POLYMORPHISMS OF KAPPA-CASEIN AND BETA-LACTOGLOBULIN GENES AND THEIR ASSOCIATION WITH MILK FAT AND PROTEIN CONTENTS IN TANZANIAN GOATS

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ABSTRACT

This study was conducted to assess the polymorphisms of beta-lactoglobulin (β-Lg) and kappa-casein (κ-Cn) genes and determine the effects of β-Lg polymorphism on percentage of milk fat and protein contents in indigenous goat breeds found in Tanzania. Blood samples were collected from 22 - 24 unrelated animals for each breed. Genomic DNA was isolated from whole blood samples using Sepa gene extraction kit with some modifications. The β-Lg and κ-Cn genes were amplified using Polymerase Chain Reaction (PCR) and Restriction Fragment Length Polymorphism (RFLP) technique was used to determine variations at the β-Lg and κ-Cn loci. The amplified products for β-Lg and κ-Cn genes had the sizes of 426 and 459 bp, respectively. Digestion of β-Lg gene using *Sac*II endonuclease restriction enzyme revealed the presence of two common alleles (A and B) in all goat populations. The frequency of B-allele (0.583) was higher than that of the A-allele (0.417) for β-Lg gene in indigenous goats, while exotic goats had higher frequency of A allele (0.688) than the B-allele (0.312). The frequencies of AA, AB and BB genotypes were, respectively, 0.250, 0.333 and 0.417 in Gogo goats, 0.318, 0.273 and 0.409 in Pare white, 0.417, 0.333 and 0.250 in Malya, 0.500, 0.375 and 0.125 in Saanen. The analysis of κ-Cn gene variation in the four goat populations indicated that there were three alleles (A, B and C) at this locus. The observed allelic frequencies of A/B and C were 0.917 and 0.083 in Gogo white, 0.955 and 0.045 in Pare white, 1 and 0.00 in Malya, 0.875 and 0.125 in Saanen goats, respectively. The effects of β-Lg genotypes on milk fat and protein percentages were analyzed using a general linear model (GLM). The results show that there was no significant effects (P>0.05) of β-Lg alleles on milk fat and protein percentages.

DECLARATION

I, Salum Omari Kuwi, do hereby declare to the Senate of Sokoine University of Agriculture, that this dissertation is my original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

_____   _____

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 (MSc. Candidate)

The above declaration is confirmed

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DEDICATION

This dissertation is dedicated to my beloved mother, Ashura Mwinyimkuu Mpamba and father, the late Omari Salum Kuwi (May God rest his soul in eternal piece) who brought me up, gave me tender love and care, my wife, Saida Suleiman Mbarouk and my daughters, Shadya and Swafaa for their moral and spiritual support and prayers, especially when I was away from them.

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LIST OF ABBREVIATIONS AND SYMBOLS

bp   Base pair

ANOVA   Analysis of variance

GLM   General linear model

MAS   Marker assisted selection

PCR   Polymerase chain reaction

RFLP   Restriction fragment length polymorphism

DNA   Deoxyribonucleic acid

K-Cn   Kappa kasein

β-Lg   Beta lactoglobulin

SDS-PAGE   Sodium Dodecyl sulfate Polyacrylamide Gel Electrophoresis

QTL   Quantitative trait loci

EDTA   ethylene diamine tetraacetic acid

IDF   International dairy federation standards

μM   Micro molar

μl   Micro litre

COSTECH   Commission for Science and Technology

U   Unit

U.S.A   United States of America

dNTP   deoxynucleotide triphosphate

SNF   Solid not fat

PH   Power of hydrogen

CHAPTER ONE

1.0   INTRODUCTION

Goats are important for subsistence, economic and social livelihoods of a large number of people in East Africa. They are especially important to women, children and the aged who are often vulnerable members of the society in terms of under nutrition and poverty (Kosgey, 2004). Goats play an important role in providing animal protein and income generation, especially to resource poor farmers in rural areas. They are particularly important for rural people who cannot afford to keep cattle and are more readily sold than cattle when only small amount of cash is needed (Dossa *et al*., 2008). Das and Sendalo (1991) have reported that a large proportion of animal protein supply in rural areas is derived from small ruminants due to their high prolificacy compared to cattle.

Goat milk, though small in quantity, provides an all year round source of animal protein for low income families, contributing to food security and nutrition improvement. Goat milk is becoming popular in some regions of Tanzania and is mainly used when cow's milk is scarce and also traditionally is used for therapeutic purposes in children and allergic persons (Das and Sendalo, 1991). Goat milk exceeds cow's milk in monounsaturated, polyunsaturated fatty acids and medium chain triglycerides, which are all known to be beneficial for human health, especially for cardiovascular conditions (Haenlein, 1992). Goat milk is richer than cow's milk in some important nutrients, e.g. vitamin A, niacin, choline, and inositol. The milk from goats is believed to be more easily digestible and less allergic than cow's milk. Goat milk contains less casein alpha 1 as human milk, which is responsible for most allergies for cow's milk. Therefore, goat milk is hypoallergenic. For this reason, in some countries it is used as the basis for the development of infant formula in place of cow milk (MNT, 2011).

However, milk production per doe is relatively low and extremely variable. The variation in milk yield and composition is due to management practices like disease control, feeding and housing and variability in genetic make-up of the goats (Nangwala, 1996). This provides an opportunity for improvement of goat milk production and composition by making changes in management practices and adoption of genetic improvement technologies.

Milk fat and protein percentages are important parameters in the dairy industry. However, in Africa milk is sold on volume basis, this necessitates the identification of high-milk yielding goats and cattle. On the other hand, in developed countries the industry pays a premium for milk fat and protein concentrations as these technically reflect the milk quality (Roger *et al*., 2011). The milk buyers pay a premium for milk fat and milk protein percentages because milk quality is greatly determined by its chemical composition in terms of fat, protein and lactose contents (Torrii *et al*., 2004; Schmidely *et al*., 2005). Goat milk is widely produced in West Africa but also in the Caribbean and Central Africa, usually for household consumption, although it is sometimes traded within the community.

A large proportion of goats in Africa is of the indigenous type characterized by low milk production potential and higher milk components. Okeyo and Ahuya (1998) reported milk fat content of $3.5 \pm 0.16\%$ in Galla goats in Kenya while Ryoba and Hansen (1998) found higher levels (6.95%) of butterfat in milk from Tanzanian goats compared to butterfat content of 3.54% in Norwegian goats. However, there is limited information on molecular characterization of Tanzanian goats, especially at κ-casein and ß-lactoglobulin loci that affect milk quality traits.

Traditionally, animal breeding programmes depend on selection based on phenotypic characteristics whereby traits are measured directly and animals with superior performance in the traits are used as breeding stock so as to achieve the highest possible average performance for that trait in the offspring of selected parents. The selection of breeding stock is carried out based on observable phenotypes without knowing which genes are actually being selected. However, there are several problems associated with phenotypic selection. These include narrowing the genetic base of a population, and the approach can only be applied to traits that are easily measured. In traits that are displayed only in adults, which include most of production traits, it is necessary to raise a large number of animals for which the trait is recorded, so that a few can be chosen for breeding. In the case of progeny testing for milk yield and quality, the costs are very high, as the test sires have to be raised and then the daughters themselves raised and bred before the trait can be measured and elite sires selected. This results into breeding programmes that are expensive.

