CHAPTER ONE

GENARAL INTRODUCTION

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Sudan has a large livestock population raised mainly in low input nomadic or semi nomadic systems of production. The origin of most local livestock is not clear despite a rich history of archaeological findings (Epstein, 1971, Maason, 1988 and Payne, 1964). The development of livestock breeds is a result of the action of a number of factors including mutation, genetic drift, adaptations, random mating or artificial selection and other agents of the evolutionary process over the centuries. Sudanese cattle have had some historical studies about their origins in contrast with other domestic animals in Sudan. Epstein (1971) suggested that cattle were introduced in to Sudan from Asia through the Nile Valley or via the horn of Africa at about 5000 BC for the hump-less African Zebu and about 2500 BC for humped shorthorns. Thereafter, large introductions continued until about 670 A.D.

Apart from the animals inhabiting the Nile valley and riverbanks in the country, migratory tribes own most of the animals. Even in the large areas of the South, few tribes lead a sedentary life (Payne and Elamin, 1978). This has created some difficulties in defining and characterizing the breeds. The large number of tribal breeds or strains in the Sudan are mainly designated by external traits, specifically conformation and sometimes size and branding. The country is so large that obviously some of these groups of animals have been separated geographically for a long time, justifying the assumption of lack of contact. Thus, each breed is expected to have a unique set of genes. It will be very useful if we can identify the relative amount of variation between and within these populations. (Nei and Takezaki, 1996).

Sudan is lacking in many areas of basic information as to animal genetic resources and development. Areas such as national recording and AI schemes, breed societies and clubs, regulations and legislation are very poor. There are large gaps in our knowledge about these breeds and/or stains in the country. Also, the war-torn Western part of the country is in no position to supply sufficient and useful information about animal genetic resources.

The current impasse of animal genetic resources in Sudan is the paucity of information about the many breeds and stains that exist in the country. The continued existence of these animals is due to their disease resistance and environmental tolerance amongst other things. However, nowadays the future of any breed is increasingly being determined by its productivity and economic value. The size of the animal population in the Sudan is presented in table 1.

Table 1 :Animal census in Sudan 2012

Animal species	Numbers
Cattle	29,840,0000
Sheep	47,382,2200
Goats	21,585,4400
Camels	61,763,0000
Total	42,913,0660

The Ministry of Animal Resources, Fisheries & Rang lands 2012

Quantitative trait loci (QTL) of milk yield:

Milk and dairy products are important products because of their high nutritive value. The profit for the dairy farmer mainly depends on the production of more milk from the dairy cows and to a far lesser extent on protein and fat content. Milk yield as well as milk components are subject to considerable inters individualvariation within particularcattle breeds. Milk fat percentage is a quantitative trait that is determined by thecollective effect of multiple genes and environmental factors. The heritability (genetic contributionto the variation) of the milk fat percentage was estimated to be between 0.45 and 0.5(Goddard *et al.* 1999). The genetic variability for a given trait is a basic prerequisite for its improvement by systemic breeding. It is possible to increase the average value of one or several traits to improve the genetic potential of animals in the population. The traditional selection methods have been used to improve traits of economic importance like milk production. These methods have not been successful for traits such as reproduction, disease resistance, duration of productive life, and some conformation traits correlated with fitness (Ashwell *et al*., 2004; Sonstegard *et al.,* 2001). In the last decade, studies have been conducted to identify genes affecting economically important traits in commercial dairy populations. The identification of such genes is important since they can then be employed in selective breeding programs.

Quantitative trait loci (QTL) are chromosomal positions delimited by genetic markers, with the marker alleles being associated with a measurable effect on a quantitative characteristic. Mapping of QTLs is a first step towards identifying genes that contribute to variation in quantitative traits. A second approach is to identify the functional candidate genes based on metabolic pathways. A major goal of dairy

cattle genomics is to identify the gene representing the QTL and subsequently to identify the polymorphic site within the gene causal for the differences in the trait phenotype - the quantitative trait nucleotides (QTNs) (Mackay, 2001).

Many candidate genes with different functions in metabolism have been proposed as affecting milk yield and composition in dairy cattle, such as*Acyl-CoA:diacylglycerol acyltransferase 1* (*DGAT1*) on chromosome 14 in cattle. *DGAT1* catalyzes the final step in triglyceride synthesis and was presumed to be rate limiting with respect to lipid metabolism (Mayorek *et al.* 1989). A study with knock-out mice lacking *DGAT1* gene emphasized *DGAT1* as a strong candidate gene for milk fat percentage (Smith *et al.* 2000). Surprisingly, *DGAT1*-deficient mice were viable, indicating the existence of alternative mechanisms and/or further genes for triglyceride synthesis. However, the crucial point was that the mice were not able to produce milk. This observation highlights the determining role of *DGAT1* in milk fat synthesis and milk production in general (Smith *et al.* 2000).

Milk fat composition has a major influence on dairy products, where a more unsaturated milk fat is preferred from human nutritional and health perspectives. However, the relationship between *DGAT1* andmilk fat is that final step in the synthesis, in which diacylglycerol is transformed to triacylglycerol, is catalysed by the enzyme acyl-CoA:diacylglycerol acyltransferase1 (DGAT1)(Grisart. *et al.*2004).

Motivation:

The large population of cattle in Sudan and the increasing demand for milk and milk products necessitate the implementation of policies designed to raise the productivity of indigenous breeds; mainly Butana

and Kenana types. However, the information about the allelic and genotypic profile at such important functional or positional loci in Sudanese native cattle breeds is hardly available. Furthermore, there is an urgent need to conserve the pure breeds and to study the genetic components of Sudanese dairy cattle.

Objectives:

The objectives of this study were:

1. To review published papers on *DGAT1* gene with regard to milk production traits in dairy cattle.
2. To Characterize Kenana and Butana cattle breeds and their production systems, adopted dairy management practices, breeding objectives as well as to prioritize constraints and opportunities for dairy development in the Butana and Kenana homelands.
3. To determine variants of the *diacylglycerol acyltransferase 1* (*DGAT1*) gene in Sudanese dairy cattle (Kenana and Butana) with the aim of characterization of *DGAT1* variants in Sudanese dairy cattle breeds.

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Chapter Two

OVERVIEW OF THE DAIRY PRODUCTION SYSTEM AND GENETIC CONSERVATION OF CATTLE BREEDS IN TROPICS

OVERVIEW OF DAIRY PRODUCTION SYSTEM AND GENETIC CONSERVATION OF CATTLE BREEDS IN TROPICS

2.1 Introduction:

Livestock production is a major activity of agricultural systems in most tropical countries. In smallholder systems, which dominate tropical agriculture, large and small ruminants are important because they provide food,; they are means of transport and power for cultivation; they produce manure; and they may be a source of cash money in times of need. Nevertheless one function, the production of milk for human food, is often the primary reason for keeping livestock, whether by pastoralists to meet their subsistence needs in arid and semi-arid regions, or by peri-urban smallholder farmers as a source of income from milk sales. Indigenous cattle play a crucial role in the livelihood system and well-being of the traditional rural farmers of Sudan. Local cattle are an integral contributor of food, agricultural power, agrarian culture and heritage and biodiversity as well (FAO, 2007a).

It is reported that almost one breed of domestic species disappeared per month within the period from 2000-2006 over the globe. Around 20 percent of the reported breeds are classified at risk (FAO, 2007b). Breed substitution of indigenous stocks by specialized high producing breeds will likely cause a linear rise of unemployment among rural people associated with traditional farming leading to an inevitable catastrophe in the rural economy.

Dairy production is a biologically efficient system that converts cheep and low quality feeds (roughages), the most abundant feed in the tropics,

to high quality feed (milk), the most nutritious food known to man. Dairying is preferred to meat production since it makes more efficient use of feed resources and provides a regular income to the producer (Walshe *et al* 1991). It is also more labour intensive and supports substantial employment in production, processing and marketing. Higher levels of production than those achieved in traditional tropical systems, whether from cattle, camels or small ruminants, often require the introduction of specialised dairy breeds and increased levels of inputs (nutrition and health care) and good access to markets, both for milk sales and input acquisition. The intensification of smallholder livestock systems through the adoption of modern production techniques and specialized breeds is generally concentrated in areas with good infrastructure close to major markets, although less intensive production may occur in other, more distant areas (Walshe *et al* 1991). Market factors, play a major role in determining the type of dairy production systems found in the tropics, and they are particularly important influences on smallholder dairy development.

The rising demand for milk and dairy products in tropical countries presents smallholders with major challenges and opportunities. Several factors are responsible for the increasing gap between local supply of milk and demand in the tropics. Rapid increases in per capita income, especially in Southeast Asia, urbanisation and high-income elasticity of demand have caused a major rise in consumption. The poor genetic potential of indigenous livestock leading to low productivity, inappropriate technologies, inadequate research and extension support, poor infrastructure and unfavourable external conditions have contributed to the poor performance of the livestock sector in general, and of the dairy sub-sector in particular (Williams *et al 1995*). Competition from

foreign inexpensive dairy products compared to domestically produced equivalents has hampered the dairy development in many tropical countries. In some other countries, for example, sub-Saharan Africa, structural adjustment programmes have improved the incentives for domestic agricultural and dairy production (Staal *et al* 1997b).

Most milk in Sudan is sold without pasteurisation or packaging with the exception of some modern dairy's in the major cities. This is probably a result of consumer reluctance to pay the extra costs of pasteurisation and packaging. For example, in Khartoum, the modern processing sector handles less than 10 per cent of total milk production; the remainder is marketed through the informal sector (vendors) where it is sold as unprocessed milk. This raises issues of hygiene related to the primitive methods of production and transportation.

Broadly, dairy production systems in the tropics are concentrated near consumption centres. It is no coincidence that cattle and rural human population intensities are highly correlated (Krus*ka et al* 1997), with specialised smallholder (and large scale) dairy farms generally located close to (peri-urban) or within (intra-urban) major markets, or more distant when there is an efficient market infrastructure. On the other hand, the systems of production and their productivity in the tropics are influenced by agro-ecological factors and traditional consumption habits.

2.2 Dairy production systems in the tropics :

2.2.1 Pastoral systems:

These systems are migratory, transhumant or sedentary. Sedentary farmers live in the same homes all year round while migratory and transhumance farmers move.

In Sudan the pastoral system is the most common system and more than 90% of cattle are owned by nomadic and semi nomadic tribes, with regular seasonal migration mostly from north to south in the dry season and from south to north in the rainy season. Despite the existence of large irrigation schemes (e.g. Gezira, Halfa Algadeda, Rahad) and large-scale mechanized rain-fed farming (Gedaref, Blue Nile, Sennar, White Nile and South Kordofan states), the majority of the rural population depends mainly on herding and small-scale rain-fed cultivation.

Market linkages are limited and live animals are sold only to obtain cash for purchase of food (Musa *et al*.2007) and to prepare for the cultivation season. Nomads in Sudan are generally camel or cattle herders. They might own some sheep and goats also for social and economic reasons. When long-settled and nomadic or bedouin communities come in contact with each other, relations are often hostile or cool, reflecting competition over limited resources, the loss of rangelands to crop production and lack of demarcation of migration routes. Along the White Nile and between the White Nile and Blue Nile, some nomadic tribes have become sedentary. This transition occurred either because of the opportunities for profitable cultivation or because nomads had lost their animals and turned to cultivation until they could recoup their fortunes and return to nomadic life.

According to Tonah (2002), dairy production by the Fulani in Ghana is characterised by migratory pattern, which is changing over time. Fulani have a uniqueness which stems from the fact that they are culturally the least known to the indigenous population and share very few practices with the host population. Fulani settlements are typically located at the outskirts of the settlement and consist of several concentric huts arranged to form a single housing unit. In a study on Fulani Agro-pastoralists in central Nigeria, Waters-Bayer (1988) found that dairy production units had modal household sizes of 7 - 13 persons with almost equal males and females and 45% of members above 18 years.

Contrary to the situation of the Fulani in Ghana, milking by Nigerian Fulani is only done once daily by boys and men and exceptionally by women. The Fulani there have a more sedentary pattern of life. The modal herd size is usually between 40 - 60 cattle with majority of families keeping sheep and all keeping poultry. During dry periods, they graze their animals further away from their homes as compared to the rainy seasons when sufficient pasture can be got closer to their homes (within 5 - 6 Km). In the dry season, arrangements are made with local farmers for stubble grazing and manuring. During such periods, a woman or one of her children have to spend up to an hour on the way to such farms, taking along cooked food for the herders and returning with milk for the household (de Leeuw *et al* 1998). In other cases, part or all of the family moves with the herd and only return home when conditions are favourable. Whenever a part of the family remains home, a few cows with younger calves are left behind, to supply milk to the household and to prevent the fragile calves from dying during the stressful transhumance period.

A variation of the pastoral system is where herders are pastoralists who act as managers of communal herds of cattle, which are entrusted to them by local farmers who each own a few heads (de Leeuw *et al* 1998).

2.2.2 Semi- intensive systems :

This system is common in peri-urban areas, having farms which are owned by business men, and private individuals who employ labour in the catering of their animals, the major objective is milk production (Diop and Mazouz 1995). Dairying is done with some degree of intensification by a combination of grazing and concentrate-feeding. In this system, graded cows or crossbreeding is used, usually between exotic bulls and local cows or through artificial insemination (AI). The aim of crossbreeding is to increase milk production and conserve the adaptability of the local breeds to environmental conditions (Bayemi *et al* 2005). Milk production here is much higher than in the pastoral systems.

