ANCESTRAL BREED GROUPING FOR IMPROVING ANIMAL MODELING IN ADMIXED POPULATIONS AND ITS USE IN LEPTIN (GENETIC AND HORMONE) ASSOCIATION STUDIES WITH PERFORMANCE TRAITS IN COMMERCIAL BEEF COW HERD

A Dissertation
Submitted to the Graduate Faculty
of the
North Dakota State University
of Agriculture and Applied Science

By

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In Partial Fulfillment of the Requirements
for the Degree of
DOCTOR OF PHILOSOPHY

Major Department:
Animal Sciences

December 2020

Fargo, North Dakota

North Dakota State University
Graduate School

Title

ANCESTRAL BREED GROUPING FOR IMPROVING ANIMAL MODELING IN ADMIXED POPULATIONS AND ITS USE IN LEPTIN (GENETIC AND HORMONE) ASSOCIATION STUDIES WITH PERFORMANCE TRAITS IN COMMERCIAL BEEF COW HERD

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ABSTRACT

The first study investigated American Aberdeen (AD) cattle's genetic structure and its genetic relationships with five American taurine beef breeds and the genetic population structure of a related admixed cattle herd for improved statistical modeling. The last two studies investigated the role of leptin *(LEP) c.73C>T* polymorphism, leptin diplotype (*LEPD*), and leptin hormone (LEPH) concentration on reproductive, growth, size, feeding, and carcass ultrasound traits in beef cows and heifers. High levels of polymorphism were observed in admixed, Red Angus, and Gelbvieh (GV) populations. The lowest level of polymorphism was found in AD followed by undetermined parentage. Pairwise fixation index displayed the largest genetic differentiation between AD and GV, Simmental, and Shorthorn breeds. Even though founder animals originated from the Angus breed, the AD breed exhibited unique genomic characteristics. Mixed animal models with or without pedigree that included the primary ancestral breed group (ABG) for admixed individuals developed from diversity analyses performed better than models without the ABG. With these improved models, novel relationships between *LEP* genotype and number of follicles and ovary size were identified. Similar relationships of the *LEP* genotype in multiple growth and weight traits that have been published before have been confirmed. However, reverse relationships were observed for feed efficiency. The *LEPD* showed associations for feed efficiency, weight traits, and body size traits. Novel relationships between LEPH categories (high vs. low) were reported on two feeding behavior traits, including number of meals per day and feed intake per meal. However, no relationship was observed between *LEP* genotype and plasma LEPH concentration in this population. Literature indicates the increased productivity of the T allele for growth and carcass attributes, so it was expected that the same increased productivity would be found for

reproductive traits. Therefore, the T allele of *LEP c.73C>T* marker could be used as a valuable marker for selecting for improved growth, body size, and reproductive performance in commercial beef heifers. Also, circulatory LEPH before the breeding season may serve as a predictor for feeding behavior, body size, and reproductive traits. Even so, not all comparisons could be statistically proven; therefore, additional investigations may be warranted.

ACKNOWLEDGMENTS

The completion of these projects and this dissertation would not have been possible without the guidance and support of many good people. I am truly blessed to have these people stick with me the whole way of my Ph.D. journey. They really deserve all the acknowledgment.

First and foremost, I would like to acknowledge and thank my advisor, Dr. Lauren Hanna, who allowed me the opportunity to pursue my doctorate in the United States. She has been a fantastic mentor, phenomenal boss, and a great role model for me. Everything I have accomplished throughout my graduate program would not have been possible without her inspiration and scholastic guidance. I am very fortunate and feel proud to have your first Ph.D. student. I have achieved invaluable lab and field skills and grant writing skills while working with you. You never felt disturbed whenever I went to your office for any help or suggestions, and for this, I am genuinely appreciative. You were also very supportive and welcoming while getting any advice on my family. Thank you so much for everything you have done for me.

My committee members: Dr. Kendall Swanson, Dr. Carl Dahlen, Dr. Alison Ward, and Dr. Ned Dochtermann, who have always been very helpful and friendly, and provided constant supports, encouragement, and valuable advice, deserve many thanks. They have also provided me insights into background information and thoughts from nutritional, reproduction, and molecular genetics aspects. I am incredibly grateful to have your willingness to guide me and work with me throughout the whole way.

I wish to express my sincere appreciation, deepest sense of gratitude and profound respect to Dr. Kris Ringwall, Extension Specialist and Ex-director of Dickinson Research and Extension Centre (DREC), for his constant inspiration and valuable suggestions throughout my research projects.

I am extremely grateful to the Beef Cattle Research Complex and DREC staff, especially Trent Gilbery, Sarah Underdahl, Garry Ottmar, and Wanda Ottmar, for their assistance and continuous support during the data collection period and without whom my projects would not have been possible.

I wish to express my gratitude to the NDSU Animal Sciences Lab and Research Specialists, Courtney Crane, Jim Kirsch, Sheri Dorsam, Wanda Keller, Jennifer Young, and Marsha Kapphahn for their continuous guidance and support while working in the lab and field. Especially, Jim Kirsch taught me blood collection through jugular venipuncture and provided me instructions while preparing packages for shipment.

I am also thankful to my lab mates and graduate student in the Animal Science department, who have become my family throughout my five years at NDSU, and worked with me in the lab and field throughout my projects.

Finally, my family has been the biggest support system throughout my whole period of graduate program. I have been blessed to have a very supportive and caring wife, and an adoring daughter. My parents, parents-in-law, brothers, sisters, sisters-in-law, and brothers-in-law always provided me inspiration and mental support during completion of this program. I am thankful to all of you.

DEDICATION

TO MY MOTHER, WHO NEVER SAW THIS ADVENTURE

A pure and gentle soul who taught me to believe in God

TO MY FATHER

The person who inspires me to do hard work and believe in myself

TO MY BELOVED WIFE

The person who always encouraged and supported me through this journey, and I cannot thank her enough for that. I would not have come to this stage without her continuous love and support.

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LIST OF ABBREVIATIONS

Å..Angstrom

AD...American Aberdeen

ADF..Acid detergent fiber

ADG..Average daily gain

ADMXI...Admixed population I

ADMXII..Admixed population II

AFC...Antral follicle count

AFLP...Amplified fragment length polymorphism

AgeFC...Age at first calving

AgRP...Agouti-related peptide

AI ....Artificial insemination

AIC..Akaike information criterion

AMOVA ....Analysis of molecular variance

AN...Angus

ANOVA ....Analysis of variance

AR ....Red Angus

ARC ....Arcuate

BCS ....Body condition score

BIC..Bayesian information criterion

BL ....Body length

bp...base pair

BTA..*Bos taurus* autosome

BW ....Body weight

Ca ....Calcium

CI..Calving interval

CP..Crude protein

CT ....Connecticut

CV ....Coefficient of variation

d...Day

DENS ....Density

df ....Degrees of freedom

DIM...Days in milk

DM ....Dry matter

DMI...Dry matter intake

DNA ....Deoxyribonucleic acid

DREC..Dickinson Research Extension Centre

ENDG ....End girth

ENDr...Radius at the end

FIM ....Feed intake per meal

FIV ....Feed intake per visit

F2 ....Second filial generation

FCR ....Feed conversion ratio

FDA...Feed bunk attendance

FG ....Flank girth

F_{IS} ....Inbreeding coefficient

F_{IT} ....Total inbreeding

FSH ....Follicle-stimulating hormone

F_{ST} ....Fixation index

G:F ....Gain to feed ratio

GC ....Genomic control

GH..Growth hormone

GL ....Gestation length

GLM..Generalized linear model

GnRH ....Gonadotropin-releasing hormone

GV..Gelbvieh

H_E ....Expected heterozygosity

HE ....Human equivalent

HG..Heart girth

HH..Hip height

H_O..Observed heterozygosity

HW...Hip width

IGF-1 ....Insulin-like growth factor 1

IMF ....Intramuscular fat

IN ....Indiana

kDa...Kilodalton

LD ....Linkage disequilibrium

LEP ....Leptin gene

LEPD..Leptin diplotype

LEPH..Leptin hormone

LEPR ....Leptin receptor gene

LH ....Luteinizing hormone

LM..Longissimus muscle

MA ....Massachusetts

MAF..Minor allele frequency

MIDr ....Radius in the middle

ML..Maximum likelihood

MO ....Missouri

NMD ....Number of meals per day

NC ....North Carolina

ND ....North Dakota

NDF..Neutral detergent fiber

NDSU...North Dakota State University

NE ....Nebraska

NPY..Neuropeptide Y

NS ....Non-significant

ob..*Obese* gene

OM ....Organic matter

P ....Phosphorus

PC..Principal component

PCA..Principal component analysis

PCR ....Polymerase chain reaction

POMC ....Proopiomelanocortin

RAPD...Random amplified polymorphic DNA

REA..Rib eye area

RF..Rib fat thickness

RFI ....Residual feed intake

RFLP ....Restriction fragment length polymorphism

RH ....Ractopamine hydrochloride

RIA..Radioimmunoassay

RFAT ....Rump fat thickness

RNA ....Ribonucleic acid

SA ....Structured association

SAS ....Statistical analysis system

SE..Standard error

SH ....Shorthorn

SM...Simmental

SNP ....Single nucleotide polymorphism

SSR ....Simple sequence repeat

TFD ....Time eating at feed bunk per day

TFM ....Time eating at feed bunk per meal

TFV ....Time eating at feed bunk per visit

UCD ....University of California Davis

UHD ....Uterine horn diameter

UP ....Undetermined parentage

US ....United States

USDA..United States Department of Agriculture

VOL ....Volume

wks ....Weeks

YG..Yield grade

ZH ....Zilpaterol hydrochloride

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CHAPTER 1. INTRODUCTION AND LITERATURE REVIEW

Introduction

Genetic diversity is an essential tool for selecting improved livestock species that can feed an ever-rising population. Maintaining genetic diversity in a population is very important because its loss limits mating choices and has adverse effects on economically important traits. Earlier genetic diversity studies in natural populations were mainly focused on easily detectable and/or quantifiable variation instead of estimating population genetic variation (Hedrick, 2011). Phenotypic appearance is not always a strong indicator of genetic variation because animals within a breed might have similar phenotypes, but they can still genetically differ among themselves. Knowledge of genetic diversity and population structure is helpful in designing effective strategies for genetic improvement, efficient management, conservation of farm animal genetic resources (Groeneveld et al., 2010), and improving the efficiency of research resources using admixed populations. There are several parameters used to estimate genetic diversity and population structure. Genetic markers are commonly used to study genetic diversity and genetic relationships in the field of agricultural research. This review will provide an insight into those parameters, genetic markers used for that purpose, and previous research on genetic diversity and relationships in taurine and indicine cattle. Some of the most common taurine beef breeds used in the US beef industry will also be highlighted in this review.

Literature review

Beef cattle production is the most significant livestock industry in the United States and throughout the world. The beef herd in the United States consists of more than 80 breeds and crosses thereof (Drouillard, 2018). According to the most recent beef breed registration's report by National Pedigreed Livestock Council, member breed associations with the greatest number

of registrations were Angus (AN), Hereford (HH), Red Angus (AR), Simmental (SM), Charolais, Gelbvieh (GV), Brangus, Limousin (LM), Shorthorn (SH), Maine-Anjou, Piedmontese, Santa Gertrudis, and Chianina (National Pedigreed Livestock Council, 2020). Commercial beef cattle populations fed for slaughter or used for research are often crossbred or admixed (i.e., the presence of multiple genetically distinct subgroups within a population [Wang et al., 2005]). The proportions of different breed genetics may differ across individuals in admixed populations. This causes allele frequency differences, leading to population stratification (Tian et al., 2008). Thus, the use of crossbred or admixed individuals in the association analysis is challenging because it could lead to false association results if not corrected properly.

According to the 2017 Census of Agriculture data, cattle and calf sales reached around $77.189 billion in the United States (US) in 2017, which was approximately 1.06% more than that of 2012. However, this sale ($1.296 billion) in 2017 was around 21.85% more than in 2012 in North Dakota. This report signifies the enormous economic impact of the beef cattle industry in North Dakota as well as the US. Thus, improved production efficiency, sustainability, and profitability with rising demand for beef greatly influence beef producers. Because feeding costs contribute to around 60% of the total production cost, improving the efficiency of nutrient utilization is crucial in beef production to increase profitability and reduce beef farm operations' environmental impact (Swanson and Miller, 2008). Additionally, cow-calf production's biological efficiency and economic efficiency are primarily dependent on successful reproduction (Dickerson, 1970). The reduction of annual replacement costs and an increase in the duration of a cow's productive life are potential advantages of increased cow longevity. Therefore, improving those economically relevant traits using indicator traits (i.e., a trait that is genetically correlated to an economically relevant trait [ERT] but not an ERT itself) would

increase both sustainability and profitability in the beef cattle industry. Indicator traits of efficiency and longevity are complex traits controlled by many genes and environmental factors. Some of these traits are moderately to highly heritable. Therefore, identifying molecular markers in or around genes controlling these traits has excellent potential to enhance the rate of genetic improvement through marker-assisted selection. For example, leptin is a hormone product of the leptin (*LEP*) gene, which regulates body weight (BW), body fat deposition, reproduction, and immune functions. Previous research showed the association of *LEP* polymorphisms, their haplotype, and leptin hormone (LEPH) with beef cattle performance traits, suggesting their suitability to apply as a genetic or biological marker in the selection program.

Genetic diversity and population structure parameters

Several parameters are used to measure genetic diversity and population structure. Genetic distance is one of the more reliable genetic divergence measures between breeds (Blott et al., 1998), which is directly proportional to the amount of difference in allele frequencies between breeds. Several measures of genetic distance have been recommended over the last few decades. Most of them are highly correlated, mainly when the differences between populations are small. Nevertheless, the genetic distance measures may differ substantially for the same data set when the differences are larger. The most commonly used genetic distance measure is the standard Nei's genetic distance (Nei, 1972). Breeds sharing identical alleles at similar frequencies are genetically closely related; however, those with identical alleles at different frequencies are distinct or distantly related (Blott et al., 1998). The level of heterozygosity is the most widely used measure of genetic variation. In diploid species, individuals are either homozygous or heterozygous at a given locus, which makes this measure biologically significant. Heterozygous animals will have much more variation than homozygous animals.

Another critical measure of genetic variation is the proportion of polymorphic loci. Polymorphic loci are those for which the most common allelic frequency is less than 0.99 or less than 0.95. Both of these cutoff points are arbitrary, but 0.99 is most commonly used when the sample size is large (i.e., about ≥ 100 individuals) (Hedrick, 2011). The number and frequency of alleles can also be used to estimate genetic variation within a breed (Blott et al., 1998). By definition, single nucleotide polymorphisms (SNPs) are those nucleotides that are polymorphic in natural populations' genomes. The frequency of the rarer allele in the SNPs, minor allele frequency (MAF), is sometimes used to measure genetic variation. The MAF is generally considered as ≥ 0.05 to exclude fewer polymorphic sites. The MAF less than 0.05 is also less informative. However, an SNP found to be polymorphic in one population may be monomorphic in another population upon examination. The number of alleles or allelic richness is another measure of variation, which counts the number of alleles observed at a particular locus in a population. However, this type of measure is strongly influenced by the sample size. Several approaches have been used to estimate the overall level of genetic divergence among subdivisions of a population. Wright (1951; 1965) developed an approach that consists of three different *F* coefficients to partition the genetic variation into population subdivisions. These *F* coefficients, F_{IS}, F_{ST}, and F_{IT} are used to allocate genetic variation to the individual (*I*), subpopulations (*S*), and total population level (*T*). The fixation index F_{ST} is a more convenient and widely used measure of genetic differentiation (Hartl and Clark, 2006). This F_{ST} test identifies those alleles that are highly differentiated among subpopulations. Analysis of molecular variance (AMOVA) is one of the most widely used methods for measuring genetic variation patterns within and among populations (Excoffier et al., 1992). The basic principle of this analysis is an extension of Wright's (1951) *F*-coefficients to consider several hierarchical levels of population structure

(Cockerham, 1973) and evolutionary distances among alleles (Excoffier et al., 1992). The genetic structure of a population is most important in determining breed conservation strategies and essential to guide future management actions that promote breeds' genetic improvement (Malhado et al., 2010). Principal component analysis (PCA) and clustering are methods of displaying genetic relationships among populations. The PCA is an ordination method of ordering sample units along with coordinate systems, commonly used for molecular marker data. This method uses a mathematical algorithm for reducing the dimensionality of the large data sets to the few principal components (PCs) that contain most of the information (i.e., variation) in the large data set (Jolliffe, 2002). Therefore, PCs are new uncorrelated variables constructed as linear combinations of the initial variables. The PCs with the largest amount of variation mean the line capturing most information of the data. In addition, clustering is the method of grouping objects in categories or classes based on their common attributes or relationships. These two methods have applications in detecting population substructure and correcting for population stratification.

Genetic markers

Genetic markers represent genetic variations between individuals or between species. These DNA markers are specific DNA sequences with a known chromosomal position on the genome used as essential tools for linkage and association studies (Benavides and Guenet, 2012). There are three major types of genetic markers used in the field of agricultural research: 1) morphological markers (such as phenotypic traits); 2) biochemical markers, which include allelic variants of enzymes called isozymes; and 3) molecular (or DNA) markers, which reveal polymorphic sites in DNA (Winter and Kahl, 1995; Jones et al., 1997). The most commonly used molecular markers in various applications are the restriction fragment length polymorphism

(RFLP), amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), microsatellites or simple sequence repeat (SSR), and SNP. During earlier periods, several different immunogenetic markers and/or biochemical polymorphisms were commonly used to genetically characterize cattle breeds (Blott et al., 1998; Gonzalez et al., 1987). Blott et al. (1998) used blood group and serum protein polymorphism to investigate the genetic relationships among European cattle breeds. As techniques advanced, genetic diversity and population structure within and among breeds have been studied using molecular markers. Initially, microsatellite markers were used to conduct these studies (MacHugh et al., 1998; Mukesh et al., 2004). Pandey et al. (2006) used 21 microsatellite markers to evaluate population structure, genetic variability, and genetic bottlenecks in Indian Kherigarh cattle. The whole-genome analyses have shown that microsatellite markers usually vary in several aspects like length and occurrence of repeat interruptions (Pardi et al., 2005; Brandström and Ellegren, 2008). The focus on most polymorphic markers during microsatellite isolation and screening introduces ascertainment bias that may lead to less sensitivity for judging genomic levels of diversity (Väli et al., 2008). Also, microsatellite markers do not occur throughout the whole genome and have a high mutation rate, which may misinterpret populations' evolutionary history (Brumfield et al., 2003). Some population differentiation measures like F_{ST} can be highly sensitive to the variation within-population, leading to suspiciously low values in many microsatellite studies (Charlesworth, 1998; Hedrick, 1999; Allendorf and Seeb, 2000). Unlike microsatellites, SNPs have a relatively low mutation rate and occur across the whole genome. Because of their bi-allelic nature, SNPs facilitate high-throughput genotyping and reduces repeated substitution at a single site. Currently, it is possible to make genome-wide population genetic analyses in cattle because of many commercial SNP marker panel availability. Although

a single SNP is less informative and sometimes introduces bias, these issues may be overcome using many SNP markers for genetic analyses and introducing many individuals in population studies. Cañas-Álvarez et al. (2015) used 735,239 autosomal SNPs and assessed genetic diversity and divergence among seven Spanish beef cattle breeds. Also, Seldin et al. (2006) conducted an admixture analysis of population structure among European and European American cattle using 5,700 SNP markers.

American taurine beef breeds

Beef cattle breeds are not native to the US. In 1493, Christopher Columbus introduced the first cattle to the New World on his second voyage. On that voyage, the Spanish settlers first brought the few long-horned Iberian cattle to the Antilles Islands (Puerto Rico, Cuba, Jamaica, and Hispaniola). In the early 1500s, Antillean settlers introduced many of those Longhorn cattle to Mexico during traveling in search of gold and other treasures (Casey, 2019). In the sixteenth century, Spanish adventurers primarily brought cattle into the New World for furnishing hides, with beef tongues as a secondary consideration or termed as a by-product of the hide-producing industry (Bowling, 1942). The SH breed was the first imported purebred cattle, which were brought to the US in the 1780s. America's early settlers valued this cattle breed for meat and milk production. By 1854, Midwestern farmers started importing this breed directly from Scotland while raising SHs strictly for beef production (Association, 2020). In 1817, the famous statesman Henry Clay of Kentucky first imported HH cattle from England. The end of the Civil War marked the beginning of a HH-dominated beef cattle market in the US. At that time, cattle drives from Texas to the Midwest (like Kansas City, MO, and Abilene, TX), and Western regions of the US occurred through famous trails, such as Shawnee, Chisholm, Western and Goodnight-Loving trails. The Great Plains for beef production was opened by the end of the

clashes with Native Americans and reduced buffalo herds. Cattle production developed west of the Mississippi River as the Great Plains' vast grasslands provided good grazing to the animals in those regions. Aberdeen AN was first imported from Scotland to the US by George Grant of Kansas in 1873. Angus cattle are known for their early maturing ability, with a high genetic potential for a significant degree of marbling. Red Angus is another beef breed in the US, which has a similar origin to (black) AN cattle. Red individuals appeared in the stock of (black) Aberdeen AN; however, the American Aberdeen Angus Association decided not to allow them to register in 1917, which left many purebred cattle unregistered (Red Angus Association of America, 2009). In 1954, seven visionary breeders gathered to establish a unique breeder organization known as the Red Angus Association of America.

The fencing of the west with barbed wire led to improved herds for more efficient production by producers who could manage them more easily. This emphasis on improving herds and production eventually led to the importation of many modern beef breeds, such as SM and LM in the 1960s. The LM cattle were first introduced into the US in the late 1960s, known for their good muscle growth efficiency. Another Continental dual-purpose breed, SM, is renowned for its young's rapid growth, which was first introduced into the US through the importation of semen from Canada in 1967. The first purebred SM bull was imported into the US in 1971. Furthermore, Carnation Genetics has introduced GV cattle into the US by importing GV semen from Germany in 1971. This breed has been developed in the US by the grading up of the foundation females (Oklahoma State University Board of Regents, 2015). Grading up is the process of successive "topcrosses" of purebred sires on other breeds, crosses, or of unknown backgrounds (Hammack, 2009), where topcross refers to the use of highly inbred males to the females of the base population or non-inbred population.

American Aberdeen (AD) cattle in the US, formerly referred to as American Lowline, descend from a closed AN herd developed through a strict selection process in a study for efficiency relative to frame and growth at the New South Wales Department of Agriculture in the Trangie Agricultural Research Centre in New South Wales, Australia, in 1929. Although the literature is still lacking on AD cattle, the breed association claims these cattle are easy calving, docile, very efficient on grass, and have excellent beef taste, texture, and tenderness attributes (American Aberdeen Association. Packer, Colorado., 2020). In 1996, Bismarck cattleman Neil Effertz first imported this breed into the US from Canada.

Earlier genetic analysis studies in taurine and indicine cattle

The Aurochs (*Bos primigenius*), a wild cattle species, is the primitive ancestor of modern cattle, which originated in the Indian subcontinent. Approximately 5,000 to 10,000 years ago, cattle's domestication process was initiated by taming the first Aurochs (Ritchie, 2009). Traditionally, cattle occur as two subspecies, *Bos taurus taurus* (humpless – European, African, and Asian) and *Bos taurus indicus* (humped – South Asian, and East African), despite their complete interfertility (Lenstra and Bradley, 1999). Several different genetic analysis studies were performed on *Bos taurus* and *Bos indicus* cattle over the past few years using either microsatellites or SNP markers (Table 1.1). In a genetic structure study among cattle breeds, Gibbs et al. (2009) found that the effective population size (*Ne*) of cattle has recently decreased rapidly from a substantial ancestral population. The reduction in *Ne* may be due to the population's bottleneck effect, which is associated with selection, domestication, and breed formation. They observed similar SNP diversity to humans within studied taurine breeds but significantly lower SNP diversity than the diversity within indicine breeds. They also suggested that these differences in diversity were due to progenitor population diversity and bottleneck

effects at and before breed formation rather than variation in the intensity of natural or artificial selection post domestication. The taurine cattle showed a greater heterozygosity level than the zebu cattle (Lin et al., 2010; Porto-Neto et al., 2013). The heterozygosity level reported using microsatellites was relatively higher than those noted using SNP markers (Wiener et al., 2004; Porto-Neto et al., 2013). A greater level of genetic differentiation was observed between taurine and zebu cattle in many previous studies (McKay et al., 2008; Edea et al., 2013; Makina et al., 2014; Edea et al., 2015; Campos et al., 2017). In an earlier study, the Ethiopian cattle population showed significant introgression from South Asian zebu cattle (Edea et al., 2015). However, Iranian cattle did not show any introgression from worldwide cattle breeds (Karimi et al., 2016). New World cattle and Southern European cattle breeds showed ancestry from taurine and indicine lineages (McTavish et al., 2013). Decker et al. (2014) further reported the indicine introgression into American cattle has occurred in America.

Table 1.1. Genetic analysis studies performed in taurine and indicine cattle[1]

Study	Population and breeds	Markers (used) and their type	Major findings in the study
Sermyagin et al., 2018	195 samples (blood/semen) of 9 local Russian cattle breeds and 746 individuals of 70 worldwide cattle breeds as a reference	54,609 (35,874) SNPs	Average H_O was 0.341, and F_{IS} ranged from -0.028 to 0.036 in these local breeds; they showed 3 distinct groups and exhibited taurine ancestry
Campos et al., 2017	141 animals of 5 locally adapted taurine breeds and 553 animals of 4 indicine breeds	~777,000 (768,506) SNPs	A smaller pair-wise F_{ST} among zebu breeds than taurine breeds, a high F_{ST} between these two subspecies
Msalya et al., 2017	3 strains of Tanzanian Short Horn zebu cattle, Boran and Friesian cattle breeds (40 unrelated animals per strain or breed)	74,157 (69,019) SNPs	A low level of inbreeding in the Tanzanian cattle than Boran and Friesian breeds; common ancestry in Boran and Tanzanian breeds; Friesian ancestry in Boran cattle
Karimi et al., 2016	75 individuals of 8 Iranian cattle breeds, and HO, JE and BR as taurine and indicine outgroups, and 1157 animals from 134 cattle breeds for global analysis	777,962 (283,028) SNPs	Average H_O was 0.301, and F_{IS} ranged from -0.023 to -0.121 in indigenous Iranian breeds; they showed an intricate admixture pattern but did not exhibit any significant introgression from worldwide cattle breeds
Zinovieva et al., 2016	116 samples (tissue/sperm) of 5 local Russian cattle breeds and 29 North American & German HO as a reference	54,609 (35,874) SNPs	Average H_O was 0.381, and F_{IS} ranged from -0.054 to -0.015 in these local breeds; they showed distinct ancestry from HO or HO-related breeds
Cañas-Álvarez et al., 2015	336 animals of 7 Spanish beef cattle breeds	777,962 (57,674) SNPs	A large degree of diversity among individuals within populations; a low degree of divergence among breeds
Edea et al., 2015	244 animals of 7 Ethiopian cattle, 45 animals of 2 Bangladeshi zebu breeds, and 18 Hanwoo cattle	74,153 (69,903) SNPs	Substantial genetic differentiation between indicine and taurine cattle; significant introgression of Ethiopian cattle from South Asian indicine cattle
Decker et al., 2014	1543 samples of 134 domesticated bovid breeds	43,043 SNPs were used	Had 3 major groups of cattle, including Asian zebu, Eurasian taurine, and African taurine; a considerable influence of Shorthorn cattle in the formation of European breeds; indicine introgression into American cattle occurred in America

Table 1.1. Genetic analysis studies performed in taurine and indicine cattle[1] (continued)

Study	Population and breeds	Markers (used) and their type	Major findings in the study
Makina et al., 2014	249 animals of 3 indigenous South African breeds and 2 *Bos taurus* breeds	54,609 (46,236) SNPs	Low to moderate genetic diversity within breeds but closer relationships among breeds; a clear genetic divergence between South African and *Bos taurus* cattle
Edea et al., 2013	166 animals of 5 indigenous Ethiopian cattle and 40 Hanwoo cattle	8,773 (4235) SNPs	Average H_O was 0.377 in local Ethiopian cattle; they showed low genetic differentiation among them and genetic distinction from Hanwoo
McTavish et al., 2013	1495 animals of 58 worldwide (European, New World, indicine, African or hybrid origin, and Japan and Korea) cattle breeds	54,609 (47,506) SNPs and 1814 SNPs	New World cattle and Southern European cattle breeds exhibit ancestry from both taurine and indicine lineages; Hybrid ancestry in New World cattle facilitated their adaptation to a new environment
Porto-Neto et al., 2013	339 animals of 10 taurines and 166 animals of 3 zebu cattle breeds	~777,000 (768,506) SNPs	A greater level of average heterozygosity in taurine (0.29) than zebu cattle (0.21); higher pair-wise F_{ST} between indicine breeds in comparison to taurine breeds
Gautier et al., 2010	1121 individuals of 47 worldwide cattle breeds	51,582 (44,706) SNPs	Had 3 distinct groups (i.e., African taurine, European taurine, and zebus), indicating 3 domestication centers
Lin et al., 2010	270 animals of 9 Eurasian cattle breeds	2641 (58) SNPs	A higher heterozygosity level in *Bos taurus* than *Bos indicus* cattle
McKay et al., 2008	383 animals of 7 *Bos taurus* breeds and 137 individuals of 2 *Bos indicus* breeds	2,670 SNPs were used	A high F_{ST} values and Nei's genetic distance between *Bos taurus* and *Bos indicus* populations; genetic differences in beef vs. dairy groups and European vs. Asian breeds among taurine breeds after excluding indicine breeds
Gautier et al., 2007	14 European and African cattle breeds	1536 (696) SNPs	Average genetic differentiation among breeds was 15.5%; European breeds were genetically closer to each other than that of African breeds; genetic distinction of European breeds from African breeds (mean $F_{ST} = >0.21$)

Table 1.1. Genetic analysis studies performed in taurine and indicine cattle[1] (continued)

Study	Population and breeds	Markers (used) and their type	Major findings in the study
MacNeil et al., 2007	11 taurine cattle breeds sampled from North America and the Chirikof Island	34 microsatellites	Chirikof Island cattle appeared to be unique and strongly differentiated relative to the other breeds; low levels of admixture in the Chirikof Island population
Wiener et al., 2004	397 animals of 8 British cattle breeds	30 microsatellites	High level of average H_O in British breeds; 87% allelic variation within breeds (based on AMOVA)
Kumar et al., 2003	7 South Asian, 11 European, and 7 Near Eastern cattle breeds	20 microsatellites	*Bos taurus* influence in the cattle breeds of the Indian subcontinent

[1]SNPs: Single nucleotide polymorphisms, HO: Holstein/Holstein Friesian, H_O: Observed heterozygosity, F_{IS}: Inbreeding coefficient, F_{ST}: Fixation index

Control of population stratification in association studies

Several approaches have been developed to control the differences in population genetic structure and substructure resulting from relatedness or admixture in either genome-wide or candidate gene association studies. Case-control studies assume population homogeneity in the face of population heterogeneity, which can cause spurious associations. Family-based sampling was used as an alternative method to that effect. However, case-control designs are still attractive as it is less expensive in comparison to family-based designs. Devlin and Roeder (1999) proposed a method called "genomic control (GC)" to prevent the population heterogeneity effects in SNP association scans or candidate gene tests. In a case-control study, it was observed that overdispersion of the chi-square test statistic for association resulting from population substructure and relatedness causes false rejections of the null hypothesis (Devlin and Roeder, 1999). Their proposed method has been developed with a Bayesian probability model that can efficiently adjust over-dispersion resulting from population stratification and relatedness. Although GC is a fast and straightforward method, it has lower power to detect an association in comparison to other methods used for adjusting for population structure (Astle and Balding, 2009). Pritchard et al. (2000) proposed a model-based clustering method, "structured association (SA)," for using multi-locus genotype data to infer population structure and assign individuals to discrete subpopulation clusters. This method assumes that each individual's ancestry is drawn from one or more of the "islands" (i.e., discrete subpopulations). This method has several applications, such as inferring population structure, population assignment of individuals, studying hybrid zones, and identifying migrants and admixed individuals (Pritchard et al., 2000). However, the SA method has some limitations, such as intensive computation cost on large data sets, and individuals' assignments to clusters are highly sensitive to the number of clusters, which

is not well defined. Price et al. (2006) proposed a method to detect and correct population stratification on a genome-wide scale, which uses principal component analysis to explicitly model ancestry differences between cases and controls using continuous axes of variation. This method can be applied to association studies with hundreds of thousands of markers. Alexander et al. (2009) proposed an ADMIXTURE algorithm for model-based estimation of ancestry in unrelated individuals, which adopts the likelihood model embedded in SA (Pritchard et al., 2000). ADMIXTURE allows the specification of known descent individuals to be used as a reference panel; however, SA does not allow this. Zhang et al. (2010) suggested a mixed linear model-based approach where population structure fits as a fixed effect and kinship among individuals is incorporated as the variance-covariance structure of the individuals' random effect.

Leptin

The *obese* autosomal recessive mutation (*ob*) was first identified in the house mouse in 1950 (Ingalls et al., 1950), where they observed *ob* mice had increased BW than normal ones after the first 4-6 wks of life. Animals with recessive *ob* mutation (*ob*/*ob*) displayed a high body mass index and elevated circulatory plasma glucose and/or insulin concentrations (Friedman et al., 1991). In 1994, Zhang et al. (1994) identified that the *obese* (*ob* or *LEP*) gene is responsible for regulating energy balance and obesity in the *ob*/*ob* mouse by positional cloning. This *LEP* gene is located on chromosome 4 in cattle. This gene is 16,824 bp long, consisting of 3 exons and 2 introns (Figure 1.1). Of the three exons, only exon 2 and 3 have coding or translated regions. The *LEP* gene sequence is 67% homologous among such diverse species as humans, gorilla, chimpanzee, orangutan, rhesus monkey, dog, cow, pig, rat, and mouse (Zhang et al., 1997). The *LEP* gene translates into a 167 amino acid peptide hormone with a molecular weight of 16 kDa, named leptin after the Greek word “leptos,” meaning lean or thin. The leptin

molecule has approximate dimensions of 20 × 25 × 45 Å. This protein is comprised of four antiparallel ∝-helices (A, B, C, and D), which are attached by two long crossover links (AB and CD) and one short loop (BC), assembled in a left-hand twisted helical bundle (Zhang et al., 2005). The leptin protein is secreted predominantly by adipocytes in mammals. However, previous studies reported the expression of LEPH in several other organs, including the stomach, placenta, testes, ovary, endometrium, mammary gland epithelium, skeletal muscle, hypothalamus, and pituitary gland (Masuzaki et al., 1997; Bado et al., 1998; Smith-Kirwin et al., 1998; Wang et al., 1998; Caprio et al., 1999; Morash et al., 1999; Jin et al., 2000; Kitawaki et al., 2000; Sobhani et al., 2000; Ryan et al., 2002; Ramsay et al., 2004). The mature leptin protein consists of 146 amino acids because it is secreted into the bloodstream following the 21 amino acid signal peptides' cleavage. This protein circulates throughout biological fluids both as a free form or in a form after binding to its receptor. Leptin usually exerts its action by binding to a receptor present on the surface of cells. There are six different isoforms of leptin receptors (*LEPR*), which result from alternative splicing (Lee et al., 1996). These isoforms include a long, fully active isoform (*LEPRb*), several short isoforms (*LEPRa*, *LEPRc*, *LEPRd*, and *LEPRf*), and a soluble form (*LEPRe*). Of six isoforms, only the *LEPRe* receptor isoform acts as a soluble circulating leptin-binding protein.

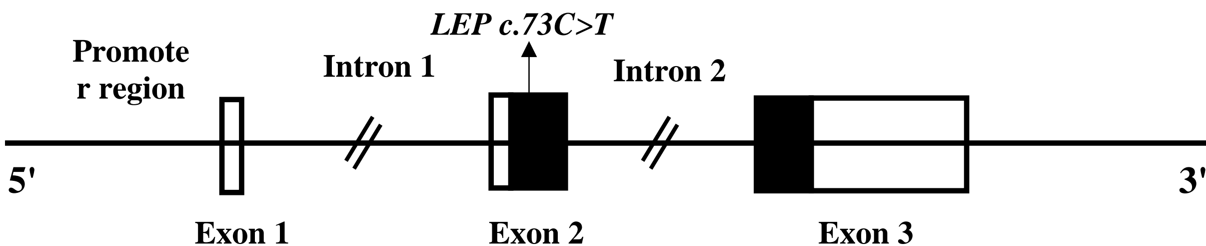

Figure 1.1. Genomic structure of the bovine leptin gene. Exons are shown as boxes (open boxes are untranslated regions, and coding regions are depicted in black), and introns are shown in lines.

Leptin's site of action

The brain, mainly the brainstem and hypothalamus, is the primary site of leptin action. As a satiety hormone, leptin has a hunger-suppressing effect and controls energy balance. Of several nuclei in the hypothalamus, the arcuate (ARC) nucleus is considered a significant site for regulating leptin's physiological processes (Rahmouni, 2012). This ARC nucleus regulates appetite and energy homeostasis through its two distinct types of neurons: orexigenic neurons and anorexigenic neurons. The orexigenic neurons produce neuropeptide Y (NPY) and agouti-related peptide (AgRP), whereas anorexigenic neurons release proopiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript. These two neurons are the principal sites of leptin receptor expression (Cowley et al., 2001). Leptin decreases appetite or feed intake in animals by acting on the ARC nucleus by stimulating POMC-containing neurons and inhibiting NPY/AgRP containing neurons. Leptin protein exerts its effect on BW by regulating feed intake and energy expenditure through the hypothalamus. Reduction in body adipose tissue level declines circulatory LEPH concentration, resulting in increased feed intake, leading to a positive energy balance and fat mass deposition and, eventually, weight gain. In contrast, excess body fat mass raises the plasma leptin level, reducing appetite, ensuing in negative energy balance, leading to loss of BW (Friedman, 2011).

Association of *LEP c.73C>T* polymorphism with performance traits

Several polymorphisms in the *LEP* coding and promoter regions have been found to be associated with cattle's performance traits. Of them, one polymorphism occurring in the coding region (exon 2) of the bovine leptin gene due to the transition of a cytosine (C) to thymine (T) residues results in an amino acid change from arginine to cysteine (Buchanan et al., 2002). This change in amino acids leads to alteration in LEPH's biological activity. It is hypothesized that

the presence of an unpaired cysteine on the A helix of the leptin molecule changes the leptin's tertiary conformation, affecting its binding to the leptin receptor and ultimately alters the signaling pathway for the LEPH action (Buchanan et al., 2002). The disulfide bridge between two existing cysteines on the leptin molecule, a critical structure for LEPH's biological function, could be destabilized by the extra unpaired cysteine resulting in a functional change of LEPH (Buchanan et al., 2002). Based on the position of nucleotides or amino acid residues or restriction enzymes used, this polymorphism has been named in different ways in several studies, such as R25C, E2FB or EXON2FB, C1180T, C73T, R4C or Arg25Cys, C305T, LEP*Kpn2*I. Our study denoted this polymorphism as *LEP c.73C>T* following the guidelines proposed by den Dunnen and Antonarakis (2000).

The associations of *LEP c.73C>T* with production and reproduction traits are summarized in Table 1.2. Other polymorphisms found in the promoter and coding regions of the bovine leptin (*LEP*) and leptin receptor (*LEPR*) genes are also encapsulated in Table A1. The *LEP c.73C>T* has displayed its association with feed intake, weight traits, average daily gain (ADG), carcass fatness traits, and some reproduction and body size-related traits in cattle. For other polymorphisms, associations have also been observed with feed intake, feed efficiency, feeding duration, weight traits, ADG, body size, carcass fatness traits, rib eye area/longissimus muscle area, and reproduction-related traits (Table A1). Since *LEP c.73C>T* has been considered as a causative mutation on the *LEP* and used in our study, further discussion will be done on this marker only.

Reproduction

The T allele of the *LEP c.73C>T* was significantly associated with shorter gestation length and easier calving than the C allele (Giblin et al., 2010). It can be hypothesized that

animals with the T allele will have less LEPH in their circulation because of less signaling, and thus, they will intake more dry matter, leading to a positive energy balance. Therefore, as an indirect measure of energy balance, body condition score (BCS) should be positively associated with the T allele of *LEP c.73C>T* genotype. An earlier study reported a greater average BCS during the first 60 days in milk in the animals with TT genotype compared with CT cows (Chebel et al., 2008). Thus, positive energy balance might contribute to improved reproductive performance, such as shorter gestation length and easier calving. However, cows with TT genotype for this marker were 25.43% less estrous cyclic by 49 days in milk than cows with the CC genotype (Chebel and Santos, 2011).

Feed intake and efficiency

Animals with the TT genotype of *LEP c.73C>T* marker had 1.2% more dry matter intake (DMI) compared to animals with the other two genotypes during the pretreatment period (McEvers et al., 2014). Also, animals homozygous for the T allele intake more dry matter than the CT animals in the entire study period. Their study also reported a 1.6% lower gain:feed (G:F) for the animals with TT genotype than the CC genotype during the pretreatment period. For the entire period, the CC genotype showed 1.4% greater G:F than the other two genotypes, CT and TT. An additive effect of the T allele was observed with increasing DMI in the steers group without ZH over the last 21 d of the feed trial (Kononoff et al., 2013). This effect of the T allele on DMI is expected as we previously discussed that low circulatory leptin in animals with the T allele would intake more dry matter. However, their study also reported a reduction in DMI with the T allele frequency for steers supplemented with zilpaterol hydrochloride (ZH). They explained that it might be due to a direct tissue effect or an indirect endocrine effect of ZH.

