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SEASONAL BODY CONDITION SCORE AND YIELD DYNAMICS OF NORMANDE CROSSBREDS COMPARED WITH PUREBREDS AND OTHER CROSSBREDS AND TELOMERE LENGTH IN PUREBRED AND CROSSBRED CATTLE

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ABSTRACT

The objectives of this study were to evaluate body condition score (BCS), milk production, reproductive performance and how performance changes across seasons and system (grazing, non-grazing) among animals sired by Normande, Ayrshire, Holstein, Jersey, and other breeds and also evaluate telomere length among crossbred and purebred cattle. Six herds with Normande crossbreds were visited once each during January, May, August, and October during 2011 to represent different seasons. Milk production and pedigree information was collected for all herds. One herd in Minnesota also provided pedigree and milk production information, but no BCS information. Cattle from one herd with Normande crossbreds provided blood for DNA extraction and telomere length analysis. Purebred Holsteins from the Penn State dairy research center, crossbred and purebred beef animals from the Penn State beef research center and a commercial Pennsylvania beef farm also provided blood for telomere length analysis.

Multiple trait mixed-models were used to analyze BCS, milk yield, fat yield and percentage, protein yield and percentage, somatic cell score, and calving interval among Normande crossbreds and their herd mates sired by Ayrshire, Holstein, Jersey and other breeds. Normande sired cattle had greater body condition than animals sired by Ayrshires, Holsteins, Jerseys and other breeds. Daily milk yield, fat yield, and protein yield were all influenced by season in herds that utilized greater amounts of pasture and were lowest during the summer months of July through August.

Normande cattle were able to mobilize more body reserves during the summer season, when pasture growth was expected to be low, than cows sired by other breeds; however, differences with Ayrshire, Holstein, and Jersey sired cattle were not significant.

Decreases in fat yield were lower for Normande (-0.08 kg/d) during the summer months when compared to Holstein sired animals (-0.11 kg/d). Cows sired by bulls of unknown origin, crossbred sires, or breeds other than Ayrshire, Holstein, Jersey or Normande had larger declines in milk, fat and protein yield during summer months in grazing herds. This reinforces the need to use proven sires, even in grazing systems that incorporate crossbreeding.

Mixed models were used to estimate telomere length for individual animals, herd, and breeds. Dairy and beef cows were evaluated separately because there was no breed overlap between herds to facilitate evaluation of breed versus herd effects. Herd was highly significant within both beef and dairy analyses ($P< 0.001$). There was a significant decline in telomere length as age increased in dairy cattle ($P<0.05$), but the decline was not significant for beef cattle ($P>0.05$). Adding breed to the model was not significant for dairy ($P = 0.71$) or beef ($P = 0.92$) animals, indicating that there was no difference in telomere length between crossbred and purebred animals. Results suggested wide variation among animals in telomere length at any given age. There was no evidence that longer telomere length explained the improved survivability reported for crossbred dairy cattle.

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LIST OF ABBREVIATIONS

AY = Ayrshire
BCS =Body Condition Score
CB = Commercial Beef Herd
CD = Commercial Dairy Herd
CINT = Calving Interval
DHI = Dairy Herd Information
DIM = Days in Milk
DKC = Dyskeratosis Congenita
DMI = Dry Matter Intake
GWAS = Genome Wide Association Study
HO = Holstein
JE = Jersey
NO = Normande
PCR = Polymerase Chain Reaction
PM = Predicted Means
POT1 = Protection of Telomeres Protein 1
PSUB = Penn State Beef Research Herd
PSUD = Penn State Dairy Research Herd
QFISH = Quantitative Fluorescent in situ Hybridization
QPCR = Quantitative Polymerase Chain Reaction
QTL = Quantitative Trait Locus
SCS = Somatic Cell Score
SNP = Single Nucleotide Polymorphism
TPP1 = Tripeptidyl-peptidase 1
TERC = Telomerase RNA Component
TERT = Telomerase Reverse Transcriptase
TIN2 = TERF Interacting Nuclear Factor 2
TRF = Terminal Restriction Fragment
TRF1 = Telomere Repeat Binding Factor 1
TRF2 = Telomere Repeat Binding Factor 2
WRN = Werner Syndrome

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Chapter 1

Review of the Literature

Selection

Cattle have undergone human selective breeding for many years. This selection has always been carried out with the intent to improve desirable traits in the animal that will benefit their human counterparts. Selection determines which individuals will become parents, the number of offspring to keep, and how long animals can stay in the population (Bourdon, 1997). Selection changes gene frequencies in the population, and response to genetic selection is dependent on three main factors: heritability of the trait, selection intensity, and the selection differential (Falconer and Mackay, 1996). Heritability is a measurement that describes the relationship between phenotypic values of a trait and the genetic breeding value of a trait (Bourdon, 1997), and is defined as the additive genetic variance divided by the phenotypic variance (Falconer and Mackay, 1996). Selection intensity is a function of the proportion of animals that are selected (Bourdon, 1997), and selection differential is the average superiority of the parents (Falconer and Mackay, 1996). Selection has caused cattle to diverge into two main groups: beef cattle (bred for carcass utilization) and dairy cattle (bred for milk utilization). There are also breeds of cattle that are considered dual-purpose breeds, which are selected for desirable carcass and dairy qualities. Normande cattle can be

considered a dual purpose breed, with more selection emphasis being placed on dairy characteristics.

Purebred dairy cattle have been intensively selected for milk production traits because milk yields are the major source of income for dairy farms. This intense selection has improved milk production in dairy cattle, but has come with a decline in health and fertility traits in dairy cattle (USDA-AIPL, 2012). Body condition score (BCS) has also declined with increased selection for dairy traits due to a negative genetic correlation between milk yield and BCS (Dechow, 2001; Veerkamp and Brotherstone, 1997). However, BCS may be a good indicator trait for health and reproductive traits. Selecting for increased BCS or crossbreeding for increased BCS could have a positive impact on important cattle traits that have suffered from unfavorable correlations to milk production traits.

Low Input Grazing Systems

Historically, dairy production in the United States has benefited from a favorable ratio between feed and milk prices, allowing for the selection of cows to maximize milk output per cow (Muller and Fales, 1998). Feed has become increasingly expensive as grain prices have increased over the past ten years, as have other farm expenditures such as fuel, land rent, and labor, (USDA-NASS, 2012). Low input forage based systems have thus become an increasingly popular way that dairy producers are looking at to reduce total farm costs and improve profitability on dairy farms.

However, dairy animals have lower milk yields when consuming a high forage diet compared to their confinement counterparts (Kolver and Muller, 1998), which

reduces the main source of revenue for dairy farms, milk yield. The reduced milk yield may be a result of lower dry matter intake (DMI) for cows on pasture (Leaver, 1985; Kolver and Muller, 1998) and cows in pasture systems may not receive enough energy to produce at their highest genetic potential.

Even with lower milk production, the decreased input associated with grazing can offset the lost income from milk production to maintain or improve profit (Parker et al., 1992). Farmers using a grazing system were shown to have increased profit per cow compared to confinement herds in the Great Lake region even with lower production per cow (Kriegl, 2006). Decreased costs in grazing herds have been shown to include machinery costs, cow replacement costs, veterinarian costs and forage production costs (Kriegl, 2006).

Certain areas or regions in the United States may provide a comparative economic advantage for low input pasture based dairy systems (Parsons, 2010). Opportunity cost of producing pasture forage rather than other crops such as corn and soybeans should taken into account, and other factors such as the local milk market and if there is a potential economic advantage for pasture raised dairy products in the region should be considered in determining if low input pasture based systems are appropriate (USDA-NRCS, 2007).

Genotype × environment interactions have been recognized for production traits when investigating genetic correlations between confinement and grazing systems, although the interaction was small (Kearney et al., 2004). However, the frequency of sire re-ranking for production traits is reported to be small and does not warrant independent genetic evaluations for dairy cattle in the differing systems (Boettcher et al., 2003;

Weigel et al., 1999; Kearney et al., 2004). Interestingly, different feeding systems have shown a scaling effect regarding genetic potential for production traits with pasture based cows having a response rate of approximately 70% when compared to cows with high concentrate supplementation (Boettcher et al., 2003; Weigel et al., 1999; Veerkamp et al., 1994). This indicates that high genetic merit cows may not obtain all of the nutritional resources required to meet their genetic potential for production traits in low input grazing systems (Kolver and Muller, 1998; Holden et al., 1995).

Dairy cattle of high genetic merit that have been selected in generous feeding systems may be impacted more severely by energy restrictions that dairy cattle can experience in pasture systems than dairy cattle that have been historically selected in grazing systems (Macdonald et al., 2007). Holsteins (HO) are more likely to direct all nutrients toward milk production when energy from pasture and supplementation is not limited and then have few body energy reserves left to mobilize when energy is limited, thus needing increased concentrate supplementation (Pryce et al., 2006). Even when feed supplementation occurs in pasture systems, it still may not prevent undesirable BCS loss in HO cows (Macdonald et al., 2008). This undesirable BCS loss may lead to negative effects on reproduction and animal health. Reproductive performance is of increased importance in many pasture based herds due to the use of seasonal calving to optimize the grazing season and reproductive inefficiencies may hinder the potential of production in pasture based systems (Evans et al., 2006).

Selection of cattle in grazing systems versus selection in intensive commercial systems also seems to impact grazing behavior. Grazing behavior in New Zealand HO (which have historically been raised on pasture) has been shown to be different than

North American HO suggesting that genetics impact grazing behavior and efficiency (Sheahan et al., 2011).

If a producer is going to take advantage of a low input grazing system, proper management of the system, including genetics, is essential for success. Breeding goals of low input grazing systems should differ from conventional breeding goals. For example, if a cow of high genetic merit cannot meet its nutrient demand for high milk production in a pasture based system, then it may be inappropriate to continue to select for increased milk production (Mayne, 1998). Other traits such as reproductive performance, feed efficiency, stature, and milk solids may play a more important role in selection (Mayne, 1998).

Crossbreeding

Crossbreeding presents another intriguing opportunity for the animal breeder to improve their herd. Crossbreeding is widely utilized in most livestock segments in the United States including beef cattle, swine, and poultry production systems. However, crossbreeding is not widely utilized in the United States dairy industry, partly due to purebred Holstein cattle being superior in yields of milk, fat and protein. Enticing dairy producers to crossbreed is also difficult due to producer concerns over marketability of crossbred offspring, maintaining uniformity in the milking herd, planning subsequent breeding of crossbreds (Welgel and Barlass, 2003), and interest in purebreed activities such as dairy cattle shows.

Heterosis, also known as hybrid vigor, is a unique advantage that crossbreeding presents. Heterosis is simply the observed effect that the offspring perform better than the average expected from the parental strains (Bourdon, 1997), and is commonly

reported as a percent difference from the parental average. Heterosis is generally attributed to dominance and epistasis (Swan and Kinghorn, 1992). Crossbreeding increases heterozygosity at loci, which may allow the individual to perform well even under a variable environment (Swan and Kinghorn, 1992). Epistatisis is the interaction between the genes at different loci (Bourdon, 1997). Generations of selection in purebred animals have created favorable epistatic interactions (Swan and Kinghorn, 1992). During crossbreeding, genes form new epistatic interactions with genes from a different breed; these may be favorable or unfavorable interactions that affect the hybrid vigor of the individual (Swan and Kinghorn, 1992). The recombination of favorable gene interactions has been associated with lower milk yield than anticipated after the first generation of crossbreeding (Dechow et al., 2007).

Inbreeding is the mating of individuals that are more closely related than the population average and causes increased homozygosity. The negative effects of inbreeding on traits such as reproduction and overall animal vigor are well known, and crossbreeding can alleviate those issues (Falconer and Mackay, 1996). Increased homozygosity can increase prepotency, increase the expression deleterious recessive alleles, and cause inbreeding depression (Bourdon, 1997). By crossbreeding we can eliminate inbreeding depression and improve traits that had reduced fitness due to inbreeding (Falconer and Mackay, 1996). Heterosis exists for most traits that are important to dairy profitability (Sorensen et al., 2008); however there is variation in the amount of heterosis observed for different traits. For example, production traits generally have heterosis levels between 0% and 10%, whereas fertility traits generally range between 5% and 25% (Swan and Kinghorn, 1992).

The level of heterosis observed can be dependent on the strains used for the mating since the level of inbreeding depression and gene frequencies are important factors (Falconer and Mackay, 1996). Animals of similar net merit should be used (Sorensen et al., 2008) to obtain the greatest benefit from crossbreeding. Using breeds that are favorable biological types and that complement each other (Bourdon, 1997) can create advantages during crossbreeding. This may be in part due to favorable genotype by environment interactions different breeds and their crosses display (Madalena et al., 1989).

Crossbreeding is not widely used in the conventional United States dairy industry, mainly because it has been reported that crossbred dairy cattle may not be able to outperform their purebred herd mates in any one production trait such as milk yield (Mcdowell, 1982). However, crossbred dairy cattle may be able to provide a net economic merit greater than that of their purebred herd mates (McDowell, 1982; McAllister, 2002) after accounting for all the advantages that crossbred cattle present.

Normande Cattle

Normande (NO) cattle, which originate from France, present a unique genetic pool that could be utilized in the United States. Normande cattle can be considered a dual purpose breed and is marketed as the "French Cheese Breed." This is because cheeses made from NO milk in the Normande region of France are highly valued and because the milk from NO cattle is thought to have improved characteristics for cheese making (Normande Genetics, 2012). The meat from NO cattle is also considered to be of a high quality and often goes for a premium price. The NO breed has historically been

raised and selected on pasture, which may present some advantages for this breed's genetics to be utilized in low input grazing systems.

Crossbreeding with Normande (NO) cattle is reported to provide several advantages, including improved reproduction, survival, and total economic merit when compared to purebreds in certain environments. The decline in fertility of purebred Holstein (HO) is an important issue, and evidence suggests that Normande×Holstein (NO×HO) crossbreds have improved fertility compared to their purebred HO counterparts (Heins et al., 2006, Walsh et al., 2008; Heins et al., 2012). NO×HO crossbreds have been shown to have fewer days to first breeding (7 fewer days), fewer days open (52% bred before 99 days open, compared to only 38% for pure HO), higher pregnancy rates and also had an improved first service conception rate compared to purebred HO (35% for NO×HO, 22% for pure HO; Heins et al., 2006; Heins et al., 2012).

Survivability of dairy cattle is of economic importance to dairy producers since replacement animals are costly. NO×HO may have improved herd survival (Heins, 2006; Walsh et al., 2008; Heins et al., 2012). NO×HO first calf heifers had greater survival to 30, 150, and 305 days postpartum compared to HO (Heins et al., 2006). NO×HO crossbreds were also more likely to survive to subsequent calvings than HO in all parities in a study done over five lactations (Heins et al., 2012). NO×HO animals were 12.9% more likely to survive to parity 2, 22.3% more likely to survive to parity 3, and 23.9% more likely to survive to parity 4 (Heins et al., 2012).

Normande crossbreds may also have fewer calving problems compared to their purebred herd mates. NO×HO heifers had significantly lower still birth rates (9.9%) compared to pure HO (14.0%; Heins et al., 2006). Additionally, NO×HO crossbred cows

had lower rates of calving difficulty (11.6%) compared to pure HO (17.7%; Heins et al., 2006).

Although the NO×HO crossbreds had better reproductive performance and survivability, they produced fewer pounds of milk, fat, and protein compared to HO, with no difference in somatic cell scores (Heins et al., 2012). The milk component percentages for fat and protein of NO cows compared to HO cows was not found to be significantly different (Walsh et al., 2007). Lifetime production estimates after the first four years from first calving favored the NO×HO crossbreds in milk (+1,680 kg), fat (+108 kg), protein (+95 kg), fat plus protein (+203) and revenue from production (+ $1,105). However, these increases in production were seen with an increase in days of herd life for the NO×HO crosses (+ 172 days), resulting in a profit per day that was significantly less (-$0.28; Heins et al., 2012). While NO×HO crossbreds do not appear to be economical in high production systems, it has been suggested that utilizing NO×HO crossbreds in low input pasture systems may improve profit due to excellent fertility and survival (Heins et al., 2012).

Several studies conducted in pasture based systems in Ireland have included NO cattle in their analysis. Purebred NO cows were found to have lower values for peak milk flow (kg/min) and average milk flow (kg/min), while having a longer milking duration when compared to purebred HO cows. However, NO×HO crossbreds had flow rates and milking durations similar to purebred HO cows (Walsh et al., 2007). Purebred NO and NO×HO crossbreds were also found to have lower milk yield than purebred HO cows in Ireland, which supports the work done in California dairy herds (Walsh et al., 2007; Heins et al., 2012; 2006).

Normande cattle were found to have greater BCS than HO cows (Walsh et al., 2008). Body condition loss from weeks 2-8 of lactation was also found to be greater for HO cows compared to purebred NO or NO×HO crossbreds (Walsh et al., 2008). This increased loss of body condition indicates that the HO cows are directing more nutrients towards milk production than the NO cows, and subsequently may be at risk of more health problems that are associated with an increased rate of body tissue mobilization. Purebred NO had greater body weight compared to purebred Holsteins, but NO×HO crossbreds had similar body weights to HO cows (Walsh et al., 2008).

