage", "polymerase slippage", or "slipped strand mispairing". In essence, this slippage is thought to occur within the complex of proteins that mediates DNA replication, as a consequence of mispairing (by one repeat unit or occasionally more) between the original template strand and the newly synthesized DNA strand. The resulting region of unpaired DNA is then forced to "loop out". If this "loop" is on the new strand the net effect is addition of a repeat unit; if it is on the template strand it is removed by enzymes, and the net effect is loss of a repeat unit. Larger order repeats would require either strand to slip further before the bases could pair correctly again, and would potentially explain why they are less common or often more stable. Other factors such as the number, location, and sequence of repeats are also likely to affect the rate and direction of slippage, although as yet no simple or universal rules have been found (Levinson and Gutman, 1987; Schlọ̈tterer, 1998).

2.18.4.2. Applications of microsatellites:

The application of microsatellites could be summarized as follows:

(A)In forensics:

The purpose of DNA typing in forensic medicine is to match a sample from the crime site with a suspect. DNA microsatellite testing has proven to be as powerful for exonerating suspects as it has for convicting them. A great diversity of criminal detection has benefited from DNA testing, and it has been especially valuable in solving rape and murder cases. Additional examples include robbery, assault, kidnapping, car accidents, extortion and blackmail. (Pestoni et al., 1995; Ghosh et al., 2003 and Forrest et al., 2004).

(B)Parentage testing:

It also has been successfully applied to parentage determination and useful in settling certain immigration disputes that hinges on proving family relationships (Mommens et al., 1999; Heyen et al., 1997; Luikart et al., 1999; Schnabel et al., 2000). A special interest was focused on horse parentage testing (Binns et al.; 1995; Bowling et al. 1995 and Tozaki et al., 2001).

(C)Construction of linkage map:

The term gene linkage map was proposed by Morgan (1911), after its observation that: Loci of two genes close together on the same chromosome do not assort independently at meiosis. For this reason gene loci on a chromosome can be mapped by measuring the frequency of recombinants produced by crossing-over. It was evident from the early work of Morgan that recombination frequencies were not always the same for two different genes, so it was suspected that it depended on the distance of the genes on the chromosome.

Microsatellites were used in construction the linkage map for cattle and other domestic species. In cattle for example the linkage map was developed rapidly during the last 10 years. The first cattle linkage map (Barendse et al., 1994; Bishop et al., 1994) was named low density map since the number of mapped markers were low. In the medium linkage map the number of mapped markers was much more the low density map (Barendse et al., 1997; Kappes et al., 1997). Now the cattle linkage map, became in a mature phase after the appearance of high density map. Due to great number of loci map, the high density linkage map is extended to map only chromosome by chromosome (Drogemuller et al., 2002; Ihara et al., 2004; Reed et al., 2004).The genetic map unit is the distance between two genes, for which one product of meiosis out of 100 is recombinant. In other words, a recombination frequency of 1% is defined as a map unit. A map unit is also called centiMorgan in honor of the discoverer of gene linkage.

The linkage map has many applications such as: Determine if trait associations are due to linkage or pleiotropy (Freyer, et al., 2004). Finding genes are determining qualitative and quantitative phenotypes. Establish evolutionary relationships: homoeology, synteny and orthology. (Hillis et al., 1996).

Homoeology: Refers to chromosomes, or chromosome segments, and which are similar in terms of the order and function of the genetic loci. Homoeologous chromosomes may occur within a single allopolyploid individual (e.g. the A,B, and D, genomes in wheat), or they may be found in related species (e.g. the 1A, 1, B, 1D series and wheat and the 1H of barley). Orthology: Refers to genes in different species which are so similar in sequence that they are assumed to have originated

from a single ancestral gene. Synteny: Refers to genetic loci that are linked on the same chromosome.

(D) Mapping quantitative trait loci:

The basic assumption underlying the use of marker loci in QTL research is that linkage disequilibrium exists between alleles at the marker locus and the liked polygene. Linkage disequilibrium is the non-random association of alleles at different loci in a population caused by selection, genetic drift and other factors. The predominant cause in primary segregating populations is physical linkage of loci. The segregating population is divided according to their marker loci, then statistical procedures are used to determine if individuals differ significantly from each other with respect to the trait being measured. If a significant difference is detected this is interpreted as a gene affecting the trait is linked to the marker locus used to subdivide. Microsatellites extensively used in mapping many important QTLs in cattle such as: ovulation and twining rate (Gonda et al., 2004; Cruickshank et al., 2004); milk production (Ashwell et al., 2004; Freyer et al., 2003; Heyen et al., 1997; Velmala et al., 1999); growth and carcass composition (Casas et al., 2004).

(E) Genetic diversity and phylogenic studies:

Microsatellites are extensively used in studying genetic diversity, identification the relationship between different breeds. It is also used in the construction of phylogenic tree and understanding the migration of breeds between countries. These studies is not limited to cattle (Bradley et al., 1996; Maudet et al., 2002; Edwards, et al., 2003; Hansen et al., 2002), but is extended to many species such as sheep (Diez-Tascon et al., 2000; Arranz et al., 2001; Jia et al., 2003; Tapio et al., 2003) and goat (Saitebekova et al., 1999; Li et al., 2002; Maudetr et al., 2002; Behl et al., 2003), buffalo (Barker et al., 1997a,b; Hassanane et al., 2000; Van Hooft et al., 2000; Moioli et al., 2001), deer (Wang and Schreiber, 2001) and camels (Lang et al., 1996; Penedo et al., 1999 and Evdotchenko et al., 2003).

Additional to the main previous applications of microsatellites there are some small applications should be taken into consideration. Microsatellites are used as a diagnostic tool to detect human diseases (e.g. Green, 1993; Lamberti et al., 1999).

Nowadays microsatellites are regularly used in population and ecological studies. Microsatellites are excellent markers for studying gene flow, effective population size (Nm), dispersal and migration related issues, and parentage and relatedness (e.g. Taylor et al.,. 1994, Coulson et al.,1998, Ciofi and Bruford, 1999, Goldstein et al.,1999, Luikart and England, 1999). Microsatellites can also be used to study the effects and level of inbreeding (Beaumont and Bruford, 1999; Pemberton et al., 1999, Sweigart et al., 1999).

Many authors show the advantages and disadvantages of SSR markers

(Bishop et al., 1994; Bishop et al,. 1995; Baron et al,. 2002; Mburu and Hanotte, 2005; Erhardt and Weimann, 2007).

2.18.4.3. Advanatages of Microsatellites:

low quantities of template DNA required (10-100 ng), highgenomic abundance, random distribution throughout the genome, high level of Polymorphism, band profiles can be interpreted in terms of loci and alleles, co dominant markers, allele sizes can be determined with high accuracy, comparison across different gels possible using size standard, high reproducibility, and different microsatellites may be multiplexed in PCR, wide range of applications, amenable to automation.

2.18.4.4 Disadvantages of Microsatellites:

initial high development costs, heterozygotes may bemisclassified as homozygotes when null-alleles occur due to mutation in the primer annealing sites, stutter bands may complicate accurate scoring of polymorphisms, underlying mutation model (infinite alleles model or stepwise mutation model) largely unknown, homoplasy due to different forward and backward mutations may underestimate genetic divergence, time-consuming and expensive to develop, microsatellite markers help to identify neutral biodiversity but do not provide information on functional traits biodiversity.

CHAPTER THREE
MATERIAL AND METHODS

3-1. Study area:

Kordofan region is situated in the central part of Sudan extending from 9.50 to 16.40^{o}N latitudes and from 27.30 to 32.25^{o}E longitudes. The region covers an area of approximately 380,000 km2, representing 24% of the total area of the country. The total population of the region is about 3.25 million, comprising 15% population of Sudan. Urban population constitutes 15%, nomads 20% and the sedentary rural population constitutes 65% of the total population.

Administratively the area is divided into two federal States, North and South Kordofan States.WSRMP Livestock Breed Characterization Study field survey (2011).

3-2 North Kordofan :

North Kordofan State is located between latitudes 11.15 and 16.45^{o}N and longitudes 27.05 to 32^{o}E. It covers an area of about 245,000 km2, representing two third of the region. It is divided into nine localities i.e. Sheikan, Um Ruwaba, Bara, Wad Banda, Sodari, Gabrat el Sheikh, EnNhud, Abu Zabad and Gubeish. It has a total population of about 2.3 million comprising 70 population of the region. WSRMP Livestock Breed Characterization Study field survey (2011).

3-3 Climate:

Annual rainfall ranges from less than 50 mm on the northern border to more than 800 mm on the southern border. The length of the rainy season varies from about one month or less in the north to about five months in the south. In North Kordofan, the rainy season does not last for more than three months. Rains occur between May-October with the peak in August. Within and between seasons variation in rainfall amount and distribution is common.

Northern part of Kordofan region characterized by extremely high rainfall variability from year to year as well as from one place to another. WSRMP Livestock Breed Characterization Study field survey (2011).

3-4 Sampling and questionnaire methodology

The survey was conducted through questionnaire guided interviews with cattle owners (household survey) in selected districts in fuga cattle habitats. One district for each group was selected according to the distribution of breed population Five villages were selected according to the clustering of villages within the district and to accessibility (150) cattle owners were selected from villags .

Table (1) the villages selected for the survey in fuga cattle areas

The villages selected for survey in fuga cattle area	Frequency	Percent(%)
Aial Erabei	35	23.3
Fuga	35	23.3
Wad banda	30	20.0
El kapara	30	20.0
Wad Abu Raceian	20	13.3
Total	150	100.0

A set of detailed structured questionnaires were prepared and used to collect information from a total of 150 cattle owners in fuga cattle areas (35 interviews for Aial Erabei and Fuga , 30 interviews for Wad banda and El kapra ,and 20 interviews from Wad AbuRaceian),in visit interviews (Table1).

The questionnaires were pre-tested to check clarity and appropriateness of the questions. Some of the information collected during interviews were supported by observation. The questionnaires were designed to obtain information on general household characteristics, livestock and herd management, breeding practices, production objectives, and production constraints.

3-5 Um.Banein Research Station for Kenana Breed:

Are located at western bank of the Blue Nile River . At latitude 14o - 24, N, and13o 04, N longitude 33o – 29 E and 33o- 57 E and altitude 407 and 435 meters above sea level.

The climate at Um-banein is mainly of Savannh zone . The annual rainfall ranged from 323 to 631 mm within a total annual average of 481mm.The respective highest and lowest daily means temperature of 44.4 and 14.3oc occurred in Abril and

January. The herd of Um-Banein had an access to sorghum stubbles and Stover and cotton stubbles during early winter and natural grazing during the wet summer and early winter .The dry livestock were fed low quality roughages and different types of hay added to any surplus of green feeds and concentrate premixes. The concentrate rations were composed of wheat barn ,crushed durra (sorghum)and or wheat grains, oil seed cakes and recently molasses with some mineral salts and urea. This was fed according to milk production during and after milking.

The animal at Um-Banein were housed in metallic fences made from local material of wood.

Hand milking was applied twice a day. Breeding was controlled using the best bulls selected according to the high merit of their dams milk productivity.

3-6 Atbara Research Station For Butana Breed:

Is situated in the eastern bank of the River Nile northern to River Atbara. At latitude 17o - 40, N, longitude 33o - 58, E and altitude 345 meters above sea level.

The climate at Atbara is mainly of the semi-desert. The annual rainfall ranged from 1.1 to 238.9 mm within annual average of 90.1mm.The highest and minimum means daily temperature were 42.8 and 14.4 oc registered in June and January successively.

The major forages cultivated in Atbara research were Abu-70 (sorghum bicolor), Sudan grass (Sorghum Sudanese), Lubia (Dolicus Lablab), Clitoria (Clitoria ternatea).Also there was some limited area of Berseem(Medicago sativa)available for the herd of Atbara .These fodders were mainly fed to the milking cows and the pre weaned livestock in addition to some highly quality roughages and hays of the abovementioned fodders.

The animal at Atbara were housed in metallic fences but those at um-Banein.

Hand milking was applied twice a day. Breeding was controlled using the best bulls selected according to the high merit of their dams milk productivity.

3-7 Blood Samples Collection :

Ninety blood samples were collected from three different regions representing the three suspected breeds under study. Randomly selected pure adult breed mainly

Dar El Reel cattle (Fuga cattle breed) Butana , and kenana.From each animal 5 ml from peripheral blood was collected from a vein in the neck of each cattle on vactounar tube containing EDTA as anti coagulant matter. The samples were transferred to the laboratory in a shadow and kept away from the direct sunlight. The samples were kept at 4 °C and processed for DNA extraction in a period not exceed 7 days from its arrival to the laboratory.

