EFFECT OF SUBCLINICAL MASTITIS AND STAGE OF LACTATION ON SOMATIC CELL COUNT, MILK COMPOSITION AND PLASMIN ACTIVITY IN GOAT MILK

By

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CHAPTER I

INTRODUCTION

Goat milk, an alternate dairy source, has become more and more important at the present date. Varieties of goat milk products such as goat cheese have been popular around the world. However, goats with subclinical mastitis produce milk of lower yield and quality, negatively affecting the ultimate quality and yield of goat cheese. Somatic cell count (**SCC**), as one of the most important indexes of the severity of subclinical mastitis, increases with the advancing stage of lactation. Based on the fact that subclinical mastitis is hard to detect even though it is related to high SCC, a better way of monitoring the health status of mammary is needed by studying the relationship between SCC, stage of lactation, and milk composition. The objective of this study was to investigate the impact of SCC and stage of lactation on goat milk composition, providing information for better detecting and controlling subclinical mastitis by means of determining milk SCC and plasmin (**PL)** activity during stages of lactation.

CHAPTER II

REVIEW OF LITERATURE

GOAT INDUSTRY

Due to the easy access to milk and meat, goats were one of the first animal species that were domesticated by humans from the Neolithic Age, which was about 10,000 years ago. About 2% of the worldwide total milk is produced by goats each year. Since the 1970's, dairy goat breeding has become a new challenge as the interest in simple and sustainable agriculture has increased in the United States. At present, goat milk can be obtained from the farm, health food stores and ethnic markets. As the demand for fresh and healthy foods exploded during the last decade, the variety and availability of goat milk and its products were growing all over the United States. Food, made from goat milk such as cheese, ice cream, and yogurt are getting popular among consumers. Meanwhile, goat cheese becomes the best known of all products, which is called "Chèvre", meaning "goat" in French (Jacobs-Welch, 1996).

Goat milk cheese manufacturing has grown nationwide and dramatically increased during the past two decades, allowing it to capture market share from imported goat cheese sources (Jacobs-Welch, 1996). Nevertheless, in France, Switzerland, Norway, Greece, Italy, Spain, and the Middle East, dairy goat industries are more developed than the United States (Haenlein, 1980; 1996).

With a world population of 750 million, goats are the fourth largest livestock population, right after cattle, sheep and swine, according to statistics from FAO (Park and Haenlein, 2006). This worldwide number went up by 52% during the past two decades, as the world human population and demand for goat milk consumption increased dramatically (Haenlein, 2001). This phenomenonal increase is even more remarkable in developed countries, and is related to the establishment of milk quota system in European Union (Morand-Fehr and Boyazoglu, 1999).

A test of a small population of U.S. dairy goats by Dairy Herd Improvement Association (**DHIA**) has indicated an annual milk production of >700 kg/goat in recent years (Haenlein, 2001). Some of the U.S. goat milk production leaders are capable of producing more than 2,000 kg of milk during one single lactation, with up to 10 kg/day (Haenlein, 2007).

Sufficient in protein and calcium content, goat milk significantly contributes to human nutrition, with a higher availability and affordability than cow milk (Haenlein, 2001). Nutritional and health benefits of milk and dairy products from goats are important for people, especially those with medical problems such as cow milk allergy (Haenlein, 2004). As the goat milk industry and business are developing fast and making commercial success in the US, people are realizing the benefit of the nutritional value from goat milk, which was overlooked in the past. Before being recognized by its excellent flavor, goat milk was originally introduced to be a medical alternative to cow milk, and mostly sold for therapeutic purpose to overcome cow milk intolerance. Cow milk contains more than 20 allergenic proteins, among which casein and whey protein are the main allergy inducers (El-Agamy, 2007). Although the gross compositions are

similar, goat milk is a profitable substitute for cow milk because goat breeds lacking α-s1-casein but with α-s2-casein, produce milk that is more digestible to humans (Haenlein, 2004). Besides the benefits of allergy prevention, goat milk is even of greater nutrient value than cow milk in an equivalent serving, with higher content of essential amino acids, calcium, potassium, magnesium, phosphorus, chlorine, manganese, riboflavin, short chain fatty acids, and vitamins A and D (Haenlein, 2004). Absorption of minerals and fatty acids is more effective from goat milk. The smaller fat globules also contribute to easy digestion, but lead to a softer curd and lower yield in cheese making (Haenlein, 2001). As goat milk has become widely recognized, the prospects of the dairy goat industry are considered to be economically optimistic due to the increasing demands for both goat milk and its products which have exceeded supplies (Haenlein, 1996).

SUBCLINICAL MASTITIS

Diagnosis

Mastitis is an inflammation of the mammary gland caused by intramammary infections (**IMI**) by bacteria (Blood and Studdert, 1999). The invasion of bacteria into the mammary gland and their accumulation above a given number induces the action of the innate mammary gland immune defense system, which may generally be defined as an inflammatory response. The inflammatory response may be localized to the mammary gland and may not include clinical signs such as edema and redness of the gland as well as pain and heat; thus, it is defined as subclinical with milk production loss and quality deterioration (Silanikove et al., 2005). Mastitis is frequently "hidden" in the form of

subclinical with less visible symptoms that precede the clinical form, which makes it more of a concern for the long term (Schultz et al., 1978).

Milk contains white blood cells which, together with a smaller amount of epithelial cells, constitute the majority of somatic cells. The white blood cells, also known as leukocytes accumulate at the inflamed site invaded by bacteria. Milk somatic cell count (SCC) is used as an indirect measure to indicate the inflammation of the mammary gland and udder health regardless of the cause, to eventually estimate the milk quality and yield (Reneau, 1986). A linear relationship between milk production loss and Log_{10} (SCC) in cows has been noted and it has been reported that milk yield is reduced at least 1.29 kg/unit increase in Log_{10} (SCC). Maximum loss could be up to 2.04 kg/day each Log_{10} (SCC) unit increase in older cows (Koldeweij et al., 1999). Somatic cell count is used to determine the individual infectious status of an animal or quarter in cows. The major factor impacting SCC is infection of the gland, while other factors such as the stage of lactation, age and season have minor effects (Eberhart et al., 1979).

Early diagnosis and treatment are essential for minimizing mastitis and its economical damage. However, it turns out to be unprofitable to cure animals by post-treatments. Therefore, early detection followed by preventive activities in controlling the infections becomes of greater importance. The relationship between milk yield and SCC is negative, so economic loss could be estimated by using SCC as an indicator. The infectious status of mammary glands could be also indicated by the SCC and bacteriological test due to the fact that SCC in milk increases throughout the infection (Harmon, 1994). According to the International Dairy Federation (**IDF**, 1971), it is recommended to take the SCC along with the microbiological status in the udder as a

basic determination method to diagnose mastitis. As of 1960's, SCC has been used as an indicator of IMI and a critical element of the definition of mastitis with the limit of SCC for a healthy quarter being 500,000 cells/mL. (IDF, 1971). Of all truly infected quarters, 50% could have SCC less than the 500,000 cells/mL cut-off, but the reliability and importance of SCC remains the primary measure for mastitis detection (Kitchen, 1981). Somatic cell count has been accepted as the most effective index of mammary inflammation in dairy herds to evaluate yield and quality of milk. When considering the diagnosis of mastitis or subclinical mastitis, it is recommended to use proteins, lactose, or bacteriological tests in addition to SCC (Pyörälä, 2003).

According to some researchers, the total number of somatic cells does not correlate with leukocytes in goat milk. This is contrary to what is observed for bovine milk. However, Dulin et al. (1982) concluded that counting methods for deoxyribonucleic acid in goat milk gave a reliable number of somatic cells. Cell content in goat milk increases with the progression of lactation and this elevation is larger with infected mammary glands. The response to udder infections and advancing lactation with a rise in SCC is greater in goat milk than in cow milk (Pettersen, 1981). Thus when predicting whether a goat is infected with mastitis by evaluating the number of somatic cells, physiological factors have to be considered as important as the infectious status.