Body Condition Score

Body condition score is a visual appraisal of an animal's energy reserves. Evidence continues to accrue on the importance and dynamics of dairy cattle body condition. Problems can arise from a dairy animal that carries too much or too little body condition. Part of the dairy cow's ability to produce milk is her ability to mobilize body reserves to provide for the neonate (Bauman et.al. 1980). In fact dairy cattle tend to lose a substantial amount of body condition postpartum for about 40 to 100 days before regenerating the lost condition later in lactation (Koenen et. al., 2001; Coffey et al., 2004). Genetic selection has impacted the body condition dynamics across breeds and within breeds of dairy cattle. Dairy cows in the United States have been selected for superior milk production; however, this has come with a negative response in fitness traits and BCS due to unfavorable genetic correlations (Dechow et al., 2001; Veerkamp and Brotherstone, 1997). Selection has also incurred many physiological responses that increase the mobilization of body energy stores (McNamara et.al., 1986; 1991). Cow

factors that influence dairy cow body condition have been shown to include: genetics (Berry et al., 2003; Roche et al., 2006), parity (Pryce et al., 2001; Coffey et al., 2004), age within parity (Pryce et al. 2001; Coffey et al., 2004), and season of calving (Pryce et al., 2001). Management factors have also been found to influence BCS and include: stocking rate (McCarthy et al., 2007; Roche et al., 2007), level of feeding (Roche et al., 2006; 2007), and diet type (Coffey et al., 2004; Roche et al., 2006; 2007). Most animal and dairy scientists agree that managing BCS is an important factor that influences animal health, milk production, and reproduction (Domecq et al., 1997; Buckley et al., 2003).

Assessing Body Condition

It is assumed that recognizing BCS was intuitive for dairy farmers to manage for a long time, although there was no standard assessment until the 1970's (Stockdale, 2001). Body weight is often not a good indicator because so many factors influence an animal's body weight at a given time including breed, frame size, parity, gestation, stage of lactation, and the feed intake of the animal (Roche et al., 2009). Voluntary dry matter intake (DMI) drops in late gestation and early lactation (Ingvartsen et al., 2000). Dry matter intake is also a complex trait influenced by many factors that cause variation such as diet, management, environment, and animal (Ingvartsen et al, 2000). With increased gut fill an animal that is actually decreasing in body tissue weight may not be perceived as losing weight. It was reported that energy reserves between cattle with similar body weight varied by as much as 40% (Andrew et al., 1994; Gibb et al., 1992), which highlights the poor representative body weight can be for BCS.

Lowman developed the first BCS scale for dairy cattle in 1973 (Roche et al., 2009), with low values indicating the animal is emaciated and high values indicating the animal is carrying excess condition. Most body condition assessments look at multiple areas including: thoracic and vertebral region of the spinal column, the ribs, the spinous processes, hook bones, pin bones, tail head, and the thigh region (Roche et al., 2009). The visual evaluation of BCS has been determined to be a good assessment of body fat reserves, with correlations of BCS assessments and dissected fat between 0.75 and 0.93 (Wright and Russell, 1984; Otto et al., 1991).

Genetics of Body Condition

Body condition score is a quantitative trait that is influenced by many factors and genes. Historically, selection in dairy cattle has focused on production type traits (Miglior et al., 2005). These selection practices have led to genetic changes in body condition of animals as well. Heritability of BCS has been estimated several times with values ranging from 0.07 to 0.6 (Berry et al., 2008), and is generally regarded as a moderately heritable trait. Phenotypic correlations between BCS and other traits have been found to be lower than the genetic correlations (Dechow et al., 2003).The heritability estimates of BCS change with on lactation and days in milk (DIM), however several authors have demonstrated a high correlation within lactation and in different lactations at the same DIM (Dechow et al., 2001; Gallo et al., 2001; Koenen et al., 2001).

Body condition score has been reported to have a negative correlation with several traits. Most importantly BCS has been negatively correlated with milk production traits including milk yield, fat yield, and protein yield (Dechow et al., 2001;

Veerkamp and Brotherstone, 1997). Interestingly, in New Zealand the correlation between BCS and milk production traits have been reported to be close to zero, which is contradictory to most research (Pryce and Harris, 2004; Harris et al., 2005). Thus, it may be inferred that the correlation between BCS and the milk production traits may be system dependent because of New Zealand's reliance on pasture availability. Body condition score has also been negatively correlated with cow angularity (-0.47 to -0.77; Veerkamp and Brotherstone, 1997), dairy form (-0.73), rear legs side view (-0.38), and dairy composite (-0.75; Dechow et al., 2003).

Body condition score has been positively correlated with several traits including strength (0.72), stature (0.20), body depth (0.40), thurl width (0.27), body size composite (0.43), and foot angle (0.38) (Dechow et al., 2003). It has been theorized that cows with greater BCS have more body fat and muscle making them appear stronger and having larger body dimensions and body weight (Dechow et al., 2003).

Different breeds and crossbreeding can influence BCS. Significant differences in BCS profiles among cows of different breeds have been reported (Koenen et al., 2001; Pryce and Harris, 2006; Walsh et.al.2008). Heterosis has also been reported to affect BCS with heterosis estimates between 0.06 and 0.07 depending on the parental breed (Pryce and Harris, 2006). There are substantial differences in body energy deposition and mobilization among breeds and parities, with a significant interaction between breed and parity (Friggens et al., 2007).

Body Condition and Reproduction

Body condition score is often cited as a useful indicator trait for improved reproductive performance. Body condition score is genetically correlated with improved reproductive performance when corrected for milk production (Dechow et al., 2001; Veerkamp et al., 2001; Pryce et al., 2001). Specifically, increased BCS during lactation has been favorably correlated with fertility traits such as days to first heat, days to first service, calving interval (Dechow et al., 2001; Pryce et al., 2001; Veerkamp et al., 2001), days to first luteal activity (Reksen et al., 2002), and first service conception rate (Buckley et al., 2003; Roche et al., 2007).

Quantity of BCS lost in early lactation has been unfavorably correlated with first service conception rate (Domecq et al., 1997; Roche et al., 2007), days open, number of inseminations per conception (Gillund et al., 2001), days to first service, days to heat, and calving interval (Pryce et al., 2001). Many of the fertility traits that are correlated with greater BCS suggest the relationship is not linear and thus an optimal BCS should be targeted (Roche et al., 2007). Body condition score can be incorporated into selection indexes to improve reproductive performance because of its favorable correlation with reproductive performance. Correlations of BCS with fertility tend to be stronger in mid to late lactation compared with early lactation (Berry et al., 2003). Reproductive performance has been noted to have increased significance in seasonal pasture based systems (Walsh et al., 2008), possibly because of seasonal calving practices and aligning peak milk production with peak forage growth.

Body Condition and Health

The health and welfare of a dairy animal is often assumed to be correlated with the body condition of the animal. Exceptionally fat or skinny are more likely to be the unhealthy animals in the herd. Regardless, BCS has been linked to health traits including metabolic disease, lameness, calving problems, and uterine infections.

Several metabolic disorders have been linked with calving and early lactation BCS loss including ketosis (Duffield, 2000; Gillund et al., 2001), milk fever (Roche and Berry, 2006), displaced abomasums (Cameron et al., 1998) and fatty liver (Drackley, 1999; Bobe et al., 2004). Cows with a BCS greater than or equal to 3.5 were 2.5 times more likely to develop ketosis than cows with a BCS lower than 3.25 (Gillund et al., 2001). This figure may be due to a tendency for heavier conditioned cows to mobilize greater amounts of body condition postpartum (Roche et al., 2007), which may cause fatty acids and ketone bodies to accumulate and cause ketosis (Roche et al., 2009). A non-linear relationship between BCS and milk fever exists (Roche and Berry, 2006). Cows with a BCS less than 2.5 or greater than 3.5 had an increased likelihood of succumbing to milk fever (Roche and Berry, 2006). Displaced abdomens are often found in dairy animals that are over conditioned (Cameron et al., 1998). This is may be due to the decrease in dry matter intake and greater negative energy balance fat cows tend to have in early lactation. With abnormal BCS being a risk factor for several dairy cow diseases it is important for dairy producers to manage and select their animals for body condition to prevent problems that could lead to financial losses incurred from unhealthy animals.

Survival in Crossbreds

It has long been noted that crossbreeding can improve the survival or livability of dairy cattle (Dickinson and Touchberry, 1961; Heins et al., 2006). Increased survival of crossbreds has also been noted in sheep (Leymaster and Jenkins, 1993), beef cattle (Nunez- Dominguez et al., 1991), pigs (Gaugler et al., 1984) and rabbits (Ozimba and Lukefahr, 1991). NO×HO crossbreds as discussed previously have also been shown to have improved survivability when compared to their purebred herd mates (Heins et al., 2006; 2012).

Recent research into telomeres in humans has indicated that telomeres may be good biomarkers for cell health and biological age (Nakagawa et al., 2004). It has also been noted that longer telomeres are a possible indicator of improved survival in birds and humans (Haussmannet al., 2005; Pauliny et al., 2006; Cawthon, 2003; Bakaysa et al. 2007). Thus, investigating telomere length in crossbred animals may provide insights into the increased survival found in crossbred animals.

Telomere Structure and Function

Telomeres are present in all eukaryotic cells at the end of the chromosome and help to maintain chromosome integrity by preventing unwanted recombination, end-to-end fusion, and exonuclease degradation (Blackburn et al., 2000). Telomeres may also play an important part in alignment and segregation of chromosomes during cell division (Blackburn, 2005). In vertebrates the telomere consists of TTAGGG at the end of the chromosome.

At the very end of the chromosome's telomere, the DNA is comprised of a segment of single stranded DNA approximately 100-200 bases long (in humans) at the 3' end, which is known as the 3' overhang (Makarov et al., 1997). A complex process known as telomere 'capping' occurs at the 3' overhang to protect the telomere. This capping process protects the 3' overhang from exonuclease degradation or being recognized as damaged DNA which could lead to cell-cycle arrest or end-to-end fusion of chromosomes. If this capping process does not occur, or occurs with errors, it can cause failures that lead to genomic instability. For capping to occur the 3'overhang associates with several proteins (collectively known as 'shelterin') and is folded into a 'T-loop' (Griffith et al., 1999). Multiple proteins interact to form the shelterin complex. Some of these proteins are TRF1, TRF2, POT1, TPP1, and TIN2. All of these proteins show strong homology in vertebrates (De Lange, 2005). In some species, telomeric sequences can be found in other parts of the chromosome, and are called interstitial telomeric elements (Meyne et al. 1990). Their function and origin are not known.

Leonard Hayflick was one of the first researchers to observe that cells had a limited replicative capacity (Hayflick, 1965). The explanation for this inability of cells to replicate indefinitely comes in part from inefficiencies in the way eukaryotic cells replicate their DNA. Watson and Crick suggested that DNA replication was semi-conservative with a parental DNA strand being copied into a daughter strand (Watson and Crick, 1953). This replication process is carried out by DNA polymerase along with a primer and copies DNA in a 5' to 3' direction. The mechanics of this process are such that the cell is incapable of fully replicating the ends of chromosomal DNA leading to the 'end replication problem' (Olovnikov, 1973; Watson, 1972). Thus, base pairs at the end

of the chromosome are lost. It was observed by Watson in 1972 that this erosion of the chromosome would ultimately lead to the loss of important genetic information and render the cell incapable of further divisions (Watson, 1972).

Fortunately, the telomere acts as a disposable barrier that can be eliminated and not result in the loss of important genetic material. The shortening rate of telomeres documented in humans has a great amount of variation in it depending on cell type and genetics; a range of 25-350 base pairs lost per cell division has been reported (Allsopp et al., 1995; Wright et al., 1996). Because base pairs at the end of the telomere are lost during cell division, telomere length tends to shorten in somatic cells as individuals age (Ehrlenbach et. al 2009, Damjanovic 2007). Interestingly though, germ and stem cells have the ability to maintain their telomere length even after numerous cell divisions. This ability is mainly due to the up-regulation in transcription for the enzyme telomerase in germ cells (Schaetzlein et al., 2005). The gradual shortening of the telomere in somatic cells, due to the 'end replication problem,' can cause the cell to reach 'Hayflick's limit,' where the telomere is too short and cellular senescence ensues (Harley, 1991; Haylflick, 1965). However, there is no given length to which the telomere will shorten that will trigger cellular senescence. This is because there is a complex interaction between the telomere and the proteins associated with it that determine the stability of the telomere (Chan and Blackburn, 2003).

Telomerase

Telomerase, first discovered in Tetrahymena (Greider and Blackburn, 1985), is an enzyme that can rebuild and maintain the telomere by synthesizing and attaching new TTAGGG nucleotide repeats at the end of the telomere (Blackburn and Greider, 1989). In cultured cells, erosion of telomeres can actually be reversed experimentally with the increased expression of telomerase (Bodnar et al., 1998; Vaziri and Benchimol, 1998). It may be expected that a lack of telomerase would have no negative effects on the telomere until cell division occurs; however, in addition to rebuilding telomeres, telomerase may also promote telomere integrity. In cells that lacked telomerase it was found that there was a significant increase in unwanted telomere fusion to DNA breaks when compared to wild type control cells (Chan and Blackburn, 2003). The way telomerase functions is by synthesizing the strand of DNA running from 5' to 3' to extend the 3' overhang found at the end of the telomere. Synthesis of the complementary strand is then assumed to be completed by lagging strand synthesis using normal cellular DNA replication methods (Chan and Blackburn, 2003). Telomerase consists of two components; a catalytic protein and a RNA template. The TERT gene codes for the protein portion of telomerase, and the TERC gene codes for a RNA template. The incorporation of the TERC RNA template into the TERT protein portion allows the enzyme to act as a reverse transcriptase and copy RNA into DNA. Telomerase is expressed differently in different cell types. Most somatic cells have extremely low or no telomerase activity (Greider, 1998). However, telomerase is expressed in stem and germ cells which allow them to be highly proliferative with little or no negative effect on their telomere length (Hohaus et al., 1997;

Lansdorp et al.1997). Telomerase activity has become a well documented contributor to the telomere length variation found in organisms.

Telomere Length and Telomere Shortening Rate

There is an enormous amount of variation found in telomere lengths of organisms and the observed variation can be attributed to numerous sources. Telomere length can be described as a quantitative trait that is influenced by both the genetic quality of an individual and the environmental conditions of the organism. Even telomere length within an individual can have a large amount of variation. Every chromosome within a cell has a different telomere length; however, there is a pattern to individual telomere lengths within a cell (Britt Compton et al., 2006).

Insight into why telomere lengths show so much variation can be gained by looking at telomere shortening rates. Even individuals from the same specie and having the same age can have large amounts of variation in their telomere length. Several longitudinal studies show a large amount of variation in telomere shortening rate between individuals followed for the same amount of time (Hall et al., 2004; Pauliny et al., 2006; Zeichner et al., 1999). Work was done in an attempt to utilize telomere length to predict chronological age in humans (Hewakapuge et al., 2008) and highlights how variation in telomere length among similarly aged individuals negatively impacts its use as a predictor.

Stage of life can also impact the rate of telomere shortening. In humans, telomere shortening in peripheral blood mononuclear cells was shown to be fourfold greater in children under 3 years old when compared to adults (Zeichner et al., 1999). A similar relationship was found in cats (Brummendorf et al., 2002). This increased rate of

telomere shortening during early stages of life may be a reflection of the accelerated rate of cell division that takes place at that time and significant changes that occur in the immune system during early life (Zeichner et al., 1999). Other sources of variation in telomere length across organisms can be attributed to factors including: age, specie, sex, breed, environment, tissue type, and disease status.

Telomere Length and Biological Age and Quality

It has been suggested that telomeres are better suited to predicting the biological age and quality of an individual than chronological age (Nakagawa et al., 2004). If two individuals of the same age have different telomere lengths, then the individual with shorter telomeres is thought to have a greater biological age. Studying the factors that impact this biological age and quality could provide important insights into determining the long-term effects life histories can have on an individual (Monaghan and Haussmann, 2006). Telomere length also seems to be a predictor of reproductive success in dunlins (Pauliny et al., 2006). Nematode worms with longer telomeres tended to be more heat resistant (Joeng et al., 2004). These reports link telomere length to a higher degree of biological quality in an individual; however, it is unknown if it is a causal relationship or simply a correlation.

Multiple studies have investigated the relationship between telomere length and survival. It was found that one year old tree swallows that had relatively short telomeres had lower survival than tree swallows of the same age with relatively long telomeres (Haussmann et al., 2005). It was also found that telomere length was a predictor of longevity in sand martins (Pauliny et al., 2006). An inverse relationship between telomere

length and the rate of mortality has been reported in unrelated individuals as well as in pairs of twins (Cawthon, 2003; Bakaysa et al., 2007). Identical twins that had the shorter mean telomere length were found to be three times more likely to die than their co-twin that had longer telomeres (Bakaysa et al., 2007). However, two separate studies failed to find this inverse relationship between telomere length and mortality rate (Martin-Ruiz et al., 2005; Bischoff et al., 2006).

Telomere Length and Aging

Aging is a natural part of life and it is widely believed that telomeres play an integral part in the aging process (Kappei et al., 2008). Some theories of aging believe that loss of function in genes that are important for DNA maintenance and repair are also important factors in explaining why individuals age (Partridge, 2001). It is also known that for an organism to successfully divide and propagate it needs to have sufficient genomic stability and telomeres are a key factor in this stability. Early work done by Barbara McClintock recognized that something at the end of chromosomes protected the chromosome from degradation and unwanted fusion (McClintock, 1941).

It has been widely documented that mean telomere length of an individual tends to decrease with age in humans (Ehrlenbach et Al, 2009, Damjanovic et al., 2007). However, no change in telomere length with age was seen in Drosophila (Walter et al., 2007), sea urchins (Francis et al., 2006), or wandering albatrosses (Hall et al., 2004). In fact, one interesting study done on storm petrels observed an increase of telomere length with age (Haussmann et al., 2003), but this unexpected trend may be due to the elevated levels of telomerase found in the storm petrel (Haussmann et al., 2007). Another

explanation to the unexpected trend of telomere length in storm petrels was observed to be the tendency for petrels with very long telomeres to survive better than individuals with short telomeres (Haussmann and Mauck, 2008).

It has long been noted that crossbreeding can improve the survival or livability of dairy cattle (Dickinson and Touchberry, 1961; Heins et al., 2006), sheep (Leymaster and Jenkins, 1993), beef cattle (Nunez- Dominguez et al., 1991), pigs (Gaugler et al., 1984) and rabbits (Ozimba and Lukefahr, 1991). It may be possible that improved telomere length or telomere maintenance explains the improved survival for crossbreds.