3.8 DNA Extraction in Central Laborotary of Ministry of Science and Technology Sudan.

The method described by Sambrook et al., 1989, was used for DNA isolation and purification, the extraction was usually performed in two successive days as follows:

1.Collect 3-5 ml blood in EDTA tube to falcon tube 25ml.

2.Add 10ml of Red Cell lysis Buffer (RCLB) centrifugation for 5 min at 6000rpm.

3.Repeat the previous step until a clear pellet of white blood.

4.Add 2ml lysis buffer.

5.Add 10 µl of proteinase k 1ml Guanidine chloride and 300µl NH4 acetate. Incubate over night at 37^{o} C over night.

6.Cool to room temperature.

7.Transfer to pre-chilled 2ml chloroform in 15 ml falcon tube.

8.Vortex and then centrifuge for 10 min at 6000 rpm.

9.Collect the upper layer into anew falcon tube and add 10ml cold absolute ethanol.

10.Shake and keep at – 20^{o}c for at least 2 hours or overnight.

11.Centrifuge at 6000rpmfor 15-20min.carefully drain the supernatant. Invert the tube on tissue paper for 5min

12.Wash the pellet with 4mlof 70% ethanol.

13.Repeat step11.

14.Centrifuge at 3000rpm for 15 min.

15.Pour off supernatant and allow the pellet to dry for 1-2 hours .

17.Resuspend the pellet in 200µl of d H2o.

17.Alquot the DNA into stock solution(store at-20^{o}c)or working solution(store at 4^{o}c).

3-9 DNA quantification in National Research Center- Cairo, Egypt- Dokki.

The DNA concentration was measured using the U.V spectrophotometer at wavelength 260 nm. The concentration was adjusted to 50 ng (nano-gram) by adding sterile double distilled water.

3.10 Molecular Markers

The microsatellites markers used in this study were chosen according to the recommendation of International Society of animal genetics (ISAG) for genetic diversity studies. Details of these markers are in the next table:

Marker name	Chromosomes location	Primer sequence	Reference
BM1824 (D1S34)	1	F. GAG CAA GGT GTT TTT CCA ATC R. CAT TCT CCA ACT GCT TCC TTG	Barendse et al.;(1994)
BM2113 (D2S26)	2	F. GCT GCC TTC TAC CAA ATA CCC R. CTT CCT GAG AGA AGC AAC ACC	Sunden et al.; (1993)
INRA023 ((D3S10)	3	F. GAG TAG AGC TAC AAG ATA AAC TTC R. TAA CTA CAG GGT GTT AGA TGA ACT C	Vaiman et al.; (1994)
SPS115 (D15)	15	F. AAA GTG ACA CAA CAG CTT CTC CAG R. AAC GAG TGT CCT AGT TTG GCT GTG	Moor and Baylor (1993)
TGLA122 (D21S6)	21	F. CCC TCC TCC AGG TAA ATC AGC R. AAT CAC ATG GCA AAT AAG TAC ATA C	Georges & Massey (1992)
TGLA126 (D20S1)	20	F. AAT CAC ATG GCA AAT AAG TAC ATA R. CTA ATT TAG AAT GAG AGA GGC TTC T	Georges & Massey (1992
TGLA227 (D18S1)	18	F.TTG GTC TCT ATT CTC TGA ATA TTC C R.GAG CAA GGT GTT TTT CCA ATC	Georges & Massey (1992
ETH10(D5S3)	5	F.CAT TCT CCA ACT GCT TCC TTG R.GCT GCC TTC TAC CAA ATA CCC	Solinas–Toldo et al.; (1993)
ETH225(D9 S1)	9	F: GATCACCTTGCCACTATTTCCT R: ACATGACAGCCAGCTGCTACT	Steffen et al. ;(1993)

3.11 DNA Amplification, and Genotyping:

The 9 microsatellite markers used are ETH 10, ETH 225, BM2113, BM1824, SPS115, TGLA122, TGLA126, TGLA227 and INRA23 were amplified in one multiplex polymerase chain reactions (PCR) using fluorescence-labeled primers. Amplicons obtained by PCR were separated by electrophoresis on an ABI 3730 instrument (Applied Biosystems) according to manufacturer recommendations and allele sizing was accomplished by using the internal size standards GeneScan_500 LIZ (Applied Biosystems). Allele nomenclature followed was that recommended by the Cattle Molecular Markers and Parentage Testing Workshop at the ISAG Conference of Cairns in 2012.

3-12 Microsatellite analysis :was carried out in Facultad de veterinaria 28040 Madrid - Spain .One PCR multiplex consists of nine fluorescence-labeled microsatellite primers were used for the analysis are (ETH 10, ETH 225, BM2113, BM1824, SPS115, TGLA122, TGLA126, TGLA227 and INRA23). The multiplex is under the recommendation of ISAG (2012). 'For amplification, 100 ng of genomic DNA was added to a reaction mixture containing 50 pMol of fluorescence-labeled forward and reverse primers; 200 μM of every dNTPs; 1.5 mM of MgCl2 and 0.5U of Taq polymerase in a final volume of 25 μl. The amplification procedure was: initial denaturation step of 1 min at 95°C, 35 cycles of 1 min at 95°C, annealing 1 min at 57°C and 1 min at 72°C and a final extension of 5 min at 72°C.

3.13 Statistical analysis of the results:

3.13.1 The SPSS Statistical Package for Social Science software (for windows, release 18) was used to analyze the data. The analysis was implemented for fuga cattle owners. Results are presented mainly in the form of descriptive tabular summaries.

3.13.2 Microsatellites analysis was used:

POPGENE software package (Yeh et al., 1999) was used to calculate allele frequencies, observed number of alleles, effective number of alleles (Kimura and Crow, 1964), observed (Ho) and expected (He) heterozygosity at each locus in the five populations under study. Polymorphism information content (PIC) value for each locus was calculated by using the method described by Bostein et al., (1980). Pair-wise alleles sharing were calculated manually from the raw results.

Using the variance-base method of Weir and Cockerham (1984), population differentiation by F-statistics was computed using FSTAT version 2.9.3.2 computer program (Goudet, 2002). Mean a standard deviations of the F-statistics program, F .f, that are analogue to Wright's (1951, 1978) Fis and Fst, were obtained across breeds by the Jackknifing procedure over loci (Weir, 1990).The extent of global inbreeding was further studied with the same software by estimated Fis value.

The effect of migration and gene flow on the genetic structure of the analyzed populations was estimated between each pair of populations according to an island

model under neutrality and negligible mutation (Slatkin 1993). Genetic distances among populations were estimated using (Ds) standard genetic distance of Nei, 1972 and the DA distance of Nei et al., 1983.

CHAPTER FOUR

RESULT

4.1 .A. Phenotypic Characterization of Fuga breed:

The result of the quetionneir of the study indicated that livestock in fuga area, cattle ranked first, then followed by sheep and goat, as illustrated in table (A1)

Table (A 1) Importance of livestock species in survey area

Importance of livestock in survey area	Frequency	Percent (%)
Cattle	102	68
Sheep	45	30
Goats	3	2
Total	150	100

The importance of livestock and crop farming in Fuga cattle area is presented in Table A2. The results showed that, the majority of Fuga cattle owners (68.7%) indicated both livestock and farming to be their main activity, and (28.7 %) of Fuga cattle owners put livestock only first, while (2.7%) of the Fuga cattle owners indicated crop farming only to be a main activity.

Table (A 2) The importance of livestock and crop farming in fuga cattle area.

The importance of livestock and crop farming in fuga cattle area	Frequency	Percent(%)
Livestock only	43	28.7
Crop farming only	4	2.7
Livestock and farming	103	68.7
Total	150	100

The important characteristics used in selection of Fuga bulls are illustrated in Table A3. The dam performance and body size was more important for Fuga cattle owners (64.7%) and other characteristics such as shape similarity to dam or sire (18%) and dam performance ranked relatively low in importance (10.7%) and the last characteristics used to select breeding bull in Fuga cattle sire performance and body size (4.7% , 2%), respectively.

Table (A 3) Characteristics used to select breeding bull in Fuga cattle area

Characteristics used to select breeding bull in Fuga cattle area	Frequency	Percent (%)
Dam performance	16	10.7
Sire performance	7	4.7
Body size	3	2
Shape similarity to dam or sire	27	18
Dam performance and Body size	97	64.7
Total	150	100

Most of breeders are keeping Fuga cattle for their milk consumption (81.3%) or as insurance against financial problems. While 18.7% of the primary reason was for keeping cattle to generate income from the sale of milk and animals Table A4.

Table (A 4) Purposes of keeping cattle

Purposes of keeping cattle	Frequency	Percent (%)
Primary reason for keeping cattle to generate income from the sale of milk and animals	28	18.7
Milk for home-consumption or as insurance against financial problems	122	81.3
Total	150	100

In the Fuga area, the major portion of income was spent to solve financial problems (30%), while (21.3%) from cattle breeders indicated reason for sales cattle financial problems - Old age and(14.7%) of cattle breeders reason of cattle sales Finance agriculture and purchase of animal feed , old age, Social activities, Investment, Purchase of animal feed -financial problems , Finance and Social activities , old age was percentage (5.3% , 8%, 9.3%, 1.3% , 6% 1.3% 2.7%), respectively in table A5.

Table (A5) Reasons of cattle sales

Reasons of cattle sales	Frequency	Percent (%)
Purchase of animal feed	8	5.3
Financial problems	45	30
Finance agriculture	22	14.7
Old age	12	8
Social activities	14	9.3
Investment	2	1.3
Purchase of animal feed, Financial problems, Finance agriculture.	9	6
Financial problems, , Old age	32	21.3
Finance agriculture, Social activities	2	1.3
Purchase of animal feed , Financial problems, Finance agriculture, Old age Social activities	4	2.7
Total	**150**	**100**

Most Fuga breeders consider that the production objective for keeping cattle to generate income from the sale of milk and animals, milk for home-consumption and Income from sale of animal ,or as insurance against financial problems. Received a lower ranking among Fuga cattle breeders. Were presented in table A6

Table (A6) Production objectives of keeping cattle and the ranking of the importance of this objective:

Production objectives of keeping cattle and the ranking of the importance of this objective	Frequency	Percent (%)
Income from sale of milk	60	40
Milk for home-consumption	61	40.7
Income from sale of animal	22	14.7
Insurance against, financial problems	7	4.7
Total	150	100

Disease prevalence -Lack of cattle feed and Disease prevalence was mentioned as the most important constraint by most of the cattle owners (33.3% and 30%, respectively). Lack of cattle feed- Disease prevalence and water shortages (24%) ware the third most important constraint particularly in Fuga area. Also considered lack of cattle feed and water shortage as a constraint (9.3%, 3.3%) respectively presented that in table A7.

Table (A **7) Production constraints, which were defined by the cattle owners in area study**

Production constraints	Frequency	Percent (%)
Lack of cattle feed	14	9.3
Disease prevalence	45	30
Water shortage	5	3.3
Disease prevalence , Lack of cattle feed	50	33.3
Lack of cattle feed, Disease prevalence, Water shortage	36	24
Total	150	100

The reproductive performance and milk performance during the beginning, middle and end of lactation are demonstrated in table A8. The lactation length of Fuga cattle averaged 208.60 ± 5.37 days, and the age at first calving in Fuga cattle averaged 40.49 ± 6.76 month. Calving interval was 15.90± 0.44 months, and number of services per conception was 2.27±0.7, and weight of birth was 22.06±0.44kg and milk yield per day was 5.26±0.29 kg, and milk yield per lactations was 1100.96±86.18 kg/lactation.

Table (A 8) Production and reproduction performance of Fuga cattle under field conditions: (Parameters study):

Parameters	No.	Mean ± S.E
Milk yield, Kg		
-Beginning of lactation	150	8.84±0.53
-Middle of lactation	150	4.93±0.33
-End of lactation	150	2. 44±0.16
Lactation period(days)	150	208.60± 5.37
Age at first calving(months)	150	40.49 ± 6.76
Calving interval(months)	150	15.90 ± 0.44
Number of service per conception	150	2.27±0.7
Weight of birth per (kg)	150	22.06±0.44
Milk yield per day (kg)	150	5.26±0.29
milk yield per lactation (kg)	150	1100.96±86.18

4-2 B. Genetic Characterization of Breeds (Fuga, Butana and Kenana) of Study:

Results of the genetic characterization in this study were shown in the following table (B1-to B18) and histograms(1 to 9).

