GENETIC MARKERS FOR INCREASED MILK PRODUCTION IN SHEEP

By

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GENETIC MARKERS FOR INCREASED MILK

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REVIEW OF LITERATURE

INTRODUCTION

Economic Importance

The sheep industry is an economically important aspect of the US economy, particularly in the area of milk production. It has been estimated that in 2007, the value of the sheep industry for milk production was $2.9 million (Shiflett, 2008). Currently in the U.S., there has been an increased demand for sheep milk products that current producers cannot meet. This has been driven in part by a growing number of ethnic groups within the U.S. that seek more sheep dairy products, such as sheep milk cheese. In 1994, the U.S. imported approximately 66 million pounds of cheese made from sheep's milk (Thomas, 1996). This number has increased to 70 million as of 2008 (Shiflett, 2008; Thomas, 2008). While we are witnessing an increase in demand for sheep milk products there is a gradual decrease in sheep livestock numbers. Trends over the last ten years show a decrease in the number of sheep and lambs (USDA, 2009). In 1995, total sheep and lamb numbers were approximately 19,889 head, decreasing 13% over the next 3 years (USDA, 2009). In 1999 there was a 36% increase to 23,494 head; since then sheep and lamb numbers have been decreasing (USDA, 2009). In 2008, sheep and lamb numbers were 19,250 head (USDA, 2009). With an increased demand but

decreased supply of sheep products, there is a need for producers to increase the sheep milk production. One way to accomplish this objective is to select for ewes with greater milk production within the producers' breeding programs.

TRADITIONAL VS MOLECULAR GENETIC SELECTION

To select for greater milk production, breeders have traditionally relied upon phenotypic evaluation and selection. Traditional selection, i.e. phenotypic selection, has been in place for centuries, starting actually with the domestication of animals some 12,000 years ago. At that time, animals were selected based upon how well the animals could handle human management, how well the animals enjoyed human companionship, how easy the animals were to care for, how much the animals tended to group together, how useful the animals were to humans, and how easy the animals were to breed and birth (Clutton-Brock, 1987). From the point of domestication and onwards, breeds were developed based on the artificial selection criterion placed on the animals by humans. The actual practice of recording pedigrees and selecting related animals to improve a particular breed did not start until the mid-1700s when Robert Bakewell sought to improve Leicester sheep and Longhorn cattle (Simm, 1998). In addition to linebreeding and progeny testing, Bakewell took measurements and saved specimens to map the progress of his animals (Simm, 1998).

Animal breeding was once viewed as more of an art form than a science; breeders would select their favorite animals to breed together and hope for the magic of champion offspring. However, today animal breeding utilizes more science and technology than art (Werf, 1999). The current traditional selection is based upon phenotypic measurements.

Phenotypic measurements include factors such as weight, height, and amount of milk produced. The phenotypic measurements can then be turned into estimated breeding values utilizing statistics. Estimated breeding values are based on the animal's performance in combination with all its relatives' performance, and compared to the population mean. By utilizing estimated breeding values, environmental influences can be accounted for and removed as a variable in selection. From here, the genetically superior animals can be selected and used for breeding.

Thus far, phenotypic selection has been successful in predicting genetically superior animals. In dairy cattle, the primary goal of the producer is to increase the profitability of their animals through high production of milk, but without causing harm to the animal's safety or health (Simm, 1998). It should be noted though, if a producer is selling their milk to a buyer who specializes in processed dairy products, the emphasis is geared more towards milk composition (Simm, 1998). The actual amount of milk produced during the lactation period is affected by several factors, including genetic differences and nutrition; other important factors include frequency of milking, season of lambing, and geographic region. A great amount of research has been directed toward nutritional factors that can influence milk production, especially milk composition. These include the level of nutrition, forage-to-concentrate ratio, forage quality (e.g. particle size), and level and type of dietary fat. Advances in the understanding of these factors and the implementation of highly specialized diets and better management practices have resulted in considerable increases in milk production. In US dairy cattle, average milk production per lactation has doubled in the last forty years (Dekkers and Hospital, 2002); in Great Britain, average milk yield has almost tripled in the last seventy years (Simm,

1998). More than half of the increase in milk production has been due to the improvement in genetics (Figure 1) (Dekkers and Hospital, 2002). Despite these advancements, phenotypic selection could be improved to give better accuracy by utilizing molecular genetic selection techniques.

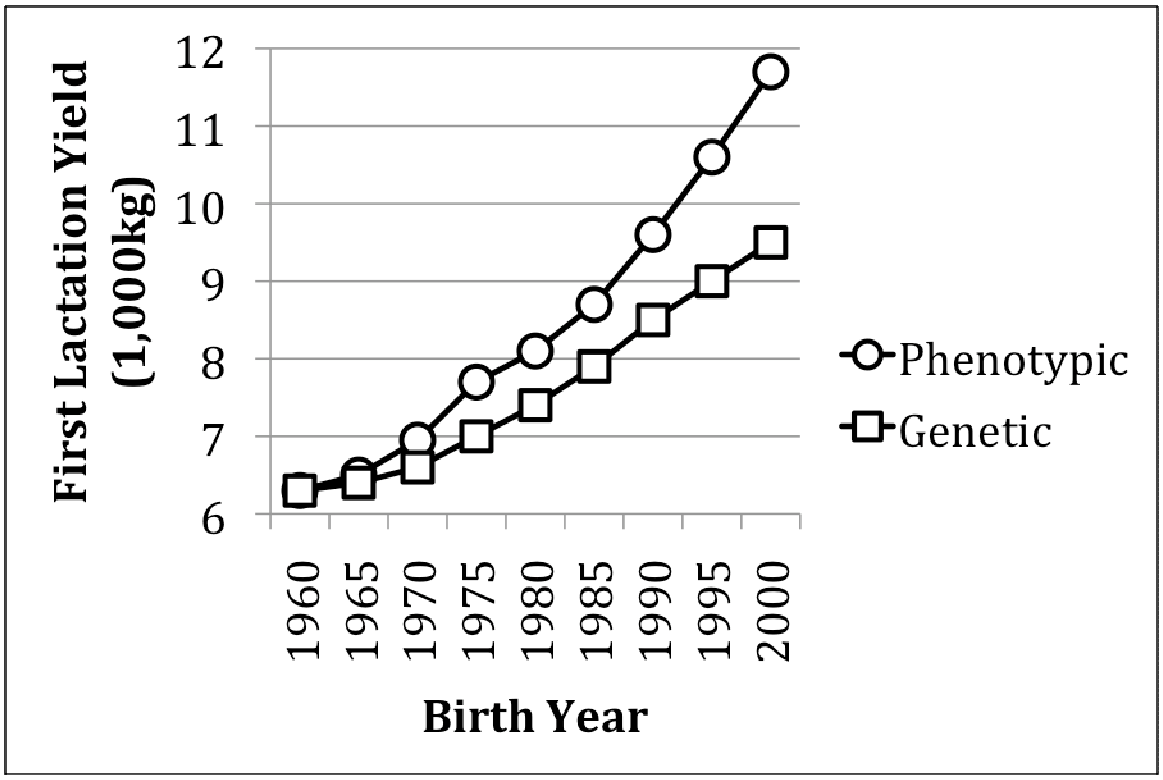

Figure 1. Milk yield in US cows.
Average milk production per lactation of US cattle has nearly doubled over the last 40 years; half of this has been due to genetics and improvements in genetic technology. Adapted from Dekkers and Hospital, 2002.

Milk production is a quantitative trait; several genes and the environment influence it. Due to the environmental influences, the estimated breeding value from traditional selection methods is not a perfect predictor of the genetic merit of an individual. In addition, milk production can only be measured in females that have reached sexual maturity; this makes it very difficult to analyze males and prepubescent

animals. However, with the recent developments and advancements in DNA technology to identify genes and quantitative trait loci, many of these pitfalls can be overcome because DNA can be obtained at any age and from any sex.

A quantitative trait locus (QTL) is defined as a region of the genome that contains one or more genes affecting a quantitative trait (Geldermann, 1975). There are two approaches to identify QTLs: genome-wide scans and the candidate-gene approach. Genome-wide scans are best for mapping the locus of a trait within a specialized population (Dekkers and Hospital, 2002), while the candidate-gene approach is best for direct detecting of loci within an unstructured population (Rothschild and Soller, 1997). Because candidate-gene markers focus on polymorphisms in a gene postulated to affect the trait, they are often tightly linked to the QTL (Dekkers and Hospital, 2002). Genome scans, on the other hand, are able to identify regions of a chromosome that affect the trait (Dekkers and Hospital, 2002).

Genome-scanning Approach

Genome scans require the use of many known markers that are evenly spread across the genome. Markers are typically spaced 10 to 25 centiMorgans apart across the genome, which represents approximately 125 to 250 markers evenly spaced across the bovine genome (Simm, 1998). This allows for the detection of previously unidentified loci that are related to the trait.

Early efforts to map QTLs for milk production using small numbers of genetic markers in cattle (Andersson-Eklund and Rendel, 1993; Bovenhuis et al., 1992; Cowan et al., 1990a; Geldermann et al., 1985; Hoeschele and Meinert, 1990; Schutz et al., 1993) did little in determining the genes responsible for the genetic variance seen in milk

production (Georges et al., 1995). This could be due to the complex relationship between the genotype and phenotype, making it difficult to identify recombinants between markers and trait loci (Andersson, 2001). The main problem encountered with milk production and QTLs is that a majority of the populations used in milk production studies have already been selected intensively for milk production; therefore it has been assumed that genes with any effect on milk production have already been fixed, while those still segregating have a minor effect, making mapping of QTLs difficult (Georges et al., 1995).

To increase the ease of mapping QTLs, two different approaches have been pursued: creating experimental crosses or using naturally available populations (i.e. commercial cattle populations) (de Koning et al., 2003; Georges, 2007). Experimental crosses are either planned backcrosses or inter se matings (F2) of F1 individuals. The F1 individuals are the product of crossing two breeds that are highly divergent for the trait of interest. While backcrosses tend to provide more detection and resolution power when the QTL is a dominant trait, inter se matings are preferred due to the design's better ability to provide a "general picture", i.e. the number of segregating QTLs and estimates of the QTLs' additive and dominance effects (Darvasi, 1998). Backcrosses only require the generation of a few F1 males that can then be mated to purebred females; inter se matings, however, require the additional generation of F1 females to mate with the F1 males (Georges, 2007). Experimental crosses are typically best suited to species with shorter reproductive cycles and higher progeny numbers, such as pigs, poultry or laboratory species, due to the high number of progeny that are required for the tests to give any power to the mapping of the QTL (Georges, 2007). Typically, mapping QTL is

only the first step in identifying the underlying genes. Fine mapping QTL to chromosome intervals that are sized to where positional cloning is possible requires the generation of thousands of additional progeny, a fact that has been well established in experimental crosses utilizing inbred strains of mice (Darvasi, 1998) and one that would not be economically feasible in the larger livestock species. In addition, backcrosses and inter se matings are designed to target the genes that underlie the phenotypic differences between breeds, but most of the ongoing breeding programs are exploiting the genetic variation that exists between breeds (Georges, 2007).

The best option for larger livestock species in QTL mapping is to utilize the naturally available populations, otherwise known as the commercial pedigrees. These populations are a good option because the pedigrees are readily available, fine mapping approaches based on population-wide linkage disequilibrium can be implemented, and the targeted QTL are still segregating within the population (Georges, 2007). Due to the extensive use of artificial insemination, some cattle populations are filled with paternal half-sibling pedigrees that are particularly well suited to QTL mapping; in dairy cattle, it is not uncommon for a bull to have anywhere from 100,000 to 200,000 milking daughters. Due to the large number of related individuals spawning from one individual, the "daughter design" and "granddaughter design", sub-sets of the progeny testing strategy, have become well-accepted strategies of mapping QTLs in commercial dairy populations (Georges et al., 1995; Heyen et al., 1999; Neimann-Sorensen and Robertson, 1961; Soller and Genizi, 1978; Weller et al., 1990). The daughter design uses marker information and phenotypic data from paternal half-sibling sisters for detection of QTL effects (Georges, 2007; Heyen et al., 1999). The granddaughter design employs marker

information and sire breeding values for quantitative traits that are estimated from the records of the granddaughters of a common sire (Heyen et al., 1999); the granddaughters are the daughters of large sets of paternal half-brothers (hence the sire breeding values) (Georges, 2007). The granddaughter design tends to be more popular as it requires 2.5 to 3 times fewer genotyped animals than the daughter design (Georges et al., 1995; Weller et al., 1990). In the granddaughter design, at least only 50 daughters are required for each paternal half-sib brother to estimate each males respective breeding value; therefore, the males' breeding values are more accurate in predicting genetic merit than the cows' breeding values that were estimated from their own performances and pedigree data (Georges, 2007). By combining the marker information with the estimated breeding values of the progeny, the QTL mapping gains more statistical power (Andersson, 2001; Dekkers, 2004; Lande and Thompson, 1990); a practice that has been proven in previous milk production studies (Georges et al., 1995; Heyen et al., 1999; Zhang et al., 1998).

Additional selection strategies for mapping QTL include selective genotyping (Lander and Botstein, 1989), progeny testing (the daughter and granddaughter design fall under this category) (Heyen et al., 1999; Lander and Botstein, 1989; Neimann-Sorensen and Robertson, 1961; Soller and Genizi, 1978; Weller et al., 1990), interval mapping (Lander and Botstein, 1989), the simultaneous search for multiple QTL (Lander and Botstein, 1989), the use of DNA pools (Arnheim et al., 1985), and the study of disease-tagged QTL (Georges et al., 1993).

Candidate-gene Approach

In the candidate-gene approach, typically a known mapped gene is chosen due to a possible function in relation to the trait of interest and that the known gene may be

linked to the trait of interest in other species (Simm, 1998). For example, in our study we are investigating beta-lactoglobulin and prolactin in sheep because studies in cattle have shown associations between these genes and milk production (see later sections). The candidate-gene approach focuses on polymorphisms within the gene of interest, therefore there is usually a tight link to the QTL and sometimes the candidate-gene marker can represent the functional variant, but this is difficult to prove (Andersson, 2001).

Detection of association in the candidate-gene approach is based on the existence of linkage disequilibrium between the marker and QTL (Dekkers and Hospital, 2002). Linkage disequilibrium is the condition in which the frequency of a particular haplotype for two loci is significantly different from the expected frequency under random mating (Dekkers and Hospital, 2002). A detected polymorphism must be in disequilibrium with the causative polymorphism in order to have an association with the phenotypic variation (Lander and Kruglyak, 1995). Markers that are tightly linked to a QTL can be in complete or partial population-wide linkage disequilibrium with the QTL, so much so that some marker-QTL haplotypes may occur more frequently than expected by chance (Dekkers and Hospital, 2002). The probability of population-wide linkage disequilibrium is higher for closely linked markers and in select populations of small effective size, which is the case for agricultural species (Farnir et al., 2000). The problem with this is when a marker and a QTL are in linkage disequilibrium, all marker-QTL haplotypes are present and in random-mating frequency, and the marker genotype does not give any information about the QTL genotype (Dekkers and Hospital, 2002). In addition, unless the functional polymorphism has been identified, the linkage phase of a candidate-gene marker with the functional variant can differ from one population to the next, and

therefore must be assessed in the population that it will be used in (Dekkers and Hospital, 2002). However, the marker and QTL will be in partial disequilibrium within a family, depending on the recombination rate; this within-family disequilibrium can be used to detect QTL and for selection on a within-family basis (Dekkers and Hospital, 2002).

Marker-Assisted Selection

With the discovery of microsatellite markers following the development of polymerase chain reaction (Weber and May, 1989), characterization of QTLs through genome-scanning or through the use of the candidate-gene approach, could lead to more efficient breeding programs by using marker-assisted selection (Soller and Beckman, 1982) especially for traits that are difficult to improve when using traditional selection (Meuwissen and Goddard, 1996; Meuwissen and Van Arendonk, 1992). All strategies for marker-assisted selection are based on the use of a molecular score to enhance traditional selection. If many QTL are known, and the favorable alleles are present in different breeds or lines, genotype building strategies can be used to design new genotypes based on combinations of the favorable alleles at all of the loci (Dekkers and Hospital, 2002). Selection is then based on the molecular score alone, which is determined by loci genotypes and any information available on linkage and linkage phase between the loci (Dekkers and Hospital, 2002). By using a cross between two parental lines, the simplest genotype building strategy is to screen a population for the individuals that are homozygous at the relevant loci (Van Berloo and Stam, 1998). Additional generational matings may be necessary to produce homozygous individuals for a greater number of loci (Charmet et al., 1999; Hospital et al., 2000).

There are two major genotype-building programs in marker-assisted selection: introgression programs and recurrent selection programs. Introgression programs are a simple form of genotype building; the target gene is introduced into a productive recipient line by crossing to an otherwise low-productive donor line that contains the gene, and then backcrossing the F1 to the recipient line to recover the recipient line's genome (Figure 2) (Dekkers and Hospital, 2002). The target gene is maintained in backcross generations through the selection of individuals that carry the target gene. The effectiveness of introgression schemes is limited by the ability to identify backcross or intercross individuals with the target gene and by the ability to identify backcross individuals that have a high proportion of the recipient genome, especially in the regions around the target gene (Tanksley et al., 1989). Recurrent selection programs are little more complex than introgression programs. The molecular score used in recurrent selection is obtained as the estimate of the statistical association between the marker genotype and phenotype (Dekkers and Hospital, 2002). In addition, the phenotypic information on the individual and/or its relatives is also used. Therefore selection can be based on molecular score alone, on molecular score, followed by phenotype, or on a combination of the molecular score and phenotype (Dekkers and Hospital, 2002). Selection on molecular score alone will result in less genetic improvement than combined selection on molecular score and phenotype, unless the molecular score captures all of the genetic variation or the phenotypic records do not provide any information that allows for selection. If the phenotypic data is available with the molecular data, then selection combining both is the most powerful strategy. Using the selection index theory, methods have been developed to derive an index for combined selection (Lande and Thompson,

1990). The index optimally weighs molecular score and phenotypic data in order to maximize accuracy of the individual's breeding value. Combined selection is most effective for traits with low heritability and when phenotypic information is limited because of the inability to record the phenotype on all selection candidates before selection (Meuwissen and Goddard, 1996). However, the ability to detect QTLs is limited when phenotypic data is not present (Moreau et al., 1998). The traits that best benefit from marker-assisted selection are those with low and moderate heritability (Dekkers and Hospital, 2002).

In dairy cattle, marker-assisted selection is being used to increase the accuracy of identifying high merit young bulls to be used in progeny testing in Britain (Simm, 1998). The markers at the loci that control the secretion of milk proteins κ-casein and β-lactoglobulin are being used; these loci serve as linked markers within sire families for QTLs that affect the production of milk and fat, and protein yields (Cowan, 1994). Sons with the genotype AA for κ-casein and BB for β-lactoglobulin had the poorest predicted transmitting ability (PTA, the dairy cattle equivalent to estimated breeding value) for milk and protein yield, and the highest fat: protein ratio (Cowan, 1994).

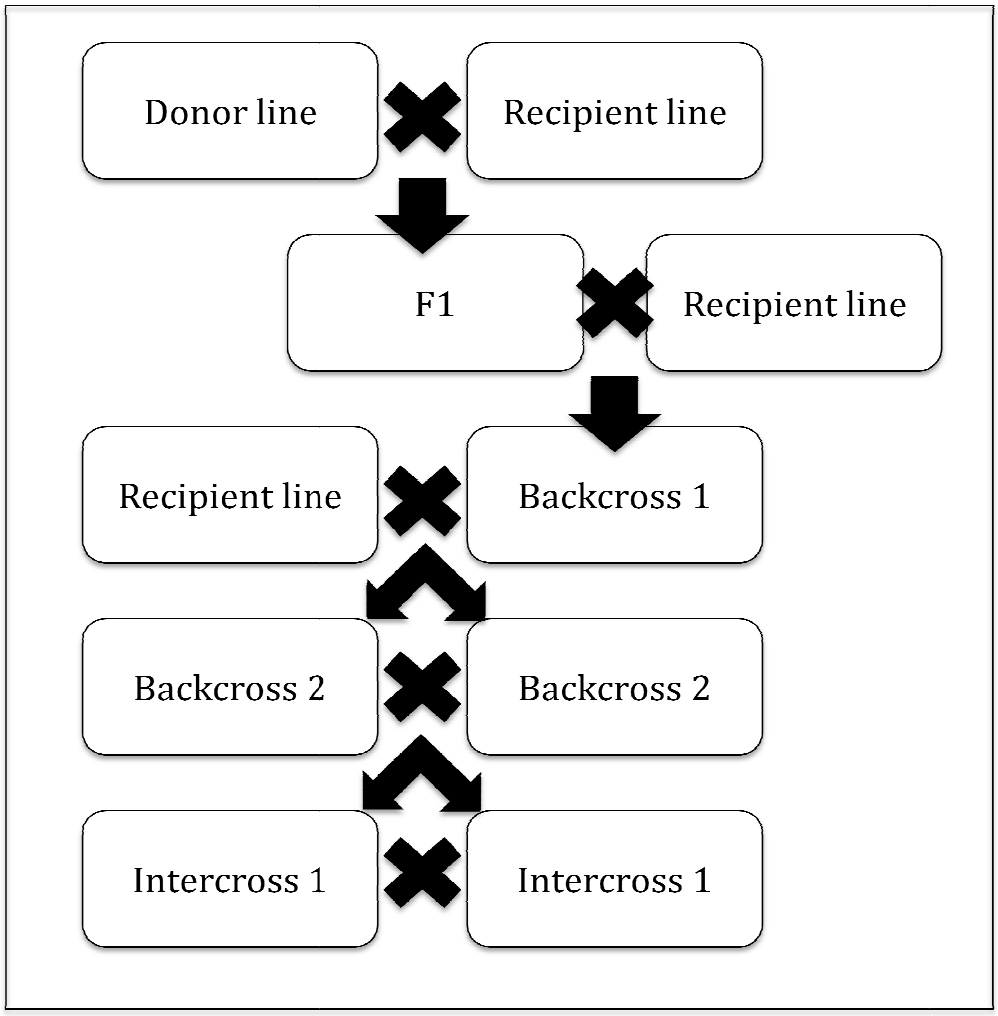

Figure 2. Introgression model.
The simplest model of genotype building strategies. The donor line contains the gene of interest, but has a poor production record. The recipient line does not contain the gene of interest, but has a good production record. The lines are crossed in hopes of capturing the gene of interest in the F1 population, which is then backcrossed to the recipient line. This is done in hopes of keeping the gene of interest and a high proportion of the recipient line. Adapted from Dekkers and Hospital, 2002.

Through the combined use of estimated breeding values and molecular data, β-lactoglobulin and prolactin are being researched as possible molecular markers to use in marker-assisted selection for increased milk production in sheep. By utilizing marker-assisted selection, selection can be made before the animal reaches maturity thereby

identifying genetically superior animals earlier, reducing the generation interval and increasing the amount of genetic progress per year.

