POLYMORPHISMS IN MAJOR MILK PROTEIN GENES (*LALBA*, *MBLG*, *CSN1S1* AND *CSN3*) AND MILK FAT GENES (*DGAT1* AND *SCD1)* AND ASSOCIATION WITH MILK PRODUCTION AND FATTY ACID TRAITS IN INDIGENOUS WHITE FULANI AND BORGOU CATTLE BREEDS IN BENIN

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Polymorphisms in major milk protein genes (*LALBA, MBLG, CSN1S1* and *CSN3*) and milk fat genes (*DGAT1* and *SCD1)* and association with milk production and fatty acid traits in indigenous White Fulani and Borgou cattle breeds in Benin

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

I dedicate this work to:

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ABBREVIATIONS AND ACRONYMS

ABCF: Africa Biosciences Challenge Fund

AMDIS: Automated Mass Spectral Deconvolution and Identification System

BecA: Bioscience eastern and central Africa

BMGF: Bill & Melinda Gates Foundation

CFSVA: Comprehensive Food Security and Vulnerability Analysis

CSIRO: Commonwealth Scientific and Industrial Research Organisation

***CSN1S1*:** α-S1 Casein gene

***CSN1S2*:** α-S2 Casein gene

***CSN2*:** β-Casein gene

***CSN3*:** Kappa-Casein gene

DAGRIS: Domestic Animal Genetic Resources Information System

DFAT: Department for Foreign Affairs and Trade

DFID: Department for International Development

DGAT1: Acyl CoA: Diacylglycerol Acyltransferase 1 gene

FAO: Food and Agriculture Organization

FAOSTAT: Food and Agriculture Organization Statistics

GC-MS: Gas Chromatography-Mass-Spectrometry

HWE: Hardy-Weinberg Equilibrium

ILRI: International Livestock Research Institute

MAEP: Ministère de l'Agriculture, de l'Elevage et de la Pêche

MUFA: Monounsaturated Fatty Acid

NCBI: National Center for Biotechnology Information

NIST: National Institute of Standards and Technology

PCA: Principal Components Analysis;

PCR: Polymerase Chain-Reaction

PUFA: Polyunsaturated Fatty Acid

JKUAT: Jomo Kenyatta University of Agriculture and Technology

LALBA**:** α-lactalbumin gene

LCFA: Long-chain fatty acids.

LDL: Low density lipoproteins

MBLG: Milk β-lactoglobulin gene

MCFA: Medium-chain fatty acids

PAFILAV: Projet d'Appuis aux Filières Lait et Viandes

PAUSTI: Pan African University Institute of Basic Sciences, Technology and Innovation

PCR: Polymerase Chain Reaction

PNAG: Programme National d'Amélioration Génétique

PUFA: Polyunsaturated Fatty Acid

RFLP - Restriction Fragment Length Polymorphism

SCD1: Stearoyl-CoA Desaturase 1

SCFA: short-chain fatty acids

SFA: Saturated fatty acids

SFSA: Syngenta Foundation for Sustainable Agriculture

SIDA: Swedish International Development Cooperation Agency

UK: United Kingdom

USDA: United States Department of Agriculture

WFP: World Food Program

ABSTRACT

Polymorphism in milk protein and fat genes is an important tool for breed characterization, genetic association as well as genetic diversity studies. However, there is no data available on genetic polymorphisms of major milk protein genes as well as their association with milk production traits in indigenous Borgou and White Fulani cattle breeds in Benin. The aim of this study was to assess the polymorphism in *LALBA*, *MBLG*, *CSN1S1*, *CSN3*, *DGAT1* and *SCD1* genes, their association with milk production and fatty acid traits as well as the genetic diversity, gene flow and phylogenetic relationships among Borgou and White Fulani cattle breeds in Benin. The genetic polymorphisms were assessed in 98 Borgou and 100 White Fulani cattle using polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) and Sanger sequencing methods. The milk components were determined using MilkoScan FT 6000 Series mid-range infrared and the fatty acid composition analysis was done by gas chromatography mass spectrometry (GC-MS). The results showed that White Fulani produced higher ($P<0.001$) test-day milk yield (1.14 vs 0.80 kg), fat content (5.49 vs 4.51 %), fat yield (57.13 vs 36.52 g) and test-day protein yield (40.4 vs 30.05 g) compared to Borgou. On the other hand, Borgou presented higher ($P<0.001$) content of milk urea nitrogen, oleic acid (C18:1 *cis*-9), linoleic acid (C18:2 *cis*-9, *cis*-12), C18 desaturation index ($P<0.05$), total index and higher ($P<0.001$) contents of polyunsaturated fatty acids (PUFA) than White Fulani. Six single nucleotide polymorphisms were found in *CSN3* gene (g.13065 C>T, g.13068 C>T, g.13104 A>C, g.13111 A>G, g.13165 A>G and g.13173 A>T). The SNPs showed significant effect in Borgou only. Thus, the *CSN3* g.13065 C>T genotypes were significantly associated with protein % ($P<0.05$), lactose % ($P<0.01$), test day fat and protein yields ($P<0.05$). The *CSN3* g.13068 C>T and *CSN3* g.13173 A>T genotypes were significantly associated with protein % ($P<0.05$) and lactose % ($P<0.01$). Moreover, the g.13111 A>G was associated with fat % and test-day fat yield ($P<0.01$) while the *CSN3*

g.13165 A>G was associated with fat% ($P<0.05$), protein % and lactose % ($P<0.01$) in Borgou. The AG genotype of *MBLG* g.5940G>A was significantly associated with higher fat (6.8% vs 4.6% vs 4.5, $P<0.05$) and total solids (16.8% vs 13.7% vs 13.5%, $P<0.05$) contents compared to AA and GG genotypes, respectively in Borgou. The AG genotype of *CSN1S1* g.10430 G>A was associated with higher ($P<0.05$) test-day milk yield (1.18 kg) compared to AA genotype (0.67 kg) and GG genotype (0.78 kg). The TT genotype of *CSN1S1* g.10359 T>C was associated with higher ($P<0.01$) test-day milk yield and higher ($P<0.05$) test-day fat and protein yields in White Fulani. Moreover, the GG genotype of *CSN1S1* g.10430 G>A was associated with higher ($P<0.01$) test-day milk yield in White Fulani. The *SCD1* VV genotype was associated with higher ($P<0.05$) protein and lactose contents and lower ($P<0.05$) C18:1 *cis*-9 content in White Fulani. The *SCD1* AV genotype was associated with higher C14 and total indices; and the *SCD1* V allele was associated with decrease in C14 index in Borgou. In White Fulani breed, the *DGAT1 K* allele was associated with increased total saturated fatty acids (SFA), and decreased C18 index, total index and total monounsaturated fatty acids. Borgou presented higher ($P<0.01$) observed heterozygosity (H_{ob}) and unbiased gene diversity ($H_{ex)}$ (0.329, 0.353) than White Fulani (0.286, 0.302). The population differentiation (θ) was 0.017 and significantly different from zero ($P<0.01$). High level of gene flow ($N_e m=14.104$), a low Nei's D_A genetic distance (0.017) and a very closer phylogenetic relationship were observed between both breeds. Borgou milk is better in terms of human health compared to White Fulani. The identified alleles and single nucleotide polymorphisms (SNPs) associated with milk production and fatty acid traits could serve as potential genetic markers to improve milk production and fatty acids traits in Borgou and White Fulani cattle breeds in Benin. To allow sustained genetic improvement, care must be taken to prevent the gene flow and loss of genetic diversity between both breeds.

CHAPTER ONE

INTRODUCTION

1.1. Background information

Indigenous cattle play a key role by providing meat and milk and hence helping to combat malnutrition and food insecurity in developing countries (FAO, 2013). Unfortunately, indigenous African livestock resources are less exploited for milk production due to lack of knowledge on their genetic potential and also because they produce very little milk. Yet, animal products such as milk and dairy products are a potential source of nutrients and vitamins, thereby important for human healthy nutrition, especially in developing countries where they are a cheap source of high quality protein. Dairy industry projects in developing countries contribute to the improvement of health and nutrition at household; provide income for poor people and thereby contributing to poverty reduction (FAO, 2013). In developing countries, the demand in livestock products has rapidly increased due to the growth of human population, income and urbanization (Thornton, 2010).

In sub-Saharan Africa, cattle produce around three-quarters of milk production (FAO, 2018). And, this production is mainly supplied by small scale-livestock holders. According to Food and Agriculture Organization (FAO), the demand for milk is expected to increase by 25% in developing countries by 2025 (FAO, 2008). In Benin, cow milk is a basic food for the rural population and is produced mainly by the indigenous cows. The cattle population of Benin was estimated at approximately 2.3 million head in 2016 (FAOSTAT, 2018). The cattle population consists mainly of indigenous *Bos taurus* cattle breeds namely, Borgou, Somba, N'dama, and Lagune and the second being *Bos indicus* namely, M'bororo, Gudali and White Fulani (DAGRIS, 2018). Borgou cattle breeds represent more than 50 % of the total cattle population in Benin (MAEP, 2007). However, the herds are not able to guarantee the full

coverage of the milk needs of the human population because of the low productivity of animals (Youssao et al., 2009). The current policy on agricultural development of Benin has mainly focused on increasing milk and meat production (PAFILAV, 2010). Thus, exotic cattle breeds such as Gir and Girolando are usually imported from Brazil into Government's farms to boost the milk production and for crossbreeding purpose. However, the majority of indigenous cattle are held by the indigenous Fulani ethnic group where the milk constitutes a source of food and income for the household. Raw milk produced in Benin is consumed at household level and mostly processed into yoghurt, curds and traditional cheese known as *Wagashi*. *Wagashi* is a rich nutritional cheese produced by women and is the most consumed among the dairy products in Benin (Aïssi et al., 2009).

There are two breeding systems in Benin; these include a traditional breeding system and a semi-modern breeding system (Youssao et al., 2009). The latter is practiced only in government farms located in Kpinnou (District of Athiémé), Samiondji (District of Zangnannado), Betecoucou (District of Dassa) and Okpara (District of Tchaourou). Thus, the traditional breeding system which is practiced throughout the rest of Benin is the most common method used. These cattle breeders in the traditional breeding system do not have clear breeding objectives that are defined by production of milk or meat (Alkoiret et al., 2011a). The indigenous cattle breeds are characterized by low milk production estimated between 0.5 and 2.5 kg per day (Adjou-Moumouni, 2006). In the meantime, the imported Girolando cows produce four to seven kilograms per day (Toukourou and Senou, 2010). The Brazilian imported breeds cannot walk the distances to obtain pasture but the local cows do. Moreover, the imported cows are more susceptible to trypanosomiasis disease than the indigenous cows.

A number of studies have focused on cattle breeding system in Benin (Alkoiret et al., 2011a; Assani et al., 2016), bovine diseases affecting milk production (Koutinhouin et al., 2003; Farougou et al., 2006), estimation of growth genetic parameters in Borgou cattle breed (Youssao et al., 2007) and intramuscular fatty acids composition of Lagune, Borgou and White Fulani cattle in Benin (Salifou et al., 2013).

Bovine milk proteins consist of caseins and whey proteins. Caseins are subdivided into αS1-CN, αS2-CN, β-CN, and κ-CN coded by *CSN1S1*, *CSN1S2*, *CSN2* and *CSN3* genes respectively, whereas whey proteins consist mainly of β-lactoglobulin (β-LG) and α-lactalbumin (α-LA) (Martin et al., 2013). On the other hand, α-LA and β-LG are coded by *LALBA* and *MBLG*, codominant autosomal genes (Hayes et al., 1993; Hayes and Petit, 1993). The studies on milk proteins variants have become a great interest to scientists worldwide since the identification of bovine β-Lactoglobulin main variants by Aschaffenburg and Drewry (1957), the pioneers of milk protein polymorphism studies. Many studies have demonstrated the effects of the polymorphism of the major milk proteins genes on milk production traits and cheese yielding capacity (Caroli et al., 2009; Martin et al., 2013). In addition, the milk protein genes polymorphism in cattle is an important tool for genetic diversity studies, breed characterization, gene evolutionary studies with many applications in human nutrition and animal breeding (Caroli et al., 2009). Therefore, milk protein polymorphisms have been thoroughly investigated in dairy and beef cattle breeds from European origin (Mahé et al., 1999) and also extended to beef breeds later.

The variability of fatty acids composition and unsaturation indices in cow milk is affected by breed, diet, stage of lactation and parity number, but the genetics also plays an important role (Kelsey et al., 2003). Thus, the two candidate genes among others that affect fatty acid traits in cows are Acyl CoA: diacylglycerol acyltransferase 1 (*DGAT1*) gene and the Stearoyl-CoA Desaturase 1 (*SCD1*) gene (Arnould and Soyeurt, 2009). There is a limited data on milk

proteins polymorphism in African breeds (Mahé et al., 1999) and this is really unfortunate because African zebu populations have presented high diversity at microsatellite and blood and milk proteins loci respectively (MacHugh et al., 1997, Ibeagha-Awemu and Erhardt, 2006)

1.2. Problem Statement

The World Food Program (WFP) conducted a comprehensive food security and vulnerability analysis in 2013 and reported that nearly 1.1 million people (11%) in Benin were food and nutrition insecure (CFSVA, 2014). In Benin, milk contributes to more than 50% of Fulani household income per year (Dossou et al., 2006). However, the local production is provided mainly by the indigenous cows characterized by low milk yield (Youssao et al., 2009). The milk consumption in Benin is estimated to 7.90 kg/capita/year lower than 30.2 kg recorded in sub-Saharan Africa (FAOSTAT, 2013). Consequently, the importation of milk and dairy products is increasing annually and was reported to have increased from approximately USD 17 million in 2003 to approximately USD 24 million in 2013 (FAOSTAT, 2018). Unfortunately, despite the insufficiency of local milk production; there are no effective cattle genetic improvement programs in Benin.

Considering the importance of cow milk in Benin and the critical deficit in milk and dairy products, it is necessary to exploit the genetic diversity of the indigenous cattle to increase milk production and improve milk composition for its ability to be processed into dairy products such as *Wagashi* (traditional cheese), yoghurt and curd milk. Therefore, understanding the genetic variation in milk proteins is important since it affects the yield and properties of different dairy products, such as cheese, yogurt and milk powder (Walstra et al., 2006). This is especially important because there is little data available on milk protein variants in African Bos *taurus* and Bos *indicus* breeds and none is available for the main

indigenous cattle from Benin. So far the available data on milk protein variants from Beninese cattle breeds did not focus on the relationship between milk proteins genes polymorphism and milk traits (Ceriotti et al., 2004). In addition, no detailed data is available either on the major milk components or on genetic variability of milk fatty acids in the main indigenous White Fulani and Borgou cattle breeds in Benin. Where data is available the focus has been on allele frequencies and no associations between the *DGAT1* polymorphisms and the milk traits (Kaupe et al., 2004).

1.3. Justification of the Study

African *Bos indicus* and *Bos taurus* cattle breeds are under threat of extinction due to uncontrolled breeding and are consequently at risk to become uniform at genetic level (Rege, 1999; Ibeagha-Awemu and Erhardt., 2006). Genetic polymorphism in major milk protein genes has been an important tool for genetic diversity studies, breed characterization, gene evolutionary studies with applications in human nutrition and animal breeding. It is well known that polymorphisms in *LALBA, MBLG, CSN1S1, CSN3*, *DGAT1* and *SCD1* genes are associated with milk yield, proteins and fat contents as well as fatty acid composition and unsaturation indices in cattle . In Benin, White Fulani and Borgou cattle breeds are well adapted to local environmental conditions but have not been characterized at the major milk protein loci. Hence, there is need to characterize the main indigenous cattle breed populations in Benin in order to improve their milk productivity as well as to conserve them. Moreover, the current agricultural policy of Benin government is to increase milk and meat production. Therefore, investigating the polymorphisms in *LALBA, MBLG, CSN1S1, CSN3*, *DGAT1* and *SCD1* genes and their association with milk traits in indigenous cattle from Benin could be used in cattle improvement programs. The current study is the first to provide scientific information on a genetic association of *LALBA, MBLG, CSN1S1, CSN3, DGAT1* and *SCD1*

variants with milk production traits and fatty acids composition in indigenous cattle breeds from Benin. In addition, the study provided information on the genetic diversity and phylogenetic relationship between White Fulani and Borgou cattle breeds based on major milk proteins and fat gene loci. The identified alleles and single nucleotides polymorphisms (SNP) associated with milk production traits and fatty acid composition could serve as potential genetic markers in breeding improvement programs. Thus, there will be a possibility to select sires for specific alleles associated with higher milk yield or particular fatty acids that are beneficial to human health. The ministry of agriculture and livestock will use the results from this study to develop a sustainable breeding program involving farmers, consumers, communities and decision-makers in Benin.

1.4. Hypotheses

1. There is no variation in major milk components and fatty acid composition between White Fulani and Borgou cattle breeds in Benin
2. There is no genetic association of *LALBA, MBLG, CSN1S1, CSN3, DGAT1 and SCD1* genes with milk production traits in white Fulani and Borgou cattle breeds in Benin
3. There is low genetic diversity within and between white Fulani and Borgou cattle populations

1.5 Objectives of the study

1.5.1. General objective

To characterize the indigenous White Fulani and Borgou cattle breeds in terms of major milk proteins, *DGAT1* and *SCD1* genes in order to contribute to the improvement of their milk production and fatty acids traits as well as their conservation in Benin

1.5.2. Specific Objectives

1. To assess genetic polymorphisms in *LALBA, MBLG, CSN1S1, CSN3, DGAT1* and *SCD1* genes in White Fulani and Borgou cattle breeds
2. To determine the fat, protein, lactose, milk urea nitrogen, total solids contents and the fatty acid profiles of milk from White Fulani and Borgou cattle breeds
3. To determine the genetic associations of polymorphisms in *LALBA, MBLG, CSN1S1, CSN3, DGAT1* and *SCD1* genes with milk production and fatty acid traits in both breeds
4. To determine the genetic diversity, gene flow and phylogenetic relationships among White Fulani and Borgou cattle breeds based on *LALBA, MBLG, CSN1S1, CSN3, DGAT1* and *SCD1* genes loci.

CHAPTER TWO

LITERATURE REVIEW

2.1. Cattle genetic resources of Benin: Production and breeding systems

The sections below describe the indigenous cattle resources in Benin that were used in this study as well as their production and breeding systems.

2.1.1. Borgou cattle breed

The Borgou cattle (Figure 2.1) breed is a taurine, derived from crossing *Bos taurus* X *Bos indicus*. Originally from the Department of Borgou (Benin), Borgou cattle are also found in Togo, Burkina Faso (Méré) and Nigeria. In Benin, this cattle breed represents more than 50% of the national cattle population (MAEP, 2007). The horns are medium in size and the face profile is flat. The body weight is between 200kg and 250kg and sometimes more. The coat is usually white, gray and sometimes piebald. The height at withers is between 120 and 130 cm for bulls and between 110 and 125 for cows. The daily milk yield in Borgou is between 0.5 and 2.5 liters (Adjou-Moumouni, 2006) and is the most productive indigenous taurine in Benin.

Figure 2.1: Borgou cattle breed of Benin (Houaga, 2016)

2.1.2. White Fulani cattle breed

It is also known as Sudanese Zebu Peulh. In Benin, this breed is found mainly in Departments of Borgou and Alibori. Zebu Fulani is medium in size with a height of 1.15 to 1.40 m. The body weight for mature animal is approximately 300 kg for male and 250 kg for female. It is characterized by a fairly developed hump and an inclined ridge. The head is long and thin. The horns are generally in lyre, strong at the base and directed forward. The dominant coats are light-gray spotted (Fulani of Samburu). The cattle have characteristically long legs, average 100 cm (Figure 2.2). They are therefore able to walk long distances. They are able to survive on during periods where there are food shortages. The cattle do not tolerate humid conditions. They are also susceptible to trypanosomiasis.

The milk production is around 2-3 liters / day. The breed is a good draft animal and is widely used for plowing. The age at first calving is around four years; the interval between calving is around 320 days. Moreover, the White Fulani cattle belong to the Zebu Peulh group and is

originated from Northern- Nigeria and Western-Cameroon, the coat is usually white with black extremities. The horns are medium to long. Their aptitudes are: meat, milk and plowing.

Figure 2.2: White Fulani cattle breed of Benin (Houaga, 2016)

2.1.3. Cattle production and breeding systems in Benin

The current policy on agricultural development of Benin has mainly focused on increasing milk and meat production (PAFILAV, 2010). In Benin, there are no national cattle breeding program ongoing. However, a national breeding program has recently been approved by the government waiting for funding (Youssao, 2015). The natural mating is the most common in Benin with trials ongoing for artificial insemination in some government farms. There are two cattle production systems in Benin; these include a traditional production system and a semi-modern production system (Youssao et al., 2009). The latter is practiced only in some government farms. Thus, the traditional production system which is practiced throughout the rest of Benin is the most common method used.

The traditional cattle production is characterized by two farming systems based on the exploitation of natural pastures: transhumance and sedentary systems. The transhumant system promotes high mobility and a weak link with agriculture. The goal is to use the best pasture at the best time and to have water. There is a permanent home; the movements are cyclical and seasonal. The herdsmen return to the permanent home each year. Depending on the distance, we can distinguish the great transhumance and the small transhumance. Thus, the great transhumance is practiced for example by Fulani Bororos pastoralists with large herds cattle and sheep over several hundred kilometers. As for the small transhumance, it is practiced on a few kilometers or tens of kilometers. The third possibility is semi-transhumance, also practiced by Fulani. The families stay in the village, only the herdsmen leave with the herd or a part of the herd. Thus, we can distinguish: displacement by whole family and whole herd, displacement by partial family and whole herd and the last being displacement by partial family and partial herd.

On the other hand, the sedentary cattle production system is associated with different crops and occupies the areas around the villages. This system is characterized by the fact that the animals are kept permanently in the village or under the guidance of the herdsmen. During the rainy season, animals graze for short distances in uncultivated areas. During the dry season their diet is supplemented with crop residues and fodder (Pagabeleguem, 2010).

At the Okpara Breeding Farm, the reference center responsible for the conservation of the Borgou breed, the production system is semi-improved. Animals (except calves) are grazing all day and at night in a park with troughs and feeders. The calves are kept in the park. Herds are constituted according to the age and sex of the animals (Youssao et al., 2000). In general, feeding is based on the exploitation of natural pastures and artificial grasslands. Animals also benefit from crop residues from crop fields. The artificial grasslands consist of *Brachiaria ruziziensis*, *Stylosanthes sp* and *Andropogon gayanus*, part of which is used as silage.

Supplementation with silage, cottonseed cake, cooking salts and licks is ensured for the animals during the transition period between the dry season and the rainy season to cover the needs for energy and digestible nitrogenous matter. The health monitoring is based on vitamino-prevention and the administration of trace elements, preventive treatments against trypanosomiasis, gastro-intestinal parasites, ticks and other arthropods. The national program for prophylaxis against major epizootics (pasteurellosis, contagious bovine pleuropneumonia) is monitored regularly. Occasional diseases are specifically treated according to the clinical cases detected.

2.2. Nutritional traits of cow milk

Milk is an important source of dietary energy, fat, protein and lactose. One kilogram of cow milk is approximately composed of 878g of water, 33g of total protein, 33g of total fat, 47g of lactose, and 7g of Ash and provides 620Kcal as dietary energy (USDA, 2009). Milk also contains directly assimilated major salts, many trace elements in the form of complexes with proteins, vitamins and several enzymes (Clinquart, 2010). Milk fat consists of many fatty acids classified on the basis of their chain length (short, medium and long) or degree of unsaturation as saturated, monounsaturated and polyunsaturated. Concentration of polyunsaturated fatty acids (PUFAs) in blood and tissue lipids is associated with several positive health benefits on cardiovascular disease, early visual, mental health, early growth and development during pregnancy (Glaser et al., 2010). Moreover, PUFAs present anticancer effects (Yang et al., 2013) and are important for allergies prevention and/or treatment (Park et al., 2013). On the other hand, it is well known that saturated fatty acids (eg: C: 14 and C: 16) are negatively associated with human health.

2.3. Polymorphism of the main milk protein genes in cattle

The milk protein variants have been extensively investigated in cattle over the last decades since the discovery of two distinct forms of β-lactoglobulin using the electrophoresis method (Aschaffenburg and Drewry, 1957). For economic reasons, most of the studies have focused on the impact on milk processing, especially cheesemaking properties. They have been also used to analyze possible relationship with quality and quantity of milk traits as well as evolutionary and biodiversity studies (Caroli et al., 2009; Martin et al., 2013).

Since 2004, the polymorphism of milk proteins genes in cattle was subject to three important reviews (Farrell et al., 2004; Caroli et al., 2009; Martin et al., 2013). The first review focused on the nomenclature in milk protein polymorphism (Farrell et al., 2004) while Caroli et al. (2009) have focused on the effect of bovine milk protein polymorphisms on animal breeding and human nutrition. The last recent review has focused on genetic polymorphism of milk protein in dairy ruminants (cattle, caprine and ovine) with emphasis on the current knowledge in the field with effort of resolving some reported sequences conflicts or misinterpretations (Martin et al., 2013).

One of the important characteristics of milk proteins genetics is the tight linkage of the four casein genes (*CSN1S1*, *CSNS2*, *CSN1S2* and *CSN3*) on chromosome 6 (Figure 2.3A) in cattle and goat. This was first highlighted by Grosclaude et al. (1965) and was confirmed later (Ferretti et al., 1990; Threadgill and Womack, 1990). Thus, there is a non-independent inheritance of casein alleles in a population which is a kind of "Linkage disequilibrium". The casein cluster is then considered as a unit of inheritance called haplotype. This notion of haplotype has been a useful tool to improve the estimation of the association of casein variants with milk production traits in cattle (Martin et al., 2013). On the other hand, the major whey proteins genes, *MBLG* and *LALBA* are mapped on chromosomes 11 (Hayes and

Petit, 1993) and 5 (Hayes et al., 1993) respectively (Figure 2.3B). The genes included in the present study are described in the sections below.