Table (B1) Microsatellites and alleles nomenclature:

Microsatellite	allele nomenclature																		
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**	**M**	**N**	**O**	**P**	**Q**	**R**	**S**
BM1824	178	108	182	-	-	188	-	192											
BM 2113	129	-	133	135	137	139	141	-	145										
ETH10	209	211	213	-	217	219	221	-	225										
ETH225	140	-	144	-	148	150	-	154	156	158									
INRA23	196	198	200	202	204	206	208	210	212	214	216								
SPS115	244	246	248	250	252	254	256	258	260										
TGLA122	137	139	-	143	145	-	151	153	-	157	159	161	-	-	165	-	-	-	173
TGLA126	115	117	119	121	123	125	127												
TGLA227	77	79	81	83	-	87	89	-	93	95	97	-	101						

Table (B1) showed that a total number of alleles were detected for the nine loci 74 , the observed number of alleles ranged from 5 (BM1824) to 11 (TGLA122) , with alleles diversity the average number of observed alleles per locus (8.22).

Table (B2) Number and size of allele's in the BM1824 microsatellite markers and their frequencies in each breed.

Allele number	Allele Size (bp)	Allele frequencies			
		Fuga	Butana	Kenana	All Breeds
1	178	0.091	0.068	0.136	0.295
2	180	0.568	0.295	0.227	1.09
3	182	0.159	0.205	0.205	0.569
4	184	0.000	0.000	0.000	-
5	186	0.000	0.000	0.000	-
6	188	0.045	0.000	0.136	0.181
7	190	0.000	0.00	0.000	-
8	192	0.136	0.431	0.295	0.862

A total number of alleles observed of this marker were eight allele domenstrated in table (B2) .Their size ranged from (178-192 bp). In Fuga breed the most frequent alleles were alleles number (2) while the allele number (4-7) were not present in this breed. In Butana breed the most frequent allele is the allele number (8) while the alleles number (4-5-6-7) were not present in this breed. In Kenana breed the most frequent allele is allele number (8) , while the alleles number (4-5-7) were not present in this breed.In all breed the most frequent allele is allele number (2).

Allele frequency distribution for microsatellite markerBM2113 in the three cattle breeds , are presented in histogram (4-1) by horizontal axes denote alleles number and vertical axes show the frequencies found for each alleles in the breeds.

Histogram (4-1) Allele frequency distribution for microsatellite markerBM2113

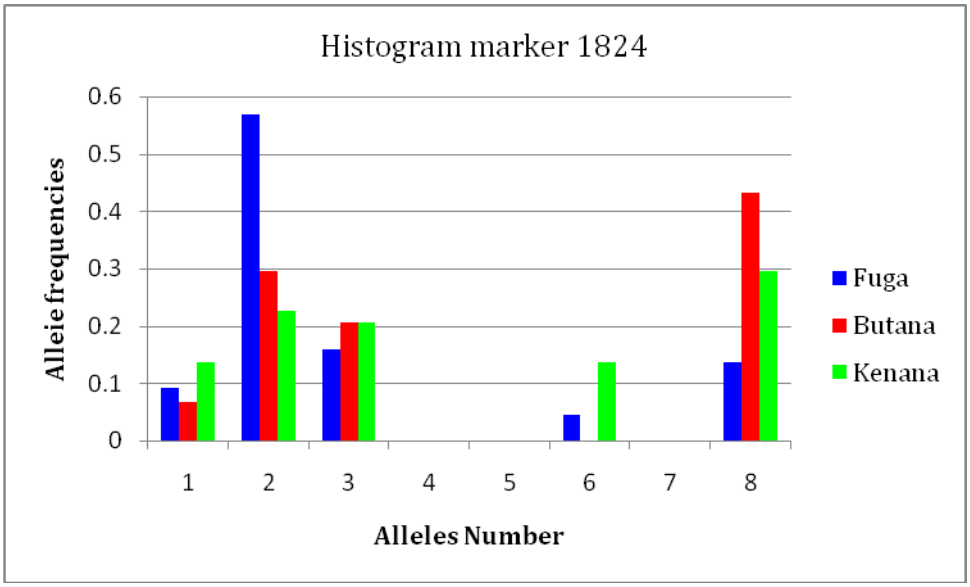


Table (B3) Number and Size of allele's in the BM2113 microsatellite markers and their frequencies in each breed.

Allele number	Allele Size (bp)	Allele frequencies			
		Fuga	Butana	Kenana	All Breeds
1	129	0.159	0.181	0. 205	0.545
2	131	0.000	0.00	0.000	-
3	133	0.068	0.023	0.023	0.114
4	135	0.205	0.409	0.341	0.955
5	137	0.227	0.023	0.045	0.295
6	139	0.045	0.250	0.273	0.568
7	141	0.227	0.114	0.114	0.455
8	143	0.068	0.000	0.000	0.068
9	145	0.000	0.000	0.000	-

The result in table (B3) indicated that a total number of alleles which observed of this marker were nine allele their size were ranged from (129-145bp). In Fuga breed the most frequent alleles were alleles number (7) while the allele number (2-9) were not present in this breed. In Butana breed the most frequent allele is the

allele number (4) while the alleles number (2-8-9) were not present in this breed . In Kenana breed, the most frequent allele is allele number (4) , while the alleles number (2-8-9) were not present in this breed. In all breed the most frequent allele is allele number (4).Allele frequency distribution for microsatellite markerBM2113 in the three cattle breeds , are presented in histogram(4-2) .Horizontal axes denote alleles number and vertical axes show the frequencies found for each alleles in the breeds.

Histogram (4-2) Allele frequency distribution for microsatellite marker BM2113

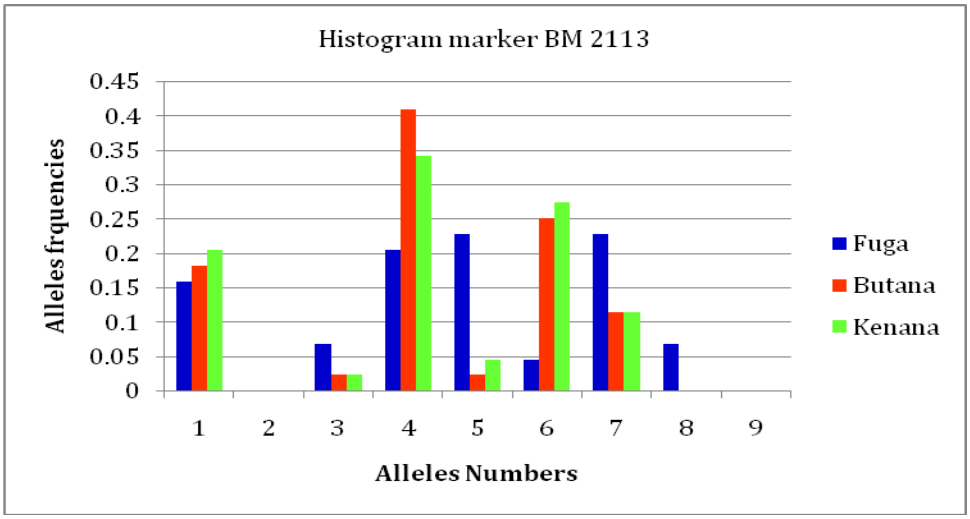


Table (B4) Number and size of allele's in the ETH10 microsatellite markers and their frequencies in each breed;

Allele number	Allele Size (bp)	Allele frequencies			
		Fuga	Butana	Kenana	All Breeds
1	209	0.591	0.500	0.750	1.841
2	211	0.068	0.159	0.091	0.381
3	213	0.205	0.273	0.114	0.592
4	215	0.000	0.000	0.000	-
5	217	0.023	0.000	0.045	0.068
6	219	0.023	0.000	0.000	0.023
7	221	0.045	0.068	0.000	0.113
8	223	0.000	0.000	0.000	-
9	225	0.045	0.000	0.000	0.045

Table (B4) showed that atotal number of alleles which observed of this marker were nine allele their size ranged from (209-225 bp). In Fuga breed the most frequent allele is allele number (1) while the alleles number (4-8) were not presnt in this breed. In Butana breed the most frequent allele is the allele number (1) while the alleles number (4-5-6-8-9)were not present in this breed . In Kenana breed the most frequent allele is allele number (1) ,while the alleles number (4-6-8-9)were not present in this breed.In all breed the most frequent allele is allele number (1).

Allele frequency distribution for microsatellite markerETH10 in the three cattle breeds, are presented in histogram (4-3). Horizontal axes denote alleles number and vertical axes show the frequencies found for each alleles in the breeds.

Histogram (4-3) Allele frequency distribution for microsatellite marker ETH10

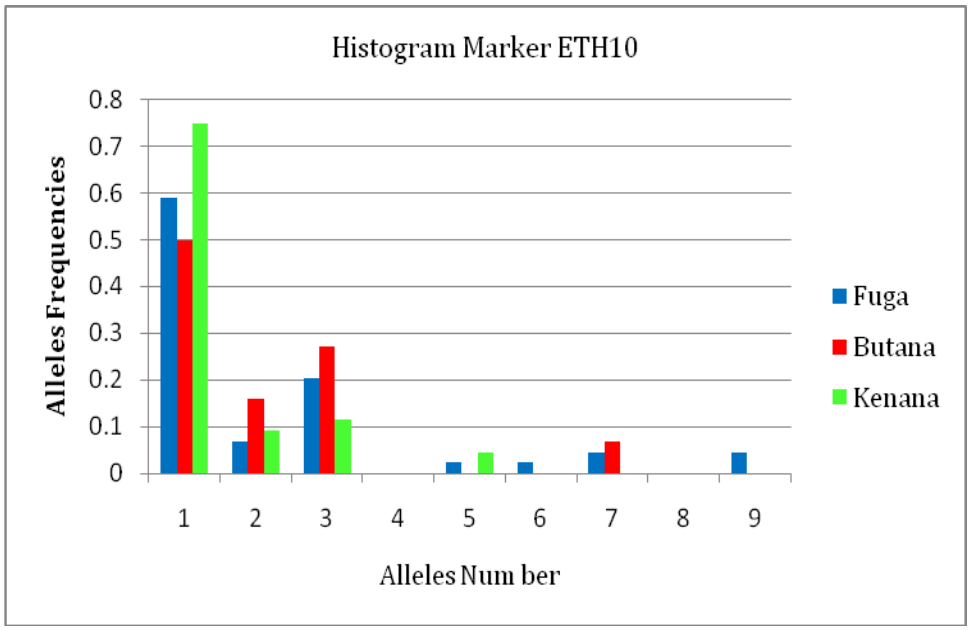


Table (B5) Number and size of allele's in the ETH225 microsatellite markers and their frequencies in each breed .

Allele number	Allele Size (bp)	Allele frequencies			
		Fuga	Butana	Kenana	All Breeds
1	240	0.136	0.205	0.091	0.432
2	242	0.000	0.00	0.000	-
3	244	0.114	0.114	0.023	0.251
4	246	0.000	0.00	0.000	-
5	248	0.136	0.023	0.045	0.204
6	250	0.023	0.00	0.023	0.046
7	252	0.000	0.00	0.000	-
8	254	0.091	0.023	0.000	0.114
9	256	0.023	0.636	0.000	0.613
10	258	0.477	0.00	0.818	1.295

The result in table (B5) pointed that a total number of alleles which observed of this marker were tan allele, their size ranged from (240-258 bp). In Fuga breed the most frequent allele is allele number (10) while the allele's number (2-4-7) were not presnt in this breed. In Butana breed the most frequent allele is the allele number (9) while the alleles number (2-4-6-7-10) were not present in this breed . In Kenana breed the most frequent allele is allele number (10), while the alleles number (2-4-7-8-9) were not present in this breed.

Allele frequency distribution for microsatellite markerETH225 in the three cattle breeds . are presented in histogram(4-4) .Horizontal axes denote alleles number and vertical axes show the frequencies found for each alleles in the breeds.