Various methods for estimation of somatic cells have been developed, including direct microscopic, Coulter counter, and Fossomatic procedure. Electro-optical Fossomatic-method is accepted to be a standard procedure. An accurate method that can be performed on SCC of milk samples is called California Mastitis Test (**CMT**), which is based on a reaction between the reagents and genetic contents of somatic cells. It was

demonstrated that CMT, somatic count, lactose and chloride contents of milk samples were informative in distinguishing milk from udders with mastitis and healthy ones. They also indicated that the microscopic total leucocyte count in association with CMT, was time consuming but of highest accuracy, followed by lactose measurement (Upadhyaya and Rao, 1993). The advantage of CMT is the relatively lower cost but it is not suitable for large-scale tests (Pyörälä, 2003).

During mastitis, indigenous enzymes related to inflammation are increased and enzymes of synthesis are decreased in milk. N-acetyl-β-D-glucosaminidase (**NAGase**), a member of enzymes stemming from phagocytes increases exponentially and its activity was found to be reliable for the detection of mastitis pathogen induced IMI (Kitchen, 1981; Mattila and Sandholm, 1986). Other enzymes including different lipases, esterases, phosphateases, and lactate dehydrogenase were also found to increase during mastitis and may help predict mastitis in cattle, although evidence demonstrating their screening capability in subclinical mastitis detection was absent (Kitchen, 1981). Politis et al. (1989) described a milk PL concentration rise in mastitis infected udders, as well as a positive correlation of milk PL activity with SCC. A 2.3-fold elevation of PL activity was due to an increase of SCC from 100,000 cells/mL to 1,300,000 cells/mL. Although milk PL concentration and activity could be affected by physiological and environmental factors (see section on Plasmin) such as stage of lactation, age of cows, and milk pH, it was suggested that PL could be used as an indicator of mastitis in cattle (Politis et al., 1989).

Based on the fact that variations of pH, fat content and concentrations of sodium and chloride induce electrical conductivity (**EC**) change in milk during inflammation,

measurement of EC provides another way to detect mastitis. Electrical conductivity is highly correlated with SCC in bovine milk (Kitchen, 1981; Korhonen and Kaartinen, 1995). It was concluded that besides mastitis inflammation, factors affecting EC also included stage of lactation and age of cow (Nielen et al., 1992). When considering stage of lactation, milk yield and parity, sensitivity of EC was higher than 55% in subclinical mastitis detection (Nielen et al., 1995). A number of studies by IDF and other authors found that the diagnosis value of EC is clearly low and not as reliable and convenient as CMT and SCC tests (IDF, 1998; Musser et al., 1998; Ruegg and Reinemann, 2002). Due to the drawbacks of measuring EC as an indicator of IMI, it is not recommended to use EC alone in detection of mastitis.

Other parameters were discussed in various studies to estimate their predictive values. Adenosine triphosphate (**ATP**), which is highly correlated with SCC, could be used as an alternative to SCC in predicting mastitis in cattle (Emanuelson, 1988). Concentration of lactose in milk decreases slightly in case of inflammation, making it promising to be applied as an indicator of IMI (Berning and Shook, 1992). Although lactose has less association with mastitis infection than NAGase and SCC, it has potential to be used as a marker of mastitis with a suggested threshold value of 4.7% (Hamann and Krömker, 1997; Hamann, 2002). If sensitivity and accuracy are the most important factors in mastitis diagnosis methods, the milk NAGase activity test and lactose content in milk together have the highest likelihood to be the most reliable (Krömker et al., 2001). However, no single method is completely reliable in detecting subclinical mastitis (Rasmussen, 2000). Thus, estimate of milk SCC combined with bacteriological test is most commonly used to diagnose subclinical mastitis.

Prevalence

The prevalence of subclinical mastitis is 5-30%, usually 15-40 times more frequent than the clinical form and clinical mastitis is lower than 5% in small ruminants. Staphylococcus spp. are the most frequently found intramammary pathogens in dairy ruminants, and coagulase-negative staphylococci (**CNS**) is the most prevalent pathogen detected in subclinical mastitis sheep and goats (White and Hinckley, 1999). In an 8-year milk quality monitoring program applied to goats in Connecticut and Rhode Island, the prevalence of both types of mastitis was 36.4% in 2911 udder half samples, with SCC equal or greater than 1,000, 000 cells/mL defined as a sample of mastitis (White and Hinckley, 1999).

As a common disease in dairy herds, mastitis occurs in the primary form of subclinical mastitis, which is transmitted between udders and is mainly caused by infection of CNS, a heterogeneous group of bacteria including a great number of species. The most commonly isolated CNS species from bovine milk were *S. chromogenes*, *S. epidermidis*, *S. haemolyticus*, *S. hyicus*, *S. simulans*, and *S. xylosus* (Jarp, 1991). The most commonly found in clinical mastitis is *S. hyicus* according to studies by Honkanen-Buzalski et al. (1994) and Waage et al. (1999). *S. simulans* was found to exist in high frequency in both clinical and subclinical mastitis (Jarp, 1991; Birgersson et al., 1992; Waage et al., 1999). Significant herd problems can be caused by CNS infections despite the relatively small udder health issues, based on the fact that CNS induces high prevalence of subclinical or clinical mastitis (Wilson et al., 1997). The prevalence of

CNS induced mastitis in cows varies on the udder quarter level, from 4% to 50% (Trinidad et al., 1990; Aarestrup, 1995). The variation may be result of different sampling and diagnosis standards, as well as other factors such as parity and stage of lactation. In one study developed by Harmon and Langlois in 1989, it was observed that the prevalence of CNS IMI was 2- to 3- fold higher for first parity cows than older ones.

The most prevalent pathogens in goat milk, Staphylococcal species were identified with various frequencies, consisting of *S. aureus, S. epidermidis, S. caprae, S. simulans, S. sciuri, S. xylosus, S. chromogenes, S. hyicus, S. haemolyticus, S. cohnii, S. saprophyticus, S. auricularis, S. hominis, S. capitis, S. intermedius, S. lentus*, and *S. wameri* (Poutrel, 1984a; Binder, 1986; Harvey and Gilmour, 1988; Kalogridou-Vassiliadou, 1991; Maisi and Riipinen, 1991). Elevated SCC was associated with staphylococcal species but certain species such as *S. lentus* was found in both mastitis and healthy mammary glands (Poutrel, 1984b). Acute mastitis could not be diagnosed in goats even when the milk was reported to be bacteria-positive (Deutz et al., 1990). In a study undertaken by Deinhofer and Pernthaner (1995), significant increases of SCC were found in milk identified with *S. aureus, S. epidermidis* and *S. simulans*, among which was found to be associated with highest SCC and prevalence of mastitis in mammary glands. Although CNS are less pathogenic than *S. aureus* reported in previous studies, Contreras et al. (1997) reported on their capability to cause persistent subclinical intramammary infection in goats during lactation. Another study carried out on a herd of lactating goats with high SCC indicated a prevalence of IMI of 34% and up to 95.7% pathogens isolated from milk sample culture were Staphylococcus spp. and 66.7% were *S. epidermidis* (Contreras et al., 1999).

Prevalence of subclinical mastitis in dairy ewes in Southern Greece increases as the lactation proceeds due to accumulation of re-occurring mastitis episodes (Fthenakis, 1994). It was also concluded that prevalence of IMI increased with age in goats because of the longer exposure to pathogens, extended duration of infection and low self-cure rate (McDougall et al., 2002). A study on CNS-related IMI in cows reported that the most frequently isolated CNS from bovine intramammary infection was *S. chromogenes*, accounting for 54.7% (Todhunter et al., 1993).