Genetics and Inheritance of Telomeres

Telomere length has been determined to be a quantitative trait that is heritable. Genetic factors could impact telomere length several ways. These could include initial telomere length inheritance, telomere shortening rate, and the degree to which an individual can maintain their telomere length. Identical twins have also been found to have a mean telomere length more similar to each other than sets of fraternal twins (Slagboom et al., 1994), indicating that genetics play an important role in telomere length. A study in mice showed that when mice with shorter than average telomere lengths were mated to each other they produced offspring with shorter than average telomere length (Armanios et al., 2009), indicating that genetics are important in determining telomere length and that there is a heritable component to telomere length.

There has been a broad range of heritability estimates for telomere length. An estimate of heritability of telomere length in humans is 36% based on a study of dizygotic twins, with a large familial effect of 49% (Andrew et al., 2006). However, this study

might overestimate the heritability of telomere length by not taking into full account of similar environments for twins. Another heritability estimate in humans was 78% based on monozygotic twins (Slagboom et al., 1994). Heritability also appears to be greater in younger individuals than in older individuals (Bakaysa et al., 2007; Slagboom et al., 1994; Bischoff et al., 2006). This is probably due to the tendency of telomeres to shorten over time and reduce variation among individuals as they age.

Human research into parent-child comparisons has uncovered a potentially strong heritable paternal factor that influences telomere length (Njajou et al., 2007; Nordjfall et al., 2005; 2009). In sand lizards, heritability estimates were much higher when estimated from sires to sons rather than dams to daughters, which may indicate an epigenetic role for telomere length (Olsson et al., 2011). The age of the father may also impact the telomere length of the offspring. One human study found that on average, offspring gained 22 base pairs for each additional year of age of the father, while indicating that the age of the mother was not significant (Unryn et al., 2005). X-linked factors of inheritance for telomere length have also been reported (Nawrot et al., 2004).

Genomic studies have been carried out to find variations in specific genes and genomic regions that could impact telomere length. Using a genome wide quantitative trait loci (QTL) study, three loci were identified in humans to be significantly correlated with telomere length (Andrew et al., 2006). One genome wide association study (GWAS) study highlighted thirteen single nucleotide polymorphisms (SNP) in four genes that were significant for telomere length (Mirabello et al., 2010). Genome wide

association studies have also provided insight into the complex and polygenic nature of telomere length.

Telomeres within Specie, Breed, and Sex

Telomeres are homologous across species, but there is a large amount of variation in telomere length among species. Interestingly, a greater amount of telomere does not seem to correlate with increased lifespan across species. For example, humans are unique among primates because they have the shortest telomeres yet they have the longest lifespan (Kakuo et al., 1999). A study done with different mice strains also found that there was no relationship between telomere length and organism lifespan (Hemann and Greider, 2000). It appears that organism lifespan may be more accurately predicted by looking at the rate at which the specie's telomere erodes. A study done on five different bird species showed that telomere shortening rate for long lived bird species was less than that of birds with short lifespan (Haussman et al., 2003).

Breed and race have also been shown to explain significant amounts of the variation in the telomere length. Racial differences have been reported in humans (Roux et al., 2009; Hewakapuge et al., 2008). Differences in telomere length were also noted between breeds of beef cattle in Italy (Tilesi et al., 2010) and in different breeds of dogs (McKevitt et al., 2002).

A difference between telomere lengths from different sexes has been determined for several species such as humans (Benetos et al., 2001), rats (Cherif et al., 2003), and ants (Jemeility et al., 2007). In humans, average telomere length is consistent between males and females at birth and then diverges with age, with males tending to have more

rapid shortening of their telomeres (Nawrot et al., 2004). Biological as well as life history factors are likely to play a part in the reason as to why telomere length diverges with age in individuals.

Telomeres in Different Tissue and Cell Types

Various tissues have been used to analyze telomere length in the literature. Work done on skin, leukocyte, and synovial tissues showed that each of the tissues from the same individual had differing relative telomere lengths (Freidrich et al., 2000). However, strong correlations were found between tissues of the same donor, suggesting that easily accessible tissues such as blood could give good indications of an individual's overall relative telomere length (Freidrich et al., 2000). Even within blood, there may be significant differences in telomere length between immune cell populations. For example, $CD4^+$ T cells were found to have shorter telomeres than naive T cells from the same donor, and differences between B cell subpopulations have also been reported (Hodes et al., 2002).

Differences in relative telomere length of multiple tissues have also been noted in the dog (Nasir et al., 2001) and the mouse (Prowse et al., 1995). Some of the variation in relative telomere length seen between tissues can be attributed to the cellular regulation of telomerase. As noted previously, somatic cells have little telomerase activity, whereas germ and stem cells have increased telomerase activity. Although in some rapidly dividing stem cell populations, increased telomerase activity is not enough to entirely impede telomere erosion (Bodnar et al., 1996; Wright et al., 1996). Work done in human

epithelial and epidermal tissue showed that the two types of tissue had significantly different rates at which the telomeric base pairs were eroded (Nakamura et al., 2002).

Environmental Stress and Telomeres

Environmental conditions have been shown to affect telomere length, although the exact mechanism of how it causes telomere shortening in the cell is unknown. Environmental factors such as psychological stress, diet, and disease have all been shown to be significant factors in premature telomere shortening. Oxidative stress may be an explanation as to how telomere length shortening is accelerated. Imposing oxidative stress on cells in culture led to accelerated telomere shortening and premature cellular senescence (von Zglinicki, 2000). Another potential mechanism by which stress can accelerate telomere shortening is through increased cortisol levels in a stressed individual. It has been shown that T lymphocytes exposed to cortisol significantly reduced telomerase activity (Choi et al., 2007), which could accelerate telomere erosion by taking inhibiting the one method cells have to maintain the telomere.

Psychological stress has been investigated and is shown to lead to premature telomere shortening in mothers of chronically ill children (found to have higher levels of perceived stress) when compared to mothers of healthy children (Epel et al., 2004). Another example was shown in caregivers of Alzheimer's patients having significantly shorter telomeres than a control group (Damjanovic et al., 2007). A unique study on chicken stocking density showed that broiler chickens that were subjected to very high stocking densities had shorter telomeres than broiler chickens house at normal and low stocking densities (Beloor et al., 2010). Finally, it was found that individuals who were

abused when they were children had shorter telomere lengths than a control group (Tyrka et al., 2010).

Diet seems to have an impact on the rate of telomere shortening. Marcon and colleagues investigated a group of individuals where detailed dietary information was available and correlated different nutrient variables to telomere length. They found that increased dietary intake of Vitamin A, Vitamin B9, Vitamin C, Vitamin E, and Beta-carotene were all significantly correlated with longer telomeres (Marcon et al., 2012). This study built on other previous studies that showed multivitamin use and greater intakes of Vitamins C and E were correlated with longer telomeres (Xu et al., 2009). An increase in Vitamin D has also been shown to have positive impact on telomere length in women (Richards et al., 2007).

Disease and Telomeres

Disease has been correlated with shorter telomere length. Incidence of cancer and mortality rate has an inverse relationship to telomere length (Willeit et al., 2010). An association between disability in the older U.S. population and shorter telomere lengths has also been reported (Risques et al., 2010). Additionally, shorter telomere lengths have been found in individuals that suffer from migraines (Ren et al., 2010), atherosclerosis (Panayiotou et al., 2010), osteoarthritis (Zhai et al., 2006), and obesity (Buxton et al., 2011). Individuals with bladder, head and neck, lung, and renal carcinomas tend to have shorter telomeres in their peripheral blood lymphocytes than age matched controls with similar risk factors (Wu et al., 2003). Shorter telomeres are also associated with increased breast cancer risk (Shen et al., 2007).

Several human diseases are known to be caused by telomerase dysfunction and these diseases drastically shorten the lifespan of individuals. For example, Dyskerata Congenita is caused by a mutation in the DKC gene which causes telomerase to not mature properly and thus telomerase cannot function properly. With telomerase not active, the stem cells reach senescence very rapidly compared to a normal individual who has functional telomerase. This leads to premature shortening of telomeres and cellular senescence in stem cells occurs, with death occurring because of bone marrow failure. Werner syndrome is a recessive genetic disorder caused by loss of function mutations in the WRN gene coding for a helicase. Werner syndrome leads to premature aging of an individual along with rapid telomere degradation (Yu et al., 1996). Deregulation of telomerase is also thought to cause oncogenesis since telomerase is up-regulated in cancer cells and the cells become 'immortal.'

Quantification of Telomeres

Three prominent techniques are utilized in the literature to quantify telomere length and they include: quantitative fluorescent in situ hybridization (QFISH), quantitative polymerase chain reaction (QPCR), and Southern blotting. The QFISH procedure generally requires intact nuclei and timely processing of samples and thus is not used in larger epidemiological studies and would not be practical for our larger study dairy cattle from different herds. QPCR has become a popular choice since Cawthon (2009) presented a novel multiplex QPCR strategy that increased throughput and decreased costs and DNA required for quantifying telomere length. Terminal restriction fragment analysis using Southern Blotting is also very popular and is considered the 'gold standard' method.

Southern blot analysis of telomeres requires a large amount of DNA (0.5-5µg/individual) and time (3-5 days) (Cawthon, 2002). The Southern blot is carried out by determining a mean terminal restriction fragments (TRF) length. However, depending on the restriction enzyme used, the mean TRF of an individual can vary by up to 5% (Cawthon, 2002). Regardless, TRF analysis using southern blotting has been considered to be the 'gold standard' of relative telomere length measurement and has been reported to have a lower inter-assay coefficient of variation (1.5-12%) compared to QPCR (2.27-28%; Aviv et al., 2011).

Cawthon (2002) published a technique that could measure relative telomere length using QPCR. It was once thought impossible to use a polymerase chain reaction (PCR) to measure the telomere, because the amplification of the TTAGGG and antisense CCCTAA repeats of the telomere using oligionucleotide primers were expected to result in only primer dimer products (Cawthon, 2002). The QPCR method created telomere primers that eliminated the dimer issue, by incorporating designed base mismatches to prevent the telomere primers from creating a stable dimer product. The method called for measuring an individual's telomere amplification in one well and using the amplification of a standard gene in a separate well to create a relative ratio comparing the amount of telomeric DNA amplified to the amount of standard reference gene amplified. Cawthon (2009) improved the original QPCR method by creating a multiplex method that allowed the measurement of the telomere product and standard reference gene in the same well, leading to savings in materials and time. Multiplexing was accomplished by creating a large difference in melting temperatures between the two amplicons.

A much greater quantity of telomere product compared to the standard gene product is expected, making the multiplex method feasible. This is because the cycle thresholds of the more abundant product is to be measured at earlier cycles while the less abundant reference gene product is still at baseline. QPCR experiments measuring telomeres generally make use of a telomere to standard gene ratio (T/S) to find the relative amount of telomere DNA to a standard reference gene. After determining T/S ratios, larger studies that were run on multiple plates or gels utilized linear models to determine a batch effect (Kim et al., 2011).

There are large amounts of variation in relative telomere lengths when several gels or plates are run. Batch variation accounted for as much as 42% of TRF variance (Slagboom et.al. 1994) in one study and 19% of TRF variance in another (Andrew, et.al, 2006). There is also known gel to gel variation in TRF for individuals (Slagboom et al., 1994; Bischoff et al., 2006). Complex experimental designs in QPCR may not be easily analyzed nor have valid inferences drawn from them using the conventional statistical methods used. It has been suggested that using linear mixed models to appropriately model all sources of variation found in the QPCR experiment are more appropriate and can be used to draw valid inferences (Steibel et al., 2009).

Conclusions

Intense selection and inbreeding in purebred dairy cattle in the United States may make crossbreeding with NO cattle beneficial to dairy producers. Crossbreeding in itself has several benefits including eliminating the deleterious effects of inbreeding and the benefit of heterosis. Crossbreeding with NO may improve BCS of the offspring that could offer benefits in cow health and reproductive performance. Use of NO genetics may also provide more marketable male offspring for beef purposes. The increased BCS may be of a greater advantage to producers that are utilizing low input grazing systems because the animals may be able to mobilize the body reserves when feed is restricted in later lactation, whereas purebred U.S. purebred cattle may not have body reserves to mobilize.

Telomere length is a quantitative trait with a large amount individual variation caused by an individual's genetics and environment. Telomere length is also thought to be related to an individual's biological quality and ability to survive. Crossbreeding has been shown to increase survival in several species and may impact telomere length in cattle. Thus, telomere length may provide insight into improved survival of cattle.

Chapter 2

Crossbred Normande cattle compared to Ayrshire, Holstein, and Jersey sired cattle for body condition and production in grazing and non-grazing herds.

Abstract

The objectives of this study were to compare crossbred Normande (NO) cattle with animals sired by Ayrshire (AY), Holstein (HO), Jersey (JE), and other breeds (OT) for production, reproductive performance, and body condition score BCS, and investigate how performance changes across seasons and management system (grazing, non-grazing). Sires and dams of unknown breed, crossbreds, or breeds other than AY, HO, JE, or NO were classified as OT. Body condition scores were assigned on 1 herd in Massachusetts, 1 herd in New York, 2 herds in Pennsylvania, and 2 herds in Vermont. Herds were visited once during each of four seasons (winter, spring, summer, and fall) of the year 2011. Herds varied from minimal confinement and high amounts of pasture utilization to herds with high confinement and minimal pasture allowance. Dairy herd information (DHI) test day and pedigree information was collected for all animals from the 6 BCS herds, in addition to 1 herd in Minnesota that milked NO crossbreds. Multiple trait mixed-models were used to analyze BCS, milk yield, fat yield and percentage, protein yield and percentage, somatic cell score (SCS), and calving interval. Normande sired cattle had significantly greater BCS (3.08) than animals sired by AY (2.78), HO (2.59), and JE (2.21). Holstein sired animals had significantly higher daily milk yield (27.95 kg) compared to animals sired by NO (23.56 kg), AY (25.12 kg), and JE (22.05 kg). There were no significant differences in daily protein yield among animals sired by

AY, HO, JE, and NO. Normande sired cattle had a fat yield (0.93 kg) that was significantly lower than HO sired cattle (1.11 kg). Normande sired cattle also had the highest SCS (2.87), which was significantly greater than HO sired animals.

A significant season and days in milk (DIM) effect for BCS and yield traits was noted, with yield (particularly fat and protein) and BCS depressed during the summer months. Normande sired cattle were able to mobilize body reserves during the summer months when energy intake from pasture was expected to be low, and lost significantly less fat yield during the summer months compared to HO sired animals. However, limited record numbers did not allow for significant breed differences to be detected in other yield traits. The exception was cows sired by bulls of OT which had significantly larger declines in milk, fat and protein yield during summer months. This reinforces the need to use proven sires of high genetic potential, even in grazing systems that incorporate crossbreeding.

Introduction

Purebred dairy cattle, particularly HO, have been intensively selected for milk production traits because yield is the major source of income for dairy farms. This intense selection has improved milk production in dairy cattle, but has come with a decline in health and fertility traits in dairy cattle (USDA-AIPL, 2012). Body condition score (BCS) has also declined with increased selection for dairy traits due to a negative genetic correlation between milk yield and BCS (Dechow, 2001; Veerkamp and Brotherstone, 1997). However, BCS may be a good indicator trait for health and reproductive traits.

Normande cattle, which originate from France, present a unique genetic pool that could be utilized in the United States. Crossbreeding with NO cattle is reported to provide several advantages, including improved reproduction, survival, BCS, and total economic merit when compared to purebreds in certain environments (Heins et al., 2006; 2012; Walsh et al., 2008). Another unique advantage of the NO breed is that they have been selected in a pasture based environment (Normande Genetics, 2012).

Dairy cattle of high genetic merit that have been selected in generous feeding systems may be impacted more severely by energy restrictions found in pasture systems than dairy cattle that have been historically selected in grazing systems (Macdonald et al., 2007). Holsteins are more likely to direct all nutrients toward milk production when energy intake from pasture and supplements is adequate and then have few body energy reserves left to mobilize when energy intake is limited, thus needing increased concentrate supplementation (Pryce et al., 2006). Even when feed supplementation occurs in pasture systems, it still may not prevent undesirable BCS loss in HO (Macdonald et al., 2008).

The objectives of this study were to compare crossbred NO cattle with animals sired AY, HO, JE, and OT for production, reproductive performance, and BCS, and investigate how performance changes across seasons and management system (grazing, non-grazing).

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Materials and Methods

Body condition scores were assigned to 1 herd in Massachusetts, 1 herd in New York, 2 herds in Pennsylvania, and 2 herds in Vermont. Herds were visited once during each of four seasons during 2011. The four seasons included winter (visit in January or February), spring (visit in late May or first week of June), summer (visit in middle of August), and fall (visit in October). The herds varied from herds with minimal confinement and high amounts of pasture utilization to herds with high confinement and only a green turnout lot with little to no grazing availability. The breed composition of the herds varied and was composed primarily of purebred AY, HO, and JE in addition to NO crossbreds and other crossbreds of varied and unknown lineage. Four herds where animals had access to pasture except during milking and that provided less than 7 kg of grain supplementation per day were grouped together as grazing herds. The grazing herds were also supplemented with stored forage in times when pasture supply was limiting. Herds that utilized only green turnout lots that provided little or no pasture were grouped as non-grazing herds, and were fed total mixed rations. One non-grazing herd allowed no outdoor access for lactating cows, and two allowed access to a barnyard with minimal pasture growth for several hours per day.

Test day milk production from DHI was collected for 7 herds. The herds included the 6 herds that were visited for body condition scoring and an additional herd from Minnesota that provided milk production data. Six of the herds participated in DHI testing prior to the start of the trial. A seventh herd with a large number of NO crossbreds (34) tested 4 times (once per season) during the trial. This herd was added to increase the number of records from NO crossbred cows; however, total lactation yields were not

available for this herd so the primary traits evaluated included test-day milk, fat (yield and percentage), protein (yield and percentage), and somatic cell score (SCS) as opposed to lactation totals. Calving interval (CINT) was calculated as the number of days between subsequent calving dates capped at 550 days and was also included in the analysis.