Histogram (4-4) Allele frequency distribution for microsatellite marker ETH225

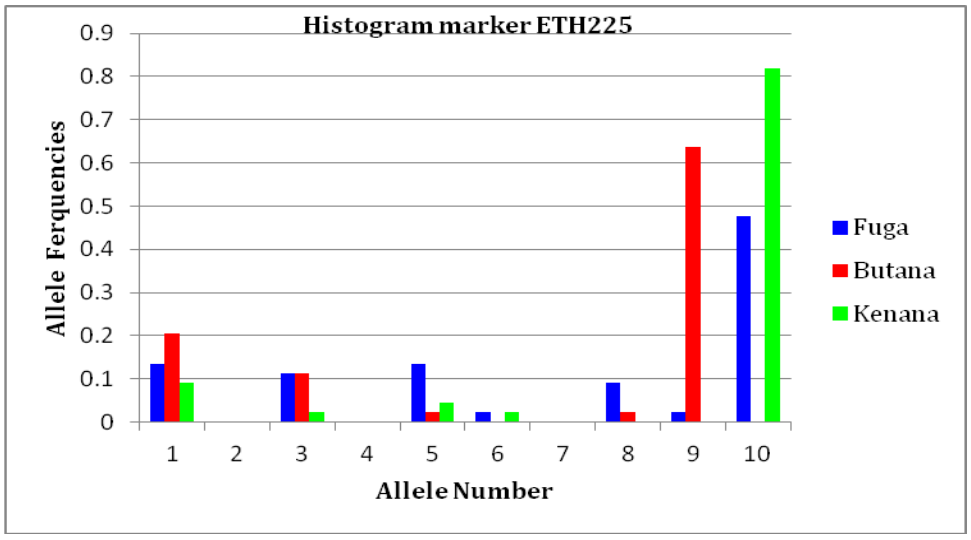


Table (B6) Number and size of allele's in INRA023 microsatellite markers and their frequencies in each breed .

Allele number	Allele Size (bp)	Allele frequencies			
		Fuga	Butana	Kenana	All Breeds
1	196	0.143	0.159	0.182	0.484
2	198	0.238	0.227	0.045	0.51
3	200	0.000	0.000	0.045	0.045
4	202	0.024	0.000	0.000	0.024
5	204	0.095	0.000	0.000	0.095
6	206	0.000	0.000	0.023	0.023
7	208	0.095	0.114	0.227	0.436
8	210	0.071	0.000	0.000	0.071
9	212	0.000	0.068	0.000	0.068
10	214	0.286	0.432	0.477	1.195
11	216	0.048	0.000	0.000	0.048

Tbale (B6) showed that atotal number of alleles which observed of this marker were eleven allele, their size ranged from (196-216 bp). In Fuga breed the most frequent allele is allele number (10) while the allele's numbers (2.6.9) were not presnt in this breed. In Butana breed the most frequent allele is the allele number (10) while the allele's numbers (3-4-5-6-8-11) were not present in this breed. In Kenana breed the most frequent allele is allele number (10) , while the alleles

number (4-5-8-9-11) were not present in this breed. In all breed the most frequent allele is allele number (10).

Allele frequency distribution for microsatellite markerINRA023 in the three cattle breeds ,presented in histogram (4-5).Horizontal axes denote alleles number while vertical axes show the frequencies found for each alleles in the breeds.

Histogram (4-5) Allele frequency distribution for microsatellite marker INRA023

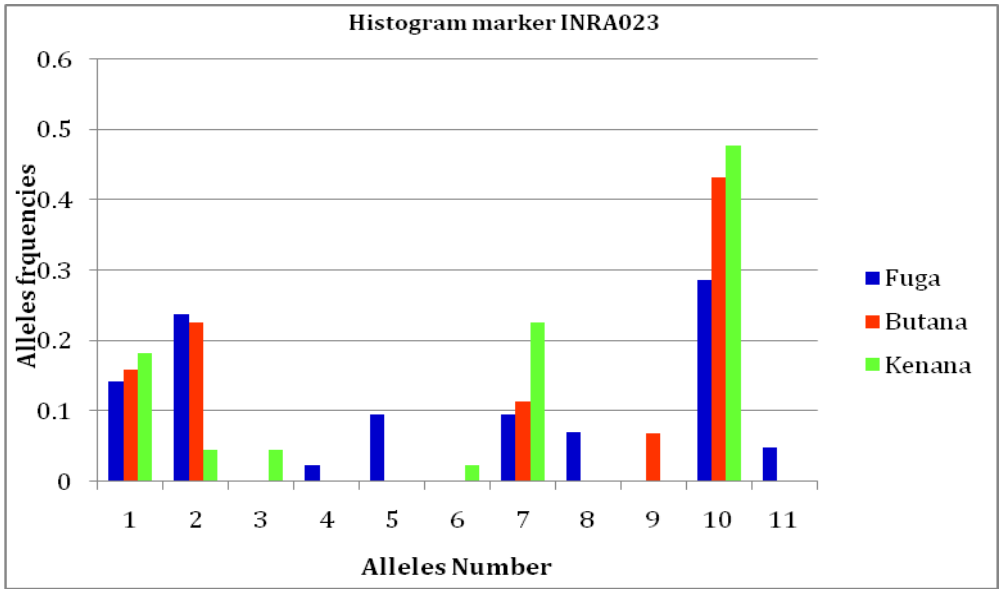


Table (B7) Number and size of allele's in the SPS115 microsatellite markers and their frequencies in each breed .

Allele number	Allele Size (bp)	Allele frequencies			
		Fuga	Butana	Kenana	All Breeds
1	244	0.045	0.000	0.023	0.068
2	246	0.727	0.068	0.136	0.931
3	248	0.000	0.682	0.432	1.114
4	250	0.023	0.000	0.023	0.46
5	252	0.045	0.114	0.000	0.159
6	254	0.068	0.000	0.114	0.182
7	256	0.045	0.136	0.250	0.431
8	258	0.045	0.000	0.000	0.045
9	260	0.000	0.000	0.023	0.023

Table (B7) domenstrated that a total number of alleles which observed of this marker were nine alleles. Their size ranged from (244-260bp). In Fuga breed the most frequent allele is allele number (2) while the alleles number (3-9) were not present in this breed. In Butana breed the most frequent allele is the allele number (3) while the alleles number (1-4-6-8-9) were not present in this breed . In Kenana breed the most frequent allele is allele number (3), while the alleles number (5-8) were not present in this breed.In all breed the most frequent allele is allele number (2). Allele frequency distribution for microsatellite marker SPS115 in the three cattle breeds, are presented in histogram (6). Horizontal axes which denote allele's number and vertical axes show the frequencies found for each alleles in the breeds.

Histogram (4-6) Allele frequency distribution for microsatellite marker SPS115

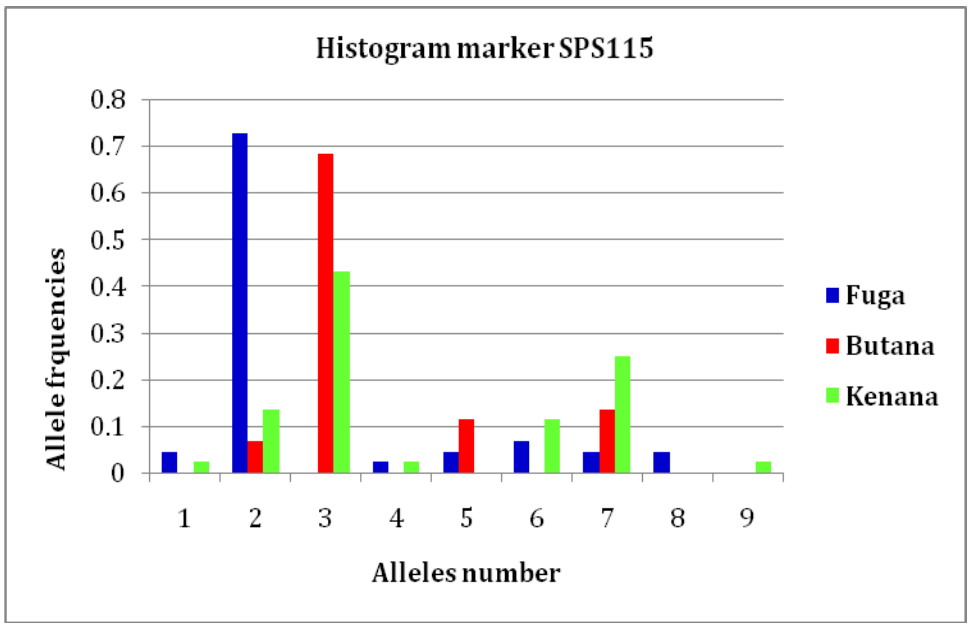


Table (B8) Number and size of allele's in theTGLA122microsatellite markers and their frequencies in each breed.

Allele number	Allele Size (bp)	Allele frequencies			
		Fuga	Butana	Kenana	All Breeds
1	137	0.000	0.114	0.023	0.137
2	139	0.000	0.000	0.023	0.023
3	141	0.000	0.000	0.000	-
4	143	0.363	0.386	0.273	1.022
5	145	0.136	0.136	0.093	0.365
6	147	0.068	0.000	0.000	0.068
7	149	0.000	0.000	0.000	-
8	151	0.136	0.000	0.341	0.477
9	153	0.023	0.182	0.000	0.205
10	155	0.000	0.000	0.000	-
11	157	0.000	0.000	0.023	0.023
12	159	0.023	0.045	0.000	0.068
13	161	0.068	0.136	0.205	0.409
14	163	0.000	0.000	0.000	-
15	165	0.068	0.000	0.000	0.068
16	167	0.000	0.000	0.000	-
17	169	0.000	0.000	0.000	-
18	171	0.000	0.000	0.000	-
19	173	0.114	0.000	0.023	0.137

The result in table (B8) domenstrated that a total number of alleles which observed of this marker were nineteen alleles their size ranged from (137-173bp). In Fuga breed the most frequent allele is allele number (4) while the alleles number (1-2-3-7-10-11-14-16-17-18) were not present in this breed. In Butana breed the most frequent allele is the allele number (4) while the alleles number (2-3-6-7-8-10-11-14-15-16-17-18-19) were not present in this breed. In Kenana breed the most frequent allele is allele number (8), while the alleles number (3-6-7-9-10-12-14-15-16-17-18) were not present in this breed.Allele frequency distribution for microsatellite marker TGLA122 in the three cattle breeds. Are presented in histogram (4-7). Horizontal axes denote allele's number and vertical axes show the frequencies found for each alleles in the breeds.

Histogram (4-7) Allele frequency distribution for microsatellite marker TGLA122

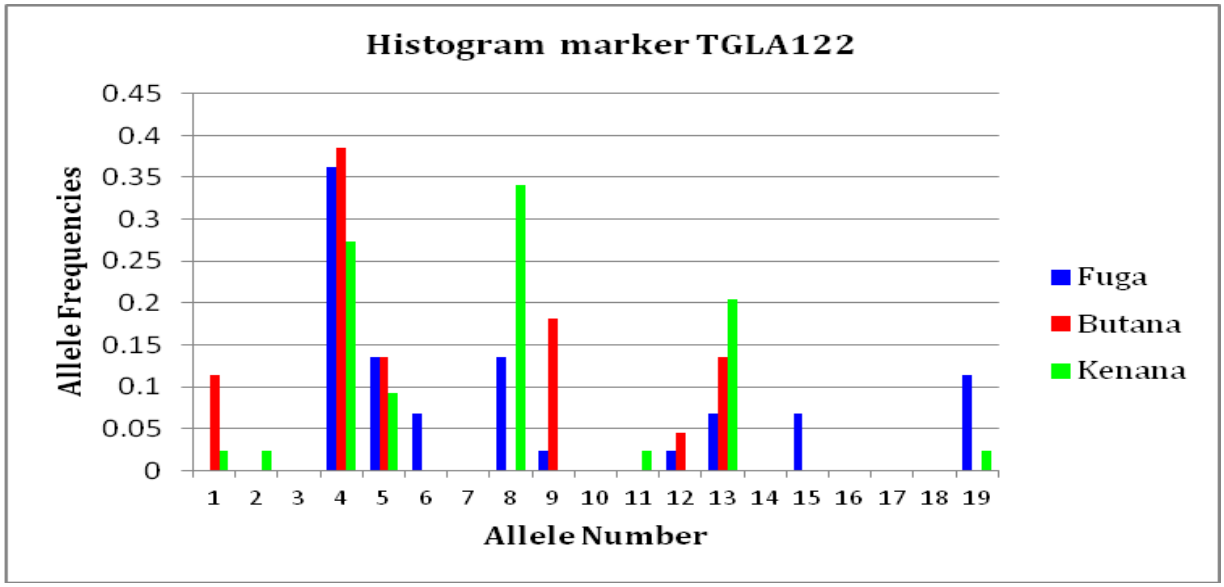


Table (B9) Number and size of allele's in theTGLA126microsatellite markers and their frequencies in each breed .