MILK PRODUCTION

Milk Production - Introduction

Milk production is the process where female mammals provide a nutrient dense food, milk, for their young. This is the basic description of milk production; however, milk production is far from a basic procedure. The process is actually quite complex, involving the interaction of genes, hormones, and neural and environmental stimulants.

Milk Production – Cytology

The bovine mammary gland is composed of a network of ducts and cisterns that could best be described in shape as a tree of sorts (Figure 3). The teat and teat cistern would be like the trunk of the tree, and the major ducts would be the larger branches that lead to the lobes of alveoli bunches. The lobes, comprised of the alveoli and terminal ducts, would be similar to the smaller branches or twigs of leaves; the terminal ducts would be the twigs and the alveolus, would be the leaves. The alveolus are spherical in shape and hollow; they are lined with alveoli cells that synthesize the milk, and the alveolar lumina, the hollow middle, holds the milk until the milk is drained. Myoepithelial cells, similar in structure to smooth muscle cells, are spindle shaped contractile cells that surround each alveolus in a mesh-like fashion (Senger, 2005b). A collagenous matrix provides further support to the lobes, and is separated from the secretory alveoli cells and the myoepithelial cells by a thin layer of basal lamina (Jimenez et al., 1984).

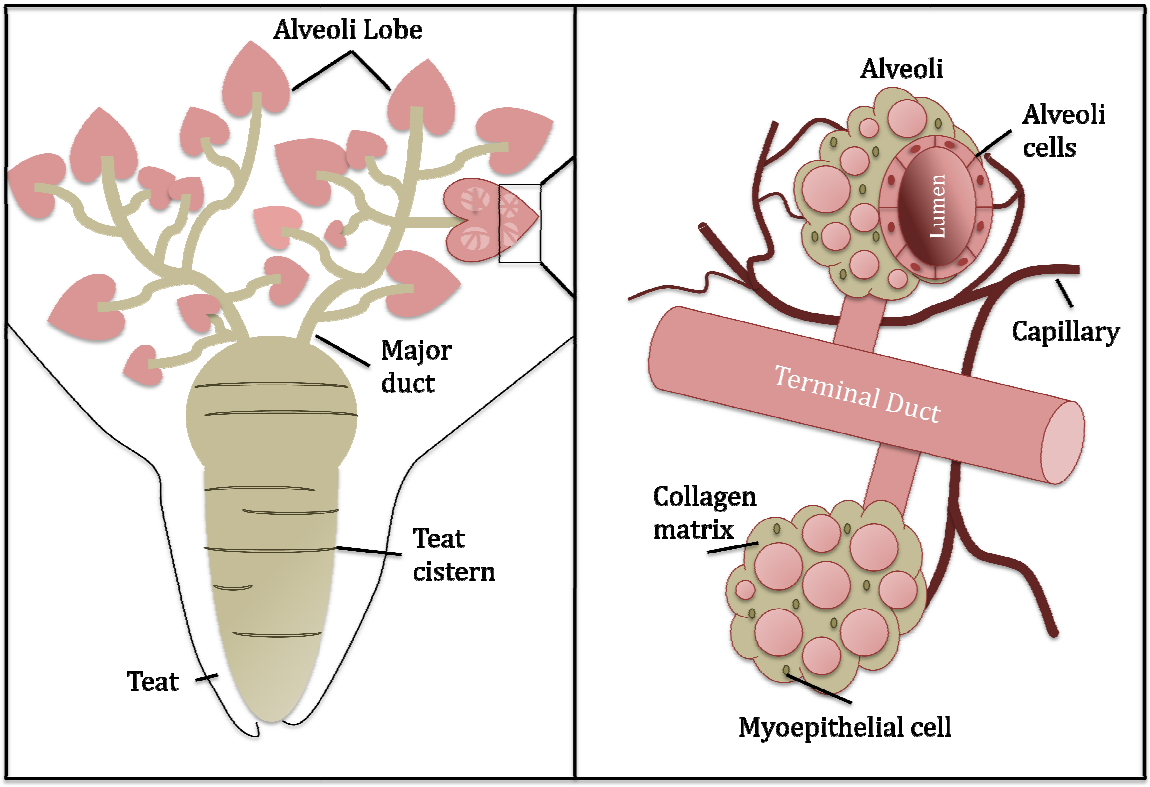

Figure 3. Bovine mammary gland.
A schematic representation of the bovine mammary gland. The alveoli cells that line the alveolus are synthesizing the milk and alveolar lumina, the hollow middle, hold the milk until the milk is drained via contractions of the myoepithelial cells. The milk passes through the ducts to the teat cistern where it is removed by suckling of the offspring. Adapted from Senger, 2005.

The mammary epithelial cell is the secretory unit of the alveoli and terminal ducts (Saacke and Heald, 1974). The cell is the site of formation for the sugar, protein, and lipids specific to milk; it also mediates the introduction of water and minerals into milk (Saacke and Heald, 1974). The cytomembranes of the cell play key roles in the synthesis and secretion of milk (Bargmann et al., 1961; Bargmann and Knoop, 1959; Bargmann and Welsch, 1969; Davis and Bauman, 1974; Hollmann, 1974, 1969; Keenan et al., 1974; Patton and Fowkes, 1967; Saacke and Heald, 1974; Stein and Stein, 1967; Wellings, 1969). The nucleus is responsible for the synthesis of DNA and RNA. The mitochondria are responsible for the production of ATP, nonessential amino acid precursors, and fatty

acid precursors. The endoplasmic reticulum, which becomes markedly larger during the onset of parturition and lactation (Davis and Bauman, 1974; Jimenez et al., 1984), is responsible for the synthesis of proteins, triglyceride, and phospholipids, and the desaturation of fatty acids (Davis and Bauman, 1974). The Golgi complex also becomes markedly larger during the onset of parturition and lactation (Davis and Bauman, 1974; Jimenez et al., 1984); it is responsible for the synthesis of lactose and glycoproteins, the phosphorylation of caseins, and the packaging of casein and lactose (Davis and Bauman, 1974). Following the increase in size of the endoplasmic reticulum and Golgi complex, the differentiated lactating epithelial cells gain polarity; this is due to the endoplasmic reticulum and the Golgi complex occupying opposite ends of the cell (Saacke and Heald, 1974), which is important to the production of proteins in the lactating epithelial cells. Another change that occurs with the onset of lactation is the presence of the casein micelles; prior to lactation the micelles are absent in the Golgi complex, whereas the micelles become present with the onset of lactation (Saacke and Heald, 1974). The presence of the casein micelles in the Golgi complex only during lactation points to their importance in the production of milk.

Milk Production – Mammary Gland Development

Milk is produced from the mammary glands in female animals. The mammary glands, specialized sweat glands, start developing in the embryo, arising from the lateral milk lines along the abdomen of the conceptus (Senger, 2005b). Between birth and puberty, the mammary glands develop at the same rate as other tissues (isometric growth); it is not until puberty that the growth rate substantially increases (allometric growth) (Senger, 2005b). The first few estrous cycles that signal the onset of puberty

allow for the duct and alveolar framework to develop in the mammary gland; the ducts begin to branch out and increase in diameter, which is followed by the formation of alveoli, the secretory elements of the mammary gland (Senger, 2005b). These changes in the growth of the mammary gland are primarily due to the influence of estrogen, progesterone, growth hormone and prolactin, hormones that all see an increase in production with the onset of puberty. However, the mammary gland does not reach its full potential until the third trimester of pregnancy (Senger, 2005b). At this point, the lobulo-alveolar structures grow until they make up approximately 90% of the cellular mass of the mammary gland at parturition (Senger, 2005b). Prolactin, adrenal cortex hormones, and placental lactogen all play a role in allowing the mammary epithelium to synthesize milk (Senger, 2005b).

Milk Production – Stimulants

The ejection of milk is the active transfer of milk from the alveoli and alveolar ducts into the larger mammary ducts, cisterns, and teats or nipples where it is then removed by the suckling offspring (Senger, 2005b). To clarify, milk secretion is the synthesis of milk by the alveolar cells and its transfer from the alveolar cells to the alveolar lumina; capillary action causes the retention of milk in the lumina (Senger, 2005b). Seventy to eighty percent of all secreted milk is stored in the alveolar lumina until suckling stimulates ejection (Senger, 2005b). Milk ejection is an active neuroendocrine reflex involving sensory neurons in the teat or nipple, release of oxytocin from the neurohypophysis, and contraction of myoepithelial cells that surround each alveolus and some of the ducts (Senger, 2005b). Milk ejection is initiated by suckling of the teat that contains sensory neurons; impulses from theses neurons travel through

afferent nerves to the hypothalamus (Senger, 2005b). Nerves in the paraventricular nuclei are stimulated by the afferent neurons, resulting in the release of oxytocin by the terminals in the posterior lobe of the pituitary gland (Senger, 2005b). Oxytocin is released into the blood and delivered to the mammary gland where the oxytocin targets the myoepithelial cells surrounding the alveolus to contract, thereby squeezing the milk out of the alveolus into the large ducts and the cistern and then into the teat (Senger, 2005b). In addition to direct teat stimulation, oxytocin release can also be triggered by sounds made by the offspring and visual sight of the offspring (Senger, 2005b).

As the need for milk by the offspring decreases, the frequency of suckling also decreases; this causes a buildup of pressure within the mammary gland, causing the secretory cells to lose functionality and undergo atrophy (Senger, 2005b). Mammary involution is an important process in the cycle of milk production; it allows for the mammary gland to recover from the lactation period and to synthesize new secretory cells for the next lactation (Senger, 2005b). In current milking parlors, induced involution is the most common practice and is more commonly studied (Wilde et al., 1999). With induced involution, there is an abrupt end to the suckling stimulus and the release of galactopoietic hormones is halted, causing milk stasis in the mammary gland, which in turn leads to a rapid decline in milk synthesis, cessation of milk secretion, down-regulation of differentiated gene expression and removal of the majority of the mammary cell population by apoptosis (Wilde et al., 1999). In ruminant mammary tissue, the response to induced involution is slower than in rat mammary tissue; within 24 hours of milk cessation, decreased levels of mRNA and enzymes can be measured in rats (Travers et al., 1996), whereas in cattle, after three days, casein and α-lactalbumin

mRNA levels are reduced, but not to the same extent as rats, and β-lactoglobulin mRNA levels did not change (Goodman and Schanbacher, 1991). After seven days, α_{S1}-casein and α-lactalbumin mRNA had dropped by 85% and 99% respectively in cattle (Wilde et al., 1997). The alveolar integrity of bovine mammary tissue remained generally intact when milking (Holst et al., 1987; Wilde et al., 1997) or suckling (Akers et al., 1990) was discontinued, indicating that there is only modest loss of secretory epithelial cells between lactations (Hurley, 1989). Indeed, the percentage of epithelial cells does not change during the non-lactating periods (Capuco et al., 1997). However, mammary DNA content decreased with induced involution (Akers et al., 1990), and apoptotic cells were detected in involuting bovine mammary tissue one week after milking was ended (Wilde et al., 1997).

Despite the presence of apoptotic cells in mammary tissue, intact alveoli have been identified in non-lactating tissues throughout involution in the dairy cow (Capuco et al., 1997; Holst et al., 1987); and measurements of tissue mass and cell number show that mammary tissue does not revert to its pre-lactational state at the end of lactation, but with the termination of milk synthesis it is believed that some de-differentiated, luminal epithelial cells remain between lactation periods (Wilde et al., 1999). Immunohistochemical characterization of the cell population in involuting goat mammary tissue, using cytokeratin markers and differentiation-specific lectin binding, supports the hypothesis that portions of the epithelial population remains between lactations; the study's findings suggest that alveolar epithelial cells can de-differentiate to ductal epithelial cells based on expression of cytokeratins, a polypeptide found predominantly in mammary ducts (Rudland and Hughes, 1989), and erbB-2, a receptor

protein associated with the development and differentiation of alveolar structures (Dati et al., 1996; Marte et al., 1995), by the alveoli (Li et al., 1999c). However, there is the possibility that incompletely differentiated myoepithelial cells could revert into ductal epithelial cells (Li et al., 1999b). Myoepithelial cells have been detected in goat mammary tissue at all stages of induced involution caused by milk stasis; it is unknown whether all myoepithelial cells present before involution are also present after, but myoepithelial cell apoptosis was not detected using *in situ* end-labeling methods during induced goat involution (Li et al., 1999b).

The survival of epithelial cells between lactation periods suggests there is the possibility that milk synthesis and secretion could be restarted after a short break in milking. A study involving bovine mammary glands found that milk secretion resumed after 12 days of milk stasis and milk yields were similar to pre-treatment levels within quarters of an udder (Hamann and Reichmuth, 1990). Following a two-week milk stasis, milk secretion was recovered within 16 days to similar levels produced prior to milk stasis; however this milk stasis was induced in only one udder half while the other half was continually milked (Hamann and Reichmuth, 1990). Another study demonstrated that involution in unmilked glands may be retarded when the contralateral glands are continuously milked (Akers and Keys, 1985). Involution can be further inhibited by treatment with lactogenic factors, such as prolactin and glucocorticoids (Feng et al., 1995), indicating that their presence caused by continuously milking one half of the udder may aid in the recovery of milk secretion in the other half by delaying involution (Noble and Hurley, 1999). In dairy cattle, mammary gland involution was partially reversed, without the aid of exogenous hormones, after 11 days of milk stasis and the full

establishment of involution in the mammary gland; however, it should be noted that the glands were only re-milked for three days, indicating that three days may not be enough time for the glands to recover full lactational function (Noble and Hurley, 1999). In beef cattle, returning the calf to the cow and allowing the calf to suckle accomplished recovery of milk secretion after a four-week milk stasis; within five weeks of returning the calf, milk composition and production were restored (Lamb et al., 1997).

Several studies have described the effect of milking frequency on milk production levels. Milking frequency is the main factor regulating milk yield and quality if feeding, welfare, health, and environmental conditions are adequate (Marnet and Komara, 2008). The effect of extended milking intervals also varies depending on the species, breed, and genetic merit of the animals used (Marnet and Komara, 2008). A dual-purpose system of suckling and milking in East Friesian ewes resulted in a reduction of milk ejection and a reduction of 40 to 60% of milk collected at each milking when compared to controls; however, the total milk yield -increased 42% for the dual-purpose system when compared to controls (Marnet and Komara, 2008). The increased total milk yield demonstrates the galactopoietic effect caused by the additional suckling period between milking periods seen in cattle (Bar-Peled et al., 1995) and goats (Hadjipanayiotou and Louca, 1976; Papachristoforou et al., 1982; Peris et al., 1997); however, these studies did not show results of a significant decrease in milk volume obtained at each milking (Papachristoforou et al., 1982; Peris et al., 1997) even though in goats the milk ejection reflex was found to be inhibited (Hernandez et al., 2002). Once-daily milking has shown a variable decrease in milk yield, anywhere from 5 to 51% (Bagdasarov, 1960; Casu and Boyazoglou, 1974; Casu and Labussière, 1972; Flamant, 1974; Labussière et al., 1983;

Labussière et al., 1974; Morag, 1968; Nudda et al., 2002; Papachristoforou et al., 1982; Partearroyo and Flamant, 1978); however, mammary gland development seems to play a role in the adaptation of ewes for once-daily milking (Marnet and Komara, 2008). Primiparous ewes appeared to be more sensitive to once-daily milking due to their lack of mammary gland development, yet it was found that the negative effect of once-daily milking on milk yield could be reduced if two weeks of twice-daily milking was used before moving the ewes to once-daily milking (Casu and Boyazoglou, 1974).

In dairy cattle, increased milking frequency resulted in increased milk production (Erdman and Varner, 1995; Hillerton et al., 1990), ranging from 10 to 15% (Svennersten-Sjauna and Olsson, 2005). When increased frequency of milking is started during the first six weeks of lactation, milk yield is increased throughout the entire lactation period (Bar-Peled et al., 1995). This is further supported by studies where increased milk yield was seen when 4x-daily milking was utilized during the first 21 days of the lactation period (Dahl et al., 2004b; Hale et al., 2003; Wall and McFadden, 2007). Factors such as increased mammary epithelial cell number and differentiation (Hale et al., 2003; Hillerton et al., 1990), increased mammary cell secretory capacity (Nørgaard et al., 2005), changes in apoptotic rate of mammary cells (Hale et al., 2003; Li et al., 1999a), and increased exposure (Wall et al., 2006) or sensitivity of the gland to prolactin (Dahl et al., 2004a) may contribute to the increased milk yield observed with increased milking frequency (Connor et al., 2008). An association was found between increased milking frequency and alterations of mammary cell extracellular matrix interactions and signaling that support milk synthesis, indicating that a remodeling of the extracellular matrix within the mammary gland should enhance mammary epithelial cell proliferation and

differentiation, promote apoptosis and cell renewal, and promote migration and invasion of endothelial cells (Connor et al., 2008). However, the changes in gene expression of the extracellular matrix could be caused by increased demand for milk production, and not itself result in increased milk yield during increased milking frequency (Connor et al., 2008).

The research into genes related to milk production has taken off in the last ten years; in the past, and still today, the predominate research of milk production has been in milk proteins and hormones. 17β-Estradiol (estrogen), progesterone, growth hormone, prolactin, placental lactogen, glucocorticoid, insulin, and thyroid hormones all play a role in mammary epithelial development and milk production (Topper and Freeman, 1980). It has been well established that mammary duct growth is stimulated by estrogen, and lobule-alveolar development is stimulated by both estrogen and progesterone (Cowie and Folley, 1961; Folley, 1952; Lyons, 1958). However, estrogen and progesterone cannot stimulate mammary growth without the presence of prolactin and growth hormone from the anterior pituitary gland (Lyons, 1958). In laboratory species with well-developed lobule-alveolar systems in the mammary gland, lactation was initiated with injections of prolactin, glucocorticoids, and estrogen (Folley, 1956; Reece, 1958). In goats, lactation was initiated by estrogen alone (Meites, 1961). Exogenous prolactin and growth hormone were observed to stimulate milk synthesis in rabbits and cows, respectively (Benson et al., 1958; Cowie and Folley, 1961; Tucker, 2000). More information about prolactin can be found later in this review.

Placental lactogen was discovered in humans in 1962 (Josimovich and MacLaren, 1962), a peptide hormone synthesized in and secreted from the placenta, and was found

to be structurally similar to prolactin and, depending upon the species, similar to growth hormone (Linzer, 1998). In rodents, by binding to the prolactin receptor, placental lactogen stimulated mammogenesis (Forsyth, 1994). In cattle, the role of placental lactogen is less clear (Tucker, 2000); administration of placental lactogen has had little effect on lactating cows' metabolism (Byatt et al., 1992), and its concentration in maternal serum is very low when compared to fetus serum (Wallace, 1993).

Cortisol is the predominant endogenous glucocorticoid in cattle whose major function at the mammary gland is to cause differentiation of the lobule-alveolar system (Tucker, 2000). Cortisol targets the endoplasmic reticulum and Golgi complex (Mills and Topper, 1970), which allows for prolactin to later induce milk protein synthesis (Juergens et al., 1965). Lactation can be induced in non-lactating cows with well-developed lobule-alveolar systems by injecting them with glucocorticoids (Tucker and Meites, 1965); however, a greater quantity of milk could be produced if the amount of prolactin secreted was concurrently increased (Collier et al., 1977). On the other hand, it has been reported that administration of adrenocorticotropic hormone, thought to be acting due to increased secretion of glucocorticoids, reduces the secretion of milk in cattle (Shaw et al., 1955). An additional study has shown that therapeutic doses of synthetic glucocorticoids have suppressed milk yields (Braun et al., 1970). Yet, in rats glucocorticoids have increased milk yield (Thatcher and Tucker, 1970). In rats, suckling-induced release of glucocorticoids decreased as lactation progressed (Ota et al., 1974), whereas in cattle, milking also induced the release of glucocorticoids, but there was no decrease as lactation progressed (Koprowski and Tucker, 1973a). In goats and cattle, mammary uptake and binding of glucocorticoids increased with the onset of lactation and

were positively correlated with the uptake of glucose by mammary tissue (Gorewit and Tucker, 1977; Paterson and Linzell, 1974).

Insulin, the hormone responsible for the uptake of glucose into cells, has been found to be essential for mammogenesis in vitro (Forsyth, 1961). This is due to insulin binding to the IGF type I receptor (Kasuga et al., 1981) possibly mimicking the mammogenic effects of IGF-I (Tucker, 2000). High doses of insulin may substitute for growth hormone-induced secretion of IGF-I and subsequent mammogenesis in the hypophysectomized animal (Jacobsohn, 1958; Tucker, 2000). Insulin, similar to glucocorticoids, seems to have dueling responses in rats versus cattle, especially in the uptake of nutrients by mammary tissue. In lactating rats, insulin increased glucose utilization and lipid uptake in the mammary gland (DaCosta and Williamson, 1994; Martin and Baldwin, 1971), but adipocytes became insulin resistant (Burnol et al., 1987), thereby channeling glucose to the mammary gland (Tucker, 2000). However, in cattle, the mammary uptake of glucose, acetate, β-hydroxybutyrate, triglycerides and amino acids did not require insulin (Laarveld et al., 1985). Yet, insulin increased the utilization of acetate for lipid synthesis and decreased lipolysis in adipose tissue (Vernon, 1980), indicating that insulin is involved in the mechanisms that moves nutrients towards body tissues and away from milk synthesis in cattle; in fact, insulin concentrations in blood were found to be negatively correlated with milk yield (Koprowski and Tucker, 1973a). By maintaining glucose concentrations in blood, via the addition of glucose, while increasing insulin concentration, protein concentrations in milk will increase (Griinari et al., 1997), indicating that it is possible to manipulate hormones, diet, and genetics to produce milk with a specific composition (Tucker, 2000).

Thyroid hormones play an interesting role in milk production. Early studies showed that thyroxine held temporary galactopeoietic effects (Tucker, 2000). Later it was discovered that thyroxine secretion rates were suppressed as milk yield increased during lactation (Lorscheider and Reineke, 1971). Thyroxine was determined to be a prohormone, due to its little inherent biological activity, that undergoes enzymatic 5'deiodination to form the biologically active hormone triiodothyronine within the thyroid and peripheral tissues (Tucker, 2000). During lactation there is decreased conversion of thyroxine to triiodothyronine in the liver and kidneys, but increased conversion in the mammary gland causing the mammary gland to reach a euthyroid state and the rest of the body to be hyperthyroidic (Jack et al., 1994; Kahl et al., 1991). In addition, large quantities of iodine, a major structural component of the thyroid hormones, are lost in milk during early lactation contributing to the hyperthyroid condition of the lactating animal (Lorscheider and Reineke, 1971). This difference in balance of thyroid hormone within the body helps to drive the metabolic priority of the mammary gland (Tucker, 2000).

Through the combination of all the above stimulants, milk is produced. Via manipulation of any of the pathways, milk production and yield will be influenced. However, in agreement with Tucker (2000), the best stimulation for milk production is pregnancy; once pregnancy has been initiated, the genes that encode the hormones and milk proteins will determine the rest.