2.2.3 Intensive systems:

Market infrastructure increases the importance of the dairy component in small farmer dairying. Rising population growth and urbanisation have led to greater intensification of dairy systems in peri urban areas in Africa which is also favoured by higher demand in such areas. The farms here are small (about 1-2 ha with 1-2 cows and generally Holstein Friesian or crosses). Feeding is mainly zero grazing with planted Lucerne and residues such as wheat bran and ground nut hulls. Most work on the majority of such dairy farms is done by the family. Contrary to pastoral systems where large proportions and sometimes all the daily milk is consumed at home, only a small portion of milk produced in this system is left for home consumption and the rest sold (de Leeuw *et al* 1998).

An example of peri urban dairy activities is the Kuku Dairy Co-operative Scheme is located in Khartoum North, Khartoum State, Sudan. . The Scheme was established in 1963 on the nucleus of small milk producers co-operatives dated from 1953.The Scheme covers an area of about 2600 acres of flat leveled land. The whole project was established by U.S.A government. The objective of the Scheme was to settle semi-nomadic animal owners and concentrate on the production of pasteurized milk.

The majority of the members practice both fodder cultivation and livestock rearing. They keep mainly cattle and may have some goats or sheep. Almost all cattle in the scheme are crossbreds (Kenana or Butana with Friesian) with different levels of foreign blood. The Kuku Dairy Cooperative offers some services and provides each member of land 5-10 feddan (Omer, 2005).

Larger intensive farms are usually owned by rich individuals, companies or the government. More investments are also made on buildings and machinery while the use of hired labour is unavoidable. There is a higher market orientation in this systems and more emphasis is laid on feeding and breeding management to assure optimal production (Diop *et al* and Mazouz 1995). In both intensive and semi-intensive systems AI plays a major role in breeding, as it is cheaper and less cumbersome than maintaining an exotic bull. Unfortunately, breeding programmes are poorly structured in some countries, leading to ineffectiveness in insemination. Farmers usually complain of poor heat detection and low success rates, leading to long inter calving periods and hence low productivity of animals (Diop *et al* and Mazouz 1995).

2.3 Conservation of Local cattle Breeds:

Itis very important to protect and keep available traditional breeds that are not in common use currently. They might be required as a resource of genes for future selection, especially for adaptation to changing production or climatic conditions, new disease challenges, etc. In many developing regions, local breeds are often not very efficient in terms of productivity, but they are particularly well adapted to environmental conditions: climate, feed, diseases and parasites.They are often able to survive where improved breeds of temperate countries cannot survive without very expensive treatments or environmental modifications (protective shelters, cooling, heavy use prophylactic treatment with antibiotics). In general, local livestock breeds tend to be more resilient and capable of tolerating the excesses of their environment. FAO.2007a

Most indigenous breeds in the tropics have a small size, and thereby require less feed than improved breeds and are better able to tolerate high temperatures. Since the price of a single animal is not very high, the loss for the owner in case of death is limited. Larger numbers compensate for the lower productivity. Some local breeds have social, religious and cultural functions as gifts, for feasts, and dowries.

Genetic variation in animals has developed during millions of years. In the course of time the usefulness of different genes and gene combinations has been under severe natural selection, especially concerning adaptability to different conditions and resistance to diseases and parasites. During the last ten thousand years man has partly influenced this evolution, and many breeds adapted to local needs and environments have been developed. The possibilities of making changes in the genetic make-up of farm animals and of concentrating on the

utilization of the breeds considered to be the best, have increased in recent decades, thanks to the availability of modern reproduction, computer and communication techniques FAO.2007a.

2.3.1 Reasons for conservation of local cattle breeds:

2.3.1.1 Economic-biological reasons:

(FAO.2007a)

1. Changes in the production environment

   i. Crossbreeding with improved European breeds is gaining ground very quickly. Many farmers have realised that crossbreeding with improved local breeds gives better milk yield than crossing with poor local breeds. Therefore, this presents the promising local breeds with greater danger.
   ii. Drought, famine and in many countries civil upheavals have led to the displacement of millions of mostly nomadic people. The breeds that these people keep are in many cases endangered.
   iii. Changes in management of animals (e.g. mechanization, milking frequencies and methods, densities, etc.) and housing conditions.
   iv. Changes in the hygienic conditions of animals (new kinds of disease agents, new vaccines and medicines)
   v. Changes in climatic conditions (temperature, humidity).

2. The demands for products and services desired from animals may change for many reasons

   a. Increased incomes and changing standards of living and new fashions in eating and clothing.

b. 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.

c. Another cause for concern is the fact that the directions of future demand cannot be predicted with any certainty.

d. Changes in international trade and trade blocs effect, costs of materials and prices of products.

e. The increased human population resulting in increased demand and the need to combat hunger. The need for compensating exhausted natural reserves of fuels, minerals, etc., with renewable plant and animal materials may become more and more topical.

f. The competition between animal species in production costs and services, as well as that between animals and plants as food producers may affect the usefulness of various kinds of animals.

g. The need of finding new ways of utilizing agricultural plant products in case of surplus problems may also increase.

h. There may appear needs to overcome selection limits and antagonisms.

2.3.1.2 Scientific reasons:

1. Research to improve the knowledge on indigenous animal genetic resources, is instrumental for increased awareness on the role of livestock and their genetic diversity and for the implementation of sustainable breeding programmes.
2. Frozen stocks are useful for the measurement of genetic trends.
3. Research in genetics, physiology, biochemistry, immunology, morphology, etc., benefits from the maintenance of a large variety of animal materials.
4. They are also useful as teaching material in animal sciences.

2.3.1.3 Cultural-historical reasons:

a) Conserved breeds can be considered to be valuable memorials of nature and culture (living cultural heritage).
b) There are ethical-moral grounds to take care of the existence of different creations of nature. FAO.2007a.

Table. 2: Population size and the threat prestige of international livestock

Breeds	Normal%	Vulnerable%	insecure%	Endangered%	Critical%
Cattle	25	15-25	5-15	2-5	Less than 2
Buffalo	30	20-30	10-20	5-10	Less than 5
Sheep	50	30-50	15-30	8-15	Less than 8
Goat	30	20-30	10-20	5-10	Less than 5
Camel	20	15-20	5-15	2-5	Less than2
Horse	20	15-20	5-15	2-5	Less than 2
Pigs	10	5-10	1-5	0.5-1.0	Less than 0.5

Source: Lecture of Animal Genetic Recourse Conservation, 2014 (By Dr.Prof. Mohammed Khair Abdallaha)

2.3.2 Steps for conservation:

2.3.2.1 Inventory:

Existing populations in danger must be identified. Definition of the status of breed as critical, endangered, insecure, vulnerable and normal depends on factors such as the number of breeding males and females, overall

numbers, number of sub-populations, and trends in population size. There is need to monitor changes in population size continuously.

2.3.2.2 Evaluation:

A description of the population is needed to allow its eventual use and conservation. This may be done with the aid of new technologies. Gene mapping approaches such as testing for single nucleotide polymorphisms (SNP's) help to track ancestry and to determine the genetic distance of one group from another. The existence of unique potentially useful traits must be investigated. Measurement of performance evaluation must be standardized, and carried out in the environment in which the herd might be used.

2.3.2.3 Choice:

In view of limited resources available for conservation choices have to be made. Choice of breeds for conservation includes cultural reasons, potential value, genetic distance from other lines or populations and threat of extinction. New techniques and economic theories assist in assessing risk of loss and potential benefits. Saving a pure breed preserve that breeds characteristics and makes a readily identifiable animal. Crossing several breeds to produce composites has the advantage of saving the genetic material from all while reducing costs. However, the total genotype of each breed is lost.

2.3.2.4 Preservation:

Populations can be saved as live animals which is expensive. Populations can be saved as pure breeds, or as composite lines. Keeping a specific breed in use is the best way to ensure the breed's adaptation to changing

production environments. In general there are two methods of conservation in-situ conservation, and ex-situ conservation.

2.3.2.4.1Conservation in situ (In situ conservation):

In situ conservation is the maintenance of live populations of animals in their adaptive environment or as close to it as it practically possible. For domestic species the conservation of live animals is normally taken to be synonymous with in situ conservation. It leads to the exploitation of diversity in the best way in the short term and protects it for the long term. Activities related to the conservation in situ include performance recording projects, the development of education and management of genetic diversity programs and steps to ensure the sustainable management of environmental systems used in agriculture and food production.

Advantage of in situ conservation:

I. The type will continue to evolve in the natural environment.
II. It helps preserve local traditional knowledge associated with breeds.
III. Usually the amount of space available for the animals is much more than what will be available in off-site conservation.
IV. It creates the potential for sustainable exploitation in rural areas and it can be economically self sustaining.

However there are problems when in situ conservation is in parks or stations it is difficult to control illegal exploitation (theft, grazing, and logging). Environment may need rehabilitation as it may be difficult to control invasive species. If the population under conservation is small there is a risk of gene drift leading to the loss of some alleles.

2.3.2.4.2 Ex-situ Conservation:

Ex situ preservation involves the conservation of animals in a situation removed from their normal habitat. It is also used to refer to the collection and freezing in liquid nitrogen of animal genetic resources. Finally it may refer to captive breeding of wild animals in zoos or other situations far removed from their indigenous environment. It includes the transfer of the animal from its natural environment and put it under human care and this usually happens when the type is threatened or the environment in which the type lives are threatened.

Ex situ conservation in live animals:

As in the case of conservation at the site, it is likely to result in improvement and natural selection to change of genes frequencies in the saved population. The fundamental question with regard to this strategy is whether there is long-term commitment and financial support to keep generations of animals.

Cryo conservation:

This involves the freezing of semen, ova or embryos. It may include the preservation of DNA segments in frozen blood or other tissues.

Advantages:

i. The relative cost of collecting, freezing and storing frozen material, as compared to maintaining large scale live populations, has been estimated to be very low (Smith, 1983).

ii. Gene banks require little space and few trained technicians. A very large number of frozen animals from a large number of populations can be stored in a single facility.

iii. Cryogenically preserved populations suffer no genetic loss due to selection or drift. Genetic resources of frozen animal can be made available to livestock breeding and research programmes throughout the world. FAO.2007b.

Disadvantages

I. Cryogenically preserved populations are not able to adapt through gradual selection, to changes in the climate or disease background of the local or global environment.
II. Little contribution in the related goals of sustainable exploitation in rural areas.
III. Multiple storage sites are needed to avoid loss in case of natural disasters (Floods, fires....).
IV. The technology necessary for semen collection and freezing, and for superovulation, ova and embryo flushing and freezing is readily transferred throughout the world, however, it is expensive for countries in which the technology is not yet established.

Ex situ and in situ conservation are not mutually exclusive. Frozen animal genetic resources or captive live zoo populations can play an important role in the support of in situ programmes. FAO.2007b.

2.4 Improvement of indigenous breeds in tropical areas by selection:

The limiting factor to increased production is not only the genetic merit of the animals but also the adverse environment, in husbandry and veterinary care which should precede the setting up of breeding schemes. The performance of most indigenous breeds is characterized by late age at maturity, short lactation, long dry period, low average daily milk yield and poor dairy temperament. Most of these characters are expressed even

under conditions of improved husbandry, thus demonstrating that poor productivity is attributable to low genetic merit. FAO.2007b.

The maximum rate of genetic gain in milk yield achievable by selection even in temperate breeds is of the order of 2.0 percent per annum. This low rate of improvement has discouraged implementation of selection schemes for indigenous breeds, since the overall increase in production attainable by selection in cows yielding 500–1000 kg is not of any great magnitude. It should, be recalled that even European breeds were as unproductive as the tropical breeds before the application of selection programmes and it is the application of planned selection programmes that has brought the advance that we see today (Cunningham, 1979).

Annual genetic gains of approximately 2.5 percent of the herd mean yield have been reported by Acharya and Lush (1968) in Haryana (zebu) cattle in India and by Franklin *et al.* (1976) in the Australian Milking Zebu (Jersey x zebu cross). The high rate of genetic gain in both populations was partly attributed to the high coefficient of variation for milk yield in the populations, which is characteristic of most dairy cattle in the tropics (Mahadevan, 1966). And on other hand populations that failed to show significant gains, it was the inefficient selection procedures that were responsible, such as, East African zebu (Kimenye, 1979).

Prior to designing a breeding programme for dairy cattle in the tropics, the objectives of the programme and the other factors such as, environment and production system under which these objectives are to be realized should be defined. Then, the objectives should make clear whether it is milk alone or milk and beef or milk and draught or all three that are to be improved. Moreover, depending on market demand, the relative importance to be related to milk composition should also be

clarified. In the same way, the production system, which can range from nomadism through small sedentary herds to large commercial farms, will determine the importance given to adaptation traits, mostly those concerned with disease resistance and reproduction.

In Large part of the tropics parts of the tropics there are lacking of infrastructural facilities for national breeding programmes. And need for such facilities has not arisen because the traditional methods of livestock husbandry practised in these areas, e.g. nomadism or backyard farming, make it impossible to operate a planned breeding scheme as is done in the developed countries. As an alternate, state-sponsored livestock farms have been set up with a view to effecting genetic improvement in these herds or flocks and passing the improved stock, especially males, to farmers.

2.4.1 Importance of selection in local breeds in the tropics:

The local breeds constitute the origin stock of adapted germ plasm, and it must be conserved for the fact that their losses mean the loss of valuable unique genes which cannot be easily replaced in future if there is changing in production conditions. Also the benefits of these unique genes that have ability to tolerate high temperatures, diseases and parasites and their ability to exist on low quality feed and limited water supply make them a valuable resource. Selection for increased productivity should raise their potential for productive traits.