The association of genetic markers with milk production and composition has stimulated interest in using genetic polymorphism of casein and beta-lactoglobulin genes in molecular marker assisted selection (MAS) to improve milk productivity in farm animals (Kumar *et al*., 2006). The use of polymorphic genes as detectable molecular markers is a promising alternative to the current methods of phenotypic selection, once these genes are proven to be associated with traits of interest in animals. Selection efficiency, however, depends on allelic frequencies in the breeds of choice and on the effect of these polymorphisms on selected traits (e.g. dairy traits and technological properties of milk) (Karim *et al*., 2009). The use of molecular genetic technology offers a way to select breeding animals at an early age, to select for a wide range of traits and improves reliability of predicting the mature phenotype of individual animals. Selection of animals

with desirable genotypes and mating them to produce the next generation has been the basis of livestock improvement.

Polymorphisms of κ-casein and β-lactoglobulin genes can be used to characterize and understand the population genetic structure and evolution of indigenous goats (Naves, 2003). Such knowledge can be used in selective breeding for improving local populations for milk production, especially when milk quality and cheese output is considered as selection criteria. The study of genetic polymorphisms of the κ-casein and β-lactoglobulin genes is of interest, since some variants could be more beneficial from the point of view of human nutrition or be associated with milk quality, composition and technological characteristics. β-lactoglobulin and κ-casein loci affect the milk production parameters and quality of milk protein. Their polymorphisms explain a part of the genetic variance and improve the estimation of breeding value. Such loci can be taken into account in selective breeding programmes as a suitable supplement to conventional breeding procedures (Přibyl, 1995).

The polymorphism of β-lactoglobulin gene was discovered in 1995 and a total of fifteen (15) alleles are known so far (Matejicek *et al*., 2007). β-lactoglobulin gene polymorphisms at the DNA level is analyzed by PCR-RFLP and two novel genetic variants of the β-lactoglobulin gene has been reported in Indian goats (Kumar *et al.,* 2006). The influence of β-lactoglobulin polymorphism on milk production traits has been reported in cows, sheep and Indian goats. Ng-Kwai-Hang *et al*. (1986) and Bovenhuis *et al*. (1992), for example, found polymorphism of β-lactoglobulin gene in dairy cattle relating to the percentage of milk protein and fat. Mroczkwski *et al*. (2004) also found that polymorphism of β-lactoglobulin gene in Merino sheep is associated with milk yield.

Studies in cattle have further indicated an association of some κ-casein alleles and milk yield, composition and quality (Strzalkowska *et al*., 2002). Three major variants (A, B and C) have been revealed in Spanish and French breeds (Yahyaoui *et al*., 2001). The κ-casein genotypes had a highly significant effect on protein content and the κ-casein BB cows produce milk with 0.8% higher protein content than the AA cows. Generally, it is believed that the κ-casein B variant is associated with higher fat, protein and casein in the milk and has a significant influence on cheese making properties of milk and superior rennet coagulation properties in comparison to AA or AB variants (Gangaraj *et al*., 2008). The high level of genetic variability observed at the κ-casein locus and its association with milk traits make it possible to select groups of animals that produce the type of milk which could be more suitable for specific processing technologies or for specific need of human nutrition (Rando *et al*., 2000).

Goat milk composition differs quantitatively and qualitatively among goat breeds and genetic groups (Chilliard *et al*., 2006). The protein and fat contents variability is genetically controlled, especially by the κ-casein and β-lactoglobulin loci, which exhibit a high degree of polymorphism (Ricordeau *et al*., 1999; Strzalkowska *et al*., 2002). Indigenous goat populations have a large variability at these loci, as it is expected at all biological levels, due to the large allelic diversity. However, most of the goat breeds in Tanzania lack molecular characterization information particularly on κ-casein and ß-lactoglobulin required for establishing selective breeding programme for improving their dairy productivity. Therefore, in this study variation of Tanzanian indigenous goats at κ-casein and ß-lactoglobulin loci was assessed and the preliminary information on the association of these genes with milk quality traits evaluated.

1.1 Main Objective

To assess the polymorphisms of κ-casein and ß-lactoglobulin genes in Gogo white, Pare white, Malya and Saanen goat breeds and their effect on milk quality.

1.2 Specific Objectives

(i)   To identify alleles and compare allele frequencies at κ-casein and ß-lactoglobulin loci in Gogo white, Pare white, Malya and Saanen goat breeds.

(ii)   To determine and compare milk composition of Gogo white, Pare white, Malya and Saanen goat breeds.

(iii)   To assess the effect of ß-lactoglobulin genotypes on goat milk quality.

CHAPTER TWO

2.0   LITERATURE REVIEW

2.1   Goat Population in Tanzania

Goats are spread over a wide range of habitats with a substantial concentration in the tropics and dry zones in developing countries (Kosgey, 2004). Therefore, they are expected to show a large amount of genetic diversity as they are adapted to various ecosystems. Goats are particularly valuable livestock species in developing countries because of their ability to utilize many types of forages and tolerate unfavorable climates. Goats play a vital role in the rural economy of many countries mainly in central and eastern Africa. They play an important role in providing animal protein and income generation, especially to resource poor farmers in rural areas. They are particularly important for rural people who cannot afford to keep cattle and are more readily sold than cattle when only small amount of cash is needed (Dossa *et al*., 2008). Das and Sendalo (1991) have reported that a large proportion of animal protein supply in rural areas is derived from small ruminants due to their high prolificacy compared to cattle. However, the contribution of goat to the people and economies of developing countries is obscured by several factors that affect their productivity, thus leading to underestimation of their true value.

Goat population is estimated to be about 744 millions in the World. About 30% of world goat population is found in Africa (FAOSTAT, 2005). The population of goat in Tanzania is estimated to be 16.7 million, of which 98% are indigenous goats mostly belonging to Small East African breed (MLFD, 2015). There are different strains of indigenous goats in Tanzania and are named after their area of origin and skin colour, for example Gogo

white, Pare white, Maasai, Newala and Mtwara. These are some of indigenous goat strains found in different parts of Tanzania.

2.2   Goat Milk Characteristics

Goat milk is characterized with its offensive odor. This is especially due to bucks whose odor floats strongly around the premises and can affect the flavor of the milk. The unpleasant odor is obvious in milk if ventilation, milking practices and cooling of milk are improper or insufficient. However, milked and cooled goat milk is odor free and hard to distinguish from cow milk in terms of odor and taste (Mowelm, 1988). Goat milk has special nutritional properties that make it attractive to many consumers. It is easier to digest than cow's milk and consumers can derive therapeutic benefits from it (Park, 1994*a*; Haenlein, 2004).

The major differences between goat and cow milk are related to the differences in casein composition and also to different structure and size of fat globules and protein micelles (Tziboula, 2003). These differences in composition affect milk processing and change the final quality of dairy products made from goat milk. The special characteristics of goat milk mean that its nutritional value is markedly higher than that of cow milk and the protein is more digestible (Park, 1994*b*; Lopez *et al*., 2003; Haenlein, 2004) and less allergenic. Similarly, the fat of goat milk is also more digestible (Alférez *et al*., 2001) and an excellent source of energy for use in various metabolic processes and even for combating metabolic diseases (Sanz-Sampelayo *et al.,* 2007). Goat milk and its products such as cheese and powder have significant importance in human nutrition, including feeding more starving and malnourished people in the developing world, and treating people afflicted with cow milk allergies and gastro-intestinal disorders (Haenlein, 2004).