Milk Proteins

The major milk proteins synthesized in the mammary gland include the caseins and whey proteins, α-lactalbumin and β-lactoglobulin. Characterization of these proteins

began in the early 1950's with the development of new technologies such as X-ray crystallography and electrophoresis (Aschaffenburg, 1964). Caseins, from cow milk, are defined as the phosphoproteins that precipitate from raw skim milk by acidification to pH 4.6 at 20°C (Farrell Jr. et al., 2004; Jenness et al., 1956). There are four casein proteins that can be differentiated according to their relative electrophoretic mobility in alkaline polyacrylamide or starch gels containing urea with or without mercaptoethanol: α_{s1}-casein, α_{s2}-casein, β-casein, and κ-casein (Farrell Jr. et al., 2004). Variations in the casein proteins have been shown to have associations with the curdling properties of milk, and could be important in the processing of dairy products (Sadler et al., 1968).

The whey proteins are the group of milk proteins that remain soluble in milk serum or whey after the precipitation of casein at pH 4.6 and 20°C (Farrell Jr. et al., 2004). This fraction includes α-lactalbumin and β-lactoglobulin, along with serum albumin, immunoglobulins, and proteose-peptone fractions; however, with the use of SDS-PAGE, lactotransferrin should also be added to the list (Farrell Jr. et al., 2004). It should be noted that commercial products termed whey protein isolates or concentrates are obtained from cheese manufacturing at a higher pH and will also contain traces of intact casein (Farrell Jr. et al., 2004), and therefore are not pure whey. α-Lactalbumin functions in the synthesis of lactose (Brodbeck et al., 1967), a disaccharide and the major osmolyte of milk (Farrell Jr. et al., 2004; Topper and Freeman, 1980). Within the Golgi apparatus of the mammary epithelial cell, α-lactalbumin interacts with the ubiquitously expressed enzyme β-1,4-galactosyltransferase to form the lactose synthase complex; α-lactalbumin modifies the substrate specificity of β-1,4-galactosyltransferase, allowing for the formation of lactose from glucose and UDP-galactose (Farrell Jr. et al., 2004).

Immunoglobulins are expected to be present in milk, especially colostrum, due of the importance of passing antibodies from the mother to the newborn. All of the five isotypes of immunoglobulins in mammals have been identified in cattle (CIgW website); IgG, IgA, and IgM have been characterized in milk (Farrell Jr. et al., 2004); and changes in the level and relative proportions of the Ig in colostrum compared with milk during lactation or resulting from immunization or infections have been presented in several studies (Butler et al., 1972; Guidry et al., 1980; Hidiroglou et al., 1992; Mackenzie and Lascelles, 1968). Lactotransferrin is a member of the family of specific iron-binding proteins that occurs in milk, but is different from the serum transferrin that also occurs in milk (Groves, 1960). While lactoferrin is of mammary origin and can be found in the milk of most species (Schanbacher et al., 1993), it is also found in the secretions of other epithelial cells (Masson et al., 1966) and polymorphonuclear leucocyctes (Baggiolini, 1972). The concentration of lactotransferrin varies, but increases in response to inflammation or infection due to its role as a first line defense (Ward et al., 2002) and its role in bacteriostasis (Bullen et al., 1978; Smith and Schanbacher, 1977). β-Lactoglobulin is the major protein in whey (Farrell Jr. et al., 2004) and will be discussed in greater detail in a different section.

PROLACTIN

Prolactin is a peptide hormone that is released into the blood from the anterior lobe of the pituitary gland (Senger, 2005a). Its primary target is the mammary epithelial cells to induce lactation (Senger, 2005a).

Prolactin was first discovered in 1928 when anterior pituitary extracts were used on rabbits with well-developed lobule-alveolar mammary glands and stimulated milk secretion (Stricter and Grueter, 1928). The extract was then applied to dogs, pigs, and cows (Riddle et al., 1933; Stricker and Grueter, 1929) and then confirmed in other species such as the rat, guinea pig, and goat (Corner, 1930; Riddle et al., 1933; Turner and Frank, 1930; Turner and Gardner, 1931). In 1933, the extract was purified and identified as prolactin (Riddle et al., 1933). With the purification of prolactin, the studies into its function and use began.

Laboratory species were the subjects of choice for the initial probing into prolactin's function. In rabbits, the development of the lobule-alveolar systems of the mammary glands increased with injections of prolactin (Lyons, 1942). This points to prolactin's role in mammiogenesis. Indeed, it was found using hypophysectomized rabbits that neither estrogen nor progesterone could stimulate mammary growth pointing towards the need for prolactin and/or growth hormone (Lyons, 1958). Milk secretion increased, especially during early lactation, in rats and rabbits when prolactin was administered (Cowie, 1969; Kumaresan et al., 1966). However, it was observed in rats that prolonged elevation of concentrations of prolactin in serum did not occur during a normal pregnancy when a large portion of mammary growth takes place (Amenomori et al., 1970). In rats, if prolactin secretion was suppressed with bromocriptine, milk secretion was also suppressed (Shaar and Clemens, 1972).

Starting around 1965, studies were initiated to determine mammary differentiation in vitro by inducting particular milk products, i.e. casein, and α-lactalbumin, using laboratory species' cells (Topper and Freeman, 1980). These studies further support the

importance of hormones in the synthesis and secretion of milk, in particular prolactin. For example, tissue freshly isolated from mid-pregnant mice can make a small amount of casein, but this synthesis is enhanced several fold within forty-eight hours in the presence of insulin, hydrocortisone and prolactin (Juergens et al., 1965; Topper and Freeman, 1980; Turkington et al., 1965). The presence of α-lactalbumin was used as an indicator of overt differentiation in the cells studied (Topper and Freeman, 1980). A different study looking at the ultrastructure of mammary alveoli in tissue also isolated from mid-pregnant mice (Mills and Topper, 1970) confirmed the finding that alveoli develop asynchronously *in vivo* (Elias, 1957). Alveoli have three cell types: A, B, and C. A cell types make up the majority of the cells, while B and C cell types make up smaller percentages (Mills and Topper, 1970). A cells have few mitochondria, little rough endoplasmic reticulum, a rudimentary Golgi apparatus that is laterally located in the cytoplasm, a centrally located nucleus, few lipid droplets, and no detectable protein granules (Mills and Topper, 1970). B cells have abundant rough endoplasmic reticulum that is distributed randomly throughout the cytoplasm, a well developed Golgi apparatus that is also laterally located in the cytoplasm, a central nucleus, and also no detectable protein granules (Mills and Topper, 1970). C cells on the other hand, are much more developed than A or B cells; the organelles are highly polarized with the rough endoplasmic reticulum and nucleus in the basal cytoplasm and the Golgi apparatus in the apical cytoplasm, the mitochondria are much more abundant, the nucleoli are enlarged, lipid droplets can be found throughout the cytoplasm, and protein granules are present (Mills and Topper, 1970). Therefore, C cells have an ultrastructure similar to lactating cells (Bargmann and Welsch, 1969; Hollmann, 1959; Wellings et al., 1966; Wellings et

al., 1960a; Wellings et al., 1960b; Wellings and Phelp, 1964) and are predicted to be the site of casein synthesis in the initial culture period (Topper and Freeman, 1980). In virgin mice, it was discovered that prolactin stimulates ductal side branching and terminal end bud regression through systemic effects; while during pregnancy it directly increases lobule-alveolar development at the mammary epithelium (Brisken et al., 1999).

Once initial studies in laboratory species pointed towards prolactin's involvement with mammogenesis, studies in ruminant species, especially cattle, also began. These studies typically mirrored the ones done in laboratory species. For example, one study utilizing three virgin goats, a dry goat and a heifer was able to show the achievement of milk synthesis with extracts from the anterior pituitary (Evans, 1932), confirming the studies done earlier in rabbits (Stricter and Grueter, 1928). In 1936, the udders of virgin goats developed after applying an estradiol benzoate ointment, and following an injection of pituitary "lactogenic" hormone, began lactating (DeFremery, 1936); this study is comparable to later studies that showed estrogen and progesterone to be important to lactation, but prolactin is a necessary ingredient to milk synthesis (Lyons, 1958). It was determined from the cultured mammary tissue of non-lactating cows forty days prepartum that insulin is required for survival, hyrodocortisone is required for differentiation, and prolactin is required for biosynthesis (Collier et al., 1976); these requirements were similar in mammary tissue samples from heifers that had been pretreated with estrogen and progesterone (Nickerson et al., 1976), further supporting prior studies (DeFremery, 1936; Lyons, 1958).

In cattle, prolactin has been discovered to be important for the initiation of lactation in the periparturient period (Tucker, 2000). Several hours before parturition, a

surge in prolactin secretion has been observed (Ingalls et al., 1973). If the prolactin surge before parturition is blocked with bromocriptine, milk yield is subsequently reduced; however, exogenous prolactin was able to reverse the effect of the bromocriptine (Akers et al., 1981). Other studies have shown that bromocriptine and cold temperatures reduce prolactin secretion, but milk yield was not affected in cows (Peters et al., 1981; Peters and Tucker, 1978; Smith et al., 1974); however, these studies took place after parturition. In addition, in vitro studies have shown that prolactin, in association with insulin and cortisol, is required to induce the secretion of milk proteins (Juergens et al., 1965). Two days after calving, blood basal concentrations of prolactin decreased (Ingalls et al., 1973; Johke et al., 1971), but suckling and milking triggered the additional release of prolactin (Convey, 1974; Koprowski and Tucker, 1973a), but as the lactation progressed, the release of prolactin declined (Koprowski and Tucker, 1973b). In fact, the concentration of prolactin in the blood of the cows was only slightly correlated with milk yield (Koprowski and Tucker, 1973b), and supplemental prolactin did not have an effect on milk yield (Plaut et al., 1987).

Prolactin Regulation

While prolactin has been shown to be important for the initiation of lactation, its role in later stages of lactation is less clear; perhaps a better understanding of prolactin's regulation will help shed some light on the matter. Prolactin binds to a specific receptor, prolactin receptor (PRLR), on the surface membrane of the mammary epithelial cell (Frantz et al., 1974). Cloning of PRLR identified the receptor as a single chain transmembrane protein that belongs to the cytokine receptor superfamily and revealed that PRLR is expressed in several tissues, including the mammary gland, choroid plexus,

testis, and liver (Boutin et al., 1988). The receptor consists of an external and internal domain (Kelly et al., 1989) and alternative splicing of the primary transcript yields two PRLR forms: one with a short cytoplasmic tail and one with a long cytoplasmic tail (Figure 4) (Boutin et al., 1988). The long and short forms are highly conserved, with the short form having a truncated cytoplasmic domain (Schuler et al., 1997). Even though the structure is conserved across multiple species, little is known about the importance of the expression of the short form of PRLR in bovine mammary tissue; however, the long form PRLR mRNA expression is increased in bovine mammary tissue during the dry period of lactation (Auchtung et al., 2005). This supports prior studies that showed increases in prolactin binding in the mammary gland as parturition approached, and that prolactin expression is down regulated in mammary tissue by pregnancy, resulting in an inverse relationship between prolactin and PRLR in the mammary gland (Kazmer et al., 1986; Smith et al., 1993). Expression of short form PRLR in bovine mammary tissue is inversely related to the long form of PRLR (Auchtung et al., 2005). The increased presence of the short form PRLR mRNA around the beginning of the dry period of lactation, combined with the decreased presence of the long form of PRLR mRNA, could limit the ability of the long form to homodimerize; instead the long form will heterodimerize (Auchtung et al., 2005). Heterodimerization is the result of a substantial decrease in ligand binding and signaling (Berlanga et al., 1997; Jabbour and Kelley, 1997). The short form of PRLR could act as a negative regulator by competing for prolactin in the system, but it is also able to overcome the deficiency of the long form of the prolactin receptor in PRLR knockout mice to the point where mammary function is practically restored in the knockout mice (Binart et al., 2003). When the short and long

forms of PRLR are absent, mice are sterile and mammary development cannot be analyzed (Ormandy et al., 1997). Greater mammary function could result due to the increased expression of long form PRLR mRNA and decreased expression of short form PRLR mRNA during the dry period of lactation (Auchtung et al., 2005).

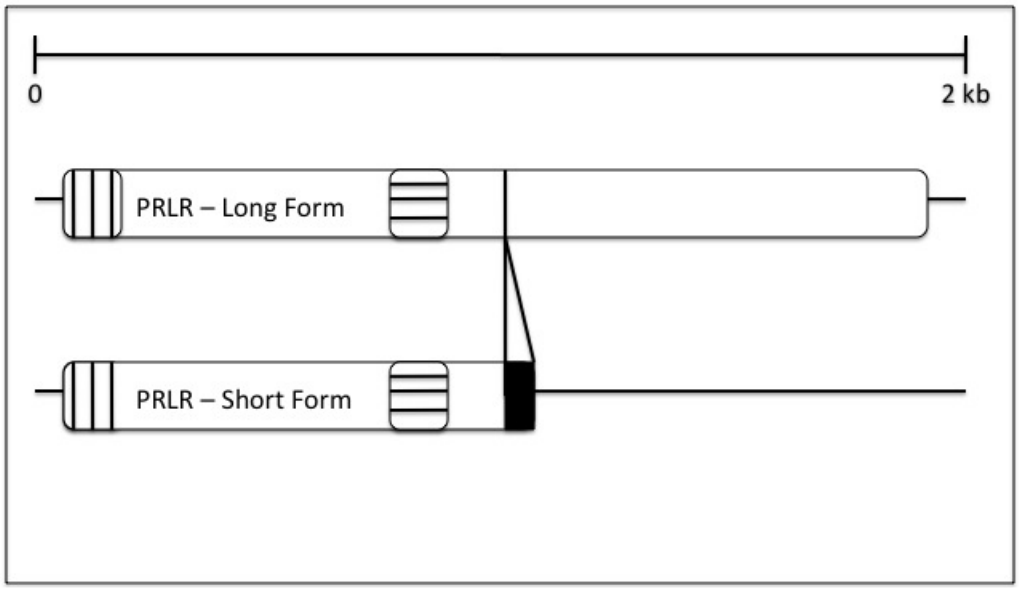

Figure 4. Prolactin receptor (PRLR) isoforms.
The signal peptide is represented by the vertical striped boxes; the transmembrane domain by the horizontal striped boxes; and the 39 bp sequence unique to the short form by the black box. The two lines that converge from the edges of the unique sequence show the point of insertion in the long form. Adapted from Bignon et al., 1997.

When prolactin binds to its receptor, it induces dimerization of the prolactin receptor and activates Janus kinase 2 (JAK2)(Liu et al., 1997), which in turn phosphorylates and activates transcription factors that belong to a family of signal transducers and activators of transcription (STAT). Specifically STAT1, -3, and -5 factors are activated and then bind to and induce transcription from promoters containing γ-interferon activation sites (GAS) (Schindler and Darnell, 1995). STAT5 is expressed at all stages of mammary gland development; however, there are slight variations in gene

expression amongst the different lactational-development stages in mice (Kazansky et al., 1995; Liu et al., 1995). STAT1 and STAT3 are also expressed during all stages of mammary gland development, but their target genes are currently unknown (Rosen et al., 1999); however, STAT3 is highly phosphorylated during involution indicating that it may play a role in the process (Liu et al., 1996; Philp et al., 1996).

STAT5 has been identified as the primary transcription factor responsible for signaling by prolactin in the mammary gland (Rosen et al., 1999). STAT5 was originally identified as a binding activity in the lactating mammary gland, known as mammary gland factor (MGF)(Schmitt-Ney et al., 1991) or milk protein binding factor (MPBF)(Watson et al., 1991); MGF was originally cloned from sheep mammary glands, and from there was determined to be a new member of the STAT protein family (Wakao et al., 1994). Once STAT5 had been identified, it was also cloned from rats, mice, and humans (Hou et al., 1995; Kazansky et al., 1995; Lin et al., 1996; Liu et al., 1995). In mice, it was determined that there are two STAT5 genes that are very similar, probably due to a gene duplication event, and have been designated STAT5a and STAT5b (Liu et al., 1995). And just like PRLR, STAT5 is not mammary specific and can be found in several different tissues (Kazansky et al., 1995; Liu et al., 1995; Wakao et al., 1994). In addition to prolactin, STAT5 can be activated by growth hormone, epidermal growth factor, cytokines, and other hormones (Azam et al., 1995; Barahmand-Pour et al., 1995; Fujii et al., 1995; Gouilleux et al., 1995; Hou et al., 1995; Ihle and Kerr, 1995; Johnston et al., 1995; Lin et al., 1996; Pallard et al., 1995; Ruff-Jamison et al., 1995; Wood et al., 1995). In mice, if the genes controlling the prolactin receptor or STAT5a were inactivated, mammary development failed because of greatly reduced differentiation of

terminal end buds of the mammary ducts resulting in no lactation (Hennighausen and Robinson, 1998; Hennighausen et al., 1997). STAT5b null mice also have decreased lubulo-alveolar development, but it is not as severe as STAT5a null mice (Rosen et al., 1999; Teglund et al., 1998; Udy et al., 1997). STAT5a/STAT5b double knockout mice also have fewer terminal end buds, appearing to have a similar phenotype as single STAT5 knockout mice (Rosen et al., 1999), which is a decreased, or a lack of lactation.

In addition to the receptor and JAK2/STAT pathway, prolactin is regulated through photoperiod. Prolactin secretion is increased in the summer and decreased in the winter, which has been observed in a wide range of mammals adapted to temperate and cold climates; these include domestic and wild ungulates, mustelids, canids, rodents, primates and marsupials (Hindes and Loudon, 1995; Lincoln, 1989). In animals with a longer lifespan (greater than 5 years), such as deer and sheep, the long-term prolactin cycle is generated endogenously and displays circannual rhythmicity under constant environmental conditions where daylength is used as the cue to time the cycle to match the environment (Jansen and Jackson, 1993). Long photoperiods stimulate, and short photoperiods inhibit prolactin secretion (Lincoln, 1999) through changes in the duration of nocturnal melatonin secretion by the pineal gland (Lincoln et al., 2003). Light passes through specialized, non-visual photoreceptors in the retina to release melatonin through two distinct pathways (Stehle et al., 2001; Tamarkin et al., 1985) that ensure melatonin is only secreted at night. The first pathway is through the suprachiasmatic nucleus (SCN) of the anterior hypothalamus; periodic light exposure every twenty-four hours synchronizes melatonin release with the circadian rhythms generated by the SCN (Stehle et al., 2001; Tamarkin et al., 1985). The second pathway is through the inhibition of

melatonin release from the pineal gland by light, via the retinal-hypothalamic-sympathetic innervation and control of the rate-limiting enzyme *N*-acetyletransferase (Stehle et al., 2001; Tamarkin et al., 1985). Specifically, the pars tuberalis of the stalk of the pituitary gland has been implicated in the photoperiodic regulation of prolactin secretion (Morgan, 2000). The pars tuberalis expresses melatonin (MT1) receptors at a higher density than other sites in the brain, and the tissue is strategically placed at the junction between the median eminence and pars distalis, to regulate neuroendocrine functions (Lincoln et al., 2003). The pars tuberalis cells secrete prolactin-releasing factors, known as tuberalins, and it is currently believed that melatonin controls the secretory function of the pars tuberalis (Hazlerigg et al., 1996; Stirland et al., 2001).

The role of the pars tuberalis in prolactin secretion is supported by studies involving hypothalamic-pituitary disconnected (HPD) Soay rams (Lincoln and Clarke, 1995, 1994; Lincoln et al., 1996). In these rams, the arcuate nucleus and median eminence of the hypothalamus are destroyed (Lincoln et al., 2001), but the pituitary gland with the pars tuberalis is left intact (Williams et al., 1997). The HPD rams show marked changes in prolactin secretion in response to switches in photoperiod and manipulation of melatonin (Lincoln et al., 2003). In addition, the rams show photoperiod-specific, long-term cycles in prolactin secretion under constant short or long days, with the timing maintained even when prolactin secretion is blocked for two to three months (Lincoln et al., 2003).

In dairy cattle, greater duration of light exposure resulted in increased milk production during lactation (Dahl et al., 2000). However, cows exposed to reduced amounts of light during the dry period of lactation, produced more milk during the

subsequent lactation when compared to cows exposed to longer days (Miller et al., 2000). Another study in dairy cattle, attempting to determine the effect of day length on prolactin and PRLR, showed that short day photoperiods are associated with a reduced amount of prolactin, but milk yield and PRLR mRNA in mammary tissue was increased (Auchtung et al., 2005). This study confirms the findings that cows exposed to short day photoperiods have greater milk production than cows exposed to long day photoperiods (Dahl and Petitclerc, 2003; Miller et al., 2000). Prolactin concentrations in blood plasma were found to be greater in long day photoperiod cows when compared to short day photoperiod cows (Auchtung et al., 2005), which is also seen in non-lactating cows (Miller et al., 2000) and pregnant heifers (Newbold et al., 1991). In addition, cows exposed to the short day photoperiod during non-lactating periods had greater expression in both short and long forms of PRLR mRNA in mammary tissue, which is inversely related to prolactin concentration (Auchtung et al., 2005).

Similar results can be found in dairy ewes; ewes exposed to short day photoperiod for at least six weeks prepartum produced more milk than ewes exposed to long day photoperiod (Mikolayunas et al., 2008). Prolactin concentrations were found to be lower in dairy ewes exposed to short day photoperiod when compared to dairy ewes exposed to long day photoperiod (Mikolayunas et al., 2008), further supporting the findings in dairy cattle. The positive effect of increased milk production prepartum caused by short day photoperiod exposure in ewes (Mikolayunas et al., 2008), in combination with dairy genetics, breed composition, and weaning systems (Bencini and Pulina, 1997; McKusick et al., 2001), may have contributed to the increase in milk production of dairy ewes reported at the Spooner Agricultural Research Station from 1996 to 2004 (Berger, 2005).

BETA-LACTOGLOBULIN

Beta-lactoglobulin is one of the major whey proteins found in milk. Initially it was believed that there was only one β-lactoglobulin synthesized from the mammary gland in cows, however two distinct β-lactoglobulins, termed β_1- and β_2-lactoglobulin, were discovered (Aschaffenburg and Drewry, 1955). The two variants differed in their isoelectric points and crystalline structure, one forming rectangular plates and the other forming diamond-shaped plates (Aschaffenburg and Drewry, 1955). In 1957, the nomenclature for the variants was changed; β_1 is now known as β-lactoglobulin A and β_2 is now known as β-lactoglobulin B (Aschaffenburg and Drewry, 1957). Preliminary studies of the β-lactoglobulin variants in cows' milk found that the concentration of each of the β-lactoglobulins varied in proportion to the casein content; in fact, if given equal yields of milk of comparable casein content, an animal that is homozygous for the A allele will produce twice as much β-lactoglobulin as an animal that is homozygous for the B allele (Aschaffenburg and Drewry, 1957). The hypothesis at this point was that β-lactoglobulin plays a nutritional role in milk, and cows carrying the A allele would better benefit calf growth (Aschaffenburg and Drewry, 1957).