Imported of exotic breeds into many tropical countries either as live animals or as frozen semen. Crossbreeding of these exotic breeds with indigenous stock poses a danger of extinction of the local breeds. In these situations, one can ensure the survival of the local strains only by raising

their genetic merit so as to minimize the competition (Cunningham, 1979).

2.4.2 Selection procedures in developed countries:

2.4.2.1 Testing stations:

In the central performance tests bulls from different herds are brought to one central location where their performance is recorded. The goal is to detect genetic differences between animals using a fair comparison under identical conditions. Central performance tests have been used for a long period in beef cattle and to a lesser extent in other meat animals.

Central performance tests have advantages but they also have weaknesses. Tests are performed under identical environmental conditions, and this gives high genetic parameters (due to lower environmental variation) and thus selection response is increased.

In this scheme it is easy to measure food consumption and take ultrasonic measurements.FAO**.** 2007a

Disadvantages:

The system is costly and therefore only a limited number of bulls can be tested. Environmental conditions may be different in herds from which the bulls came. Some studies have shown the existence of a significant impact due to the herd of origin. The impact of herd of origin may be due to genetic differences between herds or pre- selection of the bulls.

2.4.2.2 Progeny testing schemes:

The breeding value of bulls is assessed on the performance of their daughters. The test depends for its efficiency on a large progeny group per sire and on having each sire represented in several herds. AI is necessary to separate environmental from genetic effects. Milk yield

records are usually restricted to those from first-calving heifers since this gives the largest group of unselected daughters, although some countries, e.g. USA, consider records of older animals as well. Young bulls that enter the progeny test are bred from the best progeny-tested sires and selected dams. Each young bull is used on about 500 cows in milk-recorded herds to ensure that first lactation records of at least 80 daughters are obtained. Mating is done at random to ensure that the dam contribution to progeny genotype is similar for all sire progeny groups. FAO.2007b.

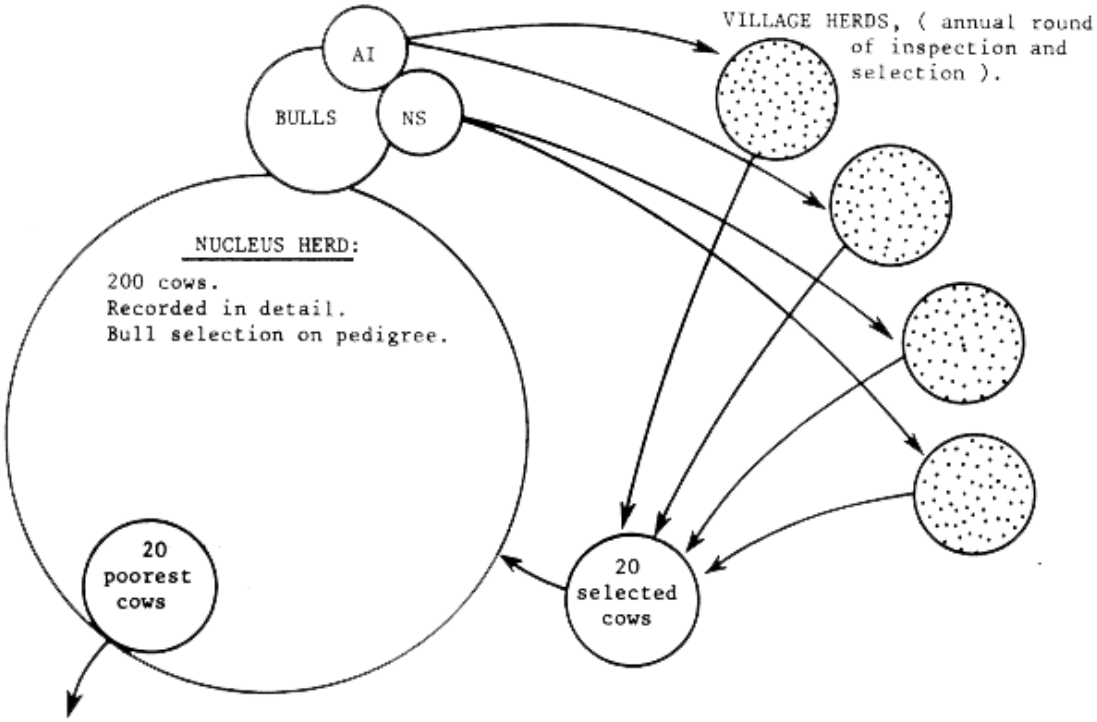
The application of progeny testing requires the existence of an efficient AI service and performance recording among other things. Where a number of herds are involved in testing, the maximum efficiency is achieved when each sire has equal number of progeny in each of the herds. The field based progeny testing is highly required when the selected bulls are to be distributed in a large area, to many farmers in different environments. Usually the breeding companies conduct progeny testing of their bulls so that they can be commercially promoted. A modification of this scheme called the reference sire scheme enables comparisons to be made also among bulls that are used only within individual herds. The reference sires, which are usually progeny tested animals and available only by AI, are used alongside the individual herd's own bulls in all herds. The breeding values of home-bred bulls are then estimated in relation to the reference sires. Comparisons among bulls in different herds are made through the linkages established by the reference sires. Thus sire replacements can be made from among all herds.FAO**.** 2007a

2. 4.2.3 Nucleus or group breeding schemes:

In each herd there is a small number of genetically superior animals which if brought together will form a nucleus whose average genetic merit is far greater than that in any of the contributing herds (Nicoll, 1976). In this scheme a group of farmers agree to pool their high performing animals. Once the nucleus herd is assembled, an efficient system of recording and selection is implemented. The best males are kept for breeding in the nucleus while other selected males are given to the base herds for breeding. By this means, improvements are quickly spread throughout the group. The nucleus may remain open to animals from the base herds, the best females from the latter being admitted periodically and compared with those in the nucleus. Only females are transferred from the base to the nucleus since sire selection will not be practicable in base herds due to managerial reasons. The main advantage in the nucleus scheme is that the genetic superiority of sire replacements coming into the base herds from the nucleus is far greater than what is achievable in each of the base herds. It is particularly attractive in situations where within-herd selection programmes are ineffective due to small population size or inadequate.

Cunningham (1979) proposed a kind of open nucleus breeding scheme (Figure.1). The plan operates around a central herd which should be under government control. The base population is the village herds which provide cows to replace about 10 percent of the cows in the central herd annually. Selection from village herds will be by simple methods include, evaluation by eye, milking ability, size, conformation and condition. The selection of these animals will be done by officers who would tour many villages to select the best cattle.

Figure .1 Open nucleus breeding scheme (Cunningham, 1979)



Source .Food and Agriculture Organization of the United Nations Rome © FAO 1982

Bulls are bred from the best cows and selected bulls in the central herd. They are then evaluated on their own growth rates and milk yields and the best are chosen as sires. The lowest yielding 10 percent of the cows are replaced with new animals drafted from village herds. The latter animals are recorded during the following year with the remainder of the central herd and re-evaluated.

The benefits to the cooperating farmers from this scheme will accrue from the sale of selected bulls from the central herd. Assuming that the central herd has 200 cows and that the best third of the bulls born annually are chosen, about 20–25 bulls will be available for sale to farmers after meeting the central herd's requirements. This may satisfy

the requirements of the cooperating farmers but will have hardly any impact among farmers outside the scheme. This is a serious shortcoming of programmes involving central herds where supporting AI services are inadequate.(Cunningham ,1979).

2.5 Molecular techniques

2.5.1 Marker assisted selection (MAS)

Nearly 1990, the time of started changing the focus of main activities in animal breeding from quantitative to molecular genetics. These activities were divided into two steps, which were, the detection of markers associated with QTL and the use of markers in Marker assisted selection (MAS) (Ignacy. 2006).

MAS is an indirect selection process where a trait of interest is selected, based on a marker linked to the trait. For example, if MAS is being used to select individuals with disease resistance, the level of disease resistance is not quantified but rather a marker allele that is linked with disease resistance is used. At a major conference in (1991), M. Georges, a major scientist in this area, claimed that in a few years there would be no need for best linear unbiased prediction (BLUP), which was a traditional method for selection based on phenotypes.

This new type of selection (MAS) has many advantage, such as, Genotypes could be determined without phenotypes, by typing animals for important markers and then calculating the breeding values by simple addition. Other advantage related to the cost, the making of selection by using phenotypic data, with public ownership of phenotypic data in many species, the results of evaluation have to be made public. The benefit of MAS selection that can be very useful for traits those are difficult or expensive to measure. And the assumption is that the marker used for

selection associates at high frequency with the gene or quantitative trait locus (QTL) of interest (Ignacy. 2006).

2.5. 2Positive and negative selectable marker:

- **Positive** selectable markers are selectable markers that confer selective advantage to the host organism.
- **Negative** selectable markers are selectable markers that eliminate or inhibit growth of the host organism upon selection.

2.5.3 Markers types:

1) **Morphological** marker - These markers are often detectable by eye, by simple visual inspection. Examples of this type of marker include the presence or absence of horn in cattle, height, grain colour, aroma of rice etc. In well-characterized crops like maize, tomato, pea, or wheat, tens or hundreds of genes that determine morphological traits have been mapped to specific chromosome locations.
2) **Biochemical marker**- A protein that can be extracted and observed; for example, isozymes and storage proteins.

3) **Cytological** - The chromosomal banding produced by different stains; for example, G banding. (Ignacy. 2006).

4) DNA-based or molecular- A unique gene (DNA sequence), occurring in proximity to the gene or locus of interest, can be identified by a range of molecular techniques such as registration fragment lenth polymorphism RFLP, microsatellite, or single-nucleotide polymorphism (SNP) detection. (Jump review MAS in plant breeding , Dubcovsky, J. 2004).

2.5.4 Genotyping methods and Marker assistant selection:

Differences among alleles caused by a single nucleotide, called SNPs, can be the basis of genotyping tests. Genotyping means using laboratory methods to determine the sequence of nucleotides in the DNA from an individual, usually a specific gene. Genetic tests based on SNPs utilize DNA derived from an individual to determine the nucleotide in the gene of interest.

Marker assisted selection is the process of using the results of DNA testing in the selection of individuals to become parents for the next generations. The information from the DNA testing combined with the observed performance records for individuals, is intended to improve the accuracy of selection and increase the possibility of identifying organisms carrying desirable and undesirable traits at an earlier.

2.5.5 Steps for Marker assistant selection:

Generally the first step is to map the gene or quantitative trait locus (QTL) of interest first by using different techniques and then using this information for marker assisted selection. It very important that, the markers to be used should be close to gene of interest (<5 recombination unit or cM) in order to ensure that only minor fraction of the selected individuals will be recombinants. Moreover, not only a single marker but rather two markers are used in order to reduce the chances of an error due to homologous recombination.

2.5. 6 Problems with MAS/QTL

1. If 100 QTLs were responsible for a trait and they acted additively, animal breeders would soon be doing simple counting. However, if interactions exist among QTLs, the QTL model becomes intractable. (W. Hill and R. Thompson n 1993).

2. (Dekkers 2004), there are three types of markers: genes, linkage equilibrium (LE), which is located very close to the gene, and linkage disequilibrium (LD), which are located farther from the gene. LD markers are easiest to find but hard to use.
3. There were no markers for low heritability traits as these require a large amount of data for estimation.

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Chapter three

DGAT1 Gene in Dairy Cattle

DGAT1 Gene in Dairy Cattle

The interest in the bovine *DGAT1* gene has increased during the last few years. *DGAT1* gene, encodes a microsomal enzyme that by using diacylglycerol and fatty acyl CoA as substrates catalyzes the terminal and committed step of triacylglycerol biosynthesis. This step is the most important storage form of energy for eukaryotic cells. *DGAT1* is also important for the physiological processes involving triacylglycerol metabolism such as absorption of intestinal fat, lipoprotein assembly, adipose tissue formation and lactation. Lactation was impaired in female mice lacking both copies of *DGAT.* This observation leads to a suggestion that *DGAT1* gene was the functional candidate gene for milk production traits. The frequency of polymorphism in *DGAT1* gene has been found to be very high in dairy cattle. Some associated studies such as, milk yield, fat content, protein yield and content have been carried out in dairy cattle. These associations will provide insight to underlying mechanism of *DGAT1* gene and polymorphisms that can be used for selection purposes in dairy cows.

Keywords: Bovine, Fat content, Lactation, Milk yield, Triacylglycerol Metabolism

Introduction:

For the last decade, molecular genetics has lead to the discovery of individual genes or candidate genes with substantial effects on the traits of economic importance.

Candidate gene strategy has been proposed by direct search for quantitative trait loci (QTL). In other words, the genetic variation in a gene affects the physiological pathways and phenotype. Moreover, the proportion of genetic and phenotypic variation would likely affect the

breeding strategy for improvement of important traits in the future. Genetic markers associated with traits of interest can be searched directly by applying molecular biology techniques. These techniques can identify genetic variation at specific loci and analyze the relationship between genetic variation at QTL and production traits. Application of molecular genetics for genetic improvement relies on the ability to genotype individuals for specific genetic loci. The information utility from candidate genes in breeding programs has potential to substantially enhance the accuracy of selection and increasing selection differences.

The QTL are chromosomal positions delimited by genetic markers, with the marker alleles being associated with a measurable effect on a quantitative characteristic. Mapping of QTLs is a first step towards identifying genes that contribute to variation in quantitative traits. A second approach is to identify the functional candidate genes based on metabolic pathways.