2.3   Milk Composition

In developed countries, milk is priced based on the composition rather than its quantity. Milk with high protein and butterfat contents has been found to fetch high price. However, milk composition varies within and between breeds. Goat's milk contains relatively more protein, fat and minerals than both cow and human milk but less than that of ewe (Table 1).

Table 1: Average composition of milk from various mammals

Species	Water	Fat	Protein	Lactose	Ash %	Non-fat solids	Total solids
Goat	87.00	4.25	3.52	4.27	0.86	8.75	13.00
Cow	87.20	3.70	3.50	4.90	0.70	9.10	12.80
Ewe	80.71	7.90	5.23	4.81	0.90	11.39	19.29
Human	87.43	3.75	1.65	4.98	0.21	8.82	12.57

Source: Harris and Frederick (2003).

Generally, the composition of goat milk falls within a specified range for each milk component. Fat is reported to be the most variable component ranging between 3.0 and 6.0%. The ranges expected for total solids, protein, lactose and ash are 12 - 16,   3 - 4, 3.8 - 4.8 and 0.70 - 0.95%, respectively (Nangwala, 1996; Harris and Frederick, 2003). A study done by Ryoba and Hansen (1998) found higher levels (6.95%) of butterfat in milk from Tanzanian goats compared to that of Friesian (4.09%) and Ayrshire (4.24%) cows. In the same study Norwegian goats had lower (3.54%) butterfat content. Other studies done by Hadjipanayiotou (1995) in Cyprus showed a range of butterfat content to be between 2.31 and 4.49% in Damascus goats. Ying *et al*. (2004) reported fat contents for Alpine, LaMnçha, Nubian, Saanen and Toggenburg in USA from 1979 to 1992 as

3.81 ± 0.47; 4.51 ± 0.048; 3.47 ± 0.063; 3.34 ± 0.059 and $2.94 \pm 0.041\%$, respectively. Among the breeds, LaMancha had the highest milk butterfat content (4.51%), followed by Alpine (3.81%), then Nubian (3.47%), Saanen (3.34%) and last Toggenburg (2.94%). Studies done by Okeyo and Ahuya (1998) in Galla goats in Kenya revealed an overall butterfat content of $3.5 \pm 0.16\%$. Estimates of butterfat contents between 4.6 and 5.54; 5.39 and 6.8; 4.4 and 4.5; 4.2 and 6.4% have been reported in Galla, Malamen, Italian Alpine, various Greek goats and Chilean goat breeds, respectively. In the same study, goat age was found to influence milk butterfat content whereby young does (<5 years) were reported to produce milk with higher butterfat content than older does (Okeyo and Ahuya, 1998).

2.4   Factors Affecting Milk Composition

Factors such as feeding, parity, breed, stage of lactation and litter size are known to affect milk yield and its composition (Milerski and Mares, 2001).

2.4.1   Feeding

Productivity of goats is fostered by the efficient utilization of nutrients which is possible with an adequate supply of energy (Morand-Fehr *et al*., 2000)**.** Energy requirements are affected by age, body size, physiological state, environmental factors, hair growth, muscular activity and relationships with other nutrients. Weather conditions such as temperature and humidity may increase or decrease energy needs (NRC, 1981). Improved feeding during pregnancy increases milk production of animals. This is because, good body conditions of the animal during late pregnancy has positive effects on early lactation milk yield (Morand-Fehr *et al*., 2000). During the early months after kidding, the doe has the tendency to mobilize body tissues for maintenance and production if they consume less dry matter feeds (Tovar-Luna *et al*., 2010).

Average daily milk yield, peak yield, time of peak yield, persistency, fat and protein contents of the milk, depend on the quantity and quality of feed eaten by the animal (Salama *et al*., 2005). Before and after parturition, feeding of good quality forages favorably affects the onset of lactation. In the middle of lactation and especially at the end of lactation, maintenance of milk production at a high level requires a slightly higher supply of concentrates than that is necessary to meet the requirements for energy.

2.4.2   Parity and breed

As parity increases from the first to the third, there is a rapid increase in milk yield and milk production increases at a decreasing rate up to the fifth parity (Hansen *et al*., 2006). During the first kidding, does have low body weights which contribute to low daily milk yield. As the number of parity increases, the animal is attaining maturity and energy competition between growth and milk synthesis is reduced, hence, high milk yield (Mellado *et al*., 2003). Schultz *et al*. (1990) reported that fat and protein percentages decrease with increase in parity.

Daily milk yield of dairy goats has been found to differ among breeds ranging from local, crosses and exotic breeds under tropical conditions (Berhane and Eik, 2006; Peacock, 2008). Breed of the dairy goats has effect on milk yield. Temperate breeds give more milk than tropical does, while crosses are intermediate in milk yields (Kendall *et al*., 2009). Genetic differences among the dairy goat breeds affect ash and fat contents of the milk (Schmidely *et al*., 2002), whereby the tropical breeds have higher percentage of milk fat than temperate breeds. Milk from the tropical breeds has higher total solids, mainly due to higher fat and protein contents. The concentration of these nutrients is associated with the relative small amount of milk produced (Abd El Gadir *et al*., 2005).

2.4.3   Stage of lactation

Milk yield and composition vary according to the stage of lactation (Table 2). Goats are more persistent milkers than cattle. They reach their peak milk yield during the 8th to 12th week after kidding. On the 5th month of lactation, milk yield decreases slowly and, thereafter, yield remains fairly constant (Salama *et al*., 2005). Antunac *et al.* (2001) reported that in the first lactation goats produce small amount of milk per day and milk yield increases progressively with the parity until the third lactation. Milk from the early stage of lactation normally contains high milk components. Fat, protein and lactose contents are high at the beginning of lactation, drop during peak milk yield and then increase slowly until the doe dries off. This observation follows the normal lactation curve of dairy goats where the total solid content is high in early lactation when milk volume is low. Conversely, when milk volume increases, the solids content decreases; and as lactating does enter the late lactation period, milk volume decreases and milk solids increase again. Similar trend in total solid (12.7 – 13.4%), fat (3.6 – 4.4%), and crude protein (3.5 – 3.8%) has been reported by Guo *et al.* (2001) from commingled goat milk in USA. Milk yield is also influenced by milking practice. For example, milking twice a day may yield up to 40 % more milk than milking once a day (Salama *et al*., 2003).

Table 2: Effect of stage of lactation on average composition of Alpine goat milk in Greece

	Week of lactation				
	8 - 12	17 - 21	26 - 30	35 - 38	39 - 42
Fat %	3.34	2.93	3.15	4.10	4.58
Protein %	2.79	2.95	3.32	3.91	4.25
Casein %	2.11	2.17	2.40	2.87	3.15
Lactose %	4.46	4.42	4.35	4.08	3.96
Mineral %	0.72	0.78	0.80	0.82	0.84
Total solids %	11.17	10.98	11.54	12.78	13.47
SNF %	7.83	8.05	8.39	8.68	8.89
PH	6.58	6.61	6.57	6.54	6.52

Source: Min *et al*. (2002).