A third rare variant was discovered in 1962, β-lactoglobulin C, in the milk of Australian Jersey cows (Bell, 1962). The C variant occurs either by itself or in combination with either A or B variants, and moves slower than A and B on a gel (Aschaffenburg, 1964). An even slower fourth variant, β-lactoglobulin D, was discovered in 1966 in cattle (Grosclaude et al., 1966). Since then, several different variants of β-lactoglobulin have been discovered in cattle. New variants H (Conti et al.,

1988; Davoli et al., 1988), I (Godovac-Zimmermann et al., 1996), J (Godovac-Zimmermann et al., 1996), and W (Godovac-Zimmermann et al., 1990) have been identified and sequenced. Variant E was isolated from yak (*Bos grunneins*) milk (Grosclaude et al., 1974). Variants E, F, and G have been observed in the milk of Bali cattle (*Bos javanicus*) (Bell et al., 1981). An additional rare variant of β-lactoglobulin, Dr, has been observed to occur in Droughtmaster cattle milk (Bell et al., 1970). It was determined to be identical to variant A except for the presence of covalently attached carbohydrates (Bell et al., 1970) which makes it a questionable new genetic variant; however, an additional study found a difference in the substitution of asparagine for aspartic acid at residue 28 (Bell et al., 1981), allowing for the Dr variant to possibly be considered a new genetic variant (Eigel et al., 1984). New variants E, F, G, and Dr have not been analyzed to clarify the precise variation in their sequences (Farrell Jr. et al., 2004). A comparison of all of the bovine variants can be found in Table 1. Table 2 contains a comparison of bovine, ovine and caprine β-lactoglobulin. The amino acid sequence of bovine beta-lactoglobulin variant B is presented in Table 3. Despite the identification of all of these different variants in cattle, variants A and B β-lactoglobulin are still the most common in bovine milk (Farrell Jr. et al., 2004).

Variant	Breed	Amino Acid Position													
		28	45	50	56	59	64	70	78	108	118	126	129	130	158
A	Bovine	D	E	P	I	Q	**D**	K	I	E	**V**	P	D	D	E
B	Bovine	D	E	P	I	Q	G	K	I	E	A	P	D	D	E
C	Australian Jersey	D	E	P	I	**H**	G	K	I	E	A	P	D	D	E
D	Bovine	D	**Q**	P	I	Q	G	K	I	E	A	P	D	D	E
E	Yak, Bali cattle	D	E	P	I	Q	G	K	I	E	A	P	D	D	**G**
F	Bali cattle	D	E	**S**	I	Q	G	K	I	E	A	P	**Y**	**Y**	**G**
G	Bali cattle	D	E	P	I	Q	G	K	**M**	E	A	P	D	D	**G**
H	Bovine	D	E	P	I	Q	**D**	**N**	I	E	**V**	P	D	D	E
I	Polish red	D	E	P	I	Q	G	K	I	**G**	A	P	D	D	E
J	Hungarian grey	D	E	P	I	Q	G	K	I	E	A	**L**	D	D	E
W	Murnau-Werdenfelser (Bavarian breed)	D	E	P	**L**	Q	G	K	I	E	A	P	D	D	E
Dr	Droughtmaster	**N**	E	P	I	Q	**D**	K	I	E	**V**	P	D	D	E

Table 1. Bovine beta-lactoglobulin variants.
Amino acids that differ from variant B are bolded.

Variant	Species	Amino Acid Position								
		1	20	53	130	148	150	158	159	162
B	**Bovine**	L	Y	D	D	R	S	E	Q	I
A	**Ovine**	I	Y	N	N	R	S	G	Q	V
B	**Ovine**	I	H	N	N	R	S	G	Q	V
C	**Ovine**	I	Y	N	N	Q	A	G	G	V
	Caprine	I	Y	N	K	R	A	G	Q	V

Table 2. Beta-lactoglobulin variants across species.
Amino acid differences between bovine, ovine, and caprine beta-lactoglobulin variants. The rest of the sequence is homologous between species.

Position	**1 – 20**
Sequence	L – I – V – T – Q – T – M – K – G – L – D – I – Q – K – V – A – G – T – W – Y
	21 – 40
	S – L – A – M – A – A – S – D – I – S – L – L – D – A – Q – S – A – P – L – R
	41 – 60
	V – Y – V – E – E – L – K – P – T – P – E – G – D – L – E – I – L – L – Q – K
	61 – 80
	W – E – N – G – E – C – A – Q – K – K – I – I – A – E – K – T – K – I – P – A
	81 – 100
	V – F – K – I – D – A – L – N – E – N – K – V – L – V – L – D – T – D – Y – K
	101 – 120
	K – Y – L – L – F – C – M – E – N – S – A – E – P – E – Q – S – L – A – C – Q
	121 – 140
	C – L – V – R – T – P – E – V – D – D – E – A – L – E – K – F – D – K – A – L
	141 – 162
	K – A – L – P – M – H – I – R – L – S – F – N – P – T – Q – L – E – E – Q – C – H – I

Table 3. Bovine beta-lactoglobulin variant B amino acid sequence.

Beta-lactoglobulin has been sequenced in several species. The protein sequence is available for bovine β-lactoglobulin A and B (Braunitzer et al., 1973), porcine β-lactoglobulin I and II (Conti et al., 1986), horse β-lactoglobulin I and II (Conti et al., 1984; Godovac-Zimmermann et al., 1985) and ovine β-lactoglobulin A (Kolde and Braunitzer, 1983), B (Gaye et al., 1986), and C (Erhardt et al., 1989). A partial nucleotide sequence of bovine β-lactoglobulin was identified in 1982 (Willis et al., 1982). The complete nucleotide sequence of bovine β-lactoglobulin A was identified in 1988 based on the amino acid sequence identified by Braunitzer et al. in 1973 (Jamieson et al., 1987). The complete nucleotide sequence of ovine β-lactoglobulin gene was identified in 1988 (Harris et al., 1988).

Lipocalin Protein Family

With the identification of the primary structure of bovine β-lactoglobulin (Braunitzer et al., 1973), β-lactoglobulin was determined to be related to the lipocalin protein family. The lipocalins are a low-molecular-mass protein family in which individual proteins show a strong affinity for different hydrophobic molecules (Godovac-Zimmermann, 1988), in other words lipocalins are hydrophobic molecule transporters. However, lipocalins also fulfill other roles; these include involvement in retinol transport, cryptic coloration, olfaction, pheromone transport, and the enzymatic synthesis of prostaglandins (Flower, 1996). In addition to their ability to bind to small hydrophobic molecules, they bind to specific cell-surface receptors and form macro-molecular complexes (Flower, 1996). The amino acid sequences of lipocalins are quite divergent, and low levels of sequence identity (below 20%) are found when comparing the overall sequence identity among some members of the family (Flower, 1996; Ganfornina et al.,

2000). Despite the low level of sequence identity, the tertiary structure is highly conserved (Ganfornina et al., 2000). Based on this information, lipocalins can be divided into two major groups: the kernel lipocalins and outlier lipocalins (Flower, 1996). The kernel lipocalins comprise the majority of the related proteins, including β-lactoglobulin, and share three conserved sequence motifs (Flower et al., 1991, 1993), which correspond to the three main structurally conserved regions of the lipocalin fold (Flower, 1996). The first motif is shared by all lipocalins and can be used to determine family membership (Flower, 1996).

The lipocalin fold is a highly symmetrical all-β protein, dominated by a single eight-stranded anti-parallel β-sheet closed back on itself to form a continuously hydrogen-bonded β-barrel (Cowan et al., 1990b; Flower, 1996; Flower et al., 1993). The cross-section of the β-barrel reveals that is has a flattened or elliptical shape and encloses an internal ligand-binding site (Cowan et al., 1990b; Flower, 1996; Flower et al., 1993). The eight β-strands of the barrel are linked by a succession of +1 connections; these seven loops, labeled L1 to L7, are all typical of short β-hairpins except for loop L1 which is a large Ω loop (Cowan et al., 1990b; Flower, 1996; Flower et al., 1993). L1 forms a lid that is folded back to partially close the internal ligand-binding site found at the end of the barrel (Cowan et al., 1990b; Flower, 1996; Flower et al., 1993).

Together with two other distinct families of ligand-binding proteins, the FABPs and the avidins, the lipocalins form part of the calycin protein superfamily (Flower, 1993; Flower et al., 1993). All families have β-barrel structures; the FABPs are a ten-stranded barrel and discontinuous while the avidins barrel is eight-stranded, like the lipocalins, but are less elliptical in cross-section (Flower, 1996). The calycin protein superfamily is

distinguished by the cup-shaped structure, a 3_{10}-helix that leads into a β-strand, common to its members (Flower et al., 1993).

Beta-lactoglobulin Physiological Roles

With the identification of β-lactoglobulin as a member of the lipocalin protein family and calycin protein superfamily, β-lactoglobulin's physiological role, especially in milk production, would point towards being a transporter of hydrophobic molecules. In fact, β-lactoglobulin is able to bind to hydrophobic and amphiphilic molecules ranging from hexane to palmitic acid to vitamin D (Hambling et al., 1992; Narayan and Berliner, 1997; Perez and Calvo, 1995; Sawyer, 2003). It has been found to be involved in retinol transport and binding, with retinoids and fatty acids binding deep in the hydrophobic pocket of β-lactoglobulin (Cho et al., 1994; Kontopidis et al., 2002; Qin et al., 1998; Wang et al., 1999). Retinol, also known as vitamin A, is essential for mammalian growth and well being (Godovac-Zimmermann et al., 1985; Sawyer, 2003). β-Lactoglobulin has also been found to play a part in the digestion of milk lipids (Perez et al., 1992), in the agonism at opioid receptors (Teschemascher and Koch, 1991), and in the acquisition of passive immunity from colostrum (Alston-Mills and Thompson, 1993). Despite all of these findings, the biological role of β-lactoglobulin is still unknown (Farrell Jr. et al., 2004); it is believed that the original biological role was related to maternal physiology, but this may have shifted to a more nutritional role in some species (Kontopidis et al., 2002). It should be noted that though β-lactoglobulin is the most abundant protein found in the whey protein fraction of milk, it is not found in the milk of all mammals; human milk does not have β-lactoglobulin (Hill et al., 1997).

Beta-lactoglobulin Regulation

Expression of β-lactoglobulin gene is restricted to the mammary gland (Mercier and Vilotte, 1993), where it is regulated by a complex interaction of hormones in conjunction with cell-to-cell and cell-to-extracellular matrix interactions (Streuli, 1993), and occurs in two phases; phase one occurs prior to parturition during pregnancy and phase two occurs after parturition during lactation (Bruce and Whitelaw, 1995). The ovine β-lactoglobulin gene is already expressed at mid-pregnancy, and the level of relevant mRNA increases slowly until parturition at which point it sharply increases (Gaye et al., 1986; Harris et al., 1991). During the second phase, prolactin has been determined to be required for the expression of β-lactoglobulin (Burdon et al., 1994b). The β-lactoglobulin promoter is sensitive to prolactin-induced signals, as demonstrated by prolactin-induction of chloramphenicol acetyltransferase (CAT) activity in Chinese hamster ovary cells cotransfected with a prolactin-receptor expression plasmid and a hybrid gene comprising the 4Kb β-lactoglobulin 5' flanking region fused to the CAT reporter gene (Lesueur et al., 1990). Prolactin activates β-lactoglobulin transcription through a member of the STAT family of transcription factors (Burdon et al., 1994a), STAT5 (Rosen et al., 1999).

In ovine β-lactoglobulin gene, a 4.3Kb 5' flanking sequence and a 7.3 or 1.6 Kb 3' flanking sequence have been efficiently and specifically expressed in the mammary gland of transgenic mice (Simons et al., 1987). By studying 5' shortened constructs (Harris et al., 1990), it was found that 0.8 Kb upstream of the transcription unit was a sufficient distance for high, tissue specific expression of β-lactoglobulin; this region, -406 to -149, appears to also be essential for achieving high, tissue-specific expression

(Whitelaw et al., 1992). By using in vitro binding assays, several binding sites have been identified in the previous region that are recognized by various nuclear effectors (Watson et al., 1991); these sites include at least five binding sites for nuclear factor 1 and three sites for milk protein-binding factor (MPBF) (Burdon et al., 1994b). Nuclear factor 1 (NF1) was originally identified as a host-encoded protein that is required for efficient adenovirus DNA replication in vitro (Nagata et al., 1982). Since then, alternative splicing has led to the identification of different NF1 isoforms with different functions that include altering chromatin topography, direct activation, or repression (Crawford et al., 1998; Osada et al., 1997a; Osada et al., 1997b). There are several NF1 isoforms, ranging in size from 46 to 114 kDa in the mammary gland; during lactation, two isoforms of 46 and 68 kDa are present (Rosen et al., 1999). During mammary involution, the smaller isoforms are lost, and a 74 kDa isoform appears (Furlong et al., 1996); the precise nature of these developmentally regulated NF1 isoforms has not been determined (Rosen et al., 1999).

Mutagenesis of the three sites for MPBF in transgenic mice revealed that MPBF mediates the effects of lactogenic hormones (prolactin, for example) in the mammary gland and therefore mediates the expression of the active β-lactoglobulin gene rather than initiating the activity of the gene (Burdon et al., 1994b). The three binding sites for MPBF are also encompassed within a strong nuclease hypersensitive site (Whitelaw et al., 1992). By using DNase1 as a probe for the β-lactoglobulin promoter, the first major elevation in β-lactoglobulin mRNA levels during pregnancy in sheep is observed (the first phase of lactogenesis as described earlier) (Ali, 1989; Gaye et al., 1986; Harris et al., 1991) indicating that the transcription complex for the promoter has formed (Bruce and

Whitelaw, 1995). However, during this stage of pregnancy, the circulating levels of plasma prolactin are very low (Ali, 1989; Smith et al., 1989) and do not increase to accompany the rise of DNase1 hypersensitivity on the β-lactoglobulin promoter (Bruce and Whitelaw, 1995). But it is possible that another of the lactogenic hormones could replace the circulating prolactin (Bruce and Whitelaw, 1995). Growth hormone, which has lactogenic properties (Caron et al., 1994) and can activate β-lactoglobulin transcription in a novel cell culture system (Goujon et al., 2994), also does not show a change in circulating levels that corresponds to the appearance of DNase1 hypersensitivity (Caron et al., 1994). Placental lactogen, on the other hand does dramatically increase during this stage of pregnancy (Chan et al., 1978), indicating that in sheep, the induction of the β-lactoglobulin gene may be regulated by placental lactogen during the first phase of lactogenesis (Bruce and Whitelaw, 1995).

REFERENCE LIST

Akers, R. M., D. E. Bauman, A. V. Capuco, G. T. Goodman, and H. A. Tucker. 1981. Prolactin regulation of milk secretion and biochemical differentiation of mammary epithelial cells in periparturient cows. Endocrinology 109: 23-30.

Akers, R. M., W. E. Beal, T. B. McFadden, and A. V. Capuco. 1990. Morphometric analysis of involuting bovine mammary tissue after 21 or 42 days of nonsuckling. Journal of Animal Science 68: 3604-3613.

Akers, R. M., and J. E. Keys. 1985. Effect of suckling intensity on human growth hormone binding, biochemical composition, and histological characteristics of ovine mammary glands. Dom Anim Endocrinol 2: 159-172.

Ali, S. 1989. Structure and expression of the gene encoding ovine beta-lactoglobulin, Univ. Edinburgh, Edinburgh, UK.

Alston-Mills, B., and M. P. Thompson. 1993. A possible mechanism of action of β-lactoglobulin. Journal of Dairy Science 76: 196.

Amenomori, Y., C. L. Chen, and J. Meites. 1970. Serum prolactin levels in rats during different reproductive states. Endocrinology 86: 506-510.

Andersson, L. 2001. Genetic dissection of phenotypic diversity in farm animals. Nature Reviews Genetics 2: 130-138.

Andersson-Eklund, L., and J. Rendel. 1993. Linkage between amylase-1 locus and a major gene for milk fat content in cattle. Anim. Genet. 24: 101-103.

Arnheim, M., C. Strange, and H. Erlich. 1985. Use of pooled DNA samples to detect linkage disequilibrium of polymorphic restriction fragments and human disease: Studies of the hla class ii loci. Proc Natl Acad Sci USA 88: 507-511.

Aschaffenburg, R. 1964. Variants of milk proteins and their pattern of inheritance 59th Annual Meeting of the American Dairy Science Association. p 128-132, Tuscon, Arizona.

Aschaffenburg, R., and J. Drewry. 1955. Occurrence of different b-lactoglobulin in cow's milk. Nature 176: 218-219.

Aschaffenburg, R., and J. Drewry. 1957. Genetics of the beta-lactoglobulins of cow's milk. Nature 180: 376-378.

Auchtung, T. L., A. G. Rius, P. E. Kendall, T. B. McFadden, and G. E. Dahl. 2005. Effects of photoperiod during the dry period on prolactin, prolactin receptor, and milk production of dairy cows. Journal of Dairy Science 88: 121-127.

Azam, M. et al. 1995. Interleukin-3 signals through multiple isoforms of stat5. EMBO J. 14: 1402-1411.

Bagdasarov, G. 1960. The milk production of fine wooled and semi fine wooled ewes (in russian). Ovcevodstovo. 6: 22-24.

Baggiolini, M. 1972. The enzymes of the granules of polymorphonuclearleukocytes and their functions. Enzyme 13: 132-160.

Bar-Peled, U. et al. 1995. Relationship between frequent milking or suckling in early lactation and milk production of high producing dairy cows. J Dairy Sci 78: 2726 - 2736.

Barahmand-Pour, F. et al. 1995. Colony-stimulating factors and interferon-γ activate a protein related to mgf-stat5 to cause formation of the differentiation-induced factor in myeloid cells. FEBS Lett. 360: 29-33.

Bargmann, W., K. Fleischhauer, and A. Knoop. 1961. Über die morphologie der milchsekretion. Ii zugleich eine kritik am schema der sekretions-morphologie. Z. Zellforsch. Mikrosk. Anat. 53: 545-568.

Bargmann, W., and A. Knoop. 1959. Über die morphologie der milchsekretion, licht- und elektronenmikroskopische studien an der milchdrüse der ratte. Z. Zellforsch. Mikrosk. Anat. 49: 344-388.

Bargmann, W., and U. Welsch. 1969. On the ultrastructure of the mammary gland. In: M. Reynolds and S. J. Folley (eds.) Lactogenesis: The initiation of milk secretion at parturition. p 43-52. University of Pennsylvania Press, Philadelphia.

Bell, K. 1962. One-dimensional startch-gel electrophoresis of bovine skimmilk. Nature 195: 705-706.

Bell, K., H. A. McKenzie, W. H. Murphy, and D. C. Shaw. 1970. B-lactoglobulin (droughtmaster): A unique protein variant. Biochim. Biophys. Acta 214: 427-436.

Bell, K., H. A. McKenzie, and D. C. Shaw. 1981. Bovine beta-lactoglobulin e, f and g bali (banteng) cattle, bos (bibos) javanicus. Aust. J. Biol. Sci. 34: 133-147.

Bencini, R., and G. Pulina. 1997. The quality of sheep milk: A review. Aust. J. Exp. Agric. 37: 485-504.

Benson, G. K., A. T. Cowie, and J. S. Tindal. 1958. The pituitary and the maintenance of milk secretion. Proc. Royal Soc. B149: 330-336.

Berger, Y. M. 2005. Evolution of milk production at the spooner agricultral research station between 1996 and 2004. 11th Annu. Great Lakes Dairy Sheep Symp. Cornell Univ. Dept. Anim. Sci., Univ. Wisc.-Madison, Dept. Anim. Sci., and Univ. Vermont Ctr. Sustainable Agric. p 6-13.

Berlanga, J. J., J. P. Garcia-Ruiz, M. Perrot-Applanat, P. A. Kelley, and M. Edery. 1997. The short form of hte prolactin (prl) receptor silences prl induction of the β-casein gene promoter. Mol Endo 11: 1449-1457.

Bignon, C., N. Binart, C. Ormandy, L. A. Schuler, P. A. Kelly, and J. Djiane. Long and short forms of the ovine prolactin receptor: cDNA cloning and genomic analysis

reveal that the two forms arise by different alternative splicing mechanisms in ruminants and rodents. Journal of Molecular Endocrinology. 19: 109-120.

Binart, N., P. Imbert-Bollore, N. Baran, C. Viglietta, and P. A. Kelley. 2003. A short form of the prolactin (prl) receptor is able to rescue mammopoiesis in heterozygous prl receptor mice. Mol. Endocrinol. 17: 1066-1074.

Boutin, J. M. et al. 1988. Cloning and expression of the rat prolactin receptor, a member of the growth hormone/prolactin receptor gene family. Cell 53: 69-77.

Bovenhuis, H., A. M. Van Arendonk, and S. Korver. 1992. Associations between milk protein polymorphisms and milk production traits. Journal of Dairy Science 75: 2549-2559.

Braun, R. K., E. N. Bergman, and T. F. Albert. 1970. Effects of various synthetic glucocorticoids on milk production and blood glucose and ketone body concentrations in normal and ketotic cows. J. Am. Vet. Med. Assoc. 157: 941-946.

Braunitzer, G., R. Chen, B. Schrank, and A. Stangl. 1973. Die sequenzanalyze des β-lactoglobulins. Hoppe-Seyler's Zeitschrift für Physiologische Chemie 354: 867-878.

Brisken, C. et al. 1999. Prolactin controls mammary gland development via direct and indirect mechanisms. Dev. Biol. 210: 96-106.

Brodbeck, U., W. L. Denton, N. Tanahashi, and K. E. Ebner. 1967. The isolation and identification of the b protein of lactose synthetase as □-lactalbumin. J Biol Chem 242: 1391-1397.

Bruce, C., and A. Whitelaw. 1995. Regulation of ovine β-lactoglobulin gene expression during the first stage of lactogenesis. Biochemical and Biophysical Research Communications 209: 1089-1093.

Bullen, J. J., H. J. Rogers, and E. Griffiths. 1978. Role of iron in bacterial infection. Curr. Top. Microbiol. Immunol. 80: 1-35.

Burdon, T. G., J. Demmer, A. J. Clark, and C. J. Watson. 1994a. The mammary factor mpbf is a prolactin-induced transcriptional regulator which binds to stat factor recognition sites. FEBS Lett. 350: 177-182.

Burdon, T. G., K. A. Maitland, A. J. Clark, R. Wallace, and C. J. Watson. 1994b. Regulation of the sheep beta-lactoglobulin gene by lactogenic hormones is mediated by a transcription factor that binds an interferon-gamma activation site-related element. Mol. Endocrinol. 8: 1528-1536.

Burnol, A.-F., P. Ferre, A. Leturque, and J. Girard. 1987. Effect of insulin on in vivo glucose utilization in individual tissues of anethetized lactating rats. Am. J. Physiol. 252: E183-E188.