Economic loss and control

Mastitis is a disease of considerable economic importance that occurs commonly in dairy herds. In general, it is reported that 70-80% of the estimated annual loss which is up to $300 per herd, is due to milk yield decrease induced by subclinical mastitis in cows (Gill et al., 1990). The national annual loss in the United States was estimated at $1.3 billion, according to a survey conducted earlier by Blosser (1979). Another estimation made by a group of European researchers indicated that with a prevalence of subclinical mastitis more than 30%, farmers could lose up to 36 euro (approximately $50.56) per goat in one single lactation (Sánchez et al., 1997). The production loss from animals with SCC of more than 800,000 cells/mL could go up to 45 euro (approximately $61.52) per goat per lactation (Contreras et al., 2003). Control plans which were applied correctly during lactation reduced the prevalence to 1% and 1-5 % in the case of clinical and subclinical mastitis, respectively (Contreras et al., 1997). The losses could be even more detrimental in developing countries based on the fact that the standard mastitis control which is encouraged by National Mastitis Council (**NMC**) is not practiced (Nickerson,

1994). Mastitis in cows is usually spread from infected to non-infected susceptible animals during the milking process. Besides milking procedures and hygiene, dry goat therapy and post milking teat dipping were found two effective control methods of goat subclinical mastitis (Poultrel et al., 1997). Dogruer et al. (2010) concluded that CNS induced subclinical mastitis in goats could be successfully treated during lactation. The best cure rates were found to be 92.5% in a group treated with intramammarian ampicillin dicloxacillin followed by 87.5% in a combination treatment group using intramammarian ampicillin dicloxacillin and intramuscular amoxicillin clavulonic acid.

Somatic Cell Count

Somatic cells in milk are a combination of the white blood cells known as leucocytes which includes macrophages (66-68%), lymphocytes and neutrophils, in milk and a relatively small amount of epithelial cells from the teat tissues (Sharif and Muhammad, 2008). Therefore, milk somatic cells play an important role in innate mammary gland immune defense system. When injured or infected by bacteria, these cells accumulate to a great amount, producing an abnormally high SCC in milk. Use of SCC is widely applied to monitor the health status of gland and milk quality. The higher the somatic cell count, the higher possibility of pathogen contamination. The fact that goat milk is normally higher in SCC than cow milk has long been a concern for goat farmers due to the regulatory standards. Compared to the standard SCC of 500,000 cells/mL for cow milk, goat milk is allowed to keep an amount of 1,500,000 cells/mL as the maximum level (Pasteurized Milk Ordinance, **PMO**, 2009). However, it is still difficult to maintain SCC below this limit for dairy goat farmers. Of all 71 goat dairies

investigated in Arkansas, Michigan, Wisconsin and California, 62% were found to have SCC higher than 1,000,000 cells/mL (Droke et al., 1993).

California Mastitis Test is a quick, simple test that accurately predicts SCC of milk from individual quarters or on composite milk samples. The CMT is accurate on cow and goat milk. Somatic cell count from a day's milk is a best indicator of the extent to which the gland is fighting against mastitis infection. The Dairy Herd Improvement (**DHI**) program provides a monthly SCC which identifies those cows with subclinical mastitis. The DHI SCC is highly correlated to losses in milk yield. The DHI SCC program assists dairy farmers in monitoring herd subclinical mastitis status, progress in mastitis control programs such as milking practices or equipment, cow environment and dry cow therapy, and can be used in making decisions regarding animal segregation and culling. Herds with bulk tank SCC above 200,000 will have varying degrees of subclinical mastitis present.

In general, as dairy herds become older, a greater percentage of the herds have higher SCC. Studies conducted at Pennsylvania State University showed that higher SCC scores in older cows were not caused by age but by increased rate of udder infections (Eberhart et al., 1979). Low SCC occurs in first parity. The stage of lactation profile will help determine when most infections develop. In uninfected cows, regardless of age, the SCC should remain below 200,000 throughout the lactation. Infection rates increase with advancing stage of lactation, especially after 200 to 250 days. Average SCC score for cows in second and later lactation increases at 101-200 days in milk, suggesting that the number of mastitis infections was increasing (Eberhart et al., 1979).

A study by Merin and coworkers (2008) demonstrated that infection of the mammary gland with S. dysgalactiae is specifically devastating to milk properties and cheese production. Moreover, excessive degradation of casein continued during cheese maturation, leading to over-mature cheese with defective texture and appearance, attributed to the excessive liberation of peptides from casein (Merin et al., 2008).

Galina and coworkers (1996) studied SCC, CMT, milk acidity and their relationship with artisan soft Chèvre-type goat cheese yield, using individual samples taken from Alpine goats over a seven-month lactation period. Lactation stage significantly affected cheese yield, CMT and SCC, although it did not affect pH. During the first 45 days SCC was high and again at the end of lactation, from days 170 to 250, but low during mid-lactation (Galina et al., 1998). High or low SCC did not have a significant correlation with clinical mastitis or udder infection. Cheese yield decreased from the second month of lactation, increased in the last 2 months of lactation and correlated negatively with CMT. In addition, high SCC resulted in inferior sensory quality of aged goat cheeses (Chen et al., 2010).

Plasmin

The Plasmin system

Plasmin (**PL**) is the predominant indigenous proteolytic enzyme in milk. Plasmin works as the most significant protease contributing the most to total proteolytic activity in dairy products (Bastian and Brown, 1996; Ismail and Nielsen, 2010). As the primary hydrolyzing enzyme in milk, it mainly associates with casein micelles as the substrate in milk. Generally in milk, PL occurs together with its inactive zymogen, plasminogen (**PG**), which is more prevalent in fresh milk. The reaction chain leading to PG activation

is mediated by PG activators (**PA**) and therefore PL is regulated by a complex network of molecular interactions between PA, specific PA inhibitors (**PAI**) and PL inhibitors (**PI**) (Politis, 1996). The PL system interwork with milk components, affecting the proteolysis in milk.

Plasmin-induced proteolysis contributes significantly to the quality of microbiological wholesome milk as well as its products. The influence of PL activity could be beneficial in a way that proper PL in casein micelle increases the ripening process during cheese making, ultimately controlling the cost. However, undesired proteolysis induced by PL has a negative effect on milk quality and shelf life as a consequence of unfavorable precipitation or gelation (Kohlmann et al., 1991; Newstead et al., 2006) and poor curding (Srinivasan and Lucey, 2002). Whether PL is beneficial or detrimental on proteolysis was concluded to be affected by PA (Ismail and Nielsen, 2010) since it had greater thermal stability (Lu and Nielsen, 1993) and the capability to convert PL from its inactivated form.

Factors affecting Plasmin activity

Milk PL system is affected by breed, stage of lactation, and the udder health, but has no relationship with daily milk yield. It was indicated that PL activity in milk increases with increased SCC in cow milk (Politis et al., 1989; Ballou et al., 1995; Baldi et al., 1996) and with stage of lactation in sheep (Theodorou et al., 2006) and goat milk (Cortellino et al., 2006). Specifically, increased levels of PL, PG and PA, and a reduced ratio of PG to PL occured as lactation advanced in cattle, indicating the enhanced

conversion of PG to PL, which together with the increased PG, may cause the increment of PL (Baldi et al., 1996). In bovine milk, significant correlations existed between PA and SCC, and PA was positively correlated with PL (Baldi et al., 1996). Research in goats indicates that PL and PG activities increase with stage of lactation (Cortellino et al., 2006) and are significantly higher in infected mammary glands associated with higher SCC in goat milk (Leitner et al., 2004b). Albenzio and coworkers found higher PL and PG activities in high SCC milk than low SCC milk, regardless of stage of lactation in ewes, and found a higher PL activity in early lactation than in later lactation in high SCC milk of ewes (Albenzio et al., 2004).

Various conditions such as processing, pH, heat treatment and storage also affect PL activity in milk and dairy products. Increased PL activity have been reported with the heat treatment in pasteurized milk due to the inactivation of PAI and PI (Prado et al., 2006), and enhanced activation of PG based on the thermal stability of PA. However, over heating could induce the inactivation of the whole system, ultimately reducing PL level. The best pH range for greatest PL activity was reported to be from pH 7.5 to 8.0 at 37 ℃ (Fox, 1981). Further information has been limited for the effect of pH. Somers and Kelly (2002) reported that increased cooking temperature caused higher PL activity due to PG activation in the cheese making system. It was also indicated that salt concentration might have a positive effect on PL activity, as well as PG and PA activities (Choi et al., 2006).