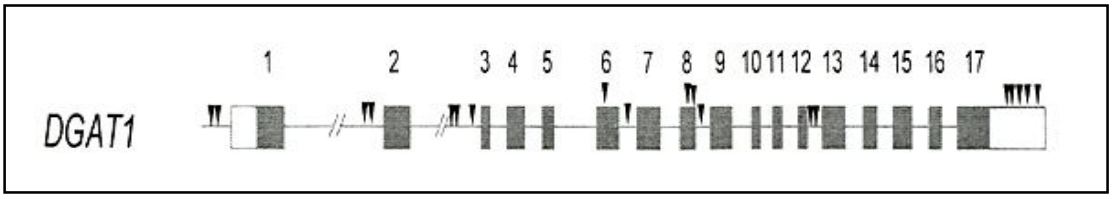
A major goal of dairy cattle genomics is to identify the gene representing the QTL and subsequently to identify the polymorphic site within the gene causal for the differences in the trait phenotype - the quantitative trait nucleotides (QTNs) (Mackay, 2001). Many candidate genes with different functions in metabolism have been proposed as affecting milk yield and composition in dairy cattle, such as, Diacylglycerol acyltransferase1 (*DGAT1*).

Many studies have reported that *DGAT1* gene is the candidate gene influence milk fat content and yield. Therefore, the aim of this article is to review the published (*DGAT1* gene) which have an influence on economic traits and could be applied for a direct search of QTL in order to plan a breeding program in the future.

Discovery of *DGAT1* gene

The *DGAT* activity was first described by Weiss and Kennedy in the 1950's (Kennedy, *et al*., 1957 Weiss, *et al*., 1956).*DGAT1* gene was localized on centromeric end of the bovine chromosome 14. A span 14,117bp consists of 17 exons.

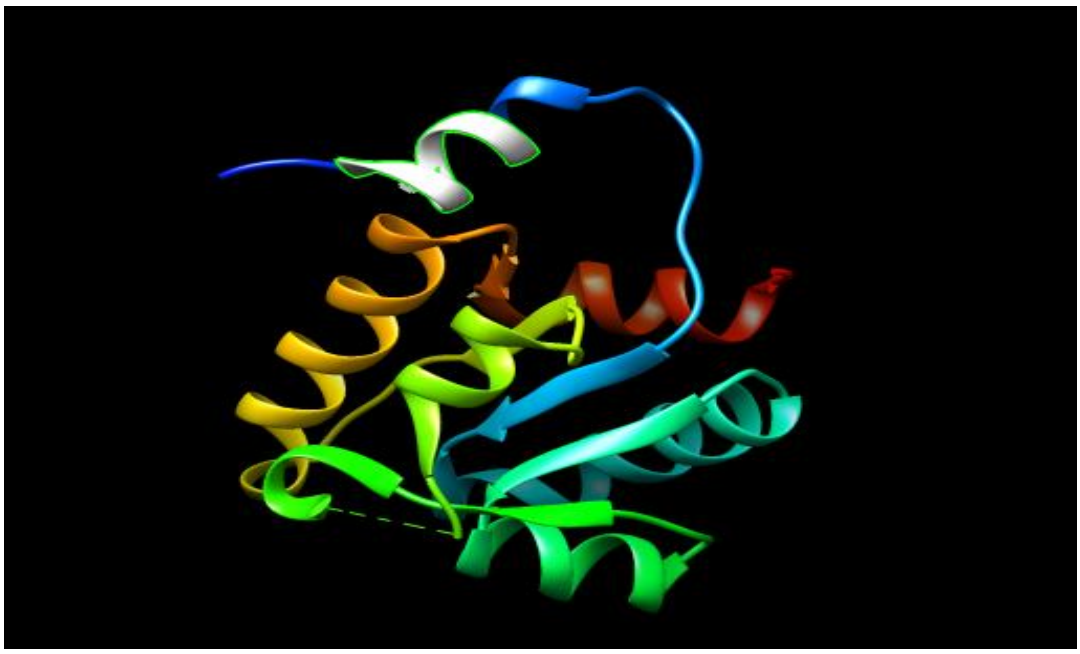
Fig 1: Gene structure of the DGAT1 gene.



Coding region Untranslated region

Accession no. AJ318490.1. Reference NCBI data base, modified

Fig 2. Three dimensional of *DGAT1* molecule



Reference: http://www.hindawi.com/journals/ecam/2014/823154/

Roles of *DGAT1* in Triacylglycerol Metabolism

The role of *DGAT1* in TG (Triacylglycerol) metabolism should be considered in the context of individual tissues:

Small intestine - In the small intestine, *DGAT* is required for the absorption of dietary TGs. Dietary TGs are non-polar molecules and are not able to be transported from the intestinal lumen to enterocytes intact. Instead, TGs in the small intestine are emulsified and digested by lipases producing 2-monoacylglycerol and unesterified fatty acids, which cross from the lumen of the gut into enterocytes. In the enterocyte, TGs are re-synthesized mainly by monoacylglycerolacyltransferase and *DGAT*. TGs are incorporated into chylomicrons in order to deliver dietary lipids through the lymphatic system to the circulation where the fatty acids are taken up by muscle, liver, adipose tissue, etc. (Stone, S.J. 2011).

Liver – In the liver, *DGAT* has a role in synthesizing TGs from either fatty acids synthesized *de novo* or from fatty acids taken up from the circulation. These TGs are incorporated into very low density lipoproteins for delivery to extrahepatic tissues where they are stored (adipose tissue) or oxidized (skeletal and cardiac muscle).

Mammary gland – TGs, a major component of milk. They are stored in adipocytes in the lactating mammary gland and provide an essential source of energy to new-borns. Fatty acids released by the hydrolysis of TGs stored in adipose tissue are re-esterified to TGs by *DGAT* in the mammary gland. (Stone, S.J. 2011).

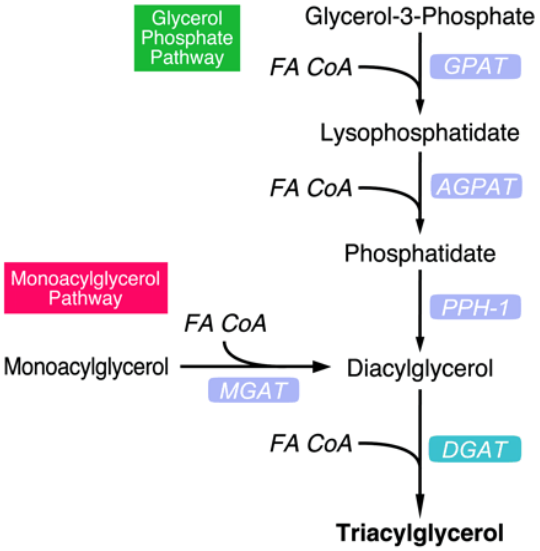
Adipose tissue – Adipose tissue has the highest content of TGs in mammals and is the main tissue for storage of TG. TGs are delivered to adipose tissue through the circulation by chylomicrons and very low density lipoproteins (VLDL). Lipoprotein lipase present in the blood capillaries in adipose tissue hydrolyze TGs contained within these lipoproteins. The unesterified fatty acids are taken up by adipocytes, re-esterified to TGs mainly by the Kennedy pathway involving *DGAT* and stored in cytosolic lipid droplets. When required, TGs in adipose tissue are hydrolyzed to fatty acids and glycerol, which are released into the

circulation. Fatty acids are then transported in an albumin-bound form to tissues such as muscle and liver where they are oxidized to promote the synthesis of ATP.(Stone, S.J. 2011).

DGAT1 Enzymes and Triglyceride Synthesis:

*DGAT1*catalyses the final step in the triglyceride synthesis (Mayorek *et al.* 1989).Diacylglycerol acyltransferase1 *(DGAT1)* gene encodes an enzyme which plays a major role in the synthesis of triglycerides. Triglycerides which are major components of fat are formed by binding of diacylglycerol to long chain fatty acyl- CoAs. This reaction is catalyzed by at least two enzymes. One of these enzymes is encoded by *DGAT1* (Cases *et al.* 1998 and Winter *et al.,* 2002). Lactation deficiency was observed in female mice lacking both copies of *DGAT,* probably as a result of deficient triglyceride synthesis in mammary gland. After this observation *DGAT1* gene was suggested as a functional candidate gene for milk production traits (Smith *et al.,* 2000 and Winter *et al.,* 2002).

Figure 3.Triglycerides synthesis and *DGAT* enzymes.



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DGAT1 and milk fat association:

Milk fat composition has a major influence on dairy products, where a more unsaturated milk fat is preferred for human nutritional and health perspectives. This may, however, render the milk fat more susceptible to oxidation, giving a 'carbon', 'metal', 'talcum' or 'fishy' flavour to the milk (Shipe *et al.,* 1978). The off-flavour in milk and results from volatile compounds that accumulate in the milk through the oxidation of the double bonds between the carbon atoms in unsaturated fatty acids (FA). Oxidation is often initiated by prooxidants such as copper and iron but these can be balanced by antioxidative substances in the milk, like β-carotene and α-tocopherol. (Barreforset *al*. 1995) found that the higher the proportions of the unsaturated FAs linoleic (C18:2) and linolenic (C18:3) in the milk, the higher is the risk of 'oxidized' flavour. The fatty acid composition varies due to factors like feed, stage of lactation, parity, season, and genotype of cow (see review by Palmqvist*et al*., 1993;

Syrstad*et al*., 1982). In studies by Renner &Kosmack (1974a) and Syrstad*et al*. (1982), the heritability (h2) estimates for individual FAs or roups of FAs were shown to vary between low and moderately high, but were generally lowest for the long chained FA.

The FA synthesis is mediated by a variety of enzymes, ending up in the triacylglyceroles being formed in the udder. In cow's milk there are the short chained FAs C4 and C6, which are unique to ruminant milk and that give its special characteristics. C4 and C6 are predominantly bound to the glycerol molecule at the *sn*-3 position according to the stereospecific numbering (Palmqvist*et al.,* 1993). The final step in the synthesis, in which diacylglycerol is transformed to triacylglycerol, is catalysed by the enzyme acyl- CoA:diacylglycerol acyltransferase1 *(DGAT1*). A dinucleotide substitution in the gene coding for DGAT1 has been shown to be the causative mutation behind an observed QTL for milk fat content. The substitution results in a replacement of the amino acid lysine (K) with alanine (A) (K232A) which in turn results in increased yields of protein and milk, and a decrease in yield of fat, and concentrations of fat and protein (Grisart *et al.,* 2002). Grisart *et al.* (2004) have shown that the enzyme encoded by the K allele is characterized by a higher velocity rate (Vmax) in producing triacylglycerols than the A allele. Due to the *DGAT1* enzyme's specific role to attach FAs to position 3, the only place on the triacylglycerol molecule where the C4 and C6 fatty acids are found, the A allele may be associated with a lower proportion of these short chained FAs.

The effect of *DGAT1*gene polymorphisms on milk production:

1. *DGAT1* K232A polymorphisms

Mapping studies in cattle resulted in the identification of an Adenine/Adenine to Guanine/Cytocine dinucleotide substitution in exon 8, which cause a Lysine K to Alanine amino acid substitution at position 232 K232A (Farnir *et al*., 2002, Grisart *et al*., 2002, Winter *et al*.,2002). This substituation of a positively charged Lysine residue with a neutral hydrophobic Alanine residue in the *DGAT1* gene has a major effect on fat content and other milk characteristics (Farnir *et al*., 2002, Rahmatalla *et al*., 2008, Sanders *et al*., 2006, Thaller et al., 2003, Winter *et al*., 2002). The lysine variant at *DGAT1* increases fat and protein contents, as well as fat yield, whereas the DGAT1Alanine variant increases milk and protein yields (Farnir *et al*.,2002, Thaller *et al*., 2003,Winter *et al*., 2002).

The effect of the *DGAT1* K232A polymorphism on fat composition has different causes: a higher activity of *DGAT1* and alteration of specificity of *DGAT1*. Expression study using a baculovirus system, shown that 232K variants has greater enzyme activity level (Vmax) than 232A in producing triglycerides, which is consistent with the in vivo effect of the K232A polymorphism (Grisart *et al*. 2004). Furthermore, the mathematical model of Shorten *et al*. (2004) predicted that an increase in fat yield because of 232K corresponds with a 120% increase in the *DGAT1* acylation rate and, consequently, is associated with a more saturated fatty acid composition. For the second, the specificity of the *DGAT1* enzyme could be altered by the K232A polymorphism.

Table (1): **Frequency of the allele and genotypes of *DGAT1* K232A in some dairy cattle:**

Breeds	Allele frequency KK	AK	AA	References
Montbéliarde	0.040	0.14	0.370	Mgautier, 2007
Normande	0.130	0.122	0.402	Mgautier, 2007
French Holstein	0.369	0.944	0.965	Mgautier, 2007
German Holstein Friesian	0.4420	0.5015	0.3072	Rahmatalla, 2010
Kenana	0.97	0.03	0	Lutfi *et al*., 2007
Butana	0.75	0.19	0.06	Lutfi *et al*., 2007
Swedish Red breed	0.91	0.16	0.83	Naslund and.Fikse, 2008
Swedish Holstein breed	0.86	0.20	0.76	Naslund and.Fikse, 2008

The lysine variant (K allele) at the K232A polymorphism frequency ranged from 0.04 in the Montbéliarde to 0.97 in Kenana breed (Table 1). The table also shows the distributions of genotypes for the 5 breeds. Only

14 out of the 384 Montbéliarde bulls were heterozygous for K232A and none was homozygous for the lysine variant (KK). Indeed, most animals were homozygous for the alanine variant (AA) in both the Montbéliarde and Normande breeds. In the French Holstein breed, where the K allele is much more frequent, approximately 10% of the bulls were homozygous (KK) and 43% were heterozygous (Mgautier 2007), while in German Holstein there were more heterozygous than homozygous AA genotypes, (Rahmatalla, 2010). In Kenana breed 28 out of 29 were homozygous for the lysine variant (KK), and only one was heterozygous, and none was homozygous for the alanine variant (AA), In the Butana breed 12 out of 16 were homozygous for the lysine variant (KK), only one was homozygous AA, and 3 were heterozygous KA (Musa, L.M.A. 2007).In Swedish Red breed, only 0.01 out of 146 were KK homozygous, 0.16 were heterozygous in KA, and 0.83 were homozygous AA. While in Swedish Holstein breed 0.03 were KK homozygous 0.20 were heterozygous in KA, and 0.76 were homozygous AA (Naslund and.Fikse., 2008).