Butler, J. E. et al. 1972. Studies on the relative synthesis and distribution of iga and iggi in various tissues and body fluids of the cow. J. Immunol. 109: 38-46.

Byatt, J. C. et al. 1992. Serum half-life and in-vivo actions of recombinant bovine placental lactogen in the dairy cow. J. Endocrinol. 132: 185-193.

Capuco, A. V., R. M. Akers, and J. J. Smith. 1997. Mammary growth in hostein cows during the dry period: Quantification of nucleic acids and histology. Journal of Dairy Science 80: 477-487.

Caron, R. W., G. A. Jahn, and R. P. Deis. 1994. Lactogenic actions of different growth hormone preparations in pregnant and lactating rats. J. Endocrinol. 142: 535-545.

Casu, S., and J. G. Boyazoglou. 1974. Effect de la suppression de la traite du soir chez la brebis sarde. In: Symposium sur la traite mécanique des petits ruminants, Paris, France. p 139-144.

Casu, S., and Labussière. 1972. Premiers résultats concernant la suppression d'une ou plusieurs traites chez les brebis sardes. Ann. Zootech. 21: 223-232.

Chan, J. S. D., H. A. Robertson, and H. G. Freisen. 1978. Distribution of binding sites for ovine placental lactogen in the sheep. Endocrine 102: 632-640.

Charmet, G. et al. 1999. Marker-assisted recurrent selection for cumulating additive and interactive qtls in recombinant inbred lines. Theor. Appl. Genet. 99: 1143-1148.

Cho, Y., C. A. Batt, and L. Sawyer. 1994. Probing the retinol-binding site of bovine β-lactoglobulin. J Biol Chem 269: 11102-11107.

Clutton-Brock, J. 1987. A natural history of domesticated mammals. British Museum (Natural History), London.

Collier, R. J., D. E. Bauman, W. J. Croom, and R. L. Hays. 1976. Lactogenesis in explant cultures of cow mammary tissue. In: 71st Ann. Meeting, Amer. Dairy, Sci. Ass., Champaign, IL. p 59 (Abstr.).

Collier, R. J., D. E. Bauman, and R. L. Hays. 1977. Effect of reserpine on milk production and serum prolactin of cows hormonally induced into lactation. Journal of Dairy Science 60: 896-901.

Connor, E. et al. 2008. Effects of increased milking frequency on gene expression in the bovine mammary gland. BMC Genomics 9: 362.

Conti, A., J. Godovac-Zimmermann, J. Liberatori, and G. Braunitzer. 1984. The primary structure of monmeric beta-lactoglobulin i from horse colostrum (equus caballus, perissodactyla). Hoppe-Seyler's Zeitschrift für Physiologische Chemie 365: 1393-1401.

Conti, A., J. Godovac-Zimmermann, F. Pirchner, J. Liberatori, and G. Braunitzer. 1986. Pig beta-lactoglobulin i (sus scrofa domestica, artiodactyla). The primary structure of the major component. Biol. Chem. Hoppe Seyler 367: 871-878.

Conti, A., L. Napolitano, A. M. Cantisani, R. Davoli, and S. Dall'Olio. 1988. Bovine β-lactoglobulin h: Isolation by preparative isoelectric focusing in immobilized ph

gradients and preliminary characterization. J. Biochem. Biophys. Methods 16: 205-214.

Convey, E. M. 1974. Serum hormone concentrations in ruminants during mammary growth, lactogenesis, and lactation: A review. Journal of Dairy Science 57: 905-917.

Corner, G. W. 1930. The hormonal control of lactation: I. Non-effect of the corpus luteum. Ii. Positive action of extracts of the hypophysis. American Journal of Physiology 95: 43-55.

Cowan, C. M. 1994. Use of genetic marker technology to select ai bulls. British Cattle Breeders Club Digest 49: 45-48.

Cowan, C. M., M. R. Dentine, R. L. Ax, and L. A. Schuler. 1990a. Structural variation around prolactin gene linked to quantitative traits in elite holstein sire family. Theor. Appl. Genet. 79: 577-582.

Cowan, S. W., M. E. Newcomer, and T. A. Jones. 1990b. Crystallographic refinement of human serum retinol binding protein at 2å resolution. Proteins: Struct. Funct. Genet. 8: 44-61.

Cowie, A. T. 1969. Variations in the yield and composition of the milk during lactation in the rabbit and the galactopoietic effect of prolactin. J. Endocrinol. 44: 437-450.

Cowie, A. T., and S. J. Folley. 1961. The mammary gland and lactation. In: W. C. Young (ed.) Sex and internal secretions No. I. p 590-642. The Williams & Wilkins Co, Baltimore, MD.

Crawford, D. R. et al. 1998. Nuclear factor 1 regulates expression of the gene for phosphoenolpyruvate carboxykinase (gtp). J Biol Chem 273: 13387-13390.

DaCosta, T. H. M., and D. H. Williamson. 1994. Regulation of rat mammary-gland uptake of arally administered [1-14c] triolein by insulin and prolactin: Evidence for bihormonal control of lipoprotein lipase activity. Biochem. J. 300.

Dahl, G., T. Auchtung, and E. Reid. 2004a. Manipulating milk production in early lactation through photoperiod changes and milking frequency. Vet Clin Food Anim 20: 675 - 685.

Dahl, G., R. Wallace, R. Shanks, and D. Lueking. 2004b. Hot topic: Effects of frequent milking in early lactation on milk yield and udder health. J Dairy Sci 87: 882 - 885.

Dahl, G. E., B. A. Buchanan, and H. A. Tucker. 2000. Photoperiodic effects on dairy cattle: A review. Journal of Dairy Science 83: 885-893.

Dahl, G. E., and D. Petitclerc. 2003. Management of photoperiod in the dairy herd for improved production and health. Journal of Animal Science 81(Suppl. 3): 11-17.

Darvasi, A. 1998. Experimental strategies for the genetic dissection of complex traits in animal models. Nat. Genet. 18: 19-24.

Dati, C., P. Maggiora, C. Puech, M. D. Bortoli, and C. Escot. 1996. Expression of the erbb-2 proto-oncogene during differentiation of the mammary gland in the rat. Cell Tissue Res 285: 403-410.

Davis, C. L., and D. E. Bauman. 1974. General metabolism associated with the synthesis of milk. In: B. L. Larson and V. R. Smith (eds.) Lactation: A comprehensive treatise No. 2. p 3-30. Academic Press, New York.

Davoli, R., S. Dall'Olio, and D. Bigi. 1988. A new β-lactoglobulin variant in bovine milk. Scienza-e-Tecnica-Lattiero-Casearia 39: 439-443.

de Koning, D. J., J. C. M. Dekkers, and C. S. Haley. 2003. Designs for qtl detection in livestock and their implications for mas. Conference marker-assisted selection: A fast track to increase genetic gain in plant and animal breeding? Session ii. Mas in animals. In: Conference of the FAO Biotechnology Forum: Molecular marker-assisted selection as a potential tool for genetic improvement of crops, forest trees, livestock and fish in developing countries, Turin, Italy. p 47-53.

DeFremery, P. 1936. On the influence of different hormones on lactation. J Physiol 87: 50 P (Abstr.).

Dekkers, J. C. M. 2004. Commercial application of marker- and gene-assisted selection in livestock: Strategies and lessons. Journal of Animal Science 82: E313-328.

Dekkers, J. C. M., and F. Hospital. 2002. The use of molecular genetics in the improvement of agricultural population. Nature Reviews Genetics 3: 22-32.

Eigel, W. N. et al. 1984. Nomenclature of proteins of cow's milk: Fifth revision. Journal of Dairy Science 67: 1599-1631.

Elias, J. J. 1957. Cultivation of adult mouse mammary gland in hormone-enriched synthetic medium. Science 126: 842-844.

Erdman, R., and M. Varner. 1995. Fixed yield responses to increased milking frequency. J Dairy Sci 78: 1199 - 1203.

Erhardt, G., J. Godovac-Zimmermann, and A. Conti. 1989. Isolation and complete primary sequence of a new ovine wild-type beta-lactoglobulin c. Biol. Chem. Hoppe Seyler 370: 757-762.

Evans, E. E. 1932. Initiation of copious milk secretion in virgin goats by anterior pituitary. Proc. Soc. Exp. Biol. Med. 30.

Farnir, F. et al. 2000. Extensive genome-wide linkage disequilibrium in cattle. Genome Res. 10: 220-227.

Farrell Jr., H. M. et al. 2004. Nomenclature of the proteins of cows' milk - sixth revision. Journal of Dairy Science 87: 1641-1674.

Feng, Z. et al. 1995. Glucocorticoid and progesterone inhibit involution and programmed cell death in the mouse mammary gland. J. Cell Biol. 131: 1095-1103.

Flamant, J. C. 1974. Queques élééments de comparaison de l'aptitude à la traite des brebis des races sardes et lacaune élevées dans la région de roquefort. In: Symposium sur la traite mécanique des petits ruminants, Paris, France. p 163-169.

Flower, D. R. 1993. Structural relationship of streptavidin to the calycin protein superfamily. FEBS Lett. 333: 99-102.

Flower, D. R. 1996. The lipocalin protein family: Structure and function. The Biochemical Journal 318 (Pt 1): 1-14.

Flower, D. R., A. C. T. North, and T. K. Attwood. 1991. Mouse oncogene protein 24p3 is a member of the lipocalin protein family. Biochem. Biophys. Res. Commun. 180: 69-74.

Flower, D. R., A. C. T. North, and T. K. Attwood. 1993. Structure and sequence relationships in the lipocalins and related proteins. Protein Sci. 2: 753-761.

Folley, S. J. 1952. Lactation. In: A. S. Parkes (ed.) Marshall's physiology of reproduction No. 2. p 525-647. Longmans, Green & Co., London, United Kingdom.

Folley, S. J. 1956. The physiology and biochemistry of lactation. Oliver and Boyd, Edinburgh, Scotland.

Forsyth, I. A. 1961. Organ culture techniques and the study of hormone effects on the mammary gland. Journal of Dairy Research 38: 419-444.

Forsyth, I. A. 1994. Comparative aspects of placental lactogens: Structure and function. Exp. Clin. Endocrinol. 102: 244-251.

Frantz, W. L., J. H. MacIndoe, and R. W. Turkington. 1974. Prolactin receptors: Characteristics of the particulate fraction binding activity. J. Endocrinol. 60: 485-497.

Fujii, H. et al. 1995. Activation of stat5 by interleukin 2 requires carboxyl-terminal region of the interleukin 2 receptor β chain but is not essential for the proliferative signal transmission. Proc Natl Acad Sci USA 92: 5482-5486.

Furlong, E. E. M., N. K. Keon, F. D. Thornton, T. Rein, and F. Martin. 1996. Expression of a 74-kda nuclear factor 1 (nf1) protein is induced in mouse mammary gland involution. Involution-enhanced occupation of a twin nf1 binding element in the testosterone-repressed prostate message2/clusterin promoter. J Biol Chem 271: 29688-29697.

Ganfornina, M. D., G. Gutiérrez, M. Bastiani, and D. Sanchez. 2000. A phylogenetic analysis of the lipocalin protein family. Molecular Biology and Evolution 17: 114-126.

Gaye, P. et al. 1986. Ovine β-lactoglobulin messenger rna: Nucleotide sequence and mrna levels during functional differentiation of the mammary gland. Biochimie 68: 1097-1107.

Geldermann, H. 1975. Investigations on inheritance of quantitative characters in animals by gene markers. I. Methods. Theor. Appl. Genet. 46: 319-330.

Geldermann, H., U. Pieper, and B. Roth. 1985. Effects of marked chromosome sections on milk performance in cattle. Theor. Appl. Genet. 70: 138-146.

Georges, M. 2007. Mapping, fine mapping, and molecular dissection of quantitative trait loci in domestic animals. Annu. Rev. Genomics Hum. Genet. 8: 131-162.

Georges, M. et al. 1993. Microsatellite mapping of the gene causing weaver disease in cattle will allow the study of an associated quantitative trait locus. Proc Natl Acad Sci USA 90: 1058-1062.

Georges, M. et al. 1995. Mapping quantitative trait loci controlling milk production in dairy cattle by exploiting progeny testing. Genetics 139: 907-920.

Godovac-Zimmermann, J. 1988. The structural motif of beta-lactoglobulin and retinol-binding protein: A basic framework for binding and transport of small hydrophobic molecules? Trends Biochem. Sci. 13: 64-66.

Godovac-Zimmermann, J. et al. 1996. Isolation and rapid sequence characterization of two novel bovine beta-lactoglobulins i and j. J. Protein Chem. 15: 743-750.

Godovac-Zimmermann, J., I. Krause, J. Buchberger, G. Weiss, and H. Klostermeyer. 1990. Genetic variants of bovine beta-lactoglobulin. A novel wild-type beta-lactoglobulin w and its primary sequence. Biol. Chem. Hoppe Seyler 371: 255-260.

Godovac-Zimmermann, J., J. Liberatori, and G. Braunitzer. 1985. The amino-acid sequence of beta-lactoglobulin ii from horse colostrum (equus caballus, perissodactyla): Beta-lactoglobulins are retinol-binding proteins. Biol. Chem. Hoppe Seyler 366: 601-608.

Goodman, R. E., and F. L. Schanbacher. 1991. Bovine lactoferrin mrna: Sequence, analysis and expression in the mammary gland. Biochem. Biophys. Res. Commun. 180: 75-84.

Gorewit, R. C., and H. A. Tucker. 1977. Lactational events related to glucocorticoid binding in bovine mammary tissue. Journal of Dairy Science 60: 889-895.

Gouilleux, F. et al. 1995. Prolactin, growth hormone, erythropoietin and granulocyte-macrophage colony stimulating factor induce mgf-stat5 DNA binding activity. EMBO J. 14: 2005-2013.

Goujon, L. et al. 2994. Proc Natl Acad Sci USA 91: 957-961.

Griinari, J. M. et al. 1997. The role of insulin in the regulation of milk protein synthesis in dairy cows. Journal of Dairy Science 80: 2361-2371.

Grosclaude, F., M.-F. Mahé, and J. C. Mercier. 1974. Polymorphisme des lactoproteines de bovines napalais. I. Mise en evidence, chez le *yak*, et caracterisation

biochimique de deau nouveaux variants: B-lactoglobuline d (yak) et caseine α_{s1} - e. Ann. Génét. Sel. Anim. 8: 461-479.

Grosclaude, F., J. Pujolle, J. Garnier, and B. Ribadeau-Dumas. 1966. Mise en évidence de deux variants supplémentaires des protéines du lait de vache: α_{s1}-cn^d et lg^d. Ann. biol anim. Biochim. Biophys. 6: 215-222.

Groves, M. L. 1960. The isolation of a red protein from milk. J. Am. Chem. Soc. 82: 3345-3350.

Guidry, A. J., J. E. Butler, R. B. Pearson, and B. T. Weinland. 1980. Iga, igg_1, igg_2, igm, and bsa in serum and mammary secretion throughout lactation. Vet. Immunol. Immunopathol. 1: 329-341.

Hadjipanayiotou, M., and A. Louca. 1976. The effects of partial suckling on lactation performance of chios sheep and damascus goats and the growth rate of the lambs and kids. J. Agric. Sci. Camb. 87: 15-20.

Hale, S., A. Capuco, and R. Erdman. 2003. Milking yield and mammary growth effects due to increased milking frequency during early lactation. J Dairy Sci 86: 2061 - 2071.

Hamann, J., and J. Reichmuth. 1990. Compensatory milk production within the bovine udder: Effects of short-term non-milking of single quarters. Journal of Dairy Research 57: 17-22.

Hambling, S. G., A. S. McAlpine, and L. Sawyer. 1992. B-lactoglobulin. In: P. F. Fox (ed.) Advanced dairy chemistry No. 1: Proteins. p 141-189. Elsevier Applied Science, London.

Harris, S., S. Ali, S. Anderson, A. L. Archibald, and A. J. Clark. 1988. Complete nucleotide sequence of the genomic ovine beta-lactoglobulin gene. Nucleic Acids Research 16: 10379-10380.

Harris, S., M. McClenaghan, J. P. Simons, S. Ali, and A. J. Clark. 1990. Gene expression in the mammary gland. J. Reprod. Fertil 88: 707-715.

Harris, S., M. McClenaghan, J. P. Simons, S. Ali, and A. J. Clark. 1991. Developmental regulation of the sheep β-lactoglobulin gene in the mammary gland of transgenic mice. Dev. Genet. 12: 299-307.

Hazlerigg, D. G., M. H. Hastings, and P. J. Morgan. 1996. Production of a prolactin releasing factor by the ovine pars tuberalis. J. Neuroendrocrinol 8: 489-492.

Hennighausen, L., and G. W. Robinson. 1998. Think globally, act locally: The making of a mouse mammary gland. Genes Dev. 12: 449-455.

Hennighausen, L., G. W. Robinson, K.-U. Wagner, and X. Liu. 1997. Prolactin signaling in mammary gland development: Minireview. The Journal of Biological Chemistry 272: 7567-7569.

Hernandez, H. et al. 2002. Maternal olfaction differentially modulates oxytocin and prolactin release during suckling in goats. Horm. Behav. 42: 232-244.

Heyen, D. W. et al. 1999. A genome scan for qtl influencing milk production and health traits in dairy cattle. Physiol Genomics 1: 165-175.

Hidiroglou, M., T. R. Batra, and K. H. Nielsen. 1992. Effect of vitamin e supplementation and of health status of mammary gland on immunoglobulin concentration in milk of dairy cows. Ann. Rech. Vet. 23: 139-144.

Hill, J. P. et al. 1997. The polymorphism of the milk protein b-lactoglobulin. In: Milk Composition, Production and Biotechnology: 173-202.

Hillerton, J., C. Knight, A. Turvey, S. Wheatley, and C. Wilde. 1990. Milk yield and mammary function in dairy cows milked four times daily. J Dairy Res 57: 285 - 294.

Hindes, L., and A. S. I. Loudon. 1995. Mechanisms of seasonality in marsupials: A comparative review. In: N. Saunders and L. Hindes (eds.) Marsupial biology. p 41-70. UNSW Press, Sydney.

Hoeschele, I., and T. R. Meinert. 1990. Association of genetic defects with yield and type traits: The weaver locus effect on yield. Journal of Dairy Science 73: 2503-2515.

Hollmann, K. H. 1959. L'ultrastructure de la glande mammaire normale de la souris en lactation. Etude en microscope électronique. J. Ultrastruct. Res. 2: 423-443.

Hollmann, K. H. 1969. Quantitative electron microscopy of sub-cellular organization in mammary gland cells before and after parturition. In: M. Reynolds and S. J. Folley (eds.) Lactogenesis: The initiation of milk secretion at parturition. p 27-41. University of Pennsylvania Press, Philadelphia.

Hollmann, K. H. 1974. Cytology and fine structures of the mammary gland. In: B. L. Larson and V. R. Smith (eds.) Lactation: A comprehensive treatise No. 1. p 3-95. Academic Press Inc., New York.

Holst, B. D., W. L. Hurley, and D. R. Nelson. 1987. Involution of the bovine mammary gland: Histological and structural changes. Journal of Dairy Science 70: 935-942.

Hospital, F., I. Goldringer, and S. Openshaw. 2000. Efficient marker-based recurrent selection for multiple quantitative trait loci. Genet. Res. 75: 357-368.

Hou, J., U. Schindler, W. J. Henzel, S. C. Wong, and S. L. McKnight. 1995. Identification and purification of human stat proteins activated in response to interleukin-2. Immunity 2: 321-329.

Hurley, W. L. 1989. Mammary gland function during involution. Journal of Dairy Science 72: 1637-1646.

Ihle, J. N., and I. M. Kerr. 1995. Jaks and stats in signaling by the cytokine receptro superfamily. Trends Genet. 11: 69-74.

Ingalls, W. G., E. M. Convey, and H. D. Hafs. 1973. Bovine serum lh, gh, and prolactin during late pregnancy, parturition and early lactation. Proc. Soc. Exp. Biol. Med. 143: 161-164.

Jabbour, H. N., and P. A. Kelley. 1997. Prolactin receptor subtypes: A possible mode of tissue specific regulation of prolactin function. Rev. Reprod. 2: 14-18.

Jack, L. J. W., S. Kahl, D. L. St. Germain, and A. V. Capuco. 1994. Tissue distribution and regulation of 5'-deiodinase processes in lactating rats. J. Endocrinol. 142: 205-215.

Jacobsohn, D. 1958. Mammary gland growth in relation to hormones with metabolic actions. Proc. Royal Soc. B149.

Jamieson, A. C., M. A. Vandeyar, Y. C. Kang, J. E. Kinsella, and C. A. Batt. 1987. Cloning and nucleotide sequence of the bovine beta-lactoglobulin gene. Gene 61: 85-90.

Jansen, H. T., and G. L. Jackson. 1993. Circannual rhythms in the ewe: Patterns of ovarian cycles and prolactin under two different constant photoperiods. Biol Reprod 49: 627-634.

Jenness, R. et al. 1956. Nomenclature of the proteins of bovine milk. Journal of Dairy Science 39: 536-541.

Jimenez, L., C. Rua, E. Muñiz, and P. Garcia. 1984. Alveolar epithelial cells of the mammary gland of lactating *myotis myotis*: An ultrastructural study. Zeitschrift fur Mikroskopisch-Anatomische Forschung 98: 465-477.

Johke, T., H. Fuse, and M. Oshima. 1971. Changes in plasma prolactin level during late pregnancy and early lactation in the goat and the cow. Japan J. Zootech. Sci. 42: 173.

Johnston, J. A. et al. 1995. Tyrosine phosphorylation and activation of stat5, stat3 and janus kinases by interleukins 2 and 15. Proc Natl Acad Sci USA 92: 8705-8709.

Josimovich, J. B., and J. A. MacLaren. 1962. Presence in the human placenta and term serum of a highly lactogenic substance immunologically related to pituitary growth hormone. Endocrinology 71: 209-220.

Juergens, W. G., F. E. Stockdale, Y. J. Topper, and J. J. Elias. 1965. Hormone-dependent differentiation of mammary gland *in vitro*. Proc Natl Acad Sci USA 54: 629-634.

Kahl, S., J. Bitman, A. V. Capuco, and J. E. Keys. 1991. Effect of lactational intensity on extrathyrodial 5'-deiodinase activity in rats. Journal of Dairy Science 74.

Kasuga, M., E. Van Oberghen, S. P. Nissley, and M. M. Rechler. 1981. Demonstration of insulin-like growth factor receptors by affinity cross-linking. J Biol Chem 256: 5305-5308.

Kazansky, A. V., B. Raught, S. M. Lindsey, Y. F. Wang, and J. M. Rosen. 1995. Regulation of mammary gland factor/stat5a during mammary gland development. Mol Endo 9: 1598-1609.