For BCS, there were 4 seasons corresponding to the winter, spring, summer and fall herd visits. Season was derived on a bimonthly basis for yield traits and SCS (January and February = Season 1, March and April = Season 2, etc.). Days in milk (DIM) classes corresponding to 28-d intervals through 252 DIM were generated, after which DIM was grouped into 48-d intervals, and a final class of >348 days, resulting in 12 total DIM groups.

Pedigree information was limited for many of the cows. Of the 1240 cows in the data set sire identification was available for 1053 cows, dam breed for 1073 cows and maternal grand-sire for 309 cows. Dam breed was coded as crossbred with no known maternal grandsire for 391 cows, whereas sire breed was missing or coded as unknown for 195 cows. If the dam was a crossbred, not HO, AY, or JE, or was unknown it was defined as OT. If the sire breed was something other than NO, HO, AY, or JE it was also categorized as OT. Breed combinations of animals sired by AY, HO, JE, NO, other known breeds, crossbreds, or if sire breed was unknown are tabulated in Table 2-1.

Statistical Model

Eight- trait models were analyzed with ASREML (Gilmour et al., 2006) as follows:

$$Y = XB + Za + \varepsilon$$

Where Y= a vector of 8-traits; X= design matrix to associate observations with fixed effects; B = a vector of the fixed effects that included sire breed, herd, lactation (1, 2, 3, 4, ≥5), DIM class, and season; Z= design matrix to associate observations with the random effects; a = a vector of random cow effects, the random interaction of sire breed and season, the interaction of herd and year of calving, and the interaction of dam and sire breeds; ε = random error.

For the first model, the 8 traits included milk (kg), fat (kg), protein (kg), BCS, SCS, and CINT. Fat and protein yield were replaced by fat and protein percentage for the second model.

The model above was also modified to investigate the differences between grazing and non-grazing environments. Herd was removed as a fixed effect and replaced with grazing class (1=grazing herd, 0=non-grazing), and an interaction between sire breed and grazing class was included as a fixed effect. Random effects for the 3-way interaction of sire breed, season and grazing class was also added. The PREDICT statement in ASREML was used to estimate Predicted Means (PM) for the eight traits and to predict differences between seasons, breeds, and grazing type.

Results

In total, 1931 BCS were available for analysis (Table 2-2) with an approximately equal number of NO sired cattle in herds that utilize large amounts of pasture and herds that utilized little or no pasture. Table 2-3 displays the breed combinations that were available for BCS analysis. In total there were 11,376 test day records available from all herds and breed combinations (Table 2-4). Holstein sired animals had the most test day records (5821); there were also 797 test day records for NO sired animals, 1376 test day records for AY sired animals, 676 test day records for JE sired animals, and 2706 records for animals sired by OT. There were no test day or BCS observations of HO×AY animals or JE×AY animals, and several other breed combinations had limited observations for test day records and BCS observations. Means for BCS, milk yield, fat yield, protein yield, SCS, and calving interval are reported in Table 2-5. Information about sire breeds, grazing, average milk yield, and average BCS for each herd is provided in Table 2-6.

Test-Day Records

Predicted means (PM) for milk yield, fat yield, fat %, protein yield, protein %, BCS, SCS, and CINT with standard errors are reported in Table 2-7. Holstein sired animals had the highest daily milk yield. Normande sired cows produced significantly less milk (23.56 kg) than HO sired cows (27.95 kg) and AY sired cows (25.12 kg), but produced more milk than JE sired cows (22.05 kg). Jersey sired cows had a significantly lower milk yield than animals sired by AY. Animals sired by OT had a daily milk yield estimate of 24.62 kg, which was significantly different than animals sired by HO, NO,

and JE. Daily milk yield and other yield traits had significantly lower estimates in grazing herds compared to non-grazing herds (Table 2-8).

Predicted means for milk yield, fat yield, protein yield, and BCS for different breed combinations are reported in Table 2-9. There was minimal variation among breed combinations within sire breed groups (AY, HO, JE, NO, OT) for milk yield and protein yield PM. There was more variation observed for fat yield and BCS, but with large standard errors.

Normande sired cattle had a numerically lower daily fat yield (0.93 kg) estimate than cows sired by other breeds, but were only significantly different than HO (1.11 kg). Normande sired cattle did not have daily protein yields that were significantly different than animals sired by other breeds. Normande sired cows had the highest SCS (2.87). HO cattle had a significantly lower SCS estimate (2.26) than cows sired by JE (2.84), AY (2.85), and NO. Animals sired by OT had a significantly lower CINT of 395 days compared to animals sired by HO, JE, and NO. There were no significant differences among animals sired by AY, HO, JE, and NO for CINT.

Body Condition Score

Normande sired cattle had a significantly greater BCS estimate (3.08) than animals sired by HO (2.59), JE (2.21), AY (2.78), and OT (2.69). Jersey sired cattle had a significantly lower BCS estimate than all other breeds. Animals sired by HO, AY and OT were not significantly different from each other. Correlation of cow effects (representing genetic and permanent environment) showed that BCS tended ($P<0.10$) to be correlated with daily milk yield (-0.28), daily fat yield (-0.30), daily protein yield (-

0.22), and SCS (0.15). Cattle that were on pasture did not have significantly different BCS than those in herds that were mainly confinement.

Seasonal Trends

Daily milk yield (Figure 2-2), fat yield (Figure 2-3), and protein yield (Figure 2-4) were all influenced by season in herds that utilized large amounts of pasture. The seasonal effect was not as apparent for daily milk yield in non-grazing herds, but there appeared to be seasonal effect for daily fat yield and protein yield in non-grazing herds.

In grazing herds, daily milk yield peaked in the period of March through June before milk yield dropped in July and August and remained lower September through December.

Protein yield showed a decline from January through February until July through August where it appears to reach its nadir, then increased through November and December. Protein yield was at its highest predicted levels during January through February and November through December in both grazing and non-grazing herds.

Daily fat yield followed a similar seasonal pattern to that of daily protein yield for grazing herds. Fat yield was greater during the winter months (November through February) with nadir daily fat yield occurring during the summer months (July through August). In similar fashion to grazing herds, fat yield in non-grazing herds appears to be greater March through June before dropping in July through August and then increasing September through December.

In grazing herds, BCS was greater during the winter and spring visits, then dropped in the summer and increased slightly in the fall (Figure 2-8); a similar trend for

BCS was observed in herds that did not utilize much pasture (Figure 2-9). There was no obvious seasonal trend for SCS in grazing and non-grazing herds.

Discussion

Normande sired cattle were found to carry more body condition than animals sired by AY, JE, and HO. Animals sire by AY and HO did not differ significantly in BCS. Jersey sired animals had a significantly lower BCS. This research supports findings by Walsh (2008) that found NO cattle and crossbred NO cattle to have greater BCS than Holsteins. Body condition score was also found to be negatively correlated with milk yield, protein yield, and fat yield which supports work done by Dechow (2001).

Previous work has shown JE animals to carry more body condition than HO (Washburn et al., 2001; Prendiville et al., 2009), while others showed no significant difference (Pryce et al., 2006) The current study found that JE sired animals carried significantly less body condition compared to HO sired animals. The NO is considered to be a dual purpose breed (Normande Genetics, 2012) and the use of NO sires appears to greatly increase the BCS of the resulting offspring. This increase is seen even if the dam is of a dairy breed, such as JE, which was found to carry significantly less body condition. With NO sired cattle carrying more body condition it could be expected for them to have advantages in health and reproductive traits. This increase in fleshing ability may also provide male offspring that are more desirable for dairy beef production.

No significant difference was found between breed BCS in grazing and non-grazing herds. This does not support research done by Washburn and colleagues (2008)

which found animals that were in grazing herds had lower BCS. However, all of the grazing herds in this study provided concentrate supplementation (4 – 7 kg/cow/day) during milking for all of their lactating cows, and the supplementation may have been at greater levels than the grazing herds previously studied. Another reason that no difference may have been found between grazing and non-grazing herds is because the two herds that were considered non-grazing and had BCS observations fed rations that may have contained greater amounts of forage than is typical in a normal commercial dairy mixed ration. The average daily milk yield of HO cows in the two non-grazing herds with BCS observations was 19.9 kg and 27.9 kg respectively. These two herds did not buy in large amounts of concentrate and depended heavily on stored forage that was produced on the farm. The commercial non-grazing herd from Minnesota that fed a more energy dense diet had HO cows with an average daily milk yield of 41.5 kg. These differences observed in the average daily milk yield between the non-grazing herds may reflect differences in the amount of concentrate feeding since milk production is expected to increase with increasing concentrate feeding (Roche et al., 2006). If the non-grazing herds that provided BCS observations in this study were not feeding high amounts of concentrate it may explain why no difference in BCS between animals in grazing and non-grazing herds was observed.

Normande sired cattle had the lowest daily fat yield and had a similar fat percent to that of Holstein sired animals. Jerseys had a relatively high fat yield when taking into account that they had the lowest daily milk yield, due to their higher milk fat percentage. Daily protein yield for NO sired cattle was not significantly different than animals sired by AY, HO, and JE. Cheese makers have been interested in NO cattle because it has

been suggested that NO cattle are desirable for cheese making. This desirability is due to an increase proportion of NO sires that are homozygous for the B kappa casein haplotype, which is thought to improve cheese yields (Normande Genetics, 2012; Keating et al., 2007).

Normande sired cattle had the highest SCS, but was only significantly greater than HO sired cattle. Heins (2012) found that NO×HO had a greater SCS in their second lactation than pure HO, but SCS between HO and NO×HO crossbreds did not differ significantly in lactations 1, 3, and 4.

The reproductive data available for analysis was limited to CINT for this study, and there were no significant differences among animals sired by AY, HO, JE and NO for CINT. This may reflect the lower heritability for reproductive related traits (Weigel and Rekaya, 2000) coupled with relatively few observations because first lactation cows do not have a CINT. Animals sired by other breeds did have a CINT that was significantly lower than animals sired by HO, JE, and NO.

Seasonal Differences

With a greater BCS estimate, NO crossbred cattle may have more body reserves to mobilize during periods when pasture growth slows. It has been previously reported that HO cows have a propensity to direct all nutrients toward milk production during periods where their energy needs are met with the available pasture and supplements and, thus, have few body resources to mobilize during periods when energy intake cannot meet their energy requirements (Pryce et al., 2006). Breeds with low BCS might require larger amounts of concentrate supplementation during these periods to maintain production.

For grazing herds in this study, it was expected that cows would have increased energy intake during the spring season (May through June), and that cows would have decreased energy intake during the summer months of July through August. During the spring months pasture would be expected to be growing more rapidly in the Northeastern United States, and it would also be expected that pasture growth would slow during the hotter and dryer summer months. Animals in grazing systems are usually provided with stored forage if there is inadequate pasture available during the summer months. .

Dry matter intake has been suggested to be a limiting factor for pasture based dairy herds (Kolver and Muller, 1998), and even if the cattle on cattle on pasture are being supplemented with stored forage in the summer months there are several factors during the summer season which could decrease DMI in grazing herds and thus energy intake during the summer season. . For example, it has been noted that hot and humid weather associated with the summer season can decrease DMI (West et al., 2003). Due to the expected drop in DMI during the summer it is recommended to increase the

nutrient density of the ration provided to dairy cattle to alleviate problems with decreased nutrient uptake and utilization that heat stress can cause (West, 1997). The effect of heat and humidity on nutrient intake may also be more significant in grazing herds compared to their conventional counterparts because it may be more difficult for grazing herds to provide common cooling methods to alleviate heat stress such as shade, fans, and misters while the cows are on pasture.

Thus, it may be expected that animals would have increased milk production and be able to maintain their body condition during the spring and early summer due to increased energy intake. Milk yield and BCS were greater during the winter and spring visits before decreasing during the summer months for cows sired by all breeds. Changes in yield traits (difference between July through August and the average of November through April) and BCS (difference between the summer visit and the average of the winter and spring visits) are reported in Table 2-10.

However, while NO sired cows had a larger decline in BCS from the average of the winter and spring visits to summer (0.27) than HO (0.17) and a smaller decline in milk yield from the average of spring and winter seasons to the summer season when compared with HO (0.04 kg and 0.52 kg, respectively) the differences were not significant (*P=0.27* for BCS and 0.*32* for milk yield).

Animals in non-grazing herds also tended have decreased fat and protein yield during the summer months, indicating that not all of the differences observed in the grazing herds can be explained by poorer pasture growth. The drop in BCS and yield may reflect reduced DMI associated with hot weather in non-grazing herds (West et al., 2003).

Daily milk yield for all breeds in grazing herds were lowest during the summer months and highest during the late spring, fall, and winter months. This supports previous research that found milk production in dairy cattle was depressed during the hot summer months (Lee et al., 1976; Ray et al., 1992). However, the trend for daily milk yield to decrease in the summer season was not as apparent in non-grazing herds. This suggests that effects other than temperature were affecting dairy animals that were in grazing herds. It may be that pasture system energy intake was reduced proportionately more during the summer months due to a diet that was not adjusted for nutrient density and thus milk production was depressed, or it could be that management factors (shade, fans, misters) to keep the animals cool were different between grazing and non-grazing herds.

Daily fat and protein yield were depressed during the summer months in grazing herds, as were fat and protein percentages. Across breeds, there was a 9.4% drop in fat and 7.2% drop in protein yield compared to average yields of November through April. Animals in non-grazing herds appeared to follow a similar trend, but changes were less pronounced.

Conclusions

Normande sired cattle had greater BCS compared to animals sired by HO, JE, AY, and OT. This may allow NO cows to mobilize additional body reserves during the summer months to support production. Cows sired by all breeds demonstrated a reduction in BCS and yield traits during summer, particularly in grazing herds. Normande sired animals did not lose as much fat yield compared to HO animals during the summer, but there were insufficient records to demonstrate significant differences among breeds in other yield traits. The exceptions were cows sired by bulls of unknown origin, crossbred sires, or breeds other than AY, HO, JE or NO; such cows had significantly smaller declines in BCS than NO sired cows and significantly larger declines in milk, fat and protein yield during summer months than cows of other sire breeds. This reinforces the need to use of proven sires of high genetic potential, even in grazing systems that incorporate crossbreeding.

Table 2-1. Known and unknown sire and dam breeds[1] for all animals in the data set

	Dam Breed						
Sire Breed	**AY**	**HO**	**JE**	**Unknown**	**OT**	**XX**	**Totals**
AY	60	3	2	15	1	24	105
HO	0	704	4	11	4	12	735
JE	0	9	55	11	0	12	87
NO	5	23	3	21	1	45	98
Unknown	6	37	12	119	0	27	201
Other	0	4	0	0	0	5	9
XX	0	0	1	0	1	3	5
Totals	71	780	77	177	7	128	1240

[1]AY = Ayrshire, HO=Holstein, JE=Jersey, NO = Normande, Other = other pure breeds, XX=Crossbred.

Table 2-2. Total number of body condition scores available for different sire breeds[1] and the type of grazing system

	Non-Grazing	Grazing	Total
AY	3	251	254
HO	744	187	931
JE	59	139	198
OT	4	377	381
NO	82	85	167
Total	892	1039	1931

[1]AY = Ayrshire, HO=Holstein, JE=Jersey, NO = Normande, OT = other.

Table 2-3. Sire and dam breed combinations available for BCS observations[1]

	Dam Breed				
Sire Breed	**AY**	**HO**	**JE**	**OT**	**Total**
AY	140	7	4	103	254
HO	0	881	8	42	931
JE	0	22	137	39	198
OT	21	64	28	268	381
NO	14	33	6	114	167
Total	175	1007	183	566	1931

[1]AY = Ayrshire, HO=Holstein, JE=Jersey, NO = Normande, OT = other.

Table 2-4. Sire and dam breed[1] combinations for test day records

	Dam Breed				
Sire Breed	**AY**	**HO**	**JE**	**OT**	**Total**
AY	784	32	30	530	1376
HO	0	5525	41	255	5821
JE	0	91	429	156	676
OT	92	457	162	1995	2706
NO	75	128	29	565	797
Total	951	6233	691	3501	11376

[1]AY = Ayrshire, HO=Holstein, JE=Jersey, NO = Normande, OT = other.

Table 2-5. Trait means, standard deviation (SD), minimum (Min) and maximum (Max) across all farms

Trait	Mean	SD	Min.	Max.
BCS	2.55	0.52	1	5
Daily milk (kg)	26.30	11.68	1.81	83.94
Daily fat (kg)	1.74	0.42	0.50	4.49
Daily Protein (kg)	1.44	0.19	0.95	3.27
SCS	2.33	2.00	0.1	9.6
Calving Interval (days)	400.20	62.99	305	550

Table 2-6. Herd information including grazing type, sire breeds[1], average daily milk yield, and average BCS

Herd	Grazing	Sire Breeds	Average Milk (kg)	Average BCS
1	Yes	AY, NO, OT	20.8	2.70
2	Yes	AY, HO, JE, NO, OT	26.2	2.48
3	Yes	AY, HO, JE, NO, OT	18.9	2.54
4	Yes	JE, NO, OT	17.1	2.64
5	No	HO, JE, NO	19.8	2.64
6	No	HO, JE, NO	27.6	2.48
7	No	HO, NO, OT	40.7	N/A

[1]AY = Ayrshire, HO=Holstein, JE=Jersey, NO = Normande, OT = other.