Table 1.2. *LEP c.73C>T* polymorphism and its phenotypic association with production and reproduction traits

Study	Population and Diets[1]	Genotype frequency	Associations found[2]	Associations not found[2]
Chebel and Santos, 2011	815 lactating HO cows fed TMR based on the stage of lactation	CC: 0.35, CT: 0.48, TT: 0.17	Proportion of cyclic cows at 49 ± 3 DIM	Proportion of cyclic cows at 62 ± 3 DIM; resumed cyclicity; AI submission rate; proportion of pregnant cows at first postpartum AI, second postpartum AI, and after 1st two postpartum AI; proportion of pregnant cows by 305 DIM
Choudhary et al., 2019	205 crossbred (½ HO × ½ Hariana) female	CC: 0.68, CT: 0.27, TT: 0.05	Birth weight	Age at first calving; BWs at 6 months, 12 months, 18 months, and 24 months of age; BW gain
Giblin et al., 2010	848 progeny tested HO dairy cattle sires	CC: 0.40, CT: 0.47, TT: 0.13	Direct calving difficulty; GL	Survival in the herd; CI; maternal calving difficulty; perinatal calf mortality; BCS; carcass fat
Komisarek and Antkowiak, 2007	219 JE cows	CC: 0.64, CT: 0.32, TT: 0.04	-	Age at first calving; days open; CI; number of AI per conception; GL
Komisarek, 2010	309 Polish HO bulls	CC: 0.29, CT: 0.52, TT: 0.19	Non-return rate in cows	Age at first insemination
Souza et al., 2010	357 NE heifers (2 selection lines for YW & a control line) raised on pasture	CC: 0.76, CT: 0.22, TT: 0.02 (overall of 3 selection lines)	Birth weight; BFT	WW; BW at 550 d; rump height; LM area; RFAT
Woronuk et al., 2012	136,286 crossbred beef steers and heifers fed standard finishing rations	CC: 0.30, CT: 0.47, TT: 0.23	BFT; BW	-
Buchanan et al., 2007	26 AN, 31 HH, and 32 CH steers fed *ad libitum* during finishing to achieve target back fat	-	Final LW; hot carcass weight; Ultrasound REA at the end of test; lean meat yield	Serum leptin hormone concentration; ADG; grade fat; average fat
McEvers et al., 2014	4,246 British and British × Continental beef steers offered a finishing diet	CC: 0.28, CT: 0.49, TT: 0.23	Final shrunk weight; DMI at pretreatment, treatment, and entire study period; ADG at treatment, and entire study period; G:F at pretreatment and entire study period; dressed yield; MS	Initial shrunk weight; shrunk weight at d 102; ADG at pretreatment period; G:F at treatment period; hot carcass weight; LM area; subcutaneous fat depth; USDA calculated YG
Larson et al., 2005	189 crossbred beef steers	CC: 0.11, CT: 0.57, TT: 0.32	Initial ADG; initial 12th rib fat; final LM area; change in LM area; YG	BW at d 71, d 160, and final weight; final ADG; change in 12th rib fat; LM area at d 0, d 160, hot carcass weight; MS

Table 1.2. *LEP c.73C>T* polymorphism and its phenotypic association with production and reproduction traits (continued)

Study	Population and Diets[1]	Genotype frequency	Associations found[2]	Associations not found[2]
da Silva et al., 2012	2162 young NE bulls (on pasture until 18 months & in feedlot until harvest)	CC: 0.88, CT: 0.12, TT: 0.00	Weight gain; LM area; RFAT	WW; YW; BFT
Nkrumah et al., 2004	144 *Bos taurus* crossbred beef steers started on a grass-based diet; however, on finishing diet during the trial period	CC: 0.23, CT: 0.50, TT: 0.27 (overall of 5 selection lines)	Gain in BFT; carcass BFT; carcass YG; carcass lean meat yield	RFI; F:G; DMI; ME intake; metabolic mid-weight; ADG; L. thoracis area; feeding duration; feeding frequency; US BFT; US L. thoracis area; US MS; carcass slaughter weight; carcass L. thoracis area; carcass MS
Kononoff et al., 2013	4,178 British × Continental beef steers fed a high-energy finishing diet	-	US fat thickness at d 0, 65, 105, 132, and their mean; average fat gain; initial fat thickness; 12th rib fat thickness; YG % (YG 1, and 4 and 5); USDA quality grade (U.S. Choice or higher, and Select)	Initial BW; final BW; ADG; DMI; F:G; hot carcass weight; LM area; MS; calculated YG; USDA quality grade (No Roll); YG % (YG 2, and 3)
Kononoff et al., 2014	2,958 British × Continental beef steers fed a high-energy finishing diet	-	DMI; 12th rib fat thickness; YG % (YG 1, and 4 and 5)	Initial BW; final BW; BW gain; ADG; F:G; hot carcass weight; LM area; MS; USDA quality grade (U.S. Choice or higher, Select, and No Roll); YG % (YG 2, and 3)
Devuyst et al., 2008	134 AN, 124 SM, 78 SH, and 259 crossbred beef cows	CC: 0.41, CT: 0.42, TT: 0.17 (Overall of 4 breeds)	Calf's WW (Shorthorn and commercial)	Calf's WW (Simmental and Agnus)
Fathoni et al., 2019	100 Indonesian Kebumen Ongole Grade cattle	CC: 0.78, CT: 0.21, TT: 0.01	Chest circumference at weaning	Birth weight; WW; YW; ADG; shoulder height at birth, weaning, and yearling; body length at birth, weaning, and yearling; chest circumference at birth, and yearling
Matteis et al., 2012	95 HO cows	*CC: 0.36, CT: 0.48, TT: 0.16	Body depth	Stature (height at hip); rump width
Banos et al., 2008	571 HO cows fed high and low concentrate diets on feed trial	CC: 0.43, CT: 0.45, TT: 0.12	-	FI; DMI; FI over milk yield; DMI over milk yield; LW; BCS
Lagonigro et al., 2003	169 second generation crossbred bull calves fed ad libitum concentrate & straw	CC: 0.44, CT: 0.42, TT: 0.14	-	FI; backfat depth; subcutaneous fat; intermuscular fat; MS

Table 1.2. *LEP c.73C>T* polymorphism and its phenotypic association with production and reproduction traits (continued)

Study	Population and Diets[1]	Genotype frequency	Associations found[2]	Associations not found[2]
Buchanan et al., 2002	60 AN, 55 CH, 22 HH, and 17 SM yearling bulls fed moderate energy ration	*AN- CC: 0.18, CT: 0.49, TT: 0.34; CH- CC: 0.44, CT: 0.45, TT: 0.12; HH- CC: 0.20, CT: 0.50, TT: 0.30; SM- CC: 0.46, CT: 0.44, TT: 0.10; Overall- CC: 0.29, CT: 0.50, TT: 0.21	Average fat; grade fat; leptin mRNA	-
Schenkel et al., 2005	43 AN, 30 LM, 11 CH, 68 SM, and 959 animals with breed composition <5/8 for all breeds (heifers/steers/bulls) fed a finishing diet	*AN- CC: 0.20, CT: 0.50, TT: 0.30; LM- CC: 0.27, CT: 0.57, TT: 0.30; CH- CC: 0.30, CT: 0.50, TT: 0.30; SM- CC: 0.35, CT: 0.48, TT: 0.17; Other- CC: 0.34, CT: 0.49, TT: 0.18	Lean yield; fat yield; grade fat	LM area; hot carcass weight; quality grade; semitendinosus muscle SF at 7 d postmortem
Corva et al., 2009	253 grazing BN (5/8 AN × 3/8 BR) steers received a grass-based diet	CC: 0.22, CT: 0.57, TT: 0.21	Carcass yield; carcass BFT	LW gain; gain in BFT; final LW; US final BFT; US final REA; carcass weight; carcass length; carcass REA; carcass IMF
Fortes et al., 2009	46 NE, 41 CC, 19 BN, 26 RG × NE, and 15 BU cattle offered a finishing diet	*NE- CC: 0.92, CT: 0.08, TT: 0.00; CC- CC: 0.37, CT: 0.48, TT: 0.15; BN- CC: 0.55, CT: 0.38, TT: 0.07; RG × N- CC: 0.85, CT: 0.15, TT: 0.01; BU- CC: 0.64, CT: 0.32, TT: 0.04; Overall- CC: 0.66, CT: 0.31, TT: 0.04	-	BFT; total lipids; REA; SF
Liu et al., 2010	422 Chinese Qinchuan steers fed normal diet	*CC: 0.21, CT: 0.50, TT: 0.29	LW; LM area	Carcass length; carcass chest depth; BFT; MS; meat tenderness

Table 1.2. *LEP c.73C>T* polymorphism and its phenotypic association with production and reproduction traits (continued)

Study	Population and Diets[1]	Genotype frequency	Associations found[2]	Associations not found[2]
Pannier et al., 2009	37 AN, 18 BB, 12 BD, 79 CH, 67 HO, 32 HH, 117 LM, 11 SA, and 57 SM	AN- CC: 0.27, CT: 0.62, TT: 0.11; BB- CC: 0.72, CT: 0.17, TT: 0.11; BD- CC: 0.50, CT: 0.50, TT: 0.00; CH- CC: 0.40, CT: 0.47, TT: 0.13; HO- CC: 0.46, CT: 0.47, TT: 0.13; HH- CC: 0.22, CT: 0.50, TT: 0.28; LM- CC: 0.55, CT: 0.36, TT: 0.09; SA- CC: 0.82, CT: 0.18, TT: 0.00; SM- CC: 0.58, CT: 0.40, TT: 0.02	-	IMF values
Lusk, 2007	1653 crossbred feedlot steers and heifers	CC: 0.32, CT: 0.49, TT: 0.19	-	Growth in BW and BFT
de Carvalho et al., 2012	201 crossbred beef bulls and cows (7 genetic groups; fed 60% concentrate & 40% forage)	CC: 0.29, CT: 0.55, TT: 0.16 (Overall of 7 genetic groups)	Carcass fat thickness; SF	Slaughter weight; hot carcass weight; REA; MS
Crews et al., 2004	433 Charolais and Charolais-cross steers fed growing & finishing diets	-	-	DMI; RFI; ADG; hot carcass weight; LM area, carcass marbling score; carcass fat thickness
de Oliveira et al., 2013	100 NE cattle (forage at 1st, forage & concentrate at 2nd, and finishing diet at 3rd periods)	CC: 0.62, CT: 0.38, TT: 0.00	Carcass fat distribution; carcass marbling; carcass fat thickness	Weights at 1st, 2nd, and 3rd periods; ADG between 1st and 2nd periods; ADG between 2nd and 3rd periods; US REA; US BFT; carcass REA
Barendse et al., 2005	Group 1: 821 AN, 101 SH, 742 others; Group 2: 256 AN, 317 BR, 149 BLR, 291 HH, 142 MG, 225 SG, and 85 SH feedlot steers	Group 1: CC: 0.48, CT: 0.38, TT: 0.14; Group 2: CC: 0.40, CT: 0.37, TT: 0.23	-	AUS-MEAT marbling; IMF detected by NIRS; BFT at the p8 position; adjusted total fat

[1]HO: Holstein/Holstein Friesian, JE: Jersey, NE: Nellore, AN: Angus, HH: Hereford, CH: Charolais, SH: Shorthorn, SM: Simmental, LM: Limousin, BR: Brahman, BN: Brangus, CC: Canchim, RG: Rubia Gallega, BU: Braunvieh, BB: Belgian Blue, BD: Blonde d'Aquitaine, SA: Salers, BLR: Belmont Red, MG: Murray Grey, SG: Santa Gertrudis, TMR: total mixed ration

[2]DIM: days in milk, AI: artificial insemination, BW: body weight, GL: gestation length, NIRS: Near-infrared spectroscopy, CI: calving interval, BCS: body condition score, WW: weaning weight, LM: Longissimus muscle, BFT: backfat thickness, RFAT: rump fat thickness, YW: yearling weight, LW: live weight, US: ultrasound, REA: rib eye area, ADG: average daily gain, G:F: gain to feed ratio, USDA: United States Department of Agriculture, YG: yield grade, MS: marbling score, RFI: residual feed intake, DMI: dry matter intake, ME: metabolizable energy, F:G: feed to gain ratio, FI: feed intake, SF: shear force, IMF: intramuscular fat, P1: 1st period, P2: 2nd period, P3: 3rd period, FCR: feed conversion ratio, U.S.: United States.

*Genotype frequencies were calculated from the allele frequencies

Growth and body size traits

Many earlier studies reported positive associations of the T allele of *LEP c.73C>T* genotype with weight traits, including weaning weight and weaning to yearling weight gain compared with the animals homozygous for the C allele (Devuyst et al., 2008; da Silva et al., 2012). Since animals with the T allele consumed more dry matter, these results were expected. However, some studies also reported conflicting results, such as an association of the C allele with greater BW at the target backfat depth in *Bos taurus* beef steers (Buchanan et al., 2007) and low ADG at the start of the feed trial in TT steers compared with CT genotype (Larson et al., 2005). Limited earlier studies have been carried out on the association of *LEP c.73C>T* with body measurement traits. Fathoni et al. (2019) reported greater chest circumference at weaning for the CT animals than the CC homozygous animals. Greater body size for animals with the T allele was expected since they will consume more feed.

Carcass traits

Earlier studies demonstrated the association of the T allele of *LEP c.73C>T* polymorphism with carcass (fat-related) traits, such as fatter carcass, ultrasound or carcass backfat thickness (BFT), marbling score, and yield grades (YG) in beef cattle, where high concentrate diet was offered in the majority of the studies (Nkrumah et al., 2004; Kononoff et al., 2005; Larson, D. M. Bauer, M. L. DeVuyst, E. A. Berg, P. T. Bullinger, 2005; Schenkel et al., 2005; Buchanan et al., 2007; Souza et al., 2010; da Silva et al., 2012; Woronuk et al., 2012; Kononoff et al., 2013; Kononoff et al., 2014; McEvers et al., 2014). These findings were anticipated because animals with the T allele for this marker will consume more dry matter, leading to positive energy balance resulting in fatness carcasses. As the T allele contributes to an increase in BFT, animals with TT genotype might have a small Longissimus muscle (LM) area

(also known as Ribeye area or *L. thoracic* area). Animals with the C allele were found to have a greater ultrasound LM area compared with the TT genotype in Nelore bulls (da Silva et al., 2012), and crossbred beef steers (Larson et al., 2005; Buchanan et al., 2007). However, Liu et al. (2010) reported a larger direct LM area for the TT genotype than that of CC and CT genotypes in Qinchuan steers.

Association of *LEP* haplotypes with performance traits

A haplotype is a group of alleles (i.e., alternative DNA sequences of a gene) of polymorphic loci located at adjacent positions on a chromosome and inherited together with high probability. The diplotype is a specific combination of two haplotypes that an individual carry within a block (or homologous haplotypes). A diplotype is usually similar to a genotype for a single polymorphic locus. A haplotype can have larger effects on a trait than individual SNPs when the haplotype has greater linkage disequilibrium (LD) with a quantitative trait loci (QTL) for that trait compared to the individual SNP alleles used to construct it (Abdel-Shafy et al., 2014). Haplotypes containing several QTL variants affecting a trait in a similar direction can also exert a more significant effect, augmenting the power of genomic region identification for the trait, albeit having their small effects (Bickel et al., 2011). However, the haplotype effect could be small if those QTL effects are in the opposite direction. Several studies have been performed using different leptin haplotypes for exploring their association with performance traits in cattle.

Reproduction

In limited previous studies, the association study between leptin haplotypes and reproductive traits have been carried out in cattle. Komisarek (2010) conducted a haplotype association study using one leptin promoter polymorphism (*LEP g.-963C>T*), one polymorphism on leptin exon 2 (*LEP c.73C>T*), and one on exon 3 (*LEP c.357C>T*) for reproductive traits in

Polish Holstein Friesian cows. They identified only CCT haplotype (SNP order: *LEP g.-963C>T*, *LEP c.73C>T*, *LEP c.357C>T*) showed significant association with non-return rate to 56 days after the first insemination in cows. In a leptin haplotype analysis, Giblin et al. (2010) used polymorphisms in the leptin gene promoter and coding regions for haplotype construction. Of the 22 possible haplotypes, 16 haplotypes with less than 1% frequency grouped together, leading to 7 different haplotypes for association analysis with reproduction. None of the leptin haplotypes were significantly associated with calving interval, direct and maternal calving difficulty. The CGGCACC (SNP order: *LEP g.-2470C>T*, *LEP g.-1457A>G*, *LEP g.-1238G>C*, *LEP g.-963C>T*, *LEP c.252A>T*, *LEP c.73C>T*, *LEP c.357C>T*) and other haplotypes showed an association with shorter gestation length (GL), though their effects were very negligible, i.e., around a one-day reduction in GL.

Growth and body size

Haplotype constructed with polymorphisms on the *LEP* showed a significant association with growth traits in dairy and beef cattle. Kulig and Kmieć (2009) carried out a haplotype association study using polymorphisms on exon 3 (*LEP c.357C>T*) and intron 2 (*LEP n.Sau3AI*) of the *LEP* with growth traits in LM cattle. Out of 9 possible diplotypes (SNP order: *LEP c.357C>T*, *LEP n.Sau3AI*), they used 5 diplotypes with high frequency. They also observed greater ADG between 3 and 210 days of age for the animals with CT/CT diplotype than the animals with CC/CC and CC/CT diplotypes. In that study, animals were not different between 5 diplotypes for other studied traits, such as BW at 3, 220, 365 days of age, ADG between 3 and 365, withers height, sacrum height, and chest girth. In a haplotype association study using 6 *LEP* SNP (Order: *LEP g.207C>T*, *LEP g.528C>T*, *LEP g.-1457A>G*, *LEP g.-963C>T*, *LEP c.252A>T*, *LEP c.73C>T*), Banos et al. (2008) did not report any association of the studied 5

haplotypes with live BW and BCS in Holstein cows, even so, the animals with CCGTTT haplotype tended to have greater live weight than the animals with other haplotypes. However, in a study with *Bos taurus* crossbred population, Nkrumah et al. (2006) reported that animals with CC/CC diplotype (SNP order: *LEP g.528C>T*, *LEP c.357C>T*) had lower ADG when compared to other 5 diplotypes. An earlier study with Brangus steers did not show any association of leptin haplotype constructed with *LEP* promoter SNP (*LEP g.528C>T*) and *LEP* exon 2 SNP (*LEP c.73C>T*) with final live weight and live weight gain (Corva et al., 2009).

Feed intake, efficiency, and carcass traits

Banos et al. (2008) used 6 SNP markers (Order: *LEP g.207C>T*, *LEP g.528C>T*, *LEP g.-1457A>G*, *LEP g.-963C>T*, *LEP c.252A>T*, *LEP c.73C>T*) of the leptin gene to construct haplotypes for investigating their effect on feed traits. They observed a positive impact of CCGTTT haplotype on feed intake and DMI. Another haplotype TCACAC negatively impacted DMI before adjusting, but this effect was not significant after Bonferroni correction. (Lagonigro et al., 2003) conducted a haplotype analysis using two SNP markers in exon 2 (*LEP c.252A>T* for the first site and *LEP c.73C>T* for the second site) and one in exon 3 (*LEP c.140C>T* for the third site) of the leptin gene in crossbred bulls. Among eight studied haplotypes, *TCC* and *ACC* haplotypes were associated with increased inter-muscular fat levels and reduced subcutaneous fat but did not affect feed intake. In another haplotype analysis using two SNPs (*LEP g.528C>T* and *LEP c.73C>T*) in the leptin gene, the CC haplotype showed to have high BFT than that of the most abundant CT haplotype (Corva et al., 2009). Pannier et al. (2009) carried out a haplotype analysis using 4 leptin polymorphisms (Order: *LEP g.207C>T*, *LEP g.528C>T*, *LEP c.73C>T*, *LEP n.Sau3AI*) to determine their haplotype effect on the intramuscular fat (IMF) levels as those four leptin loci are in LD with one another and closely located. Among 11 estimated haplotypes,

none of them were observed to be associated with the IMF level in beef × dairy crossbred cattle. In a haplotype association study with two leptin SNPs (*LEP g.528C>T* and *LEP c.357C>T*), Nkrumah et al. (2006) reported an association of the T alleles of both SNPs with higher serum leptin concentration, where TT-TT diplotype showed the highest effect. The ADG and feed conversion ratio (FCR) were different among the different leptin diplotypes. Moreover, T alleles of both SNP showed association with increased ultrasound backfat and marbling score, increased carcass backfat and marbling score, and lower lean meat yield. Schenkel et al. (2005) also conducted a haplotype analysis with 4 leptin polymorphisms (*LEP g.207C>T*, *LEP g.528C>T*, *LEP*, *LEP c.252A>T*, *LEP c.73C>T*) in crossbred heifers and steers. Of 10 haplotypes, there were three highly frequent (88%) haplotypes (TCAC, CCAT, and TTAC) that showed no association with the studied carcass traits, which were used as control (i.e., other haplotypes compared against their average). The CCTT haplotype was associated with decreased fat yield (-2.26%) and BFT (-1.84 mm), and increased lean meat yield (+2.42%) when compared to the three most common haplotypes.

Based on the above findings, it can be concluded that favorable alleles of significant SNPs within the haplotype are contributing to the significant results found in haplotype association studies. For instance, Nkrumah et al. (2006) reported a greater ADG in the animals with the T allele of *LEP c.357C>T* SNP compared to the animals homozygous for the C allele. Their study further demonstrated a low ADG for animals with the CC/CC diplotype compared to other studied five diplotypes, where the CC/CC diplotype was homozygous for the unfavorable allele (C allele) of *LEP c.357C>T* genotype. In an earlier study, the causative mutation on the *LEP*, i.e., *LEP c.73C>T*, influences the haplotype effect on a given trait (Banos et al., 2008).

Association of circulatory LEPH with performance traits

Previous studies have reported a reduction in food intake in mice, rhesus monkeys, pigs, and chickens after injecting LEPH into the body. Intracerebroventricular injection of leptin reduced food intake in *ob*/*ob* mice and high-fat diet-induced obese mice (West et al., 1994) but not in *db*/*db* obese mice (Campfield et al., 1995), rhesus monkey during the entire following day after administration (Tang-Christensen et al., 1999), and lean and *ob*/*ob* mice deprived of food for 4 h during the 48 h period post-injection (Mistry et al., 1997). Intraperitoneal injection of either mouse or human recombinant OB protein (leptin) also reduced food intake in wild-type *ob*/*ob* mice (Halaas et al., 1995). Peripheral administration of leptin showed the same effect in *ob*/*ob* and high-fat diet-induced obese mice (Campfield et al., 1995), young pigs (Ramsay et al., 2004), and chicken (Dridi et al., 2000); however, no effect of peripherally injected leptin on feed intake was observed in growing prepubertal ewe lambs (Morrison et al., 2002), and rhesus monkey (Tang-Christensen et al., 1999).

Reproduction

Crossbred (British × Brahman) beef heifers who became pregnant following fixed-timed AI showed higher circulatory plasma leptin concentrations than non-pregnant females throughout the entire experiment (Gentry et al., 2013). In their study, the mean plasma leptin concentration was also higher in pregnant females from d 2 to 9 during synchronization protocol to fixed timed AI than in the non-pregnant group. Circulatory plasma leptin concentration did not show any relationship with first postpartum luteal activity; however, higher plasma leptin concentrations showed an association with shorter intervals to first observed estrus (Liefers et al., 2003). Circulatory serum leptin concentration had a linear increase from 16 weeks before until the week of pubertal ovulation in developing heifers reaching sexual maturation from early spring or

midsummer. The leptin mRNA expression in adipose tissue significantly increased as puberty approached, though there was no correlation between serum leptin concentration and leptin mRNA expression (Garcia et al., 2002). They explained this absence of correlation might partly be due to having a relatively small number of adipose tissue samples compared to the number of serum samples. Also, the increased amount of leptin mRNA near puberty might be due to a greater number of adipocytes and increased expression of the leptin gene in adipose tissue. Leptin gene expression is regulated by several factors, such as insulin (Pratley et al., 2000), growth hormone (Houseknecht et al., 2000), prolactin (Gualillo et al., 1999), and environment, including photoperiod (Bernabucci et al., 2006). There was a marked decline in circulatory leptin concentration and leptin mRNA expression in subcutaneous adipose tissue in short-term fasting of growing peripubertal heifers, which was associated with a reduction in luteinizing hormone (LH) pulse frequency and serum concentration of insulin and IGF-1 (Williams et al., 2002). An earlier study on Brown Swiss cows demonstrated that leptin concentrations were significantly greater in the luteal-phase follicular fluid of small follicles (< 8 mm) than large follicles (≥ 8 mm). They delineated the high leptin level in small follicles resulting from ongoing atresia. Leptin concentration could be increased in the atretic follicle because leptin might play a cytokine role in follicular atresia (Spicer and Francisco, 1997, Spicer et al., 2000). Also, there was a significant correlation between serum and follicular fluid leptin concentrations in the luteal phase. Their study further reported that serum leptin was positively correlated with follicular fluid progesterone concentration in the preovulatory follicles. In Holstein dairy cows, Kadokawa et al. (2000) reported that circulatory plasma leptin concentration declined in the early postpartum period and then increased and became stable near the time of ovulation. They also suggested a delay in the recovery of leptin secretion increased the delay to the first ovulation.

Panwar et al. (2012) investigated the effect of reducing peripheral leptin concentrations by administering an anti-leptin antibody on ovarian follicular development in prepubertal mice. They demonstrated that reducing circulatory leptin concentration results in a significant increase in ovarian weight, a greater number of primary follicles but a reduced number of primordial follicles per ovarian section than the control group. They suggested leptin might inhibit the transition of primordial follicles to primary follicles, therefore, reduced leptin concentration results in increased primary follicles. They further observed that mice administered anti-leptin plus gonadotropins had a significantly greater number of Graafian follicles in their ovaries compared with other groups. Huszenicza et al. (2001) reported that circulatory plasma leptin concentration might interfere with cyclic ovarian function resumption in postpartum dairy cows. They also suggested that a minimal concentration above a supposed threshold might be the prerequisite of the onset of cyclicity. Also, high leptin level (i.e., above the physiological range) showed an inhibitory effect on the synergistic action of insulin-like growth factor-1 on follicle-stimulating hormone (FSH)-induced estradiol synthesis (Zachow and Magoffin, 1997). An inadequate ovarian response in terms of the number of follicles and retrieved oocytes has been reported in women due to increased serum LEPH concentration during FSH stimulation (Bützow et al., 1999). The high concentration of leptin in the ovary prevents estrogen production and thereby interferes with the development of dominant follicles and oocyte maturation (Mantzoros, 2000). Leptin also negatively impacted ovarian steroidogenesis in an *in vitro* study performed in rodent and bovine models (Spicer and Francisco, 1997, Spicer et al., 2000). Furthermore, increased leptin concentration exerts its inhibitory effect on mouse follicular growth over 9 days of culture in a dose-dependent manner (Swain et al., 2004). Also, LEPH concentration has been reported to be negatively correlated with endometrial thickness in humans (Chakrabarti et al.,

2012). Moreover, Duggal et al. (2000) investigated the effect of systemic leptin administration on ovulation in the rat ovary, both *in vivo* and *in vitro*, where leptin administration caused a decline in ovulation without making any significant alteration in plasma progesterone and estradiol levels. They demonstrated that leptin was associated with reduced ovulation through an indirect decrease in BW. They also explained that leptin administration might inhibit the release of oocytes from the preovulatory follicles by hindering LH action.

Growth and body size

In an *in vivo* study with rats, Watanobe and Habu (2002) demonstrated that LEPH exerts its effect on growth hormone (GH) secretion by regulating GH-releasing factors' (i.e., somatostatin) secretion through the hypothalamus. Nkrumah et al. (2007) found an association of LEPH categories with growth traits in feedlot crossbred beef steers fed a high concentrate diet. Beef steers with high serum leptin concentration had greater metabolic BW, final BW, and slaughter BW than the low serum leptin group; however, animals with different serum leptin groups showed a similar ADG. They also observed positive phenotypic correlations between circulatory serum leptin concentration and metabolic and slaughter BW in that study; even so, no phenotypic correlation with ADG was observed. Some previous studies also did not show any phenotypic correlation between circulatory LEPH concentration and ADG in AN steers (Richardson et al., 2004) and growing beef heifers (Kelly et al., 2010a). Geary et al. (2003) did not observe any phenotypic correlation between serum leptin concentration among steers and heifers and live weight in feedlot *Bos taurus* composite beef cattle. A study with *Bos taurus* composite beef breeds demonstrated a negative relationship between serum leptin concentration and ADG (Foote et al., 2015). However, their follow-up study reported a positive association of ADG with circulatory plasma leptin concentration measured at d 42, 83 and mean leptin level,

though no association with leptin measured at d 0 of the feed trial (Foote et al., 2016). They did not provide any clear explanation behind these conflicting results; rather, they discussed only the differences between these two studies (experimental units: finishing steers vs. finishing steers and heifers, and the number of animals used: 236 vs. 473). In another study with Holstein Friesian cows, Liefers et al. (2003) found high plasma leptin concentration for cows with low weight compared to heavier cows.

Feed intake and efficiency

Some feed intake and efficiency traits have been found to be associated with circulatory leptin concentration in beef cattle. Nkrumah et al. (2007) reported that crossbred steers with high serum leptin concentration (20.32 ng/mL on average) had greater DMI and residual feed intake (RFI) than the low serum leptin group (8.57 ng/mL on average). However, the FCR was not different among three different serum leptin groups. In their study, DMI showed a positive phenotypic and negative genetic relationship with serum leptin concentration. No phenotypic correlations were observed between circulatory serum leptin concentration and FCR, RFI, i.e., correlations not different from zero; however, a negative genetic correlation with the large standard error was noticed in those cases. Circulatory leptin concentration was positively correlated with DMI and RFI but negatively correlated with BW-adjusted DMI and G:F in finishing crossbred beef steers (Foote et al., 2015). They explained these results could be due to the interrelationship of BW, body fatness, leptin, and DMI. Holstein-Friesian cows with high DMI showed higher circulatory leptin concentrations when compared with low DMI cows (Liefers et al., 2003). Also, Richardson et al. (2004) reported a positive correlation between serum leptin and RFI, though no significant correlation with average daily feed intake and FCR in feedlot Angus steers. Foote et al. (2016) used leptin concentration measured at different time

points (day 0, day 42, day 83, and mean leptin concentration) during the feed trial to evaluate the leptin association with DMI, G:F, and RFI in finishing admixed beef steers and heifers. Plasma leptin measured at different periods, and their mean was positively associated with DMI calculated from the entire 84-d feed trial. They also observed the same association findings between all measures of leptin concentration and DMI measured at first 35 d and last 35 d of feed trial, except there was a tendency of a positive association between day 0 leptin and last 35 d DMI. A positive association of day 83 and mean plasma leptin was noticed with RFI; however, plasma leptin measured at day 0, and mean plasma leptin was negatively associated with G:F.

Carcass traits

The circulatory LEPH concentration also showed its association with carcass traits in several previous studies. In a study with crossbred beef steers fed a high concentrate diet, Nkrumah et al. (2007) found that animals with high serum leptin had greater ultrasound backfat, ultrasound marbling score, carcass 12th rib fat, carcass marbling score, carcass YG, and low carcass lean meat yield when compared with the low serum leptin group. However, different leptin groups did not affect the ultrasound LM area and the carcass LM area. Previous studies reported positive leptin concentration relationships with fatness carcass traits but negative with LM area and lean meat yield (Table 1.3).

Based on the above discussion, a certain LEPH level is required for maintaining reproduction in animals. In this case, leptin stimulates gonadotropin-releasing hormone (GnRH) secretion by acting centrally through the hypothalamic-pituitary-gonadal axis and regulates FSH and LH's release from the pituitary (Watanobe, 2002). Also, a low level of leptin causes more dry matter intake, resulting in the utilization of extra energy after maintenance for reproduction. However, a high concentration of LEPH can have a negative impact on reproduction by reducing

ovarian function, i.e., follicular growth in females or inhibiting testicular function in males. High LEPH can reduce feed intake, resulting in negative energy balance and thereby, cause reduced reproductive performance. Since circulatory LEPH is often associated with body fatness, obesity resulting from high LEPH might negatively affect fertility. However, some earlier studies reported a positive relationship of LEPH concentration with some reproductive traits. Since leptin reduces feed intake in animals, we anticipated that circulatory leptin concentration would negatively correlate with DMI, RFI, BW, and ADG. However, some previous studies reported positive relationships of leptin concentration in all cases. Large animals with increased adipocyte numbers might produce more leptin in their body, leading to a positive association. It was expected that LEPH concentration would positively be correlated with carcass fatness traits, including 12th rib BFT, carcass YG, marbling score, rump fat thickness, and IMF percentage because concentrations of LEPH are positively associated with the total number of adipocytes. Previous studies also reported a negative association of LEPH concentration with LM area or REA. These findings were also predicted as the LM area is negatively correlated with carcass fatness traits. Therefore, circulatory LEPH concentration has an excellent potential to utilize as a biological marker for performance traits in beef cattle.

Table 1.3. Phenotypic relationship of circulatory leptin concentration with carcass traits[1]

Study	Populations and diets	Positive relationship	Negative relationship	No relationship
Nkrumah et al., 2007	464 crossbred beef steers fed concentrate diet	US (BFT and MS), carcass (12th RF, MS, and CYG)	US LM area, carcass REA, and lean meat yield	Slaughter BW, CWT
Brandt et al., 2007	Commercial-fed beef cattle (995 steers & 757 heifers)	Carcass (CYG, 12th RF, MS), HCW	Carcass LM area	
Geary et al., 2003	179 composite *Bos taurus* steers and heifers fed growing and finishing diets	Carcass (MS, FD, QG, CYG)	-	HCW, Carcass (REA and CYG)
McFadin et al., 2003	84 beef steers	Carcass (MS, 12th RF)	-	Carcass (REA and CYG), HCW
Foote et al., 2015	473 finishing crossbred beef steers	HCW, Carcass (12th RF, CYG, MS)	Carcass LM area	-
Foote et al., 2016	236 composite beef cattle (127 steers and 109 heifers)	US (12th RF, RFAT, IMF)[2], carcass (12th RF, MS, CYG)[2]	Carcass LM area[3]	Carcass LM area[4], US LM area[2]

[1]US: Ultrasound, BFT: backfat thickness, MS: marbling score, 12th RF: 12th rib fat thickness, MS: marbling score, CYG: calculated yield grade, HCW: hot carcass weight, FD: fat depth over the 12th and 13th rib, QG: quality grade, RFAT: rump fat thickness, IMF: intramuscular fat, LM: longissimus muscle, REA: rib eye area, BW: body weight
[2]Association with plasma leptin measured at day 0, 42, 83, and its mean concentration
[3]Association with plasma leptin measured at day 42, 83, and its mean concentration
[4]Association with plasma leptin measured at day 0

Association of *LEP c.73C>T* polymorphism with LEPH concentration

A previous study reported an association of the T allele of *LEP c.73C>T* polymorphism with higher serum leptin concentration in Charolais cattle (Buchanan et al., 2007). However, Holstein Friesian heifers with CC genotype had significantly higher circulatory serum leptin concentrations during late pregnancy and the first 5 days of lactation than the TT homozygous heifers (Liefers et al., 2003). Buchanan et al. (2007) explained that these inconsistent findings might be due to the free form of the receptor (Ob-Re) binding C, whereby the T allele's faster clearance because of lower binding affinity. We previously discussed the effect of amino acid change due to the transition of C (wild type) to T (mutant type) allele in the *LEP c.73C>T*

genotype on LEPH's biological activity. The alteration in the biological function of LEPH is usually caused by changing the tertiary conformation of the leptin molecule and destabilizing the disulfide bridge between two existing cysteines on the leptin molecule (Buchanan et al., 2002). Animals with the T allele will have free circulatory leptin (i.e., unbound to leptin receptors), which would be cleared sooner from the circulation because of their significantly shorter half-life in comparison to bound leptin (Chebel et al., 2008). Therefore, it is essential to know if the T allele's biological mechanism acts through increased clearance of LEPH, or reduced signaling of LEPH due to reduced affinity for *LEPR*, or a combination of both when interpreting the effect of LEPH concentration and *LEP c.73C>T* genotypes on phenotype. If this effect is primarily due to the faster clearance of the LEPH, then plasma/serum LEPH concentration is a valid marker. However, if the effect is mainly through reduced LEPH signaling, differences in LEPH concentrations between the genotypes are not a useful marker and have less biological relevance.

Conclusions

Commercial and research beef cattle populations are often crossbred or admixed. The admixture presence results in allele frequency differences by sub-populations, leading to false positive or false negative results in association studies. This often makes the use of crossbred or admixed populations challenging in research. The presence of AD cattle in crossbreeding is increasing but is not well characterized. Understanding the AD breed's genetic structure is crucial to using this breed in crossbreeding programs or future genetic improvement programs. Research on investigating this breed's genetic structure and its genetic relationships with other beef breeds in the United States is still lacking in the literature. Also, limited studies have been performed on genetic analyses of the taurine beef breeds in the United States. Therefore, the first study focused on identifying 1) the genetic structure of AD cattle relative to other common taurine breeds in the

U.S. and 2) if fitting ancestral breed groups will improve statistical modeling in admixed populations (Study 1, Chapter 2).

The ancestral breed groups determined from the Study 1 will be applied in the last two studies (Study 2 and 3), where the associations of the *LEP* genotype, *LEPD,* and circulating LEPH concentrations with performance traits will be investigated. Based on the literature review, *LEP c.73C>T* polymorphism has been studied more than other polymorphisms in the coding and promoter regions of the bovine leptin gene. Most of the earlier association studies for performance traits using *LEP c.73C>T* polymorphism and LEPH have used steers that fed a high concentrate finishing diet, and some in heifers. Developing heifers are typically not fed high concentrate diets when being kept for the breeding herd; therefore, it is worthwhile to investigate the effect of this *LEP* marker and LEPH on some previously studied traits in developing heifers fed a forage-based diet to explore if different relationships occur between genotype, hormone and performance traits. Additionally, no information is available in the literature on the effect of the *LEP c.73C>T* genotype and circulating LEPH concentration on reproductive characteristics (e.g., antral follicle count, reproductive tract score, ovary measurements, success traits [pregnancy success, weaning success, and reproductive success over time], GL, age at first calving, and calving interval), linear and calculated body size characteristics and feeding behavior attributes in forage-fed developing beef heifers. Therefore, two studies (Studies 2 and 3) were designed to investigate the association of *LEP* genotype, *LEPD*, and LEPH with performance traits in forage-fed commercial developing beef heifers. Study 2 focused on determining the association of those leptin versions with reproductive characteristics in commercial beef cows (Chapter 3). And, study 3 focused on investigating the association of the *LEP* genotype, *LEPD,* and circulating LEPH concentrations with growth, body size, feed intake,

efficiency, feeding behavior, and ultrasound carcass traits in commercial developing beef heifers (Chapter 4).

CHAPTER 2. IMPROVING ANIMAL MODELING IN ADMIXED POPULATIONS USING ANCESTRAL BREED GROUPS: A CASE STUDY WITH AMERICAN ABERDEEN CATTLE

Abstract

Commercial and research beef cattle populations are often crossbred or admixed, resulting in allele frequency differences by sub-populations and making them challenging to use in research. American Aberdeen (AD) cattle's presence in crossbreeding is increasing but is not well characterized. Therefore, the current study aimed to 1) determine the genetic structure of AD cattle and its genetic relationship with American taurine beef breeds, and 2) understand the genetic population structure of this admixed cattle herd for improved trait analysis models. A total of 727 animals were genotyped and used based on nine sub-populations to understand genetic diversity based on expected heterozygosity (H_E), polymorphism, pairwise fixation index (F_{ST}), molecular variance, and principal components (PCs). The lowest proportion of polymorphism was observed in AD (0.8049), followed by Shorthorn (0.8523) and undetermined parentage (UP; 0.8524). However, high polymorphism levels (i.e., 95% or greater) were seen in admixed populations, Red Angus, and Gelbvieh breeds. By sub-population, H_E ranged from 0.3490 ± 0.0004 in AD to 0.3935 ± 0.0004 in Angus. The largest genetic differentiation was observed between AD and Gelbvieh, Simmental, and Shorthorn (F_{ST} = 0.169 to 0.186). Analysis of molecular variance showed that 2.839% ($P < 0.001$) of the molecular variation was accounted for among populations. The first to third PCs explained 41.09%, 25.06%, and 15.27% of the total variance, respectively, and indicated individuals grouped based on primary breed. Admixture analysis elucidated the genetic structure of AD cattle relative to five different taurine cattle breeds available from the United States populations. Regardless of including or excluding

pedigree, statistical models with primary ancestral breed groups for admixed individuals displayed better fit statistics compared to models without the ancestral group. The AD breed exhibited a unique homogeneous genetic structure even though founder animals originated from the Angus breed. Our study demonstrates the inclusion of the ancestral breed group in the mixed animal model can avoid complications with breed specification and false-positive or false-negative results and should be used in association studies when admixed populations are present.

Introduction

In the 19th century, the concept of cattle breeds led to human-mediated artificial selection imposing strong bottlenecks, which created population demes based on phenotypes (Porto-Neto et al., 2013). After breed formation, the use of artificial insemination for breed expansion decreased genetic variability within breeds, particularly in the sex chromosomes and mitochondrial DNA (Schaffner, 2004). However, in the late 20th century, the implementation of crossbreeding systems for increased productivity and profitability through efficient use of breed resources (Gregory and Cundiff, 1980; Baker, 1982) has led to the formation of crossbred or admixed cattle populations that are thought to have greater genetic diversity. Studies of genetic diversity require a detailed reference genome to catalog variations in new strains or lineages. The full assembly of the bovine genome sequence was completed in 2009 (Zimin et al., 2009), which led to the identification of several hundred-thousand single nucleotide polymorphism (SNP) markers that have been used in genetic analyses of cattle at the genome-wide level (Gibbs et al., 2009; McTavish et al., 2013; Decker et al., 2014; Sermyagin et al., 2018). These genetic studies include investigating the genetic variability across breeds and relationships between markers to uncover the history of breed origins as well as to evaluate the genomic relationship between

modern breeds. The usefulness of SNP in analyses of population diversity and genetic structure has been demonstrated in several studies (McKay et al., 2008; Lin et al., 2010).

Cattle genetic analyses have mostly been performed in the European breeds and various native breeds across the world. Many of those studies have focused on the genetic divergence between two types of cattle (*Bos taurus* and *Bos indicus*) using SNP data, where a high level of genetic differentiation between the two domestic lineages was observed (McKay et al., 2008; Gibbs et al., 2009; Lin et al., 2010; Edea et al., 2015; Campos et al., 2017). The taurine breeds have lower SNP diversity compared to the diversity within indicine breeds (Gibbs et al., 2009; Edea et al., 2015; Campos et al., 2017). Gibbs et al. (2009) suggested that these differences in diversity were because of progenitor population diversity and bottleneck effects at and before breed formation rather than variation in the intensity of natural or artificial selection post domestication. However, Lin et al. (2010) observed greater genetic diversity within European and Asian taurine populations than Asian indicine populations. A decreasing trend of mean inbreeding (-0.2% per year) has been observed in American Red Angus (AR) cattle from 10.7% in 1960 to around 3.25% in 1974 using pedigree information. New registrations that resulted from the mating of less related parents than the population average contributed to this reduction in inbreeding. However, a slight increase (0.02% per year) in inbreeding was observed from 1975 to 2005. After 1974, the percentage of nonrelated founder animals (out of animals registered each year) decreased, and matings between related individuals increased slightly, which resulted in a small rise in inbreeding. Even so, this increase in inbreeding was below critical levels for the management of genetic diversity (Márquez et al., 2010). Over the past 10 years, several studies using SNP markers have investigated the genetic variability and divergence of indigenous cattle populations (Cañas-Álvarez et al., 2015; Kim et al., 2018), their genomic

relationships with taurine populations (Edea et al., 2013; Makina et al., 2014), and combined taurine and indicine populations (Karimi et al., 2016; Sermyagin et al., 2018; Zhang et al., 2018). In analyses of global population history, New World cattle breeds exhibited ancestry from both the taurine and indicine lineages that have contributed to adapting these breeds to a novel environment (McTavish et al., 2013). Decker et al. (2014) reported that cattle migration through movement and trading that have allowed admixture of breeds had been important forces in shaping modern bovine genomic variation.

The beef cattle breeds in the United States (U.S.) are not native to this region. The first cattle were introduced to the New World by Christopher Columbus on his second voyage in 1493 (Minster, 2019). American Aberdeen (AD) cattle in the U.S., formerly referred to as American Lowline, descend from a closed Aberdeen Angus (AN) herd formed at the Trangie Agricultural Research Centre in New South Wales, Australia in 1929. Although the literature is still lacking on AD cattle production characteristics, the breed association claims these cattle are easy calving, docile, very efficient on grass, and have excellent beef taste, texture, and tenderness attributes (American Aberdeen Association, 2020). While investigating the effect of selection for growth rate on herd profitability in Trangie Agricultural Research Centre, Parnell et al. (1991) reported that high (H) line and control (C) line AN steers had slightly leaner carcasses than the low (L) line steers at a similar weight. Additionally, the H line steers had a slightly smaller average longissimus muscle area than in the C or L line steers. However, the H line heifers and cows performed very well for other traits, including earlier puberty, greater calving rate, larger and heavier calves at all ages (Parnell et al., 1991), and more efficient in utilizing feed for maintenance and calf growth (Herd, 1992) compared to the C or L line group. These L line cattle were the founder animals of today's AD breed. In another Trangie Research Center

study, it has also been claimed that the L line cattle had a greater stocking rate (54 breeding cows per 100 acres) and better retail product yield per acre off grass than other beef breeds, such as AN, Shorthorn (SH), Hereford, Simmental (SM), Wagyu, and Murray, but this is not well documented in the literature. Understanding the genetic diversity and population structure of this AD breed is therefore required to meet current production needs in various environments, allow sustained genetic improvement, and facilitate rapid adaptation to changing environments and breeding objectives (Notter, 1999; Hanotte et al., 2010). No previous studies have investigated AD cattle's genetic structure and its genomic relationship with other beef breeds.

F2 mice are traditionally used to identify genes underlying Mendelian disorders in humans. Linkage mapping with F2 populations identifies regions harboring causal variants with high statistical power (Furlotte et al., 2012); however, it does not provide enough resolution in identifying genetic variations underlying complex and polygenic traits (Bennett et al., 2010). Increasing crossover density is often used for getting a higher resolution in linkage mapping (Georges, 2007). One way of augmenting this crossover density is to use advanced intercross lines (i.e., F3, F4,…, Fn generations), which are generated by random crossing of F2 or backcross experiment (Darvasi and Soller, 1995). Commercial and research beef cattle populations are often crossbred or admixed. Admixture is the presence of multiple genetically distinct subgroups within a population (Wang et al., 2005), where admixed populations have genomes from divergent parental origins (Buerkle and Lexer, 2008) based on different selective pressures. Recently admixed populations are likely to harbor greater genetic variation than the original populations they come from (Seldin et al., 2011). Mapping with admixed populations can have a significant advantage due to admixture linkage disequilibrium generated through the interbreeding of the parental populations (Parra et al., 1998). The power of this type of mapping

is greater than the linkage analysis and comparable to the population-based association study (Montana and Pritchard, 2004). However, the proportion of admixture may differ across individuals in admixed populations. This variation makes association analysis in such populations challenging because the molecular variation can lead to population stratification rather than true associations and is often described as false-positive and false-negative association signals if not properly controlled (Wang et al., 2011). Therefore, it is crucial to define the population structure of admixed populations for performing better association studies in commercial-like populations. An admixed beef cattle population comprised of British, Continental, and Australian origin is being used in a long-term effort to understand cow longevity, efficiency (reproductive and nutritional), and their interaction. To assist with this research effort, this study aimed to 1) determine the genetic structure of AD cattle and their genetic relationship with five American taurine beef breeds, and 2) apply the use of genetic population structure of admixed populations to improve statistical trait analysis models.

Materials and methods

Animals

All procedures were reviewed and approved by the Institutional Animal Care and Use Committee of North Dakota State University. Animals used in this study were sourced from the Dickinson Research Extension Center, Dickinson, ND. The admixed base herd is comprised of two distinct groups. The first group, called the "beef herd", consists of moderate to large framed (BIF frame score ≥ 5.50; Beef Improvement Federation, 2018) AN, AR, SM, SH, or Gelbvieh (GV)-influenced cows, whereas small to moderate framed AD-influenced cows form the second group, called the "range herd". These AD-influenced cows were produced from crossing AD bulls to AN, AR, SH, SM, or GV-influenced heifers (typically produced from the beef herd).

Cows, bulls, and steers from these two herds with available DNA were included in this study (n = 252). Daughters produced from the admixed base herd from 2014 to 2017 (n = 257) are considered Cycle 1 in a long-term study focused on longevity traits, where daughters of Cycle 1 females (n = 100) are considered Cycle 2 and were sired by AR or AD bulls. Both Cycle 1 and Cycle 2 females were considered in this study. Breeding bulls and some cows from Dickinson Research Extension Center were purebred AN, AR, GV, AD, SH, and SM and were included in genetic analysis studies. The parentage of some Cycle 1 females (n = 18) could not be confirmed using DNA markers. Therefore, these heifers were grouped as "undetermined parentage" (UP) and included in this diversity analysis to describe their breed ancestry clearly. A total of 727 animals, including 6 purebred populations (8 AN, 28 AR, 23 GV, 18 AD, 12 SH, and 11 SM), 18 UP heifers, and 2 admixed populations (252 admixed population I [ADMX-I] consisted of 2 bulls, 48 steers and 202 cows from the base herd; and 357 admixed population II [ADMX-II] comprised of 257 Cycle 1 and 100 Cycle 2 females) were considered for genetic diversity analyses.

DNA and genotyping

Extraction of DNA was performed from blood samples collected via jugular venipuncture (n = 706) or ear tissue samples (n = 21) using the Qiagen DNeasy extraction kit protocol (QIAGEN N.V., Hilden, Germany). The quality of DNA was checked using a Synergy H1 microplate reader (BioTek, Winooski, Vermont), then stored at -80°C until shipment. All DNA samples were dried entirely down in 96-well plates before shipment; however, this dried DNA was rehydrated at Neogen GeneSeek for genotyping. All DNA samples were genotyped using the GeneSeek Genomic Profiler 150K for Beef Cattle (Neogen GeneSeek, Inc., Lincoln, NE). Total markers genotyped on the panel were 138,893 before quality control, including *LEP*

c.73C>T marker (as described by Buchanan et al., 2002). All SNP markers were mapped using UCD 1.2 assembly (Rosen et al., 2020). Only SNP mapped to autosomes were used in analyses (n = 132,368), which meant SNP mapped to mitochondria (8), pseudoautosomal regions (613), X and Y sex chromosomes (4,994 and 60), and without a known position in the genome (850) were excluded. The percentage of SNP markers with a call rate ≥ 95% within each of the nine subpopulations was calculated using autosomal markers (n = 132,368). Quality control was performed by removing SNP with minor allele frequency (MAF) < 0.05, call rate < 95% of the samples genotyped, and an exact Hardy-Weinberg equilibrium ($P < 0.0001$) test following Wigginton et al. (2005). After quality control analyses, 117,373 SNP were retained and were used to calculate usable loci percentage (percentage of loci with less than 5% missing data), polymorphic loci percentage (percentage of loci with more than one allele out of total loci), observed and expected heterozygosity (H_O and H_E), and inbreeding coefficient (F_{IS}) by sub-population. These analyses were accomplished using Arlequin software version 3.5 (Excoffier and Lischer, 2010). The differences between H_O and H_E in every population and the differences of H_O or H_E among populations have been checked with SAS v.9.4 (SAS Inst., Cary, NC) using the GLM procedure. The least squares means of H_O and H_E were generated for significant effects and controlled for experiment-wise error using the Tukey-Kramer method in SAS v.9.4 (SAS Inst., Cary, NC).

Before analyzing the divergence among populations, SNP in strong linkage disequilibrium (LD) (i.e., pairwise genotypic correlation, $r^2 > 0.1$) were excluded from this study using the parameter *indep (50 5 0.1)* in PLINK v.1.9 (Purcell et al., 2007) as they can affect both principal component analysis (PCA) and cluster analysis (Cañas-Álvarez et al., 2015). After this LD pruning, 6,874 markers remained for subsequent divergence analysis among populations,

where PGDSpider v.2.1.1.5 (Lischer and Excoffier, 2012) was used to convert files from PLINK to Arlequin or GENETIX format as needed.