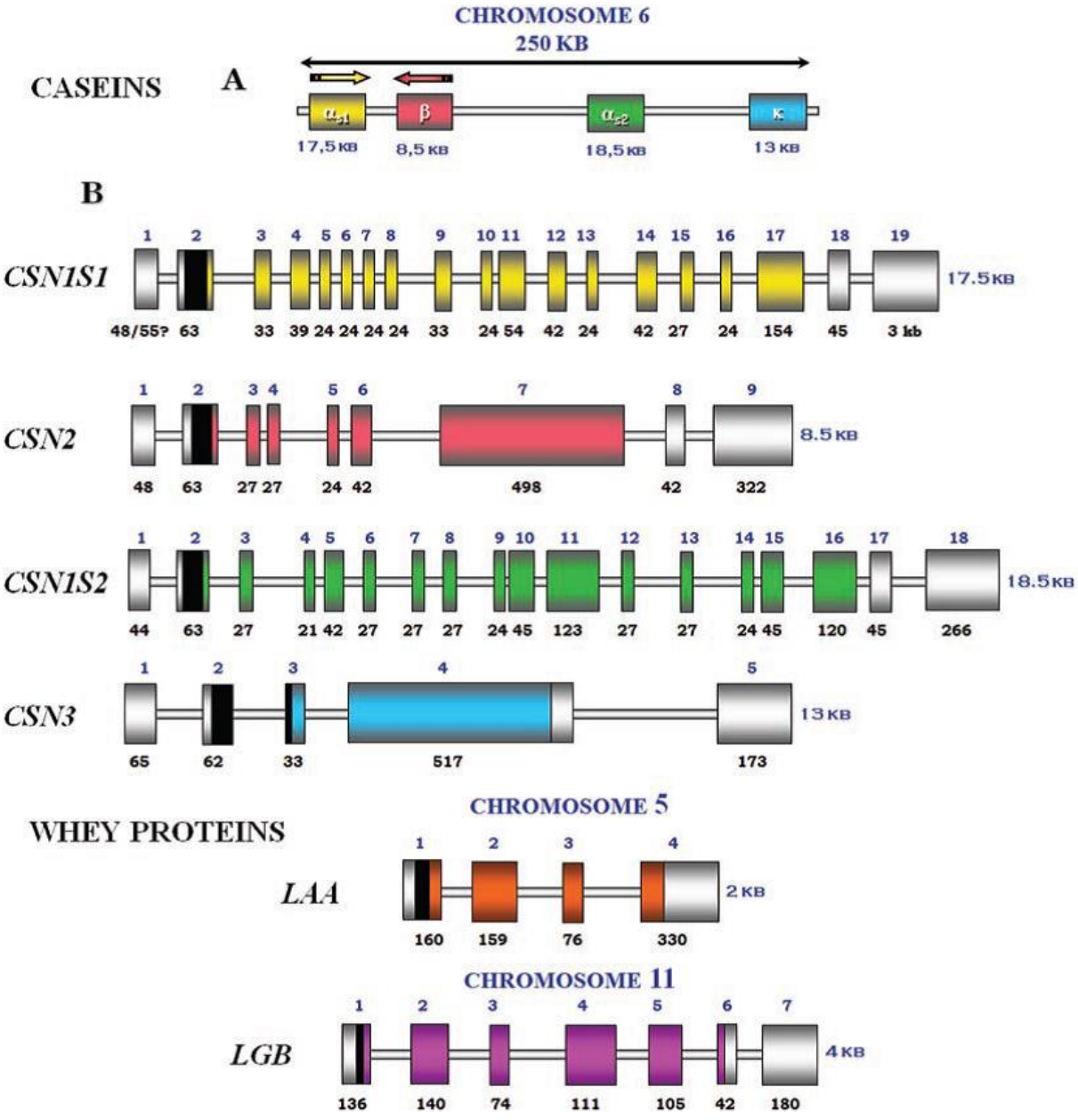


Figure 2.3: Structural organization of the transcription units encoding the 6 main milk proteins. Caseins: αs1-CN (*CSN1S1*), β-CN (*CSN2*), αs2-CN (*CSN1S2*), and κ-CN (*CSN3*). Whey proteins: α-LA (*LAA*) and β-LG (*LGB*).
A) Genomic organization of the bovine casein locus. B) Structural organization of the 6 milk protein transcription units. Open bars represent introns; exons are depicted by large, gray (5′ and 3′ untranslated regions), black (part of exon encoding the signal peptide), and colored

(exons and part of exons encoding matured proteins) boxes. Size of exons is given, in base pairs, under each exon with its number indicated on the top (Caroli et al., 2009, modified from Martin et al., 2002).

2.3.1. Bovine α_{S1}-Casein (*CSN1S1*)

The bovine αS1-Casein protein is coded by *CSN1S1* gene (17.5 KB) located on chromosome 6 (Hayes et al., 1993). The mature protein is a 199-residue polypeptide chain with ten phosphorylation sites (9 Serine and 1 Threonine) (Martin et al., 2013). It was first sequenced at the protein level by Mercier et al. (1971) and Grosclaude et al. (1973) and later at cDNA level by Nagao et al. (1984) and Stewart et al. (1984). Nine different alleles have been characterized to date for αS1-Casein protein (A, B, C, D, E, F, G, H and I). The particularity of each variant is described in Table 2.1 The bovine αS1-Casein protein variants differ mainly by single amino acid substitutions. However, the A variant is a deleted form lacking sequences (14-26) encoded by exon 4 (Grosclaude et al., 1972). Likewise, the H variant is characterized by the absence of the eight amino acids residues (51-58) encoded by exon 8 (Mahé et al., 1999). This variant H of αS1-Casein has been first found in African *Bos taurus* (Kuri) from Lake Chad basin (Mahé et al., 1999). Moreover, G variant differs from B variant by low αS1-Casein content in milk although they share the same primary structure (Rando et al., 1998). The variant F is characterized by the substitution of phosphorylated Serine to a Leucine at position 66 and loss of potential phosphorylation site at position 64 (Prinzenberg et al., 1998). The αS1-Casein I (CSN1S1*I) variant is characterized by a nucleotide substitution (A>T) in exon 11 of the coding gene which leads to amino acid exchange from Glu to Asp at position 84 in the mature protein (Lühken et al., 2009). The authors postulated that the CSN1S1*I variant originated within *Bos indicus* and subsequently spread to *Bos Taurus.* The Asp84 variant frequency was higher in Banyo Gudali from Cameroon (0.10) and scarce in European breeds (Lühken et al., 2009). In addition, polymorphism has been also reported at promoter region in α_{S1}-Casein (Koczan et al., 1993; Prinzenberg et al., 2003; Ibeagha-Awemu

et al., 2005). Thus, a novel CSN1S1 5' promoter allele (CSN1S1Prom^5) which is zebu specific has been characterized in *Bos indicus* cattle breeds from Cameroon and Nigeria and appeared to be the most variable of all described *CSN1S1* promoter alleles (Ibeagha-Awemu et al., 2005).

2.3.2. Bovine κ-Casein (*CSN3*)

Bovine κ-Casein is coded by CSN3 gene (13KB). Stewart et al. (1984) predicted the primary structure of the protein and its signal peptide by cDNA sequencing. The mature protein is a polypeptide of 169 amino acids with two cysteine and six potential phosphorylation sites (Four threonine and two serine). Fifteen genetic variants have been described to date (A, B, B2, C, D, E, F1, F2, G1, G2, H, I, J, K and L) and are presented in Table 2.2 (Martin et al., 2013). CSN3*J was described first in African taurine Baoulé, and characterized by one amino acid substitution Ser155Arg (Mahé et al., 1999). The authors postulated that it derived from the B variant.

Compared to the review by Caroli et al. (2009), B2 and D, K and L variants were missing. However, the two additional protein variants K (GeneBank accession number AF194989) and L are both detected in Exon 4 sequencing of domesticated Yak samples. Compared to the G2 variant (Asp148Ala), the K variants has two additional amino acids substitution (Pro36Leu and Pro130Arg), whereas the L variant in addition to Asp148Ala mutation carries an insertion of four AA leading to the duplication of amino acids sequence (148-151) present in bovine CSN3*B. Thus, the mature protein of the L variant contains 173 amino acids (Prinzenberg et al., 2008).

Table 2.1: Changes in bovine α_{S1}-casein variants/alleles (Martin et al., 2013)

	Position										
Variant/allele	14-26	53	51-58	59	64	66	84	192	GenBank accession	SwissProt accession	References
A	DEL										Grosclaude et al. (1972)
B		**Ala**		**Gln**	**SerP**	**SerP**	**Glu**	**Glu**	**X59856**	**P02662**	Mercier et al. (1971) ;Grosclaude et al. (1973)
C											Grosclaude et al. (1972)
D		ThrP						Gly			Grosclaude et al. (1972)
E				Lys				Gly			Grosclaude et al. (1972)
F					Ser	Leu					Prinzenberg et al. (1998)
G											Rando et al. (1998)
H			DEL								Mahé et al. (1999)
I							Asp	Gly	U862370/371 (Bos indicus) EU908730 (Bos taurus)		Lühken et al. (2009), Balteanu et al. (2008, 2010)

Variants are presented in different rows; amino acids in the *reference variant* are in boldface; amino acid modifications are given in the relevant column
DEL: internal deletion of the corresponding sequence in the mature protein

Table 2.2: Changes in Bovine k -casein variants/alleles (Martin et al., 2013)

	Position													
Variant/alle	10	36	97	104	130	135	136	148	148-151	153	155	GenBank accession	SwissProt	References
A	**Arg**	**Pro**	**Arg**	**Ser**	**Pro**	**Thr**	**Thr**	**Asp**	**_**	**Ile**	**Ser**	**AY380228**	**P02668**	Robitaille et al. (2005)
B							Ile	Ala				AY380229		Robitaille et al. (2005)
B2							Ile	Ala		Thr		M36641		Gorodetskiĭ and Kaledin (1987)
C			His				Ile	Ala						Miranda et al. (1993)
D			His									AJ619772	Q705V4	Caroli et al., (2009)
E											Gly	AF041482		Miranda et al. (1993)
F1								Val						Sulimova et al.(1992)
F2	His											AF123250		Prinzenberg et al. (1996)
G1			Cys			Ile						AF123251		Prinzenberg et al. (1996)
G2								Ala				AJ849456 AJ841941	Q5ZET1	Sulimova et al. (1996)
H							Ile					AF105260		Prinzenberg et al. (1999)
I					Ala							AF121023		Prinzenberg et al. (1999)
J							Ile	Ala			Arg			Mahé et al. (1999)
K		Leu		Arg				Ala				AH009225		
L								Ala	INS			AY095311		(Prinzenberg et al., 2008)

Variants are presented in different rows; amino acids in the *reference variant* are in boldface; amino acid modifications are given in the relevant column
INS: 4 amino acid long insertion, corresponding to a duplication of sequence 148–151 (Ala-Ser-Pro-Glu) of the bovine allele B although 147–150 (Glu-Ala-Ser-Pro) is also possible

2.3.3. Bovine Milk β-Lactoglobulin (*MBLG*)

Bovine β-Lactoglobulin is the major whey proteins in cow's milk and is coded by *MBLG* gene (4KB) mapped on chromosome 11 (Hayes and Petit, 1993). It has been sequenced first at cDNA level (Variant A) by (Alexander et al., 1989) and the mature protein contains 162-amino acid residues. Previous reviews (Farrell et al., 2004; Caroli et al., 2009) listed eleven variants of β-Lactoglobulin with the A and B variants the most frequent in cattle (Farrell et al., 2004). These A and B variants were discovered by Aschaffenburg and Drewry (1957). A recent review on genetic polymorphism of milk protein (Martin et al., 2013) listed the thirteen currently known variants of β-Lactoglobulin (A, B, B*, C, D, Dr, E, F, G, H, I, J and W) as described in Table 2.3. The A variant differs from the B variant (reference allele) by two amino acids substitution (Gly64Asp and Ala118Val). The B* and Dr variants were not included in previous reviews (Farrell et al., 2004; Caroli et al., 2009). Thus, B* and B variants share the same primary structure but the *MBLG*B** showed quantitative polymorphism effect resulting in 40% of the amount of transcripts and protein compared to MBLG*B (Kim et al., 1996) confirmed by Braunschweig and Leeb (2006). The Dr Variant is characterized by an amino acid substitution at position 28 (Aspartic to asparagine).

Recently, 50 polymorphisms was detected within the promoter regions, introns and coding sequence of bovine *MBLG* gene with 42 of them in complete linkage disequilibrium with the most frequent variants A and B (Ganai et al., 2009).

Table 2.3: Changes in Bovine milk β -Lactoglobulin variants/alleles (Martin et al., 2013)

Variant/alle	Position 28	45	50	56	59	64	70	78	108	118	126	129	158	GenBank accession	SwissProt	References
A						Asp				Val				X14712		Braunitzer et al. (1973)
B	**Asp**	**Glu**	**Pro**	**Ile**	**Gln**	**Gly**	**Lys**	**Ile**	**Glu**	**Ala**	**Pro**	**Asp**	**Glu**	**Z48305**	**P02754**	Braunitzer et al. (1973)
B*														DQ489319		Braunschweig and Leeb (2006)
C					His											As reported by Ng-Kwai-Hang and Grosclaude (2003)
D		Gln														As reported by Brignon and Ribadeau Dumas (1973)
Dr	Asn															As reported by Bell et al. (1981a)
E													Gly			As reported by Ng-Kwai-Hang and Grosclaude (2003)
F			Ser									Tyr	Gly			Bell et al. (1981a)
G								Met					Gly			Bell et al. (1981a)
H						Asp	Asn			Val						Conti et al.(1988) and Davoli et al. (1987)
I									Gly							Godovac-Zimmermann et al. (1996)
J											Leu					Godovac-Zimmermann et al. (1996)
W				Leu												(Godovac-Zimmermann et al. (1990)

Variants are presented in different rows; amino acids in the reference *variant* are in boldface; amino acid modifications are given in the relevant column

2.3.4. Bovine α-Lactalbumin (*LALBA*)

Bovine α-Lactalbumin is coded by *LALBA* gene (2KB) mapped on chromosome 5 (Hayes et al., 1993). The primary structure was determined chemically by Brew et al. (1970) and was confirmed later (Hurley and Schuler, 1987; Wang, et al., 1989). The mature protein is 123 amino acid residues displaying eight cysteines which form four intramolecular disulphide bonds. Four genetic variants have been described to date, A variant (Bhattacharya *et al*., 1963), B variant (Brew et al., 1970), C variant (Bell et.al, 1981b) and D variant (Visker et al., 2012). The description of each variant is presented in Table 2.4. The A variant is a rare allele in European cattle and the C variant has been reported in Bali Cattle (but not confirmed). The recent variant (D) is characterized by Single Nucleotide Polymorphism in exon 2 of *LALBA* gene leading to amino acid substitution(Gln65His) which is not expected to influence the protein function (Visker et al., 2012).

Table 2.4: Changes in Bovine α -Lactalbumin variants/alleles (Martin et al., 2013)

Variant/allele	Position			GenBank accession	SwissProt	References
	10	?	65			
A	Gln					(Bhattacharya et al. (1963)
B	Arg	Asp/Glu	Gln	M18780 J05147	P00711	Brew et al. (1970)
C		Asn/Gln[a]	His			Bell et.al. (1981b)
D			His			Visker et al. (2012)

Variants are presented in different rows; amino acids in the *reference variant* are in boldface; amino acid modi fi cations are given in the relevant column

a The authors suggested an Asp to Asn or Glu to Gln substitution in the sequence of the mature protein but could not establish more precisely its position

2.4. Polymorphisms in *DGAT1* and *SCD1* genes and association with milk production and fatty acid traits

Located on bovine chromosome 14 (Grisart et al., 2002), a lysine to alanine mutation in exon 8 (K232A) of *DGAT1* gene has been reported to be strongly associated with milk fatty acid composition in Holstein population (Schennink et al., 2007) and in Italian Brown cattle population (Conte et al., 2010). Moreover, five allele at the *DGAT1* promoter variable number of tandem repeat (VNTR) was described to have also major effect on milk fat content in the German Holstein population (Kühn et al., 2004). The *DGAT1* Lysine variant (DGAT1K) was associated with higher saturated fat, a larger content of C16:0 and a small fraction of unsaturated C18 and conjugated linoleic acid and C14:0 (Schennink et al., 2007). The *DGAT1* A allele was associated with higher C18, conjugated linoleic acid (CLA) and total unsaturation indices and with lower C10, C12, C14 and C16 indices in Netherlands Holstein cows (Schennink et al., 2008). Another study on *DGAT1* K232A polymorphism in Irish Holstein showed that one copy of the K allele led to 77kg decrease in milk production, 0.99 kg decrease in protein yield and 4.22 Kg increase in fat yield across first five lactations (Berry et al., 2010).

The Stearoyl-CoA Desaturase 1 (*SCD1*) gene is mapped on chromosome 26 in cattle and expressed in a variety of tissues including adipose and mammary tissues (Chung et al., 2000). Moreover, the *SCD1* enzyme contributes to the desaturation of saturated fatty acids into Δ^9 unsaturated fatty acids (Pereira et al., 2003). The bovine *SCD1* gene has two variants, A and V as a result of SNP in exon 5 (C878T) leading to Valine substitution by Alanine at amino acid position 293 in the mature protein (Ala293Val). The *SCD1* A allele was associated with higher cis-9 C18:1 and total monounsaturated content as well as C14:1/C14 ratio in Italian Holstein (Mele et al., 2007a).

In a study on *DGAT1* and *SCD1* genes polymorphisms in Dutch Holstein-Friesian heifers, both genes explained part of the genetic variation in unsaturation of milk fatty acids (Schennink et al., 2008). The authors concluded that the genetic information of both *SCD1* A293V and the *DGAT1* K232A polymorphism could be used in selective breeding to increase the milk fatty acids unsaturation indices (Schennink et al., 2008). Arnould and Soyeurt (2009), highlighted that the V variant of SCD1 increases the unsaturation of C16 and C18 while the A variant of *DGAT1* is associated with C18 unsaturation and it was concluded that both molecular and quantitative approaches should be used to help farmers to improve the nutritional quality the milk. However, data on the genetic variability of milk fatty acids composition and unsaturation indices are scarce in African indigenous cattle breeds and the few studies on *DGAT1* K232A polymorphism in African indigenous cattle did not investigate association with milk fatty acid composition (Kaupe et al., 2004; Rahmatalla et al., 2015; Houaga et al., 2017).

2.5. Association of major milk protein genes with milk production traits and fatty acid composition in cows

Using of milk protein polymorphisms as genetic markers for increasing milk production, improving milk production traits as well as altering milk composition remain a subject of interest to scientists all over the world. Unfortunately, African indigenous livestock are far from being intensively investigated compared to dairy breeds of European origin. Hence, there is lack of data on relationship between milk protein variants and milk production traits (milk yield, protein yield, fat yield, protein and fat percentages, somatic cell score and lactose content) as well as the fatty acids composition in African indigenous cows. However, many studies reported association of milk protein polymorphism with milk production traits using the haplotype concepts (Martin et al., 2013).

Thus, Nilsen et al. (2009) in their studies on Norwegian Red Cattle found that *CSN1S1-CSN2-CSN1S2* haplotype block was highly associated with protein and milk yields. Another investigation on the Dutch Holstein cows has demonstrated that milk genetic variants and haplotypes have important effect on protein composition of milk (Heck et al., 2009). The authors suggested that selection for both B allele at *MBLG* locus (*MBLG**B) and the *CSN2*A2-CSN3*B* haplotype will result in production of milk more suitable for cheese production. In addition, a study on Italian Holsteins demonstrated that *CSN2* and *CSN3* genotypes have been strongly associated with milk and protein yields, and milk coagulation traits (Comin et al., 2008). Moreover, several association studies have recently analyzed the effects of polymorphism of milk proteins genes on dairy performance in cattle (Zakizadeh et al., 2012; Grădinaru et al., 2013; Mir et al., 2014; Djedovic et al., 2015).

Djedovic et al. (2015) demonstrated that the κ -CN genotype has significantly influenced milk and milk fat yield in cattle breeds and crossbreds from Serbia. Another association study has shown that the AB genotype of kappa-casein is associated with higher milk production in Sahiwal cattle population of Pakistan and it was therefore suggested that incorporation of AB and BB genotypes for κ -CN may help to improve the milk yield in this cattle population (Mir et al., 2014). Moreover, Grădinaru et al. (2013) in their study in Romanian Spotted cattle, found that the A^1A^1 genotype of β-Casein was associated with higher milk yields, higher protein and fat contents than other genotypes whereas the A^2A^2 genotypes were associated with the highest protein percentages. Zakizadeh et al. (2012) in their association studies of polymorphism of beta-lactoglobulin coding and 5`-flanking regions, have shown that the CC genotype *MBLG* (g.435 C>G) had important effect on milk and fat yield in Holstein and on milk yield in Golpaygani breeds. In addition, the AG and GG genotypes (g.422G>A) were associated with milk yield in Holstein and native Golpaygani breed of Iran respectively.

Another interesting application of genetic polymorphisms of milk proteins genes is their relationship with bovine milk fatty acids composition. Therefore, milk proteins genes variants could serve as genetic marker to select sire to produce milk with beneficial fatty acids for humans. Thus, the association between the fatty acid composition and genetic polymorphism of milk proteins has been investigated in cattle (Bobe et al., 1999, 2004; Melia et al., 2009), in sheep (Mele et al., 2007b) and in goat (Chilliard et al., 2006; Valenti et al., 2009, 2010)

Bobe et al. (1999) examined first the relationship among individual proteins and fatty acids in Holstein cows using correlations and factor analysis. The same team later demonstrated the effect of the milk protein phenotypes on milk fatty acid composition in Holstein cows (Bobe et al., 2004). They found that K-Cn BB has presented the highest concentration of C12:0 and lowest concentration of C18:0 whereas κ -Cn AB has presented lowest concentration of C16:0. Likewise, the *MBLG* variant B was associated with higher concentration of C14:0, C16:0 and C16:1 and lower concentration of C18:0 and C18:1 fatty acids. The authors suggested that the B variant of κ –Casein and β-Lactoglobulin are associated with de novo fatty acids synthesis in the mammary gland. They concluded that the selection for the B variant of κ –Casein and β-Lactoglobulin could improve the texture and sensory properties of dairy products (Bobe et al., 2004). Five years later, Melia et al. (2009) confirmed the results of Bobe et al. (2004) in Reggiana cows and suggested the use of κ –Casein and β-Lactoglobulin variants as genetic markers to improve texture and sensory properties of dairy products (Melia et al., 2009).

2.6. Milk proteins markers for breed characterization, genetic diversity and phylogeny studies in cattle

Milk protein genetic variants as well as casein haplotypes have been intensively used in breed characterization, phylogeny and geographical diversity studies in cattle (Ng-Kwai-Hang and

Grosclaude, 2003). Thus, milk proteins markers have been used as important tools to assess the genetic diversity and relationship between West and Central African indigenous cattle populations (Ibeagha-Awemu et al., 2004; Ibeagha-Awemu and Erhardt, 2006). Moreover, casein loci have been used to investigate genetic diversity, origin and phylogeny of taurine cattle (Jann et al., 2004). A study of Caroli et al. (2010) on Original Pinzgauer cattle breed, showed that the *CSN1S1**C-*CSN2*A2 haplotype which is the most frequent in zebu (Mahé et al., 1999) also predominated in several European cattle breeds such as the Portuguese breeds and the Austrian breeds.

Arising from the previous data, milk protein polymorphisms are powerful tools for investigating of the genetic diversity, gene flow as well as relationship between indigenous cattle breeds.

CHAPTER THREE

MATERIALS AND METHODS

3.1. Sampling

3.1. 1. Description of study area

Borgou cattle were sampled from state owned farms (Samiondji Breeding Farm, Betecoucou Breeding Farm and Okpara Breeding Farm) and privately owned farms in Benin. It was critical that the government farms were included in the sampling because they are centers of conservation of the indigenous Borgou cattle breed. The White Fulani cattle were sampled from the privately owned farms in peri-urban area of Parakou District because there is no center of conservation of White Fulani cattle breed. Figure 3.1 shows locations where the sampling was conducted.

The Samiondji Breeding Farm (SBF) is located in the Department of Zou, District of Zangnanado (Benin). SBF has an area of 3,600 hectares and lies between latitudes 7/25°N and 7/30°N and longitudes 2/22°E and 2/25°E. The climate is intermediate between the subequatorial maritime and the Sudano-Guinean climate characterized by 4 seasons: a long dry season (November to March), a long rainy season (March to July), a short dry season (July to August) and a short rainy season (August to November). The average rainfall varies between 900 and 1100 mm per year. The average annual temperature is around 29 ° C. The types of fodders found are *Brachiaria ruziziensis*, *Panicum C1*, *Moringa Oleifera*, *Andropogon gayanus* and *stylosanthes guineensis*.

The Betecoucou Breeding Farm (BBF) is located in the Department of Collines and the District of Dassa (Benin). The BBF has an area of 11,127 hectares and lies between latitudes 7/45°N and 7/50°N and longitudes 2/20°E and 2/27°E. The climate is Sudano-Guinean with one rainy season from April to October and one dry season from November to March. The

average rainfall is 1100 mm per year and the temperature between 24 and 25°C. The main fodders found are *Brachiaria ruziziensis, Panicum C1 and Panicum maximum.*

The Okpara Breeding Farm (OBF) is located in the Department of Borgou, District of Tchaourou. The farm has an area of 10,000 hectares and lies between latitudes 9/6°N and 9/21°N and longitudes 2/39°E and 2/53°E. The climate is Sudanian with one rainy season from May to October and one dry season from November to April. The average rainfall is 1125 mm per year and the temperature between 26 and 27°C. The different cattle breeds found are Borgou, Azawak, N'Dama, Girolando and Girolando X Borgou and Gir X Borgou crossbreds. The main fodders found are *Brachiaria ruziziensis, Panicum maximum, Andropogon gayanus, Leucena leucocephala* and *stylosanthes sp*.

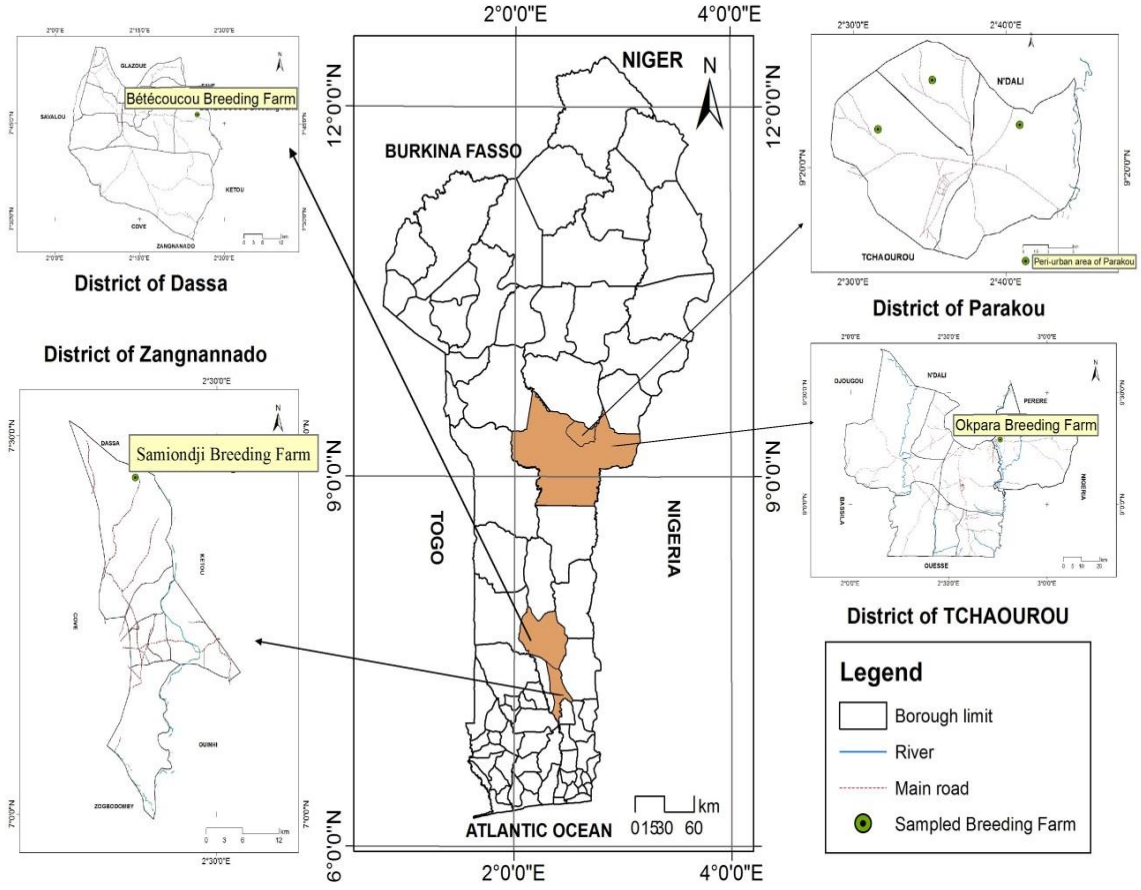


Figure 3.1: Map of Benin indicating the sampling sites

3.1.2. Breeding and production system

The production system in Benin is mostly traditional without clear breeding objective. The feeding system adopted in all the farms where samples were obtained was natural grazing without concentrate supplementation. The cows were milked manually once a day in the morning. In the private farms, there was no veterinary treatment and the cows were vaccinated only during national vaccination campaign. However, at the breeding farms of Samiondji, Betecoucou and Okpara, the cows were often treated against internal and external parasites. The sanitary prophylaxis in use at the government farms respects hygienic rules and consists of daily cleaning of the feed and water containers as well as sweeping of the stabling parks and cowsheds. The prevention against trypanosomiasis was done every two to three months. The vaccination against pasteurellosis and contagious bovine pleuropneumonia were done in rainy season. The management system practiced by White Fulani herdsmen is mainly traditional and pastoral. The system is characterized by pool management where different breeds are kept together or in the same area with movement from place to place in search of forage and water.