Table 2-7. Predicted means for body condition score (BCS), milk yield, fat (yield and percent), protein (yield and percent), somatic cell score (SCS) and calving interval (CINT) by breed of sire[1]

Sire Breed	BCS	Milk (kg)	Fat (kg)	Fat %	Protein (kg)	Protein %	SCS	Calving Interval (Days)
NO	3.08±0.13[ade]	23.56±0.66[bcd]	0.93±0.06[a]	4.11±0.17[d]	0.76±0.05	3.33±0.15	2.87±0.19[ae]	419±8.67[e]
HO	2.59±0.14[bd]	27.95±0.57[bcde]	1.11±0.06[b]	4.11±0.19[d]	0.85±0.06	3.17±0.17	2.26±0.19[bcd]	419±4.60[e]
JE	2.21±0.13[abce]	22.05±0.68[abce]	1.03±0.06	4.71±0.19[abce]	0.75±0.06	3.47±0.17	2.84±0.20[ae]	412±9.51
OT	2.69±0.12[bd]	24.62±0.61[ad]	0.98±0.06	4.12±0.17[d]	0.78±0.05	3.28±0.15	2.37±0.18[bcd]	395±6.56[abd]
AY	2.78±0.13[d]	25.12±0.69[abd]	0.98±0.06	4.06±0.18[d]	0.79±0.05	3.24±0.15	2.85±0.19[ae]	411±9.99[e]

[1]AY = Ayrshire, HO=Holstein, JE=Jersey, NO = Normande, OT = other.
[a] Significantly different from HO
[b] Significantly different from NO
[c] Significantly different from AY
[d] Significantly different from JE
[e] Significantly different from OT

Table 2-8. Predicted means for animals in grazing and non-grazing herd types by sire breed[1].

	Grazing[2]			Non-Grazing		
Sire	**Milk (kg)**	**Fat (kg)**	**Protein (kg)**	**Milk (kg)**	**Fat (kg)**	**Protein (kg)**
NO	20.90±1.60[a]	0.83±0.07[a]	0.69±0.06	27.38±1.83[ae]	1.07±0.07[ae]	0.85±0.06
HO	25.31±1.58[bcde]	1.03±0.07[bcde]	0.78±0.06	32.47±1.71[bd]	1.27±0.07[b]	0.97±0.06
JE	20.24±1.61[a]	0.87±0.07[a]	0.68±0.06	25.75±1.83[ace]	1.22±0.08	0.85±0.07
AY	22.14±1.57[a]	0.87±0.07[a]	0.72±0.06	32.20±3.15[d]	1.13±0.15	0.90±0.10
OT	21.52±1.54[a]	0.87±0.06[a]	0.70±0.06	31.33±1.91[bd]	1.25±0.08[b]	0.97±0.06

[1]AY = Ayrshire, HO=Holstein, JE=Jersey, NO = Normande, OT = other.
[2] All Grazing predictions were significantly lower than Non-grazing predictions
[a] Significantly different from HO within the column
[b] Significantly different from NO within the column
[c] Significantly different from AY within the column
[d] Significantly different from JE within the column
[e] Significantly different from OT within the column

Table 2- 9. Predicted means of daily milk yield, daily fat yield, daily protein yield and BCS for different breed[1] combinations

Sire Breed	Dam Breed	Milk(kg)	Fat(kg)	Protein(kg)	BCS
AY	AY	25.11±0.97	0.97±0.03	0.78±0.02	2.83±0.07
AY	HO	25.12±1.01	1.01±0.06	0.77±0.03	2.67±0.16
AY	JE	25.12±0.95	0.95±0.06	0.79±0.03	2.91±0.18
AY	OT	25.12±0.99	0.99±0.03	0.80±0.02	2.71±0.07
HO	AY	27.95±1.11	1.11±0.11	0.85±0.11	2.59±0.25
HO	HO	27.98±1.01	1.01±0.02	0.83±0.02	2.47±0.04
HO	JE	27.94±1.29	1.29±0.05	0.91±0.02	2.57±0.15
HO	OT	27.94±1.02	1.02±0.03	0.83±0.02	2.74±0.08
JE	AY	22.05±1.03	1.03±0.12	0.75±0.11	2.21±0.25
JE	HO	22.06±1.07	1.07±0.05	0.76±0.02	2.35±0.11
JE	JE	22.04±1.00	1.00±0.03	0.74±0.02	1.91±0.08
JE	OT	22.04±1.03	1.03±0.04	0.74±0.02	2.38±0.08
NO	AY	23.58±0.84	0.84±0.05	0.74±0.02	3.14±0.14
NO	HO	23.56±0.95	0.95±0.04	0.77±0.02	3.09±0.10
NO	JE	23.57±0.97	0.97±0.06	0.75±0.03	2.91±0.16
NO	OT	23.55±0.97	0.97±0.03	0.78±0.02	3.17±0.06
OT	AY	24.63±0.95	0.95±0.04	0.77±0.02	2.76±0.12
OT	HO	24.63±1.02	1.02±0.03	0.78±0.02	2.74±0.07
OT	JE	24.56±1.01	1.01±0.04	0.80±0.02	2.62±0.10
OT	OT	24.65±0.96	0.96±0.03	0.78±0.02	2.64±0.05

[1]AY = Ayrshire, HO=Holstein, JE=Jersey, NO = Normande, OT = other.

Table 2-10. Difference in yield from November through April minus July through August, difference in BCS from winter and spring minus summer

Sire Breed	Δ Milk	Δ Fat	Δ Protein	Δ BCS
AY	0.34±0.39[e]	0.08±0.02[e]	0.05±0.01[e]	0.17±0.06
HO	0.52±0.41[e]	0.11±0.02[d]	0.05±0.01[e]	0.17±0.07
JE	0.21±0.60[e]	0.06±0.03[e]	0.03±0.02[e]	0.17±0.07
NO	0.04±0.52[e]	0.05±0.03[be]	0.05±0.02[e]	0.27±0.07[e]
OT	1.59±0.31[abcd]	0.13±0.02[acd]	0.09±0.01[abcd]	0.13±0.05

[1]AY = Ayrshire, HO=Holstein, JE=Jersey, NO = Normande, OT = other.
[a] Significantly different from AY
[b] Significantly different from HO
[c] Significantly different from JE
[d] Significantly different from NO
[e] Significantly different from OT

Figure 2-1. Seasonal trends for daily milk yield of animals sired by Ayrshire, Holstein, Jersey, and Normande bulls in grazing herds

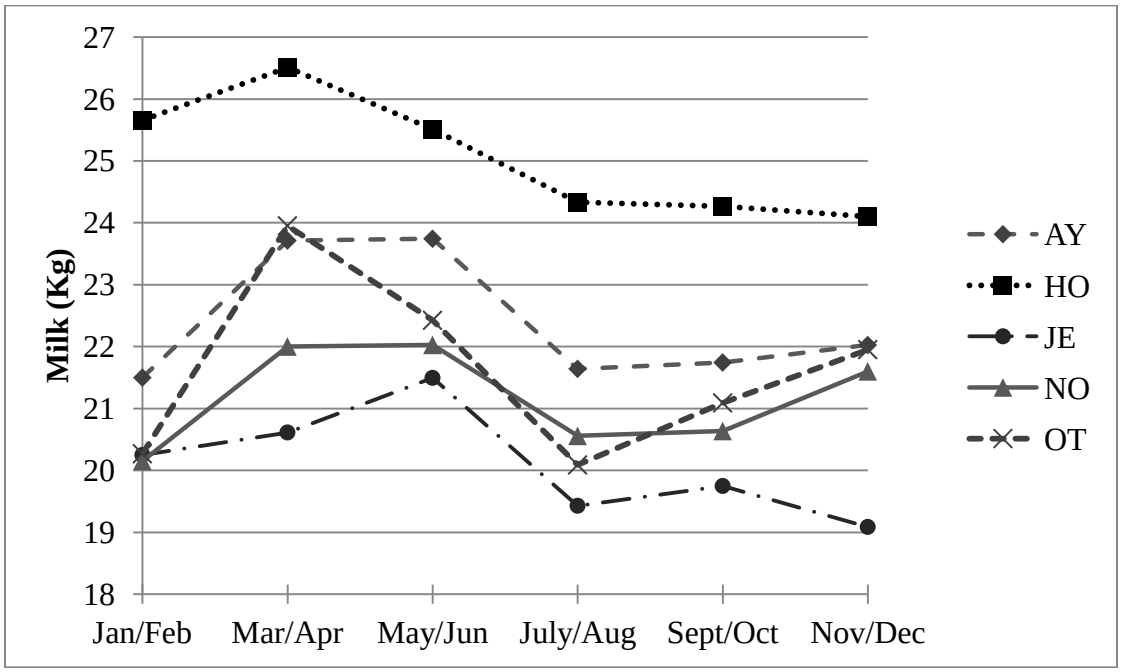


Figure 2-2. Seasonal trends for daily fat yield of animals sired by Ayrshire, Holstein, Jersey, and Normande bulls in grazing herds

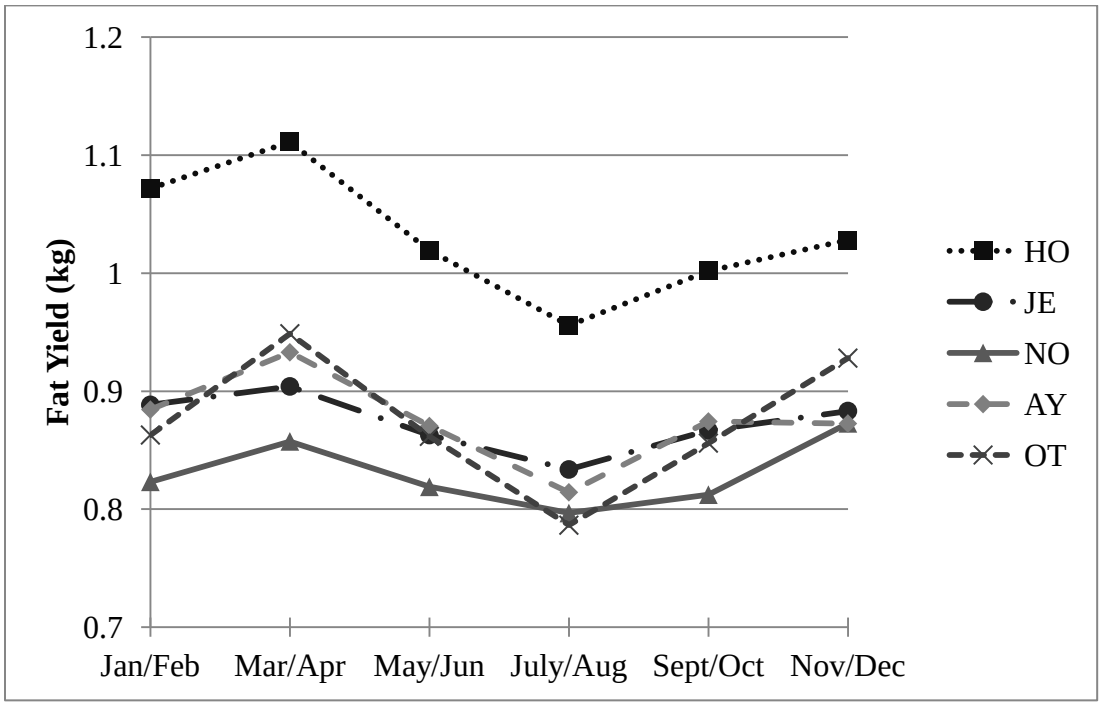


Figure 2-3. Seasonal trends for daily protein yield of animals sired by Ayrshire, Holstein, Jersey, and Normande bulls in grazing herds

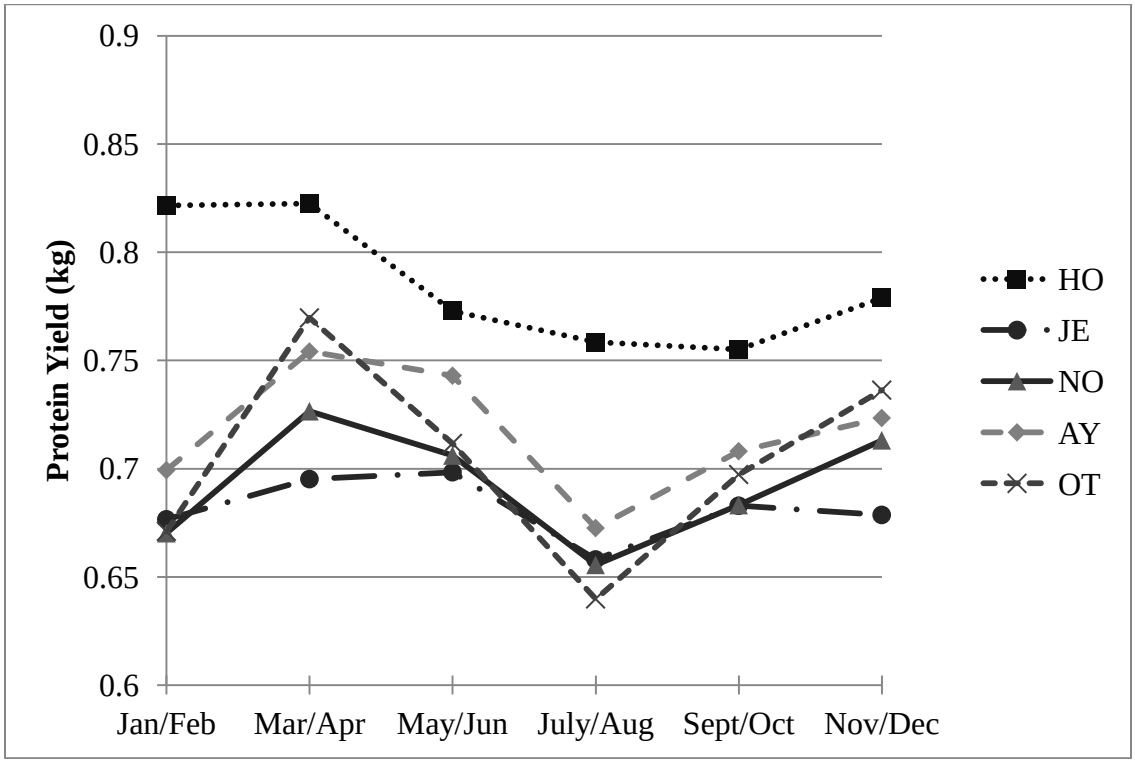
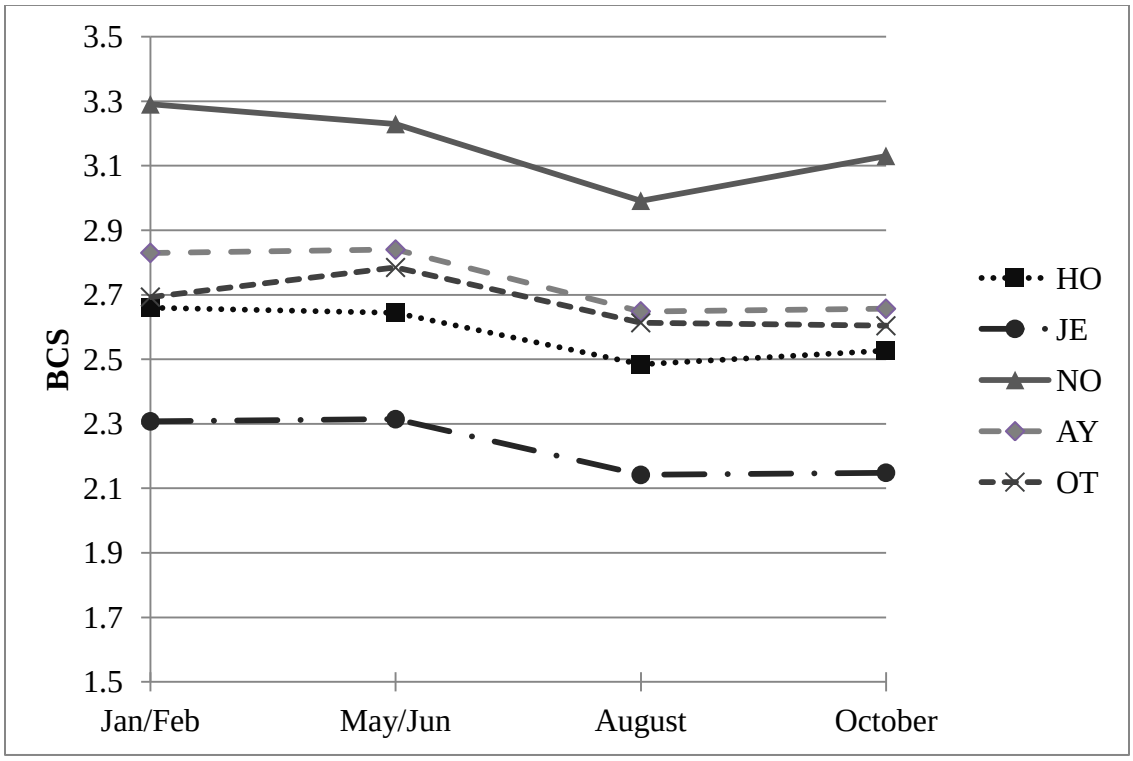


Figure 2-4. Seasonal trends for BCS of animals sired by Ayrshire, Holstein, Jersey, and Normande bulls in grazing herds



Chapter 3

Quantification of Telomeres in Purebred and Crossbred Dairy and Beef Cattle

Abstract

The objectives of this study were to evaluate differences among cattle from different herds and of different ages for telomere length and to determine if crossbreeding in dairy and beef animals had an effect on relative telomere length. Blood samples were collected from cows in two beef and two dairy herds for DNA extraction. The Penn State dairy research center provided purebred HO samples (69) and a commercial dairy herd in New York provided purebred HO samples (20) and NO×HO crossbred samples (14). The Penn State beef research center and a commercial Pennsylvania beef farm both provided purebred Angus (19 and 19, respectively) and Angus crossbred (13 and 15, respectively) samples. The telomeric repeat was quantified using a multiplex QPCR procedure. Two sets of primers were used in the same well; one for telomere amplification and a second to amplify a standard reference gene (beta-globin). Results were expressed as the relative amount of telomere product amplified (qT) to beta-globin amplification. In total, qT from169 animals were available for analysis after data editing. Mixed models and the PREDICT statement of ASREML were used to generate predicted qT for individual animals, herd, and breed. Dairy and beef cows were evaluated separately because there were no breed linkages across dairy and beef herds in order to determine breed versus herd effects. Herd was highly significant within both beef and dairy analyses ($P< 0.001$). There was a significant decline in qT as age increased in dairy cattle, but the decline was

not significant for beef cattle. Adding breed to the model was not significant for dairy (P = 0.71) or beef (P = 0.92) animals, indicating that there was no difference between crossbred and purebred animals. Results suggested wide variation among animals in qT. There was no evidence that longer telomere length explained the improved survivability reported for crossbred dairy cattle.