Allele number	Allele Size (bp)	Allele frequencies			
		Fuga	Butana	Kenana	All Breeds
1	115	0.159	0.114	0.091	0.364
2	117	0.182	0.227	0.523	0.932
3	119	0.023	0.136	0.091	0.250
4	121	0.159	0.227	0.045	0.522
5	123	0.455	0.273	0.250	0.978
6	125	0.023	0.000	0.000	0.023
7	127	0.000	0.023	0.000	0.023

Table (B9) illustrated that a total number of alleles which observed of this marker were seven alleles, their size ranged from (115-127 bp). In Fuga breed the most frequent allele is allele number (5) while the alleles number (7) were not present in this breed. In Butana breed the most frequent allele is the allele number (5) while the allele number (6) is not present in this breed. In Kenana breed the most frequent allele is allele number (2), while the alleles number (6-7) were not present in this breed.

Allele frequency distribution for microsatellite marker TGLA126 in the three cattle breeds, are presented in histogram (4-8). Horizontal axes denote allele's number and vertical axes show the frequencies found for each alleles in the breeds.

Histogram (4-8) Allele frequency distribution for microsatellite marker TGLA126

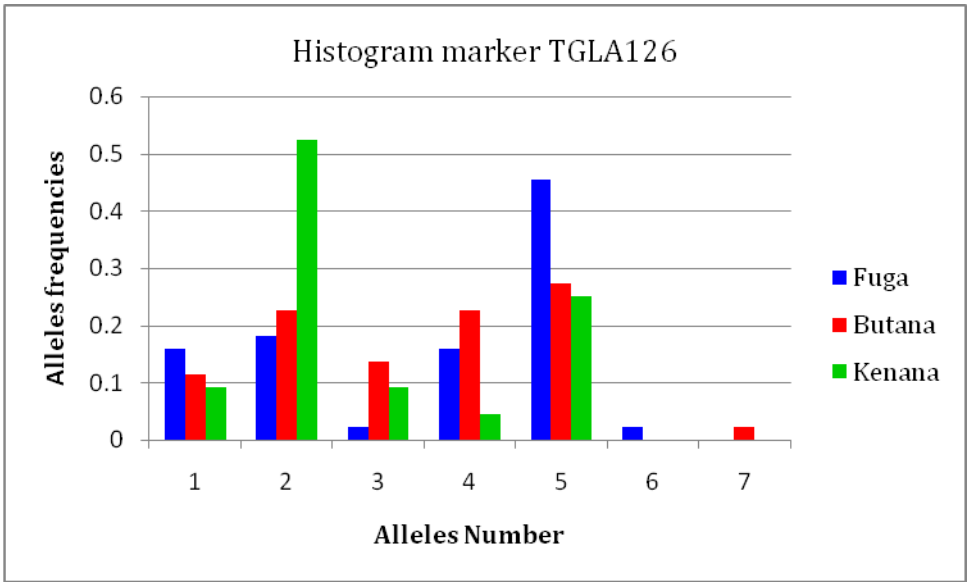


Table (B10) Number and size of allele's in theTGLA227microsatellite markers and their frequencies in each breed .

Allele number	Allele Size (bp)	Allele frequencies			
		Fuga	Butana	Kenana	All Breeds
1	77	0.432	0.456	0.545	1.433
2	79	0.023	0.205	0.205	0.433
3	81	0.023	0.000	0.045	0.068
4	83	0.023	0.000	0.000	0.023
5	85	0.000	0.000	0.000	-
6	87	0.273	0.068	0.159	0.500
7	89	0.000	0.045	0.000	0.045
8	91	0.000	0.000	0.000	-
9	93	0.159	0.205	0.045	0.409
10	95	0.000	0.023	0.000	0.023
11	97	0.023	0.000	0.000	0.023
12	99	0.000	0.000	0.000	-
13	101	0.045	0.000	0.000	0.045

For table (B10) a total number of alleles which observed of this marker were thirteen alleles, their size ranged from (77-101bp). In Fuga breed the most frequent allele is allele number (1) while the alleles numbers (5-7-10-12) were not present in this breed. In Butana breed the most frequent allele is the allele number (1) while the alleles number (3-4-5-8-11-12-13) were not present in this breed. In Kenana breed the most frequent allele is allele number (1), while the alleles number (5-8-12) were not present in this breed.

Allele frequency distribution for microsatellite marker TGLA227 in the three cattle breeds domnstrated in histogram (4-9). In which horizontal axes denote alleles number and vertical axes show the frequencies found for each alleles in the breeds.In all breed the most frequent allele is allele number (1).

Histogram (4-9) Allele frequency distribution for microsatellite marker TGLA227

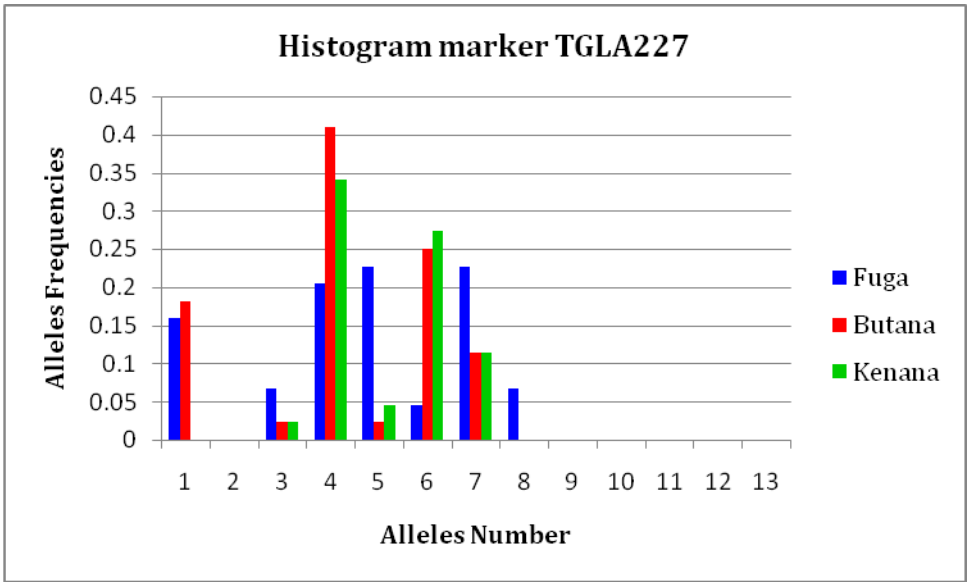


Table (B11) Number of shared alleles between the three breeds studied.

Shared alleles	Microsatellite									
	BM1824	BM2113	ETH10	ETH225	INRA23	SPS115	TGLA122	TGLA126	TGLA227	All
Fuga&Butana	**4**	**6**	**4**	**5**	**4**	**3**	**5**	**5**	**4**	**40**
Fuga&Kenana	**4**	**6**	**4**	**5**	**4**	**5**	**5**	**5**	**5**	**43**
Butana&Kenana	**5**	**6**	**3**	**3**	**4**	**3**	**3**	**5**	**4**	**36**
All the breeds	**4**	**6**	**4**	**3**	**4**	**2**	**3**	**5**	**4**	**34**
alleles per locus	**5**	**7**	**7**	**7**	**11**	**9**	**11**	**7**	**10**	**74**

The result in table (B11) illustrated that number of shared alleles in nine locus between three breeds studied and shown that totale number of alleles shared between breeds study is 34 alleles, and then alleles shared between Fuga and Kenana heiger than alleles shared between Fuag and Butana , Kenana and Butana.

Table (B12) Genetic Diversity, at each locus in the different breeds under study.

Breeds / Locus	Fuga					Butana					Kenana					Mean PIC
	Alleles		Het.		PIC	Alleles		Het.		PIC	Alleles		Het.		PIC	
	No	Ne	Ho	He		No	Ne	Ho	He		No	Ne	Ho	He		
BM 1824	**5**	**2.638**	**0.727**	**0.635**	**0.586**	**4**	**3.123**	**0.727**	**0.695**	**0.622**	**6**	**4.420**	**0.909**	**0.792**	**0.748**	**0.652**
BM 2113	**7**	**5.500**	**0.773**	**0.837**	**0.793**	**5**	**3.612**	**0.818**	**0.740**	**0.680**	**4**	**4.067**	**0.864**	**0.772**	**0.711**	**0.728**
ETH10	**7**	**2.495**	**0.636**	**0.613**	**0.562**	**4**	**2.822**	**0.773**	**0.661**	**0.589**	**4**	**1.795**	**0.546**	**0.454**	**0.387**	**0.513**
ETH225	**7**	**3.482**	**0.773**	**0.729**	**0.683**	**5**	**2.170**	**0.546**	**0.552**	**0.492**	**5**	**1.469**	**0.318**	**0.327**	**0.304**	**0.483**
INRA23	**8**	**5.867**	**0.682**	**0.849**	**0.791**	**5**	**3.559**	**0.727**	**0.736**	**0.678**	**6**	**3.153**	**0.636**	**0.699**	**0.638**	**0.702**
SPS115	**7**	**1.844**	**0.500**	**0.468**	**0.444**	**4**	**2.142**	**0.456**	**0.546**	**0.464**	**7**	**3.546**	**0.636**	**0.735**	**0.677**	**0.528**
TGLA122	**9**	**4.156**	**1.000**	**0.777**	**0.783**	**6**	**4.283**	**0.818**	**0.785**	**0.735**	**8**	**4.119**	**0.863**	**0.775**	**0.717**	**0.745**
TGLA126	**6**	**5.348**	**0.955**	**0.832**	**0.668**	**6**	**4.769**	**0.864**	**0.809**	**0.757**	**5**	**2.822**	**0.773**	**0.661**	**0.598**	**0.674**
TGLA227	**8**	**4.341**	**0.955**	**0.788**	**0.667**	**6**	**3.281**	**0.818**	**0.711**	**0.658**	**5**	**2.716**	**0.682**	**0.646**	**0.586**	**0.637**
Mean	**7**	**3.963**	**0.778**	**0.725**	**0.664**	**5**	**3.307**	**0.737**	**0.695**	**0.631**	**5**	**3.123**	**0.692**	**0.651**	**0.596**	**0.629**

No: observed Number of alleles, Ne: effective Number of alleles, Ho: observed Heterozygosity , He: expected Heterozygosity , PIC: Polymorphism Information Content.

The result in table (B12) domnestrated the Genetic Diversity and shown that the over all effective number of alleles were less than number allele observed for all locus, Observed and expected heterozygosity ranged from 0.318 (ETH225) to 1.000(TGLA122) and 0.327 (ETH225) to 0.849 (INRA023) respectively, Where there substantial variation in PIC among the marker and for each breeds the highest PIC value were found for breed of Fuga (0.664) and the lowest value was found for kenana breed (0.596) .all marker evaluated were highly informative PIC value grater than (0.5). All cattle breeds presented very high polymorphism of DNA cattle. This is testified by the considerable number of alleles identified, the high mean number of alleles and the high degree of observed (Ho) and expected (He) heterozygosity.The high value for the Ho was expected due to the absence of Hardy Weinberg equilibrium in the breeds under study.The PIC is an expected heterozygosity derived from allele frequencies in random mating populations. PIC is an indicator of how many alleles a certain marker has how much these alleles divided evenly. For example if a marker has many alleles but only one of them is frequent, the PIC will be low. PIC values were generally high and varied from 0.304 (ETH225) in Kenana to 0.793 (BM3113) in Fuga breed.

Table (B13) Gene diversity:

Microsatellite	Breed			All breeds
	Fuga	Butana	Kenana	
BMC1824	0.633	0.695	0.787	0.705
BMC2113	0.839	0.738	0.769	0.782
ETH10	0.613	0.658	0.451	0.574
ETH225	0.728	0.552	0.327	0.536
INRA23	0.838	0.736	0.700	0.767
SPS115	0.468	0.545	0.737	0.583
TGLA122	0.885	0.772	0.773	0.810
TGLA126	0.705	0.807	0.658	0.723
TGLA227	0.729	0.709	0.645	0.694
Mean estimate	0.715	0.690	0.649	0.684

Table (B13) concerning the results of the gene diversity for the nine markers in the three breeds studied, the results are presented in above table, The average gene diversity over all loci were 0.684, while for individual loci the average gene diversity ranged between 0.327 (ETH225) in Kenana breed and 0.885 (TGLA122) in Fuga breed . The diversity analysis demonstrated considerable level of genetic variation in all breeds, with Fuga breed displaying somewhat higher levels of genetic polymorphisms compared to Butana and Kenana breeds.

Table (B14) Inbreeding estimates (FST) within breeds under study.