Kazmer, G. W., M. A. Barnes, R. M. Akers, and W. D. Whittier. 1986. Lactogenic hormone recptors in mammary membrane preparations from prepartum and 60 and 180 day postpartum holstein cattle. J. Endocrinol. 109: 175-180.

Keenan, T. W., D. J. Morre, and C. M. Huang. 1974. Membranes of the mammary glands. In: B. L. Larson and V. R. Smith (eds.) Lactation: A comprehensive treatise No. 2. p 191-233. Academic Press, New York.

Kelly, P. A. et al. 1989. Purification, cloning, and expression of the prolactin receptor. Biol. Reprod. 40: 27-32.

Kolde, H. J., and G. Braunitzer. 1983. The primary structure of ovine β-lactoglobulin Milchwissenschaft 38: 18-20.

Kontopidis, G., C. Holt, and L. Sawyer. 2002. The ligand-binding site of bovine β-lactoglobulin: Evidence for a function? J. Mol. Bol. 318: 1043-1055.

Koprowski, J. A., and H. A. Tucker. 1973a. Bovine serum growth hormone, corticoids and insulin during lactation. Endocrinology 92: 645-651.

Koprowski, J. A., and H. A. Tucker. 1973b. Serum prolactin during various physiological states and its relationship to milk production in the bovine. Endocrinology 92: 1480-1487.

Kumaresan, P., R. R. Anderson, and C. W. Turner. 1966. Effect of graded levels of lactogenic hormone upon mammary gland growth and lactation. Proc. Soc. Exp. Biol. Med. 123: 581-584.

Laarveld, B., R. K. Chaplin, and R. P. Brockman. 1985. Effects of insulin on the metabolism of acetate, β-hydroxybutyrate and triglycerides by the bovine mammary gland. Comp. Biochem. Physiol. 82B: 265-267.

Labussière, J., B. Benmederbel, J. F. Combaud, and F. A. De La Chevalerie. 1983. Description des principaux paramètres caractérisant la production laitère, la morphologie mammaire et la cinétique d'émission du lait de la brebis lacuane traites deux fois par jour avec ou sans égouttage. In: III Symposium Internacional de ordeño Mecanico de Pequeños Rumiantes, Valladolid, España, Univ. Valladolid, Spain. p 625-640.

Labussière, J., J. F. Combaud, and P. Petrequin. 1974. Influence de la fréquence des traites et des tétées sur la production laitière des brebis préalpes du sud. Ann. Zootech. 23: 445-457.

Lamb, G. C. et al. 1997. Suckling reinitiated lactation in beef cows after an early postpartum hiatus of milking or suckling. Journal of Animal Science 75 (Suppl. 1): 229 (Abstr.).

Lande, R., and R. Thompson. 1990. Efficiency of marker-assisted selection in the improvement of quantitative traits. Genetics 124: 743-756.

Lander, E. S., and D. Botstein. 1989. Mapping mendelian factors underlying quantitative traits using rflp linkage maps. Genetics 121: 185-199.

Lander, E. S., and L. Kruglyak. 1995. Genetic dissection of complex traits: Guidlines for interpreting and reporting linkage results. Nat. Genet. 11: 241-247.

Lesueur, L. et al. 1990. Prolactin stimulates milk protein promoter in cho cells cotransfected with prolactin receptor cdna. Mol. Cell Endocrinol. 71: R7-R12.

Li, P., P. Rudland, D. Fernig, L. Finch, and C. Wilde. 1999a. Modulation of mammary development and programmed cell death by the frequency of milk removal in lactating goats. J Physiol 519: 885 - 900.

Li, P., P. S. Rudland, D. G. Fernig, L. M. Finch, and C. J. Wilde. 1999b. Modulation of mammary development and programmed cell death by the frequency of milk removal in lactating goats. Journal of Physiology 519: 885-900.

Li, P., C. J. Wilde, L. M. Finch, D. G. Fernig, and P. S. Rudland. 1999c. Identification of cell types in the developing goat mammary gland. The Histochemical Journal 31: 379-393.

Lin, J. X., J. Mietz, W. S. Modi, S. John, and W. J. Leonard. 1996. Cloning of human stat5b. Reconstitution of interleukin-2-induced stat5a and stat5b DNA binding activity in cos-7 cells. J Biol Chem 271: 10738-10744.

Lincoln, G. A. 1989. Significance of seasonal cycles in prolactin secretion in male mammals. In: M. Serio (ed.) Perspectives in andrology. p 299-306. Raven Press, New York.

Lincoln, G. A. 1999. Melatonin modulation of prolactin and gonadotropin secretion. Systems ancient and modern. In: J. Olcese (ed.) Melatonin after four decades. p 137-153. Kluwer Academic/Plenum Publications, New York.

Lincoln, G. A., H. Andersson, and D. Hazlerigg. 2003. Clock genes and the long-term regulation of prolactin secretion: Evidence for a photoperiod/circannual timer in the pars tuberalis. p 390-397.

Lincoln, G. A., and I. J. Clarke. 1994. Photoperiodically-induced cycles in the secretion of prolactin in hypothalamo-pituitary disconnected rams: Evidence for translation of the melatonin signal in the pituitary gland. J. Neuroendrocrinol 6: 251-260.

Lincoln, G. A., and I. J. Clarke. 1995. Evidence that melatonin acts in the pituitary gland through a dopamine-independent mechanism to mediate effects of daylength on the secretion of prolactin in the ram. J. Neuroendrocrinol 7: 637-643.

Lincoln, G. A., I. J. Clarke, and T. Sweeney. 1996. 'hamster-like' cycles in testicular size in the absence of gonadotropin secretin in hpd rams exposed to long-term changes in photoperiod and treatment with melatonin. J. Neuroendrocrinol 8: 855-866.

Lincoln, G. A., S. M. Rhind, S. Pompolo, and I. J. Clarke. 2001. Hypothalamic control of photoperiod-induced cycles in food intake, body weight, and metabolic hormones in rams. Am. J. Physiol. Reg. Int. Comp. Physiol. 281: R76-R90.

Linzer, D. I. H. 1998. Placental lactogens. In: E. Knobil and J. D. Neill (eds.) Encyclopedia of Reproduction No. 3. p 874-881. Academic Press, San Diego, CA.

Liu, X., G. W. Robinson, F. Gouilleux, B. Groner, and L. Hennighausen. 1995. Cloning and expression of stat5 and an additional homologue (stat5b) involved in prolactin signal transduction in mouse mammary tissue. Proc Natl Acad Sci USA 92: 8831-8835.

Liu, X., G. W. Robinson, and L. Hennighausen. 1996. Activation of stat5a and stat5b by tyrpsine phosphorylation is tightly linked to mammary gland differentiation. Mol Endo 10: 1496-1506.

Liu, X. et al. 1997. Stat5a is mandatory for adult mammary gland development and lactogenesis. Genes Dev. 11: 179-186.

Lorscheider, F., and E. P. Reineke. 1971. The influence of lactational intensity and exogenous prolactin on serum thyroxine levels in the rat. Proc. Soc. Exp. Biol. Med. 138: 1116-1118.

Lyons, W. R. 1942. The direct mammotrophic action of lactogenic hormone. Proc. Soc. Exp. Biol. Med. 51: 308-311.

Lyons, W. R. 1958. Hormonal synergism in mammary gland growth. Proc. Royal Soc. B149: 303-325.

Mackenzie, D. D. S., and A. K. Lascelles. 1968. The transfer of i-131-labeled immunoglobulins and serum albumin from blood into milk of lactating ewes. Aust. J. Exp. Biol. Med. Sci. 46: 285-294.

Marnet, P. G., and M. Komara. 2008. Management systems with extended milking intervals in ruminants: Regulation of production and quality of milk. Journal of Animal Science 86: 47-56.

Marte, B. M. et al. 1995. Neu differentiation factor/heregulin modulates growth and differentiation of hc11 mammary epithelial cells. Mol Endo 9: 14-23.

Martin, R. J., and R. Baldwin. 1971. Effects of insulin on isolated rat mammary cell metabolism: Glucose utilization and metabolite patterns. Endocrinology 89: 1263-1269.

Masson, P. L., J. F. Heremans, and C. Dive. 1966. An iron-binding protein common to many external secretions. Clin. Chim. Acta 14: 735-738.

McKusick, B. C., D. L. Thomas, and Y. M. Berger. 2001. Effect of weaning system on commercial milk production and lamb growth of east friesian dairy sheep. Journal of Dairy Science 84: 1660-1668.

Meites, J. 1961. Farm animals: Hormonal induction of lactation and galactopoieses. In: S. K. Kon and A. T. Cowie (eds.) Milk: The mammary gland and its secretion. p 321-367. Academic Press, New York, NY.

Mercier, J. C., and J. L. Vilotte. 1993. Structure and function of milk protein genes. Journal of Dairy Science 76: 3079-3098.

Meuwissen, T. H., and M. E. Goddard. 1996. The use of marker hapolytes in animal breeding schemes. Genet. Sel. Evol. 28: 161-176.

Meuwissen, T. H., and J. A. Van Arendonk. 1992. Potential improvements in rate of genetic gain from marker-assisted selection in dairy cattle breeding schemes. Journal of Dairy Science 75: 1651-1659.

Mikolayunas, C. M., D. L. Thomas, G. E. Dahl, T. F. Gressley, and Y. M. Berger. 2008. Effect of prepartum photoperiod on milk production and prolactin concentration of dairy ewes. Journal of Dairy Science 91: 85-90.

Miller, A. R. E., R. Erdman, L. W. Douglass, and G. E. Dahl. 2000. Effects of photoperiodic manipulation during the dry period of dairy cows. Journal of Dairy Science 83: 962-967.

Mills, E. S., and Y. J. Topper. 1970. Some ultrastructural effects of insulin, hydrocortisone and prolactin on mammary gland explants. J Biol Chem 44: 310-328.

Morag, M. 1968. The effect of varying the daily milking frequency on the milk yield of the ewe and evidence of the nature of inhibition of milk ejection by half udder milking. Ann. Zootech. 17: 351-369.

Moreau, L., A. Charcosset, F. Hospital, and A. Gallais. 1998. Marker-assisted selection efficiency in populations of finite size. Genetics 148: 1353-1365.

Morgan, P. J. 2000. The pars tuberalis: The missing link in the photoperiodic regulation of prolactin secretion. J. Neuroendrocrinol 12: 287-295.

Nagata, K., R. A. Guggenheimer, T. Enomoto, J. H. Lichy, and J. Hurwitz. 1982. Adenovirus DNA replication in vitro: Identification of a host factor that stimulates synthesis of the preterminal protein-dcmp complex. Proc Natl Acad Sci USA 79: 6438-6442.

Narayan, M., and L. J. Berliner. 1997. Fatty acids and retinoids bind independently and simultaneously to β-lactoglobulin. Biochemistry 36: 1906-1911.

Neimann-Sorensen, A., and A. Robertson. 1961. The association between blood groups and several production characters in three danish cattle breeds. Acta. Agr. Scand. 11: 163-196.

Newbold, J. A., L. T. Chapin, S. A. Zinn, and H. A. Tucker. 1991. Effects of photoperiod on mammary development and concentration of hormones in serum of pregnant dairy heifers. Journal of Dairy Science 74: 100-108.

Nickerson, S. C., C. W. Heald, and T. L. Bibb. 1976. Cytologicl effects of hormones ad plasma on bovine mammary tissue *in vitro*. In: 71st Ann. Meeting, Amer. Dairy Sci. Ass., Champaign, IL. p 59 (Abstr.).

Noble, M. S., and W. L. Hurley. 1999. Effects of secretion removal on bovine mammary gland function following an extended milk stasis. Journal of Dairy Science 82: 1723-1730.

Nørgaard, J., A. Sørensen, M. Sørensen, J. Andersen, and K. Sejrsen. 2005. Mammary cell turnover and enzyme activity in dairy cows: Effects of milking frequency and diet energy density. J Dairy Sci 88: 975 - 982.

Nudda, A., R. Bencini, S. Mijatovic, and G. Pullina. 2002. The yield and composition of milk in sarda, awassi and merino sheep milked unilaterally at different frequency. Journal of Dairy Science 85: 2879-2884.

Ormandy, C. J. et al. 1997. Null mutation of the prolactin receptor gene produces multiple reproductive defects in the mouse. Genes Dev. 11: 167-178.

Osada, S. et al. 1997a. Nuclear factor 1 family proteins bind to the silencer element in the rat glutathione transferase p gene. J. Biochem. 121: 355-363.

Osada, S., T. Ikeda, M. Xu, T. Nishihara, and M. Imagawa. 1997b. Identification of the transcriptional repression domain of nuclear factor 1-a. Biochem. Biophys. Res. Commun. 238: 744-747.

Ota, K. et al. 1974. Corticosterone secretion in response to suckling at various stages of normal and prolonged lactation in rats. J. Endocrinol. 62: 679-680.

Pallard, C. et al. 1995. Thrombopoietin activates a stat5-like factor in hematopoietic cells. EMBO J. 14: 2847-2856.

Papachristoforou, C., A. Roushias, and A. P. Mavrogenis. 1982. The effect of milking frequency on the milk production of chios ewes and damascus goats. Ann. Zootech. 31: 37-46.

Partearroyo, A. M., and J. C. Flamant. 1978. Caractéristiques moyennes de traite et de mamelle de trois génotypes de brebis laitières (lacaune, sardes et f.S.L.). In: Proc 2nd Int. Symp. Milking of small ruminants, Alghero, Sardinia, Italy. p 80-92.

Paterson, J. Y. F., and J. L. Linzell. 1974. Cortisol secretion rate, glucose entry rate and the mammary uptake of cortisol and glucose during pregnancy and lactation in dariy cows. J. Endocrinol. 62: 371-383.

Patton, S., and F. M. Fowkes. 1967. The role of the plasma membrane in the secretion of milk fat. J. Theor. Biol. 15: 274-281.

Perez, M. D., and M. Calvo. 1995. Interaction of beta-lactoglobulin with retinol and farry acids and its role as a possible biological function for this protein: A review. Journal of Dairy Science 78: 978-988.

Perez, M. D. et al. 1992. Effect of beta-lactoglobulin on the activity of pregastric lipase. A possible role for this protein in ruminant milk. Biochim. Biophys. Acta 1123: 151-155.

Peris, S. et al. 1997. Influence of reating systems on milk composition and yield of murciano-granadina dairy goats. Journal of Dairy Science 80.

Peters, R. R., L. T. Chapin, R. S. Emery, and H. A. Tucker. 1981. Milk yield, feed intake, prolactin, growth hormone, and glucocorticoid response of cows to supplemental light. Journal of Dairy Science 64: 1671-1678.

Peters, R. R., and H. A. Tucker. 1978. Prolactin and growth hormone responses to photoperiod in heifers. Endocrinology 103: 229-234.

Philp, J. A. C., T. G. Burdon, and C. J. Watson. 1996. Differential activation of stats 3 and 5 during mammary gland development. FEBS Lett. 396: 77-80.

Plaut, K., D. E. Bauman, N. Agergaard, and R. M. Akers. 1987. Effect of exogenous prolactin on lactational performance of dairy cows. Dom Anim Endocrinol 4: 279-290.

Qin, B. Y., L. K. Creamer, E. N. Baker, and G. B. Jameson. 1998. 12-bromododecanoic acid binds inside the calyx of bovine β-lactoglobulin. FEBS Lett. 438: 272-278.

Reece, R. P. 1958. Mammary gland development and function. In: J. T. Velardo (ed.) The endocrinology of reproduction. p 213-240. Oxford University Press, New York, NY.

Riddle, O., R. W. Bates, and S. W. Dykshorn. 1933. The preparation, identification and assay of prolactin - a hormone of the anterior pituitary. American Journal of Physiology 105: 191-216.

Rosen, J. M., S. L. Wyszomierski, and D. Hadsell. 1999. Regulation of milk protein gene expression. Annu. Rev. Nutr. 19: 407-436.

Rothschild, M. F., and M. Soller. 1997. Candidate gene analysis to detect traits of economic importance in domestic livestock. Probe 8: 13.

Rudland, P. S., and C. M. Hughes. 1989. Immunological identification of cell types in human mammary gland: Variations in cellular markers are dependent on glandular topography and differentiation. J. Histochem. Cytochem 37: 1087-1100.

Ruff-Jamison, S., K. Chen, and S. Cohen. 1995. Epidermal growth factor induces the tyrosine phosphorylation and nuclear translocation of stat5 in mouse liver. Proc Natl Acad Sci USA 92: 4215-4218.

Saacke, R. G., and C. W. Heald. 1974. Cytological aspects of milk formation and secretion. In: B. L. Larson and V. R. Smith (eds.) Lactation: A comprehensive treatise No. II. p 147-189. Academic Press, New York and London.

Sadler, A. M., C. A. Kiddy, R. E. McCann, and W. A. Mattingly. 1968. Acid production and curd toughness in milks of different s1-casein types. Journal of Dairy Science 51: 28-35.

Sawyer, L. 2003. B-lactoglobulin. In: P. F. Fox and P. L. H. McSweeney (eds.) Advanced dairy chemistry, vol 1 proteins. 3rd ed. Part a. Kluwer Academic/Plenum Publishers, New York, NY.

Schanbacher, F. O., R. E. Goodman, and R. S. Talhouk. 1993. Bovine mammary lactoferrin: Implications from messenger ribonucleic acid (mrna) sequence and regulation contrary to other milk proteins. Journal of Dairy Science 76: 3812-3831.

Schindler, C., and J. E. Darnell. 1995. Transcriptional responses to polypeptide ligands: The jak-stat pathway. Annu. Rev. Biochem. 64: 621-651.

Schmitt-Ney, M., W. Doppler, B. R. K., and B. Groner. 1991. B-casein gene promoter acivity is regulated by the homrone-mediated relief of transcriptional repression and a mammary-gland-specific nuclear factor. Mol. Cell. Biol. 11: 3745-3755.

Schuler, L. A., R. J. Nagel, J. Gao, N. D. Horseman, and M. A. Kessler. 1997. Prolactin receptor heterogeneity in bovine fetal and maternal tissues. Endocrinology 138: 3187-3194.

Schutz, M. M., A. E. Freeman, G. L. Lindberg, and D. C. Beitz. 1993. Effects of maternal lineages grouped by mitochondrial genotypes on milk yield and composition. Journal of Dairy Science 76: 621-629.

Senger, P. L. 2005a. Regulation of reproduction: Nerves, hormones and target tissues Pathways to pregnancy and parturition. p 102-127. Current Conceptions, Inc., Pullman, WA.

Senger, P. L. 2005b. The puerperium and lactation Pathways to pregnancy and parturition. p 326-345. Current Conceptions, Inc, Pullman, WA.

Shaar, C. J., and J. A. Clemens. 1972. Inhibition of lactation and prolactin secretion in rats by ergot alkaloids. Endocrinology 90: 285-288.

Shaw, J. C., A. C. Chung, and I. Budnding. 1955. The effect of pituitary growth hormone and adrenocorticotropic hormone on established lactation. Endocrinology 56: 327-334.

Shiflett, J. S. 2008. Sheep industry economic impact analysis, American Sheep Industry Association.

Simm, G. 1998. Genetic improvement of cattle and sheep. Farming Press, Ipswitch, UK.

Simons, J. P., M. McClenaghan, and A. J. Clark. 1987. Alteration of the quality of milk by expression of sheep β-lactoglobulin in transgenic mice. Nature (Lond.) 328: 530-532.

Smith, J. J., A. V. Capuco, W. E. Beal, and R. M. Akers. 1989. Association of prolactin and insulin receptors with mammogenesis and lobulo-alveolar formation in pregnant ewes. Int J Biochem 21: 73-81.

Smith, J. J., A. V. Capuco, I. H. Mather, and B. K. Vonderhaar. 1993. Ruminants express a prolactin receptor of mr 33,000-36,000 in the mammary gland throughout pregnancy and lactation. J. Endocrinol. 139: 37-49.

Smith, K. L., and F. L. Schanbacher. 1977. Lactoferrin as a factor of resistance to infection of the bovine mammary gland. JAVMA 170: 1224-1227.

Smith, V. G., T. W. Beck, E. M. Convey, and H. A. Tucker. 1974. Bovine serum prolactin, growth hormone, cortisol and milk yield after ergocryptine. Neuroendocrinology 15: 172-181.

Soller, M., and J. S. Beckman. 1982. Restriction fragment length polymorphism and genetic improvement. In: the 2nd World Congress on Genetics Applied to Livestock Production, Madrid. p 396-404.

Soller, M., and A. Genizi. 1978. The efficiency of experimental designs for the detection of linkage between marker locus and a locus affecting a quantitative trait in segregating populations. Biometrics 34: 47-55.

Stehle, J. H., C. von Gall, C. Schomerus, and H. W. Korf. 2001. Of rodents and ungulates and melatonin: Creating a uniform code for darkness by different signaling mechanisms. J. Biol. Rhythms 16: 312-325.

Stein, O., and Y. Stein. 1967. Lipid synthesis, intracellular transport and secretion. Ii electron microscopic radioautorgraphic study of the mouse lactating mammary gland. J. Cell Biol. 34: 251-263.

Stirland, J. A. et al. 2001. Photoperiodic regulation of prolactin gene expression in the syrian hamster by a pars tuberalis derived factor. J. Neuroendrocrinol 13: 147-157.

Streuli, C. 1993. Extracellular matrix and gene expression in mammary epithelium. Seminars Cell Biol. 4: 203-212.

Stricker, P., and F. Grueter. 1929. Recherches experimentales sur les fonctions des lobe anterieur de l'hypophysis: Influence des extraits der lobe anterieur sur l' appareil genital de la lapine et sur la montee laiteuse. Presse. Med. 37: 1268.

Stricter, P., and F. Grueter. 1928. Action du lobe anterior de l'hypophyse sur la montee laiteuse. Comp. Rend. Soc. de Biol. 99: 1978-1980.

Svennersten-Sjauna, K., and K. Olsson. 2005. Endocrinology of milk production. Dom Anim Endocrinol 29: 241 - 258.

Tamarkin, L., C. J. Baird, and O. F. X. Almeida. 1985. Melatonin: A coordinating signal for mammalian reproduction. Science 227: 714-720.

Tanksley, S. D., N. D. Young, A. H. Paterson, and M. W. Bonierbale. 1989. Rflp mapping in plant breeding: New tools for an old science. Bio/technology 7: 257-264.

Teglund, S. et al. 1998. Stat5a and stat5b proteins have essential and nonessential, or redundant, roles in cytokine responses. Cell 93: 841-850.

Teschemascher, H., and G. Koch. 1991. Opioids in the milk. Endocrine Reg. 25: 147-150.

Thatcher, W. W., and H. A. Tucker. 1970. Lactational performance of rats injected with oxytocin, cortisol-21-acetate, prolactin and growth hormone during prolonged lactation. Endocrinology 86: 237-240.

Thomas, D. L. 1996. Opportunities for genetic improvement of dairy sheep in north america Great Lakes Dairy Sheep Symposium. p 47-51, Madison, Wisconsin.