Introduction

Telomeres are the TTAGGG repeats found at the end of vertebrate chromosomes. During chromosomal replication the eukaryotic cell cannot perfectly replicate the lagging strand of the chromosome, thus a small segment of the chromosome is lost during each chromosomal replication (Olovnikov, 1973; Watson, 1972). Telomeres act as a disposable barrier that can be lost so that important genetic material is not sacrificed. Telomeres also help protect unwanted chromosome fusion and from exonuclease degradation (Blackburn et al., 2000). Telomere length has become an increasingly popular area of study because telomere length shortens with subsequent cell divisions and, thus, with increasing age.

Telomere length is highly variable among individuals at the same age and the rate at which telomeres erode appears to be different among individuals (Hewakapuge et al., 2008). This variation can be partly explained by genetics since telomere length has been shown to be a heritable trait (Slagboom et al., 1994). However, telomere length has been shown to be a quantitative trait with environmental conditions having a large impact on telomere length and rate of telomere shortening. Environmental factors that have been

found to impact telomere length include diet, psychological stress, and disease (Epel et al., 2004; Marcon et al., 2012; Willeit et al., 2010).

It has been shown that once the telomere becomes too short and reaches a critical limit, the cell undergoes senescence. The enzyme telomerase can rebuild the telomere, and is expressed at higher levels in germ and stem cells (Blackburn et al., 2005). However, telomerase is not expressed in high enough levels in somatic cells to rebuild or maintain telomere length.

Telomere length was shown to predict survival in humans (Cawthon, 2003) and birds (Haussman et al., 2005). Crossbreeding has been reported to increase survival in cattle (Dickinson and Touchberry, 1961; Heins et al., 2006). Variation in telomere length was demonstrated in two breeds of beef cattle in Italy and differences in telomere length among breeds of dogs have been reported (McKevitt et al., 2002). Improved telomere length or telomere maintenance could be one factor contributing to increased survival in crossbred cattle.

The objectives of this study were to determine if crossbreeding affected mean telomere length in cattle and to investigate age and environmental factors that may impact telomere length in cattle.

Materials and Methods

Blood was collected from 123 dairy and 87 beef animals from 4 herds using BD Vacutainer blood collection tubes (Becton Dickinson and Company, Franklin Lakes, NJ). In October 2011, blood was collected from the Penn State University beef research Center (PSUB; n=44), a commercial New York Dairy (CD; n=39), and a commercial Pennsylvania beef farm (CB; n=43). DNA was extracted using a commercial kit (Qiagen DNeasy®-96 kit; Qiagen Inc., Valencia, California), and stored at -20°C in pure water. Additionally, blood was collected from animals at the Penn State dairy research center (PSUD; n=84) in January 2012, and DNA extracted using the same commercial kit. Cow age on the day of blood collection was recorded for all cows. All DNA samples were tested for concentration and quality using the Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE); if the DNA quality was below 1.60 for the 260/280 ratio, the sample was eliminated from further analysis. Sixteen animals were eliminated from further analysis due to poor DNA quality. Pure water was used to adjust all remaining DNA samples to a concentration of 10 ng/µL.

A multiplex QPCR procedure developed by Cawthon (2009) was modified to quantify telomere length of individual cows. This multiplex method amplifies two PCR products including telomeric sequences and a standard reference gene (beta-globin).

Telomere primers developed by Cawthon (2009) were used and had the following sequences:

telg = acactaaggtttgggtttgggtttgggtttgggttagtgt, and

telc = tgttaggtatccctatccctatccctatccctatccctaaca.

These telomere primers are designed to make a specific 79 base pair product. Amplification of the TTAGGG repeat of telomeres was thought to be infeasible due to its repetitive nature and subsequent primer dimer formation during PCR. Therefore, the telomere primers were designed with specific base mismatches so that if the primers anneal to each other they are unstable and do not form dimers. The telg primer hybridizes genomic DNA and primes DNA synthesis during stage 1 of PCR cycle 2; telc also hybridizes genomic DNA but is unable to prime the PCR reaction because of a designed mismatch at its 3' end. In the second cycle of stage 2, the designed mismatch will allow telc to only bind at certain spots to the telg product synthesized in cycle 1 to create the specific 79 base pair product.

A second set of primers was specific to the bovine beta-globin gene. Primer Designer 2.0 (Scientific and Educational Software, 1991) was used to find an appropriate primer set (beta-1 and beta-2). GC-clamps were then added to primers to increase melting temperature so that the product is stable at 83°C (Cawthon, 2009). It is important to note that the GC-clamps are different from each other in sequence and length to prevent hairpin formation that would impede amplification. The beta-globin primers had the following sequences:

beta-1 = CGGCGGCGGGCGGCGCGGGCTGGGCGGgaaggcccatggcaagaagg, and

beta-2 = GCCGGCCCGCCGCGCCCGTCCCGCCGctcactcagcgcagcaaagg.

The GC-clamps are shown in capital letters. The expected product size of the beta-globin primers with GC-clamps was 144 base pairs.

QPCR reactions were set up by aliquoting 18µL of master mix into each reaction well, followed by 2µL of standardized DNA solution containing 20ng total DNA for a total volume of 20 µL per reaction well. The Applied Biosystems 7900HT Fast Real-Time PCR System in conjunction with a 384 well plate was utilized to facilitate the PCR reactions and each cow was run in triplicate. For each reaction well, the master mix contained 10 µL Quanta Green SYBR mix with Rox (Quanta Biosciences, Gaithersburg, Maryland), 1 µL each of the beta-globin and telomere primers and 4 µL pure water for a total volume of 18 µL. The telomere and beta-globin primers were diluted from a stock concentration so that 1µL of the diluted solution was added to the master mix to obtain reaction well primer concentrations of 900 nM for telc and telg and 500 nM for beta-1 and beta-2.

The thermal cycling profile was similar to that reported by Cawthon (2009) with adjustments to accommodate melting temperature differences. The thermal cycling profile was Stage 1: 15 min at 95°C; Stage 2: 2 cycles of 15 s at 94°C, 15 s at 49°C; Stage 3: 40 cycles of 15 s at 94° C, 10 s at 62°C, 15 s at 73°C with signal acquisition, 10 s at 80°C, and 15 s at 83°C with signal acquisition. Signal acquisition at 73°C provided the information to calculate Ct values (Ct_{Tel}) for the telomere product; the 83°C signal acquisition provided the information for the beta-globin product ($Ct_{\beta G}$).

During Stage 1 of the thermal cycling, the DNA polymerase is heat-activated and the genomic DNA is denatured. In Stage 2, the two cycles at 49°C allow the telomere primers to effectively anneal and extend as described above. Stage 3 begins with denaturation (94°C), annealing (62°C), and extension (73°C) with data collection. The

80°C step forces the telomere product to melt and ensures that data collection at 83°C measures only the beta-globin product.

The software program that accompanied the Applied Biosystems 7900HT Fast Real-Time PCR System (SDS 2.3, Life Technologies, Carlsbad, CA) does not compute Ct values for procedures that contain signal acquisition at multiple temperatures in the same well as is necessary for this protocol. Therefore, the multicomponent data file was exported from SDS 2.3 software which contained SYBR and background measurements for each well at both 73°C and 83°C. Ct values were calculated using the following procedure provided by Life Technologies technical support (personal communication):

1. Average SYBR readings were divided by average ROX readings to get the normalized reporter signal (Rn).
2. Rn from each cycle in the baseline range (cycle 3 to step prior to detectable amplification) was used to determine a linear regression and establish a base line.
3. The Rn from each cycle minus the correspondent value on the base line that accompanied the same cycle number was used to derive ΔRn.
4. The threshold value was set to ΔRn of 0.10.
5. The point at which the amplification curve was determined to cross the threshold was designated as the Ct value.

The SDS 2.3 program provided a Ct value at the final signal acquisition only (83°C), and the correlation between the derived Ct values for beta-globin and those estimated by the 7900HT software was 0.96

A standard DNA pool of cow genomic DNA was created using cows from each herd. The standard pool was analyzed with QPCR at eight concentrations (90ng, 70ng, 50ng, 30ng, 15ng, 7.5ng, 3ng, 1.5ng). The standard was run on each individual plate, with each concentration being run in triplicate and a standard curve was derived for telomere and beta-globin to determine primer efficiencies. Primer efficiencies were determined using the slope generated from the standard curve. The quantity of telomere and beta-globin products are expected to double each cycle; therefore, the relative quantity of telomere to beta-globin (qT) is 2^n where $n = Ct_{\beta G} - Ct_{Tel}$. The mean and coefficient of variation of qT among triplicates was calculated to identify and eliminate QPCR results that were not reliable. If the coefficient of variation was >20%, then the well that deviated the most from average was removed. If the remaining 2 wells had qT values that were similar (coefficient <10%) then those records were retained.

. Dairy cattle were stratified into maturity classes. If a dairy animals was lactating during blood collection it was considered mature and if the dairy animal had no history of lactating it was considered to be young stock. Detailed calving records were not available for all beef cows, so animals less than 2 years old were considered to be young stock and animals greater than 2 years old were considered mature which corresponded to the general management practices (heifers first calve around 2 years of age) on both farms.

ASREML (Gilmour et al., 2006) was used to analyze the QPCR data. Individual well Ct was included in the analysis as the independent effect and not the average of a cow's triplicates to better account for replicate variation. Both $Ct_{\beta G}$ and Ct_{Tel} were

evaluated with gene included in the model to accommodate the differences in Ct between them.

The mixed model used for dairy cattle was:

$$Y_{ghijklm} = G_g + H_{gh} + P_{gi} + M_{ghj} + A_{ghj} + AA_{ghj} + C_{gk} + CP_{ik} + W_{il} + \varepsilon_{ghijklm}$$

Where Y = the Ct for gene *g* in herd *h* on plate *i* in maturity class *j*, for cow *k* and well *l*. G = the effect of gene *g*; H = the gene specific effect of herd *h*; P = the gene specific effect of plate *i*; M = the gene specific interaction of maturity class *j* and herd *h*; A = the gene specific regression on age within maturity class j and herd *h*; AA = the gene specific regression on age^2 within maturity class *j* and herd *h*; C = the gene specific random effect of cow *k*; CP= the random effect of cow *k* on plate *i*; W = the random effect of well *l* on plate *i;* and e = random error. To determine breed differences, a two way interaction between gene and breed, and a three way interaction between maturity, gene, and breed were tested to predict breed differences. However, the breed effects were not significant and thus were left out of the final model.

The model explained previously was simplified using backward elimination to obtain a model for analyzing beef cattle data. The final model used for beef cattle was:

$$Y_{ghikm} = G_g + H_{gh} + P_{gi} + A_{jg} + C_{gk} + CP_{ki} + W_{ki} + \varepsilon_{ghikm}$$

Where Y = the Ct for gene *g* in herd *h* on plate *i* for cow *k* and well *m*. G = the effect of gene g; H = the gene specific effect of herd h ; P = the gene specific effect of plate i; A = the interaction between age *j* and gene *g*; C = the gene specific random effect of cow k; CP= the random effect of cow *k* on plate *i*; W = the random effect of well *m* on plate *i;* and e = random error. The interaction of gene and breed was included in the model to analyze breed differences, but was again non-significant.

This mixed model approach was utilized and is adaptable to varying QPCR experimental situations and is reported to be more accurate than the ΔC_t method (Steibel et al., 2009). The PREDICT statement in ASREML (Gilmour et al., 2006) was used to determine least-squares-means for $Ct_{\beta G}$ and Ct_{Tel} for each herd, cow and breed. The interaction of well and plate was used to control for pipetting and other experimental variation that is specific to a particular well. The random effects associated with cow allowed the estimation of $Ct_{\beta G}$ and Ct_{Tel} for each cow, which were used to derive cow qT. Cow qT were also estimated using the PREDICT statement in ASREML, and were derived at the actual age of each cow.

Results

In total, 25 animals were eliminated because of a high coefficient of variation. Of the remaining 169 animals with valid qT measurements, 57 had 2 wells available for analysis and 112 had 3 wells available for analysis. In total, 169 animals (Table 3-1) were available for analysis across the 4 herds. These 169 animals provided 936 Ct (468 $Ct_{\beta G}$ and 468 Ct_{Tel}) measurements for analysis; 340 Ct records were from beef animals and 596 Ct records were from dairy animals. Six animals were analyzed across all 3 plates. The number of animals per herd with usable qT measurements ranged from 34 to 69.

The specificity of the telomere and beta-globin primers was evaluated by agarose gel electrophoresis (Figure 3-1), which demonstrated clear bands at 79 and 144 base pairs. The dissociation curves created during QPCR also confirmed that the telomere product melted 13°C lower than the beta-globin product (Figure 3-2). The amplification efficiency for the standard curves averaged 87.9% for $Ct_{\beta G}$ and 99.5% Ct_{Tel}. However, it

is not clear whether the telomere product truly amplified more efficiently than beta-globin because beta-globin amplification is reflected to a small degree in the 73°C signal acquisition. Preliminary analyses accounting for potential efficiency differences did not have a strong influence on herd, breed or cow rankings which were of larger concern than the absolute qT differences. Therefore, efficiency corrections were not made given the inability to estimate efficiency accurately.

Telomere Analysis in Dairy Cattle

Mean qT data for dairy animals is reported in Table 3-2. The mean qT for all dairy animals was 27.61. Dairy young stock had a slightly higher average qT (28.57) than the mean qT of mature dairy animals (27.26). Herd was a highly significant variable for dairy animals ($P < 0.001$), indicating that large amounts of variation in telomere length can be explained by herd environment. In dairy cattle, maturity (lactating or young stick) of the animal within herd was found to be significant ($P < 0.001$), as was the age^2 squared effect ($P = 0.024$).

A scatter plot of predicted qT for all individual dairy animals is shown in Figure 3-3 for CD. A scatter plot of animal qT with age for animals from PSUD is shown in Figure 3-4.

The scatter plot for CD shows that qT appears to decline with increasing age across all ages. However, the scatter plot for PSUD animals does not follow the same trend. The scatter plot for PSUD indicates that young animals within the herd appeared to have a lower qT that increases slightly until approximately 2 years of age at which time qT begins to decrease with increasing age.

Figure 3-7 shows the scatter plot of dairy young stock. The standard errors for young stock in CD were much greater than that of PSUD because there were significantly fewer young stock observations for CD (n=7) compared with PSUD (n=21). Prediction for CD was also not possible at 1 year and 1 ½ years of age because no samples were available from the herd at those ages.

Figure 3-5 shows the scatter plot for mature dairy animals only for PSUD, and a decreased qT with increasing age is apparent. The same trend can be seen in mature animals from CD (Figure 3-6).

When breed was added to the model for dairy animals, the effect was not significant ($P = 0.705$). Table 3-3 shows the predicted qT for mature NO crossbreds and their purebred herd mates of HO.

Telomere Analysis in Beef Cattle

Mean qT for beef animals is reported in Table 3-2. Beef young stock had a greater mean qT (27.46) than mature beef animals (22.78). Large amounts of variation can be seen in qT between cows at the same length within individual farms. Herd was a highly significant variable for beef animals ($P < 0.001$), indicating that large amounts of variation in telomere length can be explained by herd environment in beef cattle.

The scatter plot for beef animals from the CB is shown in Figure 3-8, and the scatter plot for beef animals from PSUB is shown in Figure 3-9. Both beef herds have a similar trend for qT to decrease with age, with large amounts of variation seen at any specific age. However, the age effect was not significant in the model for beef cattle ($P =$

0.227).Maturity was also not significant when added to the beef model (P=0.522), so it was kept out of the final model.

The effect of breed was not significant for beef animals (P = 0.925). Table 3-3 shows the predicted qT for mature beef animals and their purebred Angus herd mates.

Discussion

Herd

A large amount of telomere variation can be explained by herd environment in both dairy and beef cattle. This is supported by previous work done in dairy cattle (Brown et al, 2012, *submitted*). The difference in qT between herds indicates that telomere length is a quantitative trait that is influenced by the individual's genetics as well as their environment. In human and chicken research it has been shown that an individual's environment and stressors found in the environment can decrease telomere length and increase shortening rate of telomeres (Beloor et al., 2010; Tyrka et al., 2010; Marcon et al., 2012). It is unclear whether temporary or permanent environment are greater contributors to telomere length variation. However, human research has reported multiple long-term factors causing chronic stress to shorten telomere length prematurely (Buxton et al., 2011; Ren et al., 2010; Damjanovic et al., 2007). It was also reported in broiler chickens that, when stocking density was increased above normal levels, broilers had shorter telomere lengths than broilers at lower stocking densities (Beloor et al., 2010).

Age and qT in Dairy Young-Stock

The age effect in dairy cattle was complicated by an unexpected age pattern in dairy young stock, which shows that the dairy young stock from PSUD appear to have a lower qT during the first few months that increases until they are approximately 2 years of age. This age trend is not seen in young stock from CD, which had a high qT at the beginning of life qT that was reduced by the time they were 6 months of age.

It is unknown why the young stock in PSUD had qT that appears to increase until the approximately 2 years of age. Research conducted in baboons found a rapid decrease in telomere length of nucleated blood cells during the first 50-70 weeks of life before the shortening rate stabilized and leveled off between 50-100 weeks of age (Baerlocher, 2007). In a longitudinal study of newborn children it was also found that during the first 36 weeks there was a significant decrease in telomere length of nucleated blood cells (Zeichner, 1999).

Since the young-stock and mature animals in this study represented different populations of animals as opposed to samples from the same animals at different ages, it is possible that there have been herd changes in PSUD that have resulted in lower qT for recently born animals. However, there is no known management or environmental changes that have occurred to readily explain such a circumstance.

An alternative explanation may be that qT measured from blood reflects changing immune cell populations circulating in blood. Telomere length varies with the type of immune cell (Hodes et al., 2002). T cells were found to generally have longer telomeres than B cells and different T and B cell subpopulations (naive versus memory cells) were also found to have differing telomere lengths. However, at birth calves have more T cells

and less B cells than adult animals (Woolums, 2010) so this explanation is also not straightforward. Nevertheless, the variation in cell-populations from blood may be partly responsible for the wide range of telomere length reported among individuals of the same age in studies across many species and warrants further exploration in cattle.

Colostrum intake or vaccination protocols in young PSUD animals could cause large shifts in blood immune cell populations that impact the measured telomere length of the animals in other herds. Maternal lymphocytes that are present in colostrums have been found to migrate across the intestinal wall of calves and enter the tissues of the calf (Woolums, 2010). Thus, the DNA extracted from the blood may reflect the mature colostrum donor's telomere length to some degree. However, a scatter plot investigating the predicted qT of calves along with the age of the colostrum donor in PSUD did not provide any obvious correlation (Figure 3-10).

When looking at mature dairy animals in CD and PSUD, qT seems to follow a linear decline with increasing age. This supports research done in humans, which has also noted large variation in qT among individuals of the same age despite declines with age (Ehrlenbach et Al, 2009, Damjanovic et al., 2007).

Age and qT in Beef Cattle

Age was not a significant variable in the beef cattle model. The reason age was not significant in the beef herds may be due to selection practices that favor animals with longer telomeres; if cows with short telomeres are at elevated risk for culling, then older animals are a select group that had longer than average telomeres when they were younger. It was found that lactating Holsteins that were in the lowest quartile for qT

were more likely to be culled than animals in the upper two quartiles (Brown et al., 2012, *submitted*). It is possible that cows in the beef herds experienced less environmental stress than cows in the dairy herds and thus the rate of telomere shortening was not as large as that seen in the dairy herds. It is also possible that beef cattle have favorable genetics that maintain telomeres better than their dairy counterparts. Longitudinal studies may provide additional insight into the telomere shortening rate differences that might exist between beef breeds and herds.

Crossbred Effects

Crossbred animals did not differ significantly from their purebred herd mates in dairy herds and in beef herds. Comparing beef cattle to dairy cattle for qT was not possible since a breed overlap between beef and dairy herds did not exist in any of the herds. There have been reported differences among different races and breeds of humans and dogs (Roux et al., 2009; McKevitt et al., 2002), and there has also been a reported breed difference found in beef cattle (Tilesi et al., 2010). However, the study that investigated the telomere length differences between two different breeds of beef cattle obtained the two separate breeds from independent herd environments and did consider herd environment in their analysis. Thus, the breed differences reported may have been confounded by herd environment. Breeds within the dairy and beef types may not be evolutionarily distant enough to have significant qT differences. Comparing beef animals to dairy animals within the same environment may yield breed differences in telomere length. Nevertheless, results from this study do not suggest that increased telomere length is the reason for increased survival in crossbred cattle.

Complications

Several complications arose during the project that limited the number of conclusions that could effectively be drawn from the data collected. One of complications which arose was that there is a large amount of variation in qT for animals at any given age. Because of this variation, larger numbers of samples than what we obtained in this study may be needed to obtain substantial conclusions. Specifically, in this study increasing the number of samples within one herd would have provided more power in analyzing breed differences. Although QPCR is relatively cheap and easy to perform, it also appears to be less robust than other methods (Aviv et al., 2011) and may not deliver the sensitive quantification needed to investigate telomere length without relatively large sample sizes. Using a more sensitive technique to quantify telomere length such as QFISH or Southern Blotting (Aviv et al., 2011) may have also provided more insight into breed differences and age differences if larger populations are not obtainable.

Conclusions

Large amounts of variation can be seen in qT of individuals at the same age, which supports previous research done in humans and other species. Crossbreeding did not affect animal qT in this study. It may be beneficial to investigate different types of purebreds to see if there is a breed difference. However, the samples may need to come from the same herd environment since there appears to be a large herd effect on animal qT.

There also appears to be variation in both the average telomere length and the rate of telomere shortening between herds. These differences may reflect differences in animal health and well-being if the telomeres are being shortened prematurely due to management practices, but this is outside the scope of this project.

Age was significant for dairy animals but not for beef animals. In PSUD, which provided the highest number of young stock samples, it appeared that telomere length was low in young stock and increased in length until the animals reached approximately 2 years of age. This pattern was not consistent in the other herds where young stock were predicted to have greater qT values compared to mature animals and where qT was gradually expected to decrease with age. Further longitudinal studies where individual animals are sampled at different times in their life may provide beneficial insights into how telomeres shorten in cattle in the future.

Figure 3-1. Agarose gel electrophoresis of the telomere amplicon (79 bp) and beta-globin amplicon (144 bp) following QPCR.

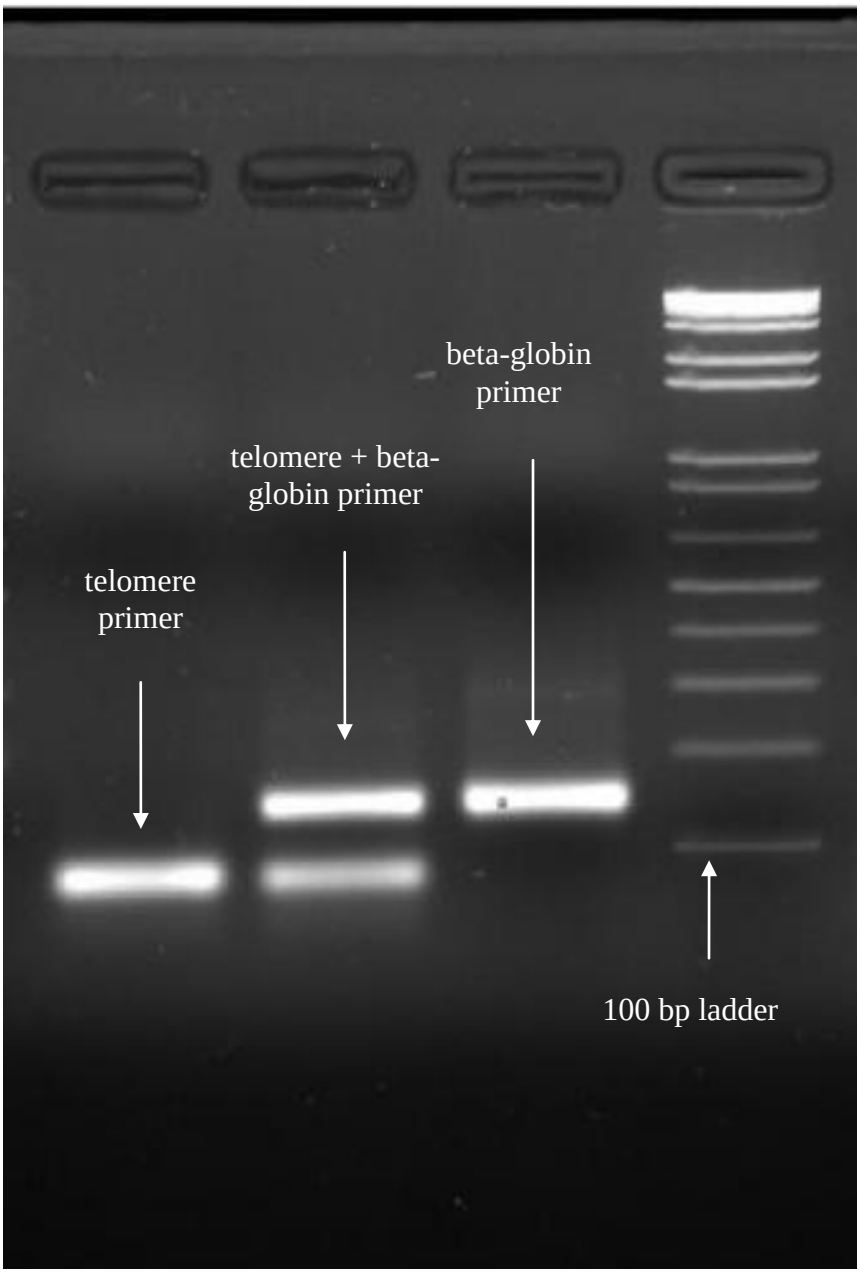


Figure 3-2. Melting curves following QPCR amplification showing the telomere amplicon melting at approximately 74.5°C and the beta-globin amplicon melting at approximately 87.5°C.

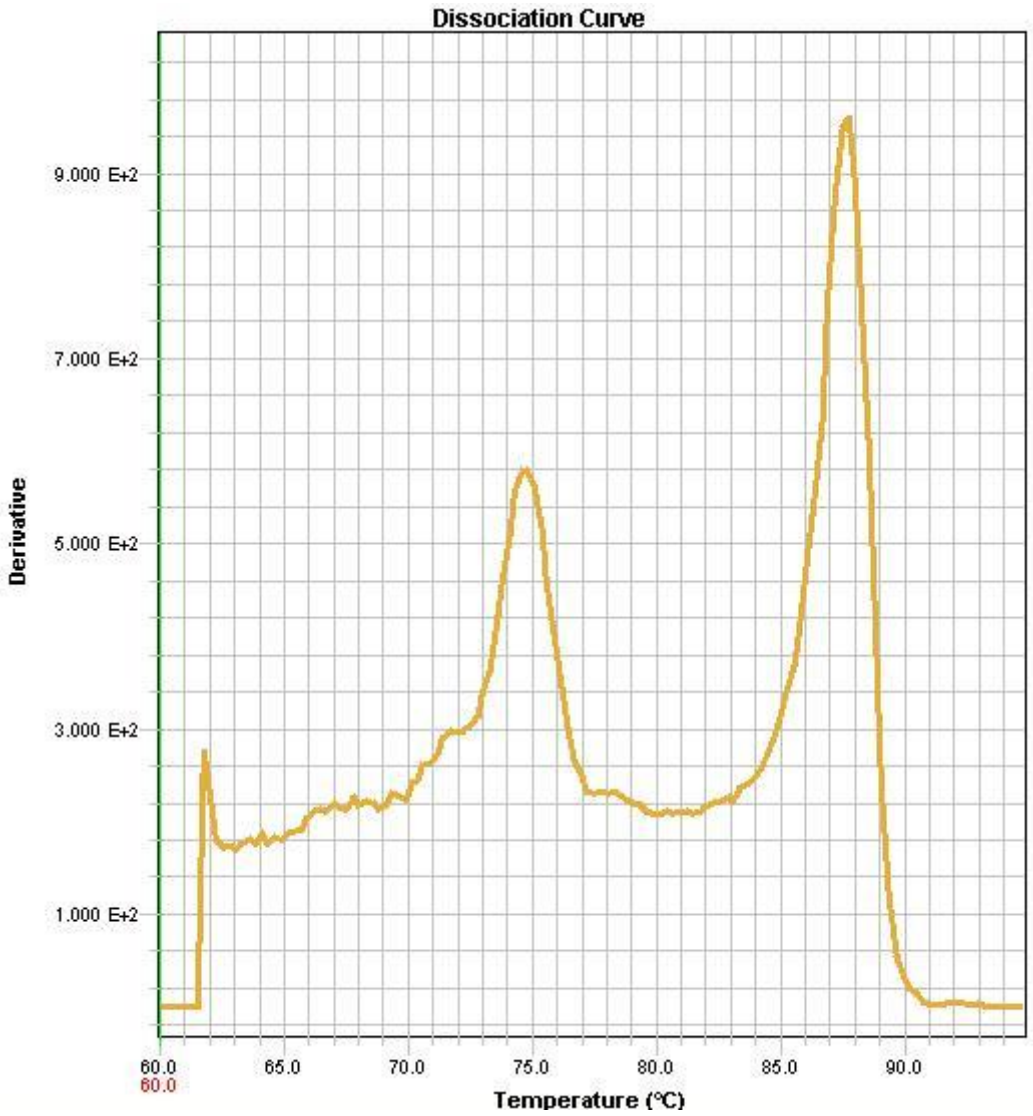


Table 3-1. Descriptive statistics by herd[1] for animals with valid qT measurements

Herd	# Animals	# Young Stock	# Mature Animals	Average Age
CB	34	12	22	4.89
PSUB	32	7	25	5.34
CD	34	8	26	4.07
PSUD	69	24	45	3.36
Totals:	169	51	118	4.19

[1]CB = Commercial Beef, PSUB = Penn State Beef, CD = Commercial Dairy, PSUD = Penn State Dairy

Table 3-2. Average Predicted Means qT for dairy animals stratified by maturity

Type	Maturity	Mean qT	# of Animals
Dairy	-	27.61	103
	Young Stock	28.57	28
	Mature	27.26	75
Beef	-	23.92	66
	Young Stock	27.46	16
	Mature	22.78	50

Figure 3-3. Individual qT estimates with age and breed for all animals in the commercial dairy herd

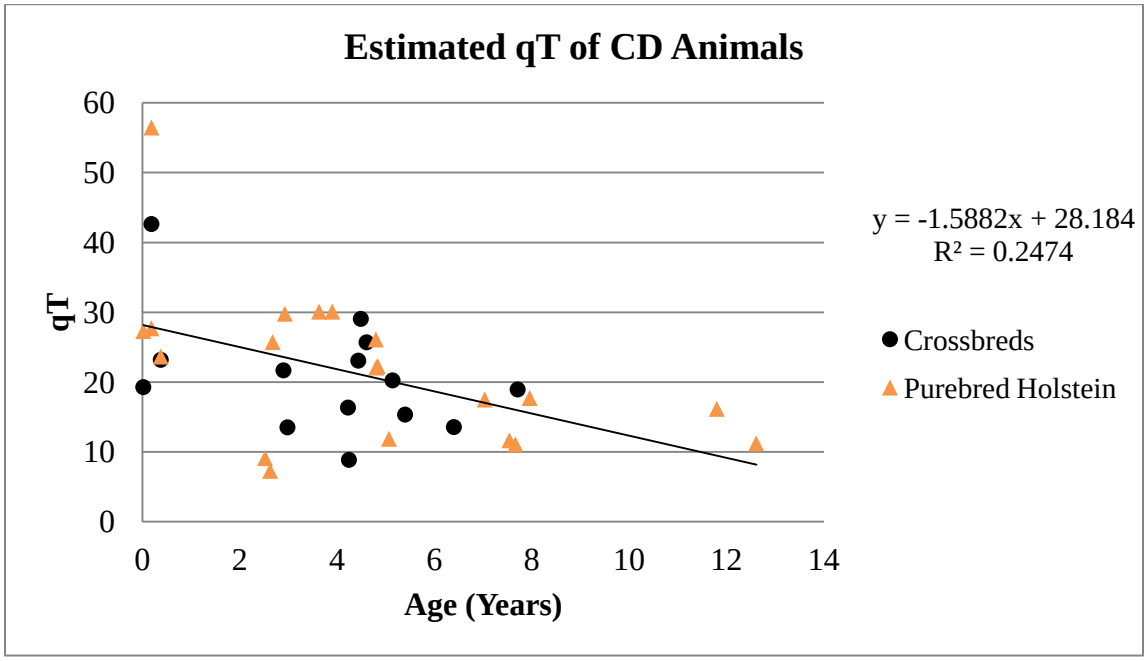


Figure 3-4. Individual qT estimates with age for all animals in the Penn State dairy herd

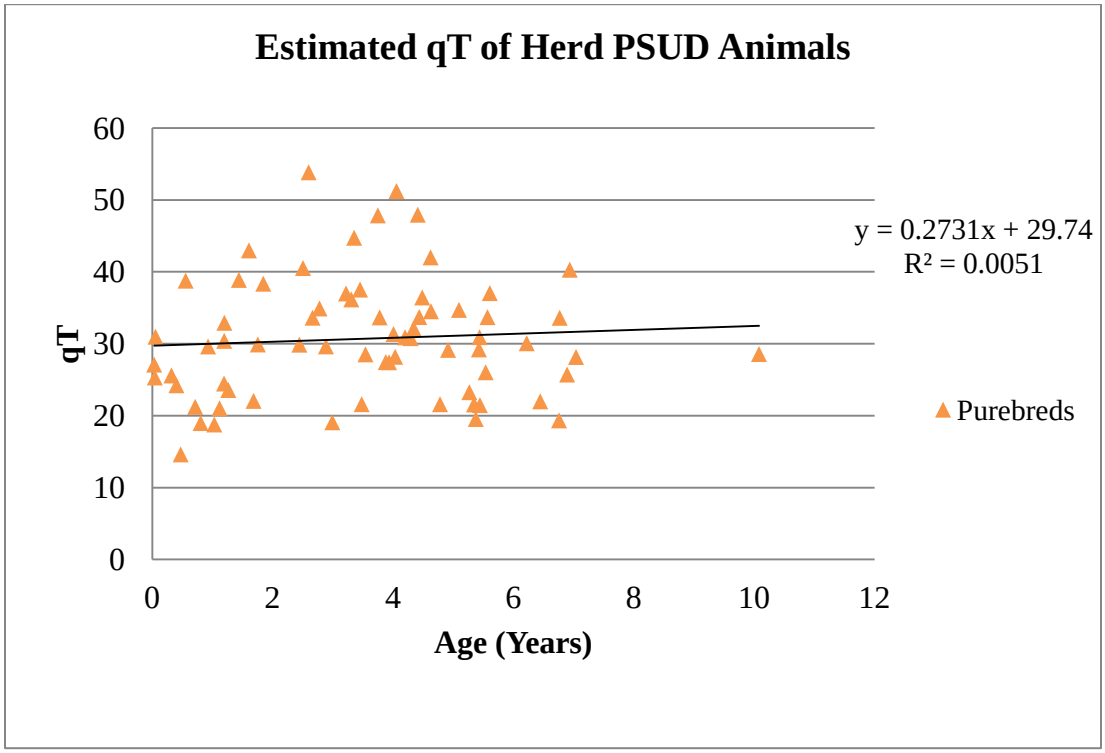


Figure 3-5. Individual qT estimates with age for mature animals in the Penn State dairy herd