Genetic distance

Two genetic distance measures (Pairwise fixation index and Nei's D genetic distance) were calculated using allele frequencies to assess the genetic divergence among populations. The pairwise fixation index (F_{ST}) was estimated in Arlequin software version 3.5 (Excoffier and Lischer, 2010) using 20,000 permutations for a significance level of 0.05. Nei's D genetic distance (Nei, 1972) between all pairs of populations was also measured using GENETIX v.4.05 (Belkhir et al., 2004). The phylogenetic tree was inferred with pairwise F_{ST} distances using the UPGMA method (Sneath and Sokal, 1973). The evolutionary analyses were conducted using MEGA X (Kumar et al., 2018).

Analysis of molecular variance

The analysis of molecular variance (AMOVA) was estimated to measure the levels of genetic variation within and among populations (Excoffier et al., 1992) using Arlequin v.3.5 (Excoffier and Lischer, 2010), where significance level for variance components was tested using 1,000 permutations.

Principal component analysis

Principal component analysis (PCA) is used to estimate the patterns of population genetic structure. This analysis assigns individuals to their respective original population using a common clustering algorithm (Patterson et al., 2006). Both eigenvectors and eigenvalues were estimated using PLINK v.1.9 (Purcell et al., 2007). Principal components with the largest percentage of the total variation were plotted for graphical representation in R (R Core Team, 2018).

Admixture analysis in unsupervised mode

A model-based admixture analysis implemented in ADMIXTURE v1.3 (Alexander et al., 2009) was performed to characterize the genetic structure across 6 purebred populations. This admixture analysis in unsupervised mode estimates ancestry fractions (*Q* matrix) and allele frequencies (*P* matrix) from the SNP matrix G without any additional information (Alexander et al., 2009). The basic *barplot* function of R was used to visualize the ancestry coefficients for K = 2 to $K = 6$ clusters (Edea et al., 2013). Cross-validation errors were estimated to identify the lowest value for K using ADMIXTURE's cross-validation procedure (Alexander et al., 2009).

Selection of unrelated animals

A subset of mutually unrelated animals (i.e., representative of overall population structure in the entire sample set) was identified using an algorithm described by Conomos et al. (2016) and implemented with the *GENESIS* package (Gogarten et al., 2019) and *SNPRelate* v1.6.4 package (Zheng et al., 2012). The *PC-Relate* algorithm in the *GENESIS* package accurately estimates measures of recent genetic relatedness in samples with unknown or unspecified population structure using ancestry representative principal components (PCs) determined by the PC-AiR method in the *GENESIS* package. The PC-AiR method accounts for known and/or cryptic relatedness in the sample and identifies PCs with accurate ancestry inference that is not confounded by family structure. This algorithm uses pruned SNP data to perform PCA and requires pairwise measures of kinship and ancestry divergence to partition the sample into an "unrelated subset" and a "related subset". These measures were calculated using the R function *snpgdsIBDKing* in the *SNPRelate* package, and a matrix was created with those kinship coefficients outputs using the *kingToMatrix* function in the *GENESIS* package. The matrix created was then used in the *pcair* function, and the top 10 PCs identified from PC-AiR analysis

were checked to determine which PCs were ancestry informative. Among them, the top 5 PCs reflecting population structure were used in a PC-Relate analysis to compute kinship estimates adjusting for ancestry (Conomos et al., 2016). Following this, a kinship matrix was created using the *pcrelateToMatrix* function in the *GENESIS* package. Finally, the *pcairPartition* algorithm in the *GENESIS* package was applied to identify the unrelated and related sets of animals. Samples in the unrelated set were selected to have pairwise kinship coefficients of less than 0.044 among them, whilst having the largest number of pairwise divergences of less than -0.044 with the rest of the samples (Conomos et al., 2016).

Admixture analysis in supervised mode

In admixture analysis, population structure due to differences in breed composition can be biased by the presence of close familial relationships and shared recent ancestry among the sample set being analyzed (Patterson et al., 2006; Conomos et al., 2016). To account for the confounding effect of known and/or cryptic familiar relationships, the population structure identified in the unrelated subset was used as a reference when inferring membership coefficients for the rest of the samples, i.e., related set (Gobena et al., 2018). The membership coefficients and breed allele frequencies were estimated from the genomic data using a maximum likelihood model implemented in ADMIXTURE v1.3 (Alexander et al., 2009). Admixture was first run in unsupervised on the unrelated subset of animals using pruned SNP data and a $K = 5$ (determined by cross-validation procedure) as inputs. Membership coefficients (Q matrix) and breed allele frequencies (*P matrix*) were obtained from the SNP matrix G. Admixture was then run in supervised mode on the related subset where population structure identified in the unrelated individuals was applied as a reference, using an additional file with a *.pop* suffix. This supervised admixture run was applied to both purebred only samples and the full population (purebred and

admixed) samples separately for *K* = 5 clusters (the best value for *K* based on the lowest cross-validation error in previous analyses). To run this analysis on the entire set of genotyped animals, UP and admixed populations were grouped based on their suspected or known pedigree primary breed percentage (50% or greater), which included categories of AN, AR, AD, GV, SM, and SH. Membership coefficients estimates were used to show the bar plot in unrelated, purebred, and full population sets of samples using the R environment. We considered ancestral breed fractions of unsupervised run in case of unrelated individuals (purebred and admixed) for plotting of purebred and full population sets of samples with breed membership coefficients from the supervised run. Four Limousin-influenced heifers and three Hereford-influenced cows were excluded from both unsupervised and supervised ADMIXTURE runs as no purebred Limousin and Hereford genotype data were available in the study.

PCA with population group created using supervised admixture (*K* = 5) outputs

This PCA was performed to check any differences between PCA plots made with population groups using pedigree-based breed composition and membership coefficients of supervised admixture run (*K* = 5). Since no purebred Hereford and Limousin animals were available in this study, individuals with ≥ 0.25 Hereford or Limousin breed fractions based on pedigree (n = 52 for Hereford, and n = 7 for Limousin) were excluded from this analysis. The remaining individuals (n = 668) of the full population were used in the PCA, where plots were created using principal components with top two larger variances for graphical representation in R (R Core Team, 2018).

Goodness-of-fit animal model comparisons

Based on unsupervised and supervised model-based admixture analysis outputs, UP and both admixed (ADMX-I and ADMX-II) individuals were assigned to their primary ancestral breed. From the output of unsupervised runs, two scenarios of ancestral breed grouping were considered for statistical model testing using the preferred levels of clusters (K = 4 to 5) due to the low cross-validation errors. In both scenarios, an individual was assigned to a breed or breed group based on their greatest proportion of breed membership; however, an individual was considered as "MIX" when the difference between the two greatest breed fractions was ≤ 0.05. From the output of the supervised run with K = 5 clusters, statistical model testing was conducted with four different scenarios of ancestral breed grouping (Category 1 to 4). In this case, we considered ancestral breed fractions of unsupervised run in case of unrelated admixed individuals for breed grouping. In the first three scenarios (C1, C2, and C3), an individual was assigned to a breed or breed group based on the proportion of membership using criteria of ≥ 0.75, ≥ 0.625, and ≥ 0.55, respectively, however animals with primary breed membership proportion less than the threshold value were grouped as "MIX". In the C4 scenario, breed assignment was done following the same procedure used in the unsupervised runs (i.e., greatest proportion). To identify how these ancestral breed groupings may influence statistical models, available feed intake (dry matter intake [DMI]) and behavior (feed intake per meal [FIM] and number of meals per day [NMD]) attributes were utilized and were only available on most of the ADMX-II population (n = 331). Feed intake and behavior data were collected using the Insentec electronic feeding system during feed trials.

Model fits were checked with ASReml 4.2 (Gilmour et al., 2015) to allow for an animal model based on the inclusion or exclusion of available pedigree and model effects. Fixed effects

for each trait included project cycle nested within birth year (n = 6), frame size grouping (n = 4), dam age (used as fixed covariate), ancestral breed group (n = 5 for C1 to 4 and unsupervised K = 4 runs or n = 6 for unsupervised K = 5 run), feed trial week × year (n = 60) as well as random effects of heifer with (additive genetic) and without (permanent environment) pedigree to account for repeated measures by heifer. An additional model was run with no additive genetic component to determine pedigree impact in the presence or absence of the ancestral breed group. Model testing of the fixed ancestral breed group effect was performed using the goodness of fit statistics (AIC and BIC). Variance components were estimated to observe the impact of including ancestral breed groups on modeling additive genetic, permanent environment, residual, and phenotype as well as heritability and repeatability. Frame size was calculated based on hip height and age at weaning using BIF equations (BIF Improvement Federation, 2018), where frame size among females was used to create 4 groups of small (SM; less than 4.00), moderately small (MS; 4.00 to 5.50), moderately large (ML; 5.51 to 6.50), and large (LG; 6.51 or greater). No attempts were made to interpret significant effects of ancestral breed groups on respective response variable evaluated, only to understand how these breed groups through various assignments may influence statistical model fits and genetic parameter estimates for these studied traits (e.g., DMI, FIM and NMD).

Results

Genetic diversity within populations

The level of polymorphism and genetic variability within the nine different cattle populations are shown in Table 2.1. The percentage of markers with call rate ≥ 95% was high (91% or greater) for all populations except the UP population. Similarly, the percentage of SNP loci with less than 5% missing data was lowest in the UP group; however, it was high (92% or

above) for all other populations. The admixed populations were not the only groups with high levels of polymorphism. Similar levels of high (95% or greater) were seen in AR and GV populations. The lowest level of polymorphism was observed in AD followed by UP animals. For all populations, observed heterozygosity was significantly greater than that of their respective expected heterozygosity ($P < 0.001$; Table 2.1). The average expected heterozygosity across all cattle populations was 0.378, with estimates ranging from 0.349 ± 0.0004 in AD to 0.393 ± 0.0004 in AN. Angus and UP populations showed significantly higher proportions of expected ($P < 0.001$) and observed ($P <0.001$) heterozygosity than other studied populations, respectively. The levels of both observed and expected heterozygosity values were significantly lower in the AD population compared to other populations ($P < 0.001$). The inbreeding level was low across all studied populations ranging from -0.112 in UP to -0.013 in AR.

Table 2.1. Genetic variability within nine different beef cattle sub-populations

Population[1]	**N[2]**	**Percentage of markers with call rate ≥ 95%**	**Usable loci (%)**	**Polymorphic loci (%)**	**Observed heterozygosity[3] (Mean ± SD)**	**Expected heterozygosity[3] (Mean ± SD)**	**Inbreeding coefficient (F_{IS})**
AN	8	97.00	98.27	89.16	$0.398 \pm 0.191^{A,c}$	$0.393 \pm 0.132^{B,a}$	-0.016
AR	28	97.98	99.66	97.48	$0.374 \pm 0.156^{A,h}$	$0.369 \pm 0.136^{B,e}$	-0.013
GV	23	95.83	96.84	95.49	$0.402 \pm 0.167^{A,b}$	$0.375 \pm 0.133^{B,d}$	-0.081
AD	18	92.72	94.50	80.49	$0.354 \pm 0.174^{A,i}$	$0.349 \pm 0.148^{B,f}$	-0.019
SH	12	91.17	92.61	85.23	$0.382 \pm 0.179^{A,g}$	$0.370 \pm 0.138^{B,e}$	-0.039
SM	11	95.95	97.25	91.26	$0.395 \pm 0.180^{A,e}$	$0.385 \pm 0.134^{B,c}$	-0.029
UP	18	83.49	85.25	85.24	$0.427 \pm 0.166^{A,a}$	$0.386 \pm 0.124^{B,c}$	-0.112
ADMX-I	252	95.55	96.70	96.70	$0.400 \pm 0.119^{A,bc}$	$0.389 \pm 0.112^{B,b}$	-0.043
ADMX-II	357	98.09	99.80	99.80	$0.392 \pm 0.119^{A,f}$	$0.386 \pm 0.114^{B,c}$	-0.019

[1]AN = Angus; AR = Red Angus; GV = Gelbvieh; AD = American Aberdeen; SH = Shorthorn; SM = Simmental; UP = Undetermined parentage; ADMX-I = Admixed population I; ADMX-II = Admixed population II.
[2]N = Number of animals used.
[3]Levels of heterozygosity were provided as proportion.
^{A,B}Superscripts that differ within a row differ ($P \leq 0.001$).
^{a,b,c,d,e,f,g,h,i}Superscripts that differ within a column differ ($P \leq 0.0001$).

Genetic distance between cattle populations

The values of pairwise genetic differentiation (F_{ST}) estimated between populations ranged from 0.005 to 0.186 (Table 2.2). The largest genetic differentiation among nine cattle populations was observed between AD and GV, SM, and SH, as F_{ST} estimates ranged from 0.169 to 0.186. The lowest F_{ST} estimate (0.005) was found between ADMX-I and ADMX-II populations, as expected. However, low F_{ST} estimates were observed between two British purebred populations, AN and AR (0.047), and two Continental purebred populations, GV and SM (0.079). The pairwise Nei's D genetic distances indicated similar relationships as the pairwise F_{ST} estimates, with values ranging from 0.006 to 0.171 (Table 2.2). The phylogenetic relationships among these populations illustrate a closed relationship of ADMX-I and ADMX-II populations (Supplementary Figure A1). A similar association was also observed between GV and SM. The UP population clustered close to AR and AN. The AD population was close to AN but distant from GV, SM, and SH populations.

Analysis of molecular variance and genetic differentiation

Analysis of the nine cattle populations showed that 2.839% ($P < 0.001$) of the molecular variation was accounted for among populations while 99.547% ($P = 1.00$) of the molecular variation was due to within-individual variation (Table 2.3). Average *F*-statistics over all loci showed a total inbreeding (F_{IT}) of 0.005 ($P = 1.00$), within-population inbreeding (F_{IS}) of -0.025 ($P = 1.00$), and a genetic variability among populations (F_{ST}) of 0.028 ($P < 0.001$). The AMOVA analysis with six purebred populations (i.e., after dropping the admixed and UP groups from the analysis) revealed an increase in genetic variation among populations from 2.839% to 12.314% ($P < 0.001$; Table 2.3). Within-individual variability (90.917%; $P < 0.001$) was still high; however, it was 8.630% lower than observed with all studied populations.

Table 2.2. Pairwise genetic differentiation (F_{ST}) values and Nei's D genetic distance among nine cattle populations[1]

Population	**AN**	**AR**	**GV**	**AD**	**SH**	**SM**	**UP**	**ADMX-I**	**ADMX-II**
AN		0.064	0.123	0.112	0.132	0.138	0.063	0.041	0.043
AR	0.047		0.099	0.100	0.113	0.115	0.041	0.027	0.021
GV	0.115	0.103		0.155	0.125	0.083	0.090	0.067	0.063
AD	0.116	0.112	0.169		0.168	0.171	0.107	0.061	0.062
SH	0.125	0.115	0.129	0.186		0.136	0.112	0.082	0.077
SM	0.125	0.113	0.079	0.184	0.137		0.109	0.086	0.075
UP	0.040	0.032	0.088	0.116	0.110	0.102		0.030	0.029
ADMX-I	0.023	0.026	0.069	0.067	0.081	0.082	0.023		0.006
ADMX-II	0.026	0.018	0.065	0.067	0.075	0.070	0.022	0.005	

[1]F_{ST} are located below the diagonal and Nei's D are located above the diagonal. Populations include: AN = Angus; AR = Red Angus; GV = Gelbvieh; AD = American Aberdeen; SH = Shorthorn; SM = Simmental; UP = Undetermined parentage; ADMX-I = Admixed population I; ADMX-II = Admixed population II.

Table 2.3. Analysis of molecular variance in the purebred populations (PP) only and entire sub-population (ES) sets

	df		Variance components		Percentage of variation		Fixation indices[1]		
Source of variation	**ES**	**PP**	**ES**	**PP**	**ES**	**PP**		**ES**	**PP**
Among populations	8	5	43.679	193.079	2.839	12.314	F_{ST}	0.028^{**}	0.123^{**}
Among individuals within populations	718	94	-36.715	-50.655	-2.386	-3.231	F_{IS}	-0.025^{NS}	-0.037^{NS}
Within individuals	727	100	1531.791	1425.575	99.547	90.917	F_{IT}	0.005^{NS}	0.091^{**}
Total	1453	199	1538.754	1567.998					

$^{**}P < 0.01$; NS = Non-significant; df = degrees of freedom.

[1]F_{ST} = proportion of genetic variability among breeds, F_{IS} = within-population inbreeding, and F_{IT} = total inbreeding.

Principal component analysis of populations

For 6,874 autosomal SNP in low LD, the first and second PCs (PC1 and PC2) accounted for 41.09% and 25.06% of the total molecular variance, respectively. The PC1 completely isolated AN, AR, and AD from SH and both Continental purebred populations, whereas PC2 clearly distinguished the AD population from both British and Continental populations (Figure 2.1a). Moreover, the PCA plot including PC1 and PC2 revealed that AN and AR form one cluster, SH and Continental breeds (GV and SM) form a second cluster, and AD stands alone in a third cluster. The third principal component (PC3) accounted for 15.27% of the total variation and separated SH from both Continental populations when paired with PC1 (Figure 2.1b). However, both Continental populations (GV and SM) remained clustered as before. Although the AD clustered tightly together, PC3 moved their cluster closer to AN and AR populations, which aligns with their origin. A PCA plot with PC2 and PC3 showed that PC2 completely separated the AD population from the other 5 purebred populations, but also demonstrated individuals that may be influenced by the AN breed due to registration techniques used in the US (Figure 2.1c). Furthermore, PCA, including all studied populations, revealed that UP individuals grouped solely with AN and AR populations (Figure 2.2).

The individuals in ADMX-I and ADMX-II populations dispersed throughout the PC plot (Figure 2.2). Since both ADMX-I and ADMX-II consisted of AN, AR, AD, SM, SH, or GV-influenced animals, these individuals are expected to be clustered with or close to their primary breed based on their pedigree and parentage breed composition. This PCA plot did not clearly reveal these clusters; therefore, ADMX-I and ADMX-II were further classified into 19 different groups using their pedigree-based primary breed composition, where F_1 individuals were grouped distinctly (Figures 2.3 to 2.4 and Supplementary Figure A2). This breed composition

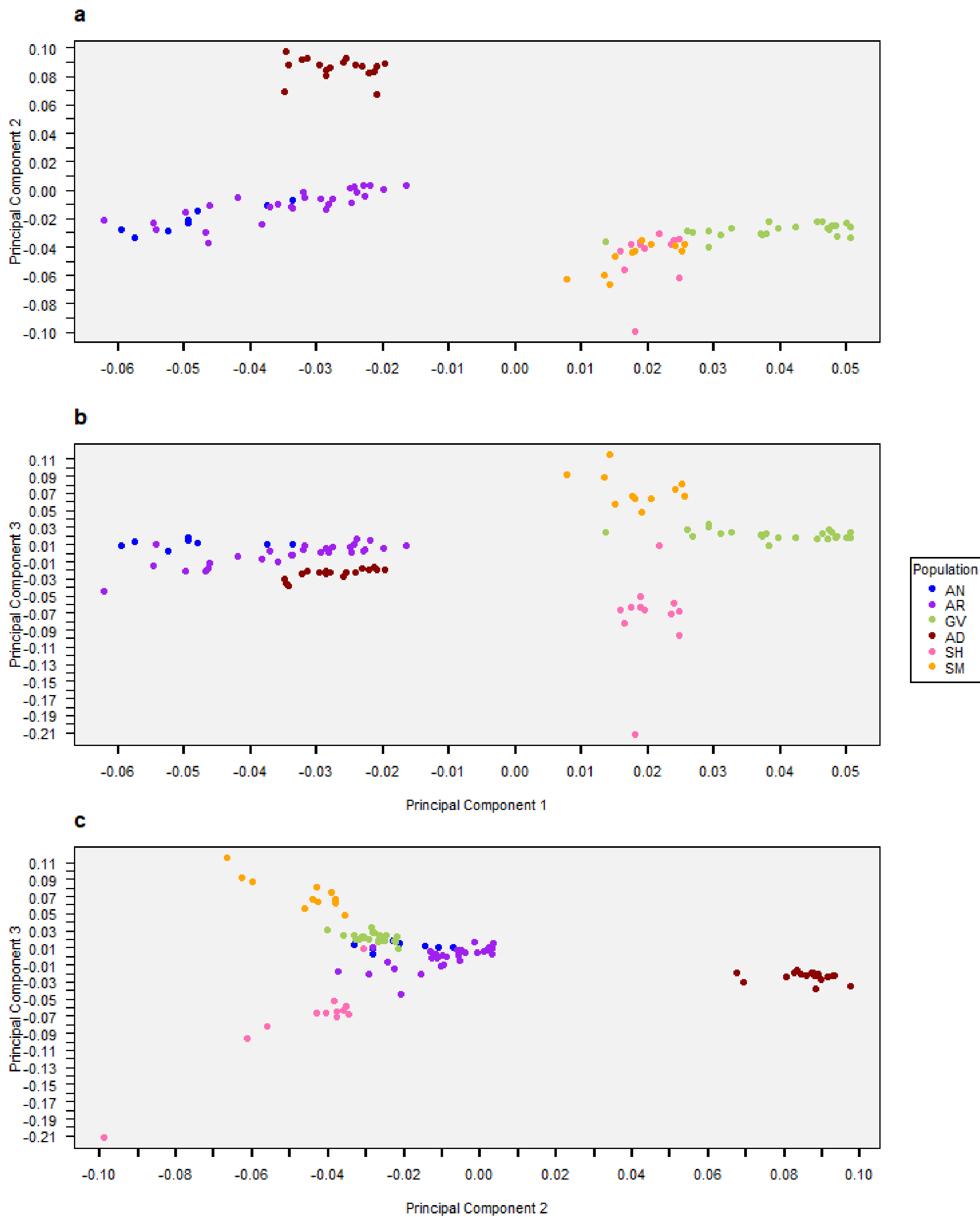

Figure 2.1. Purebred population groups defined by principal component analysis using 6,874 SNPs. Principal components a) 1 and 2, b) 1 and 3, and c) 2 and 3 define molecular variation explained within and across purebreds, including Angus (AN), Red Angus (AR), Gelbvieh (GV), American Aberdeen (AD), Shorthorn (SH), and Simmental (SM).

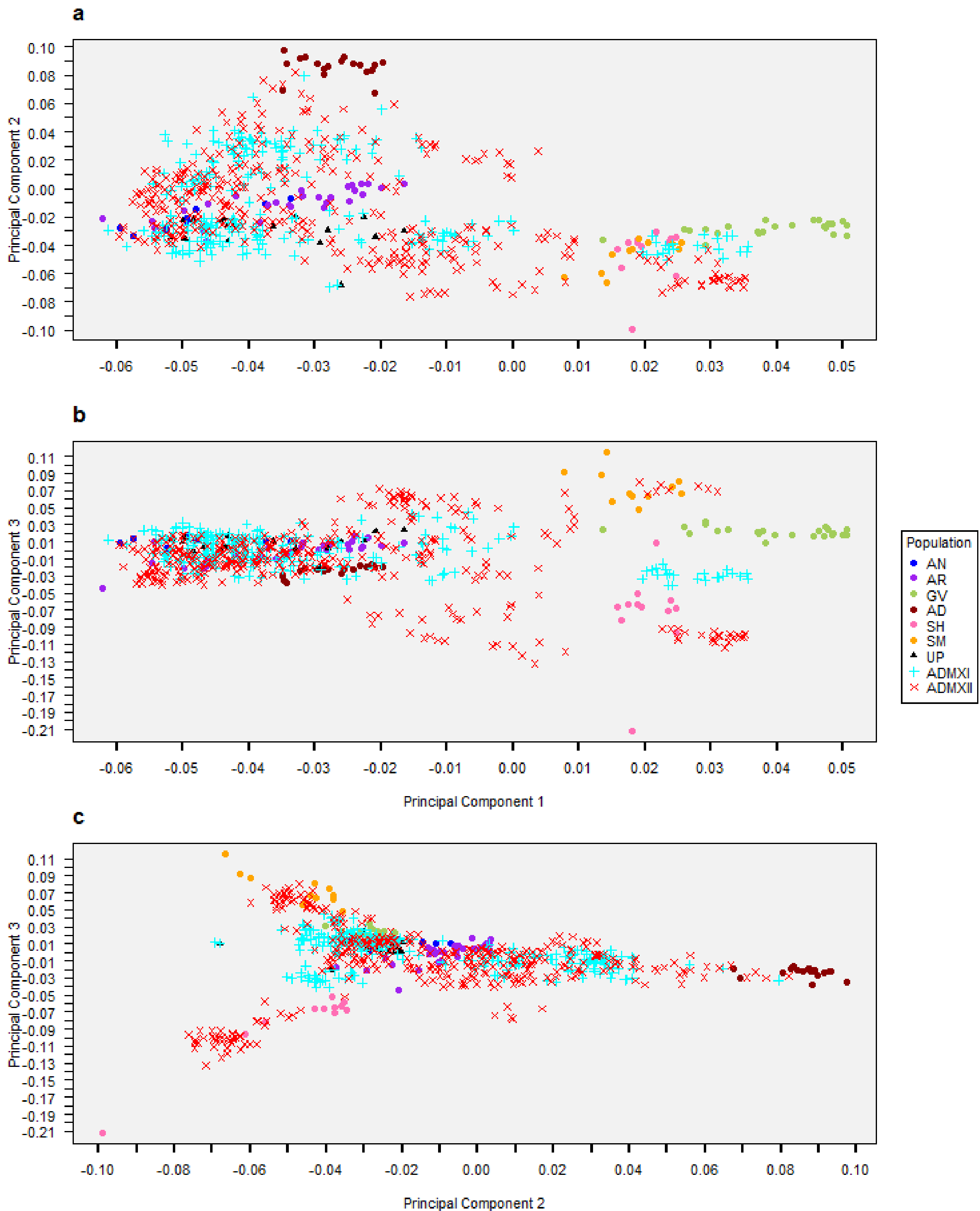

Figure 2.2. Principal component analysis of purebred and admixed populations using 6,874 SNPs. Principal components a) 1 and 2, b) 1 and 3, and c) 2 and 3 define molecular variation explained within and across populations, including Angus (AN), Red Angus (AR), Gelbvieh (GV), American Aberdeen (AD), Shorthorn (SH), Simmental (SM), Undetermined parentage (UP), Admixed population I (ADMXI), and Admixed population II (ADMXII).

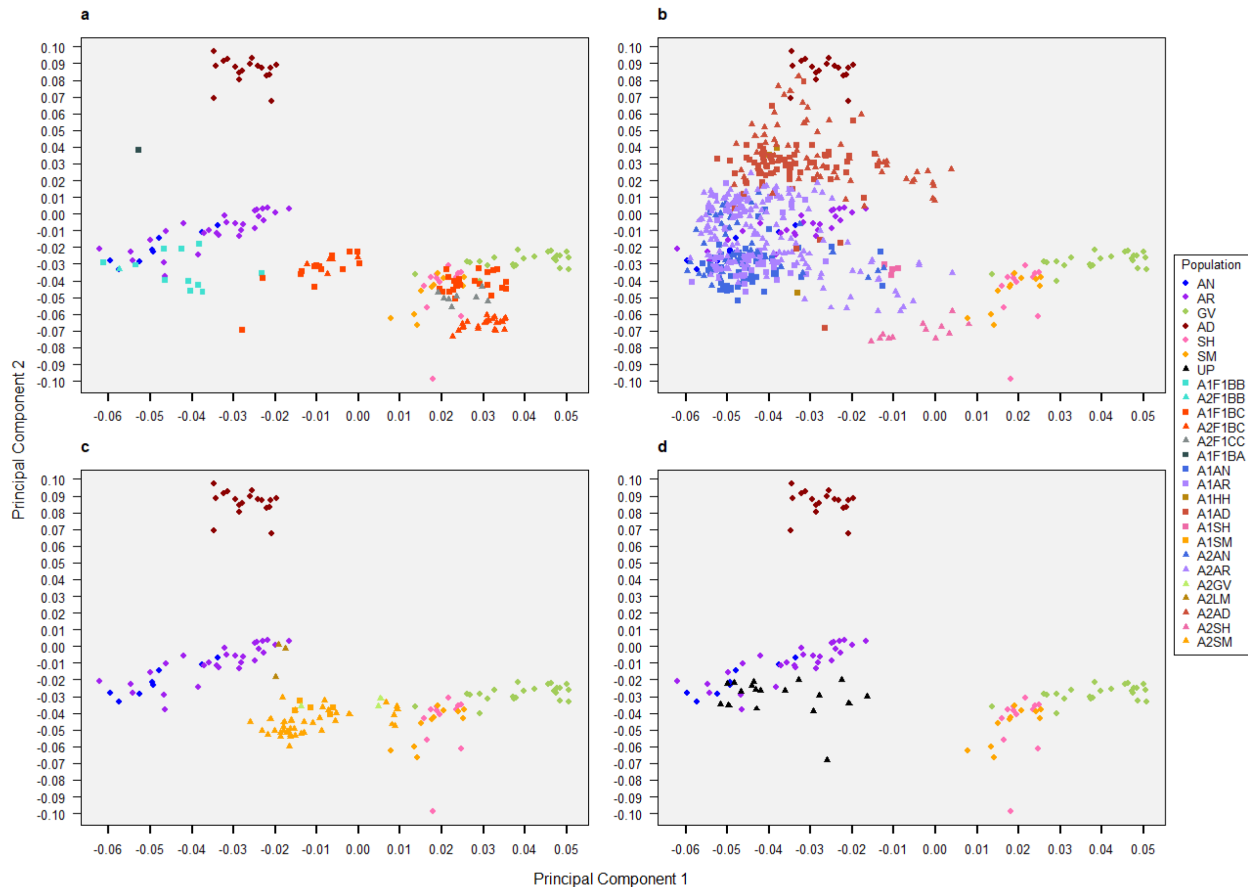

Figure 2.3. Principal components 1 and 2 of purebred and admixed populations with primary breed of admixed individuals designated based on pedigree. Molecular variation explained within and across populations using principal components 1 and 2 with purebreds (Angus, AN; Red Angus, AR; Gelbvieh, GV; American Aberdeen, AD; Shorthorn, SH; Simmental, SM) and admixed populations (ADMXI, A1 and ADMXII, A2) sub-grouped based on primary breed (≥ 50%) of a) F_1 (F_1 British×British, A1F1BB and A2F1BB; F_1 British×Continental, A1F1BC and A2F1BC; F_1 Continental×Continental, A2F1CC; F_1 British×Australian, A1F1BA), b) British and Australian breeds (Angus, A1AN and A2AN; Red Angus, A1AR and A2AR; American Aberdeen, A1AD and A2AD; Hereford, A1HH; Shorthorn, A1SH and A2SH), c) Continental breeds (Gelbvieh, A2GV; Limousin, A2LM; Simmental, A1SM and A2SM), and d) Undetermined parentage (UP).

was constructed based on the pedigree information available from previous herd records and parentage testing. In this case, an individual animal was assigned to a breed if their primary breed composition was greater than or equal to 50%. The graphical representation of 26 sub-populations clearly showed individuals' clustering patterns in the UP and admixed groups. Although all admixed individuals did not cluster directly over their purebred counterparts, they did cluster together based on primary breed and in close proximity to their respective primary breed.

Admixture analysis in unsupervised mode

The lowest cross-validation error was obtained for $K = 5$ clusters, indicating that this was the most parsimonious number of clusters of ancestral breed types (Supplementary Figure A3). For predefined $K = 2$, purebred populations were clustered into British and Continental populations (Figure 2.5), where all British populations had some level of admixture with Continental populations. The genome of AN and AR appeared to share around 42% of Continental ancestry; however, it was higher in the SH population (almost 68%). The AD population revealed nearly 98.8% of British ancestry, which supported their British origin and lack of influence from Continental breeds. Both Continental populations showed around 8.6 to 14.5% introgression from British ancestry. At $K = 3$, population clustering was nearly consistent with the grouping of the PCA using PC1 and PC2. At this level, AD wholly isolated from its British ancestry and showed its own identity (97.8%) with minimal signals of admixture with British (1.7%) and Continental (0.5%) ancestry. Shorthorn genome still appeared to share around 36.4% of Continental genetics. At $K = 4$, the SH population separated from the other two British populations, AN and AR. In contrast, AN and AR still clustered together and had some admixture levels with AD, SH, and Continental ancestries. For $K = 5$, both Continental

populations segregated from one another and showed their individual identity. However, GV had a substantial level of admixture with SM (13.6%) and a minimal amount with other population's ancestries (AN and SH). At $K = 6$, some individuals of the AR population completely showed their own identity. Even so, many AR individuals still had a substantial amount of AN genetics (53.6%).

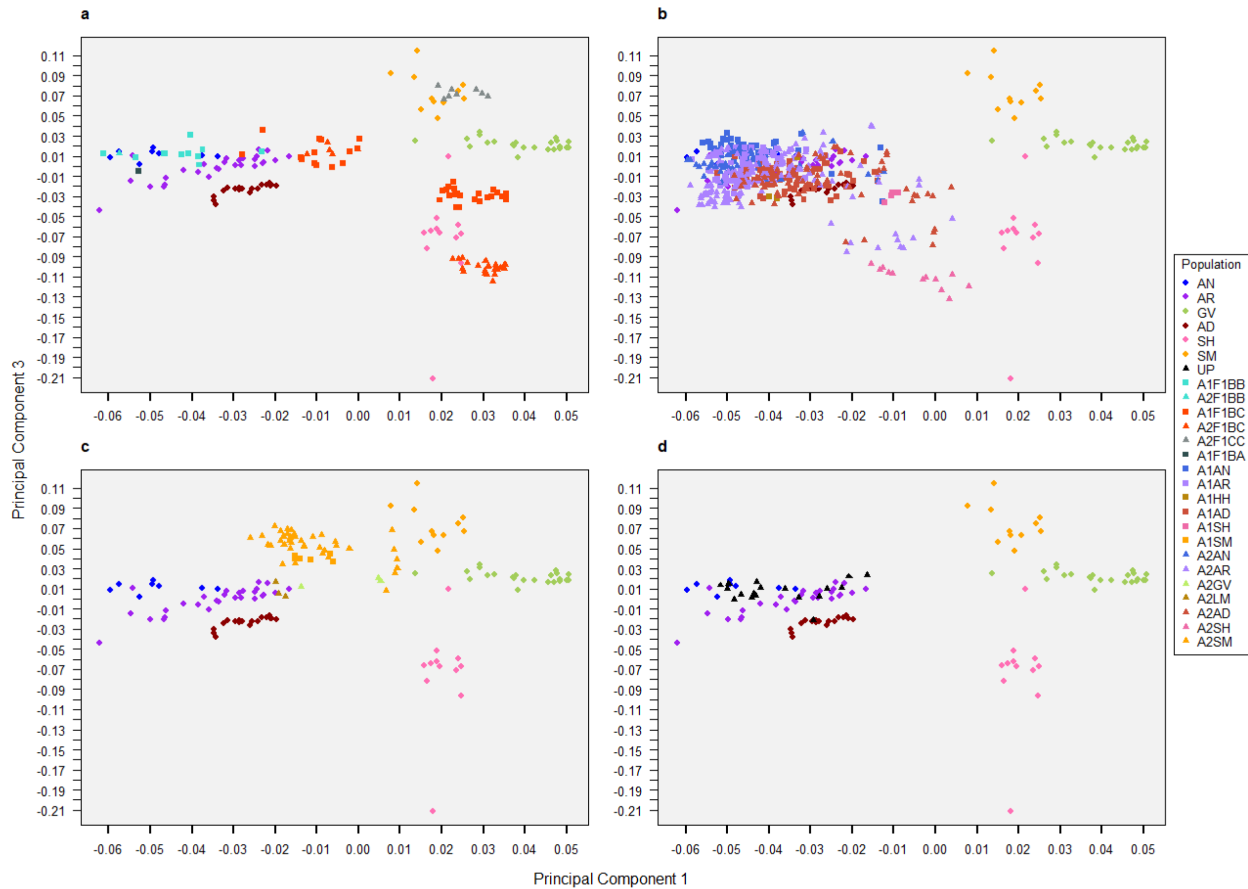

Figure 2.4. Principal components 1 and 3 of purebred and admixed populations with primary breed of admixed individuals designated based on pedigree. Molecular variation explained within and across populations using principal components 1 and 3 with purebreds (Angus, AN; Red Angus, AR; Gelbvieh, GV; American Aberdeen, AD; Shorthorn, SH; Simmental, SM) and admixed populations (ADMXI, A1 and ADMXII, A2) sub-grouped based on primary breed (≥ 50%) of a) F_1 (F_1 British×British, A1F1BB and A2F1BB; F_1 British×Continental, A1F1BC and A2F1BC; F_1 Continental×Continental, A2F1CC; F_1 British×Australian, A1F1BA), b) British and Australian breeds (Angus, A1AN and A2AN; Red Angus, A1AR and A2AR; American Aberdeen, A1AD and A2AD; Hereford, A1HH; Shorthorn, A1SH and A2SH), c) Continental breeds (Gelbvieh, A2GV; Limousin, A2LM; Simmental, A1SM and A2SM), and d) Undetermined parentage (UP).

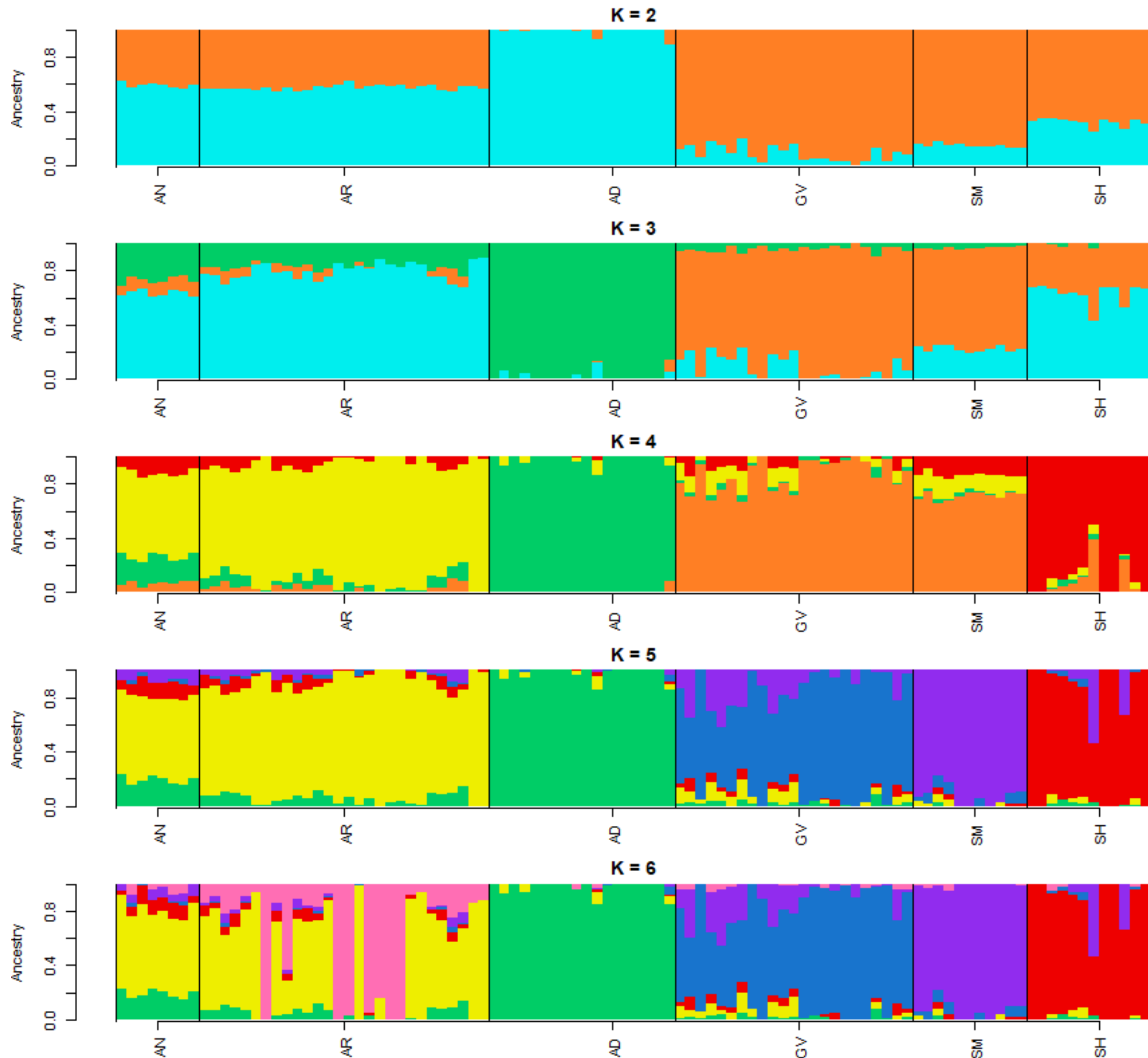

Figure 2.5. Bar plot of the *Q* matrix from an unsupervised ADMIXTURE run in purebred populations. Individuals (n = 100) are represented by a single vertical bar and segregated into *K*-colored segments. Each segment's length shows the proportion of the individual's genome for a given ancestral grouping based on cluster analysis designation (i.e., *K* = 2 to *K* = 6). Purebred populations are separated by black lines and include Angus (AN), Red Angus (AR), Gelbvieh (GV), American Aberdeen (AD), Shorthorn (SH), and Simmental (SM).

Admixture analysis in supervised mode

A subset of 95 individuals was identified as unrelated and representative of the full population; however, one Limousin-influenced heifer was excluded from further analysis as no purebred Limousin were available in this study (n = 94 for subsequent analyses).

ADMIXTURE was first run in unsupervised mode (*K* = 5) on the unrelated subset of animals, where Supplementary Figure A4 shows the proportion of the genome contributed by each ancestral breed for the 94 unrelated individuals. The unrelated set consisted of 37 purebreds and 57 admixed individuals. The average ancestral breed fractions observed in the unrelated purebred populations were AN (83.5% AN genetics), AR (85.2% AN genetics), AD (99.9% AD genetics), GV (78.6% GV genetics), SM (96.1% SM genetics), and SH (95.6% SH genetics). In the primary breed influenced admixed population groups (based on pedigree) of unrelated individuals, the average ancestral breed fractions were AN (65.4% AN genetics), AR (73.0% AN genetics), AD (61.9% AD genetics), GV (63.6% AN and 24.0% GV genetics), SM (48.2% SM genetics), and SH (50.6% SH genetics). In admixture analysis (*K* = 5) with only purebred populations, substantial differences (3.2% in SH genetics to 13.8% in GV genetics) were found between average ancestral breed fractions from unsupervised and supervised runs in AN (61.2% vs. 74.7% AN genetics), AR (86.28% vs. 92.5% AN genetics), GV (75.9% vs. 89.7% GV genetics), SM (92.5% vs. 96.8% SM genetics), and SH (87.4% vs. 90.6% SH genetics) purebred populations (Figure 2.6). However, this difference was minimal (0.9%) in AD (97.5% vs. 98.4% AD genetics). Furthermore, ADMIXTURE runs (*K* = 5) after adding UP and admixed groups with purebred populations provide distinct ancestral breeds fractions between unsupervised and supervised runs for both purebred and crossbred individuals (Figure 2.7). In the unsupervised run, AR showed its own ancestry (50%) with a large extent of admixture with AN (33.1%) and a little amount with AD (7.0%), SM (5.7%), and SH (4.1%), whereas GV displayed mainly SM and SH ancestries (54.6% and 34.2%, respectively). However, in the supervised run all purebred populations separated more clearly i.e., AR showed AN ancestry (90.7%) and GV showed its own ancestry (87.6%). Other purebreds showed their individual ancestry, where considerable

differences (3.5% in AD genetics to 21.7% in SH genetics) were observed between average breed fractions from unsupervised and supervised runs in AN (74.7% vs. 83.7% AN genetics), AD (96.0% vs. 99.5% AD genetics), SM (79.0% vs. 94.0% SM genetics), and SH (65.7% vs. 87.3% SH genetics). Distinct differences (-2.0% in SH genetics to 42.8% in AN genetics for AR individuals) were found between average ancestral breeds fractions from unsupervised and supervised runs for crossbred individuals in AN (61.8% vs. 70.9% AN genetics), AR (33.1% vs. 75.9% AN genetics), AD (56.5% vs. 62.2% AD genetics), GV (37% AN and 20.6% SM vs. 66.9% AN and 21.0% GV genetics), SM (57.4% vs. 66.8% SM genetics), and SH (63.8 vs. 61.8% SH genetics). Membership coefficients obtained from both ADMIXTURE runs were used to estimate the genomic breed composition for the 626 animals in the ADMX-I, ADMX-II, and UP populations for further model testing analyses.

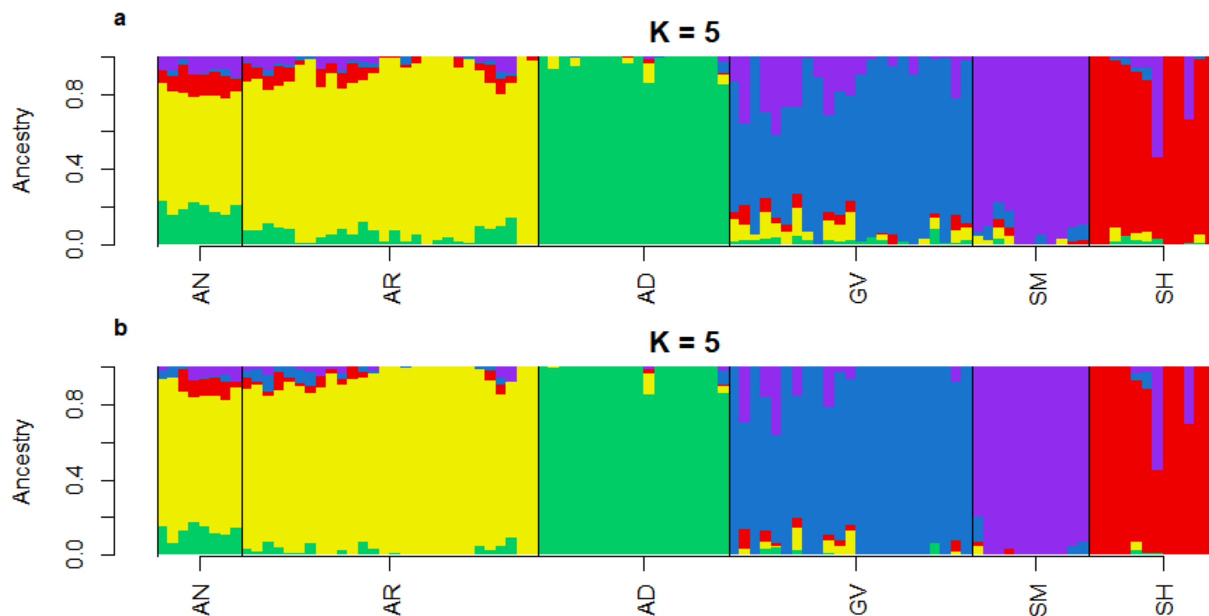

Figure 2.6. Bar plot of the Q matrix from ADMIXTURE runs in purebred animals with $K = 5$. Runs include a) unsupervised and b) supervised, where individuals (n = 100) are represented by a single vertical bar. Purebred populations are separated by black lines and include Angus (AN), Red Angus (AR), Gelbvieh (GV), American Aberdeen (AD), Shorthorn (SH), and Simmental (SM).