3.1. 3. Blood and milk sampling

A total of 98 Borgou and 100 White Fulani indigenous cows in lactation were sampled in this study between May-July 2016 during the rainy season. The sampling was done during rainy season because the White Fulani herdsmen are pastoralists and herd their animals in the forest during dry season. Blood and milk samples were randomly obtained from three to ten cows in lactation per herd from a total of 12 herds of Borgou and 9 herds of White Fulani. Blood samples were collected from the jugular vein into 10 ml EDTA vacutainer tubes, labelled and immediately transported to the laboratory in a cool box containing ice and stored at -20°C until further analysis. Milk samples were aseptically collected into 50ml falcon tubes each

containing one tablet of Bonopol milk preservative (Systems Plus, Canada), labelled and sent to Valacta laboratories (Valacta Laboratories Inc., Canada, www.valacta.com) for the analysis of milk components. The sampling-day milk yield was recorded for each cow using a 20 kg weighing scale. The sample collection procedures were approved by the Ethical Sub-committee of Laboratory of Animal Biotechnology and Meat Technology, reference 01-16/LBATV/DPSA/EPAC prior to the commencement of study (Appendix 1).

3.1.4. Data obtained from the farmers

Additional meta data on sampled cows obtained from the livestock keepers and herders following a written consent permitting sampling included: age, lactation stage (days in milking) and parity number. The stage of lactation or days in milking (DIM) was separated in three intervals as DIM <100 days (early lactation), DIM from 100 to 200 days (mid lactation) and DIM >200 days (late lactation) (Kgwatalala et al., 2009). The questionnaire used to collect the additional meta data is presented in Appendix 2.

3.2. DNA extraction, quantity and quality check

Genomic DNA was isolated from blood samples using standard phenol-chloroform method (Sambrook and Russell, 2001). The DNA extraction was carried out at the Molecular Genetics and Genome Analysis laboratory of Abomey-Calavi University (Benin). Briefly, the frozen 10ml blood sample was thawed at room temperature. The tube was inverted gently to homogenize the contents and 1ml of blood was transferred into 2ml Eppendorf tube. One ml of $T_{10}E_1$ washing buffer (10ml 1M tris HCL (pH= 8.5), 2ml 0.5M EDTA, 988 ml dH_2O) was added. The tube was vortexed then centrifuged at 7235×g for 10 min at 4°C and the supernatant discarded. The white blood cells were washed a second time. One ml of SDS (Sodium Dodecyl Sulphate) lysis buffer (193g urea, 7 g NaCl, 8g SDS, $T_{10}E_1$ added to a 400

ml final volume) was added. The tube was then incubated at 37°C overnight. One ml of phenol chloroform isoamylic alcohol (25 volume phenol, 24 volume chloroform and 1 volume isoamylic alcohol) was added. The tube was mixed thoroughly by inverting the tube for 15 min and centrifuged at 7235×g for 15 min. The upper layer containing the DNA was transferred into a new clean 2ml Eppendorf tube. Two volumes of cold 96% ethanol was added and the tube mixed by inverting several times for precipitation. The tube was centrifuged at 7235×g for 10mn at 4°C and the supernatant discarded. The DNA pellet was washed twice with 500µl of 70% Ethanol. The DNA pellet was then air dried and 75µl of T_{10} E_1 buffer added and incubated at 65°C for 30mn. The stock DNA was stored at ¯20°C. The DNA quality was checked on 0.8% agarose gel and the quantity was determined using the NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies Inc., USA).

3.3. Genotyping

3.3. 1. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) of *DGAT1* K232A, *SCD1* A293V and *LALBA* (A/B) variants

The *DGAT1* K232A and *SCD1* A293V genotypes were determined by polymerase chain reaction–restriction fragment length polymorphisms (PCR-RFLP) (Kaupe et al., 2004; Kgwatalala et al., 2009).

The primers 5'-GCACCATCCTCTTCCTCAAG-3'(forward) and 5'-GGAAGCGCTTTCGGATG-3' (reverse) (Kaupe et al., 2004) were used to amplify a 411bp fragment of bovine DGAT1 containing the lysine/alanine substitution (exon 8) while 5′-CCCATTCGCTCTTGTTCTGT-3 (forward) and 5′-CGTGGTCTTGCTGTGGACT-3' (reverse) (Kgwatalala et al., 2009) were used to amplify a 400 bp fragment containing the A293V polymorphism in exon 5 of the *SCD1* gene. The PCR reactions were carried out in a 30µL volume containing 45ng of template DNA, 15 µL of PCR Master Mix (Bioneer, Korea)

and 4.5 pmol of each primer (3pmol/µL). The PCR conditions were as follows: an initial denaturation step at 94°C for 3 min, 35 cycles of 94°C for 45s, 62°C (*DGAT1* K232A) or 64°C (*SCD1* A293V) for 60s, 72°C for 60 s, and a final extension step of 72°C for 5 min. All the PCR reactions in this study were performed in the GeneAmp PCR System 9700 (Applied Biosystems, USA). Amplification was confirmed by running the PCR products on 1.8% agarose gel and visualized with GelDoc-It^2 Imager (Ultra-Violet Products Ltd., UK). The PCR products were purified with the QIA quick PCR Purification Kit (Qiagen, Germany).

Five µL of purified *DGAT1* PCR products were digested at 37°C overnight with 10 U of *EaeI* restriction enzyme (New England Biolabs, Inc., USA). The digested PCR products were separated on 1.8% agarose gel stained with GelRed^{TM} (Biotium, UK) resulting in two fragments of 203 and 208bp (seen as a single band on gel) for AA genotype, two fragments of 203/208bp and 411bp for KA genotype and the undigested 411bp for KK genotype.

Similarly, 5µL of purified *SCD1* PCR products were digested overnight at 37°C with 10 U of *Nco*I restriction enzyme (New England Biolabs, Inc., USA). The digested products were separated on 1.8% agarose gel stained with GelRed^{TM} (Biotium, UK), visualized and scanned with GelDoc-It^2 Imager (Ultra-Violet Products Ltd., UK). The digestion patterns resulted in two fragments of 200-bp for the AA genotype, undigested 400-bp fragment for the VV genotype and 400-bp and 200-bp fragments for the AV genotypes.

The *LALBA* A/B polymorphisms were determined by PCR-RFLP. The primer sequences 5'-TTGGTTTTACTGGCCTCTCTTGTCATC-3' (forward) and 5'-TGAATTATGGGACAAAGCAAAATAGCAG-3'' (reverse) (Mitra et al., 1998) were used to amplify the 309 bp fragment in exon 1 of *LALBA* gene. The same PCR conditions described above for *SCD1* A293V were used for *LALBA*. Five µL of purified PCR products (309 bp) containing the *LALBA* A/B variants was digested overnight at 37°C with 10 U *MspI* restriction enzyme (New England Biolabs Inc., USA). The digested products were separated

on 1.8% agarose gel stained with GelRed™ (Biotium, UK), visualized and scanned with GelDoc-It^2 Imager (Ultra-Violet Products Ltd., UK). The digestion patterns resulted in two fragments of 220 and 89 bp for BB genotype, uncut 309bp for AA genotype, 309, 220 and 89bp for AB genotype (Mitra et al., 1998).

3.3. 2. *CSN1S1*, *MBLG* and *CSN3* sequencing

The single nucleotide polymorphisms in *CSN1S1*, *MBLG* and *CSN3* genes were assessed by PCR followed by Sanger Sequencing. Primers 5´- TGCATGTTCTCATAATAACC- 3´ (forward) and primer 5´- GAAGAAGCAGCAAGCTGG - 3 (reverse) (Koczan et al., 1993) were used to amplify the polymorphic region (310bp) located between 5'end and exon 1 of *CSN1S1* gene. The primers 5´-TGTGCTGGACACCGACTACAAAAAG-3´ (forward) and 5´- GCTCCCGGTATATGACCACCCTCT-3' (reverse) (Medrano and Aguilar-Cordova, 1990) were used to amplify the partial exon 4 and intron 4 of *MBLG* gene. The primers 5'-CCAACTACCATGGCACGTCA-3' (forward) and 5'-AGCCCATTTCGCCTTCTCTG-3' (reverse) were designed to amplify partial exon 4 and flanking intronic sequences (386 bp) of *CSN3* gene based on the reference sequence (GeneBank Accession N°AY380228). The NCBI Primer Blast tool was used for that purpose (**https://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi?LINK_LOC=BlastHome**). The PCR conditions were as described above except for the annealing temperature of 58°C (*CSN1S1*), 64°C (*MBLG*) and 62°C (*CSN3*).

The PCR products of *CSN1S1*, *MBLG* and *CSN3* were purified with the QIA quick PCR Purification Kit (Qiagen, Germany). 30µl (at least 25ng/µl) of the purified PCR products were sent for sequencing at Bioneer company (Bioneer, KOREA) using Sanger method (ABI machine, USA). The same forward and reverse primers used for the PCR amplification were used for the sequencing.

3.4. Nutritional analysis

3.4. 1. Milk component analysis

Test-day milk fat, protein, milk urea nitrogen, lactose and total solids contents were determined in milk samples with MilkoScan FT 6000 Series mid-range infrared Fourier transform infrared-based spectrometers (Foss, Hillerod, Denmark) by Valacta Laboratories. The test-day protein and fat yields were determined by multiplying the test-day protein and fat percentages respectively with the test-day milk yield.

3.4. 2. Fatty acids analysis and quantification

3.4. 2.1. Sample preparation (Saponification and methylation)

The milk samples were prepared for analysis using alkali hydrolysis followed by methylation as described in AOAC Method 991.39 (Latimer, 2012). Briefly, 1 ml of cow milk was transferred into a 15ml screwcap centrifuge tube in triplicate and 3 ml of 0.5N NaOH in methanol was added into each tube and mixed thoroughly by shaking for 30sec. Tubes were incubated in a water bath at 85°C for 10 min and cooled at ambient temperature. In a fume hood, 1 ml of boron trifluoride (BF_3) was added, mixed thoroughly by shaking and incubated in a water bath at 85°C for 10 minutes. Samples were allowed to cool to room temperature followed by addition of 3 ml iso-octane and 3 ml of saturated NaCl solution, mixed by shaking vigorously and centrifugation at 452 ×g for 5 minutes. The upper layer (Iso-octane) containing the fatty acid methyl esters (FAMEs) was transferred through 1g of sodium sulfate anhydrous Na_2SO_4 (placed on top of some cotton wool in a filter liner) into a test tube with a Pasteur pipette. 2g of anhydrous Na_2SO_4 was added. The FAMEs were diluted to a volume of 2 ml with hexane in a volumetric flask. 1ml of the sample was transferred into a screw cap gas chromatography (GC) sample vial.

3.4. 2.2. Chromatographic Analysis

The composition of individual FAMEs was analyzed by gas chromatography mass spectrometry (GC-MS). The fatty acids methyl esters in hexane (1µl) were injected into a 7890A GC system (Agilent Technologies, USA) coupled to a 240 ion trap mass spectrometer detector (Agilent Technologies) using the Agilent 7693A automatic liquid sampler at a split ratio of 100:1. A VF5-MS (5% phenyl methylpolysiloxane), 30 m x 0.25 mm id, 0.25 µm film capillary column was used with the injector port set at 280°C. Helium was used as carrier gas at a flow rate of 1ml/min. The oven temperature was programed to rise from 50°C to 180°C at 4°C/min followed by an increase to 250°C at 3°C/min. The ion trap mass spectrometer parameters were as follow: scan range 50-540 (m/z), ionization mode EI, filament delay time 3 mins and transfer line temperature, manifold temperature and trap temperature of 250°C, 100°C and 150°C respectively.

3.4. 2.3. Calculation of Kovats Linear retention indices

The individual fatty acid methyl ester Kovats Linear retention Indices was calculated using the formulae below for temperature programed chromatography (Strehmel et al., 2008).

$$\mathrm{I} = 100\ X\ (n + \frac{(t_{r(unknown)} - t_{r(n)}}{t_{r(N)} - t_{r(n)}})$$

Where I= Kovats retention index

n= the number of carbon atoms in smaller n-alkane.

N= the number of carbon atoms in the larger n alkane.

t_r= Retention time (min)

3.4. 2.4. Identification of FAME and expression of results

Fatty acid present in milk samples were identified as their corresponding Fatty acid methyl esters. Chromatograms and spectra representing individual FAMES were analyzed using the automated mass spectral deconvolution and identification system software (AMDIS, US). A chromatogram of the FAMEs is shown in Appendix 3. The identification of the individual FAMES was performed by comparing each of the mass spectra with the database of NIST 11(Gaithersburg, MD, USA) and Wiley 7N (John Wiley, NY, USA) and also by comparing the calculated Kovats linear retention indices using retention times of n-alkane series against the values obtained in the NIST webbook for the same capillary column stationery phase (Table 3.1) (Strehmel et al., 2008). The quantification of individual FAMEs was performed by the peak area percentage method. The fatty acid concentrations were expressed as the ratio of each individual fatty acid to the total of all fatty acids detected in the sample. The fatty acids unsaturation indices were calculated as the ratio of *cis-9* unsaturated to *cis-9* unsaturated + saturated for specific fatty acids pairs and multiplied by 100 (Kelsey et al., 2003). We calculated the following indices: C14 index= C14:1 *cis*-9/ (C14:1 *cis*-9+ C14:0) × 100 and C18 index= C18:1 *cis*-9/ (C18:1 *cis*-9+ C18:0) × 100. The total index was calculated according to Mele et al. (2007a) as total index= (C14:1 *cis*-9+ C18:1 *cis*-9)/ (C14:1 *cis*-9+ C14:0+ C18:1 *cis*-9+ C18:0) × 100.

Table 3.1: Identification of FAMES based on the Linear Retention indices

Retention time (min)	Name Fatty acid methyl ester	Corresponding fatty acid	Calculated LRI	theoretical - dbase	column
6.358	Methyl hexanoate	Caproic acid (C6:0)	923	934	db5-ms
12.72	Methyl Octanoate	Caprylic methyl ester (C8:0)	1123	1126	db5-ms
19.554	methyl caprate	capric acid (C10 :0)	1324	1326	db5-ms
25.872	Methyl laurate	lauric acid (C12 :0)	1523	1525	HP-5MS
30.582	Methyl myristoleate	Myristoleic acid (C14:1 *cis*-9)	1686	1691	db5-ms
31.288	Tridecanoate	Tridecanoic acid (C13:0)	1712	1710	db5-ms
31.603	Methyl myristate	Myristic acid (C14 :0)	1724	1726	db5-ms
34.305	Methyl pentadecanoate	pentadecanoic acid (C15 :0)	1823	1826	db5-ms
37.089	Methyl palmitate	Palmitic acid (C16 :0)	1925	1926	db5-ms
39.882	Methyl heptadecanote	heptadecanoic acid (C17 :0)	2025	2028	db5-ms
41.721	Methyl linoleate	Linoleic acid	2092	2092	db5-ms
41.931	Methyl oleate	oleic acid (C18 :1)	2100	2103	db5-ms
42.705	Methyl stearate	Stearic acid (C18 :0)	2128	2128	db5-ms
45.457	methyl nonadecanoate	Nonadecanoic acid (C19:0)	2227	NA	NA
48.228	Methyl eicosanoate	Arachidic acid (C20:0)	2329	NA	NA

NA: Not applied, LRI=Linear retention indices.

3.5. Statistical Analysis

3.5. 1. Genetic polymorphisms in *LALBA, CSN1S1, CSN3, MBLG, DGAT1* and *SCD1* genes

The *CSN1S1, MBLG, CSN3* sequencing data were analyzed using the CLC Main Workbench software version 7.8.1 (Qiagen Bioinformatics, Germany). Briefly, the sequencing reads were trimmed to eliminate reads of poor quality. Thereafter, the forward and reverse sequences were assembled to get a contiguous sequence (contig). The conflicts arisen from the assembling were found and edited manually. After editing, the consensus sequences were extracted for further analysis. The consensus sequences were aligned with the references

sequences available in NCBI Genbank: X59856, Z48305 and AY380228 for *CSN1S1*, *MBLG* and *CSN3* respectively. The positions of the SNPs were found in comparison to the reference sequences. The protein translation analysis was done to find the synonymous and nonsynonymous mutations using the CLC Main Workbench software version 7.8.1 (Qiagen Bioinformatics, Germany).

The haplotypes and linkage disequilibrium analysis was done using the PHASE algorithm of DNA sequence polymorphism (DnaSP) software version 5.10.01 (Rozas, 2009).

GENEPOP Program was used to estimate allele frequencies and test for Hardy-Weinberg Equilibrium (HWE) at all the studied loci (Raymond and Rousset, 2001).

3.5. 2. Associations of genetic polymorphisms with milk production and fatty acid traits

To investigate the effect of breed, age, stage of lactation, parity number, geographical region and the genetic polymorphisms on milk production and fatty acids traits, the dataset was analyzed with IBM SPSS version 20 software package. The following linear model was used:

$Y_{ijklmnop} = \mu + H_i + A_j + B_k + DIM_l + P_m + G_n + GRo + e_{ijklmnop}$ in which $Y_{ijklmnop}$ is the dependent variable i.e. value of each trait (Protein%, lactose%, fat%, milk urea nitrogen (mg/dl), test-day milk yield (Kg), test-day protein yield (g), test-day fat yield (g), individual fatty acid%, groups of fatty acids (saturated fatty acids [SFA], monounsaturated fatty acids [MUFA], polyunsaturated fatty acids [PUFA]) and unsaturation indices (C14 index, C18 index and total index)), μ is the population mean, Hi is the fixed effect of Herd, A_j is the fixed effect of age, B_k is the fixed effect of breed, DIM_l is the fixed effect of days in lactation, P_m is the fixed effect of Parity number, G_n is the fixed effect corresponding to genotype at *CSN1S1*, *CSN3*, *MBLG*, *DGAT1* and *SCD1* loci. The *LALBA* genotypes were included in the initial model and dropped out when no significant effect was found. GRo is the fixed effect

corresponding to the geographical region of the animal and $e_{ijklmnop}$ is the random residual term. The phenotypes were nested within breed to obtain breed specific estimates.

The allele substitution effect of *DGAT1* (K variant) and *SCD1* (V variant) was estimated following the method of Marchitelli et al. (2013) by regressing the number of copies of *DGAT1* K allele and *SCD1* V allele against each of the milk fatty acid and milk component traits separately. The results of the different effects are presented as least squares means ± standard error. The Pearson correlation indices were calculated among the various milk components and fatty acids traits using the IBM SPSS version 20 software package. The means were compared using paired t-test and differences between means were declared significant at $P < 0.05$. The principal component analysis (PCA) of the significant milk traits between breeds was done using the FactoMineR package (Husson et al., 2016) of R software version 3.4.3 (R Core Team, 2017).

3.5. 3. Genetic diversity, gene flow and phylogenetic relationship among breeds

The polymorphisms in all the studied genes were used in the genetic diversity analysis. The observed heterozygosity (H_{ob}) and expected unbiased heterozygosity or unbiased gene diversity, (H_{exp}) for each breed were determined using POPGEN Program (Yeh et al., 1999). H_{exp} was estimated using algorithm of Levene (Levene, 1949). The mean number of allele (MNA) occurred at the studied loci and the mean effective number of alleles (MNE) per population were assessed using the POPGEN program (Yeh et al., 1999). The significance differences between H_{exp}, MNA and MNE between the two breeds, and the H_{ob} and H_{exp} within each breed were tested using paired t-test with IBM SPSS version 20 software package (SPSS Inc., USA). The extent of gene flow between breeds was estimated with GENEPOP program (Raymond and Rousset, 1995, 2001). The within population inbreeding (*f*), total inbreeding (*F*); and population differentiation θ were estimated according to the variance-

based method (Weir and Cockerham, 1984). The phylogenetic tree of relationship between breeds was constructed based on the *CSN3* gene sequences (Prinzenberg et al., 2008) using the Neighbor-Joining method (Saitou and Nei , 1987) with MEGA7 software (Kumar et al., 2016). The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2004). *CSN3* sequences from Indian and Ethiopian indigenous cattle breeds were used in the phylogenetic analysis for comparison purposes.

CHAPTER FOUR

RESULTS

4.1. Polymorphisms in *LALBA, CSN1S1, CSN3, MBLG, DGAT1* and *SCD1* genes

4.1.1. Polymorphisms in *LALBA* gene

Three restrictions patterns were detected corresponding to AA, AB and BB genotypes. The AA genotype showed a single band of 309 bp, the AB genotype three bands of 309b, 220 and 89 bp, and the BB genotype showed bands of 220 and 89 bp (Figure 4.1). The genotypic frequencies of AA, AB and BB in White Fulani and Borgou breeds were 0.03, 0.52, 0.45 and 0.07, 0.87, 0.06 respectively. Frequencies of A and B alleles were 0.29 and 0.71, and 0.51 and 0.49 in White Fulani and Borgou breeds respectively (Table 4.1). The *LALBA* genotype frequencies were not in Hardy-Weinberg equilibrium in Borgou and White Fulani cattle breeds ($P<0.05$) (Table 4.1).

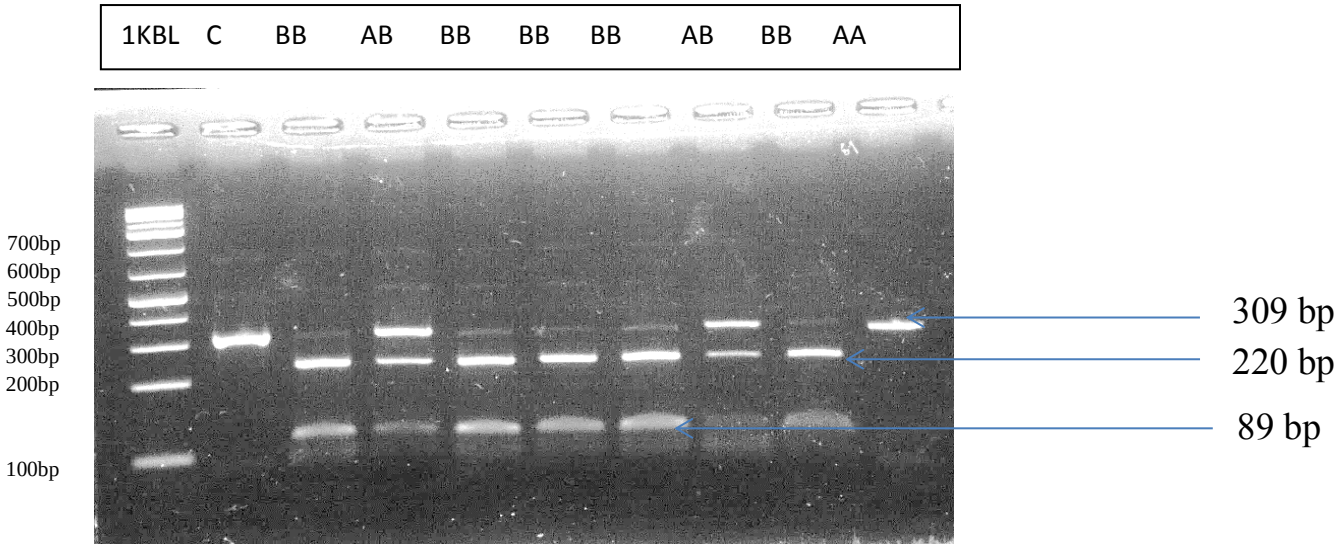


Figure 4.1: LALBA polymorphisms: agarose gel separation showing a 309 bp fragment representing AA genotype, 220 and 89 bp fragments representing BB genotype and three fragments of 309, 220 and 89 bp representing AB genotype**.** 1KBL: 100 bp DNA ladder; C: Control (undigested PCR product).

Table 4.1: Genotypes and allelic frequencies of *LALBA* polymorphisms in Borgou and White Fulani cattle breeds

Gene	Breed (Sample size)	Genotype frequencies			Allele frequencies		Heterozygosity		P-value[c] HWE
LALBA (A/B)		**AA**	**AB**	**BB**	**A**	**B**	H_{ob}[a]	H_{ex}[b]	
	Borgou (85)	0.07 (6)	0.87 (74)	0.06 (5)	0.51	0.49	0.87	0.50	0.000
	White Fulani (96)	0.03 (3)	0.52 (50)	0.45 (43)	0.29	0.71	0.52	0.41	0.010

[a]$H_{ob=}$ observed heterozygosity; [b]$H_{ex=}$ expected heterozygosity
[c] $P<0.05$, genotypes deviate from Hardy-Weinberg Equilibrium.

4.1.2. Polymorphisms in *DGAT1* and *SCD1* genes

The PCR-RFLP genotyping of *DGAT1* K232A polymorphisms showed three different genotypes on agarose gel namely KK (lysine homozygote, 411bp), AA (alanine homozygote, 203/208 fragment) and KA (lysine-alanine heterozygote, 411bp and 203/208bp fragments) (Figure 6). The frequencies of KK, KA and AA genotypes in Borgou and White Fulani breeds were 0.57, 0.37, 0.05 and 0.85, 0.14, 0.01 respectively (Table 4.2). Frequencies of K and A alleles were 0.77 and 0.23, and 0.92 and 0.08 in Borgou and White Fulani breeds respectively. The AA genotype was observed in four individuals of Borgou and in only one individual of White Fulani (Table 4.2).

Similarly the *SCD1* A293V showed three different genotypes VV, AV and AA on agarose gel The VV genotype corresponds to a single band of 400 bp, the AV genotype two bands of 400 and 200 bp and the AA genotype a single band of 200 bp (Figure 4.3). The frequencies of VV, AV and AA genotypes in Borgou and White Fulani were 0.72, 0.23, 0.05 and 0.89, 0.10, 0.01 respectively (Table 4.2). The SCD1 293V allele was the most frequent with frequencies of 0.83 and 0.94 in Borgou and White Fulani breeds respectively. The A allele was therefore the least frequent allele at *DGAT1* and *SCD1* loci in both breeds. The *DGAT1* K232A and the

SCD1 A293V genotypes frequencies did not deviate from the Hardy-Weinberg equilibrium in both breeds (P>0.05).

Comparison of the sequenced fragments of *DGAT1* K23A polymorphism with the reference sequence in GenBank (rs AJ318490) showed a rare nucleotide insertion mutation in two individuals of white Fulani and one individual of Borgou breed. The mutation is characterized by G nucleotide insertion in intron 8 at position 10515 of the *DGAT1* gene (Genebank accession N°: AJ318490.1) corresponding to the nucleotide position 271 in Figure 4.4**.** The Indel mutation seems to be in a linkage-disequilibrium with the lysine variant (KK genotype) in both breeds. The sequences were deposited in GenBank of NCBI under accession numbers MF445056 for Borgou, and MF445054 and MF445055 for White Fulani.

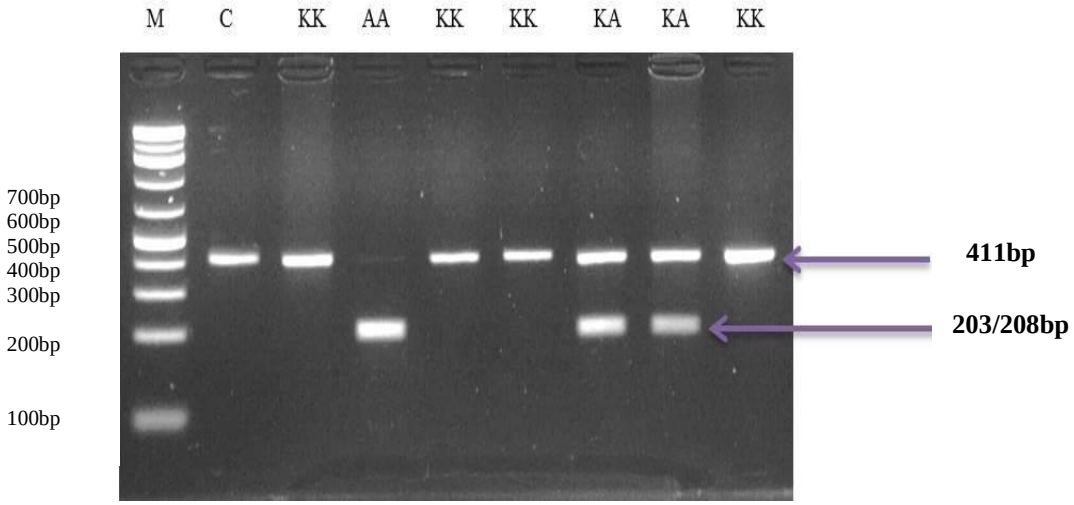


Figure 4.2: *DGAT1* K232A polymorphisms: agarose gel separation showing a 411 bp fragment representing KK genotype, 203/208 fragment representing AA genotype and 203/208 bp and 411 bp fragments representing KA genotype. M: 100 bp DNA ladder; C: Control (undigested PCR product).

Table 4.2: Allele and genotype frequencies for *SCD1* A293V and *DGAT1* K232A polymorphisms in Borgou and White Fulani cows

Gene	Breed	Genotype frequencies			Allele frequencies		Heterozygosity		P-value[c] HWE
SCD1 A293V		**VV**	**AV**	**AA**	**V**	**A**	H_{ob}[a]	H_{ex}[b]	
	Borgou (85)[1]	0.72 (61)	0.23 (20)	0.05 (4)	0.84	0.16	0.23	0.27	0.160
	White Fulani (96)	0.89 (85)	0.10 (10)	0.01 (1)	0.94	0.06	0.10	0.11	0.237
DGAT1 K232A		**KK**	**KA**	**AA**	**K**	**A**			
	Borgou (83)	0.58 (48)	0.37 (31)	0.05 (4)	0.77	0.23	0.37	0.35	0.763
	White Fulani (97)	0.85 (82)	0.14 (14)	0.01 (1)	0.92	0.08	0.14	0.15	0.603

[a]$H_{ob=}$ observed heterozygosity; [b]$H_{ex=}$ expected heterozygosity

[c] P>0.05, genotypes in Hardy-Weinberg Equilibrium. [1]Numbers in brackets correspond to sample size

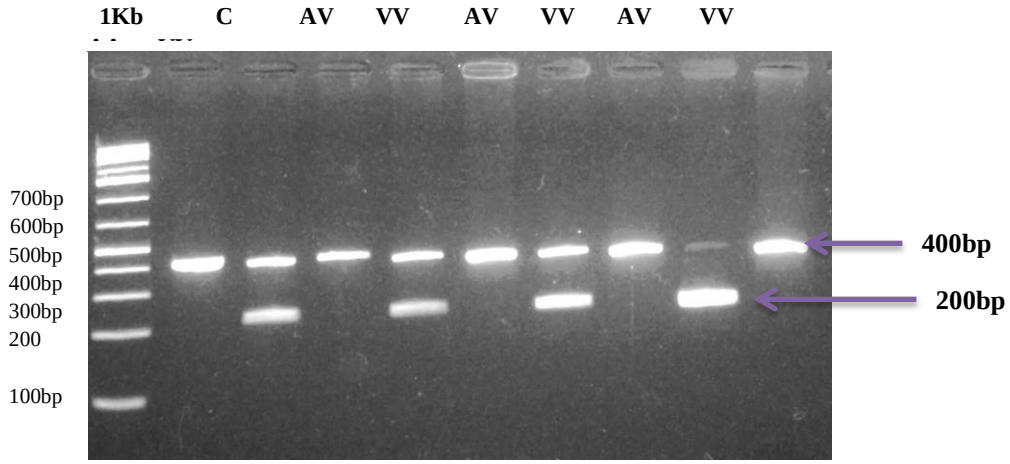


Figure 4.3*: SCD1* A293V polymorphisms: agarose gel separation showing a 200 bp fragment representing the AA genotype, undigested 400 bp fragment representing the VV genotype and 400 bp and 200 bp fragments representing the AV genotype.

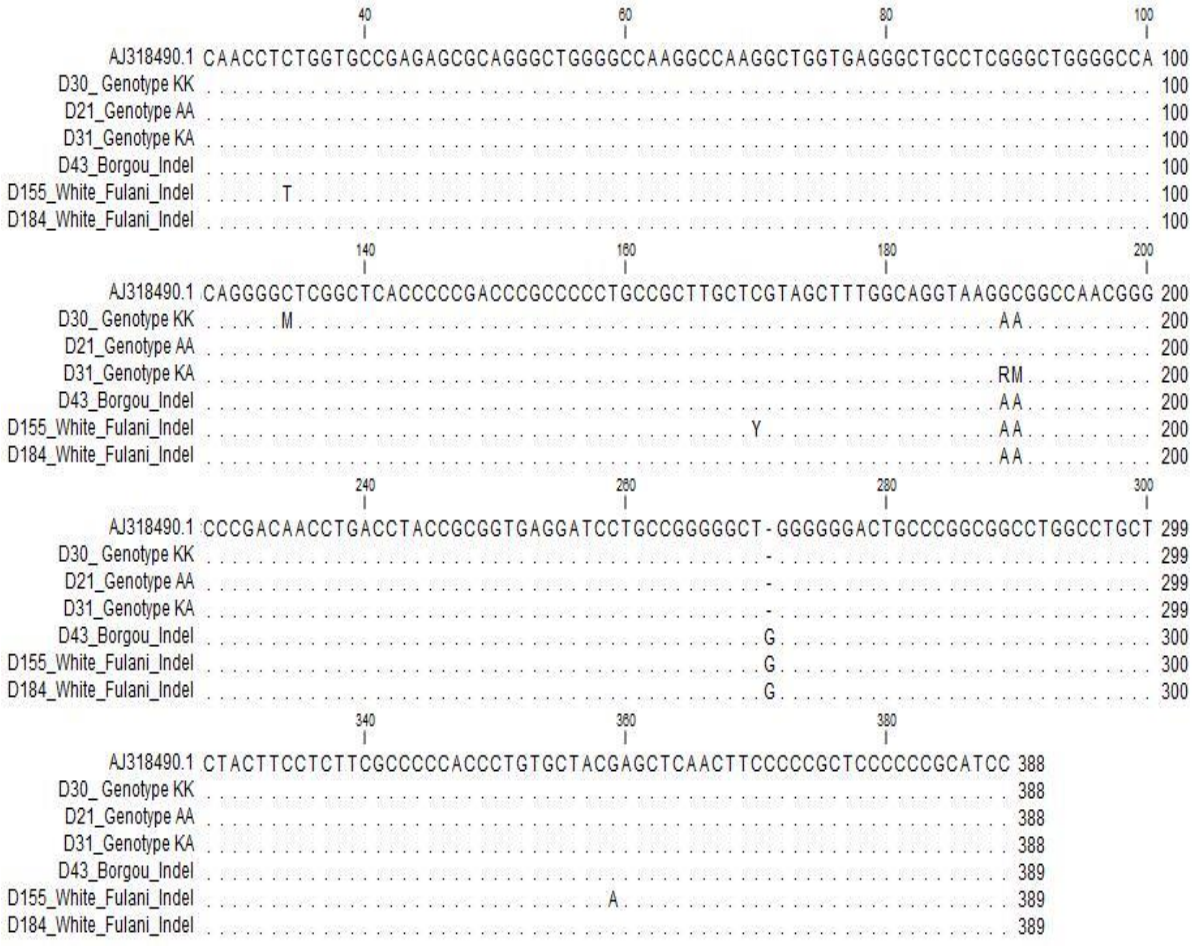


Figure 4.4: Sequence alignment of the AA genotypes, KK genotype, KA genotype and the Indel mutation (D184, D155 and D43) samples.
Matching nucleotides are replaced by a dot (.) and the substitutions are indicated by letter. The heterozygous genotype is indicated with RM (IUPAC nucleotide code). R for A or G, M for A or C and Y for C or T nucleotides.

4.1.3. Polymorphisms in *CSN1S1* gene

4.1.3.1. *CSN1S1* genotypes and alleles frequencies

Three different SNPs (g.10331 A>G, g.10359 T>C and g.10430 G>A) were discovered in *CSN1S1* 5' flanking region. The SNPs positions were based on the bovine *CSN1S1* gene reference sequence GenBank accession No.: X59856. No mutation was found in the partial *CSN1S1* exon 1 sequence. The SNPs genotypes and allelic frequencies are presented in Table 4.3.

At the *CSN1S1* g.10331 A>G locus, the AA, AG and GG genotypes were observed respectively in 76, 1 and 3 individuals of Borgou. The GG and AA genotypes were found respectively in 1 and 75 cows of White Fulani breed while the AG genotype was absent. The A allele was the most frequent with frequencies of 0.956 and 0.987 in Borgou and White Fulani respectively (Table 4.3).

At the *CSN1S1* g.10359 T>C locus, the CC, CT and TT genotypes were observed respectively in 12, 17 and 51 individuals of Borgou. The CC, CT and TT genotypes were found respectively in 18, 27 and 31 cows in White Fulani breed. The T allele was the most frequent with frequencies of 0.744 and 0.590 in Borgou and White Fulani respectively (Table 4.3).

At the *CSN1S1* g.10430 G>A, the AA, AG and GG genotypes were observed respectively in 18, 17 and 45 individuals of Borgou. The AA, AG and GG genotypes were found respectively in 20, 27 and 29 cows in White Fulani breed. The G allele was the most frequent with frequencies of 0.669 and 0.564 in Borgou and White Fulani respectively (Table 4.3).

All the genotypic frequencies at the *CSN1S1* loci were not in Hardy–Weinberg Equilibrium in Borgou and White Fulani populations ($P<0.05$).

Table 4.3: *CSN1S1* genotypes and allelic frequencies in Borgou and White Fulani cattle breeds

Polymorphisms *CSN1S1*	Borgou (BO)					White Fulani (WF)					P-value HWE[a]	
	Genotypic frequency			Allelic frequency		Genotypic frequency			Allelic frequency		BO	WF
g.10331 A>G (5'flanking region)	AA	AG	GG	A	G	AA	AG	GG	A	G	<0.001	<0.001
	76	1	3	0.956	0.044	75	0	1	0.987	0.013		
g.10359 T>C (5'flanking region)	CC	CT	TT	C	T	CC	CT	TT	C	T	<0.001	0.019
	12	17	51	0.256	0.744	18	27	31	0.410	0.590		
g.10430 G>A (5'flanking region)	AA	AG	GG	A	G	AA	AG	GG	A	G	<0.001	0.014
	18	17	45	0.331	0.669	20	27	29	0.436	0.564		

The SNPs positions were based on the bovine *CSN3* gene reference sequence GenBank accession No.: X59856. BO: Borgou, WF: White Fulani.

[a] P<0.05 population deviate from Hardy-Weinberg Equilibrium

4.1.3.2. *CSN1S1* haplotype blocks and distribution in Borgou and White Fulani

The three *CSN1S1* SNPs were in high linkage disequilibrium (D´>0.94) resulting in five different haplotypes (H1=ATG, H2=ACA, H3=ATA, H4=GTA and H5=ACG) (Table 4.4). Four haplotypes, H1, H2, H3 and H4 were found in Borgou and the H5 was found only in White Fulani cattle breed. The *CSN1S1*-H1 haplotype was the most frequent in both breeds with frequencies of 0.705 and 0.556 in Borgou and White Fulani respectively (Table 4.4). The H3 (0.026) was the least frequent haplotype in Borgou while the H4 (0.013) and H5 were the least frequent (0.013) haplotypes in White Fulani (Table 4.4).

Table 4.4: *CSN1S1* haplotype blocks and distribution in Borgou and White Fulani cattle breeds

Haplotypes Blocks		Frequency	
		Borgou	**White Fulani**
H1	ATG	0.705	0.556
H2	ACA	0.232	0.393
H3	ATA	0.026	0.025
H4	GTA	0.037	0.013
H5	ACG	0	0.013

The SNPs g.10331 A>G, g.10359 T>C and g.10430 G>A were chronologically in the *CSN1S1* haplotypes block.

4.1.4. Polymorphisms in *CSN3* gene

4.1.4.1. *CSN3* genotypes and alleles frequencies

Through screening of exon IV and partial intronic sequences of bovine *CSN3* gene, a total of six SNPs were found in this study. The SNPs positions were based on the bovine *CSN3* gene reference sequence GenBank accession No.: AY380228**.** Five of these SNPS **(**g.13065 C>T, g.13068 C>T, g.13104 A>C, g.13111 A>G and g.13165 A>G) were found in exon IV while the g.13173 A>T was found in intron IV. Three of the reported SNPs in exon IV (g.13065 C>T, g.13068 C>T, g.13104 A>C) were non synonymous leading to amino acid replacement in the resulting protein while two SNPs (g.13111 A>G and g.13165 A>G) were synonymous (Table 10). The SNP genotypes and allelic frequencies are presented in Table 4.5.

At the *CSN3* g.13065 C>T locus, the CC, CT and TT genotypes were observed respectively in 40, 43 and 11 individuals of Borgou. The CC, CT and TT genotypes were found respectively in 30, 45 and 21 cows in White Fulani breed. The C allele was the most frequent with frequencies of 0.65 and 0.55 in Borgou and White Fulani respectively (Table 4.5).

At the *CSN3* g.13068 C>T locus, the CC, CT and TT genotypes were observed respectively in 44, 38 and 12 individuals of Borgou. The CC, CT and TT genotypes were found respectively in 55, 35 and 6 cows in White Fulani breed. The C allele was the most frequent with frequencies of 0.67 and 0.76 in Borgou and White Fulani respectively (Table 4.5).

At the *CSN3* g.13104 A>C, the AA, AC and CC genotypes were observed respectively in 44, 37 and 13 individuals of Borgou. The AA, AC and CC genotypes were found respectively in 54, 36 and 6 cows in White Fulani breed. The A allele was the most frequent with frequencies of 0.66 and 0.75 in Borgou and White Fulani respectively (Table 4.5).

At the *CSN3* g.13111 A>G locus, the AA, AG and GG genotypes were observed respectively in 68, 24 and 2 individuals of Borgou. The AA and AG genotypes were found respectively in 74 and 22 cows in White Fulani breed. The GG genotype was not found in White Fulani. The A allele was the most frequent with frequencies of 0.85 and 0.89 in Borgou and White Fulani respectively (Table 4.5).

At the *CSN3* g.13165 A>G locus, the AA, AG and GG genotypes were observed respectively in 44, 39 and 11 individuals of Borgou. The AA, AG and GG genotypes were found respectively in 54, 36 and 6 cows in White Fulani breed. The A allele was the most frequent with frequencies of 0.67 and 0.75 in Borgou and White Fulani respectively (Table 4.5).

At the *CSN3* g.13173 A>T locus, the AA, AT and TT genotypes were observed respectively in 45, 39 and 10 individuals of Borgou. The AA, AT and TT genotypes were found respectively in 54, 36 and 6 cows in White Fulani breed. The A allele was the most frequent with frequencies of 0.68 and 0.75 in Borgou and White Fulani respectively (Table 4.5).

Based on Hardy–Weinberg Equilibrium exact P-values, all the genotypic frequencies in Borgou and White Fulani populations were in Hardy–Weinberg Equilibrium at *CSN3* loci ($P>0.05$).

Table 4.5: *CSN3* genotypes and allelic frequencies in Borgou and White Fulani cattle breeds

Polymorphisms *CSN3* *(Exon 4-Intron 4)*	Borgou (BO)					White Fulani (WF)					P-value[a] HWE	
	Genotypic frequency			Allelic frequency		Genotypic frequency			Allelic frequency		BO	WF
g.13065 C>T Non synonymous (Thr135Ile)	CC	CT	TT	C	T	CC	CT	TT	C	T	0.743	0.560
	40	43	11	0.65	0.35	30	45	21	0.55	0.45		
g.13068 C>T Non synonymous (Thr136Ile)	CC	CT	TT	C	T	CC	CT	TT	C	T	0.248	0.850
	44	38	12	0.67	0.33	55	35	6	0.76	0.24		
g.13104 A>C Non synonymous (Asp148Ala)	AA	AC	CC	A	C	AA	AC	CC	A	C	0141	0.958
	44	37	13	0.66	0.34	54	36	6	0.75	0.25		
g.13111 A>G synonymous(Pro150Pro)	AA	AG	GG	A	G	AA	AG	GG	A	G	0.876	0.216
	68	24	2	0.85	0.15	74	22	0	0.89	0.11		
g.13165 A>G synonymous (Ala168Ala)	AA	AG	GG	A	G	AA	AG	GG	A	G	0.404	0.958
	44	39	11	0.67	0.33	54	36	6	0.75	0.25		
g.13173 A>T intron IV	AA	AT	TT	A	T	AA	AT	TT	A	T	0.513	0.958
	45	39	10	0.68	0.32	54	36	6	0.75	0.25		

Thr: Threonine, Ile: Isoleucine; Asp: Aspartic acid, Ala: Alanine, Pro: Proline.
[a] P>0.05 population are in Hardy-Weinberg Equilibrium.

4.1.4.2. *CSN3* haplotype blocks and distribution in Borgou and White Fulani

The six SNPs showed high linkage disequilibrium (D´> 0.95) in both breeds resulting in eight different haplotypes in total. Seven haplotypes (H1, H2, H3, H4, H6, H7 and H8) were found in Borgou with the H1 (0.343) and H2 (0.313) the most frequent and H7 (0.005) and H8 (0.005) the least frequent haplotypes (Table 4.6). Five haplotypes were found in White Fulani (H1, H2, H3, H4 and H5). The H2 (0.450) was the most frequent followed by the H1 (0.240) and the H5 (0.005) was the least frequent haplotype in White Fulani (Table 4.6).

The haplotype H5 (CCCAGT) was specific to White Fulani while the haplotypes H6 (CTCAGA), H7 (TCCAAA) and H8 (CTCAAA) were specific to Borgou cattle breed.

Table 4.6: *CSN3* haplotype blocks and distribution in Borgou and White Fulani cattle breeds

Haplotypes Blocks		Frequency	
		Borgou	**White Fulani**
H1	CTCAGT	0.343	0.24
H2	TCAAAA	0.313	0.45
H3	CCAGAA	0.152	0.115
H4	CCAAAA	0.172	0.19
H5	CCCAGT	0	0.005
H6	CTCAGA	0.01	0
H7	TCCAAA	0.005	0
H8	CTCAAA	0.005	0

The SNPs g.13065 C>T, g.13068 C>T, g.13104 A>C, g.13111 A>G, g.13165 A>G and g.13173 A>T were chronologically in the *CSN3* haplotypes block.

4.1.5. *MBLG* gene

Two SNPs were found through screening of partial exon IV and intron IV of *MBLG* gene. The SNPs *MBLG* g.5864C>T was detected in exon IV and the *MBLG* g.5940G>A in intron IV. The *MBLG* g.5864C>T mutation led to the change of amino acid alanine by valine at position 118 in the resulting protein. The genotypic and allelic frequencies of *MBLG* polymorphism are presented in Table12. At the *MBLG* g.5864C>T locus, the CC, CT and TT genotypes were observed respectively in 52, 23 and 1 individuals of Borgou. The CC and CT genotypes were found respectively in 79 and 11 cows in White Fulani breed. The TT genotype was not found in White Fulani. The C allele was the most frequent with frequencies of 0.84 and 0.94 in Borgou and White Fulani respectively (Table 4.7). At the *MBLG* g.5940G>A locus, the AA, AG and GG genotypes were observed respectively in 66, 8 and 2 individuals of Borgou. The AA, AG and GG genotypes were found respectively in 70, 18 and 2 cows in White Fulani breed. The G allele was the most frequent with frequencies of 0.92 and 0.88 in Borgou and White Fulani respectively (Table 4.7). Only the *MBLG* g.5940G>A genotypes were not in Hardy-Weinberg Equilibrium and in Borgou only ($P<0.05$).

Table 4.7: *MBLG* genotypic and allelic frequencies

Polymorphisms *MBLG*	Breed										HWE P-value[a]	
	Borgou					White Fulani						
	Genotypic frequency			Allelic frequency		Genotypic frequency			Allelic frequency		BO	WF
MBLG g.5864C>T (Ala118Val)-Exon IV	CC	CT	TT	C	T	CC	CT	TT	C	T	0.404	0.557
	52	23	1	0.84	0.16	79	11	0	0.94	0.06		
MBLG g.5940G>A (intron IV)	GG	AG	AA	G	A	GG	AG	AA	G	A	0.010	0.479
	66	8	2	0.92	0.08	70	18	2	0.88	0.12		

The SNPs positions were based on the bovine *MBLG* gene reference sequence GenBank accession No.: Z48305. BO: Borgou, WF: White Fulani.
[a] P>0.05 population are in Hardy-Weinberg Equilibrium.

4.2. Characteristics of sampled animals

In total, 98 Borgou and 100 White Fulani cows were sampled from 12 and 9 different herds respectively. In Borgou, 48 cows were between 3 and 5 years old while 50 were more than 5 years old. In White Fulani, 64 cows were between 3 and 5 years old while 36 were more than 5 years old. In Borgou breed, 25, 17 and 56 cows were at their first, second and third and more parity, respectively. In White Fulani, 28, 22 and 50 cows were at their first, second and third and more parity, respectively. In Borgou, 30, 39 and 29 cows were at early lactation (DIM<100 days), mid-lactation (100<DIM<200 days) and late lactation (DIM>200 days), respectively. In White Fulani, 32, 32 and 36 cows were at early, mid and late lactation, respectively.

4.3. Effect of breed on milk production and fatty acid traits

Least square means of milk components, test-day milk yield and fatty acid profiles across breeds are reported in Table 13. White Fulani produced higher (P<0.001) test-day milk yield (1.14 vs 0.80 kg), fat content (5.49 vs 4.51 %), fat yield (57.13 vs 36.52 g) and test-day

protein yield (40.4 vs 30.05 g) compared to Borgou. On the other hand, Borgou presented higher ($P<0.001$) content of milk urea nitrogen than White Fulani. However, no significant differences ($P>0.05$) were observed for protein and lactose contents between the two breeds (Table 4.8). About fifteen different fatty acids were quantified with confidence in the milks of White Fulani and Borgou breeds, namely caproic acid (C6:0), caprylic acid (C8:0), capric acid (C10:0), lauric acid (C12:0) , 12-methyl tridecanoic acid (C13:0), myristoleic acid (C14:1 *cis*-9), myristic acid (C14:0), pentadecanoic acid (C15:0), palmitic acid (C16:0), margaric acid (C17:0), linoleic acid (C18:2 *cis*-9, *cis*-12), oleic acid (C18:1 *cis*-9), stearic acid (C18:0), nonadecanoic acid (C19:0) and arachidic acid (C20:0). The fatty acid profiles revealed that oleic acid (16.63%) and linoleic acid (15.84%) were the most abundant fatty acids in Borgou milk while stearic acid (17.88%) and palmitic acid (16.19%) were the most abundant fatty acids in White Fulani milk. The Arachidic acid and Caproic acid were the least abundant fatty acids in Borgou (0.29 %; 0.2 %) and White Fulani (0.24 %; 0.23 %). The Borgou cows produced milk with higher ($P<0.05$) contents of C8:0, C10:0, C14:0, C15:0, C17:0, C18:2 *cis*-9, *cis*-12, C18:1 *cis*-9 , and C19:0 compared to White Fulani cattle breed. On the other hand, White Fulani produced milk with higher ($P<0.001$) contents of C18:0 and C16:0 compared to Borgou breed. Moreover, Borgou had higher ($P<0.05$) C18 desaturation index, total index and higher ($P<0.05$) contents of PUFA and long-chain fatty acids (LCFA) than White Fulani. On the contrary White Fulani produced milk with higher ($P<0.001$) contents of total SFA and medium chain fatty acids (MCFA) as compared to Borgou. No significant differences ($P>0.05$) between breeds were observed for C6:0, C12:0, C13:0, C14:1 *cis*-9, C20:0 and MUFA and short-chain fatty acids (SCFA) (Table 13).

Table 4.8: Effect of breed on milk production traits and individual fatty acids composition in Borgou and White Fulani Cows

Trait	Breed				p-value
	Borgou (98)	SE	White Fulani (100)	SE	
Milk production traits					
Test-day milk yield (kg)	0.8	0.06	1.14	0.06	<0.001***
Fat (%)	4.51	0.19	5.49	0.19	<0.001***
Protein (%)	3.76	0.06	3.8	0.06	0.62
Milk urea nitrogen (mg/dl)	10.33	0.33	8.04	0.33	<0.001***
Lactose (%)	4.45	0.04	4.48	0.04	0.586
Test-day fat yield (g)	36.52	2.65	57.13	2.64	<0.001***
Test-day protein yield (g)	30.05	1.67	40.4	1.66	<0.001***
Fatty acids and unsaturation indices (%)					
Caproic acid (C6:0)	0.29	0.04	0.23	0.03	0.268
Caprylic acid (C8:0)	0.42	0.04	0.31	0.04	0.042*
Capric acid (C10:0)	1.15	0.08	0.93	0.07	0.047*
Lauric acid (C12:0)	1.57	0.11	1.46	0.11	0.47
12-Methyl Tridecanoic acid (C13:0)	0.37	0.04	0.34	0.04	0.735
Myristoleic acid (C14:1 *cis*-9)	1.02	0.08	0.87	0.08	0.21
Myristic acid (C14:0)	12.19	0.6	9.07	0.57	<0.001***
Pentadecanoic acid (C15:0)	4.29	0.24	2.62	0.23	<0.001***
Palmitic acid (C16:0)	5.27	0.95	16.19	0.89	<0.001***
Margaric acid (C17:0)	7.34	0.36	3.67	0.34	<0.001***
Linoleic acid (C18:2 *cis*-9, *cis*-12)	15.84	0.64	9.85	0.6	<0.001***
Oleic acid C18:1 *cis*-9)	16.63	0.84	14.25	0.79	0.042*
Stearic acid (C18:0)	12.96	0.9	17.86	0.84	<0.001***
Nonadecanoic acid (C19:0)	0.96	0.13	0.53	0.13	0.019*
Arachidic acid (C20:0)	0.2	0.03	0.24	0.03	0.207
C14 index[1]	9.69	1.17	8.48	1.1	0.452
C18 index[2]	55.61	2.11	45.37	1.98	<0.001***
Total index[3]	40.78	1.72	35.66	1.62	0.031*
Fatty acid groups (%)					
SFA[4]	58.46	1.03	68.19	0.97	<0.001***
MUFA[5]	21.98	1.09	19.41	1.03	0.088
PUFA[6]	19.56	0.77	12.4	0.72	<0.001***
SCFA[7]	2.3	0.18	1.84	0.17	0.065
MCFA[8]	30.76	1.12	38.97	1.05	<0.001***
LCFA[9]	66.94	1.18	59.19	1.11	<0.001***

[1] C14 index=C14:1 *cis*-9/ (C14:1 *cis*-9+ C14 :0) × 100.
[2]C18 index= C18:1 *cis*-9/ (C18:1 *cis*-9+ C18 :0) × 100.
[3]Total index= (C14:1 *cis*-9+ C18:1 *cis*-9)/ (C14:1 *cis*-9+ C14 :0+ C18:1 *cis*-9+ C18 :0) × 100.
[4]SFA= saturated fatty acid; [5]MUFA= monounsaturated fatty acid; [6]PUFA=polyunsaturated fatty acid; [7]SCFA= short-chain fatty acids, [8]MCFA=medium-chain fatty acids, [9]LCFA=long-chain fatty acids. SCFA included C6:0, C8:0 and C10:0; MCFA included all linear fatty acids from C12:0 to C16:0; LCFA included all linear fatty acids from C17:0 to C20:0. SE=standard errors; *P<0.05; *** P<0.001

4.4. Effect of geographical region on milk production and fatty acids traits in Borgou

The effect of geographical regions on milk production and fatty acid traits are presented in Table 4.9. Because the White Fulani cattle are only found in the northern part of Benin, only Borgou breed was considered in the geographical effect analysis. The Borgou cows from the South of Benin showed milk with higher ($P<0.05$) contents of C12:0, C18:2 *cis*-9, *cis*-12 and PUFA. However, the Borgou cows from the North of Benin produced milk with higher ($P<0.001$) milk urea nitrogen (11.75 vs 8.57 mg/dl), higher ($P<0.01$) contents of C14:1 *cis*-9, C18:1 *cis*-9, MUFA, and higher ($P<0.05$) C14 index, C18 index and total index compared to Borgou cows from the South of Benin (Table 4.9).