2. Variable number of tandem repeats (VNTR)

In subsequent studies, at least one additional source of variation besides the diallelic *DGAT1 K232A* mutation was postulated to be responsible for the QTL in the centromeric region at BTA14 (Winter *et al*., 2002; Bennewitz *et al*., 2004). In the German Holstein population, Kühn *et al*. (2004) described 5 alleles at a variable number of tandem repeat (VNTR) polymorphism in the *DGAT1* promoter, which showed an effect on fat content additional to the *DGAT1 K232A* mutation.

It was observed that the VNTR allele E showed significant effects for some milk production characteristics compared with all other alleles at the *DGAT1* promoter VNTR. The same results were reported by Kühn *et*

al. (2004) for the *DGAT1* VNTR allele 5. However, in contrast to Kühn *et al*. (2004), the VNTR allele E was mainly linked to the K variant at *DGAT1 K232A* (Table 2), whereas the *DGAT1* VNTR allele 5 showed up with the A variant in the German Holstein Friesian population (Kühn *et al*., 2004). It is likely that the VNTR allele E corresponds to the *DGAT1* VNTR allele 5 of Kühn *et al*. (2004).

Table2. Allele substitution effects of the K variant at *DGAT1 K232A* and of the *DGAT1* VNTR allele E on milk production traits, in German Angeln Dairy Cattle.

		K variant		**VNTR allele E**		
Trait	a	SE	*P*- value	A	SE	*P*- value
Milk yield (kg)	-77.26	20.17	<0.001	-20.74	20.29	0.31
Protein yield(kg)	-0.98	0.69	0.155	-0.68	0.70	0.33
Proteincontent %	0.03	0.005	<0.001	0.002	0.005	0.75
Fat yield(kg)	3.59	1.07	<0.001	-0.71	1.08	0.52
Fat content %	0.12	0.01	<0.001	0.007	0.01	0.54
Lactose yield (kg)	-6.46	2.66	0.015	-5.53	2.67	0.04
Lactosecontent %	0.009	0.003	0.007	-0.008	0.004	0.03
Milk energy yield (ME)	0.30	0.17	0.088	-0.29	0.17	0.10
Milk energy content(ME/kg	0.08	0.007	<0.001	0.01	0.007	0.07
SCS	-0.03	0.01	0.038	0.03	0.01	0.04

(Rahmatalla, 2010)

3. Cytochrome P450, family 11, subfamily B (*CYP11B1*)

Kuhn *et al*. 2004 reported strong evidence for segregation of at least three alleles in the promoter region of the *DGAT1* (gene up-stream of the *DGAT1*) that affects milk fat percentage. In the centromeric region of BTA14, was suggested to be the causative gene for the QTL related to fat metabolism de Roos AP, Schrooten C,. 2007. The *CYP11B1* gene was

negatively associated with milk yield and protein yield, but positively associated with fat content Kaupe B,*et al* 2007,.

In some species, the CYP11B1 gene has developed into distinct isoforms (Kawamoto *et al.*, 1992; Mellon *et al.*, 1995; Bülow *et al.*, 1996; Muller, 1998), whereas in pig, sheep, and cattle functional unity is conserved (Bülow *et al.*, 1996; Muller, 1998). In all mammals CYP11B1 pseudogenes exist (Kirita *et al.*, 1990; Mellon *et al.*, 1995). Also because the CYP11B1 coding gene has been mapped to BTA14q12 (Kaupe *et al.*, 2004a) and HSA8q21-23 (Wagner *et al.*, 1991; Taymans *et al.*, 1998), this gene can be considered as a positional candidate gene. Because CYP11B1 is involved in energy metabolism, this gene can also be considered as a functional candidate gene for milk production.

DGAT1 gene encodes a microsomal enzyme that by using diacylglycerol and fatty acyl CoA as substrates catalyzes the terminal and committed step of triacylglycerol biosynthesis, which is the most important storage from of energy for eukaryotic cells. *DGAT1* is also important for physiological process involving triacylglycerol metabolism such as intestinal fat absorption, lipoprotein assembly, adipose tissue formation and lactation (Cases *et al.*, 1998), and it was presumed to be rate limiting with respect to lipid metabolism (Nina *et al.*, 1989).

Conclusions:

Nowadays, the sophisticated use of molecular and quantitative information on an industry-wide scale will require robust systems that can cope with imperfect data as well as the development of selection indices to take full advantage of the information.

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Chapter four

Small holder dairy production systems of Kenana and Butana cattle in Sudan

Small holder dairy production systems of Kenana and Butana cattle in Sudan

Abstract:

One hundred one dairy small holders, of both Kenana and Butana cattle in nine villages in the homeland of Kenana and Butana cattle in Sudan were randomly selected, with the objective of characterizing dairy production systems, adopted management practices, breeding objectives and constraints, for dairy development in this area. The average age of household heads in study was 54.92±7.78 and 56.73± 12.0 years for Kenana and Butana, respectively. With regard to educational status, the proportion of Khalwa (Quranic schools) was high among both Kenana (56%) and Butana (62.7%) herders. The average herd size of Kenana and Butana cattle was 10 and 6 animals, respectively. Sale of milk was the main source of income in Kenana area (100%) while it was up to 50% of the total income in Butana area. All Kenana herders bred their cow to their own bulls, while all Butana owners relied bulls from other sources such as neighbors. The criteria for selection of breeding bulls were body conformation (72%) and (80.4%) for Kenana and Butana owners, respectively. The disease reported most by Kenana owners was Trypanosomosis (61.8%), while tick problems was the major concern among Butana herders. Constraints for dairy development in Kenana area included, poor pastures, unavailability and high costs of feeds and shortage of water, disease, poor animal health and lack of veterinary services, while predators and lack of veterinary services were the main constraints in the Butana region. Dairying in the studied area can be improved through the provision of services related to feed supply, use of non-conventional feed resources, improving access to water, allocating land for semi intensive farms. A sustainable genetic improvement

program and the provision of veterinary and extension services are also central to development.

Key words:breeding,production, characteristics, Kenana, Butana cattle

Introduction:

Livestock is the largest subsector of the Sudanese domestic economy and is a growing contributor to the exports. The great bulk of all livestock production – possibly 90% of the total, though the actual figure is not known – comes from small holders and migratory producers. Cattle population in Sudan was estimated to be 29,840.000 millions head (MARFR**,** 2012). Local cattle are well adapted to the local environmental conditions and they are able to survive long periods of feed and water shortage but show correspondingly poor performance levels as exemplified by low milk yield, delayed first calving and long calving intervals. Among the Sudan cattle population Kenana and Butana are the most promising indigenous milk breeds, which under improved feeding and management in research stations yield more than 1500 kg milk per lactation (Saeed et al., 1987; El-Habeeb, 1991 and Musa et al., 2005). Kenana cattle are mainly kept by the Kenana tribe in the southern central plain of the country between the Blue Nile and White Nile. Rege (1999) reported that Kenana cattle population size was1.5 million head and that the status of the population was not at risk. He also mentioned that the breed has been extensively crossed with other breeds during the past 20 years. Kenana cattle habitat is a low rainfall savanna region (300 - 800 mm) with a dry season from November to April. This zone hosts some large scale irrigated agricultural schemes such as Gezira scheme. Butana cattle are found in the Butana plain of central Sudan (between the River Nile, Atbara River and Blue Nile), a typical semi-arid ecological zone (300 mm rainfall, 8 months dry period). This breed is also found in the Gezira between the Blue Nile and the White Nile and along the River Nile in the northern region. The population size as reported by Rege (1999) was one million heads and thus, the breed is not at risk. However,

the population shows a decreasing trend due to extensive crossbreeding with European cattle (since 1956) and due to effects of recurrent droughts in 1972/73, 1983/84 and 1989/90. Many herdsmen 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 process of fast upgrading aimed at increasing local milk production in response to the rising demand in urban areas. There is concern regarding the fate of local ecotypes under this extensive crossbreeding since 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. In addition to the phenotypic characterization of the breeds, this study aims at understanding the conditions of production systems, identify breeding objectives, and production constrains as a first step toward development of a sustainable breeding programme.

Materials and methods:

Small holders were interviewed using a structured formal questionnaire. Two major dairy production systems, namely traditional nomadic system and transhumance system were identified for Kenana and Butana cattle. The main production activity for smaller holders in Kenana and Butana regions were mixed Crop-livestock production activity. Questionnaire was prepared and used to collect information from a total of 101 owners in both Kenana and Butana area (50 Kenana and 51Butana).

The villages selected for the survey in Kenana and Butana cattle areas as follow:

Kenana area

Um-Benein

Near Um-Benein

Alingaz

Um-Biaga

Butana area

Near Atabara

Alzadabsharag

Um-Alteor

Barber

Algadawab

Sampling and questionnaire methodology:

The questionnaire was pre-tested to check clarity and appropriateness of the questions. Some of the information collected during interviews was supported by observation. The questionnaire was designed to obtain information on general household characteristics, management system, farming system, purposes of keeping cattle,selection of breeding bulls, breeding practices, mating organization, animal health and production constraints.

Data analysis:

The SPSS statistical computer software (Statistical package for social sciences, ver. 17) was used to obtain descriptive statistics. Chi-square contingency tables were used for tests of independence.

Results and discussion:

Household characteristics:

Socio- economic characteristics of households in study area are shown in Tables 1 and 2. The mean herder age in Kenana and Butana areas was 55 and 57 years, respectively. The results also showed that 42% and 29.4% of Kenana and Butana owners, respectively had primary education, only

2% of Butana and none of Kenana herders had university education. About 2% and 5.9% of Kenana and Butana herders respectively were illiterate. The majority of Kenana and Butana respondents (56% and 62.6%, respectively) received informal education (Khalawi). This presents a challenge to extension services and makes the introduction and adoption of new technologies difficult. Education is an important factor which if lacking can negatively impact on future improvement of livestock production.

Management system:

Different management systems (Table 3) were identified in the two areas. The traditional nomadic system was more prevalent in Kenana area (98%), while all Butana owners used a transhumant system (100%). In Kenana area the owners moved with their animals to the northern parts in the wet season and during the dry season they move to the vicinity of irrigated agricultural schemes such as Gezira scheme, Elsuki, ELrahad and the Blue Nile State where water and pasture are available.

Farming system:

Mixed crop-livestock production system is the dominant farming system (98% and 96%) in the study area (Kenana and Butana, respectively). Livestock species kept by farmers comprise cattle, sheep and goats. Cattle are the dominant species, mainly used for draught power followed by milk production (Table 4). Kenana farmers grow alfalfa (*Medicago sativa*), sesame, sorghum (abu70) and Sudan grass in the wet season only, while Butana farmers grow crops all year round. Butana farmers practice a cereal dominated cropping system with wheat as the most important crop in addition to some fruits and dates. Vegetables and *Lawsonia inermis* (henna) are of increasing importance, and are grown by farmers

in home gardens. The major sources of feed for cattle in Kenana area were natural pastures, crop residue, conserved hay and non-conventional feeds, while Butana cattle graze on the banks of the Nile and are fed some concentrates and minerals when they return home. Market oriented dairy production opportunities exist in some parts of the study area. This requires investing in active forage production and conservation methods. Feed sources such as legumes, browse trees and agro-industrial by products (e.g. Molasses, bagass and sugar cane tops) can be integrated into improving crop residue utilization for complementing dry season feeding. In addition supplementation with concentrates can be used only if it is economically viable.

Purpose of keeping cattle:

All of Kenana breeders and 52% of Butana breeders questioned considered that the primary reason of keeping cattle was to generate income from the sale of milk. Butana herders stated that social reasons (27%) and income from milk + social reasons (21%) were important objectives (Table 5).

Breeding practices:

Bull owners in Kenana and Butana selected villages provide mating services to cattle owners. Kenana bulls were generally selected from own herd while Butana bulls were mostly purchased. Breeding bulls were kept on average for 6.5 years in service; Cows were kept for production in Kenana areas on average about 10 years (Table 6). Most Butana owners do not keep a breeding bull because of the high cost of keeping a bull in small herds and the need to sell bull calves to solve recurrent financial problems. Butana owners unlike Kenana owners tend to use in crossbreeding with exotic breeds to improve milk production. This may

be due to the more settled nature of Butana production system compared to Kenana.

Selection of breeding bulls in Butana and Kenana cattle areas:

The preferences of Kenana and Butana owners with regard to the characteristics of bulls chosen as sires are shown in table 7. The most important criterion taken into consideration by both Kenana and Butana owners (72% and 80.4%, respectively) was general appearance (body conformation, vigour, health and color). Kenana owners prefer steel grey colors while reddish is favored by Butana owners.

Mating organization:

All of Kenana and Butana owners reported that they planned to improve their herd. The options suggested for improving milk production by Kenana owners were the choice of a good sire (70%) and good feeding regime and good sire (28%). Butana owners suggested that good feeding regime and exotic blood (crossbreeding) was their main plan for improving milk production (Table 8).

Animal health and feeding management:

All Kenana and Butana cattle owners reported disease incidences within the last 12 months (table 9). Trypanosomosis was the main problem reported by Kenana herders while Butana owners complained mainly of ticks. Trypanosomosis was reported more in Kenana (61.8%) compared to Butana (6.1%). The results show that tick infestation was the most important problem (75.8%) in the Butana area, while only 5.9% of Kenana owners thought it was a major problem. Veterinary services in

the country at large have declined in recent years and in some areas have witnessed a degree of collapse. This is probably attributed to the liberalization policy of the economy and the sudden shift from complete government sponsorship to private veterinary services which provide care at market prices (El-Sammani et al 1996). As a result, the high cost of veterinary services and drugs placed the service beyond the reach of poor herders in rural areas. Most cattle breeders in both areas used the services of private veterinarians. Cattle trypanosomosis is endemic inside and outside the tsetse belt (Yagi 1968). Nomadic cattle movements maintain the transmission cycle between the parasite and the vector. All cattle keepers in Kenana area recognized trypanosomosis as the most important disease. This result was in agreement with the results reported by Abdalla et al (2005). As a result of the decline in annual rainfall and the increase in intensity, frequency and duration of droughts in the Western Sudan region, particularly the drought of 1983, a large number of displaced people of Baggara cattle keepers moved with their animals and settled in Kenana cattle area in the southern central part of the country. The Baggara tribes normally encroach deeper into the tsetse habitat; this could have compounded the problem of trypanosomosis in the Kenana area. Butana cattle are found in a relatively rich area with abundance of cultivated fodder and water but the absence of veterinary services made the tick problem worse.

Production constraints:

Production constraints defined by cattle owners in both areas, are presented in table 10. Lack of pasture and shortage of water in summer (75% and 19%, respectively) were mentioned as the most important constraints by Kenana cattle owners. This is because Kenana cattle reside in a poor savannah region and herders migrate during the wet and dry

season. Predators (such as dogs) and lack of veterinary services (80% and 20%, respectively) were the most important constraints for Butana cattle owners. They had no problem of lack of pasture since Butana area is bounded by three rivers, the Nile, Atbara River and the Blue Nile. Over all, most Kenana farmers were constrained by lack of pasture and water. Free-range is the mainstay of the production system in Kenana area. Grasses grow rapidly during the short wet season producing abundant biomass, and the body condition of cattle improves. In the dry season both quantity and quality of the pasture decline, and cattle lose body weight and compensate the loss during the next rainy season (Ryan 1990 and Barash et al., 1994). Although the two ecotypes are phenotypically distinct they are similar in productivity and adaptability to harsh environments. There are differences in the production systems adopted by Kenana and Butana herders which appear to be designed to make the maximum use of the environment in the two regions.

Table1. Educational level of owners in Kenana and Butana area

Study area	Educational level %				Total
	Illiterate	Primary	Graduate	Khalawi	
Kenana	2.0	42.0	0.0	56.0	50
Butana	5.9	29.4	2.0	62.6	51
Total					101

Table2. Owner age in Kenana and Butana area

Study area	Mean	Number
Kenana	55	49
Butana	57	51
Total	56	100

Table3. Management system in Kenana and Butana areas

Management system	Kenana area	Butana area	Total
Traditional nomadic	98	0	48
Transhumant	2	100	51
Total	100	100	100

Table4. Major activities of owners

Activities	Kenana owners %	Butana owners %
Livestock only	2	3.9
Crop farming only	0	0
Livestock and farming	98	96.1
Total	100	100

Table5. Production objectives of keeping cattle

Study area	Production objectives %			Total
	Income from sale of milk	Social reason	Income + social reason	
Kenana	100	0	0	100
Butana	52	27	21	98

Table6. Replacement of breeding sires

Items	Own herd %	Purchased %	Total
Kenana	100	0	100
Butana	0	100	100

Table7. Selection criteria of breeding bulls

Characteristics	Kenana owners %	Butana owners %
Pedigree	20	2.0
General appearance	72	80.4
Daughter performance	2.0	0
Performance of other relative	6.0	17
Total	100	100

Table8. Herd improvement plan

Study area	How do you improve milk production?		
	Ration+ exotic blood %	Good sire%	Good sire +ration%
Kenana	0	70	28
Butana	100	0	0

Table9. Prevalent diseases as reported by owners (within the last 12 months)

Items	Kenana cattle area %	Butana cattle area %	Total
Trypanasomiasis	61.8	6.1	34.3
Trypanasomiasis (Tryp)+ Babesiosis(B.B)+ Thaleriosis+ Black quarter	5.9	0	3
Tryp+ B,B+ Thaler+ Type worm + Skin disease	5.9	6.1	6
Tryp+ Rinder past+ B,B	2.9	0	1.5
Tryp+ B.B	2.9	0	1.5
B.B+ Tryp+ Skin disease +Ticks	5.9	0	1.5
Ticks	5.9	75.8	40.3
Tickts+Trypanasomiasis	5.9	0	3
Ticks+ Skin disease	5.8	3	4.4
Tryp+ Rinder	2.9	9.1	6
Total	50.7	49.3	100

Table 10.Production constraints

Study area	Lack of pasture%	High cost of feeds%	Lack of water in summer%	Veterinary service%	Predators	Total
Kenana	75	2	19	4	0	100
Butana	0	0	0	20	80	100

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Chapter five

Variants of the *diacylglycerol acyltransferase 1* (*DGAT1*) gene in Sudanese dairy cattle (Kenana and Butana)

Variants of the *diacylglycerol acyltransferase 1* (*DGAT1*) gene in Sudanese dairy cattle (Kenana and Butana)

Abstract:

The aim of the study was the characterization of DGAT1 variants in Sudanese dairy cattle breeds. In this study, we examined 94 Kenana and 91 Butana dairy cattle from two regions of Sudan. We genotyped the DGAT1 sequence variant AJ318490.1:g.10433/10434 AA>GC that leads to the Lysine – Alanine substitution at position 232 (K232A) in the protein and the VNTR polymorphism in the promoter region. Genotyping was performed by allele specific PCR and PCR fragment lengths determination, respectively. In both breeds, the DGAT1 Lysine variant (232K) that is associated with high fat and protein content as well as high fat yield in other breeds is the high frequent allele. The frequencies of the 232K allele were 96.3% and 84.6% in Kenana and Butana breeds, respectively. At the DGAT1 promoter VNTR locus, four alleles containing four to seven repeats of the 18 bp motif were found in both breeds. The highest frequent allele was the VNTR allele 3 containing five repeats with 60.4 % and 57.5 % in Kenana and Butana breeds, respectively. In conclusion, the two examined Sudanese dairy cattle breeds do not differ in allele frequencies at the DGAT1 locus.

Keywords: dairy cattle, DGAT1, Kenana, Butana

Introduction:

The cattle population in Sudan was estimated to be 29, 210, 47 head (IGAD, 2013). The increasing demand for fresh milk and milk products requires the improvement of the productivity of dairy breeds. Among them, indigenous breeds that are adapted to the local environmental conditions are of particular value for milk production. Kenana and Butana are such indigenous dairy breeds that belong to the large East African Bos indicus breeds. Kenana cattle are distributed east of the confluence of the Blue and White Niles, down the Eastern bank of the Blue Nile up to the Ethiopian border, and down the Western bank in the Gezira region South of Khartoum. The Butana breed is native to the Butana region East of Khartoum which extends to the desert area between the Blue Nile and the Atbara River.

Under high feeding and management condition of research stations in Sudan, Kenana and Butana cattle can produce more than 1500 kg milk per lactation (EL-Habeeb, 1991; Musa, 2005; Saeed et al., 1987). Among several candidate genes, the diacylglycerol acyltransferase1 (DGAT1) became a functional candidate gene for lactation traits after studies indicated that female knockout mice lacking DGAT1 did not lactate due to the interrupted triglyceride metabolism in the mammary gland (Smith et al., 2000).

The DGAT1 gene was mapped on bovine chromosome 14 close to the centromere. It spans 14,117 bp and comprises 17 exons (Winter et al., 2002). The non-conservative substitution of Lysine by Alanine K232A in the DGAT1 gene, which is caused by a sequence variation of the two bases Adenine/Adenine to Guanine/Cytosine at positions 10433 and 10434 in exon 8 (rs109234250, rs109326954) had strong effects on milk

yield and composition in several breeds and different Holstein cattle populations in New Zealand (Farnir et al., 2002; Grisart et al., 2002), the Netherlands (Farnir et al., 2002), Germany (Rahmatalla et al., 2008; Sanders et al., 2006; Thaller et al., 2003), Poland (Pareek et al., 2005; Strzałkowska et al., 2005), France (Gautier et al., 2007), Sweden (Naslund et al., 2008) and Brazil ((Lacorte et al., 2006). Cows homozygous for the Alanine variant had higher milk, protein and lactose yields than the other genotypes. Carriers of the Lysine variant had higher fat yield and higher contents of fat and protein (Rahmatalla et al., 2008; Thaller et al., 2003).

Besides the protein variants, a variable number of tandem repeat (VNTR) motive in the promoter region of the DGAT1 gene was identified as an additional source of variation for milk yield and composition, especially in milk fat content (Bennewitz et al., 2004; Kuhn et al., 2004). The VNTR polymorphism contains a SP1 transcription factor binding site motif (CCCGCC) and, therefore, could have functional relevance for the regulation of gene expression (Kuhn et al., 2004).The potential functional relevance of the *DGAT1* promoter VNTR alleles is underlined by in-vitro studies providing evidence for SP1 binding to the CCCGCC motif of the repeat and for differential gene expression activity by different VNTR alleles (Furbass et al., 2006).The most frequent allele of the DGAT1 promoter was the VNTR allele 3 (5 repeats) (Kuehn et al., 2007; Kuhn et al., 2004; Rahmatalla et al., 2008). This allele showed significant positive effects on fat yield in German Holstein cows (Rahmatalla et al., 2008).
The aim of this study was to characterize the DGAT1 gene in the two Sudanese dairy cattle breeds Kenana and Butana in order to obtain information on allele frequencies of DGAT1 polymorphisms for selection decisions to improve the genetic potential in milk production.

Materials and Methods:

Animals:

In this study, 94 Kenana and 91 Butana cattle were used. Blood and hair samples were collected from unrelated individuals according to the recommendations of (FAO, 1996). Kenana cattle were chosen from Sennar state and Butana cattle from Nile river state. For Kenana cattle, in eight villages 10 samples were collected and 14 samples were collected in one additional village. For Butana breed, 11 samples were collected from each of seven villages and 14 samples were collected in one village.

Genotyping:

DNA was extracted from blood samples with the Bioscience Kit (Bioscience GmbH, Jena, Germany) andfrom hair samples with a specific salt precipitation protocol (Reissmann 2014, personal communication).The genotyping of the DGAT1 K232A substitution (AJ318490.1:g.10433/10434 AA>GC) in exon 8 was carried out by a competitive allele specific PCR (KASP assay) that has been described in detail previously (Kreuzer et al., 2013) (and I am one of the first group that applied this method in the lab in January 2014). Primers for PCR were designed from the DGAT1 gene sequence available at GenBank (accession number AJ318490.1) using KBioscience software (www.kbioscience.co.uk). The following allele specific primers were used: 5'-GAAGGTGACCAAGTTCATGCTCGTAGCTTTGGCAGGTAAGA-3' (Primer A1) and 5'-GAAGGTCGGAGTCAACGATTCTCGTAGCTTTGGCAGGTAAGG-3' (Primer A2). The reverse primer sequence was 5`-GCTGGGCAGCTCCCCCGTT-3'. PCR was performed in a volume of

8.1 µl containing 30 ng dried genomic DNA, 4.0 µl 2X KASP reaction mix (LGC, Herts, UK), 0.11 µl primer mix (100 µM A1-primer: 100 µM A2-primer: 100 µM C-primer: water = 1: 1: 2.5: 4), 0.06 µl 50 mM MgCl2, and 4.0 µl water. The DGAT1 promoter VNTR was genotyped as described by Kuhn et al. (2004). The primers left and right of the VNTR were 5'-CAGACGTTGTAAAACGACGACCCTGGCAGCACCTCAATC-3' and 5'-AGAAGGCACGGACTGTGAAGGC-3', respectively. The PCR reaction contained 30 ng genomic DNA in a reaction volume of 15 µl with 0.2 µM of each primer, 2.5 mM MgCl2, 1.0 µl of 10X B buffer, 0.1 mM dNTP, 1.5 µl of solution S 10X, and 0.5 U Hot-FirePol taq polymerase (Solis Bio Dyne, Tartu, Estonia). We used the M13 tail technique for fluorescence labelling of the fragments during PCR. After denaturation of PCR-products, the samples were loaded on a 6% polyacrylamide gel and run on a LICOR sequencer (Licor Biosciences, Nebraska, USA). The VNTR comprises an 18 bp repetitive sequence motif (Kuehn et al., 2004). Four VNTR alleles were found, which were denoted according to the fragment length with the longest fragment having the lowest number of repeats. The VNTR allele 2 contains four repeats, VNTR allele 3 five, VNTR allele 4 six, and VNTR allele 5 seven repeats.

Statistical analysis:

Allele and genotype frequencies were calculated based on the counting method (Falconer and Mackay, 1996). The Chi square test was used to test differences of genotype frequencies between the breeds using MedCalc Software (Schoonjans et al., 1995). The Chi-square test was also used for testing Hardy-Weinberg equilibrium.

Results:

In Sudanese dairy cattle most of the animals were homozygous for the DGAT1 Lysine variant KK (Table 1). In the examined Kenana and Butana animals, the frequencies of the 232K allele were 96.3% and 84.6%, respectively. Frequencies of the different genotypes are presented in Table 1. With respect to the DGAT1 protein variants, the Chi-square test showed that the examined population of Kenana cattle was in Hardy-Weinberg equilibrium ($\chi 2 = 0.14$), while the population of Butana cattle was not ($\chi 2 = 9.59$). The differences in genotype frequencies between Kenana and Butana cows were marginal ($p=0.057$).

The DGAT1 promoter VNTR has been proposed to explain additional variance of milk yield and composition. Four different alleles (4 to 7 repeats of the 18 bp motif) at the DGAT1 promoter VNTR were segregating in Kenana and Butana cows. The most frequent allele in both breeds was the VNTR allele 3 containing five repeat elements. Frequencies of VNTR allele 3 were 60.4 % and 57.5 % in Kenana and Butana breeds, respectively. The VNTR allele 4 (6 repeats) was present with frequencies of 35.1 and 39.9% in Kenana and Butana cattle, respectively. The promoter VNTR allele 2 with four repeats and allele 5 with seven repeats were least frequent with 3.9 and 0.6%, respectively, in Kenana cows and 2.1 and 0.5%, respectively, in Butana cows (Table 2). For the DGAT1 promoter VNTR polymorphism, significant deviations from Hardy-Weinberg-equilibrium in Kenana and Butana cattle populations were observed. There are significant differences between genotypes in Kenana and Butana at DGAT1 promoter VNTR ($p<0.0001$).

Discussion:

In this study, the estimated allele frequency at DGAT1 K232A was 96.3% and 84.6% for the Lysine and 3.7% and 15.4% for the Alanine variants in Kenana and Butana cattle, respectively. The main zebu breed in Brazil, Gyr and Red Sindhi, showed high frequencies of >96% of the 232K allele, respectively (Lacorte et al., 2006). The 232K allele is fixed in Sahiwal, Rathi, Deoni, Tharparkar, Red Kandhari and Punganur Indian Bos indicus breeds (Tantia et al., 2006), Indian Nellore cattle(Kaupe et al., 2004), Brazilian Nellore and Guzerat cattle (Lacorte et al., 2006). In the Holstein Friesian breed, frequencies of DGAT1 alleles differed considerably between populations. Thaller et al. (2003) and Rahmatalla et al. (2008) reported an allele frequency of 55% and 44.2% of the Lysine variant in German Holstein sires and cows, respectively. For Dutch Holstein Friesian cows and Polish black and white Friesian cows, the allele frequency of 40% for the Lysine variant was estimated by Schennink et al. (2008) and Strzałkowska et al. (2005). Other studies estimated the allele frequencies between 30% and 70% in the Holstein population and in the Polish Black and White populations (Bovenhuis and Schrooten, 2002; Grisart et al., 2002;Pareek et al., 2005; Winter et al., 2002). The frequency of the Lysine variant was lower (12%) in Swedish Holstein cows (Naslund et al., 2008).

In different studied populations for several dairy cattle breeds, including Holstein Friesian (Bennewitz et al., 2004; Grisart et al., 2002; Kuehn et al., 2007; Rahmatalla et al., 2008; Spelman et al., 2002; Thaller et al., 2003), Jersey (Komisarek et al., 2004; Spelman et al., 2002), Ayrshire (Spelman et al., 2002), and Angeln dairy cattle (Sanders et al., 2006), the Lysine variant was consistently associated with high fat and protein contents as well as high fat yield. Although the magnitude of the effects

differed among the populations, the direction of effects was always the same.

With respect to the DGAT1 promoter VNTR alleles, the VNTR allele 3 (five repeats) had the highest frequency of 60.4 and 57.5% in Kenana and Butana cows, respectively. In German Holstein Friesian cows, the promoter VNTR allele 3 (5 repeats) was the most frequent allele with 55 % (Kuehn et al., 2007) and 62.7% (Rahmatalla et al., 2008). The highest frequent allele (VNTR allele 3) found in German Holstein, which accounted for increased fat yield (Rahmatalla et al., 2008), were also found in high frequencies in Kenana and Butana cattle. We would expect that this allele is also associated with the same direction of allele effects as in German Holstein Friesian. However, these expectations must be confirmed in Kenana and Butana cattle. Therefore, it is necessary to record milk performance and composition from animals of the examined populations.

Conclusions:

From the results obtained, it can be concluded that the Lysine variant of DGAT1 which is associated with high fat and protein content in Holstein cattle was the most frequent allele in both Kenana and Butana cattle. The frequency of VNTR allele 3 (five repeats) of the DGAT1 promoter VNTR polymorphism was high in the examined Sudanese breeds. This allele is also associated with high fat yield in Holstein cattle. The obtained genetic information can be used for studying the effect of allelic association with milk yield and composition traits in Kenana and Butana cattle, which is necessary before selection decisions of the minor allele can be drawn for improving the local breeds. Albeit milk production traits in Sudan are not recorded, the DGAT1 genotyping data generated in

this study suggest that the low milk yield with the high fat and protein content in Sudanese Bos indicus Kenana and Butana cattle compared to taurine cattle could results in part from the genetic predisposition associated with the DGAT1 gene variants.

Table 1: Genotype and allele frequencies of the DGAT1 K232A polymorphism

Breed	Number of animals	Genotype	Genotype frequency (%)	Allele	Allele frequency (%)	H.W.E (χ2-value)
Kenana	94	KK	92.5	232K	96.3	0.14NS
		KA	7. 5			
		AA	-	232A	3.7	
Butana	91	KK	75.8	232K	84.6	9.59S
		KA	17.6			
		AA	6.6	232A	15.4	

NS: No significant deviation from HWE, S: Significant deviation from HWE.

Table 2: Genotype and allele frequencies at the DGAT1 VNTR locus

Breed	Number of animals	Genotype	Genotype frequency (%)	Allele	Allele frequency (%)	H.W.E (χ2-value)
Kenana	1	22	1.3	2	3.9	
	4	23	5.2	3	60.4	
	39	33	50.6	4	35.1	43.5 S
	10	34	13.0	5	0.6	
	1	35	1.3			
	22	44	28.6			
Butana	3	23	3.2	2	2.1	
	1	25	1.1	3	57.5	
	31	33	33.0	4	39.9	
	43	34	45.7	5	0.5	45.3 S
	16	44	17.0			

S: Significant deviation from HWE.

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Chapter six

General Discussion

General Discussion

The demographic questions put to farmers in Kenana and Butana areas show that most herders were of advanced age and most of them had informal education. This indicates that young people tend to migrate to urban areas in search of better job opportunity. It also presents a challenge to extension services and makes the introduction and adoption of new technologies difficult. Education is an important factor which if lacking can negatively impact on future improvement of livestock production.

There were different management systems in the two areas. The traditional nomadic system was more prevalent in Kenana area, while all Butana owners used a transhumant system. In Kenana area the owners moved with their animals to the northern parts in the wet season and during the dry season they move to the vicinity of irrigated agricultural Schemes such as Gezira scheme, Elsuki, ELrahad and the Blue Nile State where water, agricultural by products and pasture are available.

Kenana and Butana cattle are kept in a mixed crop- livestock production systems and livestock species kept by farmers comprise cattle, sheep and goats. Cattle are the dominant species, mainly used for draught power followed by milk production. Kenana farmers grow alfalfa (Medicago sativa), sesame, sorghum (abu70) and Sudan grass in the wet season only, while Butana farmers grow crops all year round. Butana farmers practice a cereal dominated cropping system with wheat as the most important crop in addition to some fruits and dates. Vegetables and Lawsonia inermis (henna) are of increasing importance, and are grown by farmers in home gardens. The major sources of feed for cattle in Kenana area were natural pastures, crop residues, conserved hay and non-conventional feeds, while Butana cattle graze on the banks of the Nile and

are fed some concentrates and minerals when they return home. Market oriented dairy production opportunities exist in some parts of the study area. Development of dairy production in these areas requires investing in active forage production and conservation methods. Feed sources such as legumes, browse trees and agro-industrial by products (e.g. Molasses, bagass and sugar cane tops) can be integrated into improving crop residue utilization for complementing dry season feeding. In addition supplementation with concentrates can be used only if it is economically feasible.

The purpose of keeping cattle in the study areas was to generate income from the sale of milk. However, surplus milk is sold at farm gate to middlemen at low prices, and animals are sold live in the village or nearest markets.

Kenana bulls were generally selected from own herd while Butana bulls were mostly purchased. Most Butana owners do not keep a breeding bull because of the high cost of keeping a bull in small herds and the need to sell bull calves to solve recurrent financial problems. Butana owners unlike Kenana owners tend to use crossbreeding with exotic breeds to improve milk production. This may be due to the more settled nature of the Butana production system compared to Kenana.

On the other hand, Selection of breeding bulls in Kenana and Butana cattle areas is done with regard to the characteristics of bulls chosen as sires, such as body conformation, vigour, health and colour. Kenana owners prefer steel grey colours while reddish is favoured by Butana owners.

All of Kenana and Butana owners reported that they planned to improve their herd. The options suggested for improving milk production by

Kenana owners were the choice of a good sire and good feeding regime. Butana owners suggested that good feeding regime and exotic blood (crossbreeding) were their main plan for improving milk production.

Disease prevalence is important in both production systems and almost all farmers in both areas reported incidences of diseases. Trypanosomosis and ticks were the main problems reported by Kenana herders while Butana owners complained mainly of ticks. Veterinary services in the country at large have declined in recent years and in some areas have witnessed a degree of collapse. This is probably attributed to the liberalization policy of the economy and the sudden shift from complete government sponsorship to private veterinary services which provide care at market prices (El-Sammani et al 1996). As a result, the high cost of veterinary services and drugs placed the service beyond the reach of poor herders in rural areas. Most cattle breeders in both areas used the services of private veterinarians. Cattle Trypanosomosis is endemic inside and outside the tsetse belt (Yagi 1968). Nomadic cattle movements maintain the transmission cycle between the parasite and the vector. All cattle keepers in Kenana area recognized Trypanosomosis as the most important disease. This result is in agreement with the results reported by (Abdalla et al (2005). As a result of the decline in annual rainfall and the increase in intensity, frequency and duration of droughts in the Western Sudan region, particularly the drought of 1983, a large number of displaced people of Baggara cattle keepers moved with their animals and settled in Kenana cattle area in the southern central part of the country. The Baggara tribes normally encroach deeper into the tsetse habitat; this could have compounded the problem of Trypanosomosis in the Kenana area. Butana cattle are found in a relatively rich area with abundance of

cultivated fodders and water but the absence of veterinary services made the tick problem worse.

Production constraints defined by Kenana cattle owners were lack of pasture and shortage of water in summer. These were mentioned as the most important constraints by Kenana cattle owners. This is because Kenana cattle reside in a poor savannah region and herders migrate during the wet and dry season. Predators (such as dogs) and lack of veterinary services were the most important constraints for Butana cattle owners. They had no problem of lack of pasture since Butana area is bounded by three rivers, the Nile, Atbara River and the Blue Nile. Overall, most Kenana farmers were constrained by lack of pasture and water. Free-range is the mainstay of the production system in Kenana area. Grasses grow rapidly during the short wet season producing abundant biomass, and the body condition of cattle improves. In the dry season both quantity and quality of the pasture decline, and cattle lose body weight and compensate the loss during the next rainy season (Ryan 1990 and Barash et al., 1994). Although the two ecotypes are phenotypically distinct they are similar in productivity and adaptability to harsh environments. There are differences in the production systems adopted by Kenana and Butana herders which appear to be designed to make the maximum use of the environment in the two regions.

In this study, the estimated allele frequency at DGAT1 K232A was high. The frequency of the 232K allele in the two Sudanese dairy breeds is in accordance with previous investigations of (Musa et al. (2007). The main zebu breed in Brazil, Gyr and Red Sindhi, showed also high frequencies of the 232K allele, (Lacorte et al., 2006). The 232K allele is fixed in Sahiwal, Rathi, Deoni, Tharparkar, Red Kandhari and Punganur Indian Bos indicus breeds (Tantia et al., 2006), Indian Nellore cattle (Kaupe et

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In different studied populations of dairy cattle breeds, including Holstein Friesian (Bennewitz et al., 2004, Grisart et al., 2002, Kuehn et al., 2007, Spelman et al., 2002, Thaller et al., 2003, Rahmatalla et al., 2008), Jersey (Komisarek et al., 2004, Spelman et al., 2002), Ayrshire (Spelman et al., 2002), and Angeln dairy cattle (Sanders et al., 2006), the Lysine variant was consistently associated with high fat and protein contents as well as high fat yield. Although the magnitude of the effects differed among the populations, the direction of effects was always the same.

With respect to the DGAT1 promoter VNTR alleles, the VNTR allele 3 (five repeats) had the highest frequency of 60.4 and 57.5% in Kenana and Butana cows, respectively. In German Holstein Friesian cows, the promoter VNTR allele 3 (5 repeats) was the most frequent allele with 55 % (Kuehn et al., 2007) and 62.7% (Rahmatalla et al., 2008). In the study of Sudanese cattle breeds, the frequencies of the VNTR allele 3 (5 repeats) were lower than previously reported for Kenana (81.3%) and Butana (70.5%) cattle populations, which estimate in cattle from research stations in Sudan (Rahmatalla, 2010). This is consistent with effects in German Holstein cattle as reported by Kuehn et al. (2007). The allele 3 (five repeats), which is the most frequent allele in Kenana and Butana cattle in this study, had an increasing effect on fat yield in German

Holstein cows (Rahmatalla et al., 2008). In a previous study in Butana cows in the research station , the VNTR allele 3 (5 repeats) significantly increased the fat and protein content by 0.8 and 0.2%, respectively compared to allele 4 (6 repeats) and 5 (7 repeats) (Rahmatalla, 2010).

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Chapter seven

Summary and Conclusions

Summary and Conclusions

One hundred one dairy small holders, of both Kenana and Butana producers in the nine villages representing the Kenana and Butana areas in Sudan, were randomly selected, with the objective of characterizing dairy production system, adopted dairy management practices, breeding objectives as well as to prioritize constraints and opportunities for dairy development in the area. Small holders were interviewed using a structured formal questionnaire. Two major dairy production systems, namely traditional nomadic system and transhumance system were identified for Kenana and Butana cattle, respectively. The main production activity was raising livestock for smaller holders in the Kenana region, while in Butana the mixed crop-livestock production was the dominant system. The average age of the household heads in the study sites were 54.92±7.78 and 56.73± 12.0 years for Kenana and Butana, respectively, and it was within the range of the production age. With regard to educational status, the proportion of Khalawi (non systematic education) was high among Kenana (56%) and Butana (62.7%) farmers. The dominant source of labor for dairy production across the two systems is family labor while the contribution of hired labor was minimal.

The average herd size of households in Kenana was 10 animals, and 6 animals in the Butana area. Natural pasture (grazing/hay) and crop residues were the major feed resources used as a basal diet for dairy production in the two dairy systems. Husbandry practices such as feeding watering, housing, breeding, milking and waste management were also different between the two production systems. Sale of milk was the main source of income in Kenana area (100%) while it was up to 50% of the

total income in Butana area, About 48% of Kenana farmers bred their cows to their own bulls, but in Butana region farmers did not have their own bulls and they relied on bulls from other sources such as neighbors. The selection of breeding bulls was based on body conformation (72% and 80.4% of Kenana and Butana owners respectively). The most prevalent disease reported by Kenana owners was Trypanosomosis (61.8%), while Tickets problems were the major concern of Butana cattle owners. Constraints for dairy development in the two areas included, lack of pasture, availability and costs of feeds, shortage of water especially in Kenana area, and predators were important in the Butana region. Disease and poor animal health and extension services, and the knowledge gap regarding improved dairy production systems were also important constraints.

Dairying in the studied area can be improved by interventions in several key areas that are of concern to smallholder dairy producers. Intervention are required in services related to feed supply, use of non-conventional feed resources, access to water, allocating areas for organized farms, the initiation of a sustainable genetic improvement program and the provision of education, veterinary, extension and capacity building services.

With regard to the DGAT1 variants in Sudanese dairy cattle breeds 94 Kenana and 91 Butana dairy cows from two regions of Sudan were examined. We genotyped the DGAT1 sequence variant AJ318490.1:g.10433/10434 AA>GC that leads to the Lysine – Alanine substitution at position 232 (K232A) in the protein and the VNTR polymorphism in the promoter region. Genotyping was performed by allele specific PCR and PCR fragment lengths determination, respectively. In both breeds, the DGAT1 Lysine variant (232K) that is associated with high fat and protein content as well as high fat yield in

other breeds was the most frequent allele. The frequencies of the 232K allele were 96.3% and 84.6% in Kenana and Butana breeds, respectively. At the DGAT1 promoter VNTR locus, four alleles containing four to seven repeats of the 18 bp motif were found in both breeds. The most frequent allele was the VNTR allele 3 containing five repeats with 60.4 % and 57.5 % in Kenana and Butana breeds, respectively.

In conclusion, from the results obtained in this study, it can be concluded that the Lysine variant of DGAT1 which is associated with high fat and protein content in Holstein cattle was the most frequent allele in both Kenana and Butana cattle. The frequency of VNTR allele 3 (five repeats) of the DGAT1 promoter VNTR polymorphism was high in the examined Sudanese breeds. This allele is also associated with high fat yield in Holstein cattle. The obtained genetic information can be used for studying the effect of allelic association with milk yield and composition traits in Kenana and Butana cattle, which is necessary before selection decisions can be drawn for improving the local breeds. Albeit milk production traits in Sudan are not recorded, the DGAT1 genotyping data generated in this study suggest that the low milk yield with the high fat and protein content in Sudanese Bos indicus Kenana and Butana cattle compared to taurine cattle could results in part from the genetic predisposition associated with the DGAT1 gene variants.

So generally recommitting,

I. Since most of livestock in Sudan are owned by smallholders in pastoral areas, it is essential to give a high priority development. Education is an important factor in development and lack of it can negatively impact on future improvement of livestock production. Veterinary services are also important in this regard.

II. Um-Benein and Atbara Livestock Research Stations should become the nucleus herds for Kenana and Butana breeds, respectively. The herd build up need to be organized as self supporting competitive enterprise. Subsequently, simple recording schemes should be encouraged through the farmer's cooperatives.

III. The sophisticated use of molecular and quantitative information on an industry- wide scale will require robust systems that can cope with imperfect data as well as the development of selection indices to take full advantage of the information.

IV. The overall outcome of the current study showed that most of the Sudanese dairy cattle were homozygous for the Lysine variant KK in both dairy cattle Kenana and Butana (96.1% and 87.5%, respectively).

V. Allele 3 of the VNTR in the promoter of the *DGAT1* gene is the most frequant in both dairy cattle, but it was higher in Kenana dairy cattle than in Butana dairy cattle.

VI. Butana dairy cattle has low frequancy of lysine variant (*DGAT1* K232A) and low frequancy of allele 3 VNTR promoter, in addition to that Butana dairy cattle shows higher frequancy of allele 232A, compared to Kenana dairy cattle.

VII. To draw final recommendation about *DGAT1* K232A and VNTR polymorphisms, we must have phenotypic data for association analysis.

VIII. Sudanese dairy cattle could be screened for other genes that affect milk traits.

Appendix

Gene: DGAT1 ENSBTAG00000026356
Description: diacylglycerol O-acyltransferase 1

Location : Chromosome 14: 1,795,351-1,804,562 forward strand.

[Source: RefSeq peptide; Acc: NP_777118]

- All exons in this region

CCTGGGCAGGGGAGAGGTGGCCACCCTGGGAATAGGTGGGCATGGCACAAGTCCCGGAA
T
GCGAGGACTGCGGCCTTTCTCCCCCTCCGTTCTCTGACCTGGCGCGTGTTTGAACAGCCT
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CCGCGGGATGTACGTTCGGTAGACGCGTTGGGTGTCAGCCTGACGTTAACGCACTAGGCA
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GAAGCCCCGCGTGTTCCTCGTCTGTCCGGGATTGCATTTGCCAGGAGACCACAACTCCCA
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CCCGG
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CGGAA
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GCAGGAGGAGGCCTGGCTGGTGGAAGCTGCCTCCTGGCCTCCACCAGGCCTCTGCCTGAA
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ATGCTCTGGAAACCCTACAGATCTCGCCCAAGGGTCTGAATGTGTCAATAAAGTGCTGTG
CACAGTGAGCTCCCTCAGCCTCCAGGGCACAGGGCTGGCAGGAGGGGGCGGCCCTCCCAC
GTGGGGCCATGCTGTGGGAAGGAGGCCCCAGCGCCTGGAGAGGAGCTGGGGCTGTGGTG
A
CCCTCCCTGCCTCACAGGGCTCTGTGGTCAGACGTCTTGCCCTGCAAGGTGGAGACTCCA
TGCTCCAAGGCCCCCTGTGCCTGAGGTCTGCACACAAGTGGATTCAACTTGGGTCAGGCC
AGAGGCTAAGGTGTGGAAGAGGGTTGAGAATCAGGCTGACTTGAACGGCAGCAAAGACT
C
CAAGGCAAGGCTGCAGAGGTCTCAGAGGCTAT

Questionnaires used in the survey

1- General household information

Farmer's name:……

Village:…….

Level of education:…….

Age:…….....

1.1 Labor distribution in dairy production

	Dairy production					
	Feeding	**Milking**	**Breeding**	**Herding**	**Health care**	**Housing**
Husband						
Wife						
Sons						
Daughters						
Laborer						

1.2 What types and number of livestock do you keep

a) Cattle ———b) sheep: ———c) Goats ———d) other ———

1.3 If you have cattle, sheep and goats, could you rank them according to the relative importance to you?

a) Cattle ———b) sheep ———c) Goats ———

1.4 How is composition of your herd?

a) Number of cows ———b) Number of heifers ———c) Number of bulls ———d) Number of calves ———e) Number of steers ———

2- Herd management

2.1 Did you sell any cattle during the past 12 months?

2.1.1 If yes: How many? *And fill the table for each animal sold*

No	**Sex**	**Age**	**Reason why sold**	**Condition score**
				A ( ) B ( ) C ( )
				A ( ) B ( ) C ( )

Sex: (m/f); Condition score: A+ healthy, B + good for breeding
A – sick, B – weak, C – infertile

	1	2	3	4	5	6	7	8	9
Sex (m/f)									
Age (years									

2.3 Did any animals die during the past 12 months?

d you grow crops?

3.1.1 If yes: Did you sell any crops during the past 12 months?

3.1.2 If yes which crop did you sell?

3.2 What do you consider your main production activity?

a) Livestock ———b) farming ————c) livestock and farming ———

4- Breeding practices

4.1 Do you keep a breeding bull?

4.1.1 If yes: Why do you have? What is the bread and age of bull (s) you are owning?

4.1.2 How many bulls do you have?

4.1.3 If No: Why do you not have breading bull (*and on to question no. 5.6)*

4.2 Where is your breeding bull from?

a) Own herd ———b) other herd ———c) purchased ———d) other ———

4.2.1 If (a) own herd: At what age do you select your breeding bull? ——— years ———months

4.3 What do you do with bulls that are not selected for breeding purposes?

a) Castrate ———b) just leave them in the herd ————c) sell (before mature) ———d) other ———

4.4 Do you select your own bull?

4.4.1 If Yes: How do you choose a breeding bull, what are the characteristics you use to select your breeding bull?

4.5 How long do you keep a breeding bull for service? Other ————years

4.6 Where do you take the replacement breeding bull from?

a) Own herd ————b) other herd ——c) purchased ———d) other ——

4.7 Can the replacement bull be the son of the former breeding bull?

4.7.1 If No: Why not?

5- Mating organization:

5.1 Do you keep mating records of your bull (s)? If yes how?

5.2 What are the mating records you keep (observation of the records)?

5.3 In addition to your farm,

5.3.1 For how many farmers do you give service at the moment? ——— farmers

5.3.2 For how many cows do you give service at the moment? ——— cows

5.3.3 How many farmers used your bull service last year? ———farmers

5.3.4 What was the total number of cows served per year per bull last year? ———cows

5.4 Do you get feedback information from cow owners about the condition of cows after service?

5.4.1 If your answer yes, what was the number of cows that got pregnant after serve by your bull last year? ———cows

5.5 How much do you charge for one bull service? ————Dinars (*and go to question 5.8*)

5.6 If you not using your own bull, do you know the serving your cow?

5.6.1 If yes: what is what are the source, and the breed of the bull you are using for mating?

5.7 How much do you pay for one bull service? ————Dinars

5.8 How long do you keep a cow for production? ——————years

5.9 Do you have a plan to improve the milk productivity of your herd?

5.9.1 If yes: how do you want to improve the milk productivity of your herd?

5.10 What improvement in your herd do you expect from the selection of breeding bull, in may be 20 to 30 years?

5.11 Do you record or keep the performances of your breeding cattle?

5.11.1 If yes, how do you record the performance of your herd?

6- Production and reproduction performance:

6.1 What was the average quantity of milk you got from your cows last time and how long did you milk your cows?

Cow number	Daily milk yield (1)			Lactation length (months)
	Beginning of lactation	Middle of lactation	End of lactation	

6.2 What was the age of your cows when they gave birth to their first calf?

6.3 When did your cows give their last calving previous calving?

6.4 How many times have you taken your cows for bull or Al services before they get pregnant last time?

7- Production objectives:

7.1 Why do you keep cattle? ——————————————(first reply given)

7.2 From the following list, could you rank the reasons according to the degree of importance?

Reasons	Rank
Income from sale milk	
Milk for home-consumption	
Income from sale animal	
Traction (animal for work)	
Manure	
Insurance against financial problems	
Investment (like a bank)	

8- Feeding Management, Animal health and Production Constrains:

8.1.1 What do you feed your animals?

a) Grazing ————b) hay ————c) crop residues ———— d) concentrates ————e) minerals ————

8.1.1.1 If you use hay, which animals do you supplement with it? ———

8.1.1.2 If you use concentrates, which animals do you supplement with it? ————————

8.1.2 Do you consider that the feeding is constrained to your herd production?

8.2.1 What are the prevalent diseases in your area?

8.2.2 What is the most important one?

8.2.3 Did you report any diseases among your herd during past 12 months?

8.2.3.1 If yes: could you mention them?

8.2.4 If you report any case of disease, where you look for veterinary help from?

a) Government veterinary service ————b) private veterinarians ———c) drugs suppliers ————b) other ————

8.3 What do you consider a more serious constraint to your cattle production?

Kenana dairy cattle

Butana dairy cattle