Microsatellite	Breed			All
	Fuga	Butana	Kenana	
BMC1824	-0.149	-0.047	-0.040	- 0.236
BMC2113	0.079	-0.109	-0.122	- 0.310
ETH10	-0.039	-0.174	-0.209	- 0.422
ETH225	-0.061	0.012	0.026	0.099
INRA23	0.148	0.012	0.091	0.251
SPS115	-0.069	0.000	0.137	0.206
TGLA122	0.076	-0.060	-0.118	- 0.254
TGLA126	0.032	-0.070	-0.174	- 0.276
TGLA227	0.190	-0.154	-0.057	- 0.401
Means	0.035	-0.068	-0.049	- 0.273

The result in table (B14) illustrated Inbreeding estimates (FST) within breeds under study and showed that Fis value in all loci was not significantly different from zero ($p>0.05$) and the extension was in between -0.422 (ETH10) and 0.099 (ETH225) with average of -0.273 demonstrating absence in the heterozygosis

deficit and, consequently, a small probability of inbreeding occurrence in above table,and then Looking at the table in general it is noticed that most in breeding value were negative and below the zero. This value according to animal breeders should be considered zero.Meaning the absence of inbreeding within breeds study. In contrast, table B15 is showing the inbreeding rates between breeds under study.

Table (B15): Inbreeding rates between breeds under study.

Breeds	Fuga	Butana	Kenana
Fuga	--------	0.1015	0.0965
Butana	0.1015	---------	0.0353
Kenana	0.0965	0.0353	---------

The results of this table is showing also either negative values or very low values and should be also considered zero, indicating the absence of inbreeding between the populations under study.Indicates pair-wise values of genetic differentiation between breeds measured by *F*-statistics (Fst), which varie between zero (meaning no genetic differentiation) and one (meaning complete genetic differentiation).

The observed (Fst) is usually much smaller than 1.To help interpreting (Fst), Wright (1978) divided the value of (Fst) into four intervals: (1) from 0 to 0.05, indicating little genetic differentiation; (2) from 0.05 to 0.15, indicating moderate genetic differentiation; (3) from 0.15 to 0.25, indicating great genetic differentiation;(4) from 0.25 to 1, and indicating very great genetic differentiation.

The results of this table is showing also low values and should be also indicating moderate genetic differentiation of inbreeding between breeds under study ranged from (0.0353 to 0.1015) between Btana and Kenana , Fuga and Butana .

Table (B16) Estimate effective migration rate *(Nm)*.

Microsatellite	Gene flow
BMC1824	4.104
BMC2113	5.995
ETH10	8.754
ETH225	4.753
INRA23	7.606
SPS115	0.814
TGLA122	2.019
TGLA126	2.849
TGLA227	2.125
Mean	4.335

Table (B16) domenstrated the gene flow between breeds under study is calculated for each microsatellite separately. Results of this analysis presented at the above table showed that esteemed number of migration per generation (Nm) between breeds was generally low in most loci indicating that there is minimal exchange of genetic material between breeds though this level of migration is expected to maintain the genetic differentiation observed between the breeds.

Table (B17) Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

Breed	Fuga	Butana	Kenana
Fuga	****	0.618	0.628
Butana	0.482	****	0.889
Kenana	0.466	0.118	****

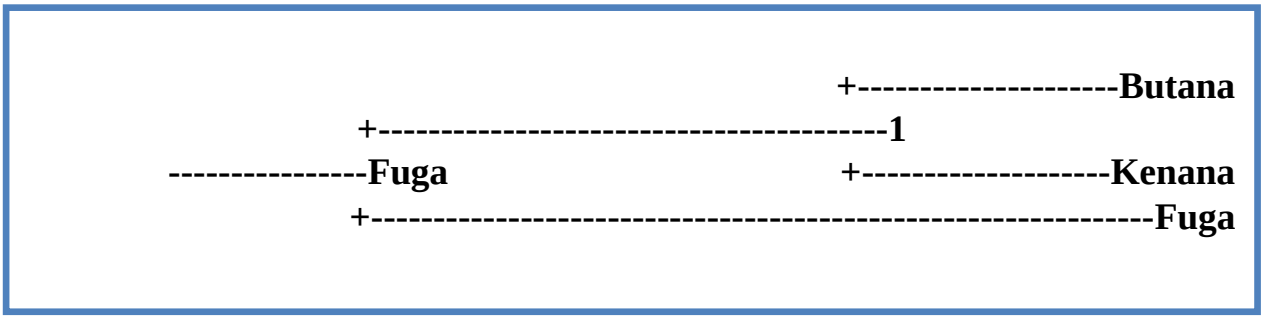
The result in table (B17) indicated that Nei's genetic identity and genetic distance between breeds under study Moreover the Nei genetic analysis and identity are presented at above table .The lowest genetic distance was found between Kenana and Butana pair (0.118) and the highest genetic distance between the Fuga and Butana pair (0.482) were significantly different from zero ($p < 0.05$) for all pair wise combination.

Table (B18) Estimated divergence time between the three breeds under study calculated according to Nei standard genetic distance and the mammalian mutation rate .

Breeds	Nei's standard genetic distance (Ds)	Mutation Rate (α)	Divergence time	
			Generations	Years
Fuga&Butana	0.618	1.2×10^{-3}	258	1806
Fuga&Kenana	0.628	1.2×10^{-3}	262	1834
Butana&Kenana	0.889	1.2×10^{-3}	370	2590

* one generation equals seven years.

Estimated divergence time between the three breeds under study calculated according to Nei standard genetic distance and the mammalian mutation rate as indicated in table (B18) Taking into consideration that one generation equals seven years, and transforming the number of generations into years. Looking at the table we could notice that the biggest divergence time (2590 years) was between the Butana and Kenana cattle. In contrast the lowest divergence time (1806 years) was between Fuga and Butana. The results of this table are confirming the phylogeny dendrogram obtained using UPGMA method. The dendrogram is based on Nei's (1978) using Genetic distance: Method UPGMA (computer software), modified from NEIGHBOR procedure of PHYLIP Version 3.5.



Dendrogram showing the genetic relationship among the different breeds using Ds genetic distance from 9 microsatellites marker indicates (expected phylogeny tree) the divergence of the different populations from each other.

Drawing phylogeny tree between the breeds proved that Butana and Kenana are within one cluster while Fuga is in another cluster, and the three breeds are then coming from one ancestor.

This dendogram is giving a primary idea about the possible migration way across breeds study in Sudan. It also shows that the migration starts from the northern east part of Sudan to the middle part in north kordufan.

Also this dendogram gives a logic estimate for migration process which happaned in the past. Only one confliction will be noted. It was the genetic distance between breeds under study. According to that all the breeds under this study may be considered as different breeds.

CHAPTER FIVE

DISCUSSION

5.1 .A. Phenotypic Characterization of Fuga breed:

Good understanding of a production system and the relative importance of the different constraints is essential prior to initiating any genetic improvement programme (Baker and Gray 2003). This study wes curried to understand the production constraints and to identify cattle breeding goals and practices of breeder in Fuga cattle area in western Sudan for developing a sustainable breed improvement programme.Cattle are the most important livestock species in fuga area table (A1) .These cattle are kept in a mixed crop farming systems and livestock production system.Cattle play multi-functional roles in all production systems with similar production objectives in fuga area. In Gambia, Steglich (2006 such practices coincides with the practical manegment of fuga herd owner for their breeding animals.Whan he studied the production objectives of agro-pastoralists. He reported that cattle have primarily saving functions. However, milk production is important, but so are manure and traction (animal for work) power. In these systems as stated by Peters (1991) the targets of breed improvement programs should not be focused on few traits such as lactation yield but overall performance including reproduction efficiency to obtain a sustainable performance.They depended on information about the performance of potential bull dams and appraisal of young bull. The knowledge about the dam of the breeding Sire and her milk yield indicates that herd owners are well aware of their stock. Selection of bulls born by high yielding cows was also reported in Gambia (Jaitner et al 2003 and Steglich 2006). In the present study the important characteristics used in selection of Fuga bulls in table (A3). The dam performance and body size was more important for Fuga cattle owners (64.7%) and other characteristics such as shape similarity to dam or sire (18%) and dam performance ranked relatively low importance (10.7%) and the last characteristics used to select breeding bull in Fuga cattle sire performance and body size (4.7% , 2%), respectively.

Most of breeders are keeping Fuga cattle for their milk consumption (81.3%) or as insurance against financial problems table (A4).In the Fuga area, the major portion of income was spent to solve financial problems (30%), while(21.3%) from cattle breeders reason for sales cattle to avoid financial problems ,and old age, table (A5).Most Fuga breeders consider that the production objective for keeping cattle to generate income from the sale of milk and animals, milk for home-consumption (40% , 47%) respectively ,received a higher ranking among Fuga cattle breeders. presented in table (A6).Production constraints in fuga area, in table (A7) owners stressed the Disease prevalence and Lack of cattle feed to be the most important limiting factor for productivity of the cattle, and indicated the importance of improving their feeding regime as an essential step towards any improvement program. Free grazing of rangelands is the most common feeding system. During the short wet season grasses grow rapidly producing abundant biomass. Water supply during the dry season is a constraint to the breeders in fuga area. However, most breeders tend to prolong watering intervals (once per day or longer).Production and reproduction performance of Fuga cattle under field conditions comparable with previous studies and the estimated lactation milk yield for Butana cattle (538.26 kg) and for Kenana cattle (598.73 kg) showed that both breeds had a comparable milk performance under field condition which was much lower than their yield under station condition. However, Butana cattle in Atbara Livestock Research Station yielded 1662.57 ± 108.96 kg/ lactation (Musa et al 2005) and Kenana cattle in Um-Benein Livestock Research Station yielded 1423.58 ± 551.70/ lactation (El-Habeeb 1991). For both breeds the lactation period was also much shorter under field condition.The findings of Abate et al. (2010) (2847 ± 632.88 kg) and Ishag (2000), and (2417.20± 921.00 kg) for crossbred Kenana x Friesian cows.

Previous studies reported by many researches (Bayoumi and Dinasoury., 1962) revealed that the average milk production for Sudanese dairy cows was (442.5) gallons with C.V of 32% while (Fangaly.,1980) reported that the 305

days milk yield for Kenana cows at Um-benein and Nesheshiba station, were (1872.5) and (1367.13)kg respectively. Butana cows at Atabra station showed milk yield of (2254.2)kg per lactations .The average milk yield is 2253 kg in 240 days lactation (Osman 1981).However, it was much higher than Milk production of fuga breed which had a comparable milk yield under field condition lower than their yield under station condition of Butana and Kenana.Ali et al.,1988 reported that the lactation yield for cross-breed(Friesian × Kenana or butana)cows having 50% , 62.5% and 75% Friesian blood were 4306 , 5733 , and 4136 Ib, respectively.

Regarding productive performance, of the present study in table (A8) showed milk yield (1100.96±86.18) per lactation was lower when comparable with previous studies and milk yield per day (kg) in table (A8) obtained in this study is 5.26±0.29 kg with those estimates obtained by El-Habeeb (1991) , and Muas et al (2005) for Knana and Butana (5.60 ± 1.77 , 6.10 ± 0.41 kg) respectively .The average age at first calving in *Bos indicus* cattle is about 44 months ,compared with about 34 months in *Bos taurus* and *Bos indicus* x *Bos taurus* crosses in the tropics . Heritabilities of age at puberty, at first conception and at first calving are generally low , indicating that these traits are highly influenced by environmental factors, Jochle (1972). For Butana breed the age at first calving is 43 months with calving intervals of 373 days.The age at first calving is 45.2 months but with good management; this could be reduced to 32 months (Khalafalla 1977).

Chhikara et al (1979) found that breed differences had a significant effect on age at first calving in Haryana, and selected Haryana, Tharparkar and Sahiwal heifers in India. In an analysis of production data covering 14 years, Aroeria et al (1977) found that Gir heifers tended to be older than Nellore or InduBrazil heifers at first calving. Breed differences probably reflect differences in management conditions. Wagenaar et al (1986) found a mean age at first calving of 50.2 ±9.1 months in 146 Fulani-type dams in Niger.

Dennis and Thiongane (1978) found that Gobra (Senegal Fulani) heifers kept on pasture and fed a balanced concentrate supplement calved first at 31 months old, compared with 40 months for unsupplemented heifers.

Weitze (1984) found that supplemental feeding during the dry season reduced the average age at first calving from 45.0 to 37.5 months in Nellore, Gir and InduBrazil cattle in Brazil. The age at first calving in the present study was (40.49± 6.76) months in table (A8) and lower when comparable with the previous studies

Defined as the number of elapsed days between two consecutive calving and calving interval is period between two consecutive calving, regular calving of dairy cows every 12-13 month is economically desirable for profitable production(Bath et al 1985).calving interval in the present study was (15.90±0.44) months in table (A8) high than, regular calving of dairy cows every 12-13 month.