Table 4.9: Effect of geographical regions on milk fatty acids composition traits in Borgou

	Geographical region				
Trait (%)	South (44)	SE	North (54)	SE	p-value
Milk production traits					
Test-day milk yield (kg)	0.72	0.07	0.9	0.07	0.070
Fat (%)	4.30	0.29	4.68	0.27	0.340
Protein (%)	3.86	0.09	3.68	0.08	0.140
Milk urea nitrogen (mg/dl)	8.57	0.45	11.75	0.40	<0.001***
Lactose (%)	4.44	0.05	4.46	0.05	0.874
Test-day fat yield (g)	31.82	4.00	40.55	3.62	0.117
Test-day protein yield (g)	27.13	2.62	32.44	2.37	0.136
Fatty acids and unsaturation indices (%)					
Caproic acid (C6:0)	0.23	0.05	0.32	0.04	0.117
Caprylic acid (C8:0)	0.33	0.07	0.48	0.05	0.068
Capric acid (C10:0)	1.18	0.12	1.12	0.09	0.717
Lauric acid (C12:0)	2.07	0.19	1.27	0.15	0.002**
12-Methyl Tridecanoic acid (C13:0)	0.36	0.08	0.37	0.06	0.944
Myristoleic acid (C14:1 *cis*-9)	0.70	0.12	1.21	0.09	<0.001***
Myristic acid (C14:0)	12.88	0.99	11.78	0.77	0.387
Pentadecanoic acid (C15:0)	4.00	0.49	4.46	0.38	0.454
Palmitic acid (C16:0)	5.76	1.43	4.98	1.11	0.667
Margaric acid (C17:0)	7.72	0.71	7.11	0.55	0.492
Linoleic acid (C18:2 *cis*-9-12)	17.48	1.03	14.85	0.80	0.046*
Oleic acid (C18:1 *cis*-9)	13.69	1.13	18.40	0.88	0.001**
Stearic acid (C18:0)	13.35	0.88	12.72	0.69	0.569
Nonadecanoic acid (C19:0)	0.79	0.27	1.06	0.21	0.436
Arachidic acid (C20:0)	0.21	0.04	0.19	0.03	0.761
C14 index[1]	5.01	2.56	12.52	1.99	0.023*
C18 index[2]	50.83	2.38	58.50	1.85	0.012*
Total index[3]	55.84	3.86	71.02	3.00	0.003**
Fatty acid groups (%)					
SFA[4]	60.63	1.58	57.15	1.22	0.086
MUFA[5]	17.87	1.43	24.46	1.11	<0.001***
PUFA[6]	21.50	1.22	18.39	0.95	0.046*
SCFA[7]	2.15	0.26	2.38	0.20	0.476
MCFA[8]	31.98	1.81	30.03	1.41	0.397
LCFA[9]	65.87	1.89	67.59	1.46	0.475

[1] C14 index=C14:1 *cis*-9/ (C14:1 *cis*-9+ C14 :0) × 100.
[2]C18 index= C18:1 *cis*-9/ (C18:1 *cis*-9+ C18 :0) × 100.
[3]Total index= (C14:1 *cis*-9+ C18:1 *cis*-9)/ (C14:1 *cis*-9+ C14 :0+ C18:1 *cis*-9+ C18 :0) × 100.
[4]SFA= saturated fatty acid; [5]MUFA= monounsaturated fatty acid; [6]PUFA=polyunsaturated fatty acid; [7]SCFA= short-chain fatty acids, [8]MCFA=medium-chain fatty acids, [9]LCFA=long-chain fatty acids. SCFA included C6:0, C8:0 and C10:0; MCFA included all linear fatty acids from C12:0 to C16:0; LCFA included all linear fatty acids from C17:0 to C20:0. SE=standard errors; *P<0.05; *** P<0.001

4.5. Principal component analysis of milk components and fatty acid composition

The principal component analysis (PCA) of the significant variables between breeds is presented in Figure 4.5, Figure 4.6 and Figure 4.7. The variation accounted for by PC1, PC2 and PC3 was 21.77%, 17.97% and 13.56% respectively. The first PC (PC1 vs PC2) had no clustering pattern (Figure 4.5). However, the second PC (PC1 vs PC3) and the third PC (PC2 vs PC3) clearly separated the White Fulani from Borgou breed. The Borgou population was on one extreme while White Fulani was on the other extreme as shown in Figure 4.6 and Figure 4.7. Therefore, the second PC (Figure 4.6) and the third PC (Figure 4.7) better discriminated the two breeds in terms of milk components and milk fatty acids composition.

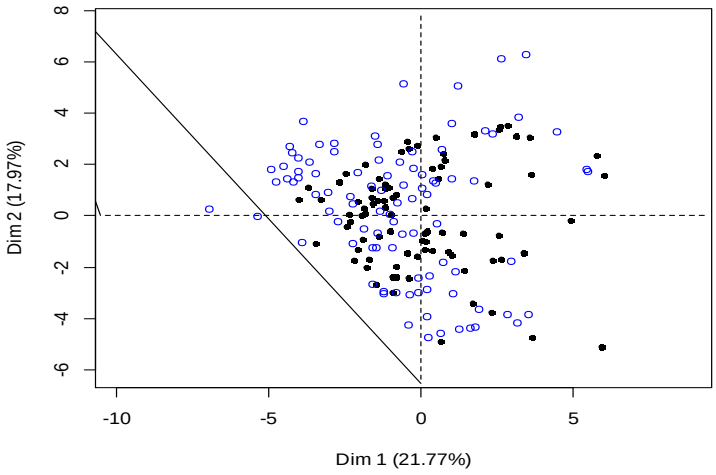


Figure 4.5: Principal Component Analysis (PC1 vs PC2) among Borgou and White Fulani cows based on milk components and Fatty acid composition. White Fulani in blue and Borgou in black.

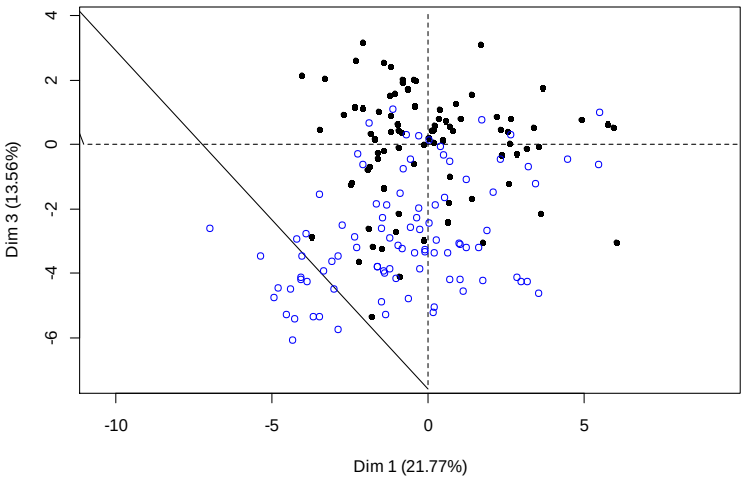


Figure 4.6: Principal Component Analysis (PC1 vs PC3) among Borgou and White Fulani cows based on milk components and Fatty acid composition. White Fulani in blue and Borgou in black.

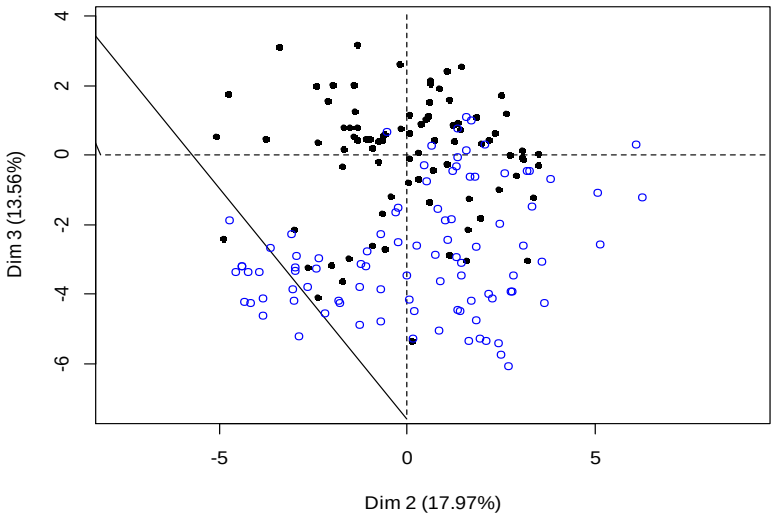


Figure 4.7: Principal Component Analysis (PC2 vs PC3) among Borgou and White Fulani cows based on milk components and Fatty acid composition. White Fulani in blue and Borgou in black.

4.6. Effect of age on milk production traits

The effect of age on milk production traits in Borgou and White Fulani is presented in Table 4.10. No significant difference (P>0.05) of age effect on milk production traits was observed in White Fulani. However, the Borgou cows of 3-5 years old presented higher test-day fat yield (44.32 vs 33.95 g, P<0.05) and higher protein yields (34.63 vs 30.05, P<0.05) than the Borgou cows of more than 5 years old (Table 4.10). No significant effect (P>0.05) of age was observed on test-day milk yield, fat, protein, milk urea nitrogen and lactose contents in Borgou.

Table 4.10: Effect of age on milk production traits in Borgou and White Fulani cattle breeds

	Borgou					White Fulani				
	3-5 years (47)		6+ years (48)			3-5 years (64)		6+ years (35)		
Trait	Mean	SEM	Mean	SE	P-value	Mean	SE	Mean	SE	P-value
Test-day milk yield (kg)	0.95	0.08	0.79	0.08	0.466	1.13	0.07	1.07	0.11	0.919
Fat (%)	4.54	0.32	4.39	0.31	0.174	5.47	0.21	5.56	0.32	0.538
Protein %	3.7	0.1	3.76	0.1	0.261	3.80	0.05	3.78	0.08	0.232
Milk urea nitrogen (mg/dl)	10.11	0.43	10.45	0.42	0.328	7.43	0.35	9.08	0.53	0.345
Lactose (%)	4.50	0.05	4.46	0.05	0.176	4.51	0.04	4.41	0.07	0.070
Test-day fat yield (g)	44.32	3.76	33.95	3.69	0.047*	57.22	3.52	57.32	5.29	0.669
Test-day protein yield (g)	34.63	1.99	30.05	1.95	0.024*	40.45	2.21	39.28	3.32	0.746

* P<0.05; ** P<0.01; *** P<0.001. SE: Standard Error

4.7. Effect of herd on milk production traits

In Borgou cattle breed, significant differences in test-day milk, protein and fat yields (P<0.01), milk urea nitrogen % (P<0.001) and lactose % (P<0.05) were observed between herds (Table 4.11). The mean test-day milk yield ranged from 0.2 kg in herd 2 to 1.8 kg in herd 11. The highest milk urea nitrogen was observed in herd 11 (14.2 mg/dl) and the lowest in herd 12 (3.6 mg/dl). The highest lactose content (4.9 %) was observed in herd 6 and herd 9 while the lowest lactose content (4.1 %) was observed in herd 10 (Table 4.11). The highest

test-day fat and protein yields were respectively 68.8 g and 61.1 g and were observed in herd 11 while the lowest test-day fat and protein yields were 20.3g and 15.3 g respectively and observed in herd 2 (Table 4.11).

In White Fulani breed, significant differences in fat% and milk urea nitrogen % ($P<0.001$), protein% ($P<0.05$) and lactose ($P<0.01$) were observed between herds in White Fulani (Table 4.12). The fat % ranged from 4.2 % (herd 15) to 9.9 % (Herd 13) (Table 4.12). The highest protein content (5.7 %) was observed in herd 13 and the lowest (3.4 %) in herd 20. The milk urea nitrogen ranged from 4.2 mg/dl in herd 18 to 14.1 mg/dl in herd 13 (Table 4.12). The lowest lactose content (3.9 %) was observed in herd 13 while the highest lactose content (4.9 %) was observed in herd 19 (Table 4.12).

Table 4.11: Effect of herd on milk production traits in Borgou (Mean±SE)

Traits	Herds												P-Value
	1	2	3	4	5	6	7	8	9	10	11	12	
Test-day milk yield (kg)	0.8±0.1	0.2±0.2	1.1±0.8	0.8±0.2	1±0.5	0.8±0.3	0.8±0.2	0.6±0.2	1±0.2	1±0.24	1.8±0.2	0.8±0.3	<0.001***
Fat (%)	4.5±0.6	4.7±0.6	4.1±0.7	3.7±0.7	5.2±1.9	4.3±1.7	5.0±0.7	4.8±0.7	4.7±0.1	5.3±0.10	3.7±0.7	6.4±1.2	0.852
Protein %	3.9±0.8	3.7±0.2	3.7±0.2	3.5±0.2	4.1±0.6	3.4±0.4	3.7±0.2	3.7±0.2	3.6±0.3	3.3±0.30	3.5±0.2	5±0.4	0.066
Milk urea nitrogen (mg/dl)	7.2±0.7	11.3±0.8	9.0±0.9	9.5±0.9	10.3±2.5	10.3±1.7	11.7±0.9	11.4±0.9	12.6±1.3	9.9±1.3	14.2±0.9	3.6±1.6	<0.001***
Lactose (%)	4.3±0.1	4.5±0.10	4.4±0.10	4.5±0.1	4.6±0.3	4.9±0.9	4.4±0.1	4.4±0.1	4.9±0.14	4.1±0.2	4.6±0.1	4.2±0.2	0.017*
Test-day fat yield (g)	32.7±6.5	20.3±7.4	46.9±1.7	30.7±8.2	42.6±22.5	35.6±13.8	33.6±7.9	26.4±8.0	49.5±11.2	50.2±11.4	68.8±8.1	44.9±13.8	0.003**
Test-day protein yield (g)	28.1±3.5	15.3±3.9	38±4.01	28.3±4.3	33.9±11.9	27.2±7.3	25.9±4.12	20.7±4.2	42.7±5.9	28.8±6.04	61.1±4.3	35.9±7.3	<0.001***

* P<0.05; ** P<0.01; *** P<0.001. SE: Standard Error

Table 4.12: Effect of herd on milk production traits in White Fulani (Mean ± Standard Error)

Traits	Herds									P-Value
	13	14	15	16	17	18	19	20	21	
Test-day milk yield (kg)	1±0.4	0.9±0.1	1.3±0.1	1.4±0.1	1.2±0.1	0.8±0.1	1.3±0.2	1±0.2	1.1±0.1	0.080
Fat (%)	9.9±1.6	6.5±0.4	4.2±0.4	5.4±0.4	5.2±0.4	5.1±0.5	4.4±0.8	5.4±0.8	6.9±0.5	<0.001***
Protein %	5.7±0.5	3.8±0.1	3.7±0.1	3.9±0.1	3.8±0.1	3.7±0.1	3.6±0.3	3.4±0.2	3.9±0.1	0.017*
Milk urea nitrogen (mg/dl)	14.1±2.4	6.4±0.6	10.4±0.6	8.5±0.6	7.7±0.6	4.2±0.7	10.8±1.2	10.8±1.1	8.1±0.7	<0.001***
Lactose (%)	3.9±0.3	4.3±0.1	4.7±0.1	4.5±0.1	4.6±0.1	4.4±0.1	4.9±0.2	4.4±0.1	4.2±0.1	0.001**
Test-day fat yield (g)	69.8±26.3	58.6±6.8	54.1±6.6	62.5±6.4	56.4±6.4	46.6±7.6	54.8±13.1	62.8±11.8	60.6±7.6	0.886
Test-day protein yield (g)	39.9±15.2	34.9±3.9	46.7±3.8	45.7±3.7	42.1±3.7	34.3±4.4	43.2±7.6	39.7±6.8	34.3±4.4	0.221

* P<0.05; ** P<0.01; *** P<0.001.

4.8. Effect of parity number on milk production traits

In Borgou Breed, cows on second parity presented higher (1.12 kg; $P<0.05$) test-day milk and protein yields (38.14 g, $P<0.05$) than cows on first and third or more parity (Table 4.13). However, no significant difference ($P>0.05$) was observed for test-day milk and protein yields between Borgou cows on first parity and the cows on third and more parity number. On the other hand, the White Fulani cows on the third and more parity presented higher (4.55 %; $P<0.05$) lactose % than the group of cows on first (4.39 %) and second parity (4.42 %) (Table 4.13). However, no significant difference was observed for lactose content of cows on first and second parity (Table 4.13). On the contrary, the White Fulani cows on the first parity produced milk with lower (34.17 g, $P<0.05$) test-day protein yield than cows on the second (43.27 g) and the third and more parity (41.55 g) (Table 4.13). The protein yield did not show significant difference ($P>0.05$) after second parity in White Fulani.

4.9. Effect of stage of lactation on milk production traits

The effect of the stage of lactation on milk production traits is presented in Table 4.14. The lactation stage did not show significant difference on milk production traits in Borgou cattle breed. However, the white Fulani cows at late lactation stage presented milk with higher fat% (6.79 vs 4.35 vs 5.11 %, $P<0.001$) and protein% (4.26 vs 3.44 vs 3.60 %, $P<0.001$) than White Fulani cows at early and mid-lactation stage respectively (Table 4.14). On the contrary, the White Fulani cows at late lactation stage showed lower content of milk urea nitrogen (6.88 vs 8.46 vs 8.60 %, $P<0.05$) and lactose content (4.33 vs 4.63 vs 4.49 %, $P<0.05$) compared to the White Fulani cows at early and mid-lactation stage respectively (Table 4.14).

Table 4.13: Effect of parity number on milk production traits in Borgou and White Fulani cattle breeds

	Borgou							White Fulani						
	Parity 1 (24)		Parity 2 (16)		Parity 3+ (55)			Parity 1 (28)		Parity 2 (22)		Parity 3+ (49)		
Trait	Mean	SE	Mean	SE	Mean	SE	P-value	Mean	SE	Mean	SE	Mean	SE	P-value
Test-day milk yield (kg)	0.84[a]	0.11	1.12[b]	0.14	0.82[a]	0.08	0.016*	1.00	0.11	1.19	0.12	1.12	0.08	0.132
Fat (%)	4.89	0.44	4.642	0.54	4.14	0.30	0.888	5.82	0.33	5.75	0.36	5.20	0.26	0.401
Protein %	3.81	0.14	3.631	0.17	3.70	0.09	0.412	3.78	0.08	3.82	0.09	3.80	0.07	0.538
Milk urea nitrogen (mg/dl)	10.6	0.59	9.678	0.73	10.2	0.40	0.182	7.53	0.54	6.66	0.59	8.77	0.42	0.836
Lactose (%)	4.54	0.07	4.4	0.08	4.49	0.05	0.084	4.39[a]	0.07	4.42[a]	0.08	4.55[b]	0.05	0.012*
Test-day fat yield (g)	42.4	5.20	50.4	6.46	34.7	3.58	0.155	52.10	5.44	64.5	5.98	56.15	4.25	0.056
Test-day protein yield (g)	30.95[a]	2.74	38.14[b]	3.41	31.96[a]	1.89	0.010*	34.17[a]	3.41	43.27[b]	3.75	41.55[b]	2.66	0.028*

* P<0.05. SE: Standard Error. Means with different superscript letters in the same row differ significantly.

Table 4.14: Effect of stage of lactation on milk production traits in Borgou and White Fulani cattle breeds

	Borgou							White Fulani						
	Early lactation (29)		Mid lactation (38)		Late lactation (28)			Early lactation (32)		Mid lactation (32)		Late lactation (35)		
Trait	Mean	SE	Mean	SE	Mean	SE	P-value	Mean	SE	Mean	SE	Mean	SE	P-value
Test-day milk yield (kg)	0.84	0.11	0.90	0.09	0.94	0.11	0.618	1.16	0.10	1.12	0.11	1.05	0.10	0.148
Fat (%)	4.24	0.41	4.07	0.35	5.35	0.44	0.095	4.35[a]	0.31	5.11[a]	0.32	6.79[b]	0.29	<0.001***
Protein %	3.55	0.13	3.65	0.11	4.02	0.14	0.096	3.44[a]	0.08	3.60[a]	0.08	4.26[b]	0.07	<0.001***
Milk urea nitrogen (mg/dl)	11.34	0.56	9.53	0.47	9.80	0.59	0.222	8.46[a]	0.51	8.60[a]	0.53	6.88[b]	0.47	0.024*
Lactose (%)	4.58	0.06	4.50	0.05	4.35	0.07	0.236	4.63[a]	0.06	4.49[a]	0.07	4.33[b]	0.06	0.047*
Test-day fat yield (g)	40.66	4.91	39.40	4.16	41.64	5.23	0.986	51.94	5.13	58.73	5.37	60.48	4.77	0.921
Test-day protein yield (g)	34.01	2.59	32.55	2.20	32.05	2.76	0.323	41.31	3.22	40.24	3.36	38.98	2.99	0.645

* P<0.05; ** P<0.01; *** P<0.001. SE: Standard Error. Means with different superscript letters in the same row differ significantly.

4.10. Correlations between milk components and fatty acid traits

The phenotypic correlations between milk component and fatty acid traits in Borgou cows are presented in Table 4.15. In Borgou, the fat percentage showed significantly (P<0.05) positive correlation with protein content (0.54), C14 index (0.45), C18:1 *cis*-9 (0.24), and MUFA (0.28) and negative correlations with C16:0 (-0.22) and PUFA (-0.25). The C14:1 *cis*-9 showed positive and moderate correlation (P<0.01) with C14 index (0.33) and C18 index (0.31) but a negative correlation with C16:0 (-0.37, P<0.001). The correlations of C16:0 were negative between all the traits except for SFA (0.48), (Table 4.15). The C18:2 *cis*-9, *cis*-12 was negatively correlated (P<0.01) with C18:1 *cis*-9, SFA and MUFA. The C18:1 *cis*-9 showed high significant and positive correlation (P<0.001) with C18 index, total unsaturation index and MUFA. On the other hand, high significant and negative correlation (P<0.001) was observed between C18:1 cis-9 and SFA. The C14 index showed moderate and positive correlation (P<0.05) with C18 index (0.27), total unsaturation index (0.52) and with MUFA (0.38). The total index showed high positive correlation (P<0.001) with MUFA (0.88) and negative correlation (P<0.001) with SFA (-0.71).

The Table 4.16 presents the phenotypic correlations between milk component and fatty acid traits in White Fulani cows. Fat content showed significant and positive correlation (P<0.01) with protein content, C14:1 *cis*-9 and C14 index (Table 4.16). The protein percentage was moderately and positively correlated (P<0.05) with C14:1 *cis*-9 and C14 index. The C18:2 cis-9-12 was negatively correlated (P<0.05) with C18 index (-0.24) and PUFA (-0.42) while positively correlated (P<0.05) to C14 index (0.24) and SFA (0.31). The total index showed high positive correlation (P<0.001) with MUFA (0.94), negative correlation (P<.0.01) with SFA (-0.82) and PUFA (-0.34) (Table 4.16).

Table 4.15: Phenotypic coefficient of correlations (Pearson) in Borgou

Trait	Fat (%)	Protein (%)	C14:1 *cis*-9	C16:0	C18:2 *cis*-9, *cis*-12	C18:1 cis-9	C14 index	C18 index	Total index	SFA	MUFA	PUFA
Fat (%)		0.54***	0.45***	-0.22*	-0.25*	0.24*	0.14	0.20	0.15	-0.07	0.28*	-0.25*
Protein (%)	0.54***		0.17	-0.17	-0.00	0.17	0.03	0.05	0.07	-0.16	0.17	-0.01
C14:1 *cis*-9	0.45***	0.17		-0.37***	-0.11	0.10	0.33**	0.31**	0.08	-0.10	0.20	-0.12
C16:0	-0.23*	-0.17	-0.37***		-0.38***	-0.19	-0.11	-0.15	-0.01	0.48***	-0.19	-0.38***
C18:2 *cis*-9, *cis*-12	-0.25*	-0.00	-0.11	-0.38**		-0.31**	-0.03	-0.13	-0.17	-0.44***	-0.35**	1.00***
C18:1 cis-9	0.24*	0.17	0.10	-0.19	-0.31**		0.37***	0.75**	0.90***	-0.72***	0.99***	-0.31**
C14 index	0.14	0.03	0.33**	-0.11	-0.03	0.37**		0.27*	0.52***	-0.34**	0.38***	-0.04
C18 index	0.20	0.05	0.31**	-0.15	-0.13	0.75**	0.271*		0.83***	-0.63***	0.76***	-0.13
Total index	0.15	0.07	0.08	-0.01	-0.17	0.90**	0.52***	0.83***		-0.71***	0.88***	-0.17
SFA[1]	-0.07	-0.16	-0.10	0.48***	-0.44***	-0.72***	-0.34**	-0.63***	-0.71***		-0.69***	-0.44***
MUFA[2]	0.28*	0.17	0.2	-0.19	-0.35**	0.99***	0.38***	0.76***	0.88***	-0.69***		-0.35**
PUFA[3]	-0.25*	-0.01	-0.12	-0.38***	1.00***	-0.31**	-0.04	-0.13	-0.17	-0.44***	-0.35**	

* P<0.05; ** P<0.01; *** P<0.001; [1]SFA= saturated fatty acid; [2]MUFA= monounsaturated fatty acid; [3]PUFA=polyunsaturated fatty acid

Table 4.16: Phenotypic coefficient of correlations (Pearson) in White Fulani

Trait	Fat (%)	Protein (%)	C14:1 *cis*-9	C16:0	C18:2 *cis*-9, *cis*-12	C18:1 cis-9	C14 index	C18 index	Total index	SFA	MUFA	PUFA
Fat (%)		0.62***	0.40***	0.08	0.19	0.00	0.29**	-0.06	-0.13	0.04	-0.11	0.12
Protein (%)	0.62***		0.34**	0.09	0.13	-0.13	0.26*	-0.05	-0.08	0.07	-0.03	-0.05
C14:1 *cis*-9	0.40***	0.34**		-0.05	-0.07	0.00	0.43***	0.11	-0.10	-0.09	-0.10	0.27**
C16:0	0.08	0.09	-0.05		0.12	0.14	0.04	-0.20*	-0.16	0.09	-0.19	0.18
C18:2 *cis*-9, *cis*-12	0.19	0.13	-0.07	0.12		-0.12	0.24*	-0.24*	-0.10	0.31**	-0.01	-0.42***
C18:1 cis-9	0.00	-0.13	0.00	0.14	-0.12		0.04	-0.162	-0.28**	0.18	-0.40***	0.37***
C14 index	0.29**	0.26*	0.43**	0.03	0.24*	0.04		-0.17	-0.17	0.13	-0.16	0.08
C18 index	-0.06	-0.05	0.11	-0.20	-0.24	-0.16	-0.17		0.93**	-0.83***	0.82***	-0.14
Total index	-0.13	-0.07	-0.10	-0.16	-0.10	-0.28**	-0.17	0.93***		-0.82**	0.94***	-0.34**
SFA[1]	0.04	0.07	-0.09	0.09	0.31**	0.18	0.13	-0.83***	-0.82**		-0.78***	-0.18
MUFA[2]	-0.11	-0.03	-0.1	-0.19	-0.01	-0.40***	-0.16	0.82***	0.94***	-0.78***		-0.49***
PUFA[3]	0.12	-0.05	0.27**	0.18	-0.42***	0.37***	0.08	-0.14	-0.34**	-0.18	-0.49***	

* P<0.05; ** P<0.01; *** P<0.001; [1]SFA= saturated fatty acid; [2]MUFA= monounsaturated fatty acid; [3]PUFA=polyunsaturated fatty acid.

4.11. Association of *CSN3* SNP genotype with milk production traits in Borgou and White Fulani cattle breeds

The estimated effect of the six SNPs in *CSN3* (g.13065 C>T, g.13068 C>T, g.13104 A>C, g.13111 A>G, g.13165 A>G and g.13173 A>T) on six milk production traits in Borgou cattle breed are shown in Table 4.17. Five SNPs (g.13065 C>T, g.13068 C>T, g.13104 A>C, g.13111 A>G, g.13165 A>G and g.13173 A>T) were found to be significantly ($P<0.05$) associated with milk production traits in Borgou. The g.13065 C>T genotypes were significantly associated with protein % ($P<0.05$), lactose % ($P<0.01$), test day fat and protein yields ($P<0.05$) (Table 4.17). The g.13068 C>T and g.13173 A>T genotypes were significantly associated with protein % ($P<0.05$) and lactose % ($P<0.01$). Moreover, the g.13111 A>G was associated with fat % and test-day fat yield ($P<0.01$) while the g.13165 A>G was associated with fat% ($P<0.05$), protein % and lactose % ($P<0.01$) as shown in Table 4.17. However, *CSN3* SNPs did not show significant association with milk production traits in White Fulani cattle breed (data not shown).

Table 4.17: Association of *CSN3* SNP genotypes with milk production traits in Borgou cattle breed

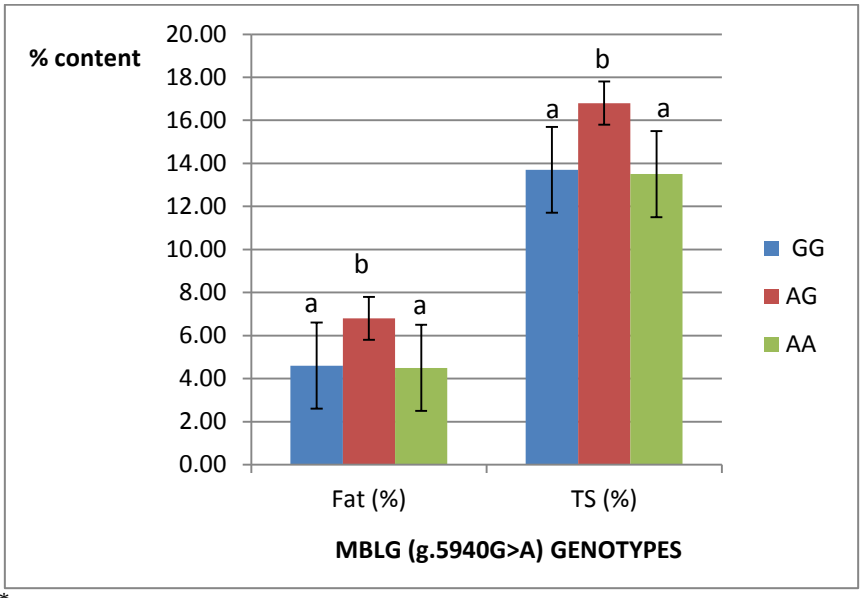
Locus	Genotype	TDMY (kg)	Fat (%)	Protein (%)	Lactose (%)	TDFY (g)	TDPY (g)
g.13065 C>T	CC	0.87 ± 0.14	5.56 ± 0.47	3.79 ± 0.18	4.31 ± 0.11	47.72 ± 6.95	31.8 ± 4.38
	CT	0.67 ± 0.16	4.71 ± 0.56	3.88 ± 0.22	4.47 ± 0.14	35.60 ± 8.28	28.35 ± 5.22
	TT	0.78 ± 0.19	3.55 ± 0.66	3.44 ± 0.26	4.55 ± 0.16	28.11 ± 9.82	26.33 ± 6.20
P value		0.179	0.107	0.047*	0.009**	0.008**	0.032*
g.13068 C>T	CC	0.85 ± 0.09	4.42 ± 0.32	3.82 ± 0.12	4.60 ± 0.08	39.5 ± 4.93	32.22 ± 2.99
	CT	0.81 ± 0.10	4.90 ± 0.36	3.78 ± 0.14	4.50 ± 0.08	39.78 ± 5.51	29.71 ± 3.34
	TT	0.95 ± 0.30	4.40 ± 1.08	3.20 ± 0.42	3.80 ± 0.26	31.60 ±16.35	26.00 ± 9.92
P value		0.752	0.171	0.033*	0.004**	0.436	0.578
g.13104 A>C	AA	0.87± 0.11	5.08 ± 0.4	4.05 ± 0.15	4.62 ± 0.10	48.47 ± 5.93	35.45 ± 3.74
	AC	0.76 ± 0.11	4.91 ± 0.4	3.80 ± 0.15	4.51 ± 0.10	38.77 ± 5.93	29.27 ± 3.74
	CC	0.72 ± 0.25	5.41 ± 0.88	3.38 ± 0.34	4.16 ± 0.22	36.30 ± 2.99	23.41 ± 8.20
P value		0.441	0.320	0.187	0.250	0.305	0.156
g.13111 A>G	AA	0.88 ± 0.12	5.17 ± 0.44	3.65 ± 0.17	4.41 ± 0.11	45.84 ± 6.55	31.67±4.13
	AG	0.65 ± 0.12	3.96 ± 0.43	3.80± 0.17	4.56 ± 0.11	26.76 ± 6.47	25.88 ± 4.08
	GG	0.50 ± 0.41	8.50 ± 1.40	5.00 ± 0.55	4.50 ± 0.35	59.50 ± 0.83	31.50 ±13.15
P value		0.069	0.004**	0.221	0.920	0.005**	0.055
g.13165 A>G	AA	0.85 ± 0.09	4.42 ± 0.32	3.82 ± 0.12	4.6 ± 0.08	39.50 ± 4.93	32.22 ± 2.99
	AG	0.91 ± 0.29	3.45 ± 1.05	2.89 ±0.41	3.75 ± 0.25	27.39 ± 5.83	24.35 ± 9.60
	GG	0.90 ± 0.18	6.80 ± 0.65	4.40 ± 0.25	4.60 ± 0.15	48.20 ± 9.86	33.00 ± 5.98
P value		0.871	0.03*	0.006**	0.003**	0.313	0.482
g.13173 A>T	AA	0.89 ± 0.13	5.50 ± 0.44	3.90 ± 0.17	4.53 ± 0.11	50.97 ± 6.60	34.24 ±4.16
	AT	0.66 ± 0.17	4.34 ± 0.61	3.48 ± 0.24	4.30 ± 0.15	29.86 ± 9.06	24.16 ± 5.72
	TT	0.88 ± 0.19	6.66 ± 0.66	4.55 ± 0.26	4.66 ± 0.16	46.22 ± 9.82	33.66 ± 6.20
P value		0.970	0.065	0.004**	0.006**	0.462	0.730

TDMY: Test day milk yield, TDFY: Test day fat yield, TDPY: Test day protein yield
*P<0.05; ** P<0.01. Different superscript letters among the genotypes indicate significant differences. Values are expressed as means ± Standard errors.

4.12. Association of *MBLG* SNP genotypes with milk production traits in Borgou and White Fulani cattle breeds

The effect of *MBLG* genotypes on milk fat and total solids contents in Borgou cattle breed is shown in Figure 4.8. The *MBLG* SNPs did not show significant (P>0.05) association with

milk production traits in White Fulani cattle breed. Only the *MBLG* g.5940G>A polymorphism showed significant association with fat content and total solids in Borgou breed. The *MBLG* AG genotype was significantly associated with higher fat (6.8% vs 4.6% vs 4.5, P<0.05) and total solids (16.8% vs 13.7% vs 13.5%, P<0.05) contents compared to AA and GG genotypes, respectively (Figure 4.8).



*P<0.05; TS: Total solids

Figure 4.8: Effect of *MBLG* (g.5940G>A) genotypes on milk fat and total solids contents in Borgou

4.13. Association of *CSN1S1* SNP genotypes with milk production traits in Borgou and White Fulani cattle breeds

The effect of the SNPs in 5'flanking region of *CSN1S1* on milk production traits in Borgou and White Fulani are presented in Table 4.18 and Table 4.19 respectively. The *CSN1S1* g.10430 G>A genotypes showed significant association with milk yield in Borgou. The AG genotype of *CSN1S1* g.10430 G>A was associated with higher (P<0.05) test-day milk yield (1.18 kg) compared to AA genotype (0.67 kg) and GG genotype (0.78 kg) (Table 4.18). However, the *CSN1S1* g.10331 A>G and g.10359 T>C SNPs did not show any significant

association with milk production traits in Borgou. On the other hand, the TT genotype of *CSN1S1* g.10359 T>C was associated with higher (P<0.01) test-day milk yield and higher (P<0.05) test-day fat and protein yields in White Fulani (Table 4.19). Moreover, the GG genotype of *CSN1S1* g.10430 G>A was associated with higher (P<0.01) test-day milk yield in White Fulani (Table 4.19).

Table 4.18: Effect of *CSN1S1* (5'flanking region) SNPs on milk production traits in Borgou cattle breed

Locus	Genotype	TDMY (kg)	Fat (%)	Protein (%)	Lactose (%)	TDFY (g)	TDPY (g)
g.10331 A>G	AA	0.87 ± 0.07	5.04 ± 0.26	3.92 ± 0.10	4.53 ± .06	42.81 ± 3.56	32.34 ± 2.14
	GG	0.33 ± 0.33	4.00 ± 1.30	3.33 ± 0.51	4.67 ± 0.31	19.00 ± 17.92	15.67 ± 10.76
P value		0.104	0.735	0.323	0.616	0.387	0.198
g.10359 T>C	CC	0.75 ± 0.17	4.08 ± .64	3.92 ± .26	4.41 ± .15	34.42 ± 8.93	30.67 ± 5.41
	CT	1.12 ± 0.14	5.06 ± .54	3.82 ± .21	4.59 ± .13	50.88 ± 7.51	37.65 ± 4.55
	TT	0.77 ± 0.08	5.20 ± .31	3.90 ± .13	4.55 ± .07	39.74 ± 4.33	29.61 ± 2.63
P value		0.081	0.299	0.945	0.671	0.314	0.311
g.10430 G>A	AA	0.67 ± 0.13a	4.06 ± .52	3.78 ± .21	4.56 ± .13	32.06 ± 7.24	28.00 ± 4.39
	AG	1.18 ± 0.14b	5.06 ± .53	3.88 ± .22	4.59 ±0.13	51.88 ± 7.45	38.71 ± 4.52
	GG	0.78 ± 0.08a	5.36 ±0.33	3.93 ±0.13	4.51 ± .08	41.02 ± 4.58	30.13 ± 2.78
P value		0.019*	0.110	0.824	0.867	0.168	0.187

TDMY: Test day milk yield, TDFY: Test day fat yield, TDPY: Test day protein yield
*P<0.05. Different superscript letters among the genotypes indicate significant differences. Values are expressed as means ± Standard errors.

Table 4.19: Effect of *CSN1S1* (5'flanking region) SNPs on milk production traits in White Fulani cattle breed

Locus	Genotype	TDMY (kg)	Fat (%)	Protein (%)	Lactose (%)	TDFY (g)	TDPY (g)
g.10331 A>G	AA	1.17 ± 0.06	4.91± 0.20	3.87± 0.10	4.79± 0.05	55.05 ± 2.97	42.69 ± 2.07
	GG	1.00 ± 0.53	7.00± 1.73	4.00± 0.84	5.00± 0.41	70.00± 25.72	45.00 ±17.91
P value		0.746	0.232	0.876	0.609	0.565	0.899
g.10359 T>C	CC	0.94^{a} ± 0.12	5.00 ±0.41	3.94 ±0.20	4.78± 0.10	47.11^{a} ± 5.85	35.67^{a} ± 4.08
	CT	1.04^{a} ± 0.09	4.82± 0.34	4.04± 0.16	4.70± 0.08	50.63^{a} ± 4.78	41.19^{a} ± 3.33
	TT	1.42^{b} ± 0.09	5.00± 0.32	3.67± 0.15	4.87± 0.07	64.00^{b} ± 4.46	48.16^{b} ± 3.11
P value		0.002**	0.907	0.243	0.303	0.040*	0.049*
g.10430 G>A	AA	0.95^{a} ± 0.11	5.15± 0.39	3.95± 0.19	4.80± 0.09	50.35 ± 5.64	37.15 ± 3.90
	AG	1.04^{a} ± 0.09	4.82± 0.34	4.04± 0.16	4.70± 0.08	50.63 ± 4.85	41.19 ± 3.36
	GG	1.45^{b} ± 0.09	4.90± 0.33	3.66± 0.15	4.86± 0.08	62.93 ± 4.68	48.00 ± 3.24
P value		0.001**	0.802	0.208	0.790	0.355	0.121

TDMY: Test day milk yield, TDFY: Test day fat yield, TDPY: Test day protein yield
*P<0.05; ** P<0.01. Different superscript letters among the genotypes indicate significant differences. Values are expressed as means ± Standard errors.

4.14. Effects of *SCD1* A293V genotypes on milk components and fatty acid traits

The effect of *SCD1* A293V genotypes on milk components and fatty acids traits in White Fulani and Borgou cattle breeds is presented in Table 4.20.Only four Borgou cows and one White Fulani cow were of AA genotype at the *SCD1* locus. The AA genotype was therefore not included in the association analysis. The *SCD1* VV genotype was associated with higher (P<0.05) protein and lactose contents and lower (P<0.05) C18:1 *cis*-9 content in White Fulani (Table 4.20). On the other hand, the *SCD1* AV genotype was associated with higher (P<0.01) C14 index and total index compared to the VV genotype in Borgou (Table 4.20).

Table 4.20: Effect of *SCD1* A293V genotypes on milk components and fatty acids traits in White Fulani and Borgou cattle breeds

Trait	SCD1 genotypes in White Fulani			SCD1 genotypes in Borgou			
	VV ± SE (n = 84)	AV ± SE (n = 10)	p-value	VV ± SE (n = 61)	AV ± SE (n = 20)	AA ± SE (n = 4)	p-value
Milk production traits							
Fat (%)	4.82 ± 0.19	4.80 ± 0.53	0.887	4.74 ± 0.28	5.7 ± 0.49	3.25 ± 1.09	0.075
Protein (%)	3.90 ± 0.09	3.2 ± 0.25	0.023*	3.84 ± 0.11	4.05 ± 0.20	3.50 ± 0.44	0.438
Lactose (%)	4.84 ± 0.04	4.60 ± 0.12	0.024*	4.44 ± 0.12	4.65 ± 0.13	4.83 ± 0.28	0.282
Fatty acids and unsaturation indices (%)							
Caproic acid (C6:0)	0.25 ± 0.04	0.07 ± 0.12	0.366	0.25 ± 0.03	0.43 ± 0.06	0.13 ± 0.12	0.010*
Caprylic acid (C8:0)	0.10 ± 0.03	0.00 ± 0.08	0.573	0.38 ± 0.05	0.59 ± 0.08	0.21 ± 0.18	0.040*
capric acid (C10:0)	0.50 ± 0.09	0.30 ± 0.27	0.671	1.14 ± 0.08	1.25 ± 0.15	0.76 ± 0.33	0.384
lauric acid (C12:0)	0.94 ± 0.11	0.50 ± 0.32	0.300	1.68 ± 0.15	1.28 ± 0.26	1.35 ± 0.58	0.386
12-Methyl Tridecanoic acid (C13:0)	0.10 ± 0.04	0.00 ± 0.10	0.646	0.38 ± 0.06	0.32 ± 0.1	0.35 ± 0.22	0.839
Myristoleic acid (C14:1 *cis*-9)	0.45 ± 0.09	0.10 ±0.25	0.365	0.95 ± 0.09	1.31 ± 0.16	0.58 ± 0.35	0.065
Myristic acid (C14:0)	4.10 ± 0.27	5.10 ± 0.80	0.469	12.54 ±0.72	11.66 ± 1.26	9.6 ± 2.82	0.536
pentadecanoic acid (C15:0)	2.13 ± 0.18	1.80 ± 0.53	0.682	4.13 ±0.35	4.97 ± 0.62	3.27 ± 1.38	0.379
Palmitic acid (C16:0)	2.14 ± 0.23	1.30 ± 0.65	0.478	5.46 ± 1.01	3.05 ± 1.76	13.60 ± 3.94	0.053
Margaric acid (C17:0)	2.82 ± 0.25	3.10 ± 0.72	0.434	7.01 ± 0.51	8.56 ± 0.89	6.26 ± 1.99	0.278
Linoleic acid (C18:2 *cis*-9, *cis*-12)	3.44 ± 0.29	3.30 ± 0.83	0.650	16.09 ± 0.76	15.11 ± 1.34	15.70 ± 2.98	0.815
Oleic acid (C18:1 *cis*-9)	2.92 ± 0.27	3.70 ±0.78	0.039*	16.08 ± 0.85	19.14 ± 1.48	12.43 ± 3.32	0.093
Stearic acid (C18:0)	2.82 ± 0.26	2.40 ± 0.74	0.821	13.32 ± 0.64	11.65 ± 1.11	13.87 ± 2.49	0.400
Nonadecanoic acid (C19:0)	0.19 ± 0.09	0.00 ± 0.79	0.753	0.88 ± 0.20	1.05 ± 0.35	1.78 ± 0.77	0.512
Arachidic acid (C20:0)	0.03 ± 0.02	0.00 ± 0.05	0.878	0.19 ± 0.03	0.16 ± 0.05	0.45 ± 0.11	0.062
C14 index[1]	8.80 ± 0.54	6.10 ± 1.57	0.269	6.93^{a} ± 1.81	18.94^{b} ± 3.15	5.57^{a}±7.05	0.005**
C18 index[2]	46.59 ± 2.53	39.77 ± 7.35	0.515	54.71 ± 1.77	59.85 ± 3.09	48.22 ± 6.90	0.199
Total index[3]	36.56 ± 2.00	31.46 ± 5.80	0.598	61.64^{a} ± 2.80	78.79^{b} ± 4.89	53.79^{a} ± 10.94	0.007**
SFA[4]	67.96 ± 1.08	68.56 ± 3.14	0.968	47.36 ± 0.88	44.96 ± 1.54	51.61 ± 3.44	0.161
MUFA[5]	20.02 ± 1.21	16.36 ± 3.52	0.499	17.03 ± 0.86	20.45 ± 1.50	13.02 ± 3.35	0.058
PUFA[6]	12.02 ± 0.78	15.08 ± 2.26	0.332	16.09 ± 0.76	15.11 ± 1.34	15.70 ± 2.98	0.815

* P< 0.05, ** P<0.01. [1] C14 index=C14:1 *cis*-9/ (C14:1 *cis*-9+ C14 :0) × 100. [2] C18 index= C18:1 *cis*-9/ (C18:1 *cis*-9+ C18 :0) × 100. [3] Total index= (C14:1 *cis*-9+ C18:1 *cis*-9)/ (C14:1 *cis*-9+ C14:0+ C18:1 *cis*-9+ C18:0) × 100. [5] SFA= saturated fatty acid; [6]MUFA= monounsaturated fatty acid; [6]PUFA= polyunsaturated fatty acid. SE = standard error of the mean. SCD1 AA genotype was not included in the analysis for While Fulani because only one individual of White Fulani breed was AA. Means with different superscript letters across genotypes differ significantly.

4.15. Effects of the *DGAT1* K232A genotypes on milk components and fatty acid traits

The *DGAT1* K232A genotypes did not significantly (P>0.05) affect milk composition, fatty acid profiles and unsaturation indices in Borgou breed and White Fulani (Appendix 4). However, the p-values for C14:0, C15:0 and C19:0 tended towards significance (P<0.1) in Borgou where the *DGAT1* KK genotype seems to show higher C14:0 and C15:0, and lower C19:0 contents (Appendix 4).

4.16. Effect of *SCD1* and *DGAT1* allele substitution on milk fatty acid traits in Borgou and White Fulani

The estimates of the *SCD1* 293V allele substitution effect in Borgou breed are presented in Table 4.21. In comparison to the A allele, the V allele was associated with decrease in C14 index (-5.68%, P<0.05). However, no significant (P>0.05) 293V allele substitution effect was observed for C18 and total indices, and MUFA in Borgou (Table 4.21).

On the other hand, the allele substitution effects indicated that the *DGAT1* 232K allele was associated with increased total saturated fatty acid (SFA, +5.41%, P<0.05), and with decreased C18 index (-12.16%, P<0.05), total index (-12.81%, P<0.01) and MUFA (-8.09%, P<0.01) in White Fulani breed (Table 4.21).

Table 4.21: Effect of *SCD1* V and *DGAT1* K alleles substitution on fatty acids unsaturation in Borgou and White Fulani cattle breeds respectively

	SCD1 V (Borgou)			*DGAT1* K (White Fulani)		
Traits	Estimates	SE	*P*-value	Estimates	SE[3]	*P*-value
C14 index	-5.68	2.7	0.048*	2.23	1.26	0.078
C18 index	-0.96	0.27	0.724	-12.16	5.84	0.040*
Total index	-2.11	0.24	0.385	-12.81	4.53	0.008**
SFA[1]	0.14	0.01	0.916	5.41	2.4	0.031*
MUFA[2]	-0.708	1.33	0.596	-8.09	2.72	0.004**

* P<0.05; ** P<0.01; [1]SFA= saturated fatty acid; [2]MUFA= monounsaturated fatty acid, [3]SE=standard errors

4.17. Genetic diversity and gene flow

The genetic diversity estimates within and between Borgou and White Fulani using 14 loci related to milk production are presented in Table 4.22 and Table 4.23. Significant differences were found between the observed and expected heterozygosities of Borgou ($P<0.01$) and White Fulani ($P<0.001$) (Table 4.22). The observed heterozygosity values per locus ranged from 0.000 (White Fulani) to 0.871 (Borgou) and the expected heterozygosity from 0.026 (White Fulani) to 0.503 (Borgou) (Table 4.22). The 0 heterozygosity was observed only at the *CSN1S1* g.10331 A>G locus and in White Fulani breed. Borgou presented higher ($P<0.01$) means of observed heterozygosity and expected heterozygosity (0.329, 0.353) than White Fulani (0.286, 0.302) (Table 4.23). The mean number of observed (MNA) alleles per locus was 2±0.000 in both breeds. Moreover, the means expected number of alleles (MNE) were similar ($P>0.05$) in Borgou (1.589 ± 0.274) and White Fulani (1.498 ± 0.332).

The Multi-locus estimates of Wright's F-statistics genetic differentiation, f (within population inbreeding), F (total inbreeding) and θ (genetic differentiation), and gene flow between Borgou and White Fulani populations are shown in Table 4.24. The f and F were respectively 0.058 and 0.074 and were not significantly different from zero (Table 4.24). Although the within population and total inbreeding coefficients were not significant, within population heterozygosity deficiency was observed *at* the studied loci except the *DGAT1, LALBA, MBLG* g.5864C>T and *CSN3* g.13111 A>G (Table 4.24). The population differentiation (θ) was 0.017 and significantly different from zero ($P<0.01$) (Table 4.24). The highest θ (0.048) and the lowest θ (0.003) were observed at *LALBA* and *CSN3* g.13111 A>G loci respectively (Table 4.24). High level of gene flow ($N_e m=14.104$) was observed between Borgou and White Fulani cattle populations at the typed loci (Table 4.24). The highest movement of genes was

observed at the *CSN3* g.13111 A>G locus (N_em=88.400) and the lowest at the *LALBA* locus (N_em= 4.975).

Table 4.22: Observed (H_{ob}) and expected (H_{ex}) heterozygosities per locus and breed

	Borgou		White Fulani	
Locus	H_{ob}[a]	H_{ex}[b]	H_{ob}	H_{ex}
SCD1 A293V	0.235	0.277	0.104	0.118
DGAT1 K232A	0.374	0.362	0.144	0.152
LALBA (A/B)	0.871	0.503	0.521	0.415
MBLG g.5864C>T	0.303	0.277	0.122	0.115
MBLG g.5940G>A	0.105	0.146	0.200	0.216
CSN3 g.13065 C>T	0.470	0.454	0.469	0.498
CSN3 g.13068 C>T	0.386	0.442	0.365	0.372
CSN3 g.13104 A>C	0.374	0.446	0.375	0.377
CSN3 g.13111 A>G	0.253	0.257	0.229	0.204
CSN3 g.13165 A>G	0.398	0.437	0.375	0.377
CSN3 g.13173 A>T	0.398	0.428	0.375	0.377
CSN1S1 g.10331 A>G	0.013	0.084	0.000	0.026
CSN1S1 g.10359 T>C	0.213	0.384	0.359	0.487
CSN1S1 g.10430 G>A	0.213	0.446	0.359	0.495
Mean (SD[c])	0.329** (0.201)	0.353 (0.126)	0.286 (0.152)	0.302*** (0.159)

[a]$H_{ob=}$ observed heterozygosity; [b]$H_{ex=}$ expected heterozygosity or gene diversity; [c]SD= standard deviation.
** P <0.01; *** P <0.001

Table 4.23: Significance of genetic diversity indices (H_{ob}, H_{ex}, MNA and MNE) between Borgou and White Fulani populations (Mean ± Standard deviation)

	Borgou	White Fulani	Significance
H_{ob}	0.329 ± 0.201	0.286 ±0.152	**
Hex	0.353 ± 0.126	0.302 ± 0.159	***
MNA	2.000 ± 0.000	2.000 ± 0.000	NS
MNE	1.589 ± 0.274	1.498 ± 0.332	NS

$H_{ex=}$ expected heterozygosity; MNA=Mean observed number of alleles; MNE= mean effective number of alleles

*** $P < 0.001$; NS= not significant

Table 4.24: Multi-locus estimates of F-statistics and gene flow in Borgou and White Fulani

Locus	*f*	*F*	θ	N_em
SCD1 A293V	0.135	0.157	0.026	9.390
DGAT1 K232A	-0.014	0.030	0.044	5.494
LALBA (A/B)	-0.524	-0.451	0.048	4.975
MBLG g.5864C>T	-0.091	-0.061	0.027	9.117
MBLG g.5940G>A	0.152	0.156	0.005	48.058
CSN3 g.13065 C>T	0.008	0.021	0.013	19.645
CSN3 g.13068 C>T	0.072	0.080	0.008	31.191
CSN3 g.13104 A>C	0.085	0.092	0.008	30.924
CSN3 g.13111 A>G	-0.051	-0.048	0.003	88.400
CSN3 g.13165 A>G	0.046	0.051	0.006	42.177
CSN3 g.13173 A>T	0.035	0.039	0.004	61.118
CSN1S1 g.10331 A>G	0.885	0.886	0.009	28.481
CSN1S1 g.10359 T>C	0.339	0.357	0.027	9.118
CSN1S1 g.10430 G>A	0.389	0.396	0.012	21.341
Mean (SE[a])	0.058^{NS}±0.041	0.074^{NS} ±0.040	0.017^{**}±0.002	14.104

f= within population inbreeding estimate; *F*= total inbreeding estimate; θ= measure of population differentiation; N_em=gene flow

[a] Standard error

** $P < 0.01$. NS: Not significant.

4.18. Nei's D_A genetic distance and Phylogenetic relationship between Borgou and White Fulani

The Nei's D_A genetic distance between Borgou and White Fulani cattle populations was 0.017 (Table 4.25). The Phylogenetic relationships among Borgou (*Bos taurus*), White Fulani (*Bos indicus*), Indian zebu (*Bos indicus*) and Ethiopian Sheko (*Bos taurus*) indigenous cattle breeds based on *CSN3* gene is shown in Figure 4.9. The water buffalo (*Bubalus bubalis*) was used as an outgroup in the phylogenetic tree construction. According to the tree, the Beninese Borgou and White Fulani were closely related. The Ethiopian Sheko separated from Borgou and White Fulani while the Indian zebu was diverged more from all other breeds (Figure 4.9).

Table 4.25: Nei's D_A genetic distance (below diagonal) and genetic identity (above diagonal) estimates between breed using 14 loci related to milk production

	Borgou	White Fulani
Borgou	-	0.983
White Fulani	0.017	-

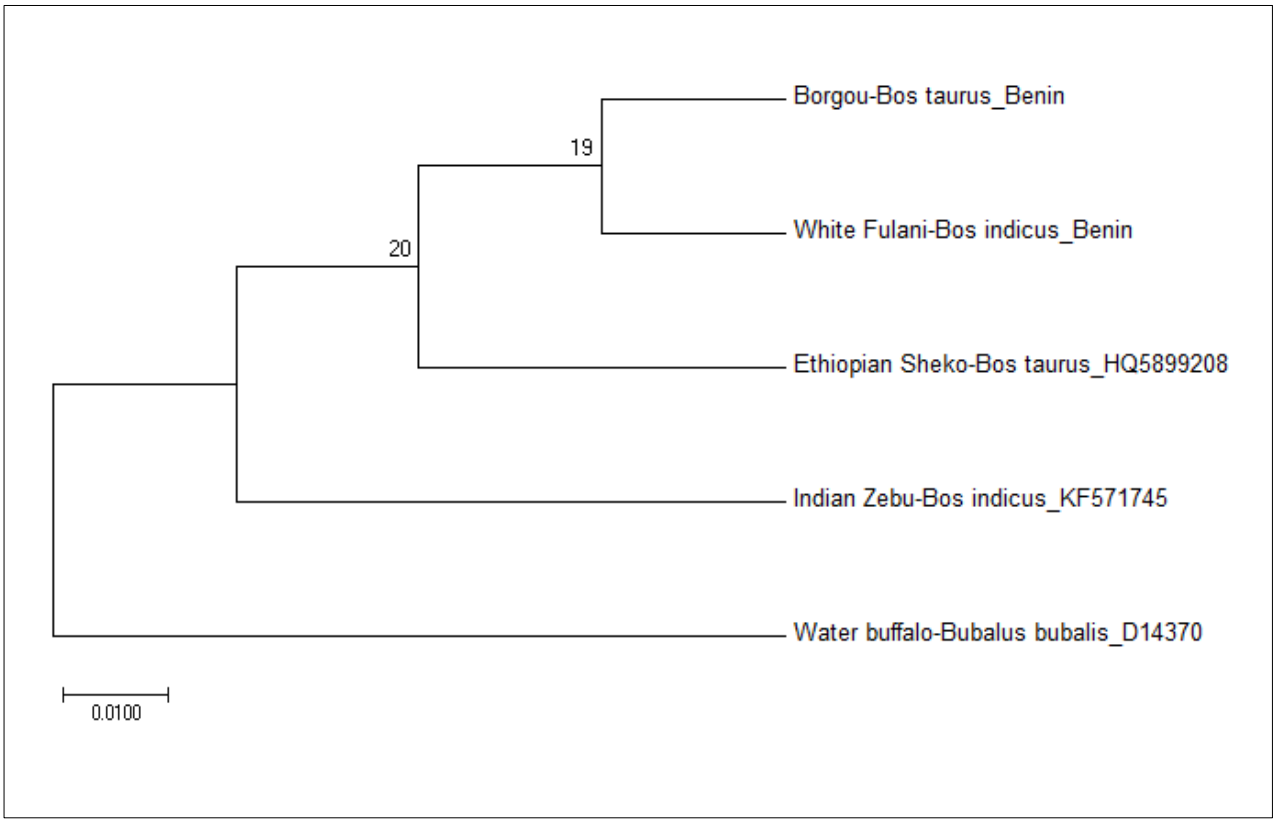


Figure 4.9: Phylogenetic tree constructed using the Neighbor-Joining method (Saitou and Nei, 1987) showing genetic relationships among Borgou, White Fulani, Indian zebu and Ethiopian Sheko indigenous cattle breeds based on *CSN3* gene.

The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (10000 replicates) are shown next to the branches (Felsenstein, 1985). The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2004) and are in the units of the number of base substitutions per site. Evolutionary analyses were conducted in MEGA7 (Kumar et al., 2016).

CHAPTER FIVE

DISCUSSION

5.1. Effect of breed on milk components and fatty acid traits

The present study showed the variation of milk components and fatty acid composition as well as fatty acid unsaturation indices in indigenous White Fulani and Borgou cows in Benin. White Fulani produced milk with higher test-day milk, fat and protein yields, and fat content than Borgou. Previous studies reported the breed effect on milk composition in cattle, sheep and goat from Nigeria (Malau-Aduli and Anlade, 2002; Ochepo et al., 2015) and South African local cattle breeds (Myburgh et al., 2012). It is important to highlight that the observed test-day milk yields in the present study do not reflect the true milk yield of Borgou and White Fulani since it was a single day record and may be affected by the person milking. Milk from White Fulani and Borgou cows showed higher fat content (5.49 and 4.51 %, respectively) than the values of 2.68%, 2.01%, and 3.79% reported in South African indigenous Boran, Tuli and Afrikaner cattle, respectively (Myburgh et al., 2012). Moreover, the protein and fat contents of Borgou (3.76 % and 4.51 %, respectively) and White Fulani (3.80 % and 5.49 %, respectively) are in the same order of magnitude and comparable to the reported in Western dairy breeds such as Brown Swiss (3.75% and 4.28%), Jersey (4.07% and 5.59%) and Holstein Friesian (3.47% and 4.01%) (Stocco et al., 2016).

Several factors influence milk fatty acid composition such as species, breed, individual variability, nutrition, stage of lactation, parity and season (Poulsen et al., 2012; Gottardo et al., 2017). In the present study, significant differences between breeds were observed for individual fatty acids, fatty acid unsaturation indices and fatty acid groups. The milk of Borgou breed had higher MUFA and PUFA content and C18 and total indices than White Fulani. Breed effect on fatty acid composition was reported in the meat of Borgou and White

Fulani cattle in Benin (Salifou et al., 2013), in the milk of South African indigenous cattle breeds (Myburgh et al., 2012) and Italian Holstein-Friesian, Brown Swiss, Simmental and Alpine cattle breeds (Gottardo et al., 2017). In this study, White Fulani presented the highest content of SFA (68.19 vs. 58.46%, $P<0.001$) and the lowest content of PUFA (12.40 vs. 19.56%) compared to Borgou, and similar MUFA content (19.41 vs. 21.98%) for both breeds. These results corroborate previous studies in Benin indicating that the meat of White Fulani bulls had higher SFA content (49.68%) compared to Borgou (43.03%) and similar MUFA content (33.60% vs. 33.43%) (Salifou et al., 2013). In the current study, Borgou presented higher C18 index and total unsaturation index than White Fulani. The unsaturation or desaturation index of a specific fatty acid represents the ratio of the concentration of the monounsaturated product to the sum of the monounsaturated and the saturated substrate (Mele et al., 2007a). Considering human health aspects, increasing the amount of unsaturated fatty acids as well as unsaturation indices is an important selection objective (Schennink et al., 2008). Borgou presented lower SFA (58.46%) than the values of 64%, 63.7%; 60.9% and 71.9% reported in milk of free-ranging South African indigenous Boran, Nguni, Tuli and Afrikaner cattle breeds, respectively (Myburgh et al., 2012). Moreover, Borgou and White Fulani presented lower MUFA content (21.98% and 19.41%, respectively) than South African indigenous cattle (MUFA content ranged from 25.7% in Afrikaner to 36.5% in Tuli breed) (Myburgh et al., 2012). On the contrary, Borgou and White Fulani produced milk with C18:2 *cis*-9, *cis*-12 of 15.84% and 9.85% respectively , which was much higher than the range of 1.3 to 1.7% observed in South African indigenous Boran, Nguni, Tuli and Afrikaner (Myburgh et al., 2012). The difference in C18:2 *cis*-9, *cis*-12 content between Borgou/White Fulani and South African indigenous cattle may be due to the lower sample size in their study being 6 Boran, 9 Nguni, 10 Tuli and 6 Afrikaner (Myburgh et al., 2012).

The suggested favorable combination of bovine milk fatty acid composition for human health enhancement is ~30% SFA, 60% MUFA, and 10% PUFA (Hayes and Khosla, 1992). In the present study, the milk fatty acid compositions were 58.48% SFA, 21.98 % MUFA and 19.56 PUFA for Borgou and 68.19 % SFA, 19.41% MUFA and 12.40% PUFA for White Fulani. It is clear that the current milk fatty acid composition of Borgou and White Fulani is far from optimal and there is need for modification towards an ideal profile. Linoleic acid (a PUFA) and oleic acid (a MUFA) have been associated with decreased serum total cholesterol and low-density lipoprotein cholesterol levels and reduced risk of coronary heart diseases in humans (Mensink et al., 2003). Oleic acid has anticancer and antiatherogenic properties (Haug et al., 2007). Linoleic acid, an essential fatty acid in the omega-6 family is associated with reduced incidence of types 2 diabetes through its ability to improve sensibility to insulin (Hu et al., 2001). On the other hand, palmitic acid (C16:0), considered as hypercholesterolemic is responsible for the increase in the concentration of low density lipoproteins (LDL) that are associated with coronary heart diseases in humans (Rotta et al., 2009). For palmitic acid, the value in White Fulani (16.19%) was significantly above the Borgou (5.27%). Borgou milk with its higher linoleic acid, oleic acid and lower total SFA contents as compared to White Fulani with higher total SFA and palmitic acid contents, may present greater health benefits to humans than White Fulani milk.

5.2. Effect of age, herd, parity and stage of lactation on milk components

The age of cow did not have significant effect on major milk component in White Fulani. This is not in agreement with previous studies which showed that the milk protein content was significantly lower in young Holstein cows than adult cows (Gurmessa and Melaku, 2012; Pratap et al., 2014). On the other hand, Pratap et al. (2014) found no significant difference

between age groups for fat and lactose contents which is similar to the results from the present study.

In the present study, high variability was observed between herds for fat, protein, lactose and milk urea nitrogen contents in White Fulani and for test-day milk, protein and fat yields in Borgou. The effect of herd on cow milk components has been reported in many studies (Jílek et al., 2006; Stocco et al., 2016). The variability of milk components between herds may be attributed to the differences in day-to-day herd's management. High variability of milk urea nitrogen between herds was reported in Czech Holstein cows (Jílek et al., 2006). Milk urea nitrogen corresponds to the portion of milk non-protein nitrogen and is an indicator of the amount of degradable protein in the rumen (Gustafsson and Palmquist, 1993). High levels (>12 mg/dl) of milk urea nitrogen in milk indicate an imbalance between the cow's intake of protein and energy and this may affect feed cost, fertility, production efficiency and the environments (Jonker et al., 2002). However, the observed milk urea nitrogen contents in Borgou (10.33 mg/dl) and White Fulani (8.04 mg/dl) were in the normal range of 8-12 mg/dl.

The present study showed that the Borgou cows on second parity presented higher test-day milk and protein yields than the cows on other parities. However, it was reported that Korean Holstein dairy cows showed the highest milk yield at the third lactation (Vijayakumar et al., 2017). This difference in results may be explained by differences in breed. The test-day milk yield observed in Borgou breed in the present study increased from first to second lactation (0.84-1.12 kg) and decreased to 0.82 kg from the third lactation. The effect of parity number on daily milk yield was reported in Borgou, N'Dama and Girolando cows in Benin (Gbangboché and Alkoiret, 2011; Alkoiret et al., 2011b). At the Okpara breeding farm in Benin, it was reported that the daily milk yield in Borgou cattle breed increased from first lactation (0.4 l) to the fifth lactation (1.1 l) (Gbangboché and Alkoiret, 2011). Similarly, the daily milk yield in indigenous N'Dama cattle breed varied from 0.5 l to 1 l for first and fifth

lactation respectively and decreased to 0.6 l at the sixth lactation (Gbangboché and Alkoiret, 2011). Another study in Girolando cattle breed in Benin showed that the daily milk yield increased from first lactation (5.9 to 6.14 l) to the third lactation (8.43 to 8.74 l) and decreased thereafter to 7.79 l in the seventh lactation (Alkoiret et al., 2011b). In the current study, the parity number did not show any significant effect on fat, protein and lactose contents in Borgou. Similar results were reported in Holstein Friesian cows (Gurmessa and Melaku, 2012; Pratap et al., 2014). However, White Fulani on parity 3 and more showed higher lactose content which was not the case in Borgou breed.

In the present study, the milk fat content was higher in late lactation than the mid lactation in White Fulani and this is similar to what was observed in Holstein cows in Ethiopia (Gurmessa and Melaku, 2012). The stage of lactation did not significantly affect the test-day milk yield and milk component in Borgou breed from this study. However, the fat content seemed to be higher in early and late lactation than mid lactation in Borgou. This is in agreement with previous studies showing that milk fat contents was lower at mid lactation compared to early and late lactation in Holstein-Friesian cows (Stoop et al., 2009; Gurmessa and Melaku, 2012; Pratap et al., 2014). However, Bohmanova et al. (2009) found that milk fat content was especially lower at late lactation in Canadian Holstein cows.

5.3. Effect of geographical region on milk fatty acid composition

Studied animals in the present study were raised in the traditional system on natural grazing without concentrate supplementation and they were sampled at the same period eliminating the effect of season. The observed difference between breeds would therefore be due to their genetic background or herd management styles that may vary from one region to another. Furthermore, the difference in major milk components and fatty acid composition between the two breeds could also be due to the composition of the forage consumed by the cows on

natural grazing. The White Fulani and Borgou are raised in different agro-ecological zones with different floristic composition (Salifou et al., 2013). The White Fulani cattle are found in the Northern part while Borgou cattle are found throughout the country. The forage species and variety, climate and stage of growth are important factors that affect fatty acid content and composition of forage (Kalač and Samková, 2010; Salifou et al., 2013) and can therefore affect the milk fatty acid composition of the cows. The Borgou cows from the North of Benin produced milk with higher C14:1 *cis*-9, C18:1 *cis*-9, C14 index, C18 index, total index and MUFA contents than Borgou cows from the South of Benin. Consequently, milk from Borgou cows raised in the North of Benin seems to be healthier than from Borgou cows raised in the South of Benin due to its higher content of MUFA and higher unsaturation indices

5.4. Genetic polymorphisms and associations with milk production and fatty acids traits

5.4.1. Effect of *LALBA* gene

In the present study, the *LABA* genotypes were not in Hardy-Weinberg Equilibrium. This may be explained by the pressure of natural selection since the indigenous cattle breeds have not been selected for any production traits in the past. The frequencies of A and B alleles of LALBA were 0.29 and 0.71 respectively and similar to the reported frequencies of 0.24 and 0.76 of *LALBA* A and B variants respectively in Nigerian White Fulani (Ibeagha-Awemu et al., 2005). Study results did not agree with fixed B allele in Muturu and N'Dama *Bos taurus* cattle breeds (Ibeagha-Awemu et al., 2005).The fixed B allele observed in Mturu and N'Dama maybe due to low sample size of 20 Muturu and 26 N'Dama used in their study (Ibeagha-Awemu et al., 2005). In the present study, the *LALBA* gene polymorphisms did not show any significant effect in the production and fatty acid traits of the studied breeds. This may be due to a breed-gene interaction and suggests investigation of the effect of the *LALBA* gene in a large number population of Borgou and White Fulani.

5.4.2. Effect of *CSN3* gene

The Kappa-casein (*CSN3*) is one of the functional candidate genes affecting milk composition and milk production traits in cattle. Polymorphisms in kappa-casein (κ-CN) have been extensively studied in cattle due to their association with milk composition and cheese-making properties (Grosclaude, 1988; Caroli et al., 2009; Martin et al., 2013). Most of the previous studies on bovine *CSN3* focused on the genotypes and allele frequencies of the two most common variants, A and B (Alim et al., 2014). The *CSN3* A variant is characterized by the threonine and aspartic acid at the positions 136 and 148 respectively while the *CSN3* B variant is characterized by isoleucine and alanine at the position 136 and 148 respectively in the mature protein (Farrell et al., 2004). Previous study showed that polymorphisms in bovine *CSN3* gene are associated with milk yield and protein contents (Morkūnienė et al., 2016) and fat, protein and lactose contents (Hamza et al., 2011). Djedovic et al. (2015) demonstrated that the *CSN3* genotypes have significantly influenced milk and milk fat yield in cattle breeds and crossbreds from Serbia. Another association study has shown that the AB genotype of kappa-casein is associated with higher milk production in Sahiwal cattle population of Pakistan and it was therefore suggested that incorporation of AB and BB genotypes for *CSN3* may help to improve the milk yield in this cattle population (Mir et al., 2014). However, there is a very limited data on *CSN3* SNPs association with milk composition and production traits in cattle. A recent study investigated the effect of the SNPs in *CSN3* on milk production and composition traits in Chinese Holstein cattle breed (Alim et al., 2014). The present study is the first to report the genetic effects of *CSN3* SNPs on milk production and composition traits in indigenous Borgou and White Fulani cattle breeds in Benin. Six SNPs were found in *CSN3* gene partial exon IV and intron IV in Borgou and White Fulani resulting in seven haplotypes in Borgou and five haplotypes in White Fulani. The haplotype diversity is high in Borgou and White Fulani compared to the four haplotypes identified in Chinese Holstein cattle (Alim et

al., 2014). In the present study g.13068 C>T showed significant association with protein content while the g.13165 A>G was associated with fat and protein contents in Borgou. These results are similar to the reported in Chinese Holstein cattle breed (Alim et al., 2014). However, the *CSN3* g.13104 A>C (g. 10924 A>C, based on NC007304 reference sequence) which showed significant association with milk fat and protein percentages in Chinese Holstein did not show any significant effect in Borgou and White Fulani cattle. Interestingly, the present study showed that the g.13068 C>T was significantly associated with lactose % in Borgou cattle. However, Alim et al. (2014) in their study did not include lactose % despite the importance of lactose content in cow milk. The identified SNPs in *CSN3* did not show any significant effect in White Fulani and no significant effect was found on milk yield in the studied breeds. This may be due to the specific genetic background in the cattle breeds. The association results in the present study suggests that *CSN3* gene is a potential candidate gene that can be used in marker assisted selection to improve the milk composition and milk production in indigenous Borgou cattle breed.

5.4.3. Effect of *MBLG* gene

Two SNPs *MBLG* g.5864C>T (Ala118Val) and *MBLG* g.5940G>A were identified in *MBLG* gene in the present study. Most of the previous studies on *MBLG* polymorphisms focused on the most common A and B variants of *MBLG* gene in cattle (Farrell et al., 2004). The *MBLG* A variant is characterized by Asp_{64} and Val_{118} in the mature protein while the B variant is characterized by the Gly_{64} and Ala_{118} in the resulting protein (Braunitzer et al., 1973). Zakizadeh et al. (2012) in their association studies of polymorphism of beta-lactoglobulin coding and 5`-flanking regions, have shown that the CC genotype *MBLG* X63139: g.435 C>G) had important effect on milk and fat yield in Holstein and on milk yield in Golpaygani breeds. In addition, the AG and GG genotypes (g.422G>A) were associated with milk yield in

Holstein and native Golpaygani breed of Iran respectively. In the current study the *MBLG* g.5864C>T (Ala118Val) corresponding to A/B variant did not show any significant effect on milk traits. However, the *AG* genotypes of *MBLG* g.5940G>A was associated with higher fat and total solid contents. This highlighted the effect of mutation in noncoding sequence on milk composition traits. Several studies have shown that allele *MBLG**B is associated with high casein % (Miceikiene et al., 2006) and fat contents (Tsiaras et al., 2005; Miceikiene et al., 2006) which would improve cheese-making properties of milk. However, contradictory results on the association of *MBLG* genetic variants with milk composition and production traits in cattle have been reported (Heidari et al., 2009). Instead of considering the *MBLG* A/B variants only in genetic association studies, the results of current study suggest the *MBLG* g.5940G>A as potential marker for milk fat and total solid contents.

5.4.4. Effect of *CSN1S1*gene

The screening of 5' flanking region and partial exon 1 of bovine *CSN1S1* gene, revealed 3 important SNPs in Borgou and White Fulani. Studies indicated significant effect of *CSN1S1* promoter variants on protein contents and postulated linked loci affecting milk yield traits in German Holsteins (Prinzenberg et al., 2003). So far, 5 promoters allele have been described in cattle, allele 1, 2, 3 and 4 being the common and allele 5 discovered lately in West and Central African Zebu (Ibeagha-Awemu et al., 2005). Previous studies on polymorphisms in *CSN1S1* 5' flanking region in African indigenous *Bos taurus* and *Bos indicus* did not focus on a single nucleotide polymorphism and did not investigate any associations with milk production traits (Ibeagha-Awemu et al., 2005). The present study is the first to uncover the effect of individual SNPs in *CSN1S1* 5'flanking region on milk traits in African indigenous *Bos taurus* and *Bos indicus*. The *CSN1S1* g.10430 G>A genotypes was associated with milk yield in Borgou and the g.10359 T>C was associated with test-day milk yield and test-day fat

and protein yields in White Fulani. Moreover the *CSN1S1* g.10430 G>A was associated with test-day milk yield in White Fulani. The identified SNPs with effect on milk yield traits could therefore be used as potential genetic markers to improve the milk production traits in Borgou and White Fulani.

5.4.5. Effect of *DGAT1* K232A and *SCD1* A293V

The frequencies of *SCD1* 293V were 0.84 and 0.94 in Borgou and White Fulani, respectively. A higher frequency of the V allele (0.82) is also reported in Italian Brown cows (Conte et al., 2010) while a higher frequency of the A allele has been reported in Dutch Holstein-Friesian heifers (0.73), Italian Holsteins (0.57), and Canadian Jersey cows (0.80) (Mele et al., 2007a; Schennink et al., 2008; Kgwatalala et al., 2009). The difference in *SCD1* A293V allele frequencies between the studied indigenous breeds and western breeds can be explained by a breed specific effect. The *SCD1* AV genotype was associated with higher C14 and total index compared to the VV genotype in Borgou breed. This result did not agree with study by Conte et al. (2010) who associated *SCD1* VV genotype with higher C14 index in Italian Brown cows. Moreover, Kgwatalala et al., (2009) showed that the AA genotype of *SCD1* was associated with higher C14 index. The effect of *SCD1* A293V genotypes on C14 index seems to vary from one breed to another. In the current study, *SCD1* genotypes did not significantly affect protein or fat percentage in Borgou which is similar to the results of Schennink et al. (2008) in Dutch Holstein-Friesian heifers. However, in the present study, the *SCD1* V allele had significant negative association (-5.68%) with C14 index compared to the A allele in Borgou. The allele A of *SCD1* is therefore significantly associated with 5.68% more C14 index in Borgou. The positive significant association between the allele A of *SCD1* and C14:1 *cis*-9 and C14 index has been reported previously (Mele et al., 2007a; Schennink et al., 2008; Kgwatalala et al., 2009). However, allele A of *SCD1* did not show significant effect on C14:1

cis-9 in this study. This may be explained by the large sample sizes used in the other studies, namely 1,725 Dutch Holstein-Friesian heifers (Schennink et al., 2008)**,** 297 Italian Holstein Friesian cows (Mele et al., 2007a) and 525 Canadian Jersey cows (Kgwatalala et al., 2009) compared to 85 Borgou and 94 White Fulani in the current study for *DGAT1* and *SCD1* genes. Also, significant associations of the *SCD1* A293V polymorphism with C10 index, C12 index, C16 index and C18 index has been reported (Kgwatalala et al., 2009; Schennink et al., 2008). However, no significant effect of *SCD1* polymorphism was observed for C18 index in this study and this could be due to difference in desaturation activity of SCD1 enzyme on stearic acid (C18:0) in Borgou and White Fulani.

The frequencies of *DGAT1* 232K were 0.77 and 0.92 in Borgou and White Fulani breeds respectively. A higher frequency of the K allele in Borgou and White Fulani breeds in Benin has been previously reported (Houaga et al., 2017). However, a lower frequency of *DGAT1* K allele (0.40) was reported in Dutch Holstein-Friesian heifers (Schennink et al., 2008).

The nucleotide BLAST analysis identified very few sequences in Genebank database with the rare *DGAT1* mutation identified in this study. However, none of the identified sequences were from African indigenous cattle. They belong to the Indian *Bos indicus* x *Bos Taurus* crossbreed (GenBank: KX965998.1) and Murrah water buffalo (GenBank DQ435292.1). Although the nucleotide insertion occurred in an intron of the *DGAT1* gene, it is important to note that introns play an important role in transcription and mRNA splicing (Zhang *et al*., 2010).

In this study, the *DGAT1* K allele was associated with lower C18 index, total unsaturation index, and MUFA, and with higher SFA in White Fulani breed. These results are similar to a reported by Schennink et al. (2007), who studied 1762 Dutch Holstein Friesian cows and found that the *DGAT1* 232K allele was associated with more saturated fatty acid. However, no significant effect of *DGAT1* 232K allele on C18 and total unsaturation indices was found

by Schennink et al. (2007) which contrast the present study. Similar to current data, Schennink et al. (2008) showed that the *DGAT1* K allele was associated with lower C18 and total indices. The majority of milk fatty acids are present in the form of triacylglycerols and the DGAT1 enzyme plays an important role during the last step of triglyceride synthesis. The *DGAT1* K232A polymorphism was reported to have significant association with milk fatty acid composition and unsaturation indices (Conte et al., 2010). However, the current study did not observe significant association of *DGAT1* K232A polymorphism with individual fatty acids but significant associations with SFA and MUFA was observed. This is conceivable because the effect of *DGAT1* on fatty acid composition and saturation may be due to a higher activity and alteration of specificity of DGAT1 enzyme (Schennink et al., 2007) which may vary between breeds. The discussion of current study results on *DGAT1* K232A polymorphism and fatty acid traits was limited to western dairy breeds because of the scarcity of data on African indigenous cattle breeds.

The Pearson correlation coefficient between total SFA and total PUFA was negative and moderate (-0.44) in Borgou. Similar correlation (-0.34) has been observed between total SFAs and total PUFAs in Canadian Holsteins (Ibeagha-Awemu et al., 2014). The fat percentage showed positive correlations with C14:1 *cis*-9 (0.45) and C18:2 *cis*-9, *cis*-12 (0.24) and negative correlation with total PUFAs (-0.25) in Borgou. Accordingly, an increase in the fat content of Borgou milk will lead to slightly higher C14:1 *cis*-9 and C18:2 *cis*-9, *cis*-12 contents and decreased total PUFA content. The fat content positively affect the price of milk in developed countries, therefore increasing PUFAs (decreasing fat content) could have negative economic impact (Arnould and Soyeurt, 2009). However, in the African context, and in Benin in particular, the price of milk is not influenced by its fat content and hence decreasing total fat content for increased PUFAs in Borgou milk will be beneficial for human health and will not negatively affect farm incomes. On the contrary, increasing fat and protein

percentage in White Fulani breed will lead to slightly higher increase in C14:1 *cis*-9 and C14 index due to the moderate positive correlation observed between the traits. In the present study, C16:0, showed significant and negative correlations with fat percentage (-0.23), C14:1 *cis*-9 (-0.37), C18:2 *cis*-9, *cis*-12 (-0.38) and positive correlation with total SFA (0.48) in Borgou breed. This implies that decreasing C16:0 will lead to increase in fat percentage, C14:1 *cis*-9, C18:2 *cis*-9, *cis*-12 and decrease in total SFAs in Borgou which would be an important breed selection goal.

5.5. Genetic diversity

High genetic diversity was reported in African indigenous *Bos taurus* and *Bos indicus* cattle breeds at microsatellites, milk proteins and blood proteins loci (MacHugh et al., 1997, Ibeagha-Awemu et al., 2004, Ibeagha-Awemu and Erhardt, 2006). Thus, Ibeagha-Awemu and colleagues (2004) reported expected heterozygosity of 0.719, 0.453 and 0.376 at microsatellites, blood proteins and milk proteins loci respectively in Nigerian White Fulani. The expected heterozygosity of 0.302 and 0.353 in Beninese White Fulani and Borgou cattle in the present study are similar to the reported expected heterozygosity in Nigerian White Fulani (0.376) and Cameroonian White Fulani (0.370) at milk protein loci (Ibeagha-Awemu and Erhardt, 2006). Although the White Fulani and Borgou cattle breeds play important role in the provision of milk and meat at the community level, there is no effective genetic improvement program for these traits in Benin. The high diversity observed in Borgou breed compared to White Fulani, would be an opportunity for a breed improvement and conservation program in Benin. To meet the growing demands of milk and dairy products in Benin, the loss of genetic diversity must be avoided as experienced in Europe through high selection pressure and also controlled gene flow between breeds (Ibeagha-Awemu and Erhardt, 2006).

5.6. Gene flow and phylogenetic relationship among Borgou and White Fulani cattle breeds

Despite the observed diversity at the studied loci, a high rate of gene flow (N_em=14.104) and a very close relationship were found between Borgou and White Fulani breeds. The genetic exchange between the two breeds is higher than the reported gene flow of 5.655 between Nigerian White Fulani and Cameroonian Red Bororo (Ibeagha-Awemu and Erhardt, 2006). High zebu introgression was reported in West and Central African cattle breeds (Ibeagha-Awemu et al., 2004). Moreover, a high zebu introgression in the taurine population was observed in Benin and the Lagune breed was the most pure taurine breed (Koudandé et al., 2009). The authors stated that it is because Lagune cattle breed is located on an island in Benin and has not gone through crossbreeding with zebu cattle. On the other hand, the high level of genetic exchanges between African indigenous cattle breeds was postulated to be due to the production system in use (Ibeagha-Awemu and Erhardt, 2005). Indeed, the cattle management system in Benin is mainly traditional and pastoral. The system is characterized by pool management where different breeds are kept together or in the same area with movement from place to place in search of forage and water. The high level of gene flow and a closer phylogenetic relationship observed between Borgou and White Fulani may be explained by the cattle management system in Benin. The high genetic exchange observed between the main two indigenous cattle breeds from Benin is a big concern. If the present situation continues unchecked and prevented, Borgou and White Fulani breeds will merge in the near future to form a single breed. This will lead to a reduction of variety of cattle breeds in Benin and consequently a loss in breed diversity at the African continent level. Therefore, to allow sustained genetic improvement, care must be taken to prevent the gene flow and loss of genetic diversity. This can be achieved by implementing an effective cattle management

system with the aims of improving productivity and conserving the current within breed diversity.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions

The aim of the study was to investigate the effect of polymorphisms in *LALBA*, *MBLG*, *CSN1S1*, *CSN3*, *DGAT1* and *SCD1* on milk production and fatty acid traits in the indigenous Borgou and White Fulani cattle breeds in Benin. The study showed that White Fulani produced higher test-day milk yield, fat content, fat yield and test-day protein yield compared to Borgou. On the other hand, Borgou presented higher content of milk urea nitrogen than White Fulani. The Borgou milk contained higher linoleic acid, higher oleic acid and lower total SFA compared to White Fulani which are beneficial traits for human health.

Five SNPs in *CSN3* gene (g.13065 C>T, g.13068 C>T, g.13104 A>C, g.13111 A>G, g.13165 A>G and g.13173 A>T) were found to be significantly associated with milk production traits in Borgou. The *CSN3* g.13065 C>T genotypes were significantly associated with protein %, lactose %, test day fat and protein yields. The *CSN3* g.13068 C>T and *CSN3* g.13173 A>T genotypes were significantly associated with protein and lactose levels in milk. The *CSN3* g.13111 A>G was associated with fat % and test-day fat yield while the *CSN3* g.13165 A>G was associated with fat%, protein % and lactose %. No significant effects of *CSN3* SNPs on milk production traits were found in White Fulani.

The *MBLG* g.5940G>A polymorphism showed significant association with fat content and total solids in Borgou breed. The *CSN1S1* g.10430 G>A genotypes showed significant association with milk yield in Borgou. The TT genotype of *CSN1S1* g.10359 T>C was associated with higher test-day milk yield and test-day fat and protein yields in White Fulani. The GG genotype of *CSN1S1* g.10430 G>A was associated with higher test-day milk yield in

White Fulani. However, the *LALBA* gene did not show significant association with milk production traits either in Borgou or in White Fulani cattle breeds.

The *CSN3, MBLG and CSN1S1* SNPs are therefore potential candidate genes that can be used in marker assisted selection to improve the milk composition and milk production traits in indigenous Borgou cattle breed. The *CSN1S1* SNPs may serve as potential genetic markers to improve milk yield traits in White Fulani and Borgou cattle breeds.

The *SCD1* AV genotype was associated with higher C14 and total indices; and the *SCD1* V allele was associated with decrease in C14 index in Borgou. In White Fulani breed, the *DGAT1 K* allele was associated with increased total SFA, and decreased C18 index, total index and total MUFA. Therefore, *SCD1* A293V and *DGAT1* K232A polymorphisms may serve as potential genetic markers to increase milk fatty acids that are beneficial to human health.

Borgou presented higher observed heterozygosity ($H_{ob)}$ and expected heterozygosity or gene diversity ($H_{ex)}$ than White Fulani. Low genetic differentiation (θ), high gene flow and very closer phylogenetic relationship were observed between Borgou and White Fulani cattle populations.

6.2. Recommendations

From the findings of the present study the following recommendations may be made:

i) The Borgou cow's milk may be recommended to the milk consumers due to its high content in beneficial fatty acids for human health

ii) This study included only 98 Borgou and 100 White Fulani. However, further studies with a large population of the Borgou and White Fulani breeds are needed to better understand the genetic variability of their milk and fatty acid traits. Similar work needs to be done in the other cattle breeds in Benin such Gudali, Lagune, Somba and Azawak. Further studies, would

be needed to assess the genetic and non-genetic factors affecting the milk yield and composition during the whole lactation period and under a well-controlled experimental conditions.

iii) Genome wide association studies in African indigenous *Bos taurus* and *Bos indicus* cattle breeds are needed for a rapid and sustainable improvement of milk production and fatty acids traits

iv) The smallholder farmers should be sensitized in Benin about the herd's management system in order to avoid uncontrolled cross breeding between the different indigenous cattle breeds leading to loss of genetic diversity

v) Proper and sustainable breeding program needs to be developed in Benin to improve the milk production and fatty acids traits in Borgou and White Fulani cattle breeds. The breeding program should involve all the stakeholders such as governments, farmers, scientists and non-governmental organizations. The roles of these parties should be a concerted effort to improve cattle productivity to meet the current and future needs while maintaining the genetic diversity. An ex-situ conservation plan should be put in place for the indigenous White Fulani cattle breed due to the observed low genetic diversity.

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APPENDICES

Appendix 1: Ethical clearance for blood and milk sampling

Republic of Benin
Polytechnic School of Abomey-Calavi
Department of Animal Health and Production
Laboratory of Animal Biotechnology and Meat Technology (LBATV)

REF: 01-16/LBATV/DPSA/EPAC **14th July 2016**

TO WHOM IT MAY CONCERN

RE: POLYMORPHISMS IN MAJOR MILK PROTEIN GENES (*LALBA, MBLG, CSN1S1* AND *CSN3*) AND MILK FAT GENES (*DGAT1* AND *SCD1*) AND ASSOCIATION WITH MILK PRODUCTION AND FATTY ACID TRAITS IN INDIGENOUS WHITE FULANI AND BORGOU CATTLE BREEDS IN BENIN 01-16/LBATV/DPSA/EPAC

This is to confirm that prior to commencement of the above research, reference **01-16/LBATV/DPSA/EPAC**, the proposal was subjected to peer-review and it was approved by the Ethic Sub-committee of Laboratory of Animal Biotechnology and Meat Technology (LBATV).

Mr Isidore Houaga registration number MB 400-0004/2015 has carried out the research in strict adherence to ethical issues dealing with animal samplings.

The Director

Prof. Dr Issaka YOUSSAO ABDOU KARIM

Professeur Issaka YOUSSAO ABDOU KARIM, 01 BP 2009 Cotonou ; E-mail : issaka.youssao@epac.uac.bj

Appendix 2: Questionnaire for sample Collection

Sheet N°…….. (one sheet/animal)

I-Identification of the Herd

1.1. Herd N°:……..

1.2.Location: Municipality of…… Village:……..

1.3.Name of the owner :

1.4.Profession of the owner:

1.5.Breeding system: Traditional ☐ Semi modern ☐

1.6.What are the objectives of production?

Milk ☐ Meat ☐ Other: Precise: ……

1.7.Crossbreeding

Controlled mating ☐ uncontrolled mating ☐

II-Identification of Cattle

2.1. What is the breed of the cow?

White Fulani ☐ Borgou ☐

2.2. Age:……..Months

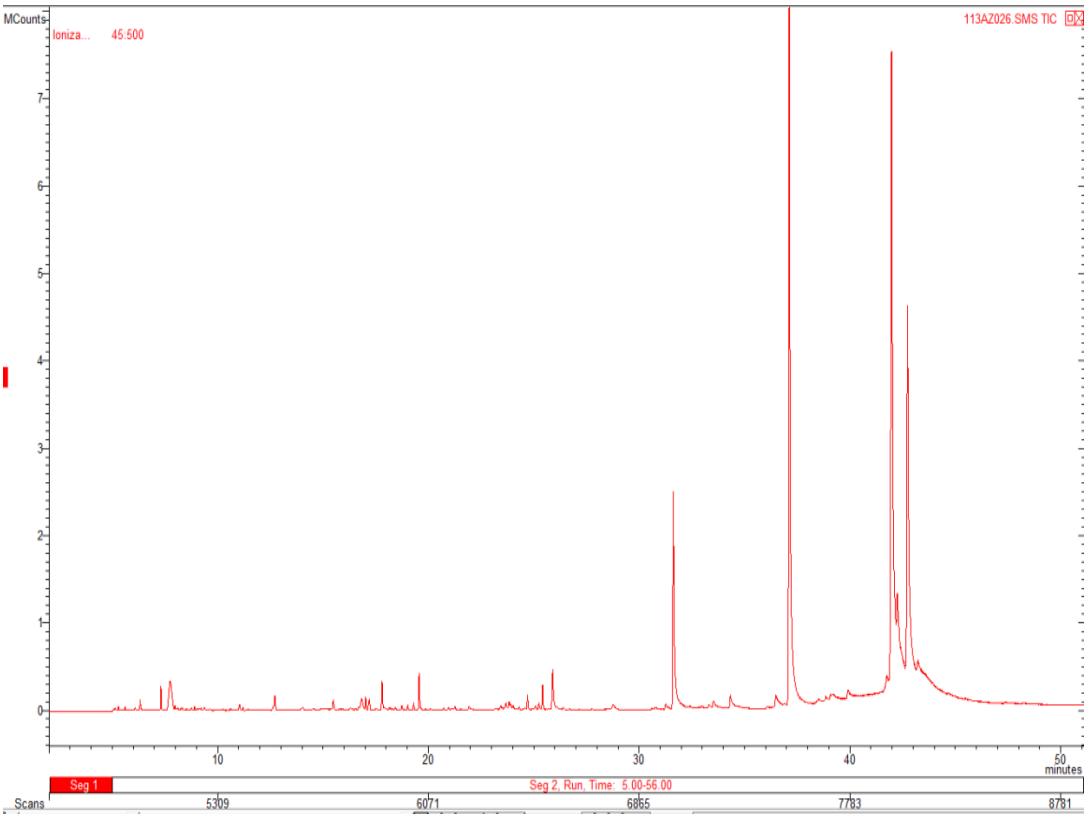
2.3. Date of Calving:……. (Month/year)

2.4. Number of parities:……..

2.5. Stage of lactation:

Early lactation ☐ mid lactation ☐ late lactation ☐

Appendix 3: Chromatogram of fatty acid methyl ester using gas-chromatography



Appendix 4: Effect of *DGAT1* K232A genotypes on milk components and fatty acids traits in White Fulani and Borgou cattle breeds

Trait	*DGAT1* genotypes in White Fulani			*DGAT1* genotypes in Borgou			
	KK ± SE (n = 80)	KA ± SE (n = 12)	p-value	KK ± SE (n = 48)	KA ± SE (n = 31)	AA ± SE (n = 4)	p-value
Milk production traits							
Fat (%)	4.88 ± 0.19	4.58 ± 0.48	0.467	4.85 ± 0.32	4.84 ± 0.40	5.5 ± 1.17	0.850
Protein (%)	3.85 ± 0.09	3.67 ± 0.24	0.471	3.94 ± 0.13	3.81 ± 0.16	4.00 ± 0.44	0.786
Lactose (%)	4.81 ± 0.05	4.75 ± 0.12	0.783	4.69 ± 0.19	4.57 ± 0.15	4.67 ± 0.31	0.870
Fatty acids and unsaturation indices (%)							
Caproic acid (C6:0)	0.26 ± 0.04	0.10 ± 0.11	0.385	0.32 ± 0.04	0.22 ± 0.05	0.28 ± 0.13	0.229
Caprylic acid (C8:0)	0.34 ± 0.04	0.18 ± 0.11	0.378	0.48 ± 0.05	0.35 ± 0.07	0.23 ± 0.19	0.181
capric acid (C10:0)	0.99 ± 0.09	0.71± 0.23	0.465	1.21 ± 0.10	1.02 ± 0.12	1.14 ± 0.33	0.476
lauric acid (C12:0)	1.51 ± 0.11	1.28 ± 0.28	0.692	1.38 ± 0.16	1.83 ± 0.20	1.94 ± 0.57	0.197
12-Methyl Tridecanoic acid (C13:0)	0.36 ± 0.04	0.35 ± 0.10	0.916	0.37 ± 0.06	0.34 ± 0.08	0.66 ± 0.22	0.397
Myristoleic acid (C14:1 *cis*-9)	0.95 ± 0.09	0.6 ± 0.23	0.271	1.13 ± 0.10	0.84 ± 0.13	1.07 ± 0.36	0.204
Myristic acid (C14:0)	9.42 ± 0.63	8.09 ± 1.62	0.637	13.33 ± 0.80	10.44 ± 1.00	12.29 ± 2.79	**0.086**
pentadecanoic acid (C15:0)	2.69 ± 0.19	2.57 ± 0.49	0.774	4.85 ± 0.39	3.75 ± 0.49	2.57 ± 1.36	**0.097**
Palmitic acid (C16:0)	16.06 ± 1.06	15. 79 ± 2.73	0.950	4.77 ± 1.18	6.48 ± 1.47	4.54 ± 4.09	0.649
Margaric acid (C17:0)	3.69 ± 0.29	4.10 ± 0.76	0.788	6.89 ± 0.57	7.82 ± 0.71	7.17 ± 1.99	0.599
Linoleic acid (C18:2 *cis*-9, *cis*-12)	10.24 ± 0.66	9.02 ± 1.70	0.500	15.80 ± 0.87	15.65 ± 1.08	17.54 ± 3.00	0.839
Oleic acid (C18:1 *cis*-9)	13.10 ± 0.92	18.52 ± 2.38	**0.065**	16.76 ± 0.99	16.46 ± 1.23	16.26 ± 3.44	0.976
Stearic acid (C18:0)	18.16 ± 1.16	16.07 ± 2.99	0.808	12.37 ± 0.72	13.85 ± 0.90	12.49 ± 2.51	0.433
Nonadecanoic acid (C19:0)	0.53 ± 0.10	0.59 ± 0.25	0.973	0.74 ± 0.21	0.99 ± 0.26	2.44 ± 0.72	**0.076**
Arachidic acid (C20:0)	0.25 ± 0.03	0.23 ± 0.08	0.836	0.18 ± 0.03	0.22 ± 0.04	0.25 ± 0.12	0.625
C14 index[1]	8.97 ± 0.55	7.13 ± 1.41	0.372	9.66 ± 2.20	10.23 ± 2.73	7.60 ± 7.61	0.946
C18 index[2]	43.33 ± 2.56	53.75 ± 6.60	0.304	57.23 ± 2.04	53.46 ± 2.54	53.81 ± 7.06	0.497
Total index[3]	33.56 ± 1.97	43.64 ± 5.10	0.138	40.58 ± 1.84	41.25 ± 2.29	40.07 ± 6.37	0.967
SFA[4]	54.25 ± 0.84	50.04 ± 2.17	0.170	58.26 ± 1.33	59.02 ± 1.66	56.86 ± 4.61	0.880
MUFA[5]	14.05 ± 0.91	19.12 ± 2.35	**0.085**	22.26 ± 1.27	21.58 ± 1.59	21.72 ± 4.41	0.945
PUFA[6]	10.24 ± 0.66	9.02 ± 1.70	0.500	19.48 ± 1.03	19.40 ± 1.28	21.43 ± 3.56	0.862

[1] C14 index=C14:1 *cis*-9/ (C14:1 *cis*-9+ C14 :0) × 100. [2] C18 index= C18:1 *cis*-9/ (C18:1 *cis*-9+ C18 :0) × 100. [3] Total index= (C14:1 *cis*-9+ C18:1 *cis*-9)/ (C14:1 *cis*-9+ C14 :0+ C18:1 *cis*-9+ C18 :0) × 100. [5] SFA= saturated fatty acid; [6]MUFA= monounsaturated fatty acid; [6]PUFA= polyunsaturated fatty acid. SE = standard error of the mean. *DGAT1* AA genotype was not included in the analysis for While Fulani because only one individual of White Fulani breed was AA.

Appendix 5: Publications

Global Science Research Journals

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Global Journal of Animal Breeding and Genetics

Full Length Research Paper

Effect of breed and Diacylglycerol acyltransferase 1 gene polymorphism on milk production traits in Beninese White Fulani and Borgou cows

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Diacylglycerol acyltransferase 1 (*DGAT1*) is a potential candidate gene with a non-conservative substitution of lysine by alanine (K232A) in exon 8 having a major effect on milk production traits in cattle. The aim of this study was to analyze the allele and genotype frequency, and investigate the association of the *DGAT1* K232A polymorphism with milk production traits in indigenous White Fulani and Borgou cattle breeds in Benin. In total, 103 White Fulani and 103 Borgou were genotyped by Polymerase Chain Reaction–Restriction Fragment Length Polymorphisms and validated by Sanger sequencing. The genotypic frequencies of KK, KA and AA in White Fulani and Borgou breeds were 0.83, 0.16, 0.01 and 0.57, 0.39, 0.04 respectively. Frequencies of K and A alleles were 0.91 and 0.09, and 0.77 and 0.23 in White Fulani and Borgou breeds respectively. The White Fulani cows showed higher daily milk yield ($P< 0.01$), lactose content ($P<0.001$), protein yield ($P< 0.01$) and fat yield ($P< 0.01$) compared to Borgou. The *DGAT1* KK genotype was significantly ($P<0.05$) associated with higher fat yield in White Fulani. Therefore, the *DGAT1* locus could serve as a genetic marker for selection of fat yield in indigenous White Fulani Cows. Further studies would be needed to investigate the effect of *DGAT1* gene on milk fatty acids variation between the two breeds.

Keywords: *DGAT1*, Milk traits, Borgou, White Fulani, Benin.

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RESEARCH ARTICLE (ACCEPTED)

EFFECT OF HERD, AGE, PARITY AND STAGE OF LACTATION ON MILK PRODUCTION TRAITS IN INDIGENOUS BORGOU CATTLE BREED IN BENIN

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Milk production traits, Indigenous, Borgou, Breed, Benin.

Abstract

Borgou cattle breed represent more than 50 % of cattle population in Benin. However, there is very little data on the variation of their major milk components. The aim of this study was to assess the effect of herd, age, parity and stage of lactation on milk production traits in Borgou cattle breed. A total of 95 Borgou cows in lactation were included in the study and the contents of major milk components were determined using MilkoScan FT 6000 Series mid-range infrared Fourier transform infrared-based spectrometers. Results showed that, differences in test-day milk, protein and fat yields ($P<0.01$), milk urea nitrogen % ($P<0.001$) and lactose % ($P<0.05$) were observed between herds. The Borgou cows of 3-5 years old presented higher test-day fat yield (44.32 vs 33.95 g, $P<0.05$) and higher protein yields (34.63 vs 30.05 g, $P<0.05$) than the Borgou cows of more than 5 years old. The cows on second parity presented higher test-day milk (1.12 kg; $P<0.05$) and protein yields (38.14 g, $P<0.05$) than cows on first and third or more parity. However, the lactation stage did not show significant effect on milk production traits in Borgou cattle breed ($P>0.05$). The test-day milk yield showed negative correlation with protein content (-0.233, $P<0.05$) and positive correlations with test-day fat yield (0.676, $P<0.001$) and test-day protein yield (0.809, $P<0.001$). The fat content showed positive and moderate correlations ($P<0.001$) with protein content (0.495) and negative correlation with lactose content (-0.379). The findings from this study will be taken into consideration when designing a breeding program to improve milk production traits in indigenous Borgou cattle breed in Benin.

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Milk fatty acid variability and association with polymorphisms in *DGAT1* and *SCD1* genes in White Fulani and Borgou cattle breeds

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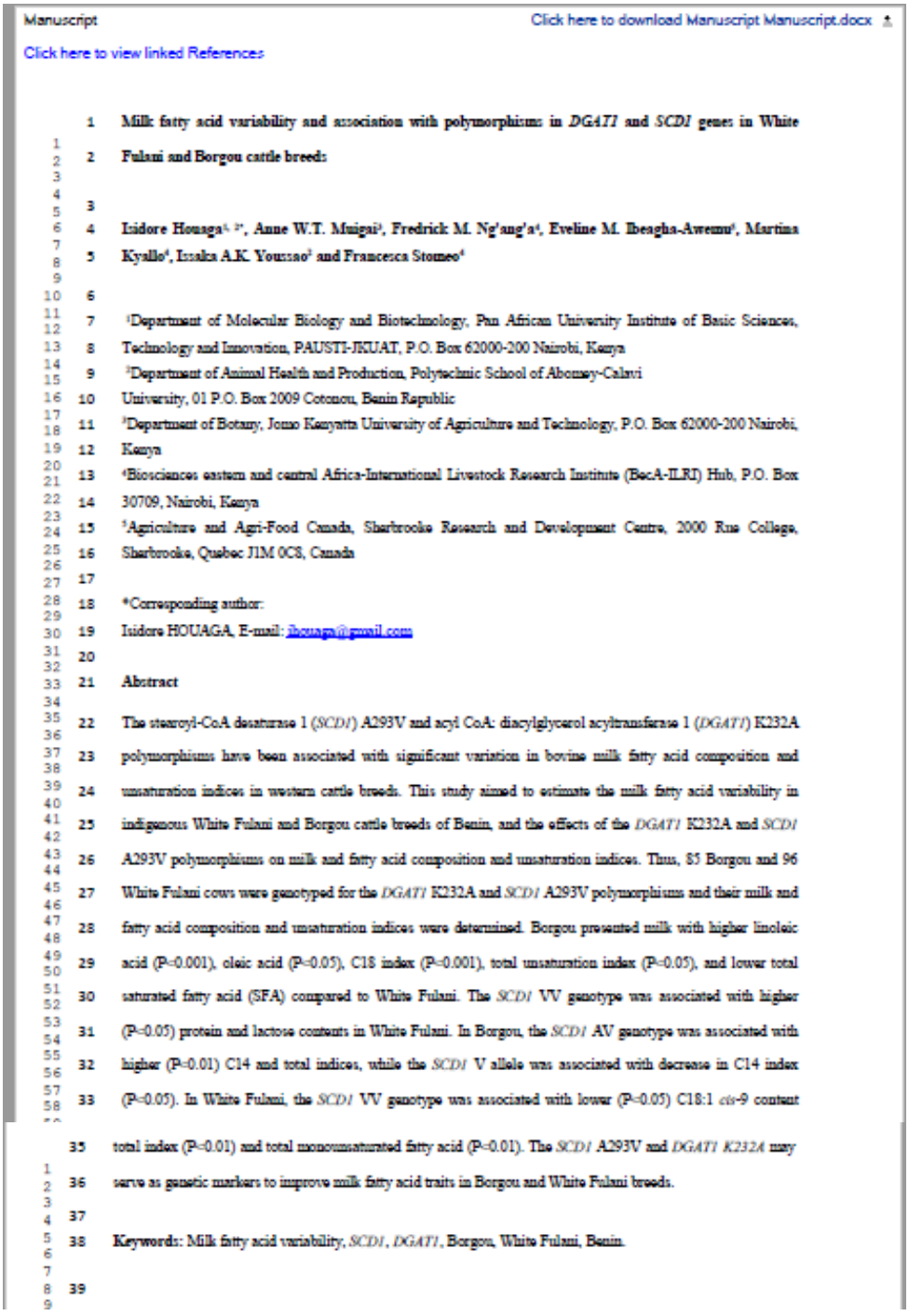

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Milk fatty acid variability and association with polymorphisms in *DGAT1* and *SCD1* genes in White Fulani and Borgou cattle breeds

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Abstract

The stearoyl-CoA desaturase 1 (*SCD1*) A293V and acyl CoA: diacylglycerol acyltransferase 1 (*DGAT1*) K232A polymorphisms have been associated with significant variation in bovine milk fatty acid composition and unsaturation indices in western cattle breeds. This study aimed to estimate the milk fatty acid variability in indigenous White Fulani and Borgou cattle breeds of Benin, and the effects of the *DGAT1* K232A and *SCD1* A293V polymorphisms on milk and fatty acid composition and unsaturation indices. Thus, 85 Borgou and 96 White Fulani cows were genotyped for the *DGAT1* K232A and *SCD1* A293V polymorphisms and their milk and fatty acid composition and unsaturation indices were determined. Borgou presented milk with higher linoleic acid ($P<0.001$), oleic acid ($P<0.05$), C18 index ($P<0.001$), total unsaturation index ($P<0.05$), and lower total saturated fatty acid (SFA) compared to White Fulani. The *SCD1* VV genotype was associated with higher ($P<0.05$) protein and lactose contents in White Fulani. In Borgou, the *SCD1* AV genotype was associated with higher ($P<0.01$) C14 and total indices, while the *SCD1* V allele was associated with decrease in C14 index ($P<0.05$). In White Fulani, the *SCD1* VV genotype was associated with lower ($P<0.05$) C18:1 *cis*-9 content total index ($P<0.01$) and total monounsaturated fatty acid ($P<0.01$). The *SCD1* A293V and *DGAT1 K232A* may serve as genetic markers to improve milk fatty acid traits in Borgou and White Fulani breeds.

Keywords: Milk fatty acid variability, *SCD1*, *DGAT1*, Borgou, White Fulani, Benin.

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Genomic selection for sustainable and rapid improvement of milk production traits in African indigenous *Bos indicus* and *Bos taurus* breeds

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Abstract

Africa is home to diverse populations of cattle breeds (*Bos indicus* and *Bos taurus* breeds and their crosses) adapted to various agro-ecological zones and production systems, and have acquired superior phenotypes that enhance their survival under tough conditions. Amongst other uses, African indigenous cattle produce milk with direct benefit for household nutrition and health, provide income and employment for farm families and contribute to sustainable poverty alleviation. The indigenous cattle breeds are the main milk producers and account for over 90% of the ruminant population in the continent. The indigenous cattle have not been systematically developed for milk production traits, and current low levels of productivity cannot meet up with demand for milk and milk products by a growing population. Although the dairy industry in Africa is presently less developed, factors like population growth, grazing land shortage, nutritional value of milk and economic interest in milk production is pushing the development of small-scale market orientated dairy systems. To sustain evolving dairy systems and local farm families, there is urgent need to develop the milk production capacity of indigenous *Bos indicus* and *Bos taurus* breeds. Interestingly, observed genetic variation associated with milk production traits and signals for adaptation to harsh environmental conditions displayed by African indigenous cattle breeds offer the opportunity for the application of genomic breeding for rapid improvements in milk traits. Recent advances in next-generation sequencing and genotyping technologies have enabled the application of genomic selection in dairy cattle in some western countries with great success. In this review, the current state of the milk production capacity of indigenous breeds and the demand and supply patterns of milk and milk products is presented. Available data on genetic variation and associations with milk production traits in African cattle is discussed. Case studies on past and current attempts to enhance the milk production capacity of indigenous breeds are presented as well as the challenges and future prospects. Finally, a case for the application of genomic selection as a sustainable way to rapidly improve milk production traits in African indigenous cattle breeds is made.

Keywords: *Bos taurus*, *Bos indicus*, milk production, Genomic selection, Africa