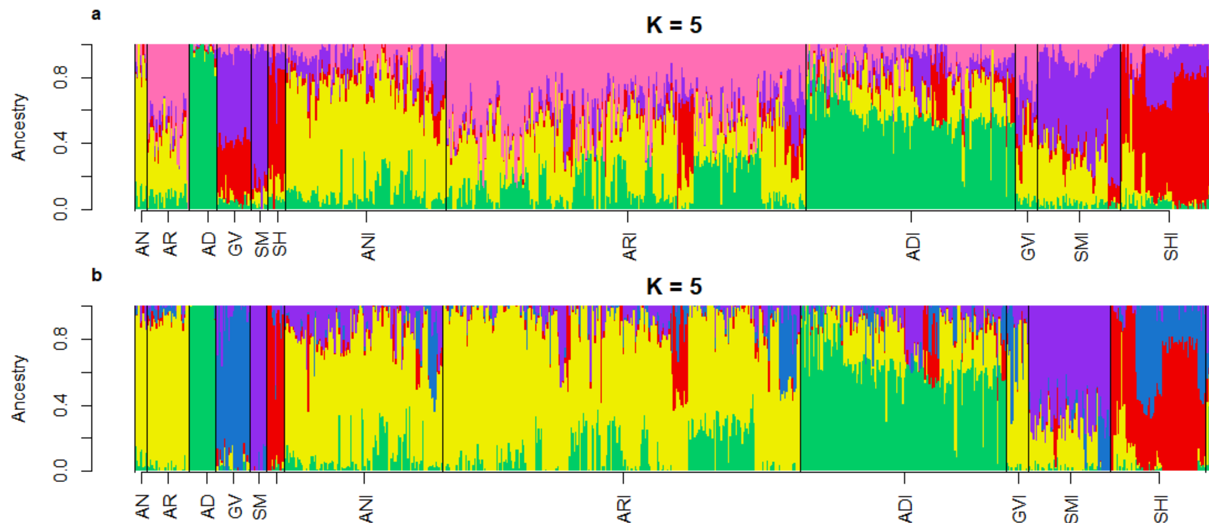

Figure 2.7. Bar plot of the *Q* matrix from ADMIXTURE runs with the entire population ($K = 5$). Runs include a) unsupervised and b) supervised, where individuals are represented by a single vertical bar. All admixed (n = 603) and undetermined parentage (n = 17) individuals are grouped and presented separately based on their primary breed composition (≥ 50%). Each purebred (n = 100) and their influenced populations are separated by black lines and include Angus (AN), Red Angus (AR), Gelbvieh (GV), American Aberdeen (AD), Shorthorn (SH), Simmental (SM), Angus-influenced (ANI), Red Angus-influenced (ARI), American Aberdeen-influenced (ADI), Gelbvieh-influenced (GVI), Simmental-influenced (SMI), and Shorthorn-influenced (SHI).

PCA with population group created using supervised admixture ($K = 5$) outputs

Based on membership coefficient estimates, individuals (n = 668) were divided into 20 different groups. An individual was assigned to a population group based on their greatest proportion of breed membership. Since AR individuals showed a large amount of AN genetics (on average 75.9%), all purebred AR or AR-influenced individuals were grouped as purebred AN or AN-influenced populations, respectively. Animals with > 40% of the two highest breed fractions were considered F1 individuals following Vanraden and Cooper (2015).

Changes to population assignment for PCA were noted (data not shown). In purebred animals, one SH individual was instead considered under the SM population in the current PCA as it displayed around 48.1% SM genetics in comparison to 36.89% SH genetics. A major change was observed from the previous PCA output for some AD-influenced individuals (n = 5) of the ADMX-I population. These individuals were found to cluster close to purebred AN or AR

individuals in the pedigree-based PCA, whereas the new PCA corrected that issue by clustering those individuals with AD-influenced individuals. Some F1 British × Continental (F1BC) individuals in the pedigree-based PCA were counted as AN (n = 6), SH (n = 26), or GV (n = 18)-influenced individuals in the supervised admixture output-based PCA since their genomic data identified that breed as having more than 50% membership. This might be possible as AR, SH, GV, and SM breed associations allow grading up. Since AR showed most of the AN genetics in the supervised admixture run, 6 F1 British × British (F1BB, i.e., F1 AN × AR) individuals were considered AN-influenced individuals in the new PCA. All F1 Continental × Continental (F1CC) individuals (n = 8) observed in the pedigree-based PCA were considered under the SM population in the new analyses as they had 69.15% SM genetics on average. Some previously considered AD-influenced individuals were grouped as F1 British × Australian (F1BA) population. Similarly, AR-influenced individuals were counted as either F1 British × British (F1 AN × SH; n = 1) or F1 British × Continental (n = 4) or F1 British × Australian (n = 1) or SH-influenced (n = 4) individuals in the updated PCA. In both cases, the two greatest breed fractions were more than 40% for those individuals. Undetermined parentage individuals (n = 16) were considered as AN-influenced individuals in this analysis. One AN-influenced cow as SH-influenced and some F1 Continental × Continental (F1CC) individuals were grouped as SM-influenced heifers in the current analysis. These assignment changes provide a cleaner and succinct alignment of purebreds to admixed individuals (data not shown).

Goodness-of-fit animal model comparisons

As was done for PCA, AN and AR-influenced heifers were considered a single breed group "ANAR" since AR individuals showed a significant amount of AN genetics (on average 33.1% and 75.9% in unsupervised and supervised runs, respectively). Due to this, the ANAR

group had the largest number of individuals in all ancestral breed categories except C1, where the "MIX" group possessed the largest number of individuals (Table 2.4). The other four breeds (AD, SH, GV, and SM-influenced heifers) were grouped separately for $K = 5$ unsupervised run and C1 to C4 ancestral breed categories. In $K = 4$, when unsupervised, GV and SM-influenced heifers were put together as "Continental". For C2 to C4, GV-influenced heifers were minimal (n = 2 or 3) and were therefore excluded from statistical analyses, which was supported by lower (i.e., better) fit statistic values after exclusion of that group (data not shown).

For DMI and FIM, models with ancestral breed group had lower (i.e., better) fit statistics compared to models without the ancestral group, regardless of fit criteria used and inclusion or exclusion of pedigree for additive genetic variance (Table 2.5). In supervised runs, using higher breed fractions (C1 and C2) was never better than moderate or highest individual fraction (C3 and C4) grouping (Table 2.5) for DMI and FIM. Similarly, when comparing the best models for breed groups based on supervised runs (C3 and C4) to models using breed groups based on unsupervised runs (*K* cluster), breed groups based on supervised runs were always better (Table 2.5). Whether C3 or C4 grouping was better was trait dependent between DMI and FIM; however, the MIX group's sample size was most likely the driving force of this difference (Table 2.4). No considerable changes of fit statistics values (around -1.9 to 4.8 difference seen) were observed between exclusion and inclusion of ancestral breed group effect for NMD, regardless of pedigree inclusion or exclusion. Regardless of trait, including pedigree to estimate additive genetic variance was always better based on fit statistics (Table 2.5).

When comparing the best fitting animal model with or without breed groups, the additive genetic variance decreased and the permanent environmental variance increased for DMI and FIM models when breed group was included (Table 2.6). Both of these traits showed increased

modeling benefit when including breed group (either C3 or C4) compared to not including it. Furthermore, heritability (ratio of additive genetic to phenotypic variance) decreased in these models, but repeatability (ratio of additive and permanent environmental to phenotypic variance) remained constant (Table 2.6). In the NMD case, which had no evidence of breed group benefit in the model, additive genetic variance increased, and the permanent environment decreased when the breed group was included (Table 2.6). This resulted in increased heritability, even though repeatability still remained constant (Table 2.6). In all cases, standard errors of heritability and repeatability estimates were similar (around 0.01 difference seen) for each trait.

Table 2.4. Sample sizes in different ancestral breed categorization groups for studied traits

Ancestral breed groups[1]	**Ancestral breed group based on unsupervised run**[2]		**Ancestral breed group based on supervised run (K = 5)**[3]			
	K = 4	**K = 5**	**C1**	**C2**	**C3**	**C4**
Continental	57	-	-	-	-	-
ANAR	179	179	109	158	172	186
AD	59	59	15	30	50	56
SH	12	12	25	31	35	36
GV	-	15	0	2	3	3
SM	-	34	17	34	38	42
MIX	24	32	165	76	33	8

[1]Grouping of heifers based on breed or ancestral breed includes Continental = Gelbvieh and Simmental; ANAR = Angus and Red Angus; AD = American Aberdeen; SH = Shorthorn; GV = Gelbvieh; SM = Simmental; MIX = Heifers with breed fraction less than the defined threshold value.
[2]Two predefined clusters (K = 4 to 5) with low cross-validation errors were used for ancestral breed grouping with membership proportion from unsupervised ADMIXTURE run; An individual was assigned to a breed or breed group based on their greatest proportion of the membership; however, an individual was considered as "MIX" if the difference between two greatest breed fractions were ≤ 0.05.
[3]C1, C2, and C3 = The threshold value for which an individual is assigned to a breed or breed group were set to ≥ 0.75, ≥ 0.625, and ≥ 0.55 (proportion of membership), respectively; however, animals with breed fraction less than the threshold value were grouped as "MIX"; C4 = Same breed assignment procedure as used in the unsupervised run.

Table 2.5. Goodness-of-fit criteria with or without ancestral breed group based on unsupervised and supervised breed fractions[1]

Traits	ASReml runs[2]	Fit criteria	Without ancestral breed group	Ancestral breed group based on unsupervised run[3]		Ancestral breed group based on supervised run (K = 5)[4]				Lowest scoring per trait
				K = 4	K = 5	C1	C2	C3	C4	
Dry matter intake (kg/day)	Including	AIC	3632.3	3603.3	3605.0	3611.3	3559.3	3536.1	3529.9	C4
		BIC	3651.8	3622.7	3624.4	3630.7	3578.7	3555.5	3549.2	
	Excluding	AIC	3675.5	3631.5	3636.8	3643.6	3588.9	3564.8	3558.9	C4
		BIC	3694.9	3650.9	3656.2	3663.0	3608.3	3584.2	3578.3	
Feed intake per meal (g of DM)	Including	AIC	57041.9	56327.7	56318.7	56322.7	55981.4	55815.5	55816.2	C3
		BIC	57061.3	56347.1	56338.1	56342.1	56000.8	55834.8	55835.6	
	Excluding	AIC	57068.7	56344.6	56345.9	56339.9	55994.9	55830.8	55834.0	C3
		BIC	57088.1	56364.0	56365.3	56359.2	56014.3	55850.2	55853.4	
Number of meals per day	Including	AIC	11594.0	11594.8	11593.9	11594.5	11597.4	11595.9	11594.0	None
		BIC	11613.4	11614.2	11613.3	11613.8	11616.8	11615.2	11613.3	
	Excluding	AIC	11608.6	11609.5	11613.4	11606.7	11609.4	11609.0	11608.7	None
		BIC	11628.0	11628.9	11632.8	11626.1	11628.7	11628.3	11628.0	

[1]Ancestral breed groups were assessed using ASReml 4.2 (Gilmour et al., 2015) to fit an animal model with fixed effects of the mean (μ), project cycle nested within birth year (n = 6), frame size grouping (n = 4), dam age (used as fixed covariate) as well as week of feed trial × year (n = 60). This included fitting pedigree or without pedigree using heifer as a random effect and repeated measures of feed intake and behavior traits were accounted for using a permanent environmental effect. Fit criteria included: AIC = Akaike information criterion; BIC = Bayesian information criterion.
[2]Including = Pedigree was included in the model; Excluding = Pedigree was not considered in the model.
[3]Two predefined clusters (K = 4 to 5) with low cross-validation errors were used for ancestral breed grouping with membership proportion from unsupervised ADMIXTURE run; An individual was assigned to a breed or breed group based on their greatest proportion of the membership; however, an individual was considered as "MIX" if the difference between two greatest breed fractions were ≤ 0.05.
[4]C1, C2, and C3 = The threshold value for which an individual is assigned to a breed or breed group was set to ≥ 0.75, ≥ 0.625, and ≥ 0.55 (proportion of membership), respectively; however, animals with breed fraction less than the threshold value were grouped as "MIX"; C4 = Same breed assignment procedure as used in the unsupervised run.

Table 2.6. Genetic parameters estimation with or without ancestral breed group[1]

Traits	Genetic parameters	Without ancestral breed group	Best-fit ancestral breed groups (C4 for DMI, C3 for FIM, and C4 for NMD)[2]
Dry matter intake (kg/day)	$\hat{\sigma}_P^2$	1.3172 ± 0.0688	1.2835 ± 0.0675
	$\hat{\sigma}_a^2$	0.5159 ± 0.1229	0.4470 ± 0.1257
	$\hat{\sigma}_{pe}^2$	0.1895 ± 0.0841	0.2289 ± 0.0896
	$\hat{\sigma}_e^2$	0.6118 ± 0.1294	0.6076 ± 0.0130
	$\hat{h}^2$	0.3917 ± 0.0792	0.3483 ± 0.0857
	$\hat{R}$	0.5355 ± 0.0245	0.5266 ± 0.0251
Feed intake per meal (g of DM)	$\hat{\sigma}_P^2$	90850.0 ± 4451.0	91750.0 ± 4624.1
	$\hat{\sigma}_a^2$	23062.0 ± 7201.9	22465.0 ± 7932.8
	$\hat{\sigma}_{pe}^2$	25862.0 ± 5816.7	26878.0 ± 6384.7
	$\hat{\sigma}_e^2$	41925.7 ± 886.9	42407.6 ± 907.1
	$\hat{h}^2$	0.2539 ± 0.0728	0.2449 ± 0.0799
	$\hat{R}$	0.5385 ± 0.0229	0.5378 ± 0.0236
Number of meals per day	$\hat{\sigma}_P^2$	7.6762 ± 0.3846	7.7254 ± 0.3996
	$\hat{\sigma}_a^2$	1.4166 ± 0.5907	1.6557 ± 0.6638
	$\hat{\sigma}_{pe}^2$	2.9137 ± 0.5307	2.7238 ± 0.5621
	$\hat{\sigma}_e^2$	3.3459 ± 0.0716	3.3459 ± 0.0716
	$\hat{h}^2$	0.1845 ± 0.0731	0.2143 ± 0.0806
	$\hat{R}$	0.5641 ± 0.0222	0.5669 ± 0.0228

[1]Variance components and genetic parameter estimates are reported for without including ancestral breed group, and two ancestral breed categories (C3 and C4). Parameters included variances of phenotype ($\hat{\sigma}_P^2$), additive genetic ($\hat{\sigma}_a^2$), permanent environment ($\hat{\sigma}_{pe}^2$), and residual ($\hat{\sigma}_e^2$), as well as heritability ($\hat{h}^2$ i.e., ratio of $\hat{\sigma}_a^2$ to $\hat{\sigma}_P^2$), and repeatability ($\hat{R}$ i.e., ratio of $\hat{\sigma}_a^2 + \hat{\sigma}_{pe}^2$ to $\hat{\sigma}_P^2$). Model effects were used in ASReml 4.2 (Gilmour et al., 2015) to fit an animal model with fixed effects of the mean (μ), project cycle nested within birth year (n = 6), frame size grouping (n = 4), dam age (used as fixed covariate) as well as week of feed trial × year (n = 60). This included fitting pedigree using heifer as a random effect, and repeated measures of feed intake and behavior traits were accounted for using a permanent environmental effect.
[2]C3 = The threshold value for which an individual is assigned to a breed or breed group was set to ≥ 0.55 (proportion of membership); however, animals with breed fraction less than the threshold value were grouped as "MIX"; C4 = an individual was assigned to a breed or breed group based on their greatest proportion of membership; however, an individual was considered as "MIX" if the difference between two greatest breed fractions were ≤ 0.05.

Discussion

Genetic differentiation within populations

The overall marker call rate for 727 animals in nine different beef cattle populations was 98.39%, which represents the high performance of genotyping. The average high marker call rate in all purebred populations is contributed by ascertainment bias from the construction of the BovineSNP150K chip assay with SNP being almost exclusively derived from sequences available in European cattle breeds. The proportion of markers genotyped on 95% of the samples was low in the UP population. Also, the usable loci percentage was found low in the same population group. Both of these might be due to errors or quality of DNA samples at the time of genotyping. Also, errors in maintaining quality control parameters may have occurred when genotyping good quality DNA samples. Previous studies reported associations of low marker call-rate and high missing data with genotyping error (Di et al., 2005; Shen et al., 2005; Moorhead et al., 2006). Some UP individuals showed a little deviation from the threshold of A_{260}/A_{280} ratio (1.8 to 2.0) on their DNA quality assessment, but genotyping was performed on those samples. These factors likely caused UP individuals to fail parentage assignments. The proportion of markers genotyped on 95% of the samples observed in the present study are similar to those previously reported by Kim et al. (2018) and Cañas-Álvarez et al. (2015). The SNP retained after the second quality control step exhibited a high degree of polymorphism (80.49 to 99.80%) in all studied populations. The highest level of polymorphism was observed in ADMX-II followed by AR, ADMX-I, and GV populations. These findings in admixed populations were similar to those reported by Gautier et al. (2010), who observed a greater level of polymorphism in crossbred populations compared to their populations of origin. The UP population also showed a low level of polymorphism but a greater level of expected heterozygosity within their

individuals. These findings might be possible since polymorphism and heterozygosity are measured in different ways. Population-wide heterozygosity refers to the average proportion of all loci that were heterozygous across individuals, whereas polymorphism denotes the polymorphic loci percentage in the population. Although they are admixed, this low polymorphism observed in UP might be affected by the low percentage of markers with ≥ 95% call rate and usable loci percentage. The degree of polymorphism observed in this study was similar to those shown in previous studies of taurine (95.21%) and African cattle populations (83.96%) (Edea et al., 2013). Gautier et al. (2007) reported a similar level of polymorphism in European cattle (93.5%), but they observed a lower degree of SNP polymorphism in African cattle (47.4 to 71.0%) using 696 SNP. In addition, the levels of polymorphism observed in Ethiopian cattle populations and Bangladeshi zebu (92 to 97%) were similar to our present study. However, the polymorphism was low in South Korean taurine cattle (71%; Edea et al., 2015). Our study's findings of observed heterozygosity indicate that there are small differences in genetic diversity between the populations. A high level of expected heterozygosity was observed in all studied populations, indicating high within-population diversity. A slight heterozygote excess was also detected by negative inbreeding coefficients in all studied populations. Linkage disequilibrium pruned SNP markers were used to check the effects of ascertainment bias on population diversity and differentiation indexes. As expected, analysis with LD pruned markers led to a slight increase in the level of within-population diversity (data not shown). The greater genetic variability within studied populations might be attributed to the presence of admixture in the studied populations (Dadi et al., 2008). This high within-population genetic diversity or heterozygosity might also result from the use of crossbreeding in our populations. The heterozygosity can be used in mate selection to maximize heterozygosity and, thereby,

performance in the offspring (de Cara et al., 2011). Thus, the presence of large genetic variation in our population will probably lead to more profitable cattle in the future. Our findings were similar to those observed in indigenous Ethiopian cattle breeds (0.370 in Danakil to 0.389 in Ambo; Edea et al., 2013), and European breeds (0.346 in Hereford to 0.370 in Japanese Holstein; Lin et al., 2010). Moreover, the results presented here are very similar to the expected heterozygosity found in European cattle breeds (0.376 in Hereford to 0.386 in AN) by using SNP (Zhang et al., 2018). However, Gautier et al. (2007) reported a lower unbiased gene diversity in European breeds (0.282 in Normande to 0.322 in French Holstein). Other studies have reported genetic diversity values slightly greater (Edea et al., 2015) or lower in taurine and/or zebu cattle (Gautier et al., 2010; McTavish et al., 2013; Porto-Neto et al., 2013; Cañas-Álvarez et al., 2015; Kim et al., 2018). This variability in genetic diversity indices is often due to ascertainment bias associated with the SNP arrays, type of molecular markers (SNP or microsatellite), and cattle (zebu or taurine) used in the study. We observed a low level of inbreeding in our studied populations. However, this may not represent the real status of inbreeding within these cattle populations as allele frequencies may be a poor estimate of inbreeding (Makina et al., 2014). The greater genetic differentiation observed within all beef cattle populations could be attributed to the absence of artificial selection pressures and the presence of certain levels of admixture in these populations (Dadi et al., 2008) causing increased heterozygosity. However, AD exhibited comparatively low polymorphism and genetic variability as expected. The trials that produced this AD breed began at Trangie Agricultural Research Centre in 1974 to evaluate selection for growth rate on herd profitability using 85 cows and a set of yearling bulls. These cows and bulls were selected based on their own yearling growth performance. From 1974, the L line herd remained closed, and all replacement heifers and bulls were selected from within the line

(Parnell et al., 1991). Having fewer population members to select from could result in greater proportional utilization of specific sires, and subsequent reduction in polymorphism and genetic variability. This scenario is not documented in current cattle literature; however, Brito et al. (2017) provided an example of such an effect in the Toggenburg goat breed that also had the lowest heterogeneity due to artificial selection and inbreeding compared to other goat populations in their study.

AMOVA and genetic differentiation between cattle populations

On average, the genetic differentiation (F_{ST}) values among populations were 0.124 and 0.017 for purebred and admixed populations, respectively. The average genetic differentiation value among breeds of European origin (British and Continental) was approximately 0.109. Analyzing 23 European populations using SNP data, a similar value of genetic differentiation (F_{ST} = 0.108) has been obtained by Gautier et al. (2010), where F_{ST} computed for each pair of populations ranged from 0.004 (for US Holstein/French Holstein pair) to 0.202 (for US Jersey/Blonde d'Aquitaine pair). Gautier et al. (2007) observed an average genetic differentiation value of 0.099, ranging from 0.035 (French breeds Salers and Aubrac) to 0.132 (Normande and Holstein). In addition, other studies conducted in European breeds reported similar genetic differentiation values using microsatellite data: 0.107 for 20 Northern European breeds (Kantanen et al., 2000) and 0.112 for seven European cattle breeds (MacHugh et al., 1998). Among purebred populations studied in this project, low genetic differentiation was observed between two British breeds, AN and AR (F_{ST} = 0.047), and two Continental breeds, GV and SM (F_{ST} = 0.079). This low genetic differentiation between these two British breeds can be expected as AR is derived from AN breed and is still currently influenced by them in the US. Low genetic distance between AN and AR cattle was also previously reported by Gautier et al. (2010) (F_{ST} =

0.029) and Porto-Neto et al. (2013) (F_{ST} = 0.024). However, we observed a slightly greater value of genetic differentiation in this study, which may be because of the presence of some levels of admixture with other breeds, such as SH, SM, AD, and GV. Furthermore, low F_{ST} value observed between GV and SM in this study revealed their genetic relationship. A close relationship between these two breeds was shown in the neighbor-jointing tree constructed with Nei's D genetic distances between 109 breeds using microsatellite data (Ginja et al., 2019). The genetic relationship between these two Continental breeds was also reported in the neighbor-net dendrogram built from a pairwise matrix of F_{ST} values based on SNP data by Sermyagin et al. (2018). American Aberdeen was the most distinct breed among six purebred populations in this study. This breed is characterized by a high degree of genetic differentiation from the other purebred populations (F_{ST} ranging from 0.112 to 0.186). The AD breed appeared to be genetically closer to the AN and AR than SH and both Continental breeds, which is attributed to the development of this breed from AN genetics. The genetic differentiation and genetic distance were lower among the admixture cattle populations (UP, ADMX-I, and ADMX-II) due to their common ancestral origin, the presence of admixture, and the strong gene flow of the populations (Edea et al., 2013).

The low genetic differentiation among populations (F_{ST} = 0.028) is likely because of a lack of selection pressure or the presence of moderate gene flow among these populations. A low level of genetic variability (F_{ST} = 0.01), a similar level of total inbreeding (F_{IT} = 0.006), and a slightly greater within-population inbreeding (F_{IS} = -0.003) in Ethiopian cattle were reported by Edea et al. (2013). The level of genetic differentiation among purebreds obtained in this study was slightly greater to those observed between locally adapted taurine breeds in Brazil using SNP markers (F_{ST} = 0.104; Campos et al., 2017), and between European breeds (including both

beef and dairy breeds) using SNP data (F_{ST} = 0.099; Gautier et al., 2007). However, McKay et al. (2008) reported a high differentiation level between European taurine breeds based on SNP data (F_{ST} = 0.170). Our study found a negative value of F_{IS} estimate, which is the correlation between mating gametes within the contemporary array of gametes that can be negative if F_{ST} is greater than F_{IT} (Wright, 1965). Wright (1965) also reported the negative value of F_{IS} indicates a systematic avoidance of consanguine mating within the subdivisions, but it was vague whether the systematic avoidance was on the part of farm managers making selection decisions or other factors. Therefore, the negative value of F_{IS} indicates that there was no increase in inbreeding within our populations. These findings are acceptable as crossbreeding program has been applied in our admixed population. A reduced level of inbreeding or increased heterozygosity is advantageous for our studied population since these allow animal selection for improved production and less chance of introducing deleterious alleles in the population.

PCA and unsupervised admixture analysis

The PC2 clearly separated AD from other purebred populations used in this study. The PC1 partitioned British and Australian origin breeds from Continental breeds; however, SH clustered with Continental because of the presence of some admixture levels that have occurred in the US based on grading up programs in specific breed associations. Grading up is the process of successive "topcrosses" of purebred sires on other breeds, crosses, or of unknown backgrounds (Hammack, 2009), where topcross refers to the use of highly inbred males to the females of the base population or non-inbred population. A 7/8 blood resulting from a third topcross is generally the minimum requirement in most associations (e.g., GV-female, SM-female) to register as a purebred. Some breed associations require 15/16 (e.g., SH, GV-male, and SM-male) or 31/32 to be considered purebred (Hammack, 2009). The Red Angus Association of

America (RAAA) also allows the offspring of animals that are not entirely AR to be registered and increase their blood percentage through a grading up program (Red Angus Association of America, 2009). The population clustering in PC2 was similar to the results of our pairwise F_{ST} estimates, where the AD breed was more genetically distant from GV, SM, and SH compared to AR and AN populations. Moreover, PC3 separated both Continental breeds from British and Australian breeds, albeit some GV individuals clustered with or close to AN and AR individuals since AR and AN breeds have influenced GV, SM, and SH to some degree in the US. The PCA confirmed that UP individuals could have a high percentage of AN or AR influence as they grouped close to the AN and AR population's cluster. These findings provide us a clear insight into their breed ancestry. Furthermore, PCA also revealed that individuals of both ADMX-I and ADMX-II populations clustered close to their primary breed (i.e., breed with greater than 50% influence). These findings will be useful for grouping of our admixed populations in future statistical and genomic association studies.

In cluster analysis with purebreds in unsupervised mode, AD showed its own ancestry from $K = 3$ to $K = 6$. This further supports their unique ancestry to be due to the continuous selection pressure encountered during the development of this breed for specific characteristics. American Aberdeen was the least admixed breed found in this study. From $K = 4$ to $K = 6$, the AR population displayed a significant amount of AN genetics, which supports the gene-flow from AN into the AR breed. In PCA plots with purebred populations, AN and AR clustered separately from other breeds, such as AD, SH, GV, and SM. Angus did not show any signal of admixture with these breeds. However, ADMIXTURE runs (unsupervised and supervised) in this study showed the presence of some level of admixtures in the AN population, which might be due to some limitation of this software in the estimation of membership proportions. Crum et

al. (2019) reported that the ADMIXTURE estimated ancestry proportions appear to be context-dependent and may vary based on the other individual included in the analysis. The American Angus Association allows only the offspring from registered animals within the breeding society. However, other breed associations, including SM, GV, and SH, allows grading up programs, which leads to AN-influenced animals being registered in their respective associations (i.e., why black coat color can be seen in such US breeds). Therefore, this might have influenced the inconsistent results found in AN with ADMIXTURE runs. Some AR cattle revealed their individual ancestry; however, the majority showed AN ancestry as expected ($K = 6$) since RAAA still allows black AN to be registered as AR.

Previous studies on population structure with either microsatellites or SNP data presented distinct clustering of British breeds from Continental breeds. MacHugh et al. (1998) observed a weak distinction between breeds from the British Isles and breeds from Continental Europe using 20 microsatellite markers. In a study on the relationship of Russian cattle with Eurasian taurine breeds using whole-genome SNP markers, cattle breeds from Great Britain were clustered separately from cattle of Central Europe (Sermyagin et al., 2018). In a similar study, admixture analysis showed that GV and SM share above 50% common genetics. Our findings were similar to those reported by Gibbs et al. (2009), who observed single clustering for AN and AR with PC and model-based clustering. At $K = 4$, clustering patterns of British and Continental breeds used in this study were found similar to those reported by McTavish et al. (2013). At $K = 12$ of their model-based population assignments, AR displayed around the two-third percentage of AN genetics and some signals of admixture with SH and Continental breeds. Model-based clustering in their study also displayed some SH cattle being admixed with AN genetics, which is due to the

grading up program in SH. In addition, their study also showed GV as more admixed breed compared to SM breed, which had the genetics of SM, Limousin, SH, and others.

Admixture analysis in supervised mode

In the unsupervised run on the unrelated individuals, all AD individuals showed their own ancestry; however, one individual had 0.71% of AN ancestry. The majority of the SM and SH individuals also showed their individual ancestry. All unrelated AR individuals displayed some signals of admixture with AD, GV, and SH. The reason of this admixture of AR breed with other beef breeds has already been discussed. Some differences of ancestral breed fractions between unsupervised and supervised model-based clustering were observed in purebred populations, with large differences for AN and GV populations. In addition, the unsupervised runs with the full population showed an unexpected outcome for two purebred populations (AR and GV). However, the supervised runs in this case showed a projected outcome for both purebred and admixed individuals. The difference between these two runs was large in AN, AR, AD, GV, and SM-influenced individuals, but it was small in SH-influenced individuals. Therefore, supervised ADMIXTURE runs with training (i.e., unrelated) set can provide more predictable outcomes while estimating genetic breed composition in an admixed population. However, ADMIXTURE analysis of purebreds can have good runs regardless of training vs. non-training set. Gobena et al. (2018) reported that unsupervised model-based clustering used for inferring genomic breed compositions runs a greater risk of confounding by sources of population structure other than heterogeneous breed ancestry. Additionally, we discussed earlier in the methods section why 18 heifers were grouped as UP population. From the supervised run ($K = 5$) with the full population, we confirmed that the UP individuals have approximately 76.3% AN genetics on average. This finding also supports our PCA results that showed UP

individuals grouped close to AN and AR individuals. During unsupervised and supervised admixture runs with the full population, all UP individuals were grouped under the GV category based on their assumed breed composition from the assumed pedigree. In admixture analysis, this GV category showed a high percentage of AN genetics as around 80% of individuals there were from the UP population. For crossbred animals, breed composition derived from genomic data should be more accurate than pedigree-based estimates since pedigrees can be incomplete or incorrect (Sölkner et al., 2010; Vanraden and Cooper, 2015). Kuehn et al. (2011) also reported that Mendelian sampling during recombination could also lead to deviation from the composition expected based on pedigree. Therefore, the breed composition estimates from membership coefficients in the non-purebred set would undoubtedly be better options for grouping our admixed populations in association studies.

PCA with population group created using supervised admixture (*K* = 5) outputs

These discrepancies between PCA plots with population groups using pedigree-based breed composition and supervised admixture's estimated breed composition may occur due to several reasons. Grouping of AD-influenced heifers as AN-influenced might be due to erroneous pedigree data. Similar impacts of incorrect pedigree information have been reflected by alteration of population assignment. For F1 individuals, changes in their orientation over the two different PCA plots might be affected by the criteria used for their grouping. An earlier study reported that pedigree-based breed composition is less accurate than that of breed fraction estimated using genomic data because pedigrees are often partially missing or incorrect (Vanraden and Cooper, 2015).

Goodness-of-fit test

This research's long-term goal is to use the admixed population in this study for genome-wide association testing, which is known to be influenced by population structure (i.e., allele frequency differences among sub-populations). We hypothesize that the inclusion of primary breed or primary ancestral breed will correct for allele frequency differences. The first step in this is to determine if the inclusion of this effect as fixed (due to population availability) improved model parameters. Testing goodness-of-fit animal models with or without pedigree confirmed that mixed models are enhanced by adding ancestral breed grouping of admixed individuals. For the purpose of ancestral breed grouping, we used membership coefficients from both unsupervised and supervised ADMIXTURE runs. Ancestral breed grouping with outputs from supervised runs provides better modeling goodness-of-fit statistics than the unsupervised runs. The best ancestral breed grouping was trait-dependent in the supervised run, which might be due to the MIX group's sample size. Our study also showed that the mixed animal model could improve by including the pedigree as expected.

Including the ancestral breed group (either C3 or C4) for DMI and FIM decreased heritability, but there was no change in repeatability. This provides evidence that the variation explained is only occurring between the additive and permanent environment variances and leads to more accurate estimates of the parameters. The standard errors of heritability and repeatability observed in all cases were similar for each studied trait. The sample size is the largest contributor to standard error in these models (i.e., less than 1,000 animals with data and pedigree often result in large standard errors). Therefore, we propose that breed group inclusion in the animal model is a good strategy for performing association studies when using crossbred or admixed populations.

In genome-wide association studies, population structure and relatedness are a concern as they provide spurious association outputs (Toosi et al., 2018). Several approaches have been developed to control confounding factors and reduce their impact on the results of genome-wide association studies. Devlin and Roeder (1999) demonstrated that the effects of cryptic relatedness and population substructure on test statistics of interest are essentially constant across the genome, under certain conditions. They suggested an approach called genomic control (GC) that uses null markers (e.g., polymorphisms unlikely to affect liability) across the genome to estimate the effect of confounding and then removing the effect from the association test statistic. Since the GC method was introduced, Pritchard et al. (2000) have proposed a model-based clustering method for using multi-locus genotype data to infer population structure and assign individuals to populations called structured association (SA). This approach uses marker loci unlinked to the candidate genes under study to infer subpopulation membership; there is neither bias nor excess variance due to population substructure. Individuals in the sample are assigned (probabilistically) to populations or jointly to two or more populations if their genotypes indicate that they are admixed. Alexander et al. (2009) proposed an ADMIXTURE algorithm for model-based estimation of ancestry in unrelated individuals, which adopts the likelihood model embedded in SA (Pritchard et al., 2000). ADMIXTURE allows the specification of known descent individuals to be used as a reference panel; however, SA does not allow this. Another approach uses PCA to explicitly model ancestry differences between cases and controls called the principal components-based method (Price et al., 2006), although it does not directly deliver admixture fractions. Zhang et al. (2010) suggested a mixed linear model-based approach where population structure is fit as a fixed effect and kinship among individuals is incorporated as the variance-covariance structure of the random effect for the individuals. Our

study used the ADMIXTURE algorithm to infer ancestral breed membership coefficients, which has been found to provide admixture coefficients with greater accuracy (Alexander et al., 2009).

Longer generation intervals can hinder research in cattle compared to other livestock and model organisms; therefore, using populations available can improve research resources' efficiency while still clarifying research questions. Furthermore, finding consistent population structures based on genetic variance from this study and previous literature indicates that as long as breed type is known, the inclusion of this effect in the model can further aid research objectives with or without the expense of genotyping an entire population. In the event that an admixed population is to be used, however, the available databases of purebred genotypes from research can serve as a resource to clarify population structure, primary breeds, and improved modeling of research objectives while keeping genotyping costs to a minimum.

Conclusions

Our findings indicate a large degree of diversity among individuals within the studied population. Our results also provide novel information about the genetic structure of AD cattle and its genomic relationship with five different taurine beef cattle breeds available from the U.S. populations. We showed that AD has a unique homogeneous genetic structure, even though founder animals originated from the AN breed. Furthermore, using ancestral breed grouping in mixed animal model resulted in goodness-of-fit criteria that were more desirable, thereby indicating it is a good strategy for correcting population sub-structures when performing association studies in admixed populations and improving the efficiency of research resources with admixed populations.

CHAPTER 3. ASSOCIATION OF LEPTIN WITH REPRODUCTIVE PERFORMANCE OF COMMERCIAL BEEF COWS AND DEVELOPING HEIFERS

Abstract

Leptin is a hormone product of the leptin (*LEP*) gene, synthesized and expressed predominantly by adipose tissues. This study aimed to determine the association of leptin genotype (*LEP c.73C>T*), leptin diplotype (*LEPD*), and plasma leptin hormone (LEPH) concentration (high vs. low) with reproductive characteristics (n = 19) in commercial beef cows and developing heifers. A total of 594 commercial females, 218 cows and 376 heifers, were genotyped for the *LEP c.73C>T* SNP. Phasing with markers within a 0.25 Mbp flanking *LEP* (n = 19) created haplotypes; however, only four SNP markers within or immediately surrounding *LEP* were used for *LEPD* association study. Circulating levels of LEPH were measured on 333 heifers prior to their first breeding season. Reproductive traits analyzed were antral follicle count (AFC), uterine horn diameter (UHD), and ovary (heifers only); however, age at first calving, calving interval, gestation length, and 3 success traits (pregnancy, weaning, and overall reproductive) were available for all females. Mixed model procedures of SAS with fixed effects of ancestral breed group (n = 6 or 5), *LEP c.73C>T* genotype (CC, CT, and TT) or *LEPD* (n = 9 or 11) or LEPH category (n = 2), frame size group (n = 4), health cull status (n = 2), age of dam (n = 4 or covariate), project cycle (n = 2), birth year (n = 4 or covariate), or cycle nested within year (n = 6) were used depending on the trait. Left ovary small follicles (*P* = 0.021), overall small follicles (*P* = 0.028), average ovarian diameter (*P* = 0.006), left ovary length (*P* = 0.023), and average ovary length (*P* = 0.013) were greater in CT heifers than that of CC heifers. Statistical differences among TT heifers and CC heifers were not observed except for average ovarian diameter (*P* = 0.045). Numerical differences indicated complete dominance of the T

allele over the C allele, which was further supported through linear contrasts. Other reproductive traits did not differ ($P > 0.074$), and no associations of *LEPD*s were found ($P \geq 0.083$). Heifers with low LEPH had greater right ovary diameter ($P = 0.033$), length ($P = 0.013$), and medium follicle count ($P = 0.039$) than those with high levels. Plasma LEPH was negatively correlated with AFC, right ovary follicles, overall medium follicles, right ovary diameter, average ovary diameter, and UHD (-0.131 to -0.170, $0.004 \leq P \leq 0.024$). Literature reports the increased productivity of the T allele for growth and carcass attributes, so it was expected the same increased productivity would be found for reproductive traits. Increased follicles as well as uterine and ovary sizes found in this study, paired with low levels of LEPH, indicate this expectation is true.

Introduction

The selection of animals for better reproductive performance is a time-consuming process. Since reproduction is a complex trait (i.e., controlled by many genes and environmental factors), some genomic locations might account for large amounts of genetic variation, but this is not well understood. Molecular markers in or around genes may be involved directly or indirectly in reproduction. Therefore, selection programs using specific genetic markers could be a good strategy for precise and improved genetic changes in these traits. However, the power of a single marker association test may be affected by ignoring the linkage disequilibrium (i.e., non-random association of alleles at two or more loci in a general population) information containing flanking markers (Akey et al., 2001). The haplotypes of tightly linked and ordered markers are intuitively more informative and powerful than the individual markers. Thus, the knowledge of a group of allelic variations in the gene and its surrounding region could provide an excellent

opportunity to select for improved reproductive performance. The diplotype is a matched pair of such haplotypes on homologous chromosomes (Lu et al., 2004).

In mammals, several regulatory substances like endogenous opioids, neuropeptide Y (NPY), ghrelin, melanin-concentrating hormones, agouti-related proteins, proopiomelanocortin (POMC), cocaine- and amphetamine-regulated transcript (CART), orexins/hypocretins, galanin, and neurotensin, etc. play a role in the regulation of feed intake, energy expenditure, and reproduction (Magni et al., 2000). Most of these neuropeptides originate and act mainly in specific hypothalamic areas of the central nervous systems to control feed intake, feeding behavior, energy expenditure (by regulating the level of spontaneous physical activity), and neuroendocrine aspects of the hypothalamic-pituitary-gonadal axis (HPG; Kalra et al., 1999). Previous studies reported associations of neurons producing NPY, POMC, and CART with gonadotropin-releasing hormone (GnRH) neurons in the medial preoptic area (Sabatino et al., 1987; Guy et al., 1988; Leranth et al., 1988; Rondini et al., 2004). This interaction facilitates hypothalamic neurons regulating energy metabolism to communicate with the HPG (Hill et al., 2008). Therefore, energy status influences reproduction at the systemic level by modulating the hypothalamic GnRH neuronal network (and subsequently pituitary gonadotropin secretion) through several hormones or neuropeptides (Garcia-Garcia, 2012). Leptin, a 16kDa protein product of the leptin (*LEP*, also known as *ob*) gene, influences feed intake and energy expenditure by interacting with neuropeptides in the hypothalamus and impacts reproduction by stimulating GnRH release (Máčajová et al., 2004) or indirectly through metabolic status regulation. *LEP c.73C>T* SNP has been reported as a missense mutation in the bovine leptin gene that results in the amino acid change from arginine to cysteine, which reduces leptin

receptor affinity and increases serum clearing rate. This causative mutation directly results in changes in biological function (Buchanan et al., 2002).

Minimal earlier association studies have been performed between *LEP* polymorphisms and reproductive traits in beef cattle. The *LEP c.73C>T* SNP did not show any association with age at first calving in F_1 *Bos taurus* × *Bos indicus* dairy cattle (Choudhary et al., 2019). However, the *LEP* polymorphisms (*LEP c.73C>T* and *LEP g.-963C>T*) have been linked with gestation length and direct calving difficulty in Holstein-Friesians sires, estimated from the performance of their daughter-parity records (Giblin et al., 2010). Also, polymorphisms in the *LEP* gene (*LEP/Sau3AI*) and *LEPR* gene (*LEPR c.115C>T*) have a known association with milk production traits, calving interval, and age at first calving in Slovak spotted and Pinzgauer cows (Trakovická et al., 2013). Earlier studies in dairy cattle also showed associations of the *LEP* SNP markers other than *LEP c.73C>T* with calving interval, weight at first calving, age at first service, the total number of artificial inseminations, days to conception, and fertility traits (Clempson et al., 2011; Trakovická et al., 2013; Jecminkova et al., 2018). Haplotypes constructed with polymorphisms on *LEP*, including *LEP c.73C>T* SNP, showed association with gestation length but did not present any association with calving interval, calving difficulty, and perinatal calf mortality. Therefore, it has been established that selection using the *LEP* markers or *LEP* markers and its flanking markers (i.e., haplotype) can be made in cattle. However, there is limited information on the association of the *LEP c.73C>T* SNP genotype or diplotype with reproductive characteristics such as gestation length, pregnancy status, weaning success, and reproductive success over time in commercial beef cows. Also, little is known on the effect of the *LEP c.73C>T* SNP genotype, leptin diplotype (*LEPD*), and circulating LEPH concentration on antral follicle count, reproductive tract score, and ovary measurements in forage-fed

developing beef heifers. Thus, this study was conducted to determine the association of the *LEP c.73C>T* genotype, *LEPD,* and circulating LEPH concentrations with reproductive characteristics in commercial beef cows and developing heifers.

Materials and methods

Animals and phenotypic data

All procedures were reviewed and approved by the Institutional Animal Care and Use Committee of North Dakota State University. Data were generated in part by 1) the base cow herd (base herd; n = 218) at Dickinson Research Extension Center (Dickinson, ND), and 2) daughters of the base herd (2 cycles) that became part of a long-term study focused on longevity traits. The base, Cycle 1, and Cycle 2 herds have already been described in Chapter 2. Only females with phenotypic observations were included in the present study. The number of females used in this study varied based on reproductive traits. Age at first calving data was available for 524 females, including 215 base herd cows, 221 Cycle 1 female, and 88 Cycle 2 females. A total of 473 observations were available for calving interval, i.e., the period between two subsequent calving events (n = 213, 217, and 43 for the base, Cycle 1, and Cycle 2 herd, respectively). Data on success traits, such as pregnancy success, weaning success, and reproductive success over time, were available on 479 females (n = 218, 216, and 45, respectively). However, gestation length (n = 223 and 88) and other reproductive measurements (n = 257 and 45) data were only available for Cycle 1 and Cycle 2 females, respectively. Frame size data were available for only Cycle 1 and Cycle 2 females. Frame size was calculated based on hip height and age at weaning using BIF equations (BIF Improvement Federation, 2018), where frame size among females was used to create 4 groups of small (SM; less than 4.00), moderately small (MS; 4.00 to 5.50), moderately large (ML; 5.51 to 6.50), and large (LG; 6.51 or greater).

Reproductive data included age at first calving, calving interval, gestation length (the number of days from the day of conception to birth of the calf), pregnancy success (pregnant or not pregnant at pregnancy check), weaning success (live calf or no calf at weaning, weaning is defined as the time point of separating the calf from the dam, typically 6 months of age), and reproductive success (0 to 3, where 0 is open, 1 is pregnant but no live calf birthed, 2 is pregnant and calved but calf died prior to weaning, and 3 is pregnant, calved, and weaned) over time were recorded for all cows with weaning seasons completed (n = 1 to 13). Pregnancy was diagnosed by transrectal ultrasonography (5.0-MHz linear array transducer, an Aloka 500V unit, Corometrics Medical Systems, Wallingford, CT) performed by two skilled persons at least 35 d after the end of the breeding season. Reproductive physiological characteristics (antral follicle count [AFC], uterine horn diameter [UHD], and ovary measurements) were collected from only Cycle 1 and Cycle 2 during feed trials leading up to their first breeding season. All physiological data were taken according to the procedure described by Cushman et al. (2009). Briefly, ovarian ultrasonography was performed prior to breeding season, while each ovary was scanned for ovarian follicles and measurements using a 7.5-MHz linear array transducer (Aloka 500V unit, Corometrics Medical Systems, Wallingford, CT). Heifers were also evaluated for UHD.