Thomas, D. L. 2008. E-mail correspondence. In: J. S. Shiflett (ed.), University of Wisconsin-Madison, Wisconsin.

Topper, Y. J., and C. S. Freeman. 1980. Multiple hormone interactions in the developmental biology of the mammary gland. Physiological Reviews 60: 1049-1106.

Travers, M. T. et al. 1996. The role of prolactin, and growth hormone in the regulation of casein gene expression and mammary cell survival: Relationships to milk synthesis and secretion. Endocrinology 137: 1530-1539.

Tucker, H. A. 2000. Symposium: Hormonal regulation of milk synthesis: Horomones, mammary growth, and lactation: A 41-year perspective. Journal of Dairy Science 83: 874-884.

Tucker, H. A., and J. Meites. 1965. Induction of lactation in pregnant heifers with 9-fluoroprednisolone acetate. Journal of Dairy Science 48: 403-405.

Turkington, R. W., W. G. Juergens, and Y. J. Topper. 1965. Hormone-dependent synthesis of casein *in vitro*. Biochim. Biophys. Acta 111: 573-576.

Turner, C. W., and A. H. Frank. 1930. The effect of estrus producing hormone on the growth of the mammary gland. Mo. Agric. Expt. Sta. Res. Bull. 145.

Turner, C. W., and W. U. Gardner. 1931. The relation of the anterior pituitary hormones to the development and secretion of the mammary gland. Mo. Agric. Expt. Sta. Res. Bull. 158.

Udy, G. B. et al. 1997. Requirement of stat5b for sexual dimorphism of body growth rates and liver gene expression. Proc Natl Acad Sci USA 94: 7239-7244.

USDA. 2009. Sheep and lamb national statistics. National Agricultural Statistics Service.

Van Berloo, R., and P. Stam. 1998. Marker-assisted selection in autogamous ril populations: A simulation study. Theor. Appl. Genet. 96: 147-154.

Vernon, R. G. 1980. Lipid metabolism in the adipose tissue of ruminant animals. Prog. Lipid Res. 19: 23-106.

Wakao, H., F. Gouilleux, and B. Groner. 1994. Mammary gland factor (mgf) is a novel member of the cytokine regulated transcription factor gene family and confers the prolactin response. EMBO J. 13: 2182-2191.

Wall, E., H. Crawford, S. Ellis, G. Dahl, and T. McFadden. 2006. Mammary response to exogenous prolactin or frequent milking during early lactation in dairy cows. J Dairy Sci 89: 4640 - 4648.

Wall, E., and T. McFadden. 2007. The milk yield response to frequent milking in early lactation of dairy cows is locally regulated. J Dairy Sci 90: 716 - 720.

Wallace, C. R. 1993. Concentration of bovine placental lactogen in dairy and beef cows across gestation. Dom Anim Endocrinol 10: 67-70.

Wang, Q., J. C. Allen, and H. E. Swaisgood. 1999. Binding of lipophilic nutrients to β-lactoglobulin prepared by bioselective adsorption. Journal of Dairy Science 82: 257-264.

Ward, P. P., S. Uribe-Luna, and O. M. Conneeley. 2002. Lactoferrin and host defense. Biochem. Cell Biol. 80: 95-102.

Watson, C. J., K. E. Gordon, M. Robertson, and A. J. Clark. 1991. Interaction of DNA-binding proteins with a milk protein gene promoter in vitro: Identification of a mammary gland-specific factor. Nucleic Acids Research 19: 6603-6610.

Weber, J. L., and P. E. May. 1989. Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. Am. J. Hum. Genet. 44: 388-396.

Weller, J. I., Y. Kashi, and M. Soller. 1990. Power of daughter and granddaughter designs for determining linkage between marker loci and quantitative trait loci in dairy cattle. Journal of Dairy Science 73: 2525-2537.

Wellings, S. R. 1969. Ultrastructural basis of lactogenesis. In: M. Reynolds and S. J. Folley (eds.) Lactogenesis: The initiation of milk secretion at parturition. p 5-25. University of Pennsylvania Press, Philadelphia.

Wellings, S. R., R. A. Cooper, and E. M. Rivera. 1966. Electron microscopy of induced secretion in organ cultures of mammary tissue of the c3h/crgl mouse. J. Natl. Cancer Inst. 36: 657-672.

Wellings, S. R., K. B. DeOme, and D. R. Pitelka. 1960a. Electron microscopy of milk secretion in mammary gland of the c3h/crgl mouse. I. Cytomorphology of the prelactating and lactating gland. J. Natl. Cancer Inst. 25: 393-421.

Wellings, S. R., B. W. Grunbaum, and K. B. DeOme. 1960b. Electron microscopy of milk secretion in the mammary gland of c3h/crgl mouse. Ii. Identification of fat and protein particles in milk and in tissue. J. Natl. Cancer Inst. 25: 423-437.

Wellings, S. R., and J. R. Phelp. 1964. The function of the golgi apparatus in lactating cells of the balb/ccrgl mouse: An electron microscopic and autoradiophic study. Z. Zellforsch. Mikrosk. Anat. 61: 871-882.

Werf, J. 1999. An overview of animal breeding programs. In: B. Kinghorn, J. Werf and M. Ryan (eds.) Animal breeding: Use of new technologies. The Post Graduate Foundation in Veterinarian Science of the University of Sydney, Sydney.

Whitelaw, C. B. A., S. Harris, M. McClenaghan, J. P. Simons, and A. J. Clark. 1992. Position-independent expression of the ovine β-lactoglobulin gene in transgenic mice. Biochem. J. 286: 31-39.

Wilde, C. J., C. V. P. Addey, P. Li, and D. G. Fernig. 1997. Programmed cell death in bovine mammary tissue during lactation and involution. Exp. Physiol. 82: 943-953.

Wilde, C. J., C. H. Knight, and D. J. Flint. 1999. Control of milk secretion and apoptosis during mammary involution. Journal of Mammary Gland Biology and Neoplasia 4: 129-136.

Williams, L. et al. 1997. Melatonin receptors in the brain and pituitary gland of hypothalamo-pituitary disconnected soay rams. J. Neuroendrocrinol 9: 639-643.

Willis, I. M., A. F. Stewart, A. Caputo, A. R. Thompson, and A. G. Mackinlay. 1982. Construction and identification by partial nucleotide sequence analysis of bovine casein and beta-lactoglobulin cdna clones. DNA (Mary Ann Liebert, Inc.) 1: 375-386.

Wood, T. J. et al. 1995. Mediation of growth hormone-dependent transcriptional activation by mammary gland factor/stat 5. J Biol Chem 270: 9448-9453.

Zhang, Q. et al. 1998. Mapping quantitative trait loci for milk production and health of dairy cattle in a large outbred pedigree. Genetics 149: 1959-1973.

CHAPTER II

THE EFFECT OF *Rsa1* POLYMORPHISM IN BETA-LACTOGLOBULIN GENE AND PROLACTIN GENE ON MILK PRODUCTION IN EAST FRIESIAN SHEEP

INTRODUCTION

Milk production is an economically important trait to the sheep industry. In recent years there has been an increase in demand for sheep milk cheese within the United States and there is such a large domestic market for dairy sheep cheese, that the current sheep industry cannot meet the demand. In addition, sheep numbers have been decreasing (USDA, 2009). Therefore there is room for expansion in milk production within the sheep industry.

Currently in the sheep industry, there are selection programs developed to increase milk production; however, milk production is a quantitative trait, i.e. influenced by genetics and the environment, which makes the selection process more difficult. Several genes control milk production; there is no one single gene that controls milk production, therefore it is difficult to see the effect of one single gene. In addition to genetics, the environment (i.e. feed, water, disease) influences milk production. A well-nourished and healthy ewe is going to produce more milk than an under-nourished and sick ewe. Another complication in the selection process is that milk production is measured repeatedly. Milk is collected over a 250-day lactation period, and the process is repeated every year that the ewe produces lambs; milk production is not a one-time measurement in the course of the ewe's lifetime, such as birth weight.

Additional problems stem from the phenotypic-based evaluation of the current selection programs. The programs lack accuracy for three major reasons. First off, milk production is a sex-limited trait; it can only be measured in ewes. In order to make a selection on rams, the daughters' records must be collected. This is a problem because rams have the most impact on the trait due to their higher reproductive rates compared to ewes. Secondly, ewes must reach sexual maturity before milk production records can be collected; this requires a lot of time. And lastly, milk production is a repeated measurement taken over a 250-day lactation.

To help enhance and improve the traditional selection of milk production, genetic selection tools can be utilized. While advances in nutritional and management practices have been very successful in increasing milk production, especially in dairy cattle, more than half of the increase in milk production has been due to improvements in genetics (Dekkers and Hospital, 2002). Milk production is a quantitative trait; therefore the multiple genes that influence milk production can be found on a quantitative trait locus (QTL). QTLs can be characterized via two methods: genome-wide scanning or candidate genes. Genome-wide scans are best for mapping the locus of a trait within a specialized population (Dekkers and Hospital, 2002) such as the dairy cattle industry where pedigree information is readily available; however, the dairy sheep industry has not yet reached the same level of organization as the dairy cattle industry and genome-wide scanning is not yet an option to characterize QTLs in dairy sheep. The candidate-gene approach is the best option in dairy sheep to characterize QTLs. In the candidate-gene approach, typically a known mapped gene is chosen due to a possible function in the trait of interest and due to the possibility that the known gene may be linked to the trait of interest in another species (Simm, 1998).

Beta-lactoglobulin was selected as one of the candidate genes for this study for several reasons. Beta-lactoglobulin is one of the major whey proteins found in ruminant milk (Perez and Calvo, 1995). The gene encoding for beta-lactoglobulin has been found to be highly and specifically expressed in the mammary gland during lactation (Mercier and Vilotte, 1993). The regulation of beta-lactoglobulin is controlled by prolactin, a hormone known to stimulate milk production (Burdon et al., 1994; Demmer et al., 1995). And finally, associations between beta-lactoglobulin variants and milk protein composition and yield have been found in previous studies utilizing bovine samples (Lunden et al., 1997; McLean et al., 1984).

Prolactin was selected as the other candidate gene for this study for several reasons as well. Prolactin was first discovered and isolated due to its ability to stimulate mammary growth and milk secretion in rabbits (Stricter and Grueter, 1928). In several species, including cattle and goats, prolactin hormone has been shown to stimulate milk production (Burdon et al., 1994; Cowie, 1969; Demmer et al., 1995; Kumaresan et al., 1966; Tucker, 2000). In mice, genetic verification that prolactin controls mammary gland development and function were done using gene deletion experiments (Horseman et al., 1997; Ormandy et al., 1997). In mice, when both the prolactin gene and prolactin receptor are deleted, the mice are infertile (Ormandy et al., 1997). The prolactin receptor can be spliced into a long and a short form (Boutin et al., 1988). The long form of prolactin receptor mRNA has increased expression in bovine mammary tissue during the dry period of lactation which may result in greater mammary function (Auchtung et al., 2005). Prolactin and prolactin receptor have an inverse relationship in the mammary gland; prolactin binding increases in the mammary gland as parturition approaches, but prolactin expression is down-regulated in mammary tissue by pregnancy (Kazmer et al.,

1986; Smith et al., 1993). In addition, a HaeIII PCR-RFLP was identified in the ovine prolactin gene (Vincent and Rothschild, 1997) and was observed to have a Mendelian inheritance pattern in nine two-generation sheep families of the AgResearch International Mapping Flock (Crawford et al., 1995).

The objective of this study was to find molecular markers linked to genes controlling milk production. Through the use of marker-assisted selection, traditional selection can be enhanced; marker-assisted selection increases the accuracy of selection by providing more information on an animal's genetic merit, or breeding value, allowing for genetically superior animals to be identified early in life, even before the animal is born, and thereby greatly reducing the generation interval and increasing the rate of genetic progress per year in a breeding program.

MATERIALS AND METHODS

ANIMALS

All animal experiments were conducted in accordance with principles and guidelines outlined in Guidelines for the Care and Use of Agricultural Animals in Agricultural Research and Teaching. The sample population for this study consisted of 676 East Friesian ewes from Old Chatham Sheepherding Company (OCSHC), the largest dairy sheep farm in the United States. OCSHC is a 600 acre farm located in Old Chatham, New York with over 1,000 sheep that are either purebred East Friesian or East Friesians crossed with Dorset/Finns. The sheep were fed a mixture of fresh grasses and whole grains without the addition of hormone supplementation or the use of antibiotics.

Milk production records were collected by OCSHC from all ewes, allowing for an estimated breeding value (EBV) to be determined; all EBVs were calculated by Cornell

University. Ewes were milked twice a day by machine, supervised by two experienced handlers, for the 250-day milk period.

DNA EXTRACTION

Blood (10 mL) was collected into a heparinized tube from the jugular vein of each sheep and stored at -20°C. Genomic DNA was extracted from the blood samples utilizing FlexiGene DNA Kits (Qiagen, Valencia, CA).

Cell Lysis

A 1.5 μL micro-centrifuge tube was labeled for each sample and then 750 μL of Buffer FG1 was added to each tube. Three hundred μL of whole thawed ovine blood was added to each labeled tube with Buffer FG1 (a lysis buffer), and then gently shaken. Tubes were centrifuged for 30 seconds at 14,000g. The supernatant was poured off while the blood pellet was retained in the tube.

DNA Isolation

One hundred fifty μL of Qiagen protease/Buffer FG2 was added to each tube, then immediately vortexed until the pellet was dissolved in the solution. Samples were then placed in a heat bath of 65°C for 5 minutes. Tubes were removed from the heat bath and 150 μL of isopropanol alcohol was added to each tube. Samples were gently rocked for 2 minutes and then centrifuged for 2 minutes at 9,000g. The supernatant was poured off, retaining the pellet in the tube.

Pellet Re-suspension

One hundred fifty μL of absolute ethanol was added to each sample and then immediately vortexed for 5 seconds. Samples were then centrifuged for 2 minutes at 9,000g. The supernatant was poured off, and then samples were re-centrifuged for 5

seconds at full speed. Any excess ethanol was removed and 200μL of Buffer FG3 (a hydration buffer) was added to each tube, followed by vortexing for 10 seconds. Samples were then incubated in a water bath at 65°C for 10 minutes, followed by brief vortexing. The previous step was repeated every 10 minutes until the pellet was completely dissolved. DNA samples were stored at -20°C.

The quality, purity, and quantity of the genomic DNA was measured using a NanoDrop spectophotometer (Thermo-Scientific Wilmington, DE). The machine was standardized with water and Buffer FG3 before measurements were taken. A volume of 1.5 μL of the suspended DNA was loaded onto the sample pedestal for measurement, and the concentration of the DNA was calculated by multiplying the dilution factor (0 in this case) by the optical density (OD) at 260nm (A_{260}) and using the relationship that A_{260} of 1.0 = 50μg/mL of pure DNA.

PRIMER DESIGN

Primers for β-lactoglobulin were obtained from the paper by Dario et al. (2007). The primers, designated BLG3-F and BLG3-R (Table 4), produce a 120 bp fragment from Intron 1 and Exon 2 of the ovine β-lactoglobulin gene.

Primers for prolactin were based upon the paper by Vincent and Rothschild (1997). The paper's primers, designated PRL-F and PRL-R (Table 4), were designed to produce a 2.5 Kb product.

Primer	Sequence (5’ to 3’)	Size (bp)	Product Size
BLG3-F	CAACTCAAGGTGCCTCTCCA	20	120 bp
BLG3-R	CTTCAGCTCCTCCACGTACA	20	120 bp
PRL-F	ACCTCTCTTCGGAAATGTTCA	21	2.5 Kb
PRL-R	CTGTTGGGCTTGCTCTTTGTC	21	2.5 Kb

Table 4. Primer sequences for beta-lactoglobulin and prolactin.

POLYMERASE CHAIN REACTION

In order to detect the polymorphism in the perspective genes, the DNA sequence of interest was amplified via polymerase chain reaction (PCR). The total volume for each PCR reaction was measured to be 10 μL. Each reaction contained 1 μL of 10X PCR buffer, 0.6 μL of 50mM $MgCl_2$, 1 μL of 2mM dNTP, 1 μL of 5uM primer mix (forward and reverse primer were at 5 μM each), 0.05 μL of Taq Polymerase (5U/μL), 1 μL of DNA (approximate concentration of 250 ng/μL), and enough water to bring the total reaction volume to 10 μL.

The thermal cycling conditions for β-lactoglobulin were 95°C for 10 minutes, followed by 34 additional cycles of 93°C for 15 seconds, 60°C for 15 seconds, and 72°C for 30 seconds, followed by a final step of 72°C for 10 minutes.

The thermal cycling conditions for prolactin were 92°C for 2 minutes, followed by 34 additional cycles of 92°C for 45 seconds, 56°C for 45 seconds, and 72°C for 3 minutes, followed by a final step of 72°C for 7 minutes.

GENOTYPING

Genotyping of prolactin polymorphism, including PRC amplification was performed by Justin Buchanan (Oklahoma State University). To determine the polymorphisms, PCR amplicons were digested with restriction enzymes. The total volume for each digestion reaction was 10 μL. Each β-lactoglobulin reaction contained 8 μL of PCR product, 1 μL of *RsaI* restriction enzyme (10,000 U/ml) and 1 μL of Reaction 1 buffer. Each prolactin reaction contained 8 μL of PCR product, 1 μL of *HaeIII* restriction enzyme (10,000 U/ml), and 1 μL of Reaction 2 buffer. Each digestion ran for 2 hours at 37°C. The digested amplicons were then separated on 8% polyacrylamide gels in TBE buffer. Beta-lactoglobulin samples were run at 250 volts for 1 hour 40 minutes against a 100 bp DNA ladder. Prolactin samples were run at 250 volts for 3 hours 40 minutes against a 1 Kb DNA ladder. The gels were then stained in an ethidium bromide bath (1.0 μg/L) for 10 minutes. De-staining was then carried out in distilled H_2O for an additional 10 minutes. The gels were visualized via UV illumination using an Alpha-Innotech AlphaImager system (San Leandro, CA).

STATISTICAL ANALYSIS

Estimation of breeding values for all animals from OCSHC was performed by Cornell University using a test-day animal model and included monthly test day production collected from the ewes in the flock from January 1st, 1998 to May 31st, 2007. The (co)variance components and genetic parameters for test day records were estimated using an autoregressive test day model and a genetic evaluation for ewes and rams in the flock were performed using all sources of information in an animal model using BLUP methodology. The following model was used to describe the data:

$Y_{ijkmnpqr} = YM_i + AGE_j + DIM_k(L_m) + A_n + LTE_p + STE_q + E_{ijklmnpqr}$ where Y is the test day observation, YM is the fixed effect of year-month, AGE is the fixed effect of the age of the ewe at lambing, DIM(L) is the fixed effect of the number of days in milking nested within the lactation, A is the random effect of the animal, LTE is the random long-term environmental effects that account for the autocorrelations generated by the ewe across lactations, STE is the random short-term environmental effects that account for the autocorrelations caused by the ewe within each lactation, and E is the random residual effect that is assumed with a normal distribution.

Once the gels had been visualized, genotypes could be called for each gene for each animal, allowing for the calculation of gene and genotypic frequencies within the sample population. A chi-square test was performed to determine if the sample population followed the hypothesis that the genes are under simple dominance with two alleles; in β-lactoglobulin, A is the dominant allele for high milk production and B is the recessive allele for low milk production, while in prolactin, A is the dominant allele for high milk production and B is the recessive allele for low milk production. If the chi-square test is not significant, then the sample population is within Hardy-Weinberg equilibrium.

The GLM procedure of SAS was used to evaluate the effect of the restriction enzyme polymorphisms on the EBV for milk production. Least Squares Means were used to separate the means, and differences were declared significant at $P < 0.05$. One degree of freedom orthogonal contrasts were constructed to compare the genotypes (AA & AB vs. BB and AA vs. AB).

RESULTS AND DISCUSSION

GENOTYPING

After the PCR amplicons were visualized on the gels, restriction sites were found in both genes. Two restriction sites were found in the β-lactoglobulin gene, however they are not in the coding region of the gene and do not cause any changes in the amino acid sequence, and are therefore not the causative mutation. The *RsaI* restriction enzyme recognizes a cut site of GTAC, cutting between the T and A nucleotides. The first restriction site found produces fragments that are 17 bp and 103 bp in length and is always present, and therefore not polymorphic; however, the second site is polymorphic. The second site cuts the 103 bp fragment from the first restriction site into two smaller fragments that are 37 bp and 66 bp in length. The A allele contains the polymorphic site, while the B allele does not (Figure 5). Alleles were determined based upon size differentiation (bp) of β-lactoglobulin (Figure 6). All three genotypes, AA, AB, and BB were detected in the East Friesian ewes. Gene frequencies were 69% for the A allele and 31% for the B allele. The genotypic frequencies of the population were 43% AA, 52% AB, and 5% BB. This population was determined to not be in Hardy-Weinberg equilibrium for the beta-lactoglobulin gene. The genotypic frequencies detected were similar to those found in Pag and Valle del Belice sheep(Cubric-Curik et al., 2002) and in East Friesian sheep from Saxony, Germany (Wessels et al., 2004), which were also not in Hardy-Weinberg equilibrium.

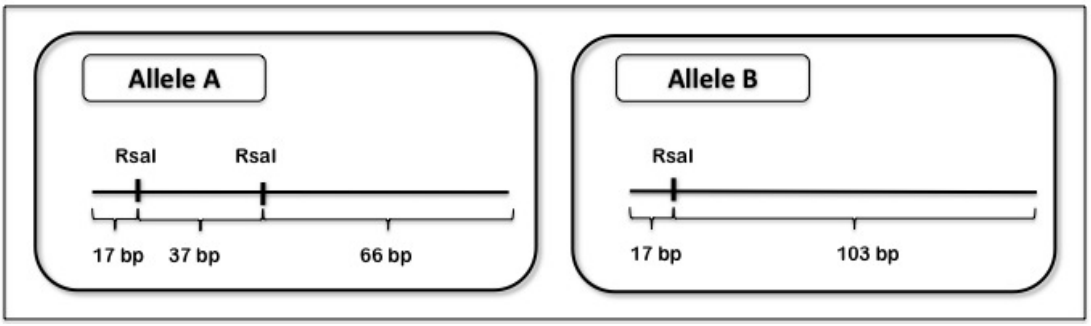

Figure 5. Restriction site maps for BLG A and B alleles.
Allele A contains both RsaI restriction sites; allele B contains only the first restriction site.