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CHAPTER III

EFFECT OF SUBCLINICAL MASTITIS AND STAGE OF LACTATION ON SOMATIC CELL COUNT, MILK COMPOSITION AND PLASMIN ACTIVITY IN GOAT MILK

ABSTRACT

A total of 91 goat milk samples from individual udders of Alpine does during early, middle and late lactations were used to investigate the impact of subclinical mastitis induced SCC increase on changes in chemical composition and plasmin (PL) activity in milk. Samples were collected and analyzed for fat, protein, lactose, solids non-fat (**SNF**) and total solids (**TS**), SCC and PL activity. Within three stages of lactation, all milk samples were sorted into three groups based on levels of SCC (low < 2.5×10^6, middle = $2.5 - 5.0 \times 10^6$, high > 5.0×10^6) and statistically analyzed in a 3×3 factorial ANOVA. There were no interactions of level of SCC and stage of lactation on variables measured ($P > 0.05$). Log_{10} (SCC) and percentage lactose in milk were negatively correlated ($r = -0.34$, $P = 0.001$). Fat, protein, SNF, TS and PL were altered by stage of lactation ($P < 0.05$). Fat content was lower in mid-lactation, whereas protein and SNF increased between early and late lactation. TS content was greater in late lactation and PL activity was greatest in early lactation. In conclusion, in high SCC milk, lactose content may be more indicative of SCC level than milk fat, protein, SNF, TS and PL activity during lactation. Stage of lactation is an important factor affecting milk composition and PL

activity in goats with infection, thus is a necessary parameter in optimizing goat milk quality in conditions of subclinical mastitis.

Keywords: Goat; Subclinical mastitis; Somatic cell count; Plasmin; Lactation

INTRODUCTION

Mastitis can be clinical with local clinical signs and milk abnormalities or subclinical signs with milk production loss and quality deterioration. Both forms result in significant economic losses to the dairy farmer due to lowered milk production and quality, early culling, drug and veterinary expenses, and increased labor costs.

The frequency of clinical and subclinical IMI in modern dairy cow herds is estimated to exceed 30-50%, which results in decreased milk yield and consequent deterioration of the quality of milk and dairy products. It is thought that altered milk composition during mastitis leads to the change of physico-chemical properties in milk. It is generally known that 70-80% of the estimated $140-300 loss per cow/year from mastitis relates to reduced milk production and decreased cheese yield caused by subclinical mastitis (Ravinderpal et al., 1990).

Goats with subclinical mastitis have reduced milk and cheese yields because of deterioration of milk quality in the infected glands (Leitner et al., 2004a, b). This effect in dairy goats could be more detrimental because of the common practice of seasonal lactation among most dairy goat herds and the fact that goat milk has naturally higher SCC than cow milk (D'Amico and Donnelly, 2010). More research is needed to identify

effective measures in minimizing the occurrence of subclinical mastitis and in improving production and quality of goat milk and cheese. This new information will assist goat milk producers and cheese manufacturers to ultimately make goat dairying more profitable and help promote the dairy goat industry as an economically viable agricultural segment.

Although systematic investigation has been initiated at Langston University, prevalence of subclinical mastitis in goats in the US has not been reported extensively. More information is needed to assist payment-by-quality schemes for both goat milk producers and cheese manufacturers. Detailed analyses of protein fractions and the gene profile of milk from goats with IMI will provide valuable information to the dairy goat industry worldwide. This study investigates the effects of subclinical mastitis and stages of lactation on changes of goat milk composition and PL activity.

MATERIALS AND METHODS

Animals

Milk was obtained from individual goat udders of lactating Alpine does in the E (Kika) de la Garza American Institute for Goat Research of Langston University and local farms (n = 91) during three different lactation stages (April, June, August-October). The selected goats were multiparous (> 2nd lactation), between 40 to 150 day in milk (**DIM**) and producing above 2 L/d of milk. Prior to animal selection, milk samples from each udder were subjected to three consecutive weekly examinations for SCC test at the

Langston University Dairy Herd Improvement (**DHI**) Lab, as well as bacterial quality on Coliform Count (**CC**) and Standard Plate Count (**SPC**).

Milk

Prior to milk collection, teats were carefully cleaned with iodine. The first three streams of foremilk were discarded and the milk afterwards was collected from each gland into sterile vials and separated into two parts: one analyzed for chemical composition (fat, protein, lactose, SNF, TS) and SCC (Foss-Combi 5000, Foss Electric, Hillerod, Denmark) at the Langston University DHI Lab for Goats the same day, the other stored frozen at -20˚C for further PL assay.

Plasmin assay

Milk was completely thawed at room temperature, mixed thoroughly, and centrifuged at 3000×g for 30 min at 4˚C. The skim milk fraction was used for PL analysis. Plasmin was analyzed as described by Politis et al. (1989). Briefly, this method involves assaying for PL activity by measuring hydrolysis rate of the chromogenic substrate (H-D-valyl-L-leucyl-Llysine-p-nitroanilide dihydrochloride (S-2251, Sigma Chemical Co, St Louis, MO). In this reaction, ε-aminocaproic acid (**ε-ACA)** (Sigma Chemical Co.) dissociates PL and PG from casein micelles and allows their transfer into the serum fraction. Formation of p-nitroanilide during cleavage of the substrate by PL was measured by absorbance at 405 nm.

An aliquot of 50 μL of skim milk was mixed with 700 μL of 50 mM Tris buffer (pH 7.4) containing 110 mM NaCl, 2.5 mM ε-ACA and 0.6 mM S-2251, incubated at 37^{o}C for 1 h, and the absorbance at 405 nm was measured in an Enzyme-Linked

Immunosorbent Assay (**ELISA**) plate reader (Multiskan Ascent, Labsystems, Barcelona, Spain) during 3 h at 30 min intervals. One unit of PL activity is defined as the amount of enzyme that produces a change in the absorbency at 405 nm of the chromogen of 0.001 unit in 1 min at 37^{o}C.

Statistical analysis

The dependent variables fat, protein, lactose, SNF, TS and PL were analyzed using the general linear (**GLM**) model procedure of SAS (version 9.2; SAS Institute Inc., Cary, NC). A 3 × 3 factorial design was applied with independent variables of three SCC levels (indicating different infectious status), and three lactation stages and their interaction. Milk samples were divided into three groups of different SCC levels. Samples with SCC below 2,500,000 cells/mL were assigned to be in the low SCC group. The middle group included milk with SCC of 2,500,000-5,000,000 cells/mL. Milk containing more than 5,000,000 cells/mL was in high SCC group. The differences among means were separated using Fisher's Protected LSD mean test. Pearson correlations between Log_{10} (SCC) and fat, protein, SNF, TS content and PL activity were analyzed. Level of significance was set at $P < 0.05$.

RESULTS

Content information of 91 samples analyzed is shown in Table 1. Somatic cell count of samples varied greatly from lowest 86,500 cells/mL to highest 18,404,000 cells/mL. Of the total samples analyzed 25.3% (n = 23) had SCC less than 2,000,000 cells/mL, 14.3% (n = 13) had extremely high SCC, (i.e., 10,000,000 cells/mL). Average

Log_{10} (SCC) for the three SCC groups is shown in Tabel 2. Log_{10} (SCC) did not change ($P > 0.60$) with stage of lactation (Table 3).

Milk fat percentage was influenced by stage of lactation ($P < 0.03$) (Figure 1), but not by SCC group ($P > 0.20$; Table 2) or interaction ($P > 0.07$) of level of SCC and stage of lactation. A significantly lower fat content of milk from mid-lactation was observed than that from early or late lactation. Milk fat percentage decreased ($P < 0.03$) from early lactation (3.95 ±0.25%) to mid-lactation (3.15 ±0.25%) and increased ($P < 0.015$) to the highest (4.02 ±0.23%) at late lactation (Table 3).

Milk protein percentage was influenced by stage of lactation ($P < 0.0001$) (Figure 2), but not by SCC ($P > 0.45$; Table 2) or their interaction ($P > 0.65$). Percentage of milk protein increased as lactation progressed, but no difference ($P > 0.10$) was found between milk from early (2.66 ±0.17%) and mid- (3.06 ±0.17%) lactation. Milk protein percentage during late lactation (3.73 ±0.16%) was higher ($P < 0.005$) than early or mid-lactation.

Milk lactose percentage was altered by level of SCC ($P < 0.006$) (Figure 3), but not by stage of lactation ($P > 0.20$; Table 3) or their interaction ($P>0.45$). Percentage lactose in milk decreased as the SCC in milk increased. Milk of low SCC group had a greater ($P < 0.03$) lactose percentage (4.43 ±0.05%) than milk of mid- (4.23 ±0.06%) and high (4.15 ±0.07%) SCC groups. Lactose percentage in mid- and high SCC groups did not differ ($P > 0.40$).