DNA and *LEP* genotyping

Blood samples were collected in 10 ml vacutainer K2-EDTA collection tubes (BD, Franklin Lakes, NJ) via jugular venipuncture on all females (n = 524) for DNA extraction using the Qiagen DNeasy extraction kit protocol (QIAGEN N.V., Hilden, Germany). The DNA quality was checked using the Synergy H1 microplate reader (BioTek, Winooski, Vermont). The threshold range of A_{260}/A_{280} ratio for pure DNA was 1.8 to 2.0, and values outside of this range were considered as contamination. The DNA samples following quality and quantity check

stored at -80°C until *LEP* genotyping. Genotyping for the *LEP c.73C>T* marker [described by Buchanan et al. (2002)] was performed using KASP by Design assay (LGC Genomics, Beverly, MA) with an Applied Biosystems 7500 Fast Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA) following the manufacturer's recommended protocol. This KASP genotyping assay consists of two components: KASP assay mix and KASP master mix. The KASP assay mix contains two different, allele-specific, competing forward primers (one for each SNP allele) with unique unlabeled tail sequences at the 5' end and one common reverse primer. On the other hand, the KASP master mix contains FAM™ and HEX™ specific FRET cassette (oligonucleotide sequences that are identical to tails on the allele 1 [T] and allele 2 [C] specific primers, respectively), Taq polymerase, and optimized buffer solution. Three non-template controls formed by using H_2O in place of DNA were included on each genotyping plate accounting for negative control.

***LEP* haplotype phasing**

All females were previously genotyped for 138,892 markers using the GeneSeek Genomic Profiler 150K for Beef Cattle (Neogen GeneSeek, Inc., Lincoln, NE), where all SNP markers were mapped using the UCD 1.2 assembly (Rosen et al., 2020). After initial processing and quality control (described in Chapter 2), 117,372 SNP markers were retained. The haplotype phasing was performed with markers within a 0.25 Mbp flanking *LEP* (n = 19) using Beagle v.3.2.2 (Browning and Browning, 2011). Initial phasing using 19 polymorphisms provided 66 possible haplotypes, which meant all possible diplotypes were not supported by our population size (i.e., number of replicates per diplotype). Therefore, the number of SNP markers was reduced to four (Table 3.1).

Table 3.1. Four single nucleotide polymorphism markers used in the haplotype and diplotype formation for association study

SNP markers	SNP order	Region[1]	BTA[2]	Position (bp)
BovineHD0400026029	1	5'-up	4	92,426,572
ARS-BFGL-NGS-59298	2	Intron 1	4	92,444,591
LEP c.73C>T	3	Exon 2	4	92,449,024
BovineHD0400026063	4	3'-down	4	92,472,469

[1]5'-up: Upstream (5' end) of the leptin gene; 3'-down: Downstream (3' end) of the leptin gene
[2]BTA: *Bos taurus* autosome

LEPH concentration

For circulating levels of LEPH, blood samples for 333 heifers were collected in 10 mL BD vacutainer heparin tubes (Franklin Lakes, NJ) via jugular venipuncture the day prior to entering their breeding season (typically July 31 or August 1 of each year). Blood samples were stored on ice after collection, centrifuged at 3000xg for 20 minutes, and then the plasma layer was extracted and stored at -20°C until hormone analysis could be completed. Plasma LEPH concentrations were determined in duplicate using the Multi-Species Leptin RIA kit (XL-85K, EMD Millipore Corporation, St. Charles, MO), which includes bovine in the species list, at the Department of Animal Science, South Dakota State University (SDSU), Brookings, South Dakota. Samples were validated for parallelism and recovery of know amounts of leptin added to various bovine samples. All values were expressed as ng/mL human equivalent (HE). The assay's sensitivity was 1.93 ng/mL and 3.44 ng/mL HE for the first (2014 to 2016 born heifers; n = 260) and second (2017 born heifers; n = 73) lot samples, respectively. The differences in assay sensitivity between two lots might be due to the samples being measured from different lots of the leptin kit. The manufacturer may have changed the titer of the antibody or the amount or specific activity of the label, affecting sensitivity. Inter-assay and intra-assay CVs of first and second lot samples were 10.8% and 8.4%, and 13.2% and 7.2%, respectively. Cycle 1 and 2

females in the study were grouped into high and low LEPH concentration groups based on the median LEPH concentration of approximately 15.96 ng/mL for all studied traits (high: heifers with LEPH > median and low: heifers with LEPH ≤ median). This median-based grouping results in an equal sample size in two different categories. In LEPH grouping, only females with both LEPH and data records determined the median value, where other females were dropped from the association analysis for a given trait.

Statistical analysis

All females were classified into six major ancestral breed groups (ANAR: Angus and Red Angus; AD: American Aberdeen; SH: Shorthorn; GV: Gelbvieh; SM: Simmental; and MIX = females with breed fraction less than the defined threshold value, i.e., ≤ 0.55) based on the best ancestral breed category observed in a population structure analysis performed under Chapter 2. For reproductive physiological data and gestation length, heifers with GV ancestral breed group were removed from the *LEP c.73C>T* genotype and *LEPD* analysis as this group had few observations (n = 3 for reproductive physiological data and n = 2 for gestation length). Additionally, females with the GV ancestral breed group were removed for all studied traits in circulatory LEPH effects analysis because of few observations. The ancestral breed group was not considered in LEPH association analysis for three success traits since the ancestral breed group's exclusion fit the model better. Some cows were culled from the herd due to health reasons rather than reproductive failure; therefore, these incidences were recorded as a potential fixed effect (health cull reason; yes or no). The dam age of all studied females was considered as a potential fixed covariate in the model. During investigating the effects of *LEPD* on UHD, dam age was included as a fixed class effect in the final model for better fit statistics instead of using as a covariate, where females were classified into four categories (W: 2 to 3 years; X: 4 to 5

years; Y: 6 to 7 years; and Z: ≥ 8 years of dam age) based on their dam age. An observation with an internally studentized residual outside the range of -3 to +3 was excluded from the analysis (n = 1 for right ovary diameter to n = 7 for right ovary height). The maximum likelihood (ML) estimation method was used for model testing of the fixed effects using goodness-of-fit statistics (AIC, AICC, or BIC) as the ML estimates are unbiased for the fixed effects (Korner-Nievergelt et al., 2015).

All statistical analyses were performed with SAS v.9.4 (SAS Inst., Cary, NC), where either the MIXED or GENMOD procedures were used based on the distribution of that trait. Fixed effects considered were ancestral breed group (n = 5 or 6), age of dam (n = 4 or covariate), frame size grouping (n = 4), health cull reason (n = 2), *LEP c.73C>T* genotype (n = 3) or *LEPD* (n = 9 for age at first calving and success traits, and n = 10 for calving interval) or LEPH category (n = 2), project cycle (n = 2), birth year (n = 4 or covariate when based herd cows were included), or cycle nested within year (n = 6) depending on the trait. Least squares means were generated for significant effects and adjusted to control for experiment-wise error using the Tukey-Kramer method. Additional linear contrasts for *LEP c.73C>T* or *LEPD* based on suggested allele or haplotype dominance were run only if pairwise comparisons could not elucidate an allele or haplotype effect. These contrast tests were performed in those cases where the original ANOVA effect was significant. The individual comparison-wise error rate was determined using the formula: $\alpha_I = 1 - (1 - \alpha_E)^{1/m}$, where α_E was set as 0.05, and m is the number of linear contrasts to be conducted. The *P*-value from the linear contrasts was only considered significant when it was below α_I to control for experiment-wise error. The CORR procedure of SAS was used to obtain Spearman correlations of LEPH with raw phenotype traits.

Results

In this study, frequencies of the three *LEP c.73C>T* genotypes (CC, CT, and TT) were distributed according to Hardy-Weinberg proportions in the entire female population. The proportion of heterozygote females were almost equal to the total proportion of both homozygote females (heterozygotes: 0.487 – 0.511 vs. homozygotes: 0.513 – 0.489). When fitting *LEPc.73C>T* genotypes as a fixed effect along with cycle nested within year, ancestral breed group, age of dam, and frame size grouping for circulating LEPH concentration, no differences between *LEP c.73C>T* genotypes were observed for either LEPH category-based or overall population ($P > 0.116$; Table 3.2). However, the average circulatory LEPH concentration was greater in heifers with high LEPH compared to those of the low LEPH group for overall and each respective genotype ($P < 0.0001$; Table 3.3).

Table 3.2. Least square means and standard errors for circulatory leptin hormone (LEPH) concentration of *LEP c.73C>T* genotype based on LEPH category in beef heifers

LEPH Category		*LEP c.73C>T* genotype		
		CC	*CT*	*TT*
Concentration, ng/mL	Low	12.76 ± 0.50 (35)	12.50 ± 0.37 (83)	12.03 ± 0.52 (48)
	High	21.58 ± 0.93 (40)	22.73 ± 0.70 (79)	24.19 ± 1.02 (47)
	Overall	17.84 ± 0.80 (75)	17.83 ± 0.57 (162)	17.99 ± 0.83 (95)

[1]Numbers in parentheses are the number of observations used

Table 3.3. Least square means and standard errors for circulatory leptin hormone (LEPH) concentration of LEPH category based on *LEP c.73C>T* genotype in beef heifers

LEP c.73C>T genotype		LEPH Category	
		Low	High
Concentration, ng/mL	CC	13.43 ± 0.80^{b} (35)	21.39 ± 0.76^{a} (40)
	CT	12.71 ± 0.60^{b} (83)	22.49 ± 0.58^{a} (79)
	TT	13.25 ± 1.22^{b} (48)	24.27 ± 1.22^{a} (47)
	Overall	12.70 ± 0.42^{b} (166)	22.36 ± 0.40^{a} (166)

[1]Numbers in parentheses are the number of observations used
[a,b]Least square means within a row without a common superscript letter differ ($P < 0.05$)

Effect of *LEP c.73C>T* polymorphism on reproduction

Average ovarian diameter ($P = 0.006$), left ovary length ($P = 0.023$), and average ovary length ($P = 0.013$) were greater in CT heifers than CC heifers. Similarly, left ovary small follicles ($P = 0.021$) and overall small follicles ($P = 0.028$) were greater in CT heifers compared with CC homozygotes. The *LEP c.73C>T* genotype showed a tendency for ANOVA effect on AFC ($P = 0.055$); however, numerical differences of LSMeans indicate the T allele's association with greater AFC. The statistical differences of TT heifers to CC heifers were not observed for these reproductive traits except for average ovarian diameter ($P = 0.045$). When focusing on average, total or overall scores for follicle counts, ovary size, or uterine horn size and *LEP c.73C>T* genotype was a significant ANOVA effect, numerical differences between TT and CT heifers were minimal but numerically different from CC heifers. Noticeably, standard errors for TT heifers were larger than CT heifers, indicating a wider range of variability that leads to statistical differences not seen between TT and CC heifers. This suggests that the T allele is dominant to the C allele, even though pairwise comparisons did not delineate this. Linear contrasts of CC vs. TT and CT did show significance (average ovarian diameter: $P = 0.002$; left ovary length: $P = 0.027$; average ovary length: $P = 0.006$; left ovary small follicles: $P = 0.034$; and overall small follicles: $P = 0.033$; Table A2). When assuming the C allele was dominant (i.e., TT vs. CC and CT), none were significant ($P > 0.191$; Table A2). No additional significances were identified (Table 3.4, 3.5, and 3.6).

Table 3.4. Association of (LSMeans ± SE) of leptin based on classification effect, and Spearman correlation (r_s) of leptin with success traits, calving interval, age at first calving, gestation length, and uterine horn diameter in cows and developing heifers

		Success[3]			Calving interval, d	Age at first calving, d	Gestation length, d	Uterine horn diameter, mm
Effect[1]	Level[2]	Preg.	Wean.	Repro.				
LEP	*P*-value	0.885	0.858	0.861	0.341	0.268	0.742	0.093
	CC	0.94 ± 0.02	0.90 ± 0.02	0.94 ± 0.01	370.4 ± 5.7	731.8 ± 3.0	274.4 ± 0.6	14.32 ± 0.25
	CT	0.93 ± 0.01	0.90 ± 0.01	0.94 ± 0.01	379.7 ± 4.5	735.3 ± 2.3	274.2 ± 0.5	14.92 ± 0.18
	TT	0.94 ± 0.01	0.90 ± 0.01	0.94 ± 0.01	378.2 ± 5.8	737.8 ± 3.0	273.8 ± 0.7	14.72 ± 0.27
LEPD[4]	*P*-value	0.302	0.486	0.438	0.854	0.862	0.185	0.122
	AA	0.93 ± 0.01	0.89 ± 0.02	0.94 ± 0.01	381.3 ± 6.7	738.5 ± 3.3	273.8 ± 0.7	14.36 ± 0.30
	BB	0.95 ± 0.02	0.90 ± 0.03	0.94 ± 0.02	372.3 ± 9.1	736.2 ± 4.7	270.5 ± 1.8	14.03 ± 0.40
	CC	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	369.5 ± 25.6	730.4 ± 13.9	277.2 ± 2.5	15.14 ± 0.93
	AB	0.93 ± 0.01	0.89 ± 0.01	0.93 ± 0.01	384.4 ± 6.2	738.2 ± 3.0	274.1 ± 0.6	14.37 ± 0.27
	AC	0.95 ± 0.02	0.93 ± 0.02	0.95 ± 0.02	370.8 ± 8.5	731.1 ± 4.3	274.3 ± 0.9	15.75 ± 0.35
	AD	0.97 ± 0.03	0.95 ± 0.04	0.97 ± 0.02	383.5 ± 15.6	736.4 ± 6.8	274.2 ± 1.4	15.22 ± 0.65
	AE	0.99 ± 0.01	0.94 ± 0.03	0.97 ± 0.02	374.4 ± 12.9	739.0 ± 6.9	271.9 ± 1.9	14.88 ± 0.70
	AF	-	-	-	-	-	269.9 ± 2.6	15.78 ± 1.00
	BC	0.91 ± 0.04	0.87 ± 0.05	0.91 ± 0.03	367.5 ± 11.4	729.6 ± 5.9	274.7 ± 1.2	14.76 ± 0.45
	BD	0.96 ± 0.04	0.95 ± 0.05	0.97 ± 0.03	380.4 ± 19.8	729.0 ± 8.8	270.5 ± 1.8	13.64 ± 0.77
	CD	-	-	-	-	728.5 ± 11.4	278.2 ± 2.1	13.87 ± 0.95
LEPH group[5]	*P*-value	0.258	0.469	0.555	0.406	0.724	0.288	0.213
	Low	0.94 ± 0.02	0.89 ± 0.02	0.92 ± 0.02	379.9 ± 7.2	730.2 ± 1.6	273.5 ± 0.5	14.88 ± 0.20
	High	0.92 ± 0.02	0.86 ± 0.02	0.91 ± 0.02	373.2 ± 6.4	729.5 ± 1.5	274.2 ± 0.5	14.60 ± 0.19
LEPH conc.	*P*-value	-	-	-	0.937	0.651	0.074	0.023
	r_s	-	-	-	0.005	-0.027	0.105	-0.132

[1]*LEP*: *LEP c.73C>T* genotype; *LEPD*: leptin diplotype; LEPH: leptin hormone; conc.: concentration
[2]Number of observations (CC: 71 – 103, CT: 147 – 260, TT: 84 – 161; AA: 75 – 138, BB: 28 – 44, CC: 04 – 07, AB: 86 – 170, AC: 43 – 59, AD: 09 – 19, AE: 08 – 18, AF: 04, BC: 20 – 26, BD: 06 – 11, CD: 04 – 06; High: 129 – 146; Low: 129 – 148; r_s: 138 – 295)
[3]Success proportion at pregnancy (preg.), weaning (wean.), and overall reproduction (repro.)
[4]AA: AATC/AATC, AB: AATC/GGCT, AC: AATC/GACT, AD: AATC/AGCT, AE: AATC/GATC, AF: AATC/AACT, BB: GGCT/GGCT, BC: GGCT/GACT, BD: GGCT/AGCT, CC: GACT/GACT, CD: GACT/AGCT
[5]The median LEPH concentrations used for LEPH grouping were 15.63 – 16.71 ng/mL

Table 3.5. Association of (LSMeans ± SE) of leptin based on classification effect, and Spearman correlation (r_s) of leptin with ovary diameter, length, and height in developing heifers

		Diameter, mm			Length, mm			Height, mm		
Effect[1]	Level[2]	Left	Right	Average	Left	Right	Average	Left	Right	Average
LEP	*P*-value	0.190	0.154	0.007	0.031	0.332	0.016	0.485	0.255	0.314
	CC	18.62 ± 0.54	19.35 ± 0.57	18.99 ± 0.34^{b}	20.91 ± 0.75^{b}	21.90 ± 0.75	21.40 ± 0.50^{b}	15.99 ± 0.63	16.85 ± 0.56	16.69 ± 0.36
	CT	19.71 ± 0.40	20.47 ± 0.41	20.14 ± 0.25^{a}	23.08 ± 0.54^{a}	22.85 ± 0.55	22.97 ± 0.37^{a}	16.48 ± 0.45	17.86 ± 0.40	17.29 ± 0.26
	TT	19.52 ± 0.59	20.57 ± 0.61	20.05 ± 0.37^{a}	22.26 ± 0.81^{ab}	23.30 ± 0.82	22.78 ± 0.54^{ab}	16.97 ± 0.67	17.64 ± 0.60	17.21 ± 0.39
LEPD[3]	*P*-value	0.048	0.561	0.057	0.024	0.902	0.041	0.306	0.148	0.602
	AA	19.96 ± 0.64	20.81 ± 0.68	20.41 ± 0.41	23.04 ± 0.87	23.51 ± 0.91	23.28 ± 0.60	17.21 ± 0.75	18.15 ± 0.66	17.32 ± 0.43
	BB	18.80 ± 0.86	20.15 ± 0.92	19.47 ± 0.55	21.76 ± 1.18	23.12 ± 1.23	22.44 ± 0.81	15.71 ± 1.01	17.56 ± 0.89	16.37 ± 0.58
	CC	14.49 ± 2.00	21.76 ± 2.15	18.19 ± 1.28	16.37 ± 2.76	20.34 ± 2.87	18.36 ± 1.89	12.94 ± 2.37	22.79 ± 2.08	18.26 ± 1.37
	AB	20.45 ± 0.58	20.56 ± 0.62	20.57 ± 0.37	23.89 ± 0.80	23.08 ± 0.84	23.49 ± 0.54	17.34 ± 0.69	18.13 ± 0.61	17.41 ± 0.40
	AC	18.87 ± 0.74	20.51 ± 0.79	19.61 ± 0.47	21.82 ± 1.01	22.82 ± 1.05	22.32 ± 0.69	15.42 ± 0.87	17.51 ± 0.78	17.19 ± 0.51
	AD	18.25 ± 1.43	19.86 ± 1.45	19.84 ± 0.87	23.06 ± 1.87	21.43 ± 1.94	22.25 ± 1.28	14.41 ± 1.69	18.23 ± 1.40	17.23 ± 0.92
	AE	18.19 ± 1.51	19.79 ± 1.62	18.95 ± 0.97	19.86 ± 2.08	22.95 ± 2.16	21.41 ± 1.42	16.20 ± 1.78	16.70 ± 1.57	16.18 ± 1.03
	AF	21.67 ± 2.14	18.29 ± 2.29	19.81 ± 1.37	25.13 ± 2.95	22.07 ± 3.07	23.60 ± 2.01	17.79 ± 2.53	14.64 ± 2.22	16.03 ± 1.46
	BC	19.95 ± 0.96	18.61 ± 1.06	19.28 ± 0.63	22.59 ± 1.32	21.57 ± 1.38	22.08 ± 0.90	17.41 ± 1.14	16.40 ± 1.02	16.98 ± 0.67
	BD	16.67 ± 1.65	18.56 ± 1.77	17.60 ± 1.06	17.23 ± 2.28	22.07 ± 2.37	19.65 ± 1.56	15.86 ± 1.95	15.55 ± 1.71	15.41 ± 1.13
	CD	18.59 ± 2.04	17.08 ± 2.19	17.91 ± 1.31	18.36 ± 2.82	18.67 ± 2.93	18.52 ± 1.93	18.80 ± 2.42	15.27 ± 2.12	17.21 ± 1.39
LEPH group[4]	*P*-value	0.111	0.033	0.733	0.386	0.013	0.235	0.098	0.593	0.130
	Low	19.08 ± 0.43	21.05 ± 0.46^{a}	20.05 ± 0.28	22.26 ± 0.60	23.84 ± 0.59^{a}	23.05 ± 0.40	16.09 ± 0.49	17.77 ± 0.43	16.95 ± 0.29
	High	19.87 ± 0.41	19.93 ± 0.43^{b}	19.94 ± 0.26	22.86 ± 0.56	22.15 ± 0.56^{b}	22.51 ± 0.37	17.00 ± 0.46	17.50 ± 0.40	17.44 ± 0.27
LEPH conc.	*P*-value	0.847	0.009	0.024	0.595	0.054	0.290	0.387	0.319	0.167
	r_s	-0.011	-0.152	-0.131	0.031	-0.113	-0.062	-0.050	-0.058	-0.081

[1]*LEP*: *LEP c.73C>T* genotype; *LEPD*: leptin diplotype; LEPH: leptin hormone; conc.: concentration

[2]Number of observations (CC: 69 – 71, CT: 143 – 147, TT: 82 – 84; AA: 73 – 75, BB: 30, CC: 04, AB: 85 – 87, AC: 41 – 44, AD: 08 – 09, AE: 08, AF: 04, BC: 20 – 21, BD: 06, CD: 04; High: 143 – 148; Low: 142 – 148; r_s: 295 – 296)

[3]AA: AATC/AATC, AB: AATC/GGCT, AC: AATC/GACT, AD: AATC/AGCT, AE: AATC/GATC, AF: AATC/AACT, BB: GGCT/GGCT, BC: GGCT/GACT, BD: GGCT/AGCT, CC: GACT/GACT, CD: GACT/AGCT

[4]The median LEPH concentrations used for LEPH grouping were 15.88 – 15.90 ng/mL

^{a,b}Least square means within a column without a common superscript letter differ ($P < 0.05$)

Table 3.6. Association of (LSMeans ± SE) of leptin based on classification effect, and Spearman correlation (r_s) of leptin with the number of follicles based on size[1] and overall (antral follicle count, AFC) for left and right ovaries in developing heifers

Effect[2]	Level[3]	AFC	Left ovary			Right ovary			Overall		
			Small	Medium	Large	Small	Medium	Large	Small	Medium	Large
LEP	*P*-value	0.055	0.027	0.389	0.514	0.214	0.778	0.357	0.040	0.655	0.989
	CC	21.89 ± 1.10	9.56 ± 0.61^{b}	1.28 ± 0.19	0.32 ± 0.06	8.84 ± 0.59	1.09 ± 0.15	0.46 ± 0.08	18.41 ± 1.04^{b}	2.39 ± 0.25	0.83 ± 0.09
	CT	24.98 ± 0.90	11.50 ± 0.53^{a}	1.16 ± 0.14	0.31 ± 0.05	10.03 ± 0.48	1.20 ± 0.13	0.44 ± 0.06	21.55 ± 0.88^{a}	2.40 ± 0.19	0.82 ± 0.07
	TT	24.02 ± 1.27	10.58 ± 0.71^{ab}	1.45 ± 0.23	0.39 ± 0.08	9.74 ± 0.68	1.17 ± 0.17	0.35 ± 0.07	20.33 ± 1.22^{ab}	2.64 ± 0.29	0.81 ± 0.10
LEPD[4]	*P*-value	0.196	0.133	0.667	0.716	0.220	0.791	0.183	0.243	0.622	0.987
	AA	25.14 ± 1.45	11.01 ± 0.80	1.64 ± 0.28	0.41 ± 0.10	10.07 ± 0.77	1.27 ± 0.21	0.38 ± 0.08	21.15 ± 1.38	2.94 ± 0.35	0.85 ± 0.12
	BB	24.56 ± 1.93	10.13 ± 1.01	1.47 ± 0.35	0.33 ± 0.10	10.52 ± 1.08	1.33 ± 0.28	0.46 ± 0.12	20.71 ± 1.85	2.81 ± 0.45	0.83 ± 0.15
	CC	15.68 ± 3.17	6.61 ± 1.78	0.42 ± 0.36	0.18 ± 0.17	6.46 ± 1.79	0.97 ± 0.56	0.83 ± 0.37	13.07 ± 3.00	1.36 ± 0.66	1.03 ± 0.38
	AB	26.45 ± 1.41	11.75 ± 0.78	1.40 ± 0.23	0.36 ± 0.08	10.89 ± 0.76	1.22 ± 0.18	0.44 ± 0.08	22.78 ± 1.37	2.67 ± 0.30	0.85 ± 0.11
	AC	22.62 ± 1.53	10.52 ± 0.89	0.88 ± 0.20	0.22 ± 0.07	8.96 ± 0.82	1.12 ± 0.21	0.48 ± 0.10	19.56 ± 1.50	2.04 ± 0.30	0.78 ± 0.12
	AD	26.02 ± 3.23	11.59 ± 1.82	1.48 ± 0.53	0.42 ± 0.19	10.11 ± 1.66	1.86 ± 0.56	0.29 ± 0.15	21.63 ± 3.05	3.30 ± 0.79	0.76 ± 0.23
	AE	24.35 ± 3.38	8.89 ± 1.61	1.24 ± 0.53	0.42 ± 0.19	12.30 ± 2.17	0.99 ± 0.41	0.23 ± 0.13	21.35 ± 3.35	2.23 ± 0.67	0.71 ± 0.21
	AF	31.42 ± 6.01	17.70 ± 4.12	0.61 ± 0.47	1.13 ± 0.74	10.84 ± 2.80	1.27 ± 0.71	0.00 ± 0.00	28.24 ± 6.07	1.87 ± 0.87	0.70 ± 0.36
	BC	20.84 ± 1.88	8.83 ± 1.03	1.22 ± 0.31	0.31 ± 0.10	8.83 ± 1.05	0.75 ± 0.21	0.47 ± 0.13	17.75 ± 1.82	2.03 ± 0.38	0.84 ± 0.16
	BD	21.05 ± 3.21	11.12 ± 2.07	1.64 ± 0.70	0.31 ± 0.21	5.95 ± 1.31	1.12 ± 0.45	0.58 ± 0.24	17.18 ± 2.98	2.75 ± 0.82	0.97 ± 0.31
	CD	21.77 ± 4.09	9.78 ± 2.33	1.16 ± 0.61	0.20 ± 0.19	8.73 ± 2.17	1.43 ± 0.62	0.21 ± 0.19	18.29 ± 3.91	2.59 ± 0.90	0.44 ± 0.26
LEPH group[5]	*P*-value	0.246	0.723	0.579	0.411	0.125	0.039	0.861	0.334	0.409	0.563
	Low	24.87 ± 1.02	10.93 ± 0.57	1.18 ± 0.15	0.37 ± 0.06	10.30 ± 0.54	1.31 ± 0.15^{a}	0.44 ± 0.07	21.24 ± 0.98	2.52 ± 0.21	0.87 ± 0.08
	High	23.57 ± 0.90	10.71 ± 0.52	1.27 ± 0.15	0.32 ± 0.05	9.39 ± 0.47	1.02 ± 0.12^{b}	0.45 ± 0.07	20.19 ± 0.87	2.33 ± 0.19	0.82 ± 0.07
LEPH conc.	*P*-value	0.020	0.096	0.193	0.997	0.051	0.004	0.231	0.065	0.006	0.514
	r_s	-0.135	-0.097	-0.076	0.000	-0.114	-0.170	0.070	-0.108	-0.160	0.038

[1]Small: 1 – 5 mm, medium: 6 – 10 mm, and large: 10 – 20 mm
[2]*LEP*: *LEP c.73C>T* genotype; *LEPD*: leptin diplotype; LEPH: leptin hormone; conc.: concentration
[3]Number of observations (CC: 71, CT: 147, TT: 84; AA: 75, BB: 30, CC: 04, AB: 86 – 87, AC: 44, AD: 09, AE: 08, AF: 04, BC: 21, BD: 06, CD: 04; High: 147 – 148; Low: 148; r_s: 295 – 296)
[4]AA: AATC/AATC, AB: AATC/GGCT, AC: AATC/GACT, AD: AATC/AGCT, AE: AATC/GATC, AF: AATC/AACT, BB: GGCT/GGCT, BC: GGCT/GACT, BD: GGCT/AGCT, CC: GACT/GACT, CD: GACT/AGCT
[5]The median LEPH concentrations used for LEPH grouping were 15.88 ng/mL
[a,b]Least square means within a column without a common superscript letter differ ($P < 0.05$)

Effect of *LEPD* on reproduction

Table 3.7 lists the genotype and minor allele frequencies, chromosomal position, and Hardy Weinberg Equilibrium *P*-value of 19 SNP markers used in the haplotype phasing. None of the SNP markers deviated from Hardy-Weinberg Equilibrium ($P > 0.05$). The minor allele frequencies of these 19 SNPs ranged from 0.075 to 0.492 (Table 3.7). The frequencies of haplotype derived using four SNP markers were presented in three categories (C1-3), where C1, C2, and C3 included 1) all 594 cows with or without records, 2) 479 cows (base herd, Cycle 1, and Cycle 2 cows with records), and 3) 303 cows (Cycle 1 and Cycle 2 cows with records), respectively (Table 3.8). The AATC haplotype occurred with the greatest frequency (≥ 50.00%; Table 3.8) in each category. However, five haplotypes (3 haplotypes for C3) occurred in less than 1% of the population. There were 25 possible *LEPD*s for C1 and C2 categories but 19 possible *LEPD*s for C3. The *LEPD*s with at least 1% frequency were considered for association analysis with studied reproductive traits.

The ANOVA tests were significant for left ovary diameter ($P = 0.048$), left ovary length ($P = 0.024$), and average ovary length ($P = 0.041$); however, *LEPD*s were not different in mean comparisons. Four different linear contrasts (CC vs. AA & AC, AA vs. CC & AC, CC vs. BB & BC, and BB vs. CC & BC) were performed for these significant traits. None of the linear contrasts were significant for these traits after correcting for experiment wise error rate. Also, none of the *LEPD*s were associated with other reproductive physiological characteristics, including calving interval, gestation length, age at first calving, and success traits ($P > 0.057$; Table 3.4, 3.5, and 3.6).

Table 3.7. Genotype and minor allele frequencies, and Hardy-Weinberg Equilibrium *P*-value of single nucleotide polymorphism (SNP) markers used in haplotype phasing

SNP markers	Region[1]	BTA[2]	Position (bp)	Genotype (Frequency)			MAF[3]	HWE[4]
Hapmap41207-BTA-22424	5'-up	4	92206455	T/T (0.008)	T/C (0.160)	C/C (0.831)	0.089	0.982
ARS-BFGL-NGS-30397	5'-up	4	92245734	T/T (0.071)	T/C (0.395)	C/C (0.534)	0.269	0.979
BovineHD0400025976	5'-up	4	92276448	A/A (0.007)	A/G (0.142)	G/G (0.852)	0.078	0.969
BovineHD0400025985	5'-up	4	92316493	A/A (0.597)	A/C (0.345)	C/C (0.058)	0.230	0.816
BovineHD0400025993	5'-up	4	92356977	A/A (0.778)	A/G (0.212)	G/G (0.010)	0.116	0.719
BovineHD0400025997	5'-up	4	92367496	T/T (0.309)	T/C (0.489)	C/C (0.202)	0.447	0.947
BovineHD0400026000	5'-up	4	92371648	T/T (0.005)	T/C (0.140)	C/C (0.855)	0.075	0.979
ARS-BFGL-NGS-101028	5'-up	4	92398977	A/A (0.427)	A/G (0.456)	G/G (0.117)	0.345	0.969
BovineHD0400026016	5'-up	4	92408134	T/T (0.108)	T/C (0.453)	C/C (0.439)	0.335	0.915
BovineHD0400026029	5'-up	4	92426572	A/A (0.307)	A/G (0.521)	G/G (0.172)	0.433	0.326
ARS-BFGL-NGS-59298	Intron 1	4	92444591	A/A (0.444)	A/G (0.444)	G/G (0.113)	0.335	0.994
LEPc.73C>T	Exon 2	4	92449024	T/T (0.298)	T/C (0.497)	C/C (0.205)	0.454	0.999
BovineHD0400026063	3'-down	4	92472469	T/T (0.197)	T/C (0.508)	C/C (0.295)	0.451	0.798
BovineHD0400026085	3'-down	4	92520445	A/A (0.247)	A/C (0.489)	C/C (0.264)	0.492	0.864
BovineHD0400026093	3'-down	4	92542300	T/T (0.002)	T/C (0.193)	C/C (0.805)	0.098	0.092
BovineHD0400026099	3'-down	4	92571770	A/A (0.091)	A/G (0.438)	G/G (0.470)	0.310	0.844
BovineHD0400026101	3'-down	4	92576547	T/T (0.349)	T/C (0.485)	C/C (0.166)	0.408	0.984
BovineHD0400026113	3'-down	4	92629043	A/A (0.157)	A/G (0.520)	G/G (0.323)	0.417	0.236
BTB-00203359	3'-down	4	92677005	T/T (0.037)	T/C (0.296)	C/C (0.667)	0.185	0.891

[1]5'-up: Upstream (5' end) of the leptin gene; 3'-down: Downstream (3' end) of the leptin gene
[2]BTA: *Bos taurus* autosome
[3]MAF: Minor allele frequency
[4]Hardy-Weinberg Equilibrium *P*-value calculated using a chi-square test

Table 3.8. Haplotype frequencies in the different set of populations

Haplotypes[1]	Code[2]	Frequency[3] (%)		
		C1	C2	C3
AATC	A	51.599	50.626	50.000
GGCT	B	29.377	30.063	29.043
GACT	C	10.859	11.273	13.531
AGCT	D	3.788	3.132	3.795
GATC	E	2.694	3.027	1.980
AACT	F	0.758	0.731	0.825
AACC	G	0.589	0.731	0.660
GGTT	H	0.168	0.209	0.165
AGTC	I	0.084	0.104	-
GGTC	J	0.084	0.104	-

[1]SNP order: BovineHD0400026029, ARS-BFGL-NGS-59298, *LEP c.73C>T*, and BovineHD0400026063
[2]Haplotype code used for the analyses
[3]C1 included base herd, Cycle 1, and Cycle 2 females with or without observations (n = 594); C2 included base herd, Cycle 1, and Cycle 2 females with observations (n = 479); and C3 included Cycle 1 and Cycle 2 females with observations (n = 303)

Effect of LEPH on reproduction

There were significant differences between heifers with low LEPH and those with high LEPH for right ovarian diameter ($P = 0.033$), right ovary length ($P = 0.013$), and right ovary medium follicles ($P = 0.039$). In all cases, the low LEPH group had greater reproductive measurements compared to the high LEPH group (Table 3.5 and 3.6). There was a tendency of greater left ovary height ($P = 0.098$) in heifers with low LEPH than that of the high LEPH group. No differences were observed between these two LEPH groups for other reproductive traits measured in this study ($P \geq 0.110$).

Concentrations of LEPH were negatively correlated with AFC, right ovary medium follicles, and overall medium follicles (-0.135 to -0.170, $0.004 \leq P \leq 0.020$; Table 3.6). There

was a tendency of negative correlation of circulatory LEPH concentration with left ovary small follicles, right ovary small follicles, and overall small follicles (0.097 to 0.114, $0.051 \leq P \leq 0.096$). There were also negative correlations between plasma LEPH concentrations and right ovary diameter, average ovary diameter, and UHD (-0.131 to -0.152, $0.010 \leq P \leq 0.024$; Table 3.4 and 3.5); however, a tendency of negative correlation was observed between circulatory LEPH concentrations and right ovary length (r_s = -0.113, P = 0.054).

Discussion

The reproductive performance of cows is highly associated with the number of follicles present in the ovary. Erickson et al. (1976) reported a higher number of primordial and growing follicles in fertile cows compared with infertile cows. Antral follicle count could be used as a good indicator of fertility because cows with a higher number of antral follicles are more fertile than those with a lower number of antral follicles (Cushman et al., 2009; Martinez et al., 2016). Cows with high AFC had a higher chance of being pregnant at first service and the end of the breeding season compared with low AFC cows in beef (Cushman et al., 2009) and dairy cattle (Mossa et al., 2012; Martinez et al., 2016). In an earlier study using crossbred (Hereford × Angus × Charolais) beef cattle, Ireland et al. (2008) further reported a greater ovary height and length in heifers with higher AFC compared to the low AFC heifers during follicular waves. This means greater ovary diameter might also be associated with more fertile cows. In this study, the T allele of *LEP* c.73C>T genotype displayed complete dominance over the C allele for increased average ovarian diameter, left ovary length, average ovary length, higher left ovary small follicles, and overall small follicles. Many previous studies have been performed using the same *LEP* marker, where researchers reported the association of T allele with greater dry matter intake, increased weight gain, greater backfat thickness and rump fat thickness, greater carcass fat yield, lower

yield grade, and lower ultrasound rib eye area in beef cattle (Buchanan et al., 2002; Nkrumah et al., 2004; Schenkel et al., 2005; Buchanan et al., 2007; de Carvalho et al., 2012; da Silva et al., 2012; Woronuk et al., 2012; Kononoff et al., 2013; Kononoff et al., 2014). Therefore, from our current study, heifers with the T allele would also be ideal for selecting animals with increased fertility along with improved production traits. The allelic substitution from C (wild type) to T (mutant type) in the *LEP c.73C>T* of leptin exon 2 results in changing amino acid from arginine to cysteine, which alters the biological function of the leptin hormone. It is hypothesized that an unpaired cysteine's presence on the A helix of the leptin molecule changes the leptin's tertiary conformation that potentially affects binding to the leptin receptor and alters the signaling pathway for the leptin hormone action (Buchanan et al., 2002). This extra unpaired cysteine could also destabilize the disulfide bridge (i.e., found critical for the biological function of leptin) between two existing cysteines on the leptin molecule, which can cause a functional change of leptin hormone (Buchanan et al., 2002). Therefore, animals with the T allele will have free circulatory leptin (i.e., unbound to leptin receptors), which would be cleared sooner from the circulation because of their significantly shorter half-life in comparison to bound leptin (Chebel et al., 2008). This also explains why different levels of circulating leptin in plasma was not seen by genotype in this study, as the genotype has no impact on presence or absence of the hormone, only its binding affinity. Association of Holstein-Friesian cows homozygous for the C allele of *LEP c.73C>T* genotype with greater serum leptin concentration has been previously reported by Liefers et al. (2003). Leptin secretion is pulsatile in nature, and Wylie et al. (2001) reported 8 to 10 pulses per 10 h in heifers. Foote et al. (2016) suggested multiple plasma leptin measures for better estimates rather than using a single time point. It can be hypothesized that the low plasma leptin in heifers with the T allele leads to improved reproductive performance, i.e., greater antral

follicles and greater ovarian diameter in different ways. The low level of leptin acts centrally through the hypothalamic-pituitary-gonadal axis and elicits the GnRH secretion, which stimulates the synthesis and release of gonadotropins (i.e., follicle-stimulating hormone [FSH] and luteinizing hormone [LH]) from the pituitary (Watanobe, 2002). These gonadotropins subsequently promote oocyte development and follicular maturation. Therefore, the influence of leptin on reproduction may be permissive, i.e., a threshold level of leptin is required to positively impact reproductive performance (Huszenicza et al., 2001). Additionally, animals with low plasma leptin would intake more dry matter, which would be more competent in utilizing extra energy after maintenance for reproduction.

Our study did not find any *LEP c.73C>T* genotype associations with gestation length, calving interval, and age at first calving. Age at first calving used in our study might be influenced by the bull turnout system, which imposed an artificial limit on older heifers in a herd vs. younger heifers in a herd. Young heifers in a herd have ample opportunity to show a reduced age at first calving vs. the older ones who are artificially limited by their age at the bull turnout. However, Giblin et al. (2010) reported associations of the T allele of *LEP c.73C>T* marker with shorter gestation length and easier calving, but no association with calving interval in Holstein-Friesian sires, estimated from the performance of daughter-parity records. Trakovická et al. (2013) studied different leptin genotypes (*LEPn.Sau3AI* and *LEPR c.115C>T*) and found their association with age at first calving and calving interval, respectively, in Slovak spotted and Pinzgauer cows. Effects of *LEPSauAI* RFLP and IDVGA51 STR markers on calving interval and weight at first calving in composite (Aberdeen Angus × Nelore) beef cattle were also described by Almeida et al. (2003). The polymorphisms in the *LEPR* gene have also been linked to the reproduction traits such as litter sizes of sows in Duroc and Yorkshire (Chen et al., 2004).

Although the ANOVA test was significant for left ovarian diameter, left and average ovarian length, the post-hoc tests among *LEPDs* using the Tukey-Kramer method were not significant. These results might be due to a lack of statistical power for small sample sizes per *LEPD* group or a greater number of factor levels (i.e., *LEPDs*). Although not statistically different, heifers with the CC diplotype (GACT/GACT) numerically had a low number of antral follicles and lesser ovary measurements for most reproductive physiological traits. The small samples and large standard error with CC (GACT/ GACT) diplotype might lead to that non-significant result. Even so, the numerical values were biologically relevant. This C haplotype contains the *LEP* marker's C allele, associated with a low number of antral follicles and lesser ovary measurements. None of the earlier studies have been performed on the association between leptin haplotypes and reproductive physiological and success traits in cattle. However, in a study with *LEP g.-2470C>T, LEP g.-1457A>G, LEP g.-1238G>C, LEP g.-963C>T, Y7F, LEP c.73C>T*, and *A80V* haplotypes, Giblin et al. (2010) did not find any association of haplotypes with fertility, i.e., calving interval in Holstein-Friesian sires, estimated from the performance of daughter-parity records.

Right ovarian diameter and length were greater in heifers with low LEPH concentration. Right ovary medium follicles were also higher in the same LEPH group heifers. These results indicate high leptin concentration might have an inhibitory effect on the ovarian size and follicular development. Also, there was a low negative correlation between circulating levels of LEPH and the majority of the reproductive traits; however, some were not significant. It can be hypothesized that an elevated level of plasma leptin directly exerts its inhibitory effect on the ovary by blocking the stimulatory effect of FSH on preantral follicular growth (Kikuchi et al., 2001). Leptin can also suppress cAMP-induced folliculogenesis of preantral follicles by blocking

events downstream to the cAMP-dependent signal transduction pathway (Kikuchi et al., 2001). Also, high leptin level (i.e., above the physiological range) showed an inhibitory effect on the synergistic action of insulin-like growth factor-1 on FSH-induced estradiol synthesis (Zachow and Magoffin, 1997). Increasing serum LEPH concentration during FSH stimulation also leads to an inadequate ovarian response in terms of the number of follicles and retrieved oocytes in women (Bützow et al., 1999). The high concentration of leptin in the ovary also prevents estrogen production and thereby interferes with the development of dominant follicles and oocyte maturation (Mantzoros, 2000). A negative effect of leptin was found on ovarian steroidogenesis in an *in vitro* study conducted on thecal and granulosa cells of rodent and bovine models (Spicer and Francisco, 1997; Spicer et al., 2000). Increasing concentrations of leptin also had an inhibitory effect on mouse follicular growth over 9 days of culture in a dose-dependent manner (Swain et al., 2004). Strauch et al. (2003) observed a negative relationship between serum LEPH and the postpartum interval in multiparous Brahman cows. Also, a negative correlation between LEPH levels and endometrial thickness in humans was reported by Chakrabarti et al. (2012). These outcomes might support the negative correlation of LEPH with most of the reproductive traits found in this study. Passive immunization (i.e., treatment with anti-leptin antibody) of cattle against leptin could be a good strategy to improve their reproductive performance. In an earlier study with mice, Panwar et al. (2012) observed greater ovarian weight and a greater number of primary Graafian follicles per ovarian section in prepubertal mice treated with anti-leptin antibodies compared to control animals. They also observed a greater number of Graafian follicles in mice treated with anti-leptin and gonadotropins compared with ovaries in the control and gonadotropin alone group. They suggested the reduced level of peripheral leptin after passive immunization against leptin

enhances the transition of primordial follicles to primary follicles, and treatment of animals with anti-leptin antibody and exogenous gonadotropin leads to Graafian follicle development in the ovary. Our study had an average plasma leptin concentration of 17.86 ng/mL (SD = 8.59 ng/mL; ranging from 1.93 to 45.76 ng/mL), which was smaller than the average concentration of leptin (overall: 22.85 ng/mL) in beef cattle previously reported by Geary et al. (2003), where average values were 18.71 ± 7.40 ng/mL, and 27.03 ± 8.24 ng/mL for composite gene combination steers, and lean beef steers and heifers, respectively. However, their study average was similar to the average leptin concentration observed in our study's high LEPH group. The average concentrations of low and high plasma leptin group in our study were higher than those previously reported in beef steers by Nkrumah et al. (2007). Additionally, our study's average was higher than that observed in Angus steers for low-RFI and high-RFI groups (Richardson et al., 2004). However, LEPH concentrations observed in pregnant (3.4 ± 0.1 ng/mL) and non-pregnant (2.4 ± 0.1 ng/mL) crossbred (British × Brahman) heifers following fixed-time artificial insemination (Gentry et al., 2013) were much lower than our present study. Brannian and Hansen (2002) reported that since circulating LEPH levels are directly related to body adiposity, high LEPH concentrations associated with obesity might negatively impact obesity on fertility. However, the high leptin concentration found in our study might be influenced by some other factors, as our developing beef heifers were not technically obese. Crossbred beef heifers showed a higher concentration of leptin than cows that might be due to sample collection time or their growth and development stage (Garcia et al., 2002). The mean leptin concentration usually remains higher during the luteal phase than the late luteal or follicular phase (Garcia et al., 2002). Several studies reported a higher concentration of leptin in females than their male counterparts (Hellström et al., 2000; Buff et al., 2002; Berg et al., 2003). This sexual dimorphism in

circulatory leptin concentration is attributed to the stimulating effect of estrogen or progesterone and suppressing effect of androgen on leptin production or divergent levels of fat distribution in males and females (Mantzoros, 2000). Ruminant leptin synthesis is also increased by feeding level and long daylength (Chilliard et al., 2001).

Conclusions

Heifers differed significantly between the *LEP c.73C>T* genotypes for small follicles and some of the ovary measurements. Heifers with the T allele showed improved reproductive performance for these characteristics compared to heifers carrying both C alleles. Literature indicates the increased productivity of the T allele for growth and carcass attributes, so it was expected that the same increased productivity would be found for reproductive traits. Therefore, it would be okay for a producer perspective to select animals for the T allele of *LEP c.73C>T* polymorphism on growth as it will not impact reproductive performance. The negative correlation of circulating LEPH with reproductive indicates that an elevated concentration of LEPH might have a negative impact on reproductive traits. Therefore, increased follicles as well as uterine and ovary sizes found in this study, paired with low levels of LEPH, indicate this expectation is valid. Even so, not all comparisons could be statistically proven; therefore, additional investigations may be warranted.