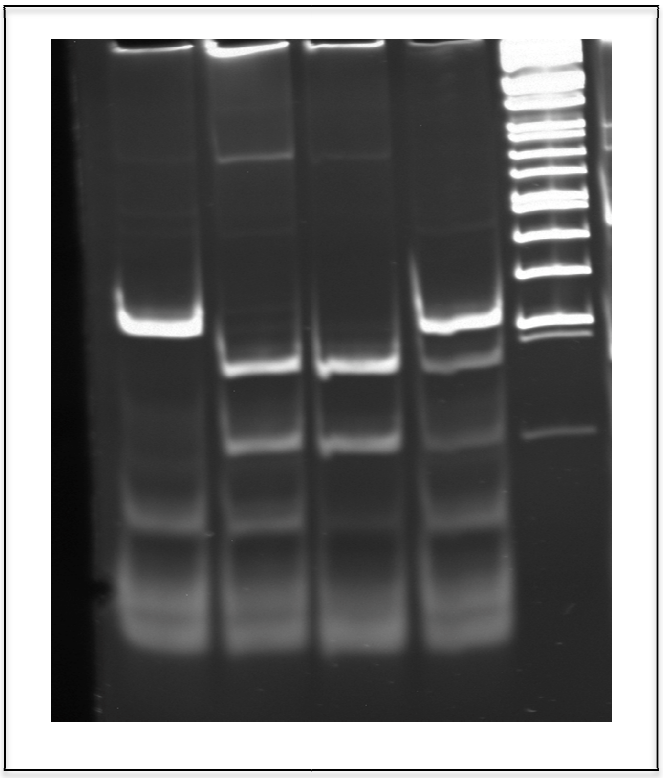

Figure 6. Representative gel showing the BLG AA, AB, and BB genotypes.
The first lane shows the BB genotype. The second and third lanes show the AA genotype. The fourth lane shows the AB genotype. The fifth lane is a 100 bp DNA standard ladder.

Three restriction sites were found in prolactin. The *HaeIII* restriction enzyme recognizes a cut site of GGCC, cutting between the G and C nucleotides. Two of the restriction sites are always present and therefore not polymorphic; they are the first and

third cut sites and produce fragments that are approximately 1.4 Kb, 360 bp, and 150 bp in length. However, the second cut site is polymorphic; individuals with the A allele have a 530 bp fragment, while individuals with the B allele have a 510 bp fragment (Figure 7). Alleles were determined based upon size differentiation (bp) of prolactin (Figure 8). All three genotypes, AA, AB, and BB were detected in the East Friesian ewes. Gene frequencies were 13% for the A allele and 87% for the B allele. The genotypic frequencies of the population were 1% AA, 23% AB, and 76% BB. This population was determined to be in Hardy-Weinberg equilibrium for the prolactin gene.

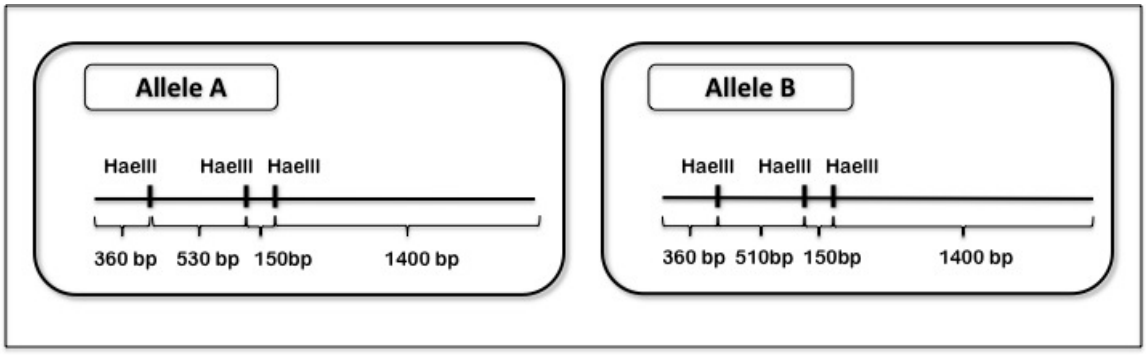

Figure 7. Restriction site maps for PRL A and B alleles.
Allele A contains all three restriction sites and produces a 530 bp fragment between sites 1 and 2; allele B also contains all three restriction sites, but produces a 510 bp fragment between sites 1 and 2.

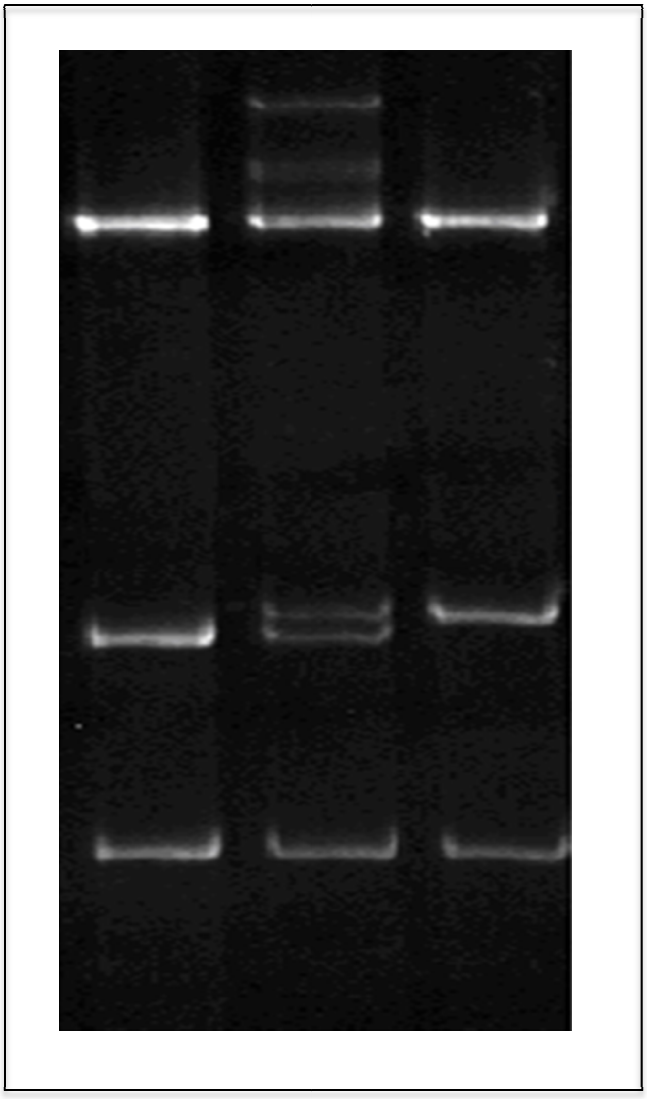

Figure 8. Representative gel showing the PRL AA, AB, and BB genotypes. The first lane shows the BB genotype. The second lane shows the AB genotype. The third lane shows the AA genotype.

GENETIC EFFECTS ON MILK PRODUCTION

Beta-Lactoglobulin

The orthogonal contrasts found no statistical difference between animals carrying one A allele versus two A alleles, and no statistical difference between animals carrying at least one A allele versus no A allele. However, the P-value for the orthogonal contrast comparing animals carrying at least one A allele versus no A alleles was lower,

indicating a trend that animals with at least one A allele have a higher EBV for milk production.

The least-squares means of EBV of milk production for AA, AB, and AB genotypes are presented in Table 5. Milk production did not differ significantly between the genotypes; however, the BB genotype tends to produce a lower mean. In fact, if the BB mean is subtracted from the AA and AB means, it can be determined that individuals with at least one A allele will produce 40g of milk per day more than individuals with no A allele; this further supports the results from the orthogonal contrasts.

Genotype	LSMean	P-Value
AA	112.16	0.99
AB	112.25	0.99
BB	72.53	0.29

Table 5. Beta-lactoglobulin EBV LSMeans.

While these results point towards the use of beta-lactoglobulin as a molecular marker, they also further add to the confusion surrounding the role of beta-lactoglobulin. Several studies have presented opposing results as to which allele produces more milk. One study presented a trend for the BB genotype in Massese ewes to have higher milk production (Rampilli et al., 1997). In Sardinian ewes, the BB genotype was also identified to have higher milk yields when compared to AB and AA genotypes (Bolla et al., 1989). In addition, in Serra da Estrela ewes, the AA genotype had the lowest milk yields, with no significant difference in milk yields between AB and BB genotypes (Ramos et al., 2009). The findings with Serra da Estrela ewes was also similar to

findings in Merino ewes where again the AB and BB genotypes produced higher milk yields (Ramos et al., 2009).

While the previous studies have presented results that the BB genotype have higher milk yield, other studies have presented results that the AA genotype have higher milk yields. In Valle del Belice ewes, the AA genotype was associated with higher milk yield despite the greater frequency of the B allele (65%) (Giaccone et al., 2000). Another study that utilized East Friesian ewes also showed that ewes with AA genotype had the highest milk yield in the first lactation, while BB genotype ewes had the highest milk yield in the following lactations (Schmoll et al., 1999). In Sarda ewes, no significant differences could be detected amongst genotypes; however, some trends could be discerned such as the AA genotype always showed the highest values for milk yield and the AB genotype was always the intermediate between the AA and BB genotypes (Pietrola et al., 2000). In Czech Fleckvieh cattle, the highest breeding values for milk production were associated with the AA genotype, however this was not significant (Kučerová et al., 2006). Additional studies have shown significant relationships between beta-lactoglobulin polymorphisms and milk production traits in sheep, such as fat and protein content, but milk yield was not examined (Dario et al., 2007; Fraghi et al., 1996; Herget et al., 1995; Nudda et al., 2000).

Prolactin

In the analysis of prolactin, 629 ewes were evaluated. The GLM procedure of SAS found that genotype was statistically significant (P=0.0006) when compared to EBV and weighted for accuracy. The orthogonal contrasts found a trend, but no statistical difference between individuals carrying at least one A allele versus no A alleles, and no trend or statistical difference between individuals carrying two A alleles versus one A

allele. However, the least squares means for the effect of genotype found a statistical significance (P = 0.0001) between individuals with genotype AB and BB. Based on the EBV least square means (Table 6), individuals with AB genotype will produce 76.3g of milk per day more than individuals with BB genotype and overall the BB genotype will produce less milk than AA and AB genotypes.

Genotype	LSMeans	P-Value
AA	153.21	0.86
AB	167.45	0.86
BB	91.17	0.0001

Table 6. Prolactin EBV LSMeans.
EBV has been weighted for accuracy.

Thus far, it appears that this is the second study to describe the influence of prolactin genotypes on milk production in dairy sheep. The first study investigated the effect of prolactin genotype on milk production traits, such as milk yield, fat content, and protein content, in Serra da Estrela and Merino sheep. Serra da Estrela are the most important dairy sheep breed in Portugal, and Merino are the most common sheep in Portugal due to their use for lamb production, although some Merino flocks are milked after weaning lambs (Ramos et al., 2009). Prolactin was found to have a significant effect on all traits analyzed in the Serra da Estrela breed, but not in the Merino breed. The authors thought that this could be due to the difference in the number of animals from each breed genotyped; 1006 Serra da Estrela ewes were genotyped while 782 Merinos were genotyped. In both breeds, the A allele occurred more frequently than the B allele which is the reverse of the results presented in this study. In addition, Serra da

Estrela ewes carrying the AA genotype had lower milk yields when compared to ewes with AB and BB genotypes (Ramos et al., 2009), which again is the opposite of the results presented in our study using East Friesian ewes. Both Serra da Estrela and Merino ewes were not in Hardy-Weinberg equilibrium. But, due to the lack of significance found in the Merino breed, it is possible that the prolactin polymorphism does not have a direct effect on milk production and is rather in linkage disequilibrium with the causative mutation (Ramos et al., 2009). Both our study and the Portugal study presented information that the *HaeIII* restriction enzyme was used to identify the polymorphism, however we found the combination of A and B allele to be the best milk producer and the study by Ramos et al. (2009) found the BB genotype to be the best milk producer. Part of this difference could be due to the different models used to explain fixed and random effects. In addition, samples for the Serra da Estrela breed was collected from seven different farms, whereas ours were collected from one farm; by collecting from different farms, a greater genetic diversity should be expected among the animals since each farm has there own unique set of criteria for selection of rams and milking ewes and there are more rams being utilized as sires.

While our results differ from the dairy sheep study, our results are similar to other studies done in cattle which are much more numerous than sheep studies. Instead of using *HaeIII* restriction enzyme, the authors of cattle studies primarily used *RsaI* to study polymorphisms in the prolactin gene. In Polish Friesian cows, the *RsaI* polymorphism was studied in three different lactations for its effect on milk production traits, and in the first lactation both AA and AB cows had higher milk yields while in the seconds lactation, AB cows produced more milk than AA cows (Dybus, 2002); in the third lactation there was not a significant difference in milk yields amongst the genotypes. In

Holstein dairy cows, an *RsaI* polymorphism also revealed that cows with AA genotype had greater milk yield than BB genotype (Chung et al., 1996). In Brown Swiss cows, no statistically significant differences between genotypes on milk yield could be determined (Chrenek et al., 1999). In fact, in Russian Red Pied cattle, the *RsaI* prolactin polymorphism indicated that the BB genotype had a higher milk yield than AA or AB genotypes (Alipanah et al., 2007). A negative association with fat content and milk production was observed for the BB genotype in Yaroslvl cattle (Khatami et al., 2005); the proportion of cows producing milk with less than 4.5% percent fat was 19-20% higher in BB genotypes than AA and AB genotypes, and the proportion of cows that were high milk producers (more than 6000 kg) was 9-10% lower in BB genotypes than AA and AB genotypes (Khatami et al., 2005).

CONCLUSIONS

A majority of the research on beta-lactoglobulin has been over the effects of BLG polymorphisms on milk composition; little research has been done over its effects on milk production. The research that has been done has found conflicting results and this could be due to differences in the designs of the studies, including breeds, population size, and restriction sites. The population in this study has been selected intensively for high milk yield and, if the BB genotype is associated with lower milk yield, the low occurrence of the BB genotype could be a consequence of this selection. Additional genotyping in a different population may be necessary to prove an association between the beta-lactoglobulin gene and milk production in sheep.

Similarly to beta-lactoglobulin, the majority of studies in prolactin have focused on the effect of PRL on milk composition or the effect of exogenous prolactin on milk

production. In addition to this, the majority of these studies have been performed on cattle instead of sheep. However, in this study an association between prolactin gene and milk production has been found. This indicates that prolactin can be used as a molecular marker for increased milk production to further assist producers in selecting animals with high milk yields. While selection should not be based entirely upon one marker, a panel of markers associated with milk production that include prolactin would be the best genetic tool for producers.

REFERENCES

Alipanah, M., L. Kalashnikova, and G. Rodionov. 2007. Association of prolactin gene variants with milk production traits in russian red pied cattle. Iranian Journal of Biotechnology 5: 158-161.

Auchtung, T. L., A. G. Rius, P. E. Kendall, T. B. McFadden, and G. E. Dahl. 2005. Effects of photoperiod during the dry period on prolactin, prolactin receptor, and milk production of dairy cows. Journal of Dairy Science 88: 121-127.

Bolla, P. et al. 1989. Milk protein markers and production in sheep. Animal Genetics 20: 78-79.

Boutin, J. M. et al. 1988. Cloning and expression of the rat prolactin receptor, a member of the growth hormone/prolactin receptor gene family. Cell 53: 69-77.

Burdon, T. G., K. A. Maitland, A. J. Clark, R. Wallace, and C. J. Watson. 1994. Regulation of the sheep beta-lactoglobulin gene by lactogenic hormones is mediated by a transcription factor that binds an interferon-gamma activation site-related element. Mol. Endocrinol. 8: 1528-1536.

Chrenek, P. et al. 1999. Genotypes of bgh and bprl genes in relationship to milk production. In: the 50th Annual Meeting of the EAAP, Zuerich. p 40.

Chung, E. R., T. J. Rhim, and S. K. Han. 1996. Associations between pcr-rflp markers of growth hormone and prolactin genes and production traits in dairy cattle. Korean Journal of Animal Science 38: 321-336.

Cowie, A. T. 1969. Variations in the yield and composition of the milk during lactation in the rabbit and the galactopoietic effect of prolactin. J. Endocrinol. 44: 437-450.

Crawford, A. M. et al. 1995. An autosomal genetic linkage map of the sheep genome. Genetics 140: 703-724.

Cubric-Curik, V., M. Feligini, J. Lukac-Havranek, I. Curik, and G. Enne. 2002. Genetic polymorphism of b-lactoglobulin in native sheep from the island of pag. Food Technol. Biotechnol. 40: 75-78.

Dario, C., D. Carnicella, M. Dario, and G. Bufano. 2007. Genetic polymorphism of b-lactoglobulin gene and effect on milk composition in leccese sheep. Small Ruminant Research 74: 270-273.

Dekkers, J. C. M., and F. Hospital. 2002. The use of molecular genetics in the improvement of agricultural population. Nature Reviews Genetics 3: 22-32.

Demmer, J., T. G. Burdon, J. Djiane, C. J. Watson, and A. J. Clark. 1995. The proximal milk protein binding factor binding site is required for the prolactin responsiveness fo the sheep beta-lactoglobulin promoter in chinese hamster ovary cells. Mol. Cell Endocrinol. 107: 113-121.

Dybus, A. 2002. Associations of growth hormone (gh) and prolactin (prl) genes polymorphisms with milk production traits in polish black-and-white cattle. Animal Science Papers and Reports 20: 203-212.

Fraghi, A., A. Carta, F. Pilla, S. R. Sanna, and A. Cappio-Borlino. 1996. Beta-lactoglobulin polymorphism in sarda dairy sheep: Influence on milk yield. In: 47th Annual Meeting of European Association for Animal Production, Lillehammer, Norway. p 42.

Giaccone, P. et al. 2000. Effect of β-lactoglobulin polymorphism on milk-related traits of dairy ewes analysed by a repeated measure design. Journal of Dairy Research 67: 443-448.

Herget, I., F. Schnoll, and K. Schellander. 1995. Beta-lactoglobulin polymorphism in east friesian milk sheep. In: 47th Annual Meeting of the EAAP, 21 Wageningen Pers, Wageningen, NL

Horseman, N. D. et al. 1997. Defective mammopoiesis, but normal hematopoiesis, in mice with a targeted disruption of the prolactin gene. EMBO J. 16: 6926-6935.

Kazmer, G. W., M. A. Barnes, R. M. Akers, and W. D. Whittier. 1986. Lactogenic hormone recptors in mammary membrane preparations from prepartum and 60 and 180 day postpartum holstein cattle. J. Endocrinol. 109: 175-180.

Khatami, S. R., O. E. Lazebny, V. F. Maksimenko, and G. E. Sulimova. 2005. Association of DNA polymorphisms of the growth hormone and prolactin genes with milk productivity in yaroslavl and black-and-white cattle. Russian Journal of Genetics 41: 167-173.

Kučerová, J. et al. 2006. Milk protein genes *csn1s1, csn2, csn3, lgb* and their relation to genetic values of milk production parameters in czech fleckvieh. Czech Journal of Animal Science 6: 241-247.

Kumaresan, P., R. R. Anderson, and C. W. Turner. 1966. Effect of graded levels of lactogenic hormone upon mammary gland growth and lactation. Proc. Soc. Exp. Biol. Med. 123: 581-584.

Lunden, A., M. Nilsson, and L. Janson. 1997. Marked effect of beta-lactoglobulin polymorphism on the ratio of casein to total protein in milk. Journal of Dairy Science 80: 2996-3005.

McLean, D. M., E. R. Graham, R. W. Ponzoni, and H. A. McKenzie. 1984. Effects of milk protein genetic variants on milk yield and composition. Journal of Dairy Research 51: 531-546.

Mercier, J. C., and J. L. Vilotte. 1993. Structure and function of milk protein genes. Journal of Dairy Science 76: 3079-3098.

Nudda, A., M. Feligini, G. Battacone, R. Campus, and G. Pulina. 2000. Effects of beta-lactoglobulin genotypes and parity on milk production and coagulation properties in sarda dairy ewes. Italian J. Anim. Sci. 26: 137-143.

Ormandy, C. J. et al. 1997. Null mutation of the prolactin receptor gene produces multiple reproductive defects in the mouse. Genes Dev. 11: 167-178.

Perez, M. D., and M. Calvo. 1995. Interaction of beta-lactoglobulin with retinol and farry acids and its role as a possible biological function for this protein: A review. Journal of Dairy Science 78: 978-988.

Pietrola, E., A. Carta, A. Fraghi, G. Piredda, and F. Pilla. 2000. Effect of β-lactoglobulin locus on milk yield in sarda ewes. Zoot. Nutr. Anim. 26: 131-135.

Ramos, A. M. et al. 2009. Candidate genes for milk production traits in portuguese dairy sheep. Small Ruminant Research 82: 117-121.

Rampilli, M., F. Cecchi, L. Giuliotti, and T. M. P. Cattaneo. 1997. The influence of β-lactoglobulin genetic polymorphism on protein distribution and coagulation properties in ewe's milk from massese breed. In: IDF Seminar, Palmerston North, New Zealand. p 311-315.

Schmoll, F. et al. 1999. Associations of beta-lactoglobulin variants with milk production, milk composition and reproduction performance in milk sheep. Wiener Tierarztliche Monatsschrift 86: 57-60.

Simm, G. 1998. Genetic improvement of cattle and sheep. Farming Press, Ipswitch, UK.

Smith, J. J., A. V. Capuco, I. H. Mather, and B. K. Vonderhaar. 1993. Ruminants express a prolactin receptor of mr 33,000-36,000 in the mammary gland throughout pregnancy and lactation. J. Endocrinol. 139: 37-49.

Stricter, P., and F. Grueter. 1928. Action du lobe anterior de l'hypophyse sur la montee laiteuse. Comp. Rend. Soc. de Biol. 99: 1978-1980.

Tucker, H. A. 2000. Symposium: Hormonal regulation of milk synthesis: Horomones, mammary growth, and lactation: A 41-year perspective. Journal of Dairy Science 83: 874-884.

USDA. 2009. Sheep and lamb national statistics. National Agricultural Statistics Service.

Vincent, A. L., and M. F. Rothschild. 1997. Rapid communication: A restriction fragment length polymorphism in the ovine prolactin gene. Journal of Animal Science 75: 1686.

Wessels, G., H. Hamann, G. Erhardt, and O. Distl. 2004. Genotype effects of milk protein polymorphisms on milk production in east friesian dairy sheep. Berliner Und Munchener tierarztliche Wochenschrift 117: 414-419.

VITA

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Scope and Method of Study: Milk production is an economically important trait for the sheep industry, however the trait is moderately heritable, expressed relatively late in life, and expressed in only one sex. The efficiency of selection for milk production in sheep would be improved by the identification of informative genetic markers. A single nucleotide polymorphism in the beta-lactoglobulin gene (BLG) and prolactin gene (PRL) were investigated with respect to the genetic merit (EBV) for milk production in East Friesian sheep.

Findings and Conclusions: A trend was found between the dominant allele of beta-lactoglobulin and increased milk production; however, this trend was not significant, possibly due to the low occurrence of the homozygous recessive individuals in the population. Ewes that carried the dominant allele for the prolactin gene produced more milk than ewes that were homozygous recessive. The results show an association between the PRL gene and milk production in sheep suggesting that this polymorphism could be used in a marker assisted selection program. However, additional genotyping in a different population may be necessary to prove an association between the BLG gene and milk production in sheep.

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