Milk SNF percentage was influenced by stage of lactation ($P < 0.0001$) (Figure 4), but not by SCC ($P > 0.30$; Table 2) or their interaction ($P > 0.75$). Percentage of milk

SNF increased ($P < 0.015$) between mid- (8.26 ±0.20%) and late (8.95 ±0.18%) stage of lactation, and did not differ ($P > 0.06$) between early (7.73 ±0.20%) and mid-lactation.

Milk TS percentage was influenced by stage of lactation ($P < 0.02$) (Figure 5), but not by SCC ($P > 0.25$; Table 2) or their interaction ($P > 0.25$). Percentage TS was greatest ($P < 0.03$) in late (12.97 ±0.39%) lactation. Milk TS content in early (11.69 ±0.41%) lactation and mid- (11.42 ±0.42) lactation did not differ ($P > 0.65$).

Plasmin activity in milk was influenced by stage of lactation ($P < 0.03$) (Figure 6), but not by SCC ($P > 0.35$; Table 2) or their interaction ($P > 0.70$). PL activity decreased ($P < 0.03$) from early (815.15 ±105.06 units) to mid- (484.27 ±105.39 units) lactation. Plasmin activity in mid- and late (459.27 ±97.60 units) lactation did not differ ($P > 0.85$).

Pearson correlation coefficients between Log_{10} (SCC) with milk components and PL activity were shown in Table 4. Fat, protein, SNF, TS, and PL activity were not significantly ($P > 0.05$) correlated to Log_{10} (SCC). Percentage lactose in milk and Log_{10} (SCC) were negatively correlated ($r = -0.34$, $P = 0.001$).

DISCUSSION

In the present study, SCC among samples varied from 86,500 cells/mL to 18,404,000 cells/mL, indicating a high prevalence (> 75%) and a wide severity range of subclinical mastitis. As infections become more severe, the level of SCC increases and may be due to increased leukocytes during infection as previously suggested (Barlett et

al., 1990; Sharif et al., 2007). Specifically, Suf Field Mastitis Test (**SFMT**) was used among 100 apparently healthy dairy buffaloes to survey the severity of subclinical mastitis as negative (N), traces (T), mild clumping (P1), moderate clumping (P2) and heavy clumping (P3), and the mean and range of milk SCC was affected by severity of subclinical mastitis, increasing as subclinical mastitis became more severe (Sharif et al., 2007).

The present study found no significant change of SCC level as stage of lactation advanced, from early (April) to late (October), and is in contrast to previous findings indicating that increased SCC is related to advancing stage of lactation (Wilson et al., 1995; Foschino et al., 2002). It is thought that lactation stage affects SCC due to increased prevalence of infection and accumulated permanent damage in glands from previous infections. Reasons for the discrepancy between the present study and previous studies could be that all animals selected for this study had abnormally high SCC and that older animals used in the present study might have more resilience to bacterial infections in spite of the accumulation of invasion. In addition, SCC may vary depending on the class of pathogen that infects the mammary gland, which likely varied among studies (Foschino et al., 2002).

The present study found a decrease of milk lactose content as the level of SCC increased and is consistent with a study in cattle (Ogola et al., 2007), sheep (Leitner, et al., 2004a) and goats (Sharif et al., 2007). Percentage lactose was higher in low SCC group indicating less severe infection of subclinical mastitis and is consistent with findings from a previous study in Pakistan, indicating a lactose decrease with the severity of subclinical mastitis in goats (Sharif et al., 2007). In middle and high SCC groups, lactose content

maintained a lower level and did not change significantly from middle to high SCC groups, but overall lactose percentage and SCC were negatively correlated ($r = -0.34$, $P = 0.001$) in the present study. However, Ying et al. (2002) found no correlation between lactose content and SCC of goat milk. A decrease of lactose associated with elevated SCC may be due to a weakened synthetic activity caused by tissue damage in infected mammary glands during subclinical mastitis (Harmon, 1994; Ben Chedly et al., 2009). In a study on lactating goats using intramammary infusion to induce cell junction disruption, there was a reduced synthetic activity in terms of decreased lactose, fat and protein, together with apoptosis induction, as the results of the disruption of mammary epithelium integrity (Ben Chedly et al., 2009). Also, it may be due to the consumption of lactose as energy source by bacteria after their invasion into the udder. Moreover, milk lactose percentage is significantly affected by dietary components in goats (Ollier et al., 2009) and cattle (Stein et al., 2006). Future studies will be required to determine if dietary factors can influence the impact of SCC on milk lactose level.

In the present study, PL activity and milk composition such as fat, protein, SNF and TS were not altered significantly by the level of SCC. This observation is consistent with a recent study in goat milk and cheese (Chen et al., 2010). In contrast, previous research conducted in dairy cows (Ballou et al., 1995; Urech et al., 1999) and dairy sheep (Leitner et al., 2004a) revealed a high PL activity in milk with high SCC. Potential subclinical mastitis with high SCC affects milk quality in terms of reduction in fat, protein, lactose. In a goat study, protein content was found to be higher in milk from more infected glands of goats (Leitner et al., 2004b), and this may be due to a decrease in milk yield. Leitner et al. (2004b) found that PL activity was significantly higher in

infected (3981 unit) glands than in the uninfected (2032 unit) ones. They did not indicate the degree of mammary infection in their study, but according to their data, infected goats were detected to have a SCC mean of 1,750,000 cells/mL. This number of SCC was much lower than the one in the present study, where only 25.3% had a SCC amount lower than 2,000,000 cells/mL, representing the low SCC group. Thus, low SCC group in the present study was in fact of large SCC amount, potentially indicating great severity of subclinical mastitis, compared with previous studies in animals lightly infected with subclinical mastitis. High SCC in goat milk is common (Zeng and Escobar, 1994; D'Amico and Donnelly, 2010), because goat milk contains a larger amount of epithelial cells than cow milk (Vihan, 1994; Oliszewski et al., 2004). According to the PMO, the standard limit for Grade "A" goat milk in the USA is revised from 1,000,000 cells/mL to 1,500,000 cells/mL (PMO, 2009), indicating that with more and more scientific research conducted in goats, goat milk SCC is reported to be higher in general. A previous study using near-infrared spectroscopy determination on 258 milk samples reported high SCC significantly influenced the accuracy of fat, protein, and lactose content (Tsenkova et al., 2001). Thus, the dissonancy of the milk composition change between the present study and previous studies may be natural, given consideration of different measurements as well.

Stage of lactation was shown to have an impact on milk fat, protein, SNF and TS in the present study. Specifically, fat and TS contents were higher at early and late stages of lactation than in middle lactation, which is consistent with previous studies in goats (Fekadu et al., 2005) and cattle (Stein et al., 2006; Lehloenya et al., 2007). Fat and TS content reached the maximum in late lactation, due to the dramatic decrease in milk

volume at that time. Lactose content in milk did not change significantly during lactation in the present study, which was in contrast with other research indicating that milk lactose increases from early to mid- lactation in cattle (Stein et al., 2006) and that lactose is greater at late lactation because of the dramatic reduction of milk yield (Fuertes et al., 1998).

In the present study, PL activity was greatest in early lactation and showed no difference between middle and late stages of lactation. This finding is consistent with an early study in which PL and PG activities were reported to be highest at early lactation and lowest at late lactation in Comisana ewe milk (Albenzio et al., 2004). In particular, PL activity in ewe milk in Italy was reported higher at early lactation than later stages of lactation in the high SCC group with over 1,000,000 cells/mL (Albenzio et al., 2004). On the contrary, other previous studies in bovine milk (Gilmore et al., 1995) and Sardinian ewe milk (Bianchi et al., 2004) reported no change in PL with stage of lactation. Compared to Leitner and coworker's study (2004b), PL activity in the present study was nearly fourfold lower. This lower PL activity may be due to the abnormally high SCC and greater severity of subclinical mastitis in the present study. According to the hypothesis by Zachos and coworkers (1992), the increment of PL activity in milk which is generally observed during later stages is mostly ascribed to the increasing SCC, and less related to the stage of lactation. In the present study, the reduction in PL activity between early and later lactation may be due to the accumulation of infection and aging of animals severely infected with subclinical mastitis.

CONCLUSIONS

Results from the current study showed that SCC negatively correlated with milk lactose, and that goat subclinical mastitis had a significant negative effect on milk lactose content whereas the other milk components were not affected. Composition of goat milk in terms of fat, protein, SNF and TS changed significantly throughout lactation in milk of goats with subclinical mastitis. However, no differences were found between SCC levels. Stage of lactation was an important factor affecting milk quality in goats with subclinical mastitis.

Plasmin activity did not change with SCC level, but decreased from early to late lactation. Whether this decrease in PL would affect quality of cheese will require further study.

Table 1. General information of milk composition and PL activity

Variables of milk	Mean ± SEM	Maximum	Minimum
Fat (%)	3.78 ± 0.14	8.49	1.72
Protein (%)	3.21 ± 0.10	6.60	1.99
Lactose (%)	4.29 ± 0.04	5.20	2.47
Solids-non-fat (%)	8.39 ± 0.11	11.71	6.81
Total solids (%)	12.17 ± 2.22	19.50	8.62
PL activity (unit)	586.25 ± 56.60	3447.02	21.94
Log_{10} (SCC)	6.47 ± 0.05	7.26	4.94
SCC ($\times 10^3$cells/mL)	4658.62 ± 475.63	18,404	86.5

SEM = standard error of the mean.

Table 2. Effect of level of SCC on milk composition and PL activity

Variables of milk	Level of SCC		
	Low (n = 36)	Middle (n = 31)	High (n = 24)
Fat (%)	3.73 ± 0.21	3.38 ± 0.24	4.01 ± 0.27
Protein (%)	3.16 ± 0.14	2.99 ± 0.17	3.30 ± 0.19
Lactose (%)	4.43^{a} ± 0.05	4.23^{b} ± 0.06	4.15^{b} ± 0.07
Solids-non-fat (%)	8.48 ± 0.17	8.12 ± 0.19	8.34 ± 0.22
Total solids (%)	12.21 ± 0.35	11.50 ± 0.40	12.36 ± 0.46
PL activity (unit)	605. 96 ± 88.85	473.66 ± 101.89	679.34 ± 115.73
Log_{10} (SCC)	6.05^{c} ± 0.04	6.54^{b} ± 0.05	7.01^{a} ± 0.06
SCC ($\times 10^{3}$cells/mL)	1,455.96 ± 400.40	3,528.09 ± 459.20	11,188.73 ± 521.57

Mean values within a row with different superscripts (abc) differ ($P < 0.05$).
SCC levels: Low < 2.5×10^{6} cells/mL; Middle = 2.5 - 5.0 $\times 10^{6}$ cells/mL; High > 5.0×10^{6} cells/mL.

Table 3. Effect of stage of lactation on milk composition and PL activity

	Stage of lactation		
Variables of milk	Early (n = 27)	Mid- (n = 32)	Late (n = 32)
Fat (%)	$3.95^{a} \pm 0.25$	$3.15^{b} \pm 0.25$	$4.02^{a} \pm 0.23$
Protein (%)	$2.66^{b} \pm 0.17$	$3.06^{b} \pm 0.17$	$3.73^{a} \pm 0.16$
Lactose (%)	4.18 ± 0.06	4.31 ± 0.06	4.32 ± 0.06
Solids-non-fat (%)	$7.73^{b} \pm 0.20$	$8.26^{b} \pm 0.20$	$8.95^{a} \pm 0.18$
Total solids (%)	$11.69^{b} \pm 0.41$	$11.42^{b} \pm 0.42$	$12.97^{a} \pm 0.39$
PL activity	$815.15^{a} \pm 105.06$	$484.27^{b} \pm 105.39$	$459.27^{b} \pm 97.60$
Log_{10} (SCC)	6.49 ± 0.05	6.56 ± 0.05	6.55 ± 0.05
SCC ($\times 10^{3}$cells/mL)	$5{,}374.15 \pm 473.45$	$5{,}648.63 \pm 474.95$	$5{,}149.97 \pm 439.85$

Mean values within a row with different superscripts (ab) differ ($P < 0.05$).

Table 4. Pearson correlation coefficients between Log_{10} (SCC), milk components and PL activity (N=91)

	Fat (%)	Protein (%)	Lactose (%)	SNF (%)	TS (%)	PL (unit)
Log_{10} (SCC)	0.13795^{NS}	0.15770^{NS}	-0.33816*	0.03306^{NS}	0.09874^{NS}	0.13937^{NS}
P	0.1922	0.1355	0.0010	0.7558	0.3517	0.1876

NS: not significantly correlated ($P > 0.05$).
* Significantly correlated ($P < 0.05$)

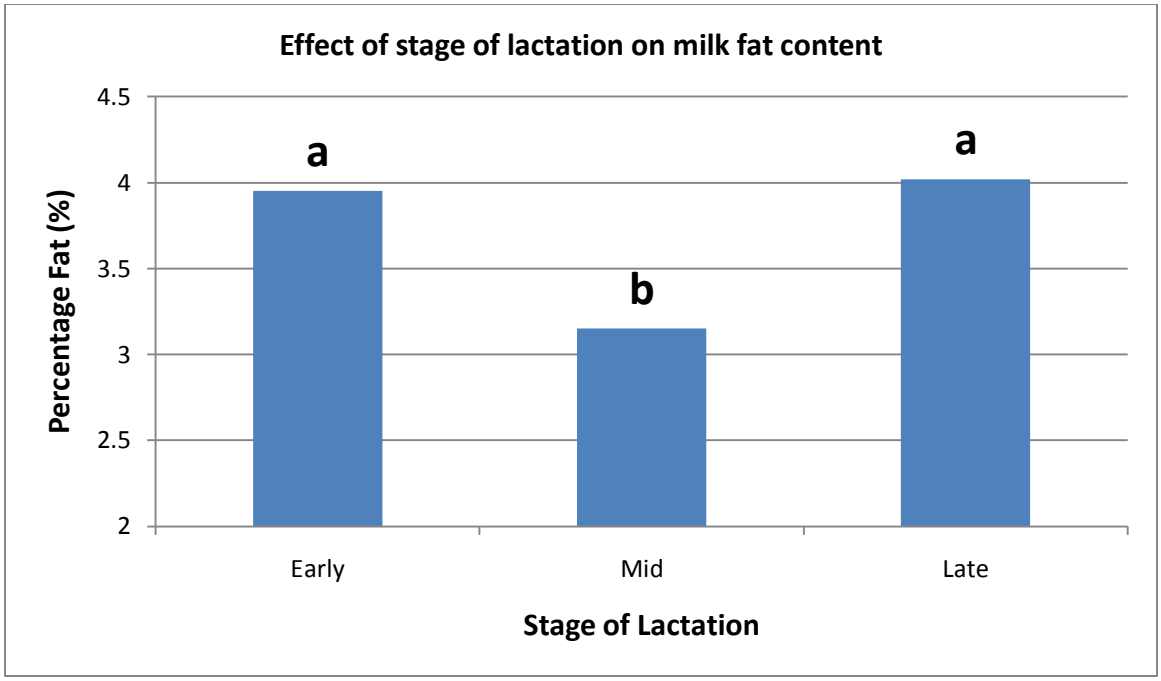

Figure 1. Percentage milk fat in early, mid-, and late lactation. Fat percentage was influenced by stage of lactation ($P < 0.03$). Means with different superscripts (ab) differ ($P < 0.05$), Pooled SEM = 0.25%.

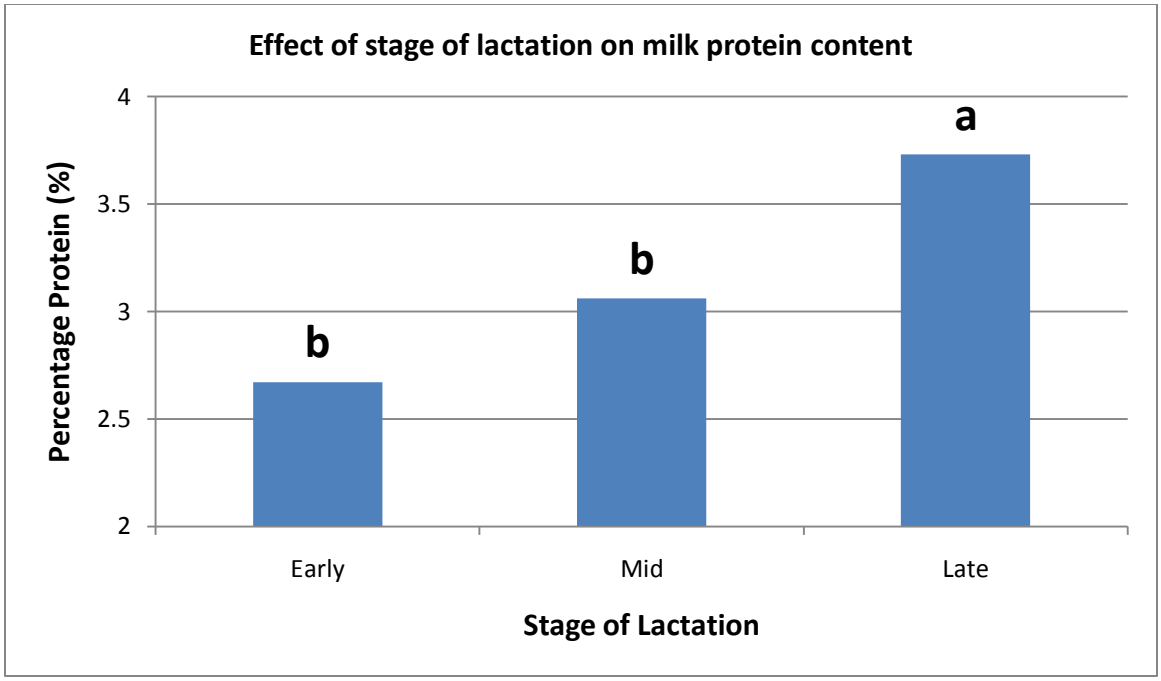

Figure 2. Percentage milk protein in early, mid-, and late lactation. Milk protein percentage was influenced by stage of lactation ($P < 0.0001$). Means with different superscripts (ab) differ ($P < 0.05$), Pooled SEM = 0.17%.

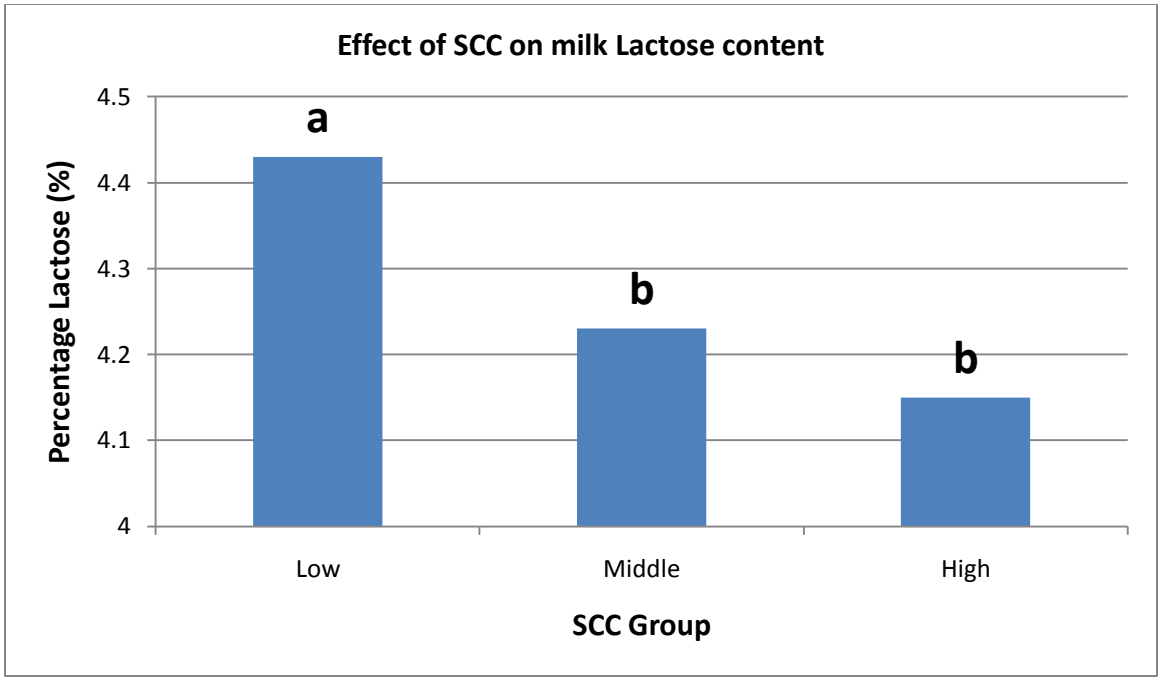

Figure 3. Percentage milk lactose in low, middle, and high SCC groups. Milk lactose percentage was altered by level of SCC ($P < 0.006$). Means with different superscripts (ab) differ ($P < 0.05$), Pooled SEM = 0.06%.

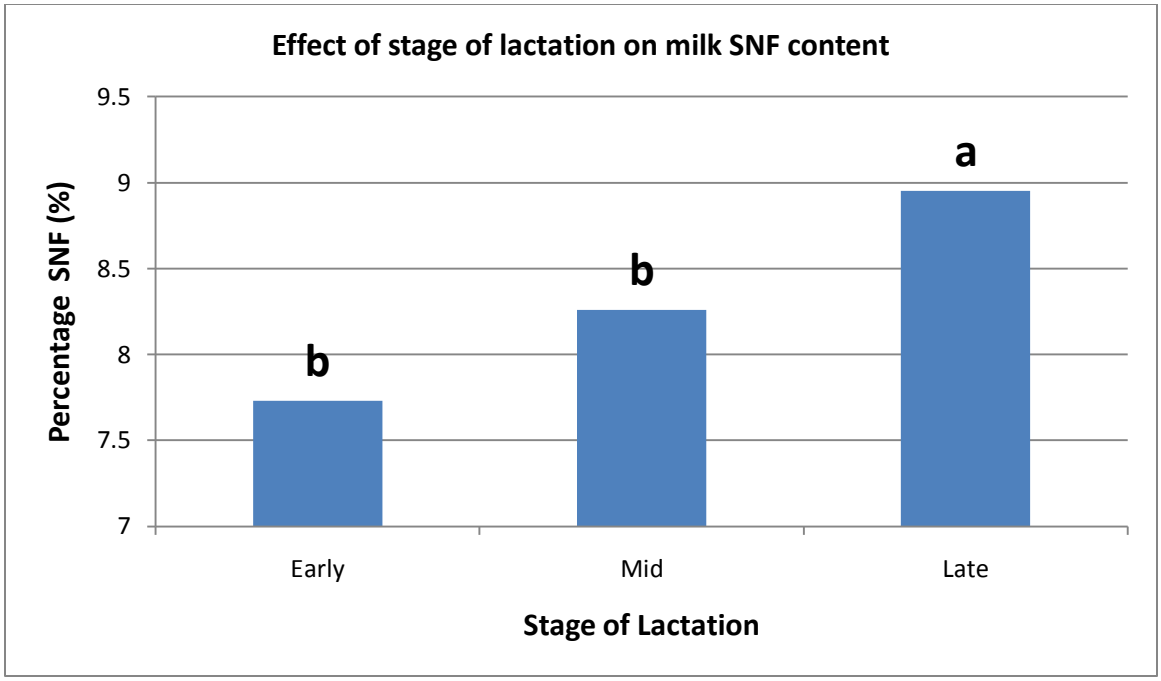

Figure 4. Percentage milk SNF in early, mid-, and late lactation. Milk SNF percentage was influenced by stage of lactation ($P < 0.0001$). Means with different superscripts (ab) differ ($P < 0.05$), Pooled SEM = 0.19%.

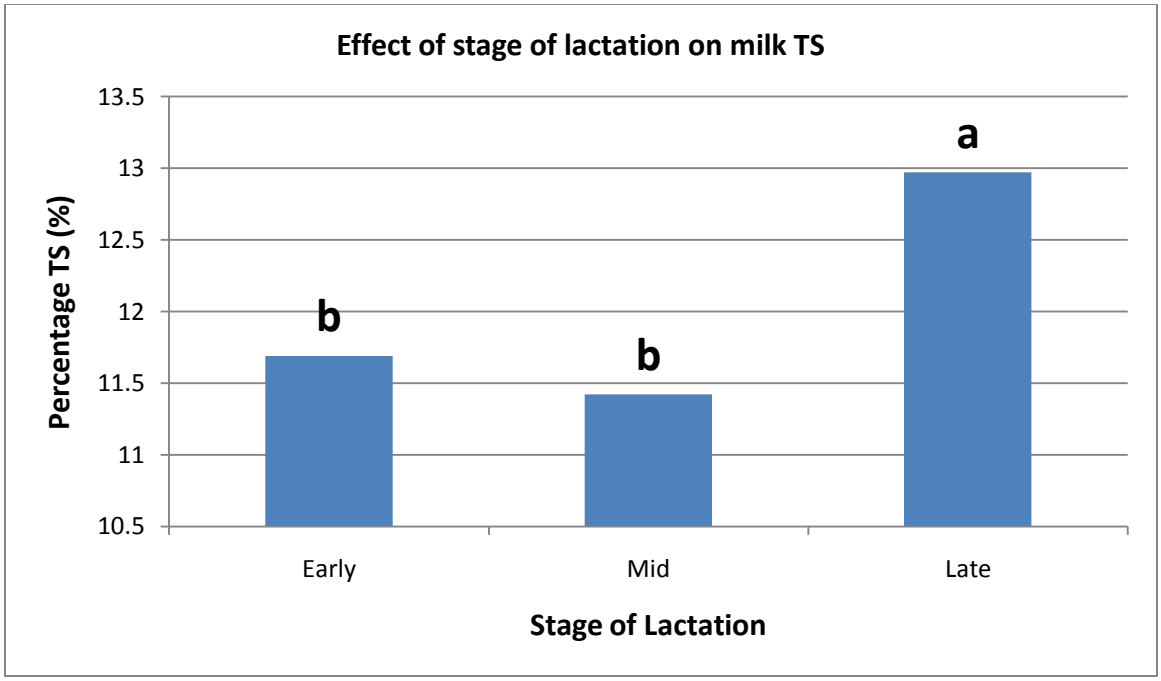

Figure 5. Percentage milk TS in early, mid-, and late lactation. Milk TS percentage was influenced by stage of lactation ($P < 0.02$). Means with different superscripts (ab) differ ($P < 0.05$), Pooled SEM = 0.41%

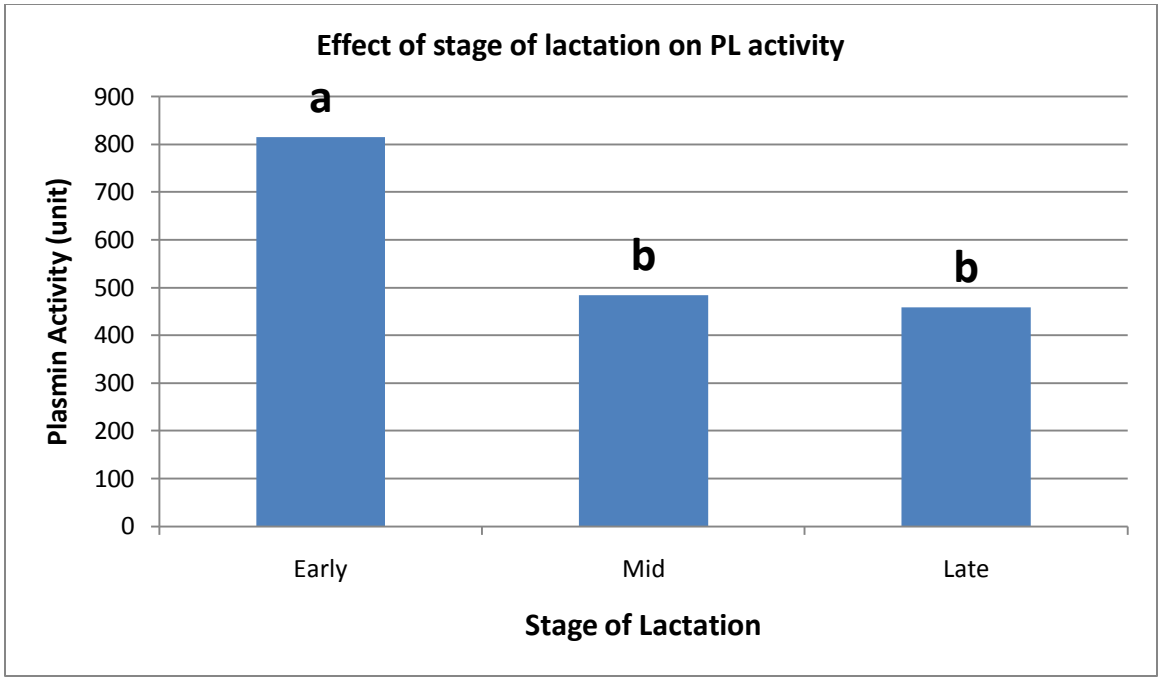

Figure 6. Milk PL Activity in early, mid-, and late lactation. PL activity in milk was influenced by stage of lactation ($P < 0.03$). Means with different superscripts (ab) differ ($P < 0.05$), Pooled SEM = 103.4.

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CHAPTER IV

CONCLUSION

Results of this study indicated a high prevalence and severity of subclinical mastitis in tested animals. Lactose content was more indicative of SCC level than fat, protein, SNF, TS and PL activity in subclinical goat milk. Although lactose content decreased with higher SCC, different levels of high SCC, representing the severity of subclinical mastitis in goats did not affect the other milk components and PL activity. In conclusion, it is not effective to control the milk quality by monitoring the milk SCC when animals are infected with subclinical mastitis, to a certain degree of severity, where PL system is on a relatively lower level than healthy goats.

Stage of lactation significantly affected the milk composition and PL activity regardless of the levels of SCC, which in this study were separated but all respectively higher than non-infected ones. We can conclude that milk composition was affected by stage of lactation during subclinical mastitis. Therefore, selection of lactation for dairy goat producers is important when herds are infected with subclinical mastitis. Milk from early lactation is of high production and proper PL activity in subclinical mastitic goats.

Composition changes due to subclinical mastitis in goat milk may affect cheese yield and quality. Thus, a better understanding of how SCC and stage of lactation impacts milk quality is needed and will ultimately lead to increased production of high quality

goat milk and dairy product. A larger number of observations and more specific work such as genetic profile of SCC are needed to give more precise insight.

APPENDICES

ABBREVIATIONS

ATP	Adenosine Triphosphate
CC	Coliform Count
CMT	California Mastitis Test
CNS	Coagulase-negative staphylococci
DIM	Day in milk
ε-ACA	ε-aminocaproic acid
EC	Electrical Conductivity
ELISA	Enzyme-Linked Immunosorbent Assay
IDF	International Dairy Federation
NAGase	N-acetyl-β-D-glucosaminidase
NMC	National Mastitis Council
PA	Plasminogen Activator
PAI	Plasmin Activator Inhibitor
PG	Plasminogen
PI	Plasmin Inhibitor
PMO	Pasteurizad Milk Ordinance
PL	Plasmin

SCC	Somatic Cell Count
SFMT	Suf Field Mastitis Test
SNF	Solids-Non-Fat
SPC	Standard Plate Count
spp.	Plural abbreviation of species
TS	Total Solids

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Scope and Method of Study: Research thesis

Findings and Conclusions: Somatic cell count had an influence on goat milk lactose content. Percentage lactose declined with increased SCC in subclinical mastitis, without differences in milk fat, protein, SNF, TS content and PL activity. Stage of lactation affected fat, protein, SNF, TS and PL activity in goat milk associated with subclinical mastitis. Somatic cell count of subclinical mastitic goat milk did not differ with stage of lactation. A better understanding of how SCC and stage of lactation impacts milk quality is needed and will ultimately lead to increased production of high quality goat milk and dairy products.

ADVISER'S APPROVAL: Leon Spicer