In most modern dairy farms, a lactation length of 305 days is commonly accepted as a standard. However, such a standard lactation length might not work for smallholder dairy cows in which the lactation length is extended considerably in most cases (Teodoro and Madalena,2003;Masama et al.,2003; Msangi, Bryant &Thorne, 2005)., Osman and Elaminm.(1971)reported lactation length of (294) days with coefficient variation of (31%) for northern zebu Sudanese cattle for butana cattle at Atbara . Alim,(1962) reported lactation length of (253±103)days (Alim.,1960)reported length 224±85)days for kenana at the Gazira Agriculture research station herd. In the present study lactation period (208.60±5.37) days in table (A 8) is lower than lactation period of the previous reports.Such difference might be due to the type of management of production.In the different region.

The birth weights for Butana breed in male are 25.64 kg and for female 24.29 kg respectively and the calves weight of kenana cattle was 24.8 kg and 23.5 kg at birth respectively for male and female,(Khalifa 1966).All ware higher when compared, with the present study.The average birth weight of fuga cattle breed were 22.06±0.44 kg.The number of services per conception (NSC) depends largely on the breeding system used. It is higher under uncontrolled natural breeding and low

where artificial insemination is used. NSC values greater than 2.0 should be regarded as poor, and some of the factors contributing to high NSC values as elaborated by. Ageeb and Hayes, (2000) working in the pure bred Holstein kept near Khartoum in Sudan reported number of services per conception, as 4.2.

Table (A8) shows Number of service per conception 2.27±0.7 in fuga cattle while El-Amin et al (1981) concluded that NSC did not differ significantly between Red Butana and Red Butana crosses (average 2.6) but was influenced by month of calving. NSC increased over the study period, probably due to changes in management. This agreed with Azage Tegegn et al (1981), using 3 local Ethiopian breeds, the Barca, Horro and Boran, and found that NSC was lower for animals from wet areas than for those from drier areas (1.74 ±0.6 vs 1.98 ±0.07). Crossbred cows required 0.12 and 0.14 fewer services per conception than local zebu cows in wet and dry areas. Respectively the NSC values greater than 2.0 should be regarded as poor in the previous studies and present study.

5.2.B. Genetic Characterization of Breeds (Fuga, Butana and Kenana) of Study:

This is the first study performed in Sudan on the genetic diversity of different three cattle breeds raised in Sudan. The results provide an indication of the feasibility of the methodology as well as information on differentiation between and within the three cattle breeds studied.

The International Society of Animal Genetics (ISAG) has chosen nine microsatellites (international marker set) that should be included in all paternity testing, were evaluated for the first time for their use in paternity testing and pedigree verification. These are BM1824, BM2113, INRA023, SPS115, TGLA122, TGLA126, TGLA227, ETH10, and ETH225.

This were analyzed in three different breeds of Sudan cattle raised in western kordufan (Fuga), Atabar station research (Butana), and Kneana in Um-Benein station reasearch. The purpose of this study is to measure the genetic

diversity between these breeds as well as measuring the purity or the heterogenecity of each breed.

Two components are used to characterize variability in animal genetic resources: phenotypic variability, which is easy to see and can be measured directly on individuals, and genetic variability, which can be measured in the variations in DNA sequence by means of different genetic markers. In both cases, there are mathematical tools that allow us to adequately characterize the variability within and between breeds. The use of marker, in genetic diversity studies extended also to differentiate between the populations of the same breed. Hanslik et al. (2000) used it to differentiate between new world and old world Holstein Friesian cattle populations.

Genetic diversity: According to Barker(1994), the standard is that selected microsatellite loci ought to have at least four alleles to be considered useful for the evaluation of genetic diversity.Diversity at the nine locus, which indicates a rich gene pool within these breeds under study**.**Highly polymorphic: BM1824, ETH10, SPS115 (9 alleles each); ETH225 (10 alleles),BM2113 (8 alleles) ,INRA023 (11 alleles) TGLA227 (13 alleles) TGLA126(19 alleles) while the marker TGLA126 (7 alleles) was shown in tables (B1 to B9).In the present study of three Sudanese indigenous breeds(Fuga, Butana and Kenana) based on this criterion, the 9 microsatellites loci that were used in the present study are suitable for population genetic analyses. In the present study the *number of alleles* per locus shown in table (B1) ranged from 5 to 11. The mean *number of alleles* per locus was 8.22 this number lies within the range of 6-9 alleles, which was reported in many cattle breeds from Europe, West Africa and Brazil (*Lubieniecka et al., 2001; Machado et al.,* 2003 and *Ibeagha-Awemu et al., 2004*). This is an indication for the high ratio of heterozygosity which arises from the absence or weak selection or organized breeding programs for the Breed under study, and the total number of alleles found in the present study is 74, in table B1 higher than those found in European breeds(MNA 6.5) (Canon et al., 2001) or South eastern European breeds(MNA

6.5) (Beja- Pereira et al.,2003).The mean number of alleles per locus in the present study equal 8.22 and the average observed heterozygosity (Ho) pointed that in table (B12) ranged from(0.778 to 0.692). This levels between breeds under study is higher than that obtained in the Mbarara population (MNA 6.8, Ho 73.7%, Ndumu et al., 2008b) West African cattle (MNA 6.7, Ho 64.3%, MacHugh et al., 1997) but higher than Bhutan cattle breeds (MNA 7.2, Ho 67%, Dorji et al., 2003), and lower than those found in Chinese yellow cattle(MNA 16.55)(Zhang et al.,2007).

The high mean value of expected heterozygosity He (0.72 5- 0.651)in this study domenstrated in table (B12)was higher in level of gene diversity . This result is in agreement with studies of indigenous populations from developing countries such as He 0.73 in Chinese yellow cattle(Zhang et al.,2007) , (He 0.73) indicates that Vietnamese cattle population shavea high level of gene diversity. or Ethiopian cattle (Dadi et al.,2008) while He in breeds of developed countries L.D. Pham Ranged from 0.61 to 0.71 in 18 European local breeds(Canon et al.,2001) or He 0.41 in Japanese Black cattle(Kim et al., 2002). However, He values of this study do not differ from those in Ankole populations and Kagera in Tanzania (Ndumu et al., 2008b). The He (0.725- 0.651) for the present study was much higher than 0.662±0.030 which was observed within the Spanish Alberes cattle breed aa(Casellas et al., 2004). and 0.45–0.69 (Kantanen et al., 2000) reported among African and European cattle breeds respectively.

In other Creole cattle populations such as the Uruguayan Creole breed (He 0.67) (Armstrong et al., 2004), Florida Craker, Pineywoods Texas Longhorn in USA (He 0.7) (McNeil et al., 2006) or the Creole breed Casanare in Colombia (He 0.82) (Barrera et al., 2006) is higher than that present in this study.

The values of genetic diversity or He in breeds under study ware higher than that found in other breeds such as Simmental (He 0.58) (Eedwards et al., 2000), Nelore (He 0.51) (Lara et al., 2005), the Highland breed (He 0.57), Hereford (He 0.60), and Shorthorn (He 0.58) (McNeil et al., 2006). The result in the present study

indicated that there is high genetic diversity between breeds under study when compared with previous studies.

Polymorphism information content (PIC): When a process of genetic isolation persists for several generations, the main consequence, from the point of view of population genetics, is inbreeding and genetic drift (Falconer and Mackay, 1996).

PIC values indicate the in formativeness of the microsatellite loci studied. In the present study the PIC values for all the 9 microsatellite loci ranged from 0.483 to 0.745 in table (B12) with the mean value of 0.629 High number of alleles and PIC values were observed for TGLA122,.the nine locus included the present study are highly informative (PIC > 0.5) and thus will be useful to evaluate the genetic diversity in breeds under study. The low PIC in Kenana breeds may be due to the high selection pressure applied in this preed Not only the narrow genetic background of this breed but also the inbreeding and higher selection pressure have contributed to the loss of genetic variation which is revealed by reduced allele numbers and lower PIC values, which is the required level of discrimination in a parentage analysis (Vankan and Faddy, 1999;Perez-Miranda et al., 2005).

In general the observed high values of average gene diversity and PIC values could be attributed to the absence of homozygozity and the large number of alleles or heterozygosity observed (Kalinowski, 2002;). In other words, the high value of observed heterozygosity could be due to the absence of selection and the animals were subject to random mating (Weir, 1996), and this is actually the situation in our cattle populations,and in general higher than reported in some Italian cattle breeds (0.55-0.63), which reported by Del Bo et al. (2001).

The result obtained in this study are similar to those described by Arranz (1994) and Rodellar et al. (1996) for Spanish cattle populations, where the ETH 225 marker was found to be highly informative in the Galician Blonde breed, which is consistent with the results obtained in this study,and comparable with the results obtained for the same set of loci in other Simmental breeds, mean PIC value was 0.757 in Czech Pied cattle, is lower and PIC 0.642 in Slovakian Pied

cattle (Czerneková et al., 2006) and 0.641 in Simmental cattle from Poland (Choroszy et al., 2006) is lower than PIC in present study.

Genetic differentiation:Gene diversity over all loci were 0.684, while for individual loci average gene diversity ranged between 0.536 (ETH225) and 0.810 (TGLA122) . As expected, the microsatellite loci show very high level of genetic diversity, (A high values for the average gene diversity were noticed for all the markers and all the breeds studied) with overall mean 0.684, this value is all most near to the previously reported by Loftus et al., (2002), which was 0.78 during their study concerning the identification of zebu alleles in some cattle breeds including breeds under study. There was a significant positive relationship between averages within population gene diversity for each locus. Regarding the inbreeding within the breeds itself, most of the observed values were negative, so they also should be considered zero, and indicating that there is small probability of inbreeding within breeds it self. The FIS value in table (B14) in all loci was not significantly different from zero ($p>0.05$) and the extension was in between -0.422 (ETH10) and 0.099 (ETH225) with and average of -0.273 demonstrating absence in the heterozygosis deficit and, consequently, a small probability of inbreeding occurrence within the same breed under study.The non-significant FIS estimates in breeds indicate that generally, there was no inbreeding in the breeds under study. After interviewing the owner, we can likely attribute inbreeding to the selection of replacement bulls from within the breeds in attempt to select for red and white colour in fuga breeder.The non-significance of the FIS-parameters could also be a result of gene flow with other breeds. The genetic differentiation analysis (FST) showed that the breeds under study were genetically distinct.

This situation of such case is usually named as out breeding. Out breeding is first defined by Falconer (1989), as the mating of animals less closely related to each other than the average relationship within the breed or population concerned. In other words it the preferential mating between non-related individuals. The other names for out breeding such as out crossing; breed crossing, species crossing and

genus crossing are less used in the recent days. And the observed (Fst) is usually much smaller than 1.To help interpreting (Fst),Wright (1978) divided the value of (Fst) into four intervals: (1)from 0 to 0.05, indicating little genetic differentiation; (2) from 0.05 to 0.15, indicating moderate genetic differentiation; (3) from 0.15 to 0.25, indicating great genetic differentiation; (4) from 0.25 to 1, indicating very great genetic differentiation.the inbreeding values between different breeds under study ranged from (0.0353 to 0.1015) between Butana and Kenana , Fuga and Butana table (B15) indicating that moderate genetic differentiation of inbreeding between those breeds.

Genetic distance:In the table (B18),it was notice that the biggest divergence time (2590 years) was between the Butana and Kenana cattle, in contrast the lowest divergence time (1806 years) was between Fuga and Butana.

According to some previous studies for the genetic diversity the low values of years or genetic distances could be neglected and the populations or breeds under study may be considered as one breed (Hansen et al., 2002). In fig (3) Dendrogram showing the genetic relationship among the different breeds using Ds genetic distance from 9 microsatellites marker that indicates (expected phylogeny tree) the divergence of the different populations from each other.The dendogram is giving a primary idea about the possible migration way a cross breeds study in Sudan. It is showing that the migration starts from the northern east part of Sudan to the middle part in north kordufan.

This indicates that the genetic distance does not necessarily depend on the geographic distance between breeds, although the breeds under investigation are known to interbreed across common borders. Unlike plants, livestock movement, in strict sense gene flow, is highly influenced by human migration due to the strong and long-term association of humans with livestock. Historically pastoral cattle keepers traverse long distances across borders along with their cattle in search of more conducive environments for themselves and their cattle. During these movements there could be possibility for exchange of breeding animals (genetic

material) to allow gene flow from one breed to another. This contributes to reduction in genetic divergence.This dendogram in this work gives a logic estimate for migration process which happened in the past. Only one confliction will be noted. It was the genetic distance between breeds under study. According to that all the breeds under study may be considered as different breeds. The allele sharing results did not show any obvious results, unique or specific alleles for specific breed. This is may come from the admixing with imported Friesian cattle breed which is hybridized with Sudanese cattle breed for genetic improvement.