2.4.4   Litter size

Litter size has been reported to have an effect on lactation performance of an animal. Litter size increases with age and is more related to the weight of the doe at conception than age (Sangare and Pandey, 2000). Does with twins produce more milk than does with single. The high milk yield in does with twins is a result of high lactogenic activities during prepartum stage which cause greater development of mammary gland potential for milk synthesis and, hence, high milk yield during early postpartum. Sangare and Pandey (2000), working with local Sahelian goats found that does with twin kids produce significantly more milk than the does with singles and milk yield declines rapidly. Other reports by other workers (Browning *et al.*, 1995; Milerski and Mares, 2001), show lower milk production in does with single litters while does with three and more kids produce

more milk and that goats with single litters produce milk with highest protein and lower fat contents.

2.5   κ-casein and β-lactoglobulin Genes in Goats

β-lactoglobulin gene in goats is located on chromosome 11q28 and encodes the main protein of whey in the milk of ruminants (Hayes *et al*., 1993). This encoded protein is also found in the milk of other mammals, but absent from the milk of rodents, lagomorphs and humans (Hambling *et al*., 1992). The biological functions of this protein are still not known. However, studies have shown that it could have a role in metabolism of phosphate in the mammary gland and the transport of retinol and fatty acids in the gut (Reddy *et al*., 1998). The fact that the protein is quite resistant to gastric proteases *in vitro* and *in vivo* suggests that the primary function of β-lactoglobulin is not nutritive (Reddy *et al*., 1998). The β-lactoglobulin locus affects mainly milk composition and milk quality, especially B allele which is associated with milk quality in European cattle breeds (Strzalkowska *et al*., 2002).

Apart from β-lactoglobulin gene, κ-casein gene is another gene of interest in this study. Four tightly linked and clustered genes, covering an area of approximately 250 kb genomic DNA fragment encode caseins. The structure and organization of casein gene locus have been described in human, mouse and bovine (Fujiwara *et al*., 1997) and mapped on chromosome 6 in bovine and caprine species (Hayes *et al*., 1993). The casein genes are tightly linked and inherited as a cluster, hence, they have a potential value and can play an important role in marker assisted selection for milk traits (Lien and Rogne, 1993). The order of the casein genes in the cluster is αS1-β-αS2-κ, and is considered as one genetic unit in which alleles are tightly linked together and transmitted as a haplotype

rather than individual alleles (Yahyaoui *et al*., 2001). The existence of genetic linkage has to be taken into account when selecting for an allele at given casein locus, or when studying the association between polymorphisms and production traits. The κ-casein gene has been intensively studied due to its vital role in the processing properties of milk by providing colloidal stability to the casein micelle.

2.6   Polymorphisms of κ-casein and β-lactoglobulin Genes in Goats

Polymorphism refers to the difference in DNA sequences among individuals, group or populations and can be caused by mutations ranging from single nucleotide base change to variations in several hundred bases (Yahyaoui *et al*., 2003). Extensive polymorphism is present in structural genes mostly with co-dominant expression. The existence of genetic polymorphism at the β- lactoglobulin locus came into light in the first report on the occurrence of two forms of β-lactoglobulin in cows by Aschaffenburg and Drewry (1955). The electrophoretic pattern of the β-lactoglobulin gene in goats has been analyzed by SDS-PAGE by different workers (Grosclaude *et al.,* 1987; Prakash *et al.,* 2002). β-lactoglobulin gene polymorphism at the DNA level has also been analyzed by PCR-RFLP and two novel genetic variants of the β -lactoglobulin gene have been reported in Indian goats (Kumar *et al*., 2006).

Analysis of goat κ-casein has shown that the κ-casein gene is highly polymorphic (Caroli *et al*., 2001; Yahyaoui *et al*., 2001; Angiolillo *et al*., 2002). The polymorphism seems to be associated with milk production traits (Angulo *et al*., 1994). The effect of κ-casein variants on milk production traits has been extensively studied in different animals. For example, in cows κ-casein BB genotype contains higher proportions of fat, proteins and caseins than milk derived from κ-casein AA genotype (Ng-kwai-Hang, 1998; Di Stasio and Mariani, 2000). The B allele is significantly associated with higher casein and

lower whey protein contents. The BB genotype has been associated with the production of milk with superior manufacturing properties e.g. shorter rennet coagulation time, formation of a firmer curd and higher cheese yield (Ng-Kwai Hang *et al*., 1986). This effect is associated with the milk casein micelles.

2.7   Genotype Assisted Selection

Selection is a key step in genetic improvement of livestock. The goal of selection is to screen out individuals with superior genotypes from a segregating population. Therefore, the most efficient approach of selection is to select individuals with desirable traits according to their genotypes directly. However, identification of genotypes is usually difficult (especially for quantitative traits). That is why selection in traditional breeding is generally performed based on the phenotypes. DNA markers provide a possibility of conducting selection based on genotypes directly. This is called marker assisted selection (MAS). In principle, no matter what trait is concerned, the genotype of a target gene or quantitative trait locus (QTL) can be deduced in light of its flanking markers and, therefore, genotype-based selection (GS) of the target gene or QTL can be conducted (WU *et al*., 2002).

2.8   Other Candidate Genes Associated with Milk Production in Goats

Casein genes are tightly linked and inherited as a cluster, hence, they have a potential value and can play an important role in marker assisted selection for milk traits (Lien and Rogne, 1993). The order of the casein genes in the cluster is αS1-β-αS2-κ, and is considered as one genetic unit in which alleles are tightly linked together and transmitted as a haplotype rather than individual alleles (Yahyaoui *et al*., 2001). The caprine αS1-casein gene is highly polymorphic in goats with 17 alleles that can be classified as strong (A, B1, B2, B3, B4, C, H, L and M), medium (E and I), low (F, D and G) and null

(01, 02 and N) (Yahyaoui *et al.*, 2001). Genotyping techniques have been developed to characterize the polymorphism of the αS1-casein gene in diverse goat populations. Initially, αS1-casein variants were typed through the analysis of milk samples by SDS-polyacrylamide gel electrophoresis combined with isoelectric focusing (Grosclaude *et al.*, 1987). PCR-RFLP and PCR-SSCP protocols are also used for typing of αS1-casein locus. In African breeds, strong αS1-casein alleles are the most frequent ones and significantly influence milk fat and protein contents (Trujillo *et al.*, 1998).

In caprine αS2-casein gene, five variants (A, B, C, E and F) encoding "normal" levels of αS2-casein have been found (Boulanger *et al.*, 1984). Yahyaoui *et al.* (2001) have also reported five β-casein variants (A, B, C, D and E) and have been identified by means of PCR-RFLP and PCR-SSCP protocols, but association studies with milk yield and milk contents are still lacking in both exotic and indigenous goat breeds in Africa. There is also a limited information on polymorphism of these candidate genes in indigenous goats found in Tanzania.

CHAPTER THREE

3.0   MATERIALS AND METHODS

3.1   Animal Blood Samples Collection and DNA Extraction

The study involved a total of 94 goats belonging to Gogo white (24), Pare white (22), Malya (24) and Saanen (24) breeds. The Gogo white, Malya and Saanen goats were found in Kongwa district, Dodoma region, in central Tanzania, while Pare white was found in Siha district, Kilimanjaro region in the northern part of Tanzania. A total of 22 - 24 blood samples from each breed were collected from unrelated goats by puncturing the jugular vein. A total of 10 mls of blood were collected per sample using vacutainer tubes with EDTA blood anticoagulant. An effort was made to collect samples from unrelated individuals based on the information provided by the farmers and animal records. The samples were brought to the laboratory within 24 hours in an ice-cooled box, where they were kept under −20°C in a deep freezer until DNA isolation. Genomic DNA was isolated from blood samples using Sepa gene extraction kit (Inqaba Biotec, Pretoria, South Africa) according to the manufacturer's instructions with some modifications. The quality of DNA was assessed through electrophoretic analysis of isolated DNA using 1.2% agarose gel, followed by visualization of DNA bands on a ultra violet transilluminator. The viewing of the gel revealed sharp high molecular weight bands of DNA, indicating that the DNA was of good quality and suitable for PCR-RFLP analysis. Poor quality DNA was re-extracted.

3.2   Milk Sample Collection and Analysis

Milk samples were obtained from 94 goats belonging to four genetic groups in their natural habitats. A total of 24, 24, 24 and 22 samples were collected from the same animals of Gogo white, Malya, Saanen and Pare white goats, respectively, and

transported in ice-cooled box to the laboratory for further analysis at the Department of Animal Science and Production, Sokoine University of Agriculture. Crude protein content was analyzed using the Kjeldahl method according to the International Dairy Federation Standards (IDF, 1993). The Gerber method (IDF, 1993) was used to determine milk fat content. All these methods are based on the procedure of the International Dairy Federation standards and all measurements were made in duplicate.

3.3   Polymerase Chain Reaction – Restriction Fragment Length Polymorphism (PCR-RFLP) Analysis of κ-casein and β -lactoglobulin Genes

Amplification of the β-LG gene (426 bp) from exon 7 to the 3' flanking region was done using the Polymerase chain reaction (PCR). The PCR was carried out in a total volume of 25 μL reaction mixture containing: 100 - 150 ng genomic DNA, 0.5 μM of each primer (forward: F   5'-CGGGAGCCTTGGCCCTCTGG   -   3',   reverse: 5'- CCTTTGTCGAGTTTGGGTGT-3'), 1.00 U of Taq DNA Polymerase, 2.5 μL of 10 × PCR assay buffer (1.5 mM $MgCl_2$, four dNTPs each at 100 μM) as described by Kumar *et al.* (2006). The amplification was carried out using a programmed thermal cycler (Applied Biosystems, California, U.S.A) with the following conditions: an initial cycle at 95°C for 5 min, followed by 35 cycles of the three steps including denaturation at 95°C for 30 s, annealing at 60°C for 1 min and extension at 72°C for 1 min and final extension at 72°C for 5 min. For κ-casein gene, a 459 bp fragment of goat κ-casein exon 4 was amplified by PCR from genomic DNA samples using primers with the following sequence: forward primer: 5'-TGTGCTGAGTAGGTATCCTAGTTATGG-3', reverse primer: 3'-GATTCCTCTGTAGTTTCTCCTGTTGCG-5' as described by Yahyaoui (2001). The PCR reaction was performed in 25 μL final volume containing 0.625 units of Taq DNA polymerase, 1×PCR buffer (1.5 mM-$Mgcl_2$, 200 μM of each of the four dNTP), 0.4 μM of each primer and 100 ng of goat genomic DNA. Thermal cycling conditions

were: 95°C for 5 min, 10 cycles of 97°C for 15 s, 63°C for 1 min and 72°C for 1 min 30 s, followed by 25 cycles of 95°C for 30 s, 63°C for 1 min and 72°C for 1 min 30 s, with a final extension at 72°C for 5 min. To confirm the amplification, the PCR products were resolved by agarose gel electrophoresis with ethidium bromide staining.

The PCR products were analyzed through restriction enzyme digestion. A total volume of 15 μL of PCR products of β-lactoglobulin gene were digested with 10 U of *Sac*II endonuclease (Inqaba Biotec Ltd, Kenya) for 9 hours at 37°C. For κ-casein gene, 10 μL of PCR products were digested with 10 U of *Alw*441 enzymes (Inqaba Biotec Ltd, Kenya) at 37°C overnight and with *Bse*NI (Inqaba Biotec Ltd, Kenya) at 65°C for 6 hours. After digestion, the resultant fragments were separated by gel electrophoresis using a 2% agarose gel stained with ethidium bromide. The different genotypes were scored manually and frequency of each genotype was determined by counting the number of individuals with one particular genotype and dividing by the total number of individuals involved in analysis. The allelic frequency for each variant was estimated using the formula: [Allelic frequency = {(2x number of homozygotes) + (number of heterozygotes)}/(2 x total number of individuals)].

3.4   Statistical Analysis

The effect of β-lactoglobulin genotypes on milk fat and protein contents was assessed using a general linear model (GLM) procedure of SAS (2004) programme. The following statistical model was used:

$$Y_{ijk} = \mu + B_i + G_j + (B*G)_{ij} + e_{ijk}$$

Where Y_{ijk} is the observation on each trait (milk fat and protein contents) of the kth animal from i^{th} breed and j^{th} genotype, μ is the general mean of each trait, B_i is the fixed effect of breed/genetic group, G_j is the fixed effect of jth β-Lg genotype and e_{ijk} is the random error.

The multiple linear regression analysis was used for assessing the association of breeds and genotypes with milk quality parameters (fat and protein contents). The following model for multiple linear regression was used.

$Y = \alpha + \beta_1 X_1 + \beta_2 X_2$

Where;

Y = dependent variable (fat contents)

α = intercept

β_1 = partial slope coefficient (Breed)

β_2 = partial slope coefficient (Genotype)

X= predictor

The chi-square (χ^2) test was used to compare variations of genotype and allele frequencies among the breeds using the following formula:

$$\chi^2 = \Sigma \frac{(O_{ij} - E_{ij})}{E_{ij}}$$

Where;

O_{ij} = observed frequencies of the cell in i^{th} row and j^{th} column

E_{ij} = expected frequencies of the cell in i^{th} row and j^{th} column

Σ = summation

CHAPTER FOUR

4.0 RESULTS

4.1 Polymorphisms of Kappa-casein and Beta-lactoglobulin Genes

The regions spanning from exon 4 to the 3' flanking region of the goat κ-Cn gene and the region spanning from exon 7 to the 3' flanking region of the β-Lg gene were amplified from genomic DNA samples belonging to the four different goat populations (Gogo white, Pare white, Malya and Saanen goats). The PCR amplified product of the κ-Cn gene had a size of 459 bp, while that of β-Lg gene was 426 bp (Figure 1).
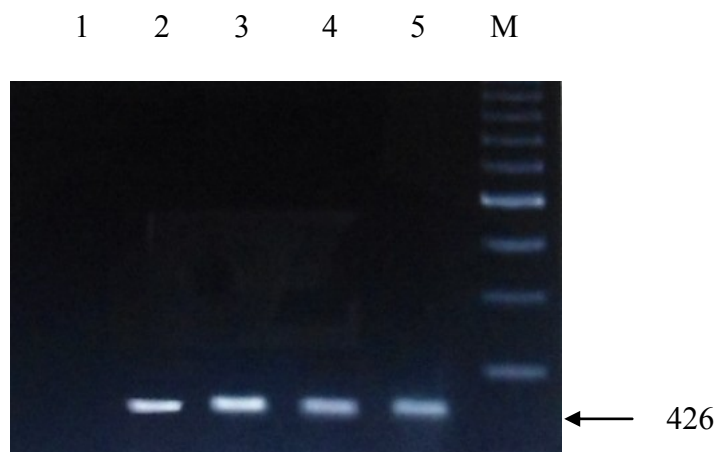
Figure 1: **Products of PCR amplification of β-lactoglobulin gene (426 bp). Lanes 2, 3, 4 and 5 represent amplified products from Gogo white, Pare white, Malya and Saanen goats, respectively, while lane 1 is a negative control (Nuclease free water). Lane M is a molecular size marker (100 bp DNA ladder).**

The restriction digestion of PCR products of κ-Cn gene with *Bse*NI produced three fragments of 51, 54 and 354 bp for A and B alleles, and two fragments of 54 and 405 bp for allele C (Figure 2). The use of *Alw*44I endonuclease produced only two fragments of 78 and 381 bp for allele C.
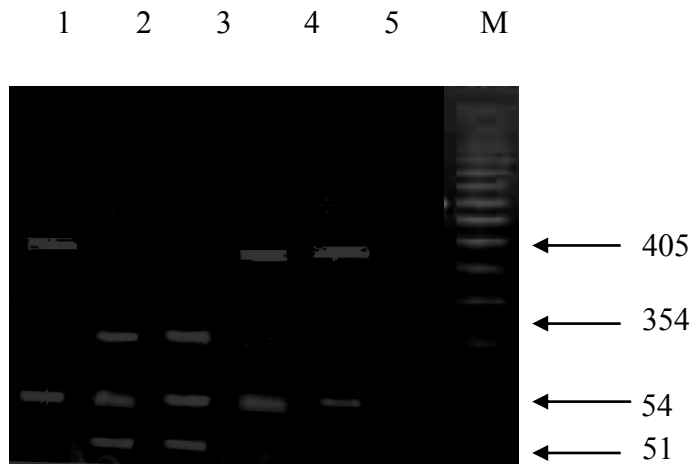
Figure 2: **Products of restriction digestion of κ-Cn gene using *Bse*NI endonuclease. (Lanes 1, 4 and 5 represent allele C (54 and 405 bp) belonging to Gogo white, Pare white and Saanen goats, respectively, while lanes 2 and 3 represents allele A/B (51, 54 and 354 bp) belonging to Malya goats. Lane M is a molecular size marker (100 bp DNA ladder).**

The restriction digestion of β-Lg gene with *Sac*II endonuclease revealed the presence of two alleles (A, B) in the goat populations under study. Three genotypes were observed i.e AA (undigested 426 bp band), BB (349 and 77 bp bands) and AB (426, 349 and 77 bp bands) (Figure 3).
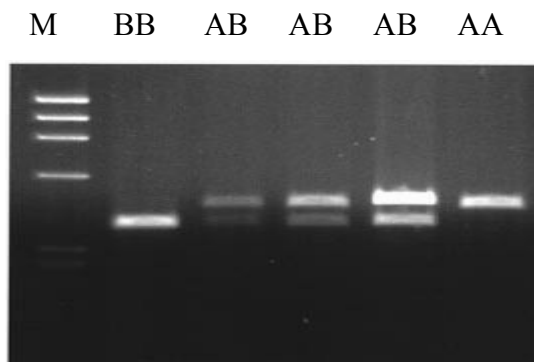
Figure 3: Products of restriction digestion of β-Lg gene using *Sac*II endonuclease in goats (Lanes AA, AB and BB represent genotypes in Gogo, Pare white, Malya and Saanen goats. M is a molecular size marker, 50 bp)

4.2 Allelic and Genotypic Frequencies of Kappa-casein and Beta-lactoglobulin Genes

The allelic and genotypic frequencies of the κ-Cn and β-Lg gene polymorphisms in the Gogo white, Pare white, Malya and Saanen goats are presented in Table 3 and 4, respectively. For the κ-Cn system, three major alleles (A, B and C) were observed in the populations under study. The allelic frequencies ranged between 0.875 and 1.0 and 0.00 and 0.125 for alleles A/B and C, respectively. The method by Yahyaoui *et al.* (2001) which was used in this study could not differentiate between alleles A and B for κ-Cn gene. The chi-square test was performed to assess if there was significant difference of allele frequencies among the breeds. No significant difference (P>0.05) of κ-Cn allele frequencies was observed among the breeds.

Table 3: Frequencies of A+B and C alleles obtained after digestion of κ-Cn gene in Malya, Gogo white, Pare white and Saanen goats

Breed/genetic group	Number of animals (N)	Allelic frequencies		Chi-square test	
		A+B	C	χ^2 (df=3)	p-value
Malya	24	1.0	0.0		
Gogo white	24	0.917	0.083	3.416	0.332
Pare white	22	0.955	0.045		
Saanen	24	0.875	0.125		

In β-Lg system, three genotypes (AA, AB and BB) were identified in goat populations under the study. The frequencies of each genotype in Gogo white, Pare white, Malya and Saanen goats are shown in Table 4. Saanen breed had the highest AA genotype frequency (0.500), while the Gogo white had the lowest (0.250). With regard to the AB genotype frequency, Malya had the highest frequency (0.417) while Pare white had the lowest

(0.273). For the BB genotype, Gogo white had the highest frequency (0.417) while Saanen had the lowest (0.125). The chi-square analysis indicated that, there was no significant difference (P>0.05) in the frequencies of these genotypes among the breeds. The allelic frequencies of A and B alleles are also presented in Table 4. The Saanen breed had the highest frequency of A allele (0.688) while the Gogo white had the lowest frequency (0.417). For the B allele, Gogo white had the highest frequency (0.583), while Saanen had the lowest frequency (0.312). The chi-square analysis indicated that, there was no significant difference (P>0.05) of the frequencies of these alleles among the breeds.

Table 4: **Genotypic and allelic frequencies of β-lactoglobulin gene after *Sac*II digestion of PCR products for fragment of the DNA region spanning from 7 exon**

Breeds

		Malya	Gogo white	Pare white	Saanen	Chi-square χ^2-value	p-value
Genotype	AA	0.333	0.250	0.318	0.500		
frequencies	AB	0.417	0.333	0.273	0.375	7.326	0.292
	BB	0.250	0.417	0.409	0.125		
Allele	A	0.542	0.417	0.455	0.688	4.818	0.186
frequencies	B	0.458	0.583	0.545	0.312		

4.3 Effect of Breeds or Genetic Groups and β-Lg Genotypes on Milk fat and Protein Percentages

Table 5 shows the least squares means for fat and protein contents in milk of different breeds and β-Lg genotypes. The milk fat content was significantly (P<0.05) influenced by breeds. Gogo goats were superior for milk fat content (4.56%) compared to Malya (3.92%), Pare white (4.24%) and Saanen (3.68%) goats. The protein content also varied

among breeds. Saanen goats were found to be superior in terms of milk protein content (4.16%) compared to the other breeds or genetic groups studied. However, there was no statistical significant differences (P>0.05) of protein contents among the goat breeds studied. The means of the analyzed traits (fat and protein contents) for each β-Lg genotypes are also shown in Table 5. Milk from goats with the β-Lg BB genotype had the highest fat (4.15%) and protein (4.01%) contents, but the differences of fat and protein contents among the genotypes were not significant (P>0.05).

Table 5: Least squares means (+SE) of fat and protein contents in milk of different breeds and β-Lg genotypes

Breed/Genetic group	Percentage	
	Fat	**Protein**
Gogo white	4.56 ± 0.15^a	3.71 ± 0.11
Malya	3.92 ± 0.15^{bc}	3.85 ± 0.11
Pare white	4.24 ± 0.16^{ab}	3.78 ± 0.11^b
Saanen	3.68 ± 0.18^c	4.16 ± 0.13^a
P-value	0.0115	$0.0576^{n.s}$
β-Lg genotype		
AA	4.14 ± 0.13	3.80 ± 0.09
AB	4.01 ± 0.13	3.81 ± 0.10
BB	4.15 ± 0.15	4.01 ± 0.11
P-value	$0.6981^{n.s}$	$0.3164^{n.s}$

Least squares means with different superscript in a column for each parameter differ significantly (P<0.05), n.s = not significant (P-value>0.05).

The multiple regression analysis result for the assessment of the influence of breeds and genotypes on milk fat contents are presented in Table 6. The regression coefficients on

milk fat contents were 0.070 and 0.079 for breed and β-Lg genotype, respectively. The coefficients of regression of both breed and β-Lg genotype were not significantly different from zero (P>0.05).

Table 6: **The coefficients of multiple regression for breed and β-Lg genotype on milk fat contents of goat populations studied**

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		β	Std. Error	β		
1	(Constant)	3.428	0.611		5.611	0.000
	Breed	0.070	0.072	0.101	0.972	0.334
	Genotype	0.079	0.100	0.082	0.787	0.434

CHAPTER FIVE

5.0   DISCUSSION

5.1   Polymorphisms of Kappa-casein and Beta-lactoglobulin Genes

The β-Lg and κ-Cn genes were successively amplified by Polymerase chain reaction (PCR). The amplified fragments had the sizes of 426 and 459 bp for β-Lg and κ-Cn genes, respectively. Restriction digestion of β-Lg gene using *Sac*II endonuclease revealed the presence of two common alleles (A, B) in all analyzed goat populations. The frequency of the β-Lg A allele was found to be lower than that of the β-Lg B allele in indigenous populations (Gogo and Pare white goats). The indigenous goats had higher frequency for the β-Lg B allele, whereas, exotic and improved goats i.e Saanen and Malya were found to have higher frequency of β-Lg A allele. The higher frequency of β-Lg B allele in indigenous goats is most likely to be associated with production traits which have being selected for within populations, by the local communities, over many generations. The β-Lg B allele has been shown to be superior for milk contents, whereas β-Lg A allele is associated mainly with milk yield (Strzalkowska *et al*., 2002). It has indeed been observed that goats with lower milk production level had higher milk fat and protein contents, this negative correlation between milk yield and milk contents is known as the dilution effect (Landau *et al*., 1993). It is also possible that the higher distribution of β-Lg A allele in exotic goat breeds is a result of selection for milk production. The frequencies of β-Lg alleles obtained in this study are within the range reported by Prinzenberg *et al*. (2005). The earlier report by this author in goat breeds from Europe, Africa and Asian part of Turkey showed that β-Lg B allele is the most common allele with frequencies ranging from 0.260 (Hair goat) to 0.674 (Angora goat). The second most common allele in their study was β-Lg A allele with frequencies ranging from 0.151 (Angora goat) to

0.414 (Borno goat). Similar results have been reported in Indian goats by Kumar *et al.* (2006).

The analysis of κ-Cn gene variation in four goat populations indicated that three alleles (A,B and C) were present, although allele C was inconsistent in that it was not found in one of the goat population (Malya goats). This inconsistency might be due to low number of samples analyzed and / or low frequency of this allele in most of the studied goat populations. However, the method used in this study failed to differentiate alleles A and B. It is obvious from the results of this study that allele A/B was the most frequent compared to allele C in all goat populations analyzed. The frequency of allele A/B in the breeds studied ranged from 0.875 to 1.00, while for allele C ranged from 0 to 0.125. These findings are consistent with the results reported by various workers. Veress *et al.* (2004) reported allelic frequencies of 0.85 and 0.15 for alleles A+B and C in Hungarian milk goats, respectively. Another report by Yahyaoui *et al.* (2001) showed that, the allelic frequencies of A+B ranges from 0.89 to 1.00, whereas that of C ranges from 0 to 0.11 in different goat breeds found in Spain. In goat populations from Eastern Africa, nine alleles for κ-Cn gene have been inferred (A, B, D, L, M, N, O, P and Q) with allele B being the most common amongst the majority of the breeds from various geographical locations (Kiplagat, *et al*., 2009). But the variation at the κ-Cn locus in Eastern African goat populations seems to have no influence on milk yield (Kiplagat, *et al*., 2009). Therefore, polymorphism was found to be present in the β-Lg and κ-Cn loci of the goat populations studied, and the PCR-RFLP analysis can be used as a valuable tool to identify desirable genotypes for better milk quality.

5.2 Effect of β-Lg Genotypes on Milk Fat and Protein Contents

The present study did not confirm the significant effect of β-Lg genotypes on milk fat and protein contents in all the goat populations, although β-Lg B allele tended to show a favorable effect. These results are similar to the findings of several previous studies (Ojala *et al*., 1997; Bobe e*t al*., 1999; Tsiaras *et al*., 2005). Bruno *et al*. (2008) also found no significant association between milk composition (fat and protein contents) and β-Lg genotypes (AA, AB and BB) in Bovine. On the contrary, Bovenhuis *et al*. (1992) suggested that the AA genotype might be associated with higher production of protein in milk. The results of the present study also indicate that there is no association between β-Lg genotypes and breed on milk fat and protein. The milk fat contents were 4.14, 4.01 and 4.15% for the AA, AB and BB genotypes, respectively, while for protein content, the average values were 3.80, 3.81 and 4.01% for the same genotypes. The average values for milk fat and protein contents for the AA, AB and BB genotypes did not differ significantly. However, the results show that animals with β-Lg BB genotype had slightly higher amount of milk fat and protein compared to the AA and AB genotypes, although their differences were not statistically significant. The results of the present study concur with the findings reported by Cece *et al*. (2008), who found no significant effect of β-Lg genotypes on fat and protein contents in sheep breed. However, this observation is contrary to the findings reported by Bovenhuis *et al*. (1992). Other studies found that the β-Lg locus affects mainly milk composition and quality and the B allele has been recognized to be associated with milk quality in cattle breeds, whereas allele A is associated with yield parameters (Strzalkowska *et al*., 2002). The results from Ng-Kwai-Hang *et al*. (1986), Aleandri *et al*. (1990), Bovenhuis *et al*. (1992) and Hill (1993) reported higher milk fat concentration in milk of Holstein cows with B allele of β-L gene. Hill (1993) observed significantly lower concentrations of fat and total solids in AA Holstein milk. Several authors have reported significant effects of β-Lg on milk fat

percentage and in all these studies, the β-Lg BB genotype was found to be associated with higher milk fat content (Bobe *et al*., 1999; Van der Berg *et al*., 1992). The β-Lg BB homozygote individuals produce milk rich in fat and protein which is very valuable in the process of cheese making, while the AA homozygote ones produce milk with low percentage of fat (Balcan *et al*., 2007). This trend has also been observed in sheep studies. According to Dario *et al*. (2005) the breed differences, population size, frequency distributions of genetic variants, the structure of data analyzed, the model used for statistical analysis and a failure to consider the relationship among animals are possible reasons for this inconsistency.

5.3   Effect of Breed on Milk Fat and Protein Contents

This study found a significant effect of breed or genetic group on milk fat content, but no significant effect of breed on content of milk protein was observed. The effect of breed or genetic group of goats on milk fat content observed in the present study is similar to the findings by Schmidely *et al*. (2002) who observed significant differences among the goat breeds on ash and fat contents of the milk. In their study the tropical breeds had higher percentage of fat than temperate breeds. Milk from the tropical breeds has higher total solids, mainly due to higher fat and protein contents. The concentration of these nutrients is associated with the relative small amount of milk produced (Abd El Gadir and Ibtisam El Zubeir, 2005). It is obvious from the present study that indigenous goat breeds (Gogo and Pare white) had higher milk fat contents than Saanen goats. The concentration of these nutrients in local goat breeds could be attributed to relative small amount of milk produced. This observation is supported by many scholars as described above.

CHAPTER SIX

6.0   CONCLUSIONS AND RECOMMENDATIONS

6.1   Conclusions

The sizes of the fragments for β-Lg and κ-Cn genes were 426 and 459 bp, respectively. The β-Lg locus has the two common alleles (A and B) in all goat populations analyzed. The β-Lg-B allele is the most frequent allele in local goat populations compared to β-Lg-A allele.

Three β-Lg genotypes were observed, namely, AA, AB and BB in the goat populations studied. Local goats have higher frequency of BB genotype than AA genotype, whereas exotic and improved goats have higher frequency of AA genotype than BB.

Local goats (Gogo and Pare white) have higher milk fat content than improved and exotic breeds (Malya and Saanen). The β-Lg genotypes do not influence / affect milk fat and protein contents in the goat populations studied. Breed has significant effect on milk fat contents.

The κ-Cn gene in the four goat populations studied has three alleles (A, B and C). The κ-Cn A/B allele is the most frequent compared to allele C in all goat populations studied.

6.2   Recommendations

i.   Further studies should be done using larger number of animals with more goat breeds from different geographic regions in Tanzania in order to get more precise information on polymorphisms of these two candidate genes for milk production traits.

ii. Since the method used in this study to analyze the variation at the κ-Cn locus failed to distinguish between alleles A and B, it is recommended that some modifications of the method should be done or other markers should be used in future studies.

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APPENDICES

Appendix 1: Primer sequences for kappa-casein gene

Forward primer: 5'-TGTGCTGAGTAGGTATCCTAGTTATGG-3'

Reverse primer: 5'-GCGTTGTCCTCTTTGATGTCTCCTTAG- 3'

Primer sequences for beta-lactoglobulin gene

Forward primer: 5'-CGGGAGCCTTGGCCCTCTGG -3'

Reverse primer: 5'- CCTTTGTCGAGTTTGGGTGT-3'

Appendix 2: Dependent Variable: Fat   ANOVA TABLE 1

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	14.04288397	1.27662582	2.41	0.0119
Error	82	43.38717667	0.52911191		
Corrected Total	93	57.43006064			

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Breed	3	8.90166673	2.96722224	5.61	0.0015
Geno	2	0.38200804	0.19100402	0.36	0.6981
Breed*Geno	6	1.73296129	0.28882688	0.55	0.7718

Appendix 3: Dependent Variable: Protein   ANOVA TABLE 2

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	3.87537166	0.35230651	1.26	0.2646
Error	82	22.99150175	0.28038417		
Corrected Total	93	26.86687340			

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Breed	3	2.18724727	0.72908242	2.60	0.0576
Geno	2	0.65447451	0.32723726	1.17	0.3164
Breed*Geno	6	1.11110443	0.18518407	0.66	0.6816

Appendix 4: Chi-square test for kappa casein alleles

Breed* Alleles Crosstabulation

Count

Breed		Alleles		Total
		A+B	C	
Saanen		21	3	24
Gogo		22	2	24
Pare	Malya	21	1	22
Total		24	0	24
		88	6	94

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	3.416[a]	3	.332
Likelihood Ratio	4.638	3	.200
Linear-by-linear Association	3.376	1	.066
N of valid Cases	94		

a. 4 cells (50.0%) have expected Count less than 5. The minimum expected Count is 1.40

Appendix 5: Chi-square test for Beta lactoglobulin alleles
Breed* Alleles Cross tabulation

Count

Breed	Alleles		Total
	A	B	
Saanen	17	7	24
Gogo	10	14	24
Pare	10	12	22
Malya	13	11	24
Total	50	44	94

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	4.818[a]	3	.186
Likelihood Ratio	4.932	3	.177
Linear-by-linear Association	0.994	1	.319
No. of valid Cases	94		

a. 0 cells (0%) have expected Count less than 5. The minimum expected Count is 10.30

Appendix 6: Chi-square test for Beta lactoglobulin genotypes
Count   Breed* Genotype Crosstabulation

	Genotype			Total
	AA	AB	BB	
Breed Saanen	12	9	3	24
Gogo	6	8	10	24
Pare	7	6	9	22
Malya	10	8	6	24
Total	35	31	28	94

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	7.326[a]	6	.292
Likelihood Ratio	7.830	6	.251
Linear-by-linear Association	0.514	1	.473
No. of valid Cases	94		

a. 0 cells (.0%) have expected Count less than 5. The minimum expected Count is 6.55

Appendix 7: Multiple Regression analysis of breeds and Beta lactoglobulin genotypes on milk fat content

ANOVA (b)

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	1.045	2	.523	.844	.434 (a)
	Residual	56.385	91	.620		
	Total	57.430	93			

a Predictors: (Constant), Genotype, Breed
b Dependent Variable: Fat

Coefficients (a)

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	Correlations		
		B	Std. Error	Beta			Zero-order	Partial	Part
1	(Constant)	3.428	.611		5.611	.000			
	Breed	.070	.072	.101	.972	.334	.107	.101	.101
	Genotype	.079	.100	.082	.787	.434	.089	.082	.082

a Dependent Variable: Fat

Appendix 8: Genotype frequencies in each goat breed/genetic group studied
   a) Gogo white goat

Genotype	Frequency	Percentage
AA	6	25.0%
AB	8	33.3%
BB	10	41.7%
Total	24	100.0%

b) Malya goat

Genotype	Frequency	Percentage
AA	10	41.7%
AB	8	33.3%
BB	6	25.0%
Total	24	100.0%

c) Pare white goat

Genotype	Frequency	Percentage
AA	7	31.8%
AB	6	27.3%
BB	9	40.9%
Total	22	100.0%

d) Saanen goat

Genotype	Frequency	Percentage
AA	12	50.0%
AB	9	37.5%
BB	3	12.5%
Total	24	100.0%