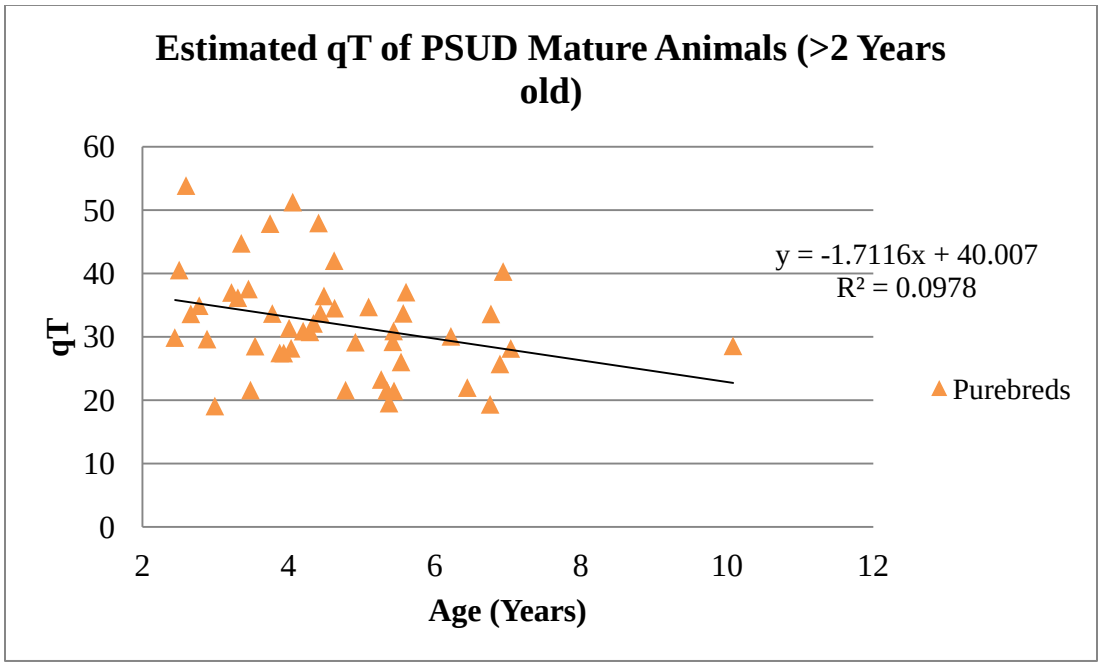


Figure 3-6. Individual qT estimates with age and breed for mature animals in the commercial dairy herd

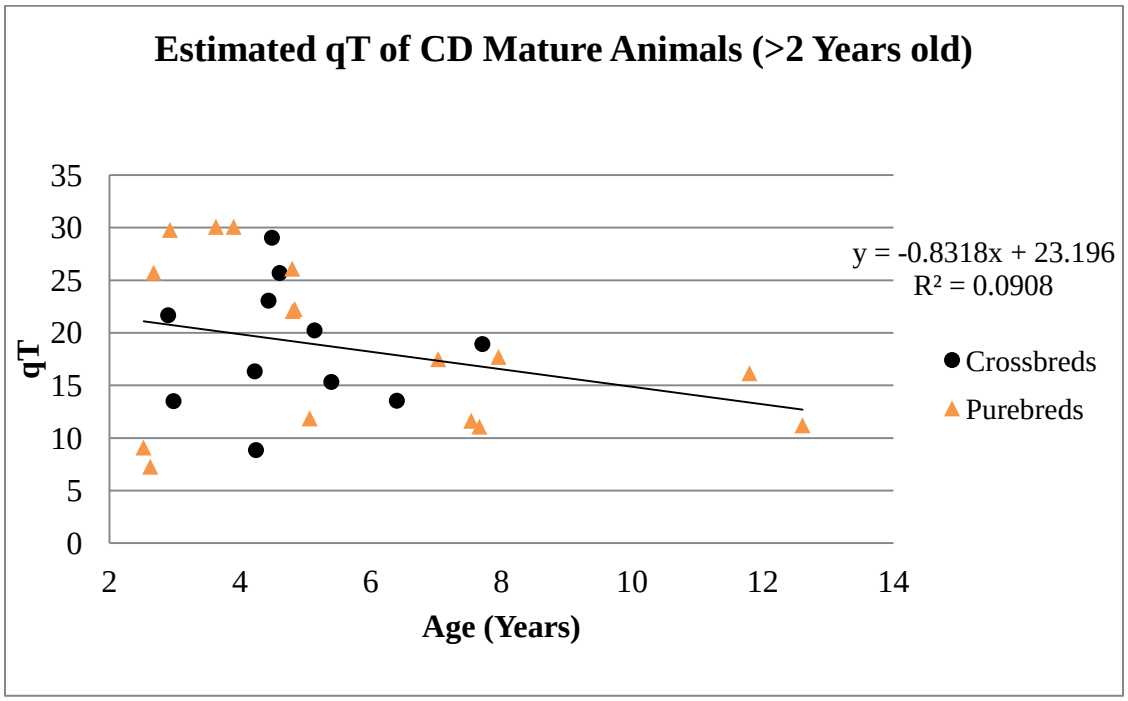


Figure 3-7. Individual qT estimates with age and for young stock in the Penn State and commercial dairy herds

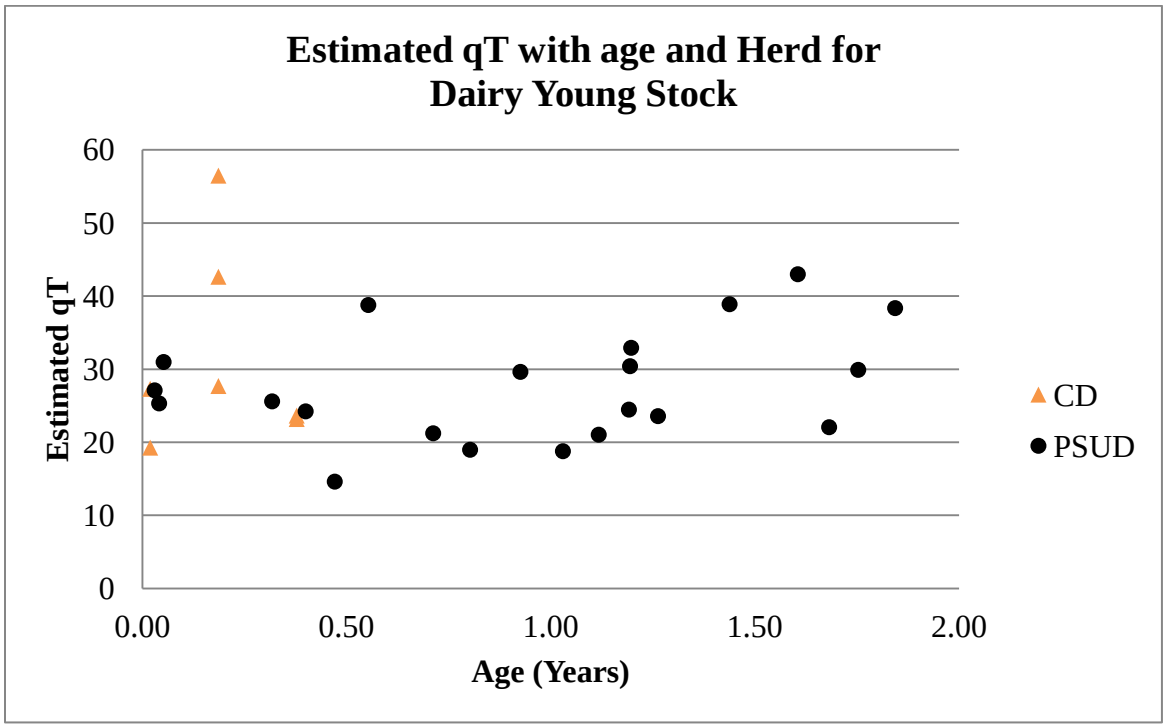


Figure 3-8. Individual qT estimates with age and breed for all animals in the commercial beef herd

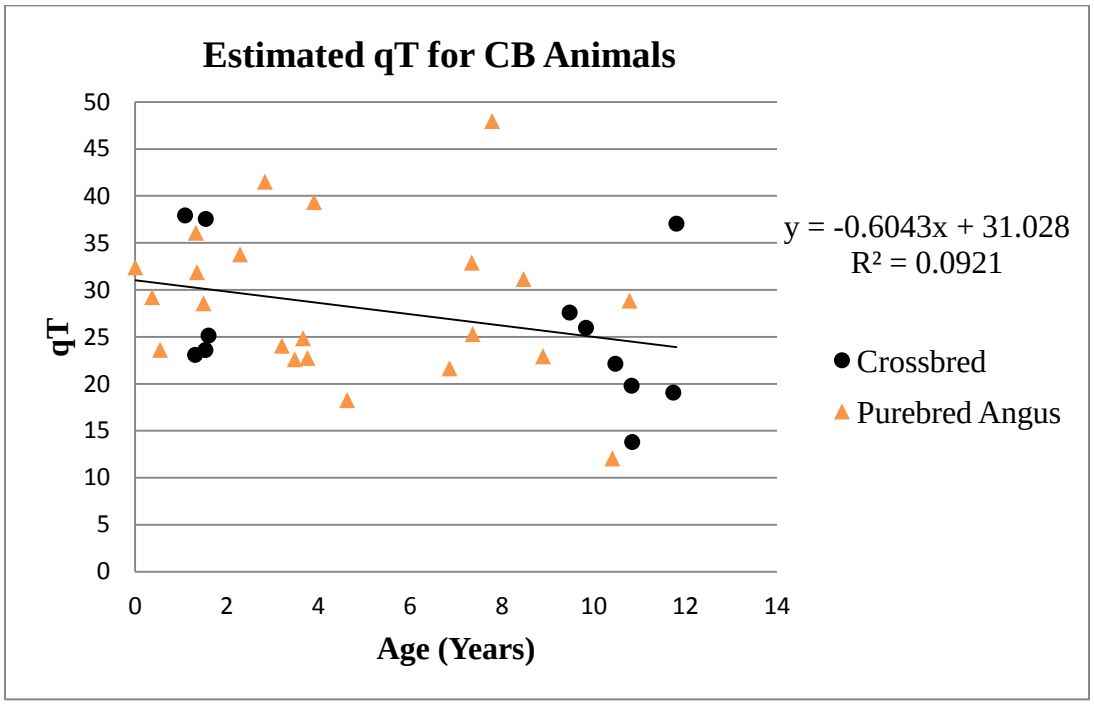


Figure 3-9. Individual qT estimates with age and breed for all animals in the Penn State beef herd

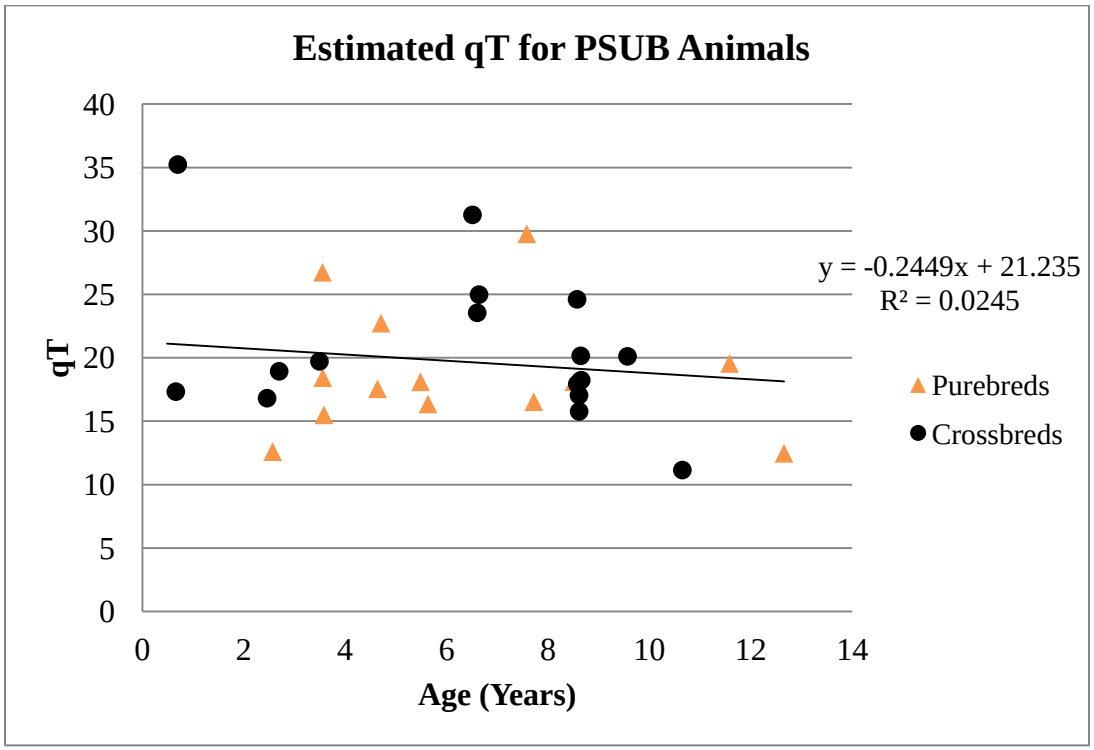


Figure 3-10. Predicted Calf qT with colostrum donor age

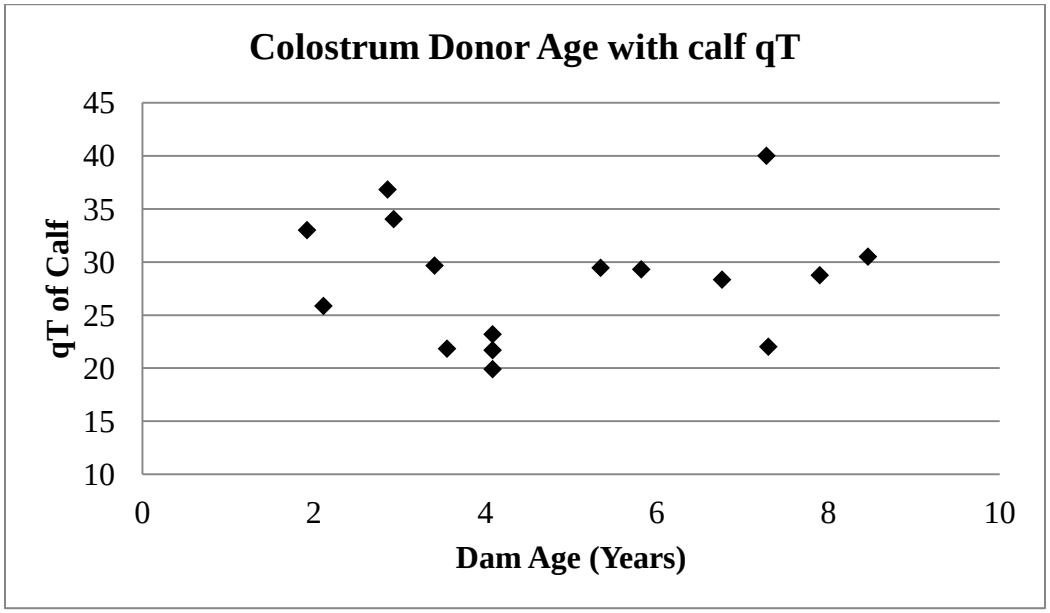


Table 3-3. Predicted qT and upper and lower confidence limits (CL) of crossbreds and purebreds for mature dairy and beef animals at the average age of all dairy and beef animals

Type	Breed	Average Age (Years)	Predicted qT	Lower CL	Upper CL
Dairy	Holsteins	3.63	24.57	22.89	26.28
	Crossbreds	3.63	24.16	20.89	27.95
Beef	Angus	5.60	22.05	20.60	23.60
	Crossbreds	5.60	22.83	21.39	24.37

Chapter 4

Conclusions and Future Research

Crossbreeding with Normande for BCS and Production

Normande sired cattle in this study had a lower daily milk yield than animals sired by AY and HO. It was previously found that NO×HO cattle had lower milk yields than their purebred HO herd mates (Heins et al., 2006; Walsh et al., 2008). Normande sired cattle did have a greater daily milk yield compared to JE. However, this increase in milk yield compared with E may not make up for the expected rise in maintenance costs that NO sired animals may present. Normande cattle have been found to have a greater body weight than HO and NO×HO crossbreds were found to have similar body weights to HO (Walsh et al., 2008). Normande sired cattle would be expected to be less efficient milk production animals compared other dairy animals because increased the Normande's increased BCS is negatively associated with milk yield (Dechow et al., 2001).

Normande and JE may be better breed compliments for grazing herds than NO×HO crossbreds. The NO×JE crosses could potentially have a more moderate body size and weight since Jerseys are small and body weight and body size are considered to be highly heritable traits (Bourdon, 1997). Genetics from the JE may also improve the daily fat yield in the crosses because the heritability of fat percent protein percent is high (Bourdon, 1997). Normande would provide additional BCS in such a system for potential mobilization during periods of slow pasture growth.

Another breed that may be of interest in future crossbreeding studies may be the AY which tended to carry more body condition than HO and JE sired animals in this

study. There is little literature available on AY cattle, which may make further investigations into utilization of this breed intriguing.

Telomeres as biomarkers

When focusing on mature animals there were significant differences among herds in qT and the rate of qT change with age among herds. Looking strictly at mean telomere length between herds may not give a clear indication of how environmental stress between herds is impacting telomere erosion, especially if animals from one herd genetically have increased telomere lengths. One would expect increased environmental stress to rapidly decrease telomere length in animals living in a high stress environment; in contrast we would expect slower telomere erosion in animals that do not have large amounts of environmental stress. Thus, investigating differences in the rate of telomere shortening between herds may provide insight into animal stress between herds.

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