CHAPTER 4. THE ROLE OF LEPTIN IN FEED INTAKE, EFFICIENCY, GROWTH, AND CARCASS PERFORMANCE OF COMMERCIAL DEVELOPING BEEF HEIFERS

Abstract

This study was aimed to investigate the effect of leptin genotype (*LEP c.73C>T*), leptin diplotype (*LEPD*), and plasma leptin hormone (LEPH) concentration (high vs. low) on feed intake, efficiency, growth and ultrasound carcass traits in commercial developing beef heifers. Females (n = 335) were genotyped for the *LEP c.73C>T* marker using KASP by Design assay with a real-time PCR system. The haplotype phasing was performed with markers within a 0.25 Mbp flanking on *LEP* (n = 19); however, only four SNP markers within or immediately surrounding *LEP* were used for the *LEPD* association study. Circulating levels of LEPH were measured using an RIA kit in 333 beef heifers prior to their first breeding season. Traits analyzed were feed intake and efficiency, feeding behavior, growth, direct linear and calculated body measurements and their growth, and ultrasound carcass traits. Data analyses were performed using the SAS MIXED procedure; however, a repeated measures model was used for dry matter intake (DMI), gain:feed, and feeding behavior traits. Fixed effects included in the final model were ancestral breed group (n = 5), age of dam as a fixed class effect (n = 4) or covariate, frame size group (n = 4), birth year (n = 2; used only for ultrasound carcass traits), cycle nested within the year (n = 5 and 6 for body measurement and other studied traits, respectively), and either *LEP c.73C>T* (n = 3) or *LEPD* (n = 8, and 11 for ultrasound carcass traits, and all other studied traits, respectively) or LEPH (n = 2). Heifers with the TT genotype were heavier at weaning (P = 0.012) and had greater initial middle girth (iMG; P = 0.048) than CC heifers. Final body weight (BW) was also greater in TT heifers than heterozygous CT heifers (P = 0.037). However, heifers with the CC genotype had greater gain:feed than the heifers with CT genotype (P = 0.038). The

LEPD showed associations for weight traits and body size traits. Both A and B haplotypes were associated with greater WW, initial and final BW, initial middle girth, initial heart girth, and initial end girth. However, the presence of the C haplotype was associated with reduced performance for those traits. Heifers with CC diplotype were associated with greater gain:feed in the current study. The low LEPH group's heifers consumed fewer meals per day and had greater DMI per meal than the high LEPH ($P < 0.05$). Heifers with high plasma LEPH concentration had greater initial REA ($P = 0.030$) than the low LEPH group for ultrasound carcass traits. Circulatory plasma LEPH was positively correlated with 12 out of total 50 studied traits (r_s = 0.108 to 0.182, $0.004 \leq P \leq 0.050$) except final density ($r_s = -0.193$; $P = 0.003$). The T allele of *LEP c.73C>T* marker could be used as a valuable marker for selecting for improved growth and body size in commercial beef heifers. Also, circulatory LEPH before the breeding season may serve as a predictor for feeding behavior and body size traits. Long-term implications continue to be explored.

Introduction

The cost of feeding accounts for approximately 55 to 75% of the total costs associated with commercial beef production (Basarab et al., 2002). Thus, reducing feed costs can significantly benefit beef cattle producers by providing more profits. Gibb and McAllister (1999) reported a 5% decrease in feed efficiency could have about four times the economic impact as a 5% increase in average daily gain (ADG). In a feedlot study using steers, Fox et al. (2001) also demonstrated a 10% improvement in ADG due to a 7% increase in feed intake (FI), improved profitability by 18%. In contrast, a 10% improvement in feed efficiency by improving metabolizable energy efficiency with constant FI had 43% more profits. Therefore, improvement in beef cattle feed efficiency can be an excellent strategy to reduce production input costs and

enhance profitability. Technological advances in electronic feed monitoring systems provide an excellent opportunity to measure individual animal feed consumption and feeding behavior patterns. Feeding behavior data are now being incorporated into the genetic evaluation program by some breed associations. However, cost and data reporting are major limiting factors in implementing this type of program. The carcass traits are economically impactful in beef cattle production systems because they determine value (de Carvalho et al., 2012) due to their association with carcass yield, prime cuts proportion (Magnabosco et al., 2006), carcass quality (da Silva et al., 2012), juiciness, flavor, and tenderness of the meat (Crouse et al., 1989). Selecting cattle for the desirable carcass traits would return more profits to beef producers.

Previous studies have been established that FI, feed conversion ratio (FCR), and residual feed intake (RFI) are moderately heritable traits in beef cattle (Arthur et al., 2001; Schenkel et al., 2004 among others). However, Freetly et al. (2020) observed high heritability for FI in growing beef heifers (0.84 ± 0.12). Feeding behavior traits were also reported moderately heritable in composite beef steers (Nkrumah et al., 2007a) and moderate to highly heritable in adapted or temperate heifers and steers (Robinson and Oddy, 2004). Moreover, FI, feed efficiency, and feeding behavior traits were highly repeatable between the growing and finishing stages in Limousin × Friesian heifers (Kelly et al., 2010b). An earlier study reported moderate heritabilities for various carcass traits, such as backfat thickness (BFT), longissimus muscle area (LM area), and marbling score (MS; Utrera and Van Vleck, 2004). Moreover, it has also been established that growth and body size traits are moderate to highly heritable. Therefore, it would be more efficient to make rapid genetic improvement in the above-discussed traits through genetic marker-assisted selection.

In recent years, the leptin (*LEP*) gene and its hormone product have been widely studied to investigate their suitability using as a genetic marker or biological marker of FI, feed efficiency, feeding behavior, growth, and carcass traits in cattle. The majority of previous association studies using *LEP* polymorphisms and LEPH have used steers that are fed a high-concentrate finishing diet, and some in heifers. A polymorphism located at cytosine to thymine 305 bp from the start of exon 2 in the *LEP* was found to be associated with FI or DMI (Kononoff et al., 2013; Kononoff et al., 2014; McEvers et al., 2014), gain to feed ratio (G:F; McEvers et al., 2014), body weight (BW; Woronuk et al., 2012; Buchanan et al., 2007), weaning weight (WW) of calves (Devuyst et al., 2008), initial ADG (Larson et al., 2005), fatter carcasses (Buchanan et al., 2002), ultrasound or carcass BFT (da Silva et al., 2012; Buchanan et al., 2007; Kononoff et al., 2014, 2013 among others), LM area (da Silva et al., 2012; Buchanan et al., 2007; Larson et al., 2005), yield grades (YG; Buchanan et al., 2007; Kononoff et al., 2005 among others), rump fat thickness (RFAT; da Silva et al., 2012), and MS (Kononoff et al., 2014; McEvers et al., 2014). Some haplotype association studies, including *LEP c.73C>T* and other markers in the *LEP,* have found associations with FI, inter-muscular fat levels, BFT, and lean meat yield (Lagonigro et al., 2003; Schenkel et al., 2005; Banos et al., 2008) with *LEP*. Furthermore, polymorphisms on the *LEP* and their haplotypes also showed association with growth and body size traits (Nkrumah et al., 2006; Kulig and Kmieć, 2009). Plasma leptin hormone (LEPH; a protein product of the *LEP* gene) concentrations collected at day 42 and day 83 of the feeding trial were positively associated with ADG (Foote et al., 2016); however, a negative association of LEPH concentration with ADG was also reported by Foote et al. (2015). Moreover, LEPH concentration showed an association with FI, feed efficiency, and carcass traits in crossbred beef steers (Nkrumah et al., 2007b; Foote et al., 2015). Therefore, these above discussed previous

studies have established that selection using the *LEP c.73C>T* marker, *LEP* diplotypes (*LEPD,* i.e., a specific combination of two *LEP* haplotypes), and/or LEPH concentration could be useful in cattle.

To our knowledge, no previous studies have examined the association of *LEP c.73C>T* genotype or *LEPD* or LEPH concentration with feeding behavior traits in commercial developing beef heifers. Although many studies were performed on *LEP* genotype and LEPH association with FI, feed efficiency, growth, and carcass traits in concentrate-based fed crossbred cattle, the research in grass-fed *Bos taurus* crossbred developing heifers remains limited. Thus, it is necessary to investigate this marker's effect on these traits in developing heifers fed a forage-based diet to explore any dietary influence as compared to previous studies. No previous studies have been conducted on the association of *LEP* haplotypes, including *LEP c.73C>T* with growth or body size traits in developing beef heifers. Thus, this study aimed to investigate the association of the *LEP c.73C>T* genotype, *LEPD,* and circulating LEPH concentrations with FI, feed efficiency, feeding behavior, growth, body measurement, and ultrasound carcass attributes in developing beef heifers.

Materials and methods

Animals and phenotypic data

All procedures were reviewed and approved by the Institutional Animal Care and Use Committee of North Dakota State University. Data were generated in developing beef heifers, including Cycle 1 and Cycle 2 females, that were part of a long-term study focused on longevity traits. Detailed information about the Cycle 1 and Cycle 2 females has been described previously in Chapters 2 and 3. These heifer calves were born and raised at the Dickinson Research Extension Center, Dickinson, ND. Before the first breeding season, animals were brought to

NDSU Beef Cattle Research Complex for a 105-d feed trial. All females were grouped into four different frame size groups, which have been detailed in Chapter 3.

Growth attributes were available from 2014 to 2017 born heifers (n = 374 or 336 for WW, ADG, and BW). Per year, data was available on 99, 81, 100, and 94 heifers, respectively. Heifer's BW was taken on d 0/1, 14/15, 28/29, 42, 56, and 105 across years. Linear body measurement traits were taken at the start and end of the feeding trial. Body measurement data from 2015 to 2017 born heifers (n = 247) were only included in the analysis as there were errors with measurement data collected in 2014.

Linear body measurement traits recorded directly from the live animal

a. Body length (BL, cm): Measured as the horizontal distance from the shoulder's highest point to the pin bones' end using a measuring tape.
b. Hip height (HH, cm): Measured as the vertical distance from the ground level to the hip bone with the animal standing on a level surface using an adjustable ruler.
c. Hip width (HW, cm): Measured at the widest point of the hip, the distance between the tuber coxae' lateral surfaces using a measuring tape.
d. Heart girth (HG, cm): Measured from a point slightly behind the shoulder blade, down the fore-ribs, and under the body behind the elbow all the way around using a measuring tape.
e. Middle girth (MG, cm): Measured from the middle point between the front leg and back leg using a measuring tape.
f. Flank girth (FG, cm): Measured on the rear flank region immediately in front of the back leg using a measuring tape.

Calculated measurements from direct linear measurements

a. Initial end girth (iENDG, cm): Calculated from the average of initial heart girth and initial flank girth.

b. Final end girth (fENDG, cm): Calculated from the average of final heart girth and final flank girth.

c. Initial radius at the end (iENDr, cm): Calculated using the formula,

$$\text{iENDr} = \text{iENDG}/2\pi$$

d. Final radius at the end (fENDr, cm): Calculated using the formula,

$$\text{fENDr} = \text{fENDG}/2\pi$$

e. Initial radius in the middle (iMIDr, cm): Calculated using the formula,

$$\text{iMIDr} = \text{iMG}/2\pi$$

f. Final radius in the middle (fMIDr, cm): Calculated using the formula,

$$\text{fMIDr} = \text{fMG}/2\pi$$

g. Initial volume (iVOL, L): Calculated using the formula,

$$\text{iVOL} = ((\pi \times \text{Initial BL} \times (\text{iENDr}^2 + (2 \times \text{iMIDr}^2)))/3)/1000$$

h. Final volume (fVOL, L): Calculated using the formula,

$$\text{fVOL} = ((\pi \times \text{Final BL} \times (\text{fENDr}^2 + (2 \times \text{fMIDr}^2)))/3)/1000$$

i. Initial density (iDENS, kg/L): Calculated using the formula,

$$\text{iDENS} = \text{Initial body weight}/\text{iVOL}$$

j. Final density (fDENS, kg/L): Calculated using the formula,

$$\text{fDENS} = \text{Final body weight}/\text{fVOL}$$

Body measurement growth traits

a. Daily hip height gain (HHdg, cm/d): Calculated from the difference of initial and final hip height measurements divided by the number of days on feed trial.
b. Daily hip-width gain (HWdg, cm/d): Calculated from the difference of initial and final hip-width measurements divided by the number of days on feed trial.
c. Daily heart girth gain (HGdg, cm/d): Calculated from the difference of initial and final heart girth measurements divided by the number of days on feed trial.
d. Daily middle girth gain (MGdg, cm/d): Calculated from the difference of initial and final middle girth measurements divided by the number of days on feed trial.
e. Daily flank girth gain (FGdg, cm/d): Calculated from the difference of initial and final flank girth measurements divided by the number of days on feed trial.
f. Daily end girth gain (ENDGdg, cm/d): Calculated from the difference of initial and final end girth divided by the number of days on feed trial.
g. Daily volume gain (VOLdg, L/d): Calculated from the difference of initial and final volume divided by the number of days on feed trial.
h. Density gain (DENSg, kg/L): Calculated from the ratio of ADG and daily volume gain.

Daily FI and feeding behavior data were recorded 24 h a day over the 105-d feed trial using the Insentec automated feeding system (Hokofarm group B.V., Marknesse, Netherlands) from a total of 335 heifers. Feed traits analyzed include DMI, gain to feed ratio (G:F), and RFI. Each heifer's daily DMI was calculated by multiplying the total daily FI with the dry matter percentage of the supplied feed for the 105-d feed trial period. For RFI, the expected DMI was obtained from the linear regression of DMI on mid-test $BW^{0.75}$ and ADG. The mid-test $BW^{0.75}$

was calculated by multiplying the mid-test BW measured at d 53 of the feed trial with 0.75, and ADG was obtained as the slope from the regression of BW on day of feeding trial. The REG procedure in SAS was used to calculate the equation intercept (β_0) and coefficients of the equation (β_1 and β_2 are coefficients of mid-test $BW^{0.75}$ and ADG, respectively), where DMI was used as a dependent variable and mid-test $BW^{0.75}$ and ADG as independent variables. Finally, RFI was calculated from the difference between actual DMI and expected DMI for each animal (Koch et al., 1963). The G:F was computed as the ratio of ADG and DMI. Weekly measurements of DMI and G:F were used for the association analyses, whereas the average value was considered for RFI. Feeding behavior characteristics were studied as described by Sitorski et al. (2019) and Montanholi et al. (2010), including number of daily visits to the feed bunk (VF; events), feed intake per meal (FIM; g DM), number of meals per day (NMD; events), feed intake per visit (FIV; g DM), time eating at feed bunk per day (TFD; min), time eating at feed bunk per meal (TFM; min), and time eating at feed bunk per visit (TFV; min). A visit was defined as each time the Insentec system detected a heifer at the feed bunk. A meal was defined as distinct eating periods that might include short breaks separated by intervals of no longer than 7 min (Forbes, 1986; Montanholi et al., 2010; Sitorski et al., 2019). Weekly measurements available for each feeding behavior traits were used in association analyses.

Ultrasound measurements of intramuscular fat percentage (IMF), rib eye area (REA), rib fat thickness (RF), RFAT, and YG were recorded by a single skilled person with an Aloka 500V unit (Corometrics Medical Systems, Wallingford, CT) equipped with a 3.5-MHz, 17-cm linear array transducer following procedures detailed by Wall et al. (2004). These measurements were taken at the start and end of the feed trial. A total of 161 – 164 ultrasound carcass measurements were available from 2014 and 2015 born heifers for association analyses.

Diet

Heifers were fed *ad libitum* intake of a forage-based diet comprised of grass hay, corn silage, dry rolled corn, fined ground corn, and dried distiller's grains with solubles. Urea, salt, monensin (176.4 g/kg premix, Elanco, Greenfield, IN), vitamin premix, and a trace mineral premix were supplemented. The ingredients and nutrient composition of diets supplied to growing heifers are presented in Table 4.1 and Table 4.2, respectively. A similar mixed ration diet was provided to heifers from the period of 2014 to 2017. The CP percentage was slightly lower in 2015 because of the low hay quality. However, it did not have any effect on heifer's growth. Corn silage was not available for a portion of the experiment in 2014, so the corn silage was replaced with dry rolled corn and grass hay to simulate the composition of corn silage. Therefore, diet 1 was supplied to heifers up to the first 55 days of the feed trial and rest period with diet 2. (Table 4.1).

Table 4.1. Ingredient of diets used in growing heifers

Item (% DM)	2014		2015 – 2017
	Diet 1	Diet 2	Diet 1
Ingredient			
Grass hay	78.50	88.40	78.50
Corn silage	16.50	0.00	16.50
Corn, dry rolled	0.00	6.60	0.00
Corn, fine ground	1.92	1.92	1.92
DDGS[1]	1.93	1.93	1.93
Supplement[2]	2.00	2.00	2.00

[1]Dried distiller's grains with solubles.
[2]Supplement contained urea, salt, monensin (176.4 g/kg premix, Elanco, Greenfield, IN), vitamin premix, and a trace mineral premix.

Table 4.2. Nutrient composition of diet used in growing heifers

Item[1] (% DM)	2014	2015	2016	2017	Overall
OM	90.0	88.5	89.0	90.1	89.4
CP	11.8	7.7	12.4	12.0	11.0
NDF	63.6	64.3	62.2	62.9	63.3
ADF	36.7	38.8	35.7	36.9	37.0
Ca	0.43	0.40	0.59	0.62	0.51
P	0.31	0.28	0.21	0.16	0.24

[1]Chemical composition included organic matter (OM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), calcium (Ca), phosphorus (P).

DNA, *LEP* genotyping, and haplotype phasing

The procedure of blood collection, DNA extraction and quantification, and DNA sample storage were previously described in Chapter 2. Animal genotyping for *LEP c.73C>T* was performed using KASP by Design assay (LGC Genomics, Beverly, MA) with an Applied Biosystems 7500 Fast Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA). A forward primer (5’ GGAAGGAAAATGCGCTGTGG 3’) and a reverse prime (5’ AGCTGTCTTTATGCCAGGGG 3’) having 284 fragment length was designed to flank the thymine to cytosine single nucleotide polymorphism in exon 2 of the *LEP*. Other markers used in the haplotype analysis were available from previously genotyping animals for GeneSeek Genomic Profiler 150K panel for beef cattle. The KASP assay method has already been discussed in Chapter 3. Markers processing, quality control, and haplotype phasing information have been provided in Chapter 2 and Chapter 3.

LEPH concentration

The procedure and time of blood collection, processing, blood plasma storage, and leptin hormone assay have been explained in Chapter 3. All heifers in the study were grouped into high

and low LEPH concentration groups based on the median LEPH concentration of 15.30, 16.75, 15.78, and 15.86 ng/mL for body measurement, ultrasound carcass attributes, RFI, and all other studied traits, respectively (i.e., high: heifers with LEPH ≤ median and low: heifers with LEPH > median). The sensitivity of the assay and inter-assay and intra-assay CVs have been described in Chapter 3. In LEPH grouping, only females with both LEPH and data records determined the median value, where other females were dropped from the association analysis for a given trait.

Statistical analysis

Based on the population structure analysis outputs from Chapter 2, all heifers were classified into six major ancestral breed groups (ANAR: Angus and Red Angus; AD: American Aberdeen; SH: Shorthorn; GV: Gelbvieh; SM: Simmental; and MIX = Heifers with breed fraction less than the defined threshold value, i.e., ≤ 0.55). Heifers with the "GV" ancestral breed group were removed from the analysis as this group had limited observations (n = 3, 1, 2, and 3 for growth, body measurement, RFI, and all other feed and behavior traits, respectively), and excluding the GV group improved the final model. No GV-influenced heifers were available for ultrasound carcass characteristics. Data were checked for normality and outliers using residual diagnostics. Any values that greatly influenced outcomes on their own (i.e., outliers) were removed from the dataset for a given trait. Dam age of heifers was used to create four groups of W (2 to 3 years), X (4 to 5 years), Y (6 to 7 years), and Z (≥ 8 years) to test as a fixed class effect in the model. The maximum likelihood (ML) estimation method was used for model testing of the fixed effects using goodness-of-fit statistics (AIC, AICC, or BIC) as the ML estimates are unbiased for the fixed effects (Korner-Nievergelt et al., 2015).

All statistical analyses were performed with the MIXED procedure of SAS v.9.4 (SAS Inst., Cary, NC). However, the repeated measures model with the MIXED procedure of SAS

v.9.4 was used for feed intake, ADG, G:F, and feeding behavior data as these data were available daily over 15 consecutive weeks. Covariance structures were evaluated to determine the best fit when using repeated measures. Fixed effects considered were ancestral breed group (n = 5), age of dam as a fixed class effect (n = 4) or covariate, frame size group (n = 4), birth year (n = 2; used only for ultrasound carcass traits), cycle nested within the year (n = 5 and 6 for body measurement and other studied traits, respectively), week of the feed trial × birth year (n = 60; used for repeated measures model), LEPH × week of the feed trial (n = 30; used for repeated measures model with LEPH main effect), and either *LEP c.73C>T* (n = 3) or *LEPD* (n = 8, and 11 for ultrasound carcass traits, and all other studied traits, respectively) or LEPH (n = 2). Least squares means were generated for significant effects and adjusted to control for experiment-wise error using the Tukey-Kramer method. Additional linear contrasts for *LEP c.73C>T* or *LEPD* based on suggested allele or haplotype dominance were run only if pairwise comparisons could not elucidate an allele or haplotype effect. These contrast tests were performed in those cases where the original ANOVA effect was significant. The individual comparison-wise error rate was determined using the formula: $\alpha_I = 1 - (1 - \alpha_E)^{1/m}$, where α_E was set as 0.05, and m is the number of linear contrasts to be conducted. The *P*-value from the linear contrasts was only considered significant when it was below α_I to control for experiment-wise error. The PROC CORR of SAS was used to obtain Spearman correlations of LEPH with raw phenotype records of traits used in this study. The 205 day WW was adjusted for the age of dam using BIF guidelines (Beef Improvement Federation, 2018) when used for correlation.

Results

The frequency of the T allele (0.50 – 0.53) of the *LEP c.73C>T* SNP was higher than that of the C allele (0.50 – 0.47) in the population when considering the studied traits (Table 4.3).

Table 4.3. Allele and genotype frequencies of *LEP c.73C>T*, and diplotype frequency in the populations of beef heifers studied

Parameters		Frequency[1]					
		Body size traits (247)	WW (374)	Other growth traits[2] (336)	DMI, G:F, RFI, and feeding behavior traits (335)	IRFAT (161)	Other US carcass traits (162)
Allele	T	0.53	0.52	0.53	0.51	0.50	0.51
	C	0.47	0.48	0.47	0.49	0.50	0.49
Genotype	CC	0.22	0.23	0.23	0.26	0.25	0.26
	CT	0.50	0.49	0.49	0.50	0.50	0.49
	TT	0.28	0.28	0.28	0.24	0.25	0.25
Diplotype[3]	AB	26.72	28.07	27.98	28.06	26.09	25.93
	AA	26.32	24.87	25.60	25.67	19.25	19.75
	AC	15.79	14.44	14.58	14.63	17.39	17.28
	BB	8.1	9.63	9.82	9.55	11.80	11.73
	BC	6.88	6.68	6.55	6.57	8.07	8.02
	AD	4.86	4.01	3.87	3.88	3.11	3.09
	BD	2.43	2.41	2.38	2.39	1.86	1.85
	AE	1.62	2.41	2.38	2.39	4.35	4.32
	AF	1.62	1.07	1.19	1.19	1.24	1.23
	CD	1.62	1.6	1.19	1.19	0.62	0.62
	CC	1.21	1.34	1.19	1.19	1.24	1.23
	BE	0.4	0.8	0.3	0.30	-	-
	BG	0.4	0.27	0.3	0.30	-	-
	CE	0.4	0.53	0.6	0.60	1.24	1.23
	CG	0.4	0.8	0.89	0.90	1.24	1.23
	DD	0.4	0.27	0.3	0.30	0.62	0.62
	DE	0.4	0.27	0.3	0.30	0.62	0.62
	DF	0.4	0.27	0.3	0.30	0.62	0.62
	AH	-	0.27	0.3	0.30	0.62	0.62

[1]Number in parenthesis is the number of observations used.
[2]Growth traits included average daily gain and body weight measured on feed trial.
[3]SNP order: BovineHD0400026029, ARS-BFGL-NGS-59298, *LEP c.73C>T*, and BovineHD0400026063; AA: AATC/AATC; AB: AATC/GGCT; AC: AATC/GACT; AD: AATC/AGCT; AE: AATC/GATC; AF: AATC/AACT; AH: AATC/GGTT; BB: GGCT/GGCT; BC: GGCT/GACT; BD: GGCT/AGCT; BE: GGCT/GATC; BG: GGCT/AACC; CC: GACT/GACT; CD: GACT/AGCT; CE: GACT/GATC; CG: GACT/AACC; DD: AGCT/AGCT; DE: AGCT/GATC; and DF: AGCT/AACT.

This is due to a reduced population compared to Chapter 3. The distribution of the three *LEP* genotypes (CC, CT, and TT) frequency was in Hardy-Weinberg equilibrium. For all studied traits, the proportion of heterozygote heifers was almost equal to the total proportion of both homozygote heifers (heterozygotes: 0.49 – 0.50 vs. homozygotes: 0.51 – 0.50).

A total of 17, 18, and 19 different *LEPD*s were observed in the population of ultrasound carcass, body measurement, and all other studied traits, respectively (Table 4.3). Of them, three *LEPD*s, including AB (AATC/GGCT), AA (AATC/AATC), and AC (AATC/GACT) occurred with greater frequency (i.e., 59.62 to 71.78 % together) in all populations, followed by *LEPD*s BB (GGCT/GGCT), BC (GGCT/GACT), AD (AATC/AGCT), and so on (Table 4.3).
The *LEPD*s with at least one frequency (for ultrasound carcass traits with a small sample set, this frequency threshold was 1.85 for having at least 3 animals in the analysis for a given *LEPD*) were considered for association study, leading to 8, and 11 *LEPD*s for ultrasound carcass traits, and other studied traits, respectively.

Growth and feed efficiency

Fixed effects included in the final model were ancestral breed group, frame size group, age of dam as fixed covariate (used as a fixed class effect for initial BW with LEPH main effect, and WW), cycle nested within the year, week of the feed trial × birth year (used for ADG and G:F), and either *LEP c.73C>T* or *LEPD* or LEPH. Autoregressive (order 1) and Toeplitz covariance structures were used in the final repeated measures model of ADG and G:F, respectively. Kenward-Roger degrees of freedom method was also included in both cases. The interaction effect of LEPH and week of the feed trial was significant for ADG, but the interaction of birth year and week of the feed trail displayed significance in all cases. Due to the week effect and number of levels, both interactions are just artifacts of the data structure rather than being

real or useful interactions. Thus, they were not included in the final model. Our study demonstrated the *LEP* genotype's impact on the growth traits in growing beef heifers (Table 4.4). Heifers with the TT genotype were heavier at weaning (*P* = 0.012) and tended to have greater final BW (*P* = 0.057) than CC heifers. The TT heifers also had a greater final BW (*P* = 0.037) and tended to have a greater WW (P = 0.093) than heterozygous CT heifers. However, no differences were observed between CT and CC heifers for these two traits. Linear contrasts of CC vs. CT and TT, and TT vs. CT and CC were found significant (*P* = 0.017 and 0.005) for WW. Also, linear contrasts of TT vs. CC and CT for final BW did show significance (*P* = 0.008), but CC vs. TT and CT were not significant (*P* = 0.133) The *LEP c.73C>T* genotype significantly impacted G:F, where heifers homozygous for the C allele had greater G:F than that of heifers with CT genotype (*P* = 0.025). However, no differences were observed between CC and TT or CT and TT genotypes (*P* ≥ 0.174). Linear contrast CC vs. TT and CT was significant for G:F (*P* = 0.015). Heifers with different *LEP c.73C>T* genotypes did not differ in RFI and other studied growth traits, such as average daily gain, initial BW on feed trial, and BW gain after feed trial (*P* ≥ 0.169; Table 4.4).

The *LEPD*s showed a significant effect on WW, initial and final BWs measured on feed trial, and gain to feed ratio (Table 4.4). Heifers with BC diplotype were lighter at weaning compared to the heifers with AA, BB, AB, and AE diplotypes (*P* = 0.001, *P* = 0.002, *P* = 0.014, and *P* = 0.047, respectively). Numerical differences were observed between CC, and AA and BB diplotypes. Similarly, heifers with AA and BB diplotypes were heavier at the start of the feed trial than the BC diplotype (*P* = 0.036, *P* = 0.0497). No significant differences were observed between the other studied *LEPDs* for WW and initial BW (*P* > 0.076). The *LEPD* showed a significant effect on final BW; however, none of the *LEPD*s were different in mean comparisons.

Four different linear contrasts (CC vs. AA & AC, AA vs. CC & AC, CC vs. BB & BC, BB vs. CC & BC) were performed for these significant traits. Linear contrasts of AA vs. CC & AC, and BB vs. CC and BC diplotypes were significant for WW and initial BW ($0.0007 \leq P \leq 0.010$). Linear contrast of AA vs. CC and AC was significant for final BW ($P = 0.007$). The effect of *LEPD*s was also significant for G:F. Heifers with the CD diplotype exhibited greater G:F compared to the heifers of AA ($P = 0.003$), AB ($P = 0.001$), AC ($P = 0.004$), AE ($P = 0.014$), BB ($P = 0.004$), and BD ($P = 0.005$) diplotypes. The effect of C and D haplotypes on G:F could not investigate since there were no animals with DD diplotype in the current study. The BC heifers also had a greater G:F than that of AB heifers ($P = 0.008$) and tended to have greater G:F than the AA heifers ($P = 0.069$). None of those four linear contrasts were significant for G:F ($P \geq 0.125$). The *LEPD* did not show any associations with ADG, BW gain during feed trial, and RFI (Table 4.4).

Our study did not find any effect of LEPH grouping on growth and feed efficiency traits (Table 4.4). However, low LEPH heifers tended to gain greater BW throughout the feed trial period and G:F than high LEPH heifers ($P = 0.065$, 0.078). Adjusted WW ($r_s = 0.161$, $P = 0.003$) had positive correlation with plasma LEPH concentration. There was a tendency of positive correlation of plasma LEPH concentration with initial BW ($r_s = 0.094$, $P = 0.087$) of the feeding trial. The phenotypic correlations of plasma LEPH concentration with other growth traits and feed efficiency traits were not different from zero ($P > 0.10$).

Table 4.4. Association (LSMeans ± SE) of leptin based on classification effect, and Spearman correlation (r_s) of leptin with growth and feed efficiency traits in developing heifers

Effect[1]	Level[2]	Average daily gain, kg/day	WW[3], lb	BW on feed trial, kg		BW gain, kg	Gain to feed ratio	Residual feed intake
				Initial	Final			
LEP	*P*-value	0.902	0.015	0.135	0.028	0.378	0.032	0.883
	CC	0.611 ± 0.038	520.4 ± 6.0^{b}	354.2 ± 3.7	413.4 ± 4.1^{ab}	59.10 ± 2.46	0.078 ± 0.002^{a}	0.088 ± 0.074
	CT	0.597 ± 0.027	528.9 ± 4.4^{ab}	355.3 ± 2.6	414.4 ± 2.9^{b}	59.22 ± 1.76	0.072 ± 0.001^{b}	0.078 ± 0.052
	TT	0.614 ± 0.039	542.9 ± 6.3^{a}	362.7 ± 3.9	425.7 ± 4.2^{a}	62.82 ± 2.57	0.073 ± 0.002^{ab}	0.044 ± 0.077
LEPD[4]	*P*-value	0.998	0.002	0.005	0.037	0.520	0.0001	0.681
	AA	0.613 ± 0.043	545.5 ± 6.6^{a}	367.2 ± 4.1^{a}	429.1 ± 4.6	61.69 ± 2.78	0.071 ± 0.002^{bc}	0.025 ± 0.082
	BB	0.600 ± 0.062	549.6 ± 9.2^{a}	370.1 ± 5.8^{a}	426.0 ± 6.5	55.69 ± 3.95	0.070 ± 0.003^{bc}	0.131 ± 0.118
	CC	0.606 ± 0.155	498.1 ± 22.5^{ab}	329.2 ± 14.8^{ab}	395.8 ± 16.5	66.57 ± 10.04	0.074 ± 0.008^{abc}	-0.085 ± 0.296
	AB	0.589 ± 0.039	534.9 ± 5.8^{a}	362.4 ± 3.7^{ab}	421.1 ± 4.1	58.52 ± 2.52	0.068 ± 0.002^{b}	0.093 ± 0.074
	AC	0.596 ± 0.052	518.6 ± 8.2^{ab}	348.2 ± 5.0^{ab}	407.2 ± 5.6	58.99 ± 3.39	0.072 ± 0.003^{bc}	0.069 ± 0.100
	AD	0.643 ± 0.089	511.6 ± 13.6^{ab}	339.2 ± 8.4^{ab}	401.5 ± 9.8	65.05 ± 5.95	0.083 ± 0.005^{abc}	-0.034 ± 0.169
	AE	0.613 ± 0.115	555.8 ± 17.2^{a}	354.1 ± 10.9^{ab}	422.9 ± 12.2	68.59 ± 7.42	0.070 ± 0.006^{bc}	0.310 ± 0.219
	AF	0.609 ± 0.165	506.6 ± 25.9^{ab}	336.0 ± 15.5^{ab}	391.3 ± 17.2	55.41 ± 10.49	0.084 ± 0.009^{abc}	-0.322 ± 0.309
	BC	0.656 ± 0.072	489.8 ± 10.9^{b}	340.6 ± 6.8^{b}	404.9 ± 7.6	63.85 ± 4.65	0.085 ± 0.004^{ac}	-0.075 ± 0.137
	BD	0.520 ± 0.111	517.4 ± 16.7^{ab}	355.3 ± 10.6^{ab}	402.9 ± 11.8	47.44 ± 7.17	0.067 ± 0.006^{bc}	0.386 ± 0.211
	CD	0.587 ± 0.158	505.0 ± 20.6^{ab}	350.6 ± 15.1^{ab}	410.5 ± 16.8	59.96 ± 10.21	0.111 ± 0.009^{a}	0.054 ± 0.301
LEPH group[5]	*P*-value	0.407	0.630	0.835	0.351	0.065	0.078	0.420
	Low	0.619 ± 0.029	529.0 ± 5.0	358.8 ± 3.0	418.3 ± 3.2	62.14 ± 1.89	0.075 ± 0.002	0.101 ± 0.056
	High	0.590 ± 0.028	531.7 ± 4.7	359.5 ± 2.8	414.8 ± 3.0	57.95 ± 1.80	0.072 ± 0.002	0.047 ± 0.053
LEPH conc.	*P*-value	0.493	0.003	0.087	0.106	0.471	0.721	0.392
	r_s	0.038	0.161	0.094^{t}	0.089	0.040	-0.020	0.047

[1]*LEP*: *LEP c.73C>T* genotype; *LEPD*: leptin diplotype; LEPH: leptin hormone; conc.: concentration
[2]Number of observations (CC: 76 – 87, CT: 163 – 184, TT: 95 – 103; AA: 86 – 93, BB: 32 – 36, CC: 04 – 05, AB: 94 – 105, AC: 49 – 54, AD: 13 – 15, AE: 08 – 09, AF: 04, BC: 22 – 25, BD: 08 – 09, CD: 04 – 06; High: 165 – 166; Low: 165 – 166; r_s: 259 – 332)
[3]Adjusted WW was used to estimate Spearman correlation
[4]SNP order: BovineHD0400026029, ARS-BFGL-NGS-59298, *LEP c.73C>T*, and BovineHD0400026063; AA: AATC/AATC, AB: AATC/GGCT, AC: AATC/GACT, AD: AATC/AGCT, AE: AATC/GATC, AF: AATC/AACT, BB: GGCT/GGCT, BC: GGCT/GACT, BD: GGCT/AGCT, CC: GACT/GACT, CD: GACT/AGCT
[5]The median LEPH concentrations used for LEPH grouping were 15.78 – 15.86 ng/mL
[a,b]Least square means within a column without a common superscript letter differ ($P < 0.05$)

Direct linear and calculated body measurements and their growth

In the final model, fixed effects included were ancestral breed group, frame size group, age of dam as covariate (used as a class effect for initial body length), cycle nested within the year, and either *LEP c.73C>T* or *LEPD* or LEPH. The *LEP c.73C>*T genotypes displayed associations with some of the direct linear body measurement traits considered in the study (Table 4.5, 4.6). The ANOVA effect for *LEP c.73C>T* genotype on initial body length showed a tendency, but the numerical difference was present between CT and CC genotypes in mean comparison. Similarly, numerical differences existed between heifers with TT and CC genotypes for initial hip height and between TT and CT genotypes for initial heart girth; however, their ANOVA effect for *LEP c.73C>T* genotype showed a tendency. Moreover, heifers homozygous for the T allele had greater initial middle girth ($P = 0.048$) than CT heterozygous heifers. The contrast test was performed only for the significant trait, i.e., initial middle girth. Linear contrast of TT vs. CC and CT for this trait was significant ($P = 0.018$); however, CC vs. TT and CT was not significant ($P = 0.252$). Our study did not find any associations between *LEP c.73C>T* genotypes and other direct linear body measurements ($P > 0.183$), calculated body measurements ($P > 0.205$), and body measurement growth traits ($P > 0.056$; Table 4.5, 4.6, and 4.7).

The *LEPD* displayed a significant effect on initial middle girth, initial heart girth, initial end girth, and heart girth daily gain (Table 4.5, 4.6, and 4.7). Heifers with AA and BB diplotypes had greater initial heart girth than heifers with CC ($P = 0.006$ and $P = 0.003$, respectively) and AD diplotypes ($P = 0.016$ and $P = 0.012$, respectively), and tended to have greater initial heart girth than that of BC diplotype ($P = 0.092$ and $P = 0.051$, respectively). The AB diplotype had greater heart girth at the start of the feed trial than heifers homozygous for the haplotype C (P = 0.018) but showed a tendency to have greater initial heart girth than the AD diplotype ($P =$

0.093). A tendency was also observed between BB and AC diplotypes for the same body measurement ($P = 0.098$). In the case of calculated body measurement traits (Table 4.6), the BB diplotype had greater initial end girth compared to the BC and AD diplotypes ($P = 0.040$ and $P = 0.013$, respectively); however, it tended to be greater than CC diplotype for the same trait ($P = 0.057$). The diplotypes AA and AD showed a tendency for initial end girth ($P = 0.090$). Although the ANOVA effect of *LEPD* was significant for initial middle girth and heart girth daily gain, none of the *LEPD*s were different in mean comparisons. Four different linear contrasts (CC vs. AA & AC, AA vs. CC & AC, CC vs. BB & BC, and BB vs. CC & BC) were performed for the significant traits. All linear contrast tests were significant for initial heart girth ($<0.0001 \leq P \leq 0.001$). Linear contrasts of CC vs. AA & AC, and AA vs. CC & AC were also significant for initial middle girth and heart girth daily gain ($0.001 \leq P \leq 0.008$). Furthermore, the linear contrast of BB vs. CC & BC was observed as significant for initial middle girth and initial end girth ($P = 0.001, 0.0001$). However, linear contrasts of AA vs. CC & AC, and CC vs. BB & BC were significant for initial end girth, and heart girth daily gain, respectively ($P = 0.003, 0.001$). The *LEPD*s did not affect other studied direct linear body measurements, calculated body measurement traits, and body measurement growth (Table 4.5, 4.6, and 4.7).

Table 4.5. Association (LSMeans ± SE) of leptin based on classification effect, and Spearman correlation (r_s) of leptin with body length, hip height, hip width, and middle girth in developing heifers

		Body length, cm		Hip height, cm		Hip width, cm		Middle girth, cm	
Effect[1]	Level[2]	Initial	Final	Initial	Final	Initial	Final	Initial	Final
LEP	*P*-value	0.064	0.185	0.068	0.457	0.775	0.507	0.050	0.402
	CC	110.5 ± 0.9	121.6 ± 0.9	117.9 ± 0.6	125.0 ± 0.6	43.91 ± 0.42	46.17 ± 0.48	196.5 ± 1.2[ab]	212.3 ± 1.5
	CT	112.7 ± 0.7	120.4 ± 0.7	118.5 ± 0.4	125.3 ± 0.4	43.98 ± 0.31	46.53 ± 0.36	196.4 ± 0.9[b]	211.9 ± 1.1
	TT	112.1 ± 0.9	121.8 ± 0.9	119.5 ± 0.6	125.8 ± 0.6	44.24 ± 0.41	46.84 ± 0.47	199.3 ± 1.2[a]	213.9 ± 1.5
LEPD[3]	*P*-value	0.504	0.089	0.166	0.321	0.869	0.153	0.021	0.236
	AA	112.1 ± 1.0	121.4 ± 0.9	119.6 ± 0.6	126.1 ± 0.6	44.18 ± 0.44	46.76 ± 0.48	199.2 ± 1.2	213.7 ± 1.5
	BB	111.4 ± 1.5	123.6 ± 1.3	118.9 ± 0.9	126.1 ± 0.9	44.74 ± 0.66	47.29 ± 0.73	199.7 ± 1.8	216.8 ± 2.3
	CC	107.8 ± 3.3	120.6 ± 3.1	116.5 ± 2.0	124.8 ± 2.2	41.68 ± 1.54	42.70 ± 1.70	185.9 ± 4.3	205.3 ± 5.4
	AB	112.4 ± 0.9	119.6 ± 0.8	119.3 ± 0.5	126.0 ± 0.6	43.91 ± 0.40	46.58 ± 0.44	196.8 ± 1.1	212.4 ± 1.4
	AC	114.0 ± 1.3	121.9 ± 1.1	117.1 ± 0.7	123.6 ± 0.8	43.68 ± 0.56	46.56 ± 0.61	195.5 ± 1.5	210.2 ± 2.0
	AD	110.7 ± 1.9	118.6 ± 1.7	117.8 ± 1.1	125.1 ± 1.2	44.19 ± 0.81	45.63 ± 0.93	193.2 ± 2.2	208.8 ± 2.9
	AE	113.3 ± 2.9	124.9 ± 2.8	120.3 ± 1.8	125.8 ± 1.9	44.40 ± 1.37	47.34 ± 1.51	196.2 ± 3.8	217.0 ± 4.8
	AF	112.7 ± 3.3	123.2 ± 3.0	118.5 ± 1.9	126.3 ± 2.1	43.35 ± 1.49	44.47 ± 1.64	188.8 ± 4.1	204.3 ± 5.2
	BC	111.0 ± 1.6	120.4 ± 1.5	117.1 ± 0.9	124.2 ± 1.1	43.48 ± 0.73	44.92 ± 0.80	193.8 ± 2.0	208.6 ± 2.6
	BD	109.2 ± 2.4	121.2 ± 2.3	117.6 ± 1.5	123.6 ± 1.6	43.23 ± 1.12	46.29 ± 1.23	196.5 ± 3.1	209.1 ± 3.9
	CD	111.5 ± 2.9	117.6 ± 2.8	116.6 ± 1.8	123.1 ± 1.9	43.05 ± 1.36	44.37 ± 1.50	195.6 ± 3.8	212.8 ± 4.8
LEPH group[4]	*P*-value	0.174	0.834	0.502	0.753	0.706	0.555	0.885	0.508
	Low	111.6 ± 0.7	120.9 ± 0.7	118.7 ± 0.4	125.4 ± 0.5	43.9 ± 0.3	46.4 ± 0.4	197.0 ± 0.9	212.0 ± 1.2
	High	112.6 ± 0.7	121.1 ± 0.7	118.4 ± 0.4	125.3 ± 0.5	44.1 ± 0.3	46.6 ± 0.4	197.2 ± 0.9	212.9 ± 1.1
LEPH conc.	*P*-value	0.053	0.291	0.892	0.942	0.071	0.136	0.038	0.016
	r_s	0.124	0.068	-0.009	-0.005	0.116	0.096	0.133	0.155

[1]*LEP*: *LEP c.73C>T* genotype; *LEPD*: leptin diplotype; LEPH: leptin hormone; conc.: concentration

[2]Number of observations (CC: 53 – 54, CT: 122 – 124, TT: 67 – 69; AA: 63 – 65, BB: 20, CC: 03, AB: 65 – 66, AC: 39, AD: 11 – 12, AE: 04, AF: 04, BC: 16 – 17, BD: 06, CD: 04; High: 121 – 122; Low: 119 – 122; r_s: 243 – 244)

[3]SNP order: BovineHD0400026029, ARS-BFGL-NGS-59298, *LEP c.73C>T*, and BovineHD0400026063; AA: AATC/AATC, AB: AATC/GGCT, AC: AATC/GACT, AD: AATC/AGCT, AE: AATC/GATC, AF: AATC/AACT, BB: GGCT/GGCT, BC: GGCT/GACT, BD: GGCT/AGCT, CC: GACT/GACT, CD: GACT/AGCT

[4]The median LEPH concentrations used for LEPH grouping were 15.30 ng/mL

[a,b]Least square means within a column without a common superscript letter differ ($P < 0.05$)

Table 4.6. Association (LSMeans ± SE) of leptin based on classification effect, and Spearman correlation (r_s) of leptin with heart girth, flank girth, end girth, volume, and density in developing heifers

		Heart girth, cm		Flank girth, cm		End girth, cm		Volume, L		Density, kg/L	
Effect[1]	Level[2]	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
LEP	*P*-value	0.081	0.183	0.738	0.653	0.326	0.425	0.205	0.237	0.728	0.389
	CC	167.4 ± 0.8	177.0 ± 1.1	175.9 ± 1.2	201.3 ± 1.4	171.6 ± 0.9	189.3 ± 1.1	318.2 ± 4.8	413.6 ± 7.0	1.156 ± 0.014	1.032 ± 0.011
	CT	167.4 ± 0.6	178.2 ± 0.8	175.4 ± 0.9	200.7 ± 1.0	171.4 ± 0.7	189.5 ± 0.8	322.6 ± 3.6	407.3 ± 5.2	1.145 ± 0.011	1.045 ± 0.008
	TT	169.2 ± 0.8	179.3 ± 1.0	176.3 ± 1.2	202.0 ± 1.4	172.8 ± 0.9	190.8 ± 1.1	328.5 ± 4.8	418.8 ± 6.9	1.152 ± 0.014	1.047 ± 0.011
LEPD[3]	*P*-value	0.0001	0.255	0.210	0.235	0.006	0.191	0.140	0.148	0.506	0.259
	AA	169.4 ± 0.8^{a}	179.4 ± 1.1	176.7 ± 1.2	201.4 ± 1.4	173.1 ± 0.9^{abc}	190.6 ± 1.2	328.4 ± 5.0	416.4 ± 7.2	1.155 ± 0.015	1.053 ± 0.011
	BB	170.7 ± 1.2^{a}	178.5 ± 1.7	180.1 ± 1.8	202.9 ± 2.2	175.4 ± 1.4^{a}	191.4 ± 1.7	330.5 ± 7.5	435.0 ± 10.8	1.158 ± 0.022	1.014 ± 0.016
	CC	158.1 ± 2.9^{b}	177.3 ± 3.8	170.7 ± 4.3	201.2 ± 5.0	164.4 ± 3.2^{abc}	189.0 ± 4.0	282.3 ± 17.4	392.6 ± 25.0	1.208 ± 0.052	1.034 ± 0.038
	AB	168.5 ± 0.7^{ac}	178.3 ± 1.0	176.2 ± 1.1	200.3 ± 1.3	172.4 ± 0.8^{abc}	189.3 ± 1.0	323.3 ± 4.5	405.0 ± 6.5	1.160 ± 0.013	1.059 ± 0.010
	AC	165.9 ± 1.0^{abc}	176.9 ± 1.4	175.0 ± 1.5	202.5 ± 1.8	170.4 ± 1.1^{abc}	189.7 ± 1.5	325.0 ± 6.3	410.7 ± 9.0	1.110 ± 0.019	1.024 ± 0.014
	AD	163.7 ± 1.5^{bc}	177.9 ± 2.1	171.8 ± 2.2	195.0 ± 2.7	167.8 ± 1.7^{bc}	186.4 ± 2.2	306.3 ± 9.2	387.9 ± 13.6	1.151 ± 0.027	1.055 ± 0.021
	AE	168.4 ± 2.5^{abc}	177.0 ± 3.4	175.9 ± 3.8	204.3 ± 4.4	172.2 ± 2.8^{abc}	190.6 ± 3.6	323.3 ± 15.5	433.5 ± 22.2	1.169 ± 0.046	1.015 ± 0.034
	AF	166.3 ± 2.8^{abc}	172.3 ± 3.7	174.4 ± 4.1	194.1 ± 4.8	170.4 ± 3.1^{abc}	182.7 ± 3.9	307.4 ± 16.9	386.4 ± 24.2	1.138 ± 0.050	1.057 ± 0.037
	BC	164.9 ± 1.4^{abc}	173.7 ± 1.8	172.8 ± 2.0	198.1 ± 2.4	168.9 ± 1.5^{bc}	185.4 ± 2.0	312.6 ± 8.2	396.5 ± 12.1	1.116 ± 0.024	1.047 ± 0.019
	BD	164.8 ± 2.1^{abc}	174.4 ± 2.8	174.6 ± 3.1	200.3 ± 3.6	169.7 ± 2.3^{abc}	187.3 ± 2.9	313.6 ± 12.7	397.7 ± 18.1	1.178 ± 0.037	1.034 ± 0.028
	CD	168.0 ± 2.5^{abc}	180.6 ± 3.4	173.7 ± 3.8	205.2 ± 4.4	170.8 ± 2.8^{abc}	192.8 ± 3.6	317.5 ± 15.4	408.8 ± 22.1	1.156 ± 0.046	1.030 ± 0.034
LEPH group[4]	*P*-value	0.586	0.691	0.793	0.834	0.934	0.973	0.402	0.639	0.425	0.125
	Low	168.0 ± 0.7	178.4 ± 0.8	175.5 ± 0.9	201.2 ± 1.1	171.8 ± 0.7	189.7 ± 0.9	321.4 ± 3.8	410.1 ± 5.4	1.154 ± 0.011	1.050 ± 0.008
	High	167.7 ± 0.6	178.0 ± 0.8	175.8 ± 0.9	201.0 ± 1.1	171.7 ± 0.7	189.8 ± 0.9	324.7 ± 3.7	412.7 ± 5.3	1.145 ± 0.011	1.036 ± 0.008
LEPH conc.	*P*-value	0.017	0.024	0.012	0.004	0.011	0.006	0.012	0.009	0.090	0.003
	r_s	0.152	0.144	0.160	0.182	0.163	0.178	0.160	0.167	-0.109	-0.193

[1]*LEP*: *LEP c.73C>T* genotype; *LEPD*: leptin diplotype; LEPH: leptin hormone; conc.: concentration

[2]Number of observations (CC: 52 – 54, CT: 122 – 124, TT: 66 – 69; AA: 62 – 65, BB: 19 – 20, CC: 03, AB: 66, AC: 38 – 39, AD: 11 – 12, AE: 04, AF: 04, BC: 16 – 17, BD: 06, CD: 04; High: 119 – 122; Low: 119 – 122; r_s: 242 – 244)

[3]SNP order: BovineHD0400026029, ARS-BFGL-NGS-59298, *LEP c.73C>T*, and BovineHD0400026063; AA: AATC/AATC, AB: AATC/GGCT, AC: AATC/GACT, AD: AATC/AGCT, AE: AATC/GATC, AF: AATC/AACT, BB: GGCT/GGCT, BC: GGCT/GACT, BD: GGCT/AGCT, CC: GACT/GACT, CD: GACT/AGCT

[4]The median LEPH concentrations used for LEPH grouping were 15.3 ng/mL

[a,b]Least square means within a column without a common superscript letter differ ($P < 0.05$)

Table 4.7. Association (LSMeans ± SE) of leptin based on classification effect, and Spearman correlation (r_s) of leptin with body measurement growth traits[1] in developing heifers

Effect[2]	Level[3]	HHdg, cm/d	HWdg, cm/d	HGdg, cm/d	MGdg, cm/d	FGdg, cm/d	ENDGdg, cm/d	VOLdg, L/d	DENSg, kg/L
LEP	*P*-value	0.803	0.916	0.339	0.753	0.438	0.862	0.237	0.056
	CC	0.063 ± 0.005	0.024 ± 0.005	0.085 ± 0.009	0.147 ± 0.013	0.243 ± 0.012	0.165 ± 0.009	0.889 ± 0.057	1.113 ± 0.147
	CT	0.060 ± 0.004	0.024 ± 0.004	0.097 ± 0.006	0.144 ± 0.009	0.233 ± 0.009	0.169 ± 0.006	0.794 ± 0.042	1.307 ± 0.115
	TT	0.059 ± 0.005	0.022 ± 0.005	0.089 ± 0.008	0.136 ± 0.012	0.247 ± 0.012	0.169 ± 0.009	0.839 ± 0.056	1.631 ± 0.181
LEPD[4]	*P*-value	0.944	0.922	0.016	0.881	0.499	0.143	0.581	0.098
	AA	0.060 ± 0.005	0.021 ± 0.005	0.087 ± 0.009	0.135 ± 0.013	0.240 ± 0.012	0.163 ± 0.009	0.817 ± 0.059	1.601 ± 0.190
	BB	0.065 ± 0.008	0.023 ± 0.007	0.071 ± 0.013	0.159 ± 0.020	0.240 ± 0.018	0.151 ± 0.013	0.976 ± 0.089	1.028 ± 0.228
	CC	0.078 ± 0.018	0.011 ± 0.017	0.174 ± 0.030	0.182 ± 0.046	0.280 ± 0.041	0.230 ± 0.031	1.028 ± 0.206	0.837 ± 0.453
	AB	0.060 ± 0.005	0.024 ± 0.004	0.088 ± 0.008	0.145 ± 0.012	0.225 ± 0.011	0.159 ± 0.008	0.761 ± 0.054	1.233 ± 0.145
	AC	0.057 ± 0.006	0.029 ± 0.006	0.096 ± 0.011	0.137 ± 0.017	0.241 ± 0.015	0.180 ± 0.011	0.799 ± 0.074	1.702 ± 0.274
	AD	0.068 ± 0.010	0.013 ± 0.009	0.129 ± 0.016	0.151 ± 0.025	0.221 ± 0.023	0.176 ± 0.017	0.804 ± 0.112	0.905 ± 0.274
	AE	0.050 ± 0.016	0.027 ± 0.015	0.074 ± 0.027	0.194 ± 0.041	0.262 ± 0.037	0.173 ± 0.028	1.029 ± 0.182	1.051 ± 0.515
	AF	0.073 ± 0.017	0.013 ± 0.017	0.047 ± 0.029	0.145 ± 0.045	0.171 ± 0.040	0.113 ± 0.030	0.736 ± 0.199	1.266 ± 0.702
	BC	0.056 ± 0.008	0.023 ± 0.008	0.073 ± 0.014	0.139 ± 0.022	0.223 ± 0.020	0.152 ± 0.015	0.774 ± 0.100	1.707 ± 0.403
	BD	0.056 ± 0.013	0.029 ± 0.013	0.082 ± 0.022	0.117 ± 0.034	0.234 ± 0.030	0.164 ± 0.023	0.783 ± 0.149	1.322 ± 0.508
	CD	0.067 ± 0.016	0.013 ± 0.015	0.110 ± 0.027	0.160 ± 0.041	0.292 ± 0.036	0.204 ± 0.027	0.852 ± 0.182	0.591 ± 0.267
LEPH group[5]	*P*-value	0.778	0.408	0.641	0.549	0.923	0.658	0.882	0.068
	Low	0.060 ± 0.004	0.022 ± 0.004	0.094 ± 0.007	0.141 ± 0.010	0.238 ± 0.009	0.170 ± 0.007	0.830 ± 0.044	1.203 ± 0.114
	High	0.061 ± 0.004	0.025 ± 0.004	0.090 ± 0.007	0.147 ± 0.010	0.239 ± 0.009	0.167 ± 0.007	0.823 ± 0.043	1.463 ± 0.125
LEPH conc.	*P*-value	0.906	0.766	0.894	0.567	0.461	0.554	0.367	0.062
	r_s	0.008	-0.019	0.009	0.037	0.048	0.038	0.058	-0.120

[1]Daily hip height gain (HHdg), daily hip width gain (HWdg), daily heart girth gain (HGdg), daily middle girth gain (MGdg), daily flank girth gain (FGdg), daily end girth gain (ENDGdg), daily volume gain (VOLdg), and density gain (DENSdg)

[2]*LEP*: *LEP c.73C>T* genotype; *LEPD*: leptin diplotype; LEPH: leptin hormone; conc.: concentration

[3]Number of observations (CC: 52 – 54, CT: 121 – 123, TT: 65 – 69; AA: 61 – 65, BB: 19 – 20, CC: 03, AB: 64 – 66, AC: 37 – 39, AD: 11, AE: 04, AF: 04, BC: 16 – 17, BD: 06, CD: 04; High: 119 – 122; Low: 117 – 122; r_s: 242 – 244)

[4]SNP order: BovineHD0400026029, ARS-BFGL-NGS-59298, *LEP c.73C>T*, and BovineHD0400026063; AA: AATC/AATC, AB: AATC/GGCT, AC: AATC/GACT, AD: AATC/AGCT, AE: AATC/GATC, AF: AATC/AACT, BB: GGCT/GGCT, BC: GGCT/GACT, BD: GGCT/AGCT, CC: GACT/GACT, CD: GACT/AGCT

[5]The median LEPH concentrations used for LEPH grouping were 15.3 ng/mL

Our study did not find any effect of LEPH grouping on direct linear body measurements, calculated body measurements, and body measurement growth traits (Table 4.5, 4.6, and 4.7). However, there was a tendency to have a greater density in high LEPH heifers compared to low LEPH. The majority of the direct linear body measurement traits had positive correlations with plasma LEPH concentration, including initial heart girth, final heart girth, initial middle girth, final middle girth, initial flank girth, and final flank girth (r_s = 0.133 to 0.182, 0.004 ≤ P ≤ 0.038). Initial body length and initial hip width tended to be positively correlated with circulatory LEPH concentration (r_s = 0.124, 0.116; P = 0.053, 0.071). Moreover, all calculated body measurements, such as initial end girth, final end girth, initial volume, and final volume, were positively correlated with circulatory LEPH concentration (r_s = 0.160 to 0.178, 0.006 ≤ P ≤ 0.012). However, plasma LEPH concentration was negatively correlated with final density (r_s = -0.193; P = 0.003), and tended to be negatively correlated with initial density (r_s = -0.109, P = 0.090). None of the body measurement growth traits were correlated with circulatory LEPH concentration (P ≥ 0.367); however, density gain tended to be negatively correlated with LEPH concentration (r_s = -0.120, P = 0.062).

Feed intake, feeding behavior, and ultrasound carcass traits

The final mixed model of these traits included fixed effects of ancestral breed group, frame size group, age of dam as covariate (used as a class effect for initial and final REA), birth year (used for ultrasound carcass traits), cycle nested within the year (used for feed intake and behavior traits), and either *LEP c.73C>T* or *LEPD* or LEPH. Toeplitz covariance structures and Kenward-Roger degrees of freedom method were used in the final repeated measures model of feed intake and feeding behavior traits. The interaction effect of LEPH and week of the feed trial was significant for time eating at feed bunk per visit; however, the other interaction (birth year ×

week of the feed trail) was found significant in all cases used. Due to the week effect and number of levels, both interactions are just artifacts of the data structure rather than being accurate or useful interactions. Therefore, they were dropped from the final model. The *LEP c.73C>T* genotype did not show any effect on DMI and feeding behavior traits ($P \geq 0.132$; Table 4.8). Similarly, no association was found between *LEP c.73C>T* genotype and ultrasound carcass traits ($P \geq 0.069$; Table 4.9).

No effect of the *LEPD*s was observed for DMI, feeding behavior, and ultrasound carcass traits ($P \geq 0.211$; Table 4.8, and 4.9).

Heifers with low LEPH ate fewer meals per day ($P = 0.027$) but had 5.60% more DMI ($P = 0.049$) per meal compared to those with high plasma LEPH (Table 4.8). No differences were observed between heifers with low and high plasma LEPH concentrations for DMI and other feeding behavior traits ($P \geq 0.246$). For ultrasound carcass traits, heifers with high plasma LEPH concentration had greater initial REA ($P = 0.030$) than that of the low LEPH group (Table 4.9). Heifers were not different between low and high LEPH groups for other ultrasound carcass traits ($P > 0.356$). DMI and feed intake per visit had positive phenotypic correlations ($r_s = 0.108$, 0.176; $P = 0.050$, 0.001) with plasma LEPH concentration. The phenotypic relationships of plasma LEPH concentrations with other feeding behavior traits were not different from zero ($P > 0.10$). Also, no phenotypic correlations were observed between circulatory LEPH concentration and ultrasound carcass traits ($P > 0.10$).

Table 4.8. Association (LSMeans ± SE) of leptin based on classification effect, and Spearman correlation (r_s) of leptin with dry matter intake (DMI) and feeding behavior traits in developing heifers

Effect[1]	Level[2]	DMI , kg/d	Events, per d		DMI, g		Time eating at the feed bunk, min		
			Visits	Meals	Per visit	Per meal	Per visit	Per meal	Per day
LEP	*P*-value	0.311	0.593	0.210	0.944	0.132	0.995	0.772	0.995
	CC	8.499 ± 0.113	97.52 ± 3.46	11.01 ± 0.26	108.6 ± 6.6	849.3 ± 26.7	2.200 ± 0.187	16.34 ± 0.77	159.2 ± 4.7
	CT	8.530 ± 0.080	93.53 ± 2.46	10.65 ± 0.18	110.3 ± 4.7	885.2 ± 19.0	2.217 ± 0.133	16.78 ± 0.55	159.5 ± 3.4
	TT	8.699 ± 0.117	94.79 ± 3.59	10.41 ± 0.27	111.4 ± 6.8	919.7 ± 27.7	2.201 ± 0.194	17.06 ± 0.80	159.0 ± 4.9
LEPD[3]	*P*-value	0.704	0.449	0.569	0.880	0.465	0.834	0.958	0.738
	AA	8.711 ± 0.127	92.66 ± 3.83	10.42 ± 0.29	114.7 ± 7.4	923.1 ± 30.1	2.262 ± 0.210	16.97 ± 0.87	159.3 ± 5.3
	BB	8.683 ± 0.182	97.38 ± 5.51	10.91 ± 0.42	116.1 ± 10.7	869.6 ± 43.2	2.357 ± 0.302	16.23 ± 1.25	159.3 ± 7.6
	CC	8.422 ± 0.460	95.04 ± 13.84	12.94 ± 1.05	90.8 ± 26.9	680.1 ± 108.7	1.630 ± 0.759	12.77 ± 3.14	146.9 ± 19.1
	AB	8.582 ± 0.115	89.58 ± 3.47	10.61 ± 0.26	115.5 ± 6.7	904.7 ± 27.2	2.246 ± 0.190	17.17 ± 0.79	162.1 ± 4.8
	AC	8.447 ± 0.156	98.26 ± 4.69	10.71 ± 0.36	100.4 ± 9.1	856.6 ± 36.9	1.936 ± 0.257	15.94 ± 1.06	151.6 ± 6.5
	AD	8.369 ± 0.266	96.87 ± 7.94	10.38 ± 0.60	114.2 ± 15.4	886.6 ± 62.5	2.340 ± 0.436	17.23 ± 1.80	161.8 ± 11.0
	AE	8.898 ± 0.337	111.89 ± 10.19	10.34 ± 0.77	95.0 ± 19.8	976.9 ± 79.8	1.852 ± 0.558	18.45 ± 2.31	158.6 ± 14.1
	AF	7.901 ± 0.483	96.10 ± 14.67	11.63 ± 1.11	124.3 ± 28.5	759.2 ± 114.6	3.043 ± 0.804	17.57 ± 3.33	188.7 ± 20.3
	BC	8.314 ± 0.212	95.21 ± 6.40	11.28 ± 0.49	98.8 ± 12.4	817.5 ± 50.1	1.957 ± 0.351	16.49 ± 1.45	162.3 ± 8.8
	BD	8.250 ± 0.330	97.25 ± 9.90	10.73 ± 0.75	120.9 ± 19.3	863.7 ± 77.9	2.597 ± 0.543	16.32 ± 2.24	160.3 ± 13.7
	CD	8.179 ± 0.483	118.82 ± 14.25	10.41 ± 1.09	79.5 ± 27.8	898.4 ± 113.2	1.274 ± 0.783	14.36 ± 3.22	128.0 ± 19.6
LEPH group[4]	*P*-value	0.421	0.679	0.027	0.492	0.049	0.502	0.246	0.605
	Low	8.601 ± 0.087	95.62 ± 2.68	10.40 ± 0.20[b]	112.3 ± 5.1	909.5 ± 20.5[a]	2.270 ± 0.141	17.20 ± 0.60	160.6 ± 3.6
	High	8.517 ± 0.083	94.30 ± 2.54	10.93 ± 0.19[a]	108.1 ± 4.8	861.3 ± 19.4[b]	2.158 ± 0.134	16.37 ± 0.56	158.3 ± 3.5
LEPH conc.	*P*-value	0.050	0.537	0.127	0.001	0.831	0.554	0.360	0.991
	r_s	0.108	-0.034	0.084	0.176	-0.012	0.033	-0.050	0.001

[1]*LEP*: *LEP c.73C>T* genotype; *LEPD*: leptin diplotype; LEPH: leptin hormone; conc.: concentration

[2]Number of observations (CC: 76 – 77, CT: 164, TT: 95; AA: 86, BB: 32, CC: 04, AB: 94, AC: 49, AD: 13, AE: 08, AF: 04, BC: 22, BD: 08, CD: 04; High: 165 – 166; Low: 166; r_s: 332)

[3]SNP order: BovineHD0400026029, ARS-BFGL-NGS-59298, *LEP c.73C>T*, and BovineHD0400026063; AA: AATC/AATC, AB: AATC/GGCT, AC: AATC/GACT, AD: AATC/AGCT, AE: AATC/GATC, AF: AATC/AACT, BB: GGCT/GGCT, BC: GGCT/GACT, BD: GGCT/AGCT, CC: GACT/GACT, CD: GACT/AGCT

[4]The median LEPH concentrations used for LEPH grouping were 15.86 ng/mL

[a,b]Least square means within a column without a common superscript letter differ ($P < 0.05$)

Table 4.9. Association (LSMeans ± SE) of leptin based on classification effect, and Spearman correlation (r_s) of leptin with ultrasound carcass traits in developing heifers

		Intramuscular fat, %		Rib eye area, cm^2		Yield grade		Rib fat thickness, cm		Rump fat thickness, cm	
Effect[1]	Level[2]	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
LEP	*P*-value	0.489	0.717	0.839	0.462	0.471	0.070	0.444	0.069	0.640	0.762
	CC	3.485 ± 0.122	3.391 ± 0.122	50.33 ± 1.04	56.82 ± 1.15	2.681 ± 0.024	2.804 ± 0.026	6.890 ± 0.243	8.141 ± 0.264	4.250 ± 0.225	5.043 ± 0.308
	CT	3.391 ± 0.089	3.448 ± 0.088	50.76 ± 0.75	58.11 ± 0.83	2.661 ± 0.017	2.739 ± 0.019	6.679 ± 0.176	7.476 ± 0.191	4.161 ± 0.163	4.807 ± 0.222
	TT	3.554 ± 0.137	3.518 ± 0.136	51.09 ± 1.15	58.44 ± 1.28	2.694 ± 0.027	2.759 ± 0.029	7.018 ± 0.272	7.680 ± 0.295	4.407 ± 0.255	4.963 ± 0.344
LEPD[3]	*P*-value	0.852	0.970	0.211	0.550	0.987	0.324	0.984	0.321	0.992	0.278
	AA	3.495 ± 0.170	3.452 ± 0.168	51.49 ± 1.40	58.77 ± 1.58	2.667 ± 0.032	2.763 ± 0.036	6.757 ± 0.330	7.720 ± 0.366	4.443 ± 0.321	5.077 ± 0.428
	BB	3.518 ± 0.201	3.290 ± 0.197	51.16 ± 1.63	58.05 ± 1.84	2.669 ± 0.038	2.821 ± 0.042	6.766 ± 0.389	8.318 ± 0.432	4.364 ± 0.375	5.676 ± 0.504
	AB	3.309 ± 0.162	3.363 ± 0.160	51.58 ± 1.31	58.50 ± 1.48	2.642 ± 0.031	2.749 ± 0.034	6.501 ± 0.314	7.577 ± 0.349	4.166 ± 0.303	4.679 ± 0.408
	AC	3.357 ± 0.172	3.435 ± 0.169	50.23 ± 1.42	56.54 ± 1.61	2.639 ± 0.033	2.714 ± 0.036	6.442 ± 0.334	7.230 ± 0.371	4.272 ± 0.323	4.856 ± 0.433
	AD	3.125 ± 0.314	3.304 ± 0.309	45.32 ± 2.64	56.63 ± 2.98	2.675 ± 0.060	2.750 ± 0.066	6.815 ± 0.608	7.598 ± 0.676	4.501 ± 0.586	5.560 ± 0.789
	AE	3.453 ± 0.289	3.607 ± 0.285	52.09 ± 2.35	56.73 ± 2.65	2.641 ± 0.055	2.731 ± 0.061	6.472 ± 0.560	7.394 ± 0.622	4.176 ± 0.540	4.330 ± 0.727
	BC	3.255 ± 0.227	3.421 ± 0.223	51.55 ± 1.86	57.32 ± 2.10	2.645 ± 0.043	2.726 ± 0.048	6.539 ± 0.439	7.350 ± 0.487	4.312 ± 0.423	4.089 ± 0.569
	BD	3.587 ± 0.409	3.594 ± 0.402	44.39 ± 3.33	50.47 ± 3.76	2.674 ± 0.078	2.907 ± 0.087	6.803 ± 0.792	9.186 ± 0.880	4.500 ± 0.764	5.071 ± 1.028
LEPH group[4]	*P*-value	0.919	0.556	0.030	0.356	0.551	0.979	0.575	0.963	0.771	0.922
	Low	3.432 ± 0.094	3.476 ± 0.093	49.74 ± 0.79[b]	57.40 ± 0.88	2.665 ± 0.018	2.757 ± 0.020	6.719 ± 0.187	7.661 ± 0.206	4.149 ± 0.175	4.874 ± 0.236
	High	3.443 ± 0.095	3.412 ± 0.094	51.69 ± 0.79[a]	58.32 ± 0.89	2.677 ± 0.018	2.758 ± 0.020	6.841 ± 0.187	7.672 ± 0.206	4.314 ± 0.181	4.900 ± 0.236
LEPH conc.	*P*-value	0.161	0.767	0.367	0.674	0.788	0.481	0.763	0.481	0.348	0.482
	r_s	-0.111	0.024	0.072	-0.033	-0.021	-0.056	-0.024	-0.056	-0.075	-0.056

[1]*LEP*: *LEP c.73C>T* genotype; *LEPD*: leptin diplotype; LEPH: leptin hormone; conc.: concentration
[2]Number of observations (CC: 42, CT: 80, TT: 39 – 40; AA: 31 – 32, AB: 42, AC: 28, AD: 05, AE: 07, BB: 19, BC: 13, BD: 03; High: 80; Low: 80 – 81; rs: 160 – 161)
[3]SNP order: BovineHD0400026029, ARS-BFGL-NGS-59298, *LEP c.73C>T*, and BovineHD0400026063; AA: AATC/AATC, AB: AATC/GGCT, AC: AATC/GACT, AD: AATC/AGCT, AE: AATC/GATC, AF: AATC/AACT, BB: GGCT/GGCT, BC: GGCT/GACT, BD: GGCT/AGCT, CC: GACT/GACT, CD: GACT/AGCT
[4]The median LEPH concentrations used for LEPH grouping were 16.75 ng/mL
[a,b]Least square means within a column without a common superscript letter differ ($P < 0.05$)

Discussion

The T and C allele frequencies of the *LEP c.73C>T* observed in our study are similar to those reported in Brangus steers (50.4% C and 49.6% T, Corva et al., 2009), crossbred finishing heifers (50.19% C and 49.81% T, Kononoff et al., 2005), and crossbred steers (48% C and 52% T, Nkrumah et al., 2004). However, some previous publications found a greater frequency of the C allele in crossbred cattle of seven distinct genetic groups (56.7%, de Carvalho et al., 2012), *Bos taurus* crossbred cattle (57.6%, Schenkel et al., 2005), and Nellore and *Bos taurus* × *Bos indicus* crossbred cattle (81% C and 19% T, Fortes et al., 2009). Buchanan et al. (2002) reported a higher frequency of T allele in British breeds than Continental breeds (57% vs. 33%) and observed an association of this allele with fatter carcasses.

The allelic substitution from C (wild type) to T (mutant type) in the *LEP c.73C>T* of leptin exon 2 results in changing amino acid from arginine to cysteine, which alters the biological function of the leptin hormone. It is hypothesized that an unpaired cysteine's presence on the A helix of the leptin molecule changes the leptin's tertiary conformation that potentially affects binding to the leptin receptor and alters the signaling pathway for the leptin hormone action (Buchanan et al., 2002). This extra unpaired cysteine could also destabilize the disulfide bridge (i.e., found critical for the biological function of leptin) between two existing cysteines on the leptin molecule, which can cause a functional change of leptin hormone (Buchanan et al., 2002). Therefore, heifers with the T allele will have free circulatory leptin (i.e., unbound to leptin receptors), which would be cleared sooner from the circulation because of their significantly shorter half-life in comparison to bound leptin (Chebel et al., 2008). Animals carrying the T allele for this SNP might also have high serum leptin levels because their leptin gene will

produce more leptin due to the leptin molecule's reduced biological activity (Buchanan et al., 2002).

Growth and feed efficiency

Our study observed the T allele's association of *LEP c.73C>T* genotype with greater WW. The contrast findings revealed the incomplete dominance effect of the T allele over the C allele for greater weight at weaning. WW is a maternally influenced trait, which is determined by the calf's genotype for preweaning growth (i.e., direct effect), the dam genetics for milking and mothering ability (i.e., maternal effect), and environmental effects (Garrick, 1990). Therefore, WW in our study might also be influenced by the *LEP* genotype of their dam. It can be assumed that calves homozygous for the T allele are born from either CT or TT dams. There is also a high possibility of CT calves being born from dams with CT or TT genotypes. Dairy cows with a T allele were observed to produce more milk than cows homozygous for the C allele (Buchanan et al., 2003). Thus, CT or TT-genotype cows might contribute to weaning heavier calves by providing more milk from birth to weaning. Devuyst et al. (2008) reported that crossbred CT and TT beef cows weaned heavier calves than calves from crossbred CC beef cows. They also noted that Shorthorn cows with CT genotype had heavier calves at weaning compared to CC Shorthorn cows. However, our study didn't find any association between this *LEP* SNP and the birth weight of these studied heifers (data not shown here). We observed an association of the TT genotype of *LEP c.73C>T* SNP with greater final BW of the feed trial. However, the C allele showed its likely complete dominance effect over the T allele for smaller final BW. Our results are in contrast to the observations of Woronuk et al. (2012) and Buchanan et al. (2007). Woronuk et al. (2012) reported that crossbred steers and heifers with CC and CT genotypes were heavier than animals with the TT genotype. Similarly, CC steers showed greater final live weight

compared to the TT steers fed a finishing diet (Buchanan et al., 2007). These discrepancies between these previous studies and the present study may be due to differences in diets (high-concentrate diet vs. forage diet). An earlier study reported that heifers fed a high energy diet for a longer period had increased fat deposition compared to the low energy diet (Petitclerc et al., 1984). Serum LEPH concentrations have been positively correlated with body fat mass in the commercial-fed beef cattle (Brandt et al., 2007), indicating high energy diet ultimately leads to increased circulating LEPH concentration which might cause the differences observed between these studies. Also, there could be an interaction of genotype × diet playing a role in this scenario. In addition, the sex of experimental animals might influence these distinct findings because we conducted our study with heifers, whereas steers were used in those previous studies. McEvers et al. (2014) reported an association of *LEP c.73C>T* genotype with ADG during the treatment period (d 102 to 125) and entire study period (d 1 to 125), where they observed greater ADG for both CC and TT genotypes than that of CT heterozygote in British and British × Continental crossbreed steers. An association between the *LEP c.73C>T* genotype and weaning to yearling weight gain was reported by da Silva et al. (2012) in Nellore cattle. Our study did not show any effect of *LEP c.73C>T* genotype on initial BW and average daily gain. Our study's current findings were similar to those reported by some previous studies with the same SNP (Nkrumah et al., 2004; Kononoff et al., 2013; Kononoff et al., 2014). The *LEP* polymorphisms other than *LEP c.73C>T* also affected BW and ADG in beef cattle. Kulig and Kmieć (2009) reported the effect of *LEP c.357C>T* polymorphism in exon 3 on BW measured at 210 days of age, and ADG between 3 and 210 days of age in Limousin cattle. Association of the same *LEP* polymorphism with ADG was also reported by Nkrumah et al. (2006) in crossbred beef steers. However, Silva et al. (2014) did not observe any association of *LEP c.357C>T* with BW and

ADG gains measured on feed trial in Nellore cattle. Polymorphisms in the bovine *LEP* promoter region (*LEP g.528C>T,* and *LEP g.1759C>G*) showed association with metabolic BW and ADG, and metabolic BW and final BW, respectively, in crossbred steers (Nkrumah et al., 2005). An interaction of *BM1500* microsatellite located 3.6 kb downstream of the *LEP* with BW measured at the third period of feed trial was also reported by Silva et al. (2014) in Nellore cattle. Studies on Holstein heifers did not show any association of the *LEP* polymorphisms (*LEP n.Sau3AI* in intron 2, *LEP c.357C>T* in exon 3) or *BM1500* microsatellite with live weight measured during lactation (Liefers et al., 2002). Our study showed a likely complete dominance effect of the T allele of *LEP c.73C>T* marker over the C allele for smaller G:F. The findings in our study are similar to those reported by McEvers et al. (2014) during the pretreatment and the entire study period of zilpaterol hydrochloride supplementation in British and British × Continental crossbred beef steers fed a concentrate rich diet. This effect of *LEP c.73C>T* marker on G:F might be expectable since animals with the T allele consuming more dry matter may be less feed efficient. However, it is completely undesirable in the selection point of view. Further research investigating this marker's effect on G:F may be warranted. Some previous studies did not observe any association of this SNP with feed efficiency traits in crossbred beef steers (Nkrumah et al., 2004; Kononoff et al., 2013; Kononoff et al., 2014).

The *LEPD* has been observed to be associated with WW, initial and final BW. Based on linear contrast findings, the C (GACT) haplotype showed its incomplete dominance over the A (AATC) haplotype and overdominance effect over the B (GGCT) haplotype for lighter weight at weaning. However, the A and C haplotype might exert their additive or codominance effect for initial BW. The C haplotype showed its incomplete dominance over the B haplotype for initial BW. Furthermore, the C haplotype showed its incomplete dominance effect over the A haplotype

for lighter final BW. A significant association of *LEPD*s was also observed with G:F in the present study. Heifers with CD (GACT/AGCT) diplotype were more feed efficient compared to heifers with 6 different diplotypes used in the study. This diplotype is homozygous for the C allele of *LEP c.73C>T* marker. Our study also observed greater G:F for CC homozygous heifers than that of CT and TT heifers. Therefore, the favorable C allele of *LEP c.73C>T* contributes to greater G:F for the CD diplotype. Similarly, BC (GGCT/GACT) diplotype homozygous for the C allele also showed greater G:F than AB heifers. These two findings confirm an association of the GACT haplotype with greater G:F in our admixed population. The GACT haplotype has been shown as a rare haplotype (1.31%) in our population. Since this haplotype showed an association with greater feed efficiency, it would be interesting to validate these finding by investigating further the effect of the GACT haplotype using a larger sample set. Associations of *LEPD* used in the present study with growth and feed efficiency traits have not been studied elsewhere. No significant *LEP* haplotype associations were observed with live BW in Holstein cows, where haplotypes were constructed using 6 *LEP* SNPs, including *C207T*, *C528T*, *A1457G*, *C963T*, *A252T*, and *C305T* (Banos et al., 2008). Their study reported a tendency to associate the CCGTTT haplotype with heavier heifers and increased total body energy content. Kulig and Kmieć (2009) reported an association of *LEP c.357C>T*/*LEP n.Sau3AI* haplotypes with average daily gain between 3 and 210 days of age; however, no associations were found with BW measured at 210 and 356 days of age in Limousin cattle. Additionally, a significant association of *LEP g.528C>T* and *LEP c.357C>T* haplotypes with ADG was observed in crossbred beef steers (Nkrumah et al., 2006).

Adjusted WW had a positive phenotypic relationship with plasma LEPH concentration. Nkrumah et al. (2007) reported a low positive phenotypic relationship between serum LEPH

concentration and metabolic BW in synthetic beef steers. They also observed greater final BW and metabolic BW in the steers with high serum LEPH than the low serum LEPH group. However, no partial relationship was found between serum LEPH concentration and live weight in crossbred *Bos taurus* steers and heifers (Geary et al., 2003). Our study showed an association of LEPH concentration with WW; however, no associations were found with either initial or final BW on the feed trial. This might be due to the differences in growth environments when suckling cows or management overwinter before starting feed trial. However, heifers were on an almost similar forage-based diet before starting their feed trial. Plasma LEPH concentration was not correlated phenotypically with ADG in the current study, which is consistent with previous studies' findings (Richardson et al., 2004 in Angus steers; Kelly et al., 2010 in growing beef heifers; Nkrumah et al., 2007 in synthetic beef steers). However, both the positive and negative relationships between circulatory LEPH concentration and ADG were reported in finishing beef steers and heifers (Foote et al., 2015; Foote et al., 2016). The phenotypic relationships between plasma LEPH hormone concentration and G:F and RFI in our study are similar to the findings of Nkrumah et al. (2007b), where they did not report any phenotypic correlations of serum LEPH concentration with these traits. No phenotypic correlation between plasma LEPH concentration and RFI was also observed by Kelly et al. (2010a). However, Foote et al. (2015) reported circulatory LEPH was positively correlated with RFI, but not correlated with G:F in finishing crossbred beef steers. Also, Foote et al. (2016) observed a positive relationship of plasma LEPH concentration measured at different time points with RFI (day 83 and mean LEPH concentration), but a negative association with G:F (day 0 and mean LEPH concentration). Similarly, Richardson et al. (2004) reported a positive correlation between serum LEPH and RFI, though no significant correlation with FCR in feedlot Angus steers. In contrast, plasma LEPH

was highly positively correlated with FCR in growing crossbred beef heifers fed a forage-based diet (Kelly et al., 2010a).

Direct linear and calculated body measurements and their growth

The *LEP c.73C>T* marker only showed its effect on initial middle girth among direct linear body measurement traits; however, significant differences between *LEP c.73C>T* genotypes could not be detected for calculated body measurement and body measurement growth traits. The contrast findings confirmed the complete dominance effect of the C allele over the T allele for a smaller initial middle girth. In previous studies, the T allele has been found to be associated with the greater backfat thickness (Nkrumah et al., 2004; Buchanan et al., 2007; Kononoff et al., 2013; Kononoff et al., 2014; Woronuk et al., 2012), rump fat thickness (da Silva et al., 2012), and carcass fat yield (Buchanan et al., 2002; Schenkel et al., 2005; De Carvalho et al., 2012). Since body fat depots have a high positive relationship with biometric measurements (Fonseca et al., 2017), an association of the T allele with greater body sizes can be expected. Our inability to show significant differences in other direct linear, calculated body measurements and body measurement growth traits may be due to the small data set used in this study or measurement error in data collection. Leptin polymorphisms (*LEP c.357C>T* and *LEP g.-1457A>G*) had an effect on crown-rump length and height at withers in Holstein cows (Clempson et al., 2011). An interaction of *LEP Sau3AI* with body dimension traits (circumference of the round and chest depth) was noted in Black and White (Friesian) cattle (Oprzadek et al., 2003). The association of an *LEP* polymorphism on exon 3 with body measurement traits (e.g., withers height, body length, and heart girth) in Chinese indigenous cattle have been reported by Yang et al. (2007).

Based on contrast findings, the A haplotype showed its incomplete dominance over the C haplotype for greater initial middle girth, initial heart girth, and initial end girth. However, B and C haplotypes showed their likely codominance effect for these traits. The T allele of *LEP c.73C>T* marker might contribute to the greater performance for those body size traits by the A (AATC) haplotype. Furthermore, A and B haplotypes might exert their incomplete dominance effects over the D (AGCT) haplotype; however, the sample size was a limitation to show this effect. No differences in *LEPD*s were found for any other linear body measurements and calculated body measurement traits. The *LEPD*s did not show any interaction with body measurement growth traits except heart girth daily gain. The A and B haplotypes also showed their incomplete dominance over the C haplotype for reduced heart girth daily gain. Associations of *LEP* haplotypes used in the present study with body measurement traits have not been studied elsewhere. Kulig and Kmieć (2009) did not find any associations of *LEP c.357C>T*/*LEP n.Sau3AI* haplotypes with withers height, sacrum height, and chest circumference in Limousin cattle.

Plasma LEPH showed positive correlations with most of the studied linear and calculated body measurement traits. However, a negative relationship was observed between plasma LEPH concentration and body density. None of the body measurement growth traits displayed a relationship with plasma LEPH concentration in this study. Serum LEPH concentration had a positive relationship with waist and hip circumference in women and men (Ruhl and Everhart, 2001). Luke et al. (1998) did not find any association between plasma LEPH concentration and height in men.

Feed intake, feeding behavior, and ultrasound carcass traits

Our study did not observe any effect of *LEP c.357C>T* genotype on DMI in growing heifers. Previous studies also reported similar findings for DMI (Lagonigro et al., 2003; Nkrumah et al., 2004; Banos et al., 2008). However, a significant association of the T allele with increased feed intake was found in *Bos taurus* purebred and crossbred steers (McEvers et al., 2014). Others have also reported conflicting results on the T allele's negative and positive effects for DMI in two different studies supplemented with zilpaterol hydrochloride (Kononoff et al., 2013) and ractopamine hydrochloride (Kononoff et al., 2014). Leptin down-regulates feed intake by acting on the satiety center in the hypothalamus through specific receptors (leptin receptor [ObR], Seufert, 2004). When less is present, animals will consume more feed. Although we did not observe any effect of the *LEP c.73C>T* genotype on DMI, our study showed a tendency of eating more dry matter per meal by the heifers with TT genotype. The limited sample size might be one reason for not observing significant effects of *LEP c.73C>T* genotype on DMI. Diet may be another vital factor because previous studies observed significant associations with DMI for this marker offered concentrate-based diets, whereas our study used a forage-based diet. Moreover, no *LEP c.73C>T* genotype associations with any other feeding behavior traits were observed in our study. In a feedlot study with crossbred beef steers, Nkrumah et al. (2004) did not report any effect of *LEP c.73C>T* on their studied feeding behavior traits (feeding duration and feeding frequency). Therefore, this *LEP* marker might not be useful to account in a marker-assisted selection program for feeding behavior traits.

It can be hypothesized that animals with the T allele would intake more dry matter because of having low circulatory plasma LEPH concentration, leading to a positive energy balance, fat mass deposition, and eventually, weight gain. Thus, our study observed the T allele

associations with greater WW and final BW measured on feed trial. However, we did not find any association of *LEP c.73C>T* genotype with DMI in the present study.

Several association studies have been conducted on *LEP c.73C>T* marker and carcass traits. In a population of purebred *Bos taurus* beef cattle, Barendse et al. (2005) did not observe any significant effect of this marker on near-infrared spectrophotometry measured or visual intramuscular fat and BFT at the P8 position (rump fat). Furthermore, animals were not different between *LEP c.73C>T* genotypes for carcass BFT in Nellore and its influenced cattle (Fortes et al., 2009) and marbling score in purebred Chinese Qinchuan cattle (Liu et al., 2010). In contrast, in studies with purebred populations (*Bos taurus* or *Bos indicus*), the T allele has shown its association with increased BFT and RFAT in purebred Nellore bulls (da Silva et al., 2012), intramuscular fat in purebred Angus bulls (Anton et al., 2011) and fatter carcasses (Buchanan et al., 2002). Also, da Silva et al. (2012) reported an association of the C allele with a larger LM area in purebred Nellore bulls. However, studies on crossbred (*Bos taurus* × *Bos taurus* or *Bos taurus* × *Bos indicus*) populations did not show any significant association between *LEP c.73C>T* genotype and BFT (Nkrumah et al., 2004; Corva et al., 2009), IMF% (Lagonigro et al., 2003; Schenkel et al., 2005; Corva et al., 2009; Pannier et al., 2009), marbling score (Crews et al., 2004; de Carvalho et al., 2012; Larson et al., 2005; Nkrumah et al., 2004; Lagonigro et al., 2003), LM area (Nkrumah et al., 2004; Schenkel et al., 2005; Corva et al., 2009; Kononoff et al., 2013; Kononoff et al., 2014; McEvers et al., 2014), and yield grades (Kononoff et al., 2013; McEvers et al., 2014). Similarly, our study focusing on *Bos taurus* admixed beef heifers did not detect any significant association of the *LEP* genotype with any studied ultrasound carcass traits. The absence of divergence in fat traits in the present study might be due to the forage-based diet offered to the developing heifers. On the contrary, the T allele of *LEP c.73C>T* genotype

displayed a dominance effect over the C allele for greater 12th rib backfat thickness (Kononoff et al., 2014; Schenkel et al., 2005; Buchanan et al., 2007; da Silva et al., 2012; Kononoff et al., 2013; Larson et al., 2005; Woronuk et al., 2012), and greater marbling score (Kononoff et al., 2014; McEvers et al., 2014) in *Bos taurus* crossbred populations. Furthermore, the C allele of this marker has been found to be associated with increased LM area (Buchanan et al., 2007; Larson et al., 2005) and greater lean meat yield, i.e., the lower numerical value of the yield grade (Buchanan et al., 2007; Kononoff et al., 2005; Larson et al., 2005; Nkrumah et al., 2004; Schenkel et al., 2005) in commercial crossbred beef cattle. However, Corva et al. (2009) observed a surprising result in Brangus steers fed a grass-based diet, where the C allele was associated with higher carcass backfat thickness. They partially explained it as a result of removing subcutaneous fat during mechanical hide pulling. Furthermore, Shin and Chung (2007) reported that animals with CC genotype had greater marbling scores and BFT than TT animals in Korean native (Hanwoo) steers. In our study, heifers with the CC genotype also tended to have a greater rib fat thickness and a higher numerical value of yield grade than the CT heterozygous heifers. Diet supplied to the heifers during the feed trial might lead to this type of unpredictable results.

The most contributing factor behind the non-significant results observed in our study might be the environment, i.e., the type of feed supplied to the experimental heifers before starting and on the feed trial, and the feed supply period. Since most carcass traits are related to fatness, feeding regimes are likely to play a significant role in regulating these traits. Therefore, failing to control this vital factor could lead to non-significant results (Pannier et al., 2009). The size of the current study data set might or might not be a cause for these results because some previous studies have reported significant associations with some of these carcass traits using a

sample size of 144 (Nkrumah et al., 2004), 154 (Buchanan et al., 2002), and 173 (Anton et al., 2011). Thus, variation among the population might contribute to this *LEP* SNP's relationship with those carcass traits. Moreover, we observed an almost similar C and T allele frequency in our study using admixed populations that might influence getting these association findings with carcass traits. An earlier study where the *LEP c.73C>T* showed an association with carcass fat content reported higher frequencies of T and C alleles in British and Continental breeds, respectively (Buchanan et al., 2002). British breeds carry more fat at a younger age because of their early maturity compared to the Continental breeds.

Several *LEP* haplotype association studies have been carried out with DMI and carcass traits in beef cattle, where *LEP c.73C>T* was used for haplotype construction. Banos et al. (2008) reported an association of the CCGTTT haplotype (SNP order: *LEP g.207C>T*, *LEP g.528C>T*, *LEP g.-1457A>G*, *LEP g.-963C>T*, *LEP c.252A>T*, *LEP c.73C>T*) with feed intake and DMI. In an *LEP* haplotype analysis with Holstein × Charolais crossbred bull calves, (Lagonigro et al., 2003) observed that the TCC and ACC haplotypes (SNP order: *LEP c.252A>T*, *LEP c.73C>T*, *LEP c.140C>T*) are linked with increased inter-muscular fat levels and reduced subcutaneous fat but did not affect feed intake. Furthermore, Corva et al. (2009) stated an association of the CC haplotype (SNP order: *LEP g.528C>T*, *LEP c.73C>T*) with greater backfat thickness than that of the most abundant CT haplotype in Brangus steers fed a grass-based diet. The haplotype association studies with 4 *LEP* polymorphisms (*LEP g.207C>T*, *LEP g.528C>T*, *LEP c.252A>T*, *LEP c.73C>T*) have been found an influence of the CCTT haplotype with decreased fat yield and backfat thickness and increased lean meat yield when compared to the three most common (88%) haplotypes (TCAC, CCAT, and TTAC; Schenkel et al., 2005). However, Pannier et al. (2009) did not report any association of studied *LEP*

haplotypes (SNP order: *LEP g.207C>T*, *LEP g.528C>T*, *LEP c.73C>T*, *LEP n.Sau3AI*) with intramuscular fat levels in beef × dairy crossbred cattle.

The phenotypic relationship between plasma LEPH and DMI observed in the present study agreed with Foote et al. (2015) and Nkrumah et al. (2007b). Additionally, Foote et al. (2016) investigated the association of plasma LEPH concentrations with finishing beef steers and heifers' production measures. Their study observed a significant positive association of plasma LEPH concentration measured at different time points with DMI (day 0, day 42, day 83, and mean LEPH concentration). Richardson et al. (2004) did not observe any significant correlation between LEPH concentration and average daily feed intake in feedlot Angus steers. However, plasma LEPH was highly positively correlated with DMI in growing crossbred beef heifers offered a forage-based diet (Kelly et al., 2010a). As a satiety hormone, leptin has a hunger-suppressing effect. Several earlier studies reported a decreased appetite on post-injection of LEPH in the body of mice, rhesus monkey, pigs, and chicken (Campfield et al., 1995; Mistry et al., 1997; Tang-Christensen et al., 1999; Dridi et al., 2000; Ramsay et al., 2004). The positive relationships of LEPH with DMI observed in the present and previous studies are opposite to what we expected. Foote et al. (2016) suggested that this might result from the slight leptin resistance developed in the beef cattle on a finishing ration.

The LSMeans and standard errors of plasma LEPH concentrations for the high and low plasma LEPH groups were 22.36 ± 0.40 and 12.70 ± 0.42 ng/mL, respectively, in the present study. These average LEPH concentrations were greater than those reported by Nkrumah et al. (2007b), where they demonstrated average serum LEPH concentrations of 20.32 and 8.57 ng/mL in high and low serum LEPH groups, respectively. In addition, our study observed a higher average plasma LEPH concentration (17.99 ± 0.83 ng/mL) compared to their study (13.91 ± 5.74

ng/mL). These differences in LEPH concentration might be due to sexual dimorphism (Saad et al., 1997) because Nkrumah et al. (2007b) used crossbred beef steers in their study. Saad et al. (1997) reported higher serum LEPH concentration in women than men after correcting BW and fat mass. Time of blood collection in our study, i.e., before the breeding season, might be another contributing factor for this elevated circulatory LEPH concentration level. In a study with feedlot crossbred steers, Nkrumah et al. (2007b) observed greater DMI and RFI for the animals with high serum LEPH concentration compared to the low serum LEPH group. Animals with high serum LEPH also had the greater backfat thickness and marbling score (direct and ultrasound measurements), carcass yield grade, and lower carcass lean meat yield than the animals with low serum LEPH; however, no association was observed with ultrasound or carcass LM area. In our study, heifers with high plasma LEPH showed greater initial REA compared to low plasma LEPH. Also, our study is the first reporting a significant association of plasma LEPH category with feeding behavior attributes, where heifers with low LEPH consumed fewer meals per day and had greater DMI per meal than those with high LEPH. However, we did not observe any significant association of LEPH grouping with DMI, ultrasound rib fat thickness, rump fat thickness, IMF%, or yield grade. This lack of significant association might be due to the limited sample size used in the study. The sample-set used by Nkrumah et al. (2007b) was around 3 times larger than our study. Regardless of sample size, animals grouping with plasma LEPH concentration collected before breeding season may not be beneficial as biomarker of feed intake, efficiency, and ultrasound carcass traits in developing admixed beef heifers fed forage-based diets.

Our study is the first to report a positive correlation between circulatory LEPH concentration and eating rate per visit. However, we did not observe any significant phenotypic

correlation of plasma LEPH concentration with any studied feeding behavior and ultrasound carcass traits. In contrast, several previous studies with commercially-fed beef steers and heifers reported positive phenotypic relationships of circulatory plasma LEPH concentration with backfat thickness, marbling score, and carcass yield grade (Geary et al., 2003; McFadin et al., 2003; Brandt et al., 2007; Nkrumah et al., 2007b; Foote et al., 2015), but negative correlations with LM area (Geary et al., 2003; Brandt et al., 2007; Nkrumah et al., 2007b; Foote et al., 2015) and lean meat yield (Nkrumah et al., 2007b). Foote et al. (2016) also reported positive associations of plasma LEPH concentration measured at different times points with ultrasound 12th rib fat thickness, ultrasound rump fat, ultrasound IMF%, carcass 12th rib fat thickness, marbling score, and USDA-calculated yield grade (day 0, day 42, day 83, and mean LEPH level); however, a negative association with carcass LM area (day 42, d 83, and mean plasma LEPH level). The reasons for discrepancies between our results and previous studies could be nutritional management (i.e., diet) and/or analyzed plasma LEPH hormone timepoint.

Conclusions

This study revealed significant differences between *LEP c.73C>T* genotypes for WW, final BW, G:F, and initial middle girth in commercial growing beef heifers. In all of those traits except G:F, the TT genotype of *LEP c.73C>T* SNP was associated with improved performance. The CC genotype of *LEP c.73C>T* showed its association with greater feed efficiency in developing beef heifers, which is an undesirable outcome that needs to be investigated further. Therefore, this genetic information potentially could be used to make selection and marketing decisions. Circulating LEPH concentration also had a positive relationship with most linear and calculated body measurement traits. Heifers with low LEPH concentration consumed fewer meals per day but had greater DMI per meal than those with high LEPH. Further research is

needed to understand LEPH concentration and its role in feed behavior and body measurement traits. However, circulatory LEPH before the breeding season may serve as a predictor for those attributes.

CHAPTER 5. GENERAL CONCLUSIONS AND FUTURE DIRECTIONS

This dissertation's findings, specifically the genetic structure of the AD cattle breed and its genomic relationship with American taurine beef breeds, are novel. Also, we are the first to report novel associations of *LEP c.73C>T* genotype with reproductive characteristics in developing heifers. Lastly, we provide novel relationships between eating rate per meal and meals per day based on the LEPH category.

In Chapter 2, the genetic architecture of AD and its genetic relationship with American taurine beef breeds were investigated. Also, the genetic population structure of the admixed population was examined for improved trait analysis models. Whole genome-wide SNP markers were used to obtain and evaluate intra-population and inter-population genetic diversity parameters, population structure, and admixture analysis. The admixed populations were not the only groups with high levels of polymorphism. Similar levels of high (95% or greater) were seen in AR and GV populations. The lowest level of polymorphism was observed in AD followed by undetermined parentage. Pairwise fixation index displayed the greatest genetic differentiation between AD and GV, SM, and SH breeds. Admixture analysis elucidated the genetic structure of AD cattle relative to five different taurine cattle breeds in the United States. The AD breed exhibited its unique genomic characteristics, even though founder animals originated from the AN breed. The mixed animal models with or without pedigree that included the primary ancestral breed group for admixed individuals developed from diversity analyses performed better in comparison to models without the ancestral breed group. For some studied traits, ancestral breed group inclusion reduced heritability estimates, but no change in repeatability estimates, indicating variation explained occurs only between the additive and permanent environment variances that leads to more accurate estimates.

Chapter 3 discussed the effect of the *LEP* genotype, leptin diplotype (*LEPD*), and plasma leptin hormone (LEPH) concentration on reproductive characteristics in commercial beef cows. The average calving interval, age at first calving, and success traits (pregnancy, weaning, reproductive) did not show any association or tendency with *LEP* genotype, *LEPD,* or circulatory LEPH concentration. Numerical differences observed for ovary size and number of follicles indicated dominance of the T over the C allele, as previously reported, which was supported through linear contrasts. However, no association or tendency of the *LEPD* or LEPH category was observed for these traits. Negative correlations were found between plasma LEPH concentration and uterine horn diameter, average ovary diameter, and antral follicle counts, indicating that as LEPH concentration increased, the number of follicles and size of ovary and uterine horn declined to a low degree.

Chapter 4 evaluated the association of the *LEP* genotype, *LEPD*, and circulatory LEPH concentration growth, body size, feed intake, efficiency, feeding behavior, and ultrasound carcass traits in developing beef heifers. The T allele of *LEP c.73C>T* marker showed its complete dominance over the C allele for greater weight at weaning. However, the C allele showed its likely complete dominance effect over the T allele for smaller final BW. Thus, heifers with TT genotype would be good to select for better growth performance. However, the T allele of *LEP c.73C>T* SNP showed its complete dominance over the C allele for smaller G:F. The *LEPD* showed associations for weight traits and body size traits. Both A and B haplotypes were associated with greater WW, initial and final BW, initial middle girth, initial heart girth, and initial end girth. However, the presence of the C haplotype was associated with reduced performance for those traits. No other association or tendency was found with the *LEP* genotype or *LEPD* for feeding and carcass ultrasound traits. Our study provides novel relationships

between eating rate per meal and meals per day based on the LEPH category. Heifers with low LEPH consumed fewer meals per day and had greater dry matter intake per meal than those with high LEPH. Animals with two LEPH categories did not differ for other studied traits. Low positive relationships were observed between LEPH concentration and WW, DMI, feed intake per visit, and most body size traits, but there was a negative relationship between LEPH concentration and body density.

In summary, the *T* allele of *LEP c.73C>T* polymorphism demonstrated its effect on growth, body size, and reproduction traits, which aligns with the allele's general understanding in several previous studies with growing cattle. Therefore, the T allele of *the LEP c.73C>T* marker could be used as a valuable marker for selecting for improved performance of these traits in commercial beef heifers. Also, circulatory LEPH before the breeding season may serve as a predictor for feeding behavior, body size, and reproductive characteristics. Even so, not all comparisons could be statistically proven; therefore, additional investigations may be warranted.

Every animal study has some limitations and shortcomings, and there is certainly room for improvement by either increasing the data set or repetition of the experiment. We confirmed that some individuals used in the admixture analysis had a considerable fraction of the Hereford or Limousin genetics from the pedigree information. The inclusion of purebred Hereford and Limousin in PCA or admixture analysis could provide more refined outputs. However, we excluded individuals with ≥ 0.25 Hereford or Limousin breed fractions in further analysis. We also noticed the effect of the small sample size used in this study on genetic parameters estimation, resulting in large standard errors. In the leptin association study with performance traits, the statistical differences could be disproven in some cases due to inadequate sample size and variability. This effect of sample size was high in the case of leptin diplotype analysis.

Furthermore, our study used plasma LEPH concentration collect at a single time point, i.e., before the breeding season. Since leptin is secreted in a pulsatile pattern in heifers (Wylie et al., 2001), plasma leptin analyzed at multiple time points could provide stronger results.

These projects have the prospective both for future research as well as benefit to the livestock industry. Based on the first study's findings, the ancestral breed's inclusion may be an excellent strategy to prevent false association results from population structure in the commercial-like populations. Furthermore, we evaluated the inbreeding coefficient in different populations. However, inbreeding estimates with runs of homozygosity (ROH) using SNP markers may provide a more accurate measure of inbreeding levels. The haplotype-based local admixture analyses could be performed to reveal more details on the genetic relationship between AD cattle and American taurine breeds. The genomic relationship analyses, including the AD breed and American indicine beef breeds (e.g., Brahman, Nellore, etc.), might be conducted to investigate interpopulation genetic diversity and admixture analysis. Based on current findings and earlier published literature, the relationships of *LEP* polymorphisms and circulatory LEPH concentrations with some performance traits are not consistent. As quantitative economic traits, many biallelic polymorphisms like *LEP c.73C>T* linked with other genes (probably most unknown) are causing the cause and effect. Therefore, future research to better understand biological pathways could be performed before extensive economic weight is placed on this genotype for selection purposes. The genome-wide association study on some of the novel traits included in the present study, such as antral follicle count, size traits of the ovary and uterine horn, success traits (pregnancy, weaning, and reproductive), and feeding behavior attributes could useful as many genes control these traits.

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APPENDIX. SUPPLEMENTARY TABLES AND FIGURES

Table A1. Single nucleotide polymorphisms in the promoter and coding region of the bovine leptin (*LEP*) and leptin receptor (*LEPR*) genes and their phenotypic associations with production and reproduction traits

Polymorphism, their location and Study[1]	Population and Diets[2]	Genotype frequency	Associations found[3]	Associations not found[3]
LEP c.252A>T (E2JW, A252T, Y7F, LEP*Cla*I), Exon 2 of the *LEP*				
Lagonigro et al., 2003	169 second generation crossbred bull calves fed ad libitum concentrate & straw	AA: 0.73, AT: 0.26, TT: 0.01	FI	backfat depth; subcutaneous fat; intermuscular fat; MS
Banos et al., 2008	571 HO cows fed high and low concentrate diets on feed trial	AA: 0.95, AT: 0.05, TT: 0.00	Milk yield	FI; DMI; FI over milk yield; DMI over milk yield; LW; BCS
Schenkel et al., 2005	43 AN, 30 LM, 11 CH, 68 SM, and 959 animals with breed composition <5/8 for all breeds (heifers/steers/bulls) fed a finishing diet	*AN- AA: 0.90, AT: 0.10, TT: 0.00; LM- AA: 0.90, AT: 0.10, TT: 0.00; CH- AA: 0.83, AT: 0.16, TT: 0.01; SM- AA: 0.96, AT: 0.04, TT: 0.00; Other- AA: 0.92, AT: 0.08, TT: 0.00; Overall- AA: 0.92, AT: 0.08, TT: 0.00	Lean yield; fat yield; grade fat	LM area; hot carcass weight; quality grade; semitendinosus muscle SF at 7 d postmortem
Clempson et al., 2011	509 HO heifers offered concentrate and forage diets	AA: 0.95, AT: 0.05, TT: 0.00	-	Height at withers; crown rump length; heart girth; age at first service; number of services; age at calving; CI; days to first service; days to conception; prevalence of having calf 100 d postpartum
de Oliveira et al., 2013	100 NE cattle (forage at 1st, forage & concentrate at 2nd, and finishing diet at 3rd periods)	AA: 0.20, AT: 0.80, TT: 0.00	Carcass fat distribution; carcass marbling; carcass fat thickness	Weights at 1st, 2nd, and 3rd periods; ADG between 1st and 2nd periods; ADG between 2nd and 3rd periods; US REA; US BFT; carcass REA;

Table A1. Single nucleotide polymorphisms in the promoter and coding region of the bovine leptin (*LEP*) and leptin receptor (*LEPR*) genes and their phenotypic associations with production and reproduction traits (Continued)

Polymorphism, their location and Study[1]	Population and Diets[2]	Genotype frequency	Associations found[3]	Associations not found[3]
LEP c.357C>T (A59V, A80V, Lep*Hph*I), Exon 3 of the *LEP*				
Komisarek and Antkowiak, 2007	219 JE cows	*CC: 0.64, CT: 0.32, TT: 0.04	-	Age at first calving; days open; CI; number of AI per conception; GL
Clempson et al., 2011	509 HO heifers offered concentrate and forage diets	CC: 0.61, CT: 0.33, TT: 0.06	Crown-rump length; age at first service; age at calving	Height at withers; heart girth; number of services; CI; days to first service; days to conception; prevalence of having calf 100 d postpartum
Liefers et al., 2002	613 HO heifers fed forage and concentrate diets	CC: 0.58, CT: 0.33, TT: 0.09	-	FI; dry matter intake; LW; commencement of luteal activity
Komisarek, 2010	309 Polish HO bulls	CC: 0.50, CT: 0.43, TT: 0.07	Non-return rate in cows	Age at first insemination
Jecminkova et al., 2018	786 Czeck Fleckvieh cows	CC: 0.58, CT: 0.37, TT: 0.05	-	CI, age at first calving; calving to the 1st insemination; days open; pregnancy after the 1st service
Kulig and Kmieć, 2009	129 Limousin calves fed grass-based diet	CC: 0.54, CT: 0.39, TT: 0.07	BW at 210 days of age; ADG between 3 and 210 days of age	BW at 3 and 365 days of age; ADG between 3 and 365 days of age
Nkrumah et al., 2006	464 crossbred beef steers offered feedlot diet	CC: 0.06, CT: 0.38, TT: 0.56	Serum leptin level; ADG; FCR; US backfat; US LM area; average carcass backfat; carcass LM area; carcass lean meat yield	DMI; US MS; carcass MS; carcass grade fat; carcass YG
Silva et al., 2014	100 NE cattle fed grass and mineralized salt (P1), grass and concentrate (P2), and concentrate (P3)	CC: 0.00, CT: 0.30, TT: 0.70	BFT	BW at P1, P2 and P3; ADG between P1 and P2, and between P2 and P3; subcutaneous fat thickness; REA; marbling
Dandapat et al., 2009	30 SW and 70 *Bos indicus* × *Bos taurus* cattle	CC: 0.36, CT: 0.57, TT: 0.07	Birth weight; weight at 12-month	First CI; First service period

Table A1. Single nucleotide polymorphisms in the promoter and coding region of the bovine leptin (*LEP*) and leptin receptor (*LEPR*) genes and their phenotypic associations with production and reproduction traits (Continued)

Polymorphism, their location and Study[1]	Population and Diets[2]	Genotype frequency	Associations found[3]	Associations not found[3]
*LEP n.*Sau3AI, Intron 2 of the *LEP*				
Trakovická et al., 2013	296 SS and 85 P cows	SS- AA: 0.70, AB: 0.27, BB: 0.03; P- AA: 0.45, AB: 0.49, BB: 0.06	Age at first calving	CI; days open; insemination interval
Almeida et al., 2003	96 synthetic (5/8 AN × 3/8 NE) females raised on pasture	*AA: 0.40, AB: 0.46, BB: 0.14	-	CI; weight at first calving
Almeida et al., 2003	149 synthetic (5/8 AN × 3/8 NE) females raised on pasture	*(-/-): 0.88, (+/-): 0.12, (+/+): 0.00	CI	Weight at first calving
Oikonomou et al., 2009	497 primiparous HO cows fed a TMR	AA: 0.78, AB: 0.22, BB: 0.00	Presence of metritis	Body condition score; no. of inseminations per conception; calving to conception interval; interval between 1st and 2nd calving; conception rate in the 1st 305 days of 1st lactation, following 1st insemination of 1st or 2nd lactation
Liefers et al., 2002	613 HO heifers fed forage and concentrate diets	AA: 0.81, AB: 0.19, BB: 0.00	-	FI; DMI; LW; commencement of luteal activity
Oprzadek et al., 2003	145 Black-and-White growing bulls fed forage & concentrate up to 15 m but only concentrate on performance test	-	DMI; crude protein intake; fat of carcass-side; chest depth	LW; FI; withers height; chest girth; chest width
Kulig and Kmieć, 2009	129 Limousin calves fed grass-based diet	CC (AA): 0.65, CT (AB): 0.33, TT (BB): 0.02	-	BWs at 3, 210, and 365 days of age; ADG between 3 and 210 days, 3 and 365 days of age
LEP g.207C>T (UASMS1), Leptin promoter				
Clempson et al., 2011	509 HO heifers offered concentrate and forage diets	CC: 0.18, CT: 0.45, TT: 0.37	prevalence of having calf 100 d postpartum in lactation 1; days to conception and CI in lactation 2	Height at withers; crown-rump length; heart girth; number of services; total number of AI services; age at first service; age at calving in lactation 1; days to first service in lactation 2

Table A1. Single nucleotide polymorphisms in the promoter and coding region of the bovine leptin (*LEP*) and leptin receptor (*LEPR*) genes and their phenotypic associations with production and reproduction traits (Continued)

Polymorphism, their location and Study[1]	Population and Diets[2]	Genotype frequency	Associations found[3]	Associations not found[3]
da Silva et al., 2012	2162 young NE bulls (on pasture until 18 months & in feedlot until harvest)	CC: 0.96, CT: 0.04, TT: 0.00	LM area	WW; YW; weight gain; BFT; RFAT
Banos et al., 2008	571 HO cows fed high and low concentrate diets on feed trial	CC: 0.13, CT: 0.46, TT: 0.42	-	FI; DMI; FI over milk yield; DMI over milk yield; LW; BCS
Schenkel et al., 2005	43 AN, 30 LM, 11 CH, 68 SM, and 959 animals with breed composition <5/8 for all breeds (heifers/steers/bulls) fed a finishing diet	*AN- CC: 0.24, CT: 0.50, TT: 0.26; LM- CC: 0.23, CT: 0.50, TT: 0.27; CH- CC: 0.21, CT: 0.49, TT: 0.30; SM- CC: 0.12, CT: 0.45, TT: 0.43; Other- CC: 0.15, CT: 0.47, TT: 0.38; Overall- CC: 0.15, CT: 0.48, TT: 0.37	Carcass fat yield percentage	Grade fat; chemical fat; BFT, lean meat yield percentage, LM area, quality grade; hot carcass weight; LM SF; SM SF
Pannier et al., 2009	37 AN, 18 BB, 12 BD, 79 CH, 67 HO, 32 HH, 117 LM, 11 SA, and 57 SM	AN- CC: 0.11, CT: 0.57, TT: 0.32; BB- CC: 0.06, CT: 0.11, TT: 0.83; BD- CC: 0.17, CT: 0.50, TT: 0.33; CH- CC: 0.17, CT: 0.44, TT: 0.39; HO- CC: 0.07, CT: 0.42, TT: 0.51; HH- CC: 0.28, CT: 0.50, TT: 0.22; LM- CC: 0.10, CT: 0.36, TT: 0.54; SA- CC: 0.09, CT: 0.27, TT: 0.64; SM- CC: 0.02, CT: 0.31, TT: 0.67	-	IMF values
LEP g.528C>T (UASMS2), Leptin promoter				
Clempson et al., 2011	509 HO heifers offered concentrate and forage diets	CC: 0.74, CT: 0.24, TT: 0.02	Number of services; total number of AI services	Height at withers; crown-rump length; heart girth; age at first service; age at calving and prevalence of having calf 100 d postpartum in lactation 1; days to first service, days to conception and CI in lactation 2

Table A1. Single nucleotide polymorphisms in the promoter and coding region of the bovine leptin (*LEP*) and leptin receptor (*LEPR*) genes and their phenotypic associations with production and reproduction traits (Continued)

Polymorphism, their location and Study[1]	Population and Diets[2]	Genotype frequency	Associations found[3]	Associations not found[3]
Nkrumah et al., 2005	150 crossbred animals (131 steers & 19 bulls; they started on background diet but on finishing diet during test period)	Experimental- CC: 0.63, CT: 0.32, TT: 0.05; Commercial- CC: 0.63, CT: 0.34, TT: 0.03	Serum leptin concentration; metabolic BW; ADG; DMI; feeding duration; US BFT; US MS	Final BW; RFI; F:G; feed bunk attendance; US LM area
Nkrumah et al., 2006	464 crossbred beef steers offered feedlot diet	CC: 0.67, CT: 0.30, TT: 0.03	Serum leptin level; DMI; US backfat; US MS	ADG; FCR; US LM area; average carcass backfat; carcass LM area; carcass lean meat yield; carcass grade fat; carcass YG
Banos et al., 2008	571 HO cows fed high and low concentrate diets on feed trial	CC: 0.82, CT: 0.17, TT: 0.01	-	FI; DMI; FI over milk yield; DMI over milk yield; LW; BCS
Pannier et al., 2009	37 AN, 18 BB, 12 BD, 79 CH, 67 HO, 32 HH, 117 LM, 11 SA, and 57 SM	AN- CC: 0.11, CT: 0.57, TT: 0.32; BB- CC: 0.06, CT: 0.11, TT: 0.83; BD- CC: 0.17, CT: 0.50, TT: 0.33; CH- CC: 0.17, CT: 0.44, TT: 0.39; HO- CC: 0.07, CT: 0.42, TT: 0.51; HH- CC: 0.28, CT: 0.50, TT: 0.22; LM- CC: 0.10, CT: 0.36, TT: 0.54; SA- CC: 0.09, CT: 0.27, TT: 0.64; SM- CC: 0.02, CT: 0.31, TT: 0.67	-	IMF values
Corva et al., 2009	253 grazing BN (5/8 AN × 3/8 BR) steers received a grass-based diet	CC: 0.55, CT: 0.30, TT: 0.15	US final REA	LW gain; gain in BFT; final LW; US BFT; carcass weight; carcass BFT; carcass REA; carcass IMF

Table A1. Single nucleotide polymorphisms in the promoter and coding region of the bovine leptin (*LEP*) and leptin receptor (*LEPR*) genes and their phenotypic associations with production and reproduction traits (Continued)

Polymorphism, their location and Study[1]	Population and Diets[2]	Genotype frequency	Associations found[3]	Associations not found[3]
Schenkel et al., 2005	43 AN, 30 LM, 11 CH, 68 SM, and 959 animals with breed composition <5/8 for all breeds (heifers/steers/bulls) fed a finishing diet	*AN- CC: 0.54, CT: 0.39, TT: 0.07; LM- CC: 0.43, CT: 0.45, TT: 0.12; CH- CC: 0.60, CT: 0.35, TT: 0.05; SM- CC: 0.49, CT: 0.42, TT: 0.09; Other- CC: 0.55, CT: 0.38, TT: 0.07; Overall- CC: 0.54, CT: 0.39, TT: 0.07	-	Lean yield; fat yield; grade fat; chemical fat; BFT, lean meat yield percentage, LM area, quality grade; hot carcass weight; LM SF; SM SF
LEP g.1759C>G (UASMS3), Leptin promoter				
Nkrumah et al., 2005	150 crossbred animals (131 steers & 19 bulls; they started on background diet but on finishing diet during test period)	Experimental- CC: 0.18, CG: 0.46, GG: 0.36; Commercial- CC: 0.26, CG: 0.52, GG: 0.22	Metabolic BW; final BW; DMI; feeding duration; US BFT	Serum leptin concentration; ADG; RFI; F:G; feed bunk attendance; US MS; US LM area
LEP g.-963C>T (C963T), Leptin promoter				
Komisarek and Antkowiak, 2007	219 JE cows	*CC: 0.69, CT: 0.28, TT: 0.03	-	Age at first calving; days open; CI; number of AI per conception; GL
Liefers et al., 2005	613 HO heifers fed forage and concentrate diets	*CC: 0.45, CT: 0.44, TT: 0.11	FI; DMI; Energy balance; first observed estrus	LW; first postpartum luteal activity
Giblin et al., 2010	848 progeny tested HO dairy cattle sires	CC: 0.41, CT: 0.46, TT: 0.14	Direct calving difficulty; GL	Survival in the herd; CI; maternal calving difficulty; perinatal calf mortality; BCS; carcass fat
Komisarek, 2010	309 Polish HO bulls	CC: 0.28, CT: 0.55, TT: 0.17	Non-return rate in cows	Age at first insemination
Jecminkova et al., 2018	786 Czeck Fleckvieh cows	CC: 0.64, CT: 0.30, TT: 0.06	Age at first calving	CI, calving to the 1st insemination; days open; pregnancy after the 1st service

Table A1. Single nucleotide polymorphisms in the promoter and coding region of the bovine leptin (*LEP*) and leptin receptor (*LEPR*) genes and their phenotypic associations with production and reproduction traits (Continued)

Polymorphism, their location and Study[1]	Population and Diets[2]	Genotype frequency	Associations found[3]	Associations not found[3]
da Silva et al., 2012	2162 young NE bulls (on pasture until 18 months & in feedlot until harvest)	CC: 0.96, CT: 0.04, TT: 0.00	LM area	WW; YW; weight gain; BFT; RFAT
Banos et al., 2008	571 HO cows fed high and low concentrate diets on feed trial	CC: 0.42, CT: 0.45, TT: 0.13	-	FI; DMI; FI over milk yield; DMI over milk yield; LW; BCS
LEP g.-1457A>G, Leptin promoter				
Clempson et al., 2011	509 HO heifers offered concentrate and forage diets	AA: 0.23, AG: 0.47, GG: 0.30	Height at withers; total number of AI services; days to conception and CI in lactation 2	Crown-rump length; heart girth; age at first service; Number of services; age at calving and prevalence of having calf 100 d postpartum in lactation 1; days to first service in lactation 2
Liefers et al., 2005	613 HO heifers fed forage and concentrate diets	* AA: 0.29, AG: 0.50, GG: 0.21	First postpartum luteal activity	LW; FI; DMI; energy balance; first observed estrus
da Silva et al., 2012	2162 young NE bulls (on pasture until 18 months & in feedlot until harvest)	AA: 0.60, AG: 0.34, GG: 0.06	-	Weight gain; WW; YW; BFT; LM area; RFAT
Banos et al., 2008	571 HO cows fed high and low concentrate diets on feed trial	AA: 0.27, AG: 0.51, GG: 0.22	DMI	FI; FI over milk yield; DMI over milk yield; LW; BCS
Silva et al., 2014	100 NE cattle fed grass and mineralized salt (P1), grass and concentrate (P2), and concentrate (P3)	AA: 0.05, AG: 0.54, GG: 0.41	-	BW at P1, P2 and P3; ADG between P1 and P2, and between P2 and P3; subcutaneous fat thickness; REA; marbling; BFT
Giblin et al., 2010	848 progeny tested HO dairy cattle sires	AA: 0.27, AG: 0.50, GG: 0.23	-	Survival in the herd; CI; maternal calving difficulty; Direct calving difficulty; GL; perinatal calf mortality; BCS; carcass fat
Matteis et al., 2012	95 HO cows	AA: 0.18, AG: 0.49, GG: 0.33	Rump width	Stature; angularity; body depth;

Table A1. Single nucleotide polymorphisms in the promoter and coding region of the bovine leptin (*LEP*) and leptin receptor (*LEPR*) genes and their phenotypic associations with production and reproduction traits (Continued)

Polymorphism, their location and Study[1]	Population and Diets[2]	Genotype frequency	Associations found[3]	Associations not found[3]
LEP g.-1238G>C, Leptin promoter				
Giblin et al., 2010	848 progeny tested HO dairy cattle sires	CC: 0.14, CG: 0.45, GG: 0.40	GL	Survival in the herd; CI; maternal calving difficulty; Direct calving difficulty; perinatal calf mortality; BCS; carcass fat
Liefers et al., 2005	613 HO heifers fed forage and concentrate diets	*CC: 0.11, CG: 0.44, GG: 0.45	-	LW; FI; DMI; energy balance; first postpartum luteal activity; first observed estrus
LEP g.-2470C>T, Leptin promoter				
Giblin et al., 2010	848 progeny tested HO dairy cattle sires	CC: 0.75, CT: 0.24, TT: 0.01	Perinatal calf mortality	Survival in the herd; CI; GL; maternal calving difficulty; Direct calving difficulty; BCS; carcass fat
LEP g.-578C>G, *LEP g.-415G>ΔG*, *LEP g.-292T>C*, *LEP g.-282G>T*, *LEP g.-272G>A*, *LEP g.-211A>G*, *LEP g.-201C>T*, *LEP g.-197A>C*, *LEP g.-170C>T*, *LEP g.-147C>T*, *LEP g.-105C>G*, Leptin promoter				
Liefers et al., 2005	613 HO heifers fed forage and concentrate diets	*CC: 0.31, CG: 0.49, GG: 0.19; GG: 0.53, GΔG: 0.40, ΔGΔG: 0.07; TT: 0.28, CT: 0.50, CC: 0.22; GG: 0.81, GT: 0.18, TT: 0.01; GG: 0.40, AG: 0.46, AA: 0.14; AA: 0.81, AG: 0.18, GG: 0.01; CC: 0.77, CT: 0.21, TT: 0.02; AA: 0.65, AC: 0.31, CC: 0.04; CC: 0.36, CT: 0.48, TT: 0.16; CC: 0.28, CT: 0.50, TT: 0.22; CC: 0.34, CG: 0.49, GG: 0.17	-	LW; FI; DMI; energy balance; first postpartum luteal activity; first observed estrus
LEPR c.115C>T (*T945M*), Exon 22 of the *LEPR*				
Trakovická et al., 2013	296 SS and 85 P cows	SS- CC: 0.92, CT: 0.08; P- CC: 0.82, CT: 0.18	CI	Age at first calving; days open; insemination interval

Table A1. Single nucleotide polymorphisms in the promoter and coding region of the bovine leptin (*LEP*) and leptin receptor (*LEPR*) genes and their phenotypic associations with production and reproduction traits (Continued)

Polymorphism, their location and Study[1]	Population and Diets[2]	Genotype frequency	Associations found[3]	Associations not found[3]
Komisarek, 2010	309 Polish HO bulls	CC: 0.84, CT: 0.15, TT: 0.01	Age at first insemination	Non-return rate in cows
da Silva et al., 2012	2162 young NE bulls (on pasture until 18 months & in feedlot until harvest)	CC: 0.77, CT: 0.21, TT: 0.02	Weight gain	WW; YW; BFT; LM area; RFAT
Clempson et al., 2011	509 HO heifers offered concentrate and forage diets	CC: 0.85, CT: 0.15, TT: 0.00	-	Height at withers; crown-rump length; heart girth; number of services; total number of AI services; age at first service; age at calving and prevalence of having calf 100 d postpartum in lactation 1; days to first service, days to conception and CI in lactation 2
Liefers et al., 2004	323 HO cows	CC: 0.93, CT: 0.07, TT: 0.00	Circulatory leptin levels during late pregnancy	Circulatory leptin concentration during lactation
Giblin et al., 2010	848 progeny tested HO dairy cattle sires	CC: 0.83, CT: 0.17, TT: 0.01	-	Direct calving difficulty; GL; survival in the herd; CI; maternal calving difficulty; perinatal calf mortality; BCS; carcass fat
LEPR g.134260C>T, *LEPR* promoter				
Matteis et al., 2012	95 HO cows	CC: 0.67, CT: 0.30, TT: 0.03	Angularity	Stature; body depth; rump width
LEPR c.138780T>G, Exon 2 of *LEPR* gene				
Matteis et al., 2012	95 HO cows	TT: 0.21, TG: 0.50, GG: 0.29	Stature	Angularity; body depth; rump width

Table A1. Single nucleotide polymorphisms in the promoter and coding region of the bovine leptin (*LEP*) and leptin receptor (*LEPR*) genes and their phenotypic associations with production and reproduction traits (Continued)

Polymorphism, their location and Study[1]	Population and Diets[2]	Genotype frequency	Associations found[3]	Associations not found[3]
LEPR g.134261G>C, *LEPR* promoter; *LEPR c.184626G>C*, Exon 11 of the *LEPR*; *LEPR c.209779C>T*, Exon 18 of the *LEPR*				
Matteis et al., 2012	95 HO cows	CC: 0.01, CG: 0.18, GG: 0.81; CC: 0.01, CG: 0.16, GG: 0.83; CC: 0.81, CT: 0.18, TT: 0.01	-	Stature; angularity; body depth; rump width
LEPR c.210413A>C, Exon 18 of the *LEPR*				
Matteis et al., 2012	95 HO cows	AA: 0.86, AC: 0.13, CC: 0.01	Angularity	Stature; body depth; rump width

[1]Codes in parentheses have been used for a given SNP marker in previous studies
[2]HO: Holstein/Holstein Friesian; JE: Jersey; NE: Nellore; AN: Angus; HH: Hereford; CH: Charolais; SM: Simmental; LM: Limousin; BR: Brahman; BN: Brangus; BB: Belgian Blue; BD: Blonde d'Aquitaine; SA: Salers; SW: Sahiwal; SS: Slovak Spotted; P: Pinzgau; TMR: total mixed ration
[3]AI: artificial insemination; BW: body weight; GL: gestation length; CI: calving interval; BCS: body condition score; WW: weaning weight; LM: Longissimus muscle; BFT: backfat thickness; RFAT: rump fat thickness; YW: yearling weight; LW: live weight; US: ultrasound; REA: rib eye area; ADG: average daily gain; YG: yield grade; MS: marbling score; RFI: residual feed intake; DMI: dry matter intake; F:G: feed to gain ratio; FI: feed intake; SF: shear force; IMF: intramuscular fat; P1: 1st period; P2: 2nd period; P3: 3rd period; FCR: feed conversion ratio
*Genotype frequencies were calculated from the allele frequencies

Table A2. *P*-value from contrast tests of the *LEP c.73C>T* genotypes for significant reproductive traits

	Contrast test *P*-value	
Traits	CC vs. CT and TT	TT vs. CC and CT
Ovary		
Diameter, mm		
Average	0.002	0.191
Length, mm		
Left	0.027	0.743
Average	0.006	0.277
Follicles		
Antral follicle count	0.035	0.613
Left		
Small	0.034	0.887
Overall		
Small	0.003	0.732

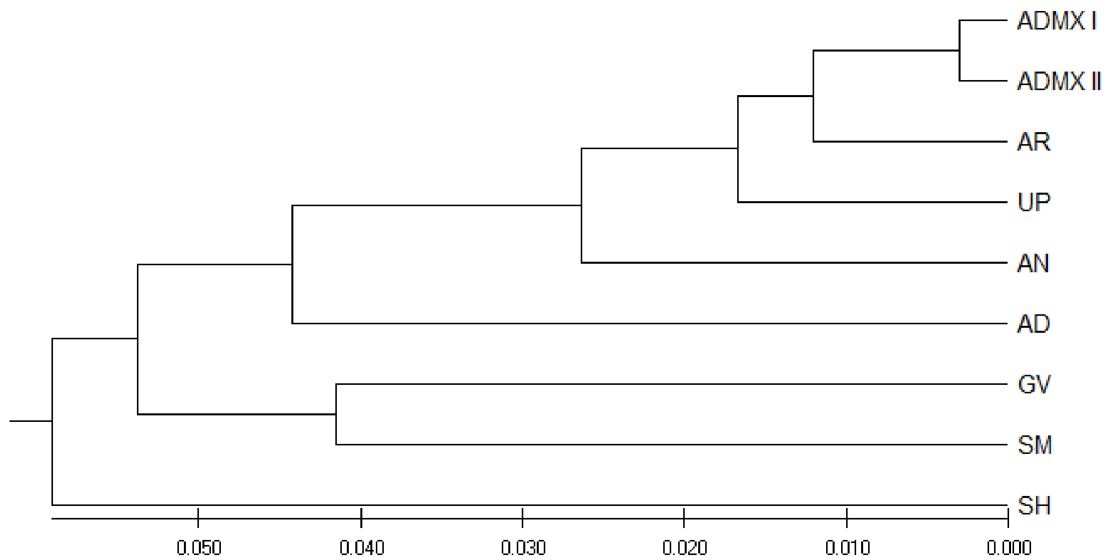

Figure A1. Phylogenetic construction for nine beef cattle sub-populations. Unweighted pair group method with arithmetic mean (UPGMA) tree using pairwise F_{ST} distances. The tree is drawn to scale, with branch lengths in the same units as those of the pairwise F_{ST} distances used to infer the phylogenetic tree. Beef cattle sub-populations include Angus (AN), Red Angus (AR), Gelbvieh (GV), American Aberdeen (AD), Shorthorn (SH), Simmental (SM), Undetermined parentage (UP), Admixed population I (ADMX I), and Admixed population II (ADMX II).

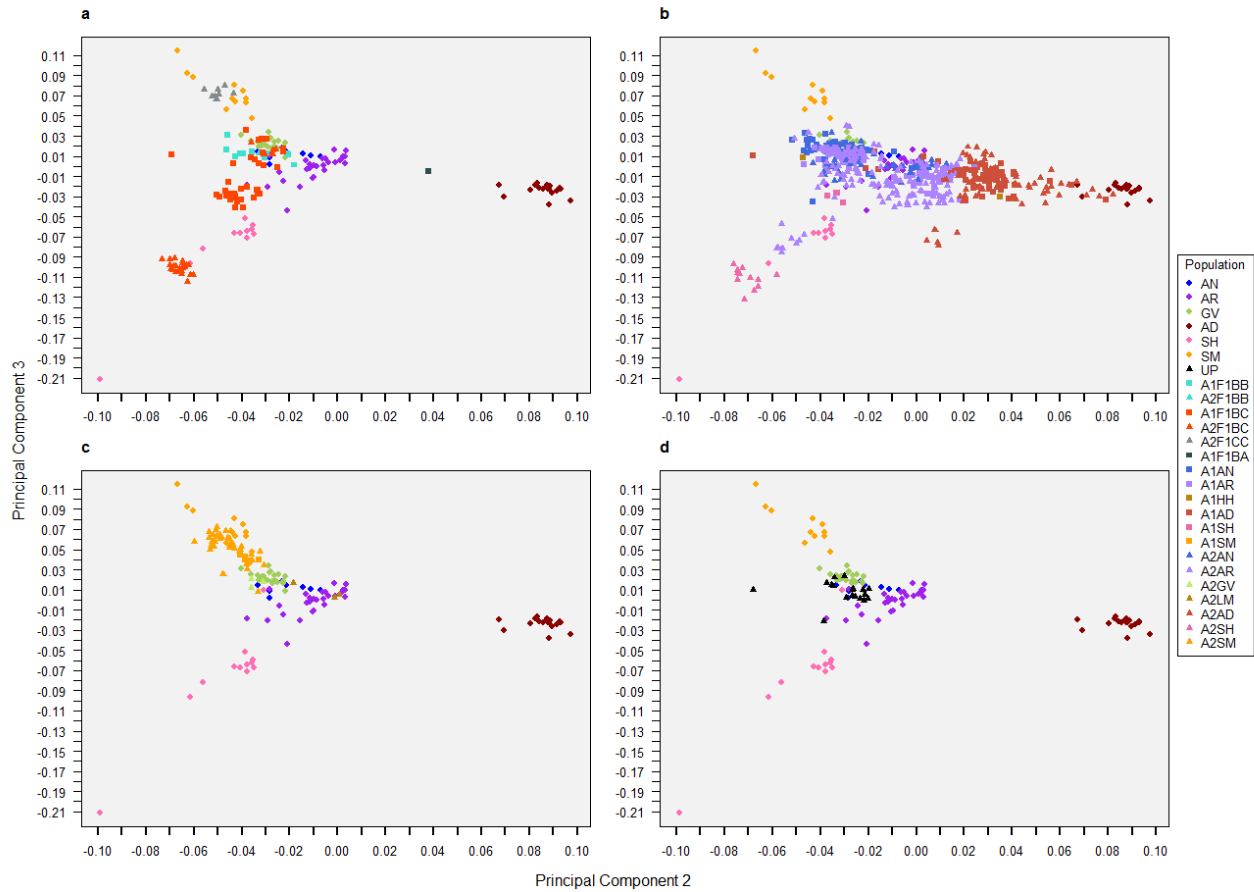

Figure A2. Principal components 2 and 3 of purebred and admixed populations with primary breed of admixed individuals designated based on pedigree. Molecular variation explained within and across populations using principal components 2 and 3 with purebreds (Angus, AN; Red Angus, AR; Gelbvieh, GV; American Aberdeen, AD; Shorthorn, SH; Simmental, SM) and admixed populations (ADMXI, A1 and ADMXII, A2) sub-grouped based on primary breed (≥ 50%) of **a)** F_1 (F_1 British×British, A1F1BB and A2F1BB; F_1 British×Continental, A1F1BC and A2F1BC; F_1 Continental×Continental, A2F1CC; F_1 British×Australian, A1F1BA), **b)** British and Australian breeds (Angus, A1AN and A2AN; Red Angus, A1AR and A2AR; American Aberdeen, A1AD and A2AD; Hereford, A1HH; Shorthorn, A1SH and A2SH), **c)** Continental breeds (Gelbvieh, A2GV; Limousin, A2LM; Simmental, A1SM and A2SM), and **d)** Undetermined parentage (UP).

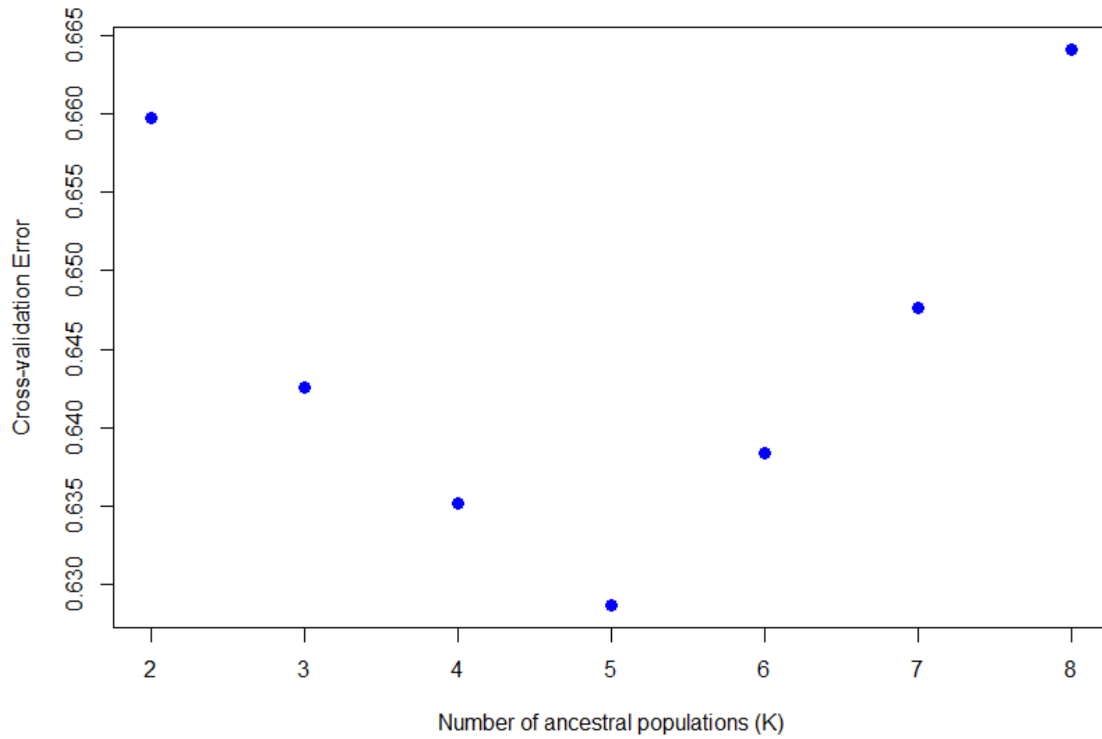

Figure A3. Cross-validation error plot for ancestral populations. Potential ancestral populations (*K*) explored were 2 to 8 based on population structure.

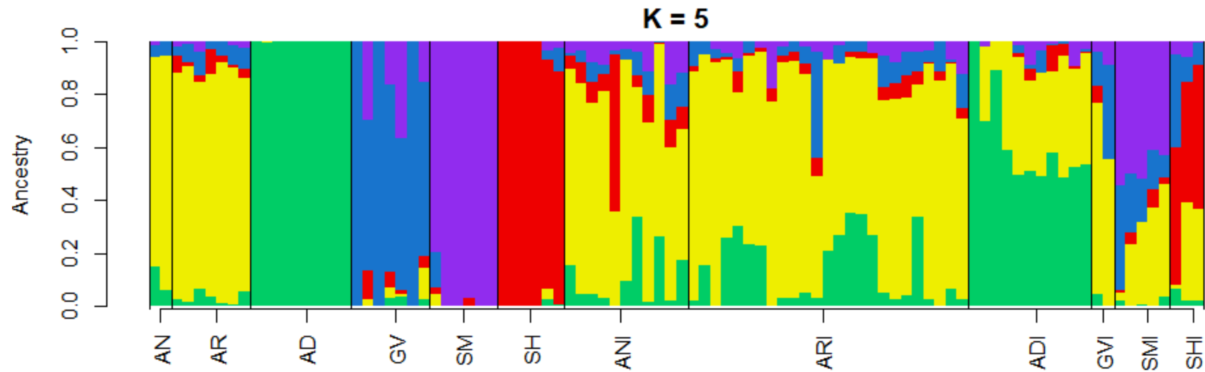

Figure A4. Bar plot of the *Q* matrix from an unsupervised ADMIXTURE run in unrelated individuals. Individuals (n = 94) are represented by a single vertical bar and segregated into *K* = 5 colored segments with each segment's length showing the proportion of the individual's genome for a given ancestral grouping. Purebred populations are separated by black lines and include Angus (AN), Red Angus (AR), American Aberdeen (AD), Gelbvieh (GV), Simmental (SM), Shorthorn (SH), Angus-influenced (ANI), Red Angus-influenced (ARI), American Aberdeen-influenced (ADI), Gelbvieh-influenced (GVI), Simmental-influenced (SMI), and Shorthorn-influenced (SHI).