5.3. Conclusion and Recommendation

5.3.1 Conclusion:

Cattle play an important role in the livelihood of people in area study, and they have potential for greater contribution through better feeding, health management and genetic improvement. Production conditions determine the rather low milk yield of Fuga herd in their habitat which does not meet production objectives.

Given the highly unfavorable production condition (seasonality of feed, water shortage and difficulties in managing diseases), adaptive traits next to milk yield are very important in the harsh production environment.

Our study is the first to provide insight into diversity of indigenous cattle breeds in Sudan at the molecular level. Molecular diversity is relatively high compared to cattle from Europe, West Africa .So this study contributes to the knowledge of genetic structure and molecular characterization of these indigenous breeds.

In the present study, genetic variation within and between three Sudanese cattle breeds named: Fuga, Butana, and kenana were estimated using genotypic data of 9 microsatellite markers recommended by ISAG (2012) for such studies. The total numbers of animals genotyped were 90 animals, 30 animals from each breed.

All the microsatellites studied showed polymorphism in the breeds studied. A total of 74 microsatellite alleles were identified with number of alleles at one locus ranging from 5 (BM1824) to 11 (INRA023& TGLA122). Due to the large numbers of alleles observed for these markers, they could be fruitfully used in further researches on quantitative trait loci (QTL) detection and subsequently marker assisted selection (MAS).

5.3.2 Recommendation:

According to the results of this study, the following recommendition may be suggested:

1. Although admix is occurred due to hybridization with Friesian breed, a high genetic diversity shown in the three studied breeds, can be used in designing good programs for genetic improvement in Sudanese zebu cattle.

2. The microsatellites used in this study showed and recommended to be excellent tools for monitoring the risks of genetic variability loss in Sudanese cattle breeds studies.

3. More emphasis needs to be placed on the improvement of Fuga cattle due to their significant contribution to the family income and food and their ability to survive and reproduce in the extreme environments.

4. To reduce losses due to diseases, there should be an urgent attention by development sectors and partners to strengthen veterinary services including training, credit facilities, and formation of farmers cooperative to facilitate drugs supply and distribution.

5. Curative treatments for the prevailing diseases should be confirmed through identification and diagnosis.

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APPENDIX

Appendix (1) Questionnaire

University of Sudan and Technology

College of graduate studies

Department of animal production

Questionnaire about the phenotype and genotype characterization of fuga cattle in west Sudan

1. The villages selected for the survey in fuga cattle areas:

……..

2. The importance of livestock and crop farming in fuga cattle area.

a. Livestock only b. Crop farming only c. Livestock and farming

3. Importance of livestock species in survey area

a. Cattle, b. Sheep c. Goats

4. Characteristics used to select breeding bull in fuga cattle area

a. Dam performance b. Sire performance c.Body size d. Shape (similarity to dam or sire)

5. Purposes of keeping cattle

a. Primary reason for keeping cattle to generate income from the sale of milk and animals,

b. Milk for home-consumption or as insurance against financial problems.

6. Reasons of cattle sales:

a. Purchase of animal feed b. Financial problems

c. Finance agriculture d. Old age

e. Social activities f. Investment

7. Production objectives of keeping cattle and the ranking of the importance of these objectives

a. Income from sale of milk b. Milk for home-consumption

C.Income from sale of animal d. Traction (animal for work)

e. Insurance against financial problems f. Investment (like a bank)

8. Production and reproduction performance of fuga cattle under field conditions: (Parameters study)

8-1 Milk yield (Kg)

a. Beginning of lactation ……

b. Middle of lactation ……

c. End of lactation……..……..

8-2 Lactation length, (months)……..……

8-3 Age at first calving, months)……..……

8-4 Calving interval, (months)……..……

8-5 Number of service per conception……

8-6 Weight of birth……

8-7 Pregnancy rat……

9. Production constraints in fuga cattle area.

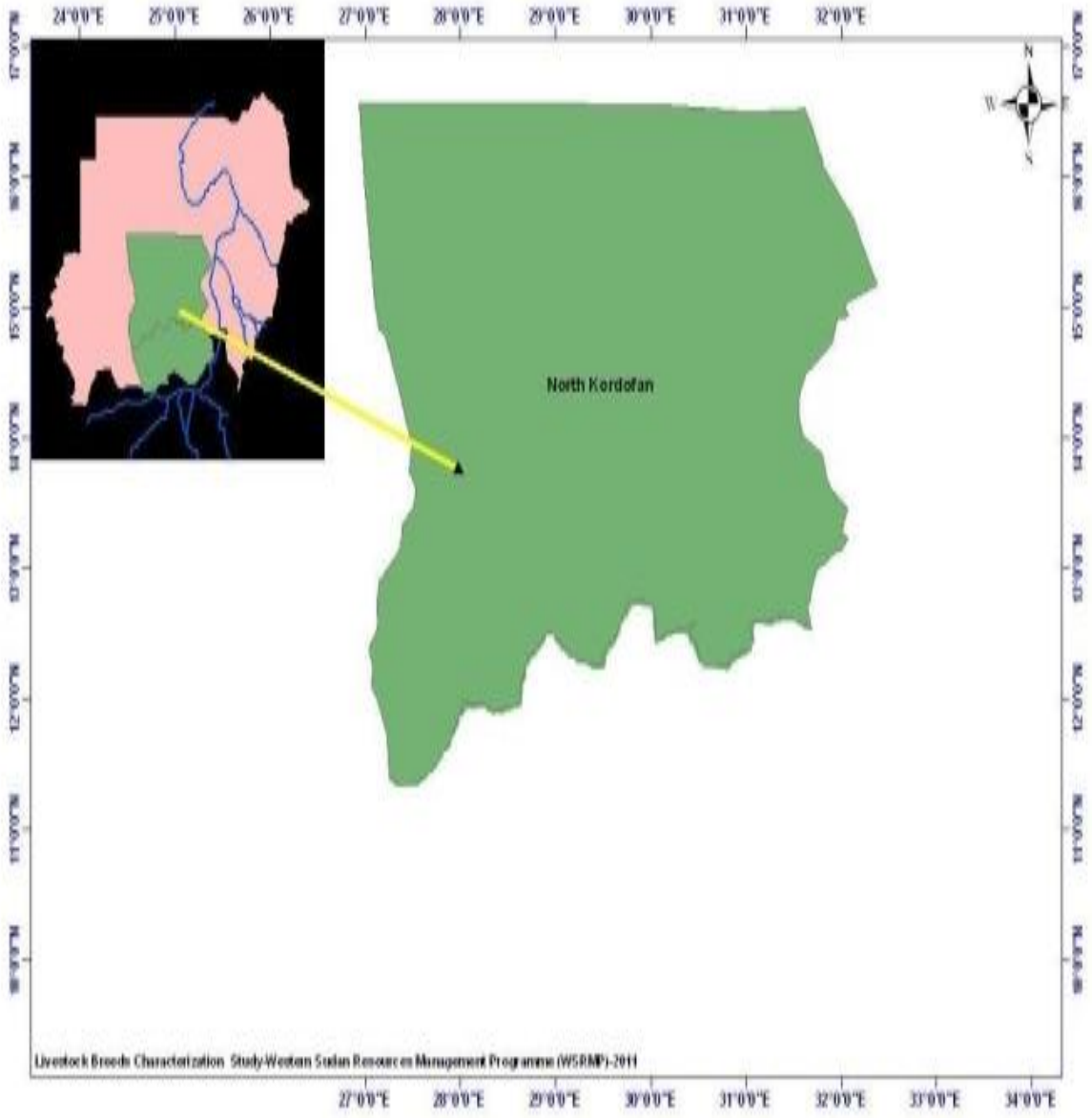
a. Lack of cattle feed

b. Disease prevalence

c. Water shortage

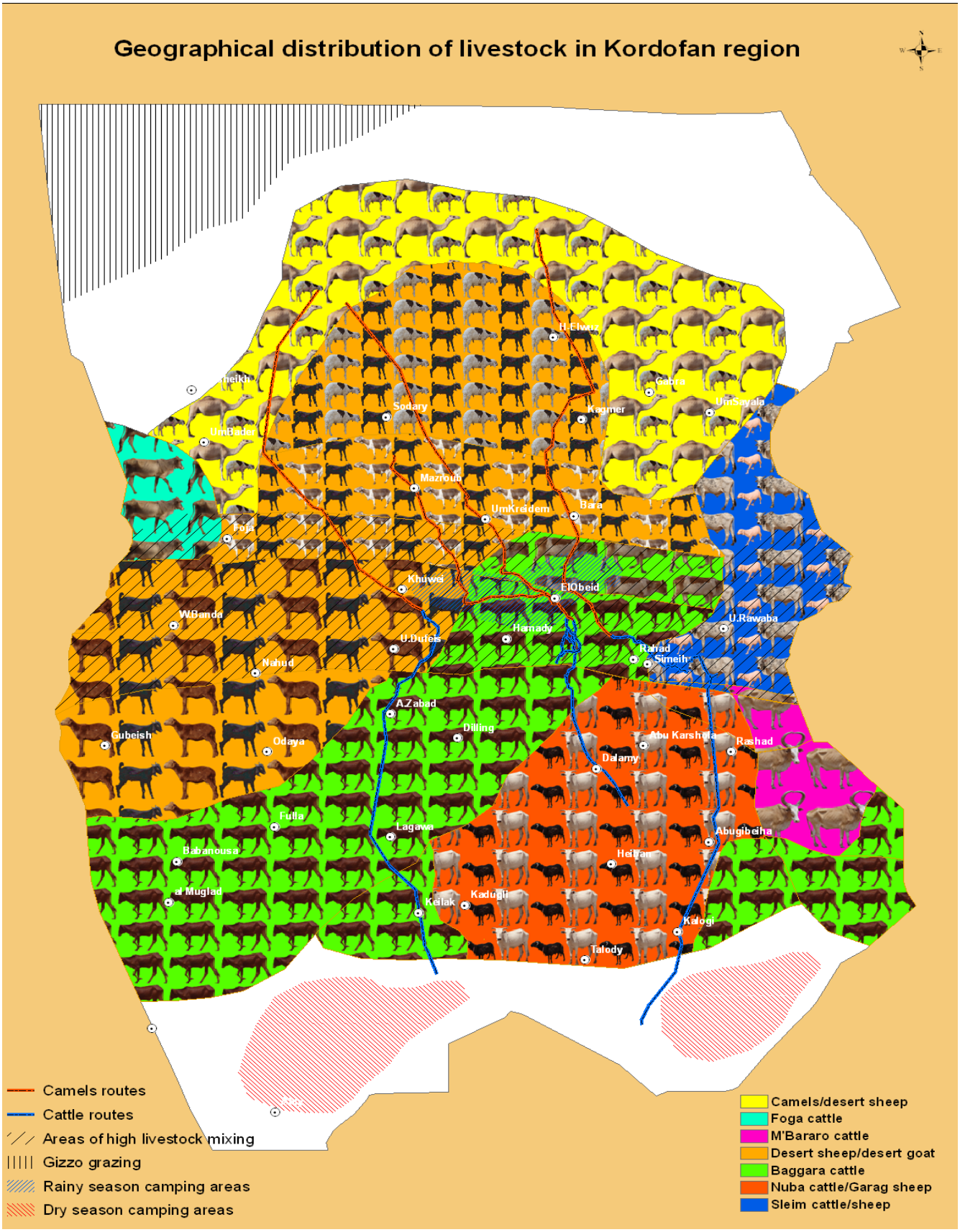
Appendix (2)

Fig (1) the study area (North Kordofan, Sudan)



Appendix (3)

Fig (2) Distribution of livestock classes in Kordofan region:



Appendix (4)

Photo:(A) Fuga breed study :

Photo (a1) Fuga Bull

Photo (a2) Fuga cows

Photo (a3)*Fuga Bull*

Photo (a4) *Fuga cows*

Photo (a5) Fuga Bull

Photo (a6) Fuga cows

Photo (a7)Fuga cows

photo (a8) Fuga cafes

Appendix (5)

Photo:(2-3) Knana breed study :

Photo (2) *Kenana cow*

Photo (3) *Kenana Bull*

Appendix (6)

Photo :(4-5) Butana breed study:

Photo (4)*Butana cows*

Photo (5)*Butana **B**ull*