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STUDY OF THE EFFECTS OF SUB CLINICAL MASTITIS AND OTHER PRODUCTION FACTORS ON COW MILK FAT AND PROTEIN CONCENTRATION IN AND AROUND GONDAR NORTHWESTERN ETHIOPIA

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ABBREVIATIONS

BST = Bovine somatotropin

CBO=Clot on boiling test

FO = Fish oil.

FA = Fatty acid

SNF = Solids not fat

TS = Total solids

SCC = Somatic cell count

BCS = Body condition score

ABSTRACT

The impact of sub clinical mastitis and other production factors on cow milk protein and fat concentrations in dairy cows were studied from October 2006 to June 2007 in and around Gondar. By way of cross sectional survey data were collected from 236 milking cows of 105 smallholder dairy farmers. Instruments used for data collection were questionnaire survey, farm inspection, animal examination and laboratory analysis. Descriptive statistics, linear regression and correlation coefficients between variable were computed.

Mastitis prevalence at herd and cow levels was respectively 37.1% and 33.1%. From the total mastitis cases 2.6% was clinical and the rest 30.5% sub clinical mastitis.

Sub clinical mastitis reduced milk fat and protein concentrations by 15.4% and 8.2% respectively; the differences were statistically significant ($P<0.05$). The influence of three other factors, namely breed, feed and management, on fat and protein concentrations were also examined both in mastitic and healthy cows.

Breed effect was significant for fat (($P=0.031$) and protein ($P=0.024$). Indigenous breeds had higher fat and protein concentrations compared to 50% and above crosses. The effect of sub clinical mastitis was found more pronounced in 50% crosses.

Cows fed on, relatively, low quality feeding stuffs (grazing with very minimum supplementation) had significantly low level of fat ($P=0.008$) and protein ($P=0.05$). In poorly fed cows, the effect of sub clinical mastitis in modifying milk composition concentrations was quite high.

Management was also very much related to milk composition. Improved management significantly increased fat ($P=0.004$) and protein ($P=0.012$) concentration. Mastitic cows subjected to poor management had more depressed fat and protein levels.

The regression analysis made after grouping milking cows by breed allowed the fitting of mathematical models that estimated, with good precision, fat and protein concentrations in healthy and mastitic cows.

Key words: Udder health, cow breed, husbandry practice, nutrition

1. INTRODUCTION

Milk is a complete diet which nature has provided the newly born to thrive on at the time when the offspring is not capable of fetching its own food. Milk is also said to be a perfect food for chilling, it contains the entire essential nutrient materials needed for body growth and normal functioning of the body (Shafei *et al*., 1988). Milk is a staple diet among the nomads and is supplemental diet among consumers who mostly feed on grain products. It is also a source of cash for small-scale dairy farms.

Milk is composed of fat, protein, carbohydrate (lactose), vitamins, minerals and enzymes. Fat is a high source of energy and carrier of fat and water soluble vitamins (vitamin A, D, E, K, B complex and C). Among the minerals found in milk, calcium and phosphorous are involved in mineralization of bones (Goff and Griffithis, 2006).

The commercial value of milk historically has been influenced by milk components. When milk was marketed in the form of butter, the importance of butterfat to any dairy producer's income was overwhelming. Consequently, one of the major advances in cow side recording of production data in early years was the development of the Babcock test for determining the fat content of milk. As scientific advancement produced ways to determine protein content, and as processing plants began to observe greater returns from cheese manufacturing, protein also became a more important milk component to be tested. With the development of rapid, accurate and automated methods of determining fat, protein and solids-not-fat (SNF), contents of milk ever increasing number of dairy producers have had access to such component information on cows in their herds.

The use of component information for within-herd management requires that the dairy producer understand the economic importance of those components to the profitability of the dairy farm. Breeders of purebred cattle see immediate economic return from the sale of cattle with known pedigree production of higher level of milk components.

Sources of variations of milk components are many, the major one being udder infection (Harmon, R. J. 1994). Cows contract udder infection at different ages and at different stages of the lactation cycle. Cows also vary in their ability to overcome an infection once it has been established. Therefore, the cow plays an active role in the development of mastitis.

The cows' environment influences both the number and types of bacteria they are exposed to and their ability to resist these microorganisms. However, through appropriate management practices, the environment can be controlled to reduce this exposure and enhance resistance to udder disease (Schroeder, J. W. 1997).

In Ethiopia liquid milk is marketed on volume basis and there were no systematic studies conducted to determine milk compositional quality under different production factors (breed, management and feeding condition) and udder health. Therefore, the purpose of this study is to bridge the gap by studying the effects of the stated factors and mastitis, on cow milk fat and protein percentage.

OBJECTIVE

The general objective of this study is therefore, to determine the effects of sub clinical mastitis on cow's milk fat and protein percentage.

1: Determining protein and fat percentage of milk in cows of different breeds and kept under different nutritional and husbandry conditions.

2: Determine the effect of udder infection on milk protein and fat percentage.

3: Establishing the existence or absence of relationship between different factors (mastitis, breed, nutrition and management) in influencing milk fat and protein percentage.

2. LITERATURE REVIEW

2. 1. Composition, nutritive value and properties of milk

2.1.1. Composition

Milk is fresh and clean lacteal secretion obtained by complete milking of one or more healthy cows. Milk is composed of fat, protein, lactose, vitamins and minerals (Rai *et al*., 1980).

Table 1: Milk composition of different lactating animals and man

Animal	Fat (%)	Protein (%)	Lactose (%)	Ash (%)
Man	4	0.9	7	0.2
Cow	3.7	3.4	4.7	0.7
Goat	3.5	3.4	4.1	0.8
Zebu cow	4.7	3.2	4.9	0.7
Camel	4.5	3.6	5	0.7
Sheep	6.5	5.8	4.6	1.0

Source: (Barbano and Lynch, 2001)

Milk fat

This is the most valuable component of milk and it exists in milk in the form of minute globules. The fat globules are found in dispersed form. Milk fat is mainly composed of triglycerides (Barbano and Lynch, 2001). The fat content of milk and cream is the most important single factor in determining the price to be paid for milk supplied by farmers in many countries. Further more the butterfat percentage in the milk of individual animal must be known in many breeding programs for the purposes such as selecting best animals, to calculate the correct amount of feed ration of dairy cows and to make accurate adjustments of the butterfat percentage in standardized milk and milk products (Khorasani *et al*., 1991; Connon, 1993).

Milk fat is not only a single chemical compound, but also a variable mixture of several different glycerides (Gilmore and Gaunt, 1962). Each glyceride is the result of the union of glycerol and

one or more organic acids. The organic acids contained in milk fat are commonly termed as fatty acids (Simopoulos, 1991; Gibson *et al*., 1991; Olabi and Barbano, 2006).

Milk fat contains more than 400 different types of fatty acids and fatty acid derivatives. The fatty acids found in milk fat ranges from butyric acid with 4 carbon atoms to fatty acids with 26 carbon atoms. Milk fat is characterized not only by numerous and different fatty acids, but also by the chain length, (Simopoulos, 1991). Among animal fats, milk fat is unique, because it contains a relatively high proportion of short chain and medium chain fatty acids. Milk fat contains 7 % short chain fatty acids (C4 - C8), 15 to 20% medium chain fatty acids (C10 - C14) and 73 to 78% long chain fatty acids (C16 and higher. Milk fatty acids are approximately, 65 % saturated 32% monounsaturated and 3 % polyunsaturated.

The saturated fatty acids present in the largest amount in milk fat are myristic (C14: 0), palmitic (C16: 0) and stearic (C18:0). While the major unsaturated fatty acids are oleic (18:1), linoleic (18: 2) and linolenic (18:3) acids. Milk fat also contains fat-soluble vitamins (A, D, E, K) and cholesterol.

Milk fat composition can be modified by direct addition of fatty acids (FA) to milk or milk products, by genetic manipulation or by feeding special diets to dairy cows. Direct supplementation has the advantage of simplicity and theoretically, all levels of desired FA could be added to milk fat (Moore *et al*., 1992). The major disadvantages are the fishy flavor associated with FA supplement such as fish oil (FO) and the need for antioxidants to stabilize long- chain unsaturated fatty acids (Pennington and Davis, 1975). Further, more supplemental FA must be blended with milk and then homogenized, a process that is undesired for cheese making, because homogenization produces cheese with excessive moisture content and reduced firmness (Spain and Polan, 1995; Chillier and Doreau, 1997; Baer *et al*., 2001).

Modest differences in fatty acid composition have been observed between breeds of cows and substantial difference exists between species (Paimquist, 1993; Kelly, 1998). Genetic changes can be accomplished by conventional breeding methods or by transgenic alteration. However, the change is slow relative to the large changes required to meet current consumer needs. Thus, in the

short-term the modification of milk fatty acid composition could be sought through changing the FA composition of dairy cows diets (Gibson *et al*., 1996; Fox *et al*., 2000).

Protein

Proteins are chains of amino acid molecules connected by peptide bonds. There are many types of proteins and each has its own amino acid sequence. There are 22 different amino acids that can be combined to form protein chains. There are 9 amino acids that the human body cannot make and must be obtained from the diet. These are called the essential amino acids.

The amino acids within protein chains can bond across the chain and fold to form 3-dimensional structures. Proteins can be relatively straight or form tightly compacted globules or be somewhere in between. The term "denatured" is used when proteins unfold from their native chain or globular shape (Fox and McSweeney 1998).

Milk proteins are synthesized in the mammary gland, but 60% of the amino acids used to build the proteins are obtained from the cow's diet. Total milk protein content and amino acid composition varies with cow breed and individual animal genetics.

There are 2 major categories of milk protein that are broadly defined by their chemical composition and physical properties. The casein family contains phosphorus and will coagulate or precipitate at pH 4.6. The serum (whey) proteins do not contain phosphorus, and these proteins remain in solution in milk at pH 4.6. The principle of coagulation, or curd formation, at reduced pH is the basis for cheese curd formation. In cow's milk, approximately 82% of milk protein is casein and the remaining 18% is serum, or whey protein. The casein family of protein consists of several types of caseins (α-s1, α-s2, ß, and 6 caseins) and each has its own amino acid composition, genetic variations, and functional properties (Fox and McSweeney 1998; Roseler *et al*., 1993).

The high phosphate content of the casein family allows it to associate with calcium and form calcium phosphate salts. The abundance of phosphate allows milk to contain much more calcium than would be possible if all the calcium were dissolved in solution, thus casein proteins provide a good source of calcium for milk consumers. The serum (whey) protein family consists of approximately 50% ß-lactoglobulin, 20% α-lactalbumin, blood serum albumin, immunoglobulins, lactoferrin, transferrin and many minor proteins and enzymes. Like the other

major milk components, each whey protein has its own characteristic composition and variations. Whey proteins do not contain phosphorus by definition, but do contain a large amount of sulfur-containing amino acids.

Proteins can be degraded by enzyme action or by exposure to light. The predominant cause of protein degradation is brought by enzymes called proteases. Milk proteases come from several sources: the native milk, airborne bacterial contamination, bacteria that are added intentionally for fermentation or somatic cells present in milk (Fox and Mc Sweeney 1998; Roseler *et al*., 1993).

Lactose

Lactose is a disaccharide made up of 2 simple sugars: glucose and galactose. Lactose comprises 48 to 52 % of milk solid not fat (SNF), and 70 % of whey solids. Lactose is not as sweet as succhrose. When bacteria act on lactose, lactic acid is produced and this is responsible for milk sourness and coagulation of casein (Goff and Griffithis, 2006).

Vitamins

Milk contains fat-soluble vitamins (Vitamin A, D, E, and K) and water-soluble vitamins such as Vitamin B1 (thiamine), B2 (riboflavin), B6 (pyridoxine), B12 (Cyanocobalamin), niacin and panthotenic acid. There is also a small amount of vitamin C (ascorbic acid) present in raw milk, but is very heat- labile and is easily destroyed by pasteurization (Goff and Griffithis, 2006; William; 2006; Weber, 2001).

Minerals

Milk contains 22 types of minerals, which are found to be very essential in human nutrition. The major minerals found in milk are sodium chloride, phosphorous, calcium and magnesium (Goff and Griffithis, 2006).

2.1.2. Nutritive value of milk

Milk in its natural form is a highly nutritious food. It is a source of energy, protein, minerals and trace elements, vitamins, enzymes, phosopholipids and pigments. It is an excellent source of high quality protein for children, adults and elderly, supplying the essential amino acids (Mahoney and Peter, 1987). Milk protein contains relative surplus amount of some essential amino acids (lysine, threonine, methionine and isoleucine) that can supplement vegetable proteins, which often are limited in those amino acids. The nutritive values of milk constituents are summarized in Tables 2 and 3 (Fedele, 2001; Goff and Griffithis, 2006; Ambrish *et al*., 1998)

Table 2: Nutrients in Milk

Nutrient	Function
Protein	Builds and repairs cells and body tissues, including bones and muscle, helps in the production of antibodies and is the source of energy.
Fat	Source of energy, carrier of fat soluble vitamins, source of essential fatty acids,
Lactose	Source of energy, helps in fat utilization
Vitamin A	Helps to maintain healthy skin, plays vital role in vision, involved in bone growth and reproduction
Vitamin D	Essential for optimal calcium and phosphorous absorption
Thiamine	Important for the production of energy in body cells
Riboflavin	Keeps tissues such as the skin, eyes and nerves healthy
Vitamin B6	Helps protein utilization, assists in the production of red cells.
Vitamin B12	Involved in red blood cell production
Panthothnate	Required for tissue formation
Calcium	Strengthens bones and teeth, helps muscle contraction, transmission of impulses, blood clotting, involved in hormone production
Phosphorous	Involved in bone formation

Source: (Goff and Griffithis, 2006; Ambrish *et al*., 1998)

Table 3: Nutritive Value of Milk and Milk products

Milk or milk product	Energy (KCal)	Protein (g)	Carbohydrate (g)	Fat (g)
Milk (cow)/liter	117	4.3	5	6.5
Butter milk/liter	15	0.8	0.5	1.1
Skimmed milk/liter	29	2.5	4.6	0.1
Cheese/kg	348	24.1	6.3	25.1
Milk powder(skimmed) /kg	357	38	51	0.1
Milk powder (Whole) /kg	496	25.8	38	26.7

Source: (Goff and Griffithis, 2006); (Ambrish *et al*., 1998)

Milk composition also influences milk processing; for example, the type of fatty acid in milk will influence the quality of dairy products such as butter, ice cream, and cheese. Changes in milk protein fraction can influence the moisture, texture, flavor and yield of various types of cheese. Changes in milk minerals can influence the heat stability of milk protein, (Barbano and Lynch, 2001).

2.2. Factors affecting milk composition

Milk composition varies according to health status, breed (Barkema *et al*., 1988), genetics, body condition during calving, stage of lactation (Chouinard *et al*., 1999), season (Simas *et al*., 1997), method of milking, interval between milking, weather, stress, exercise, excitement, udder health, feeds and feeding (Wright *et al*., 2003, Onetti *et al*., 2004).

2.2.1 Health status

Mastitis reduces not only milk yield, but also influences milk composition. The degree of alteration of milk constituents due to mastitis depend on the severity of the udder infection, which in turn is influenced by the types of pathogens causing mastitis. Cows with clinical mastitis will have more dramatic change in milk composition than cows with sub clinical mastitis (Pamela,

2004). The most notable changes in milk composition associated with mastitis are decreased concentration of fat, lactose, casein and calcium; increased concentration of albumin, sodium and chloride. Concentration of enzymes such as lipase; protease; oxidase; plasmin and planimnogen, increase that may adversely influence milk stability and milk flavor (Oliver and Calvinho, 1995).

Contagious mastitis

Mastitis is inflammation of the mammary gland caused by microorganisms, usually bacteria that invade the udder, multiply, and produce toxins that are harmful to the mammary gland. Is a complex disease, because it has numerous causative bacteria, and no single control measure will prevent infections by all pathogens however, the basic concepts for the control of the contagious pathogens *Staphylococcus aureus* and *Streptococcus agalactiae* have been known for over 30 years. Despite this fact, mastitis continues to cause losses in the dairy industry of nearly $2 billion in the United States each year. This translates to an annual loss of approximately US$18,000 in a 100 cow herd. The majority of this loss is in reduced milk production due to the presence of infections. Effective programs of post-milking use of germicidal teat dips, strict milking time hygiene, dry cow therapy, and culling can result in the eradication of *Streptococcus agalactiae* from dairy herds and a markedly reduced incidence of *Staphylococcus aureus*. These management approaches, directed at preventing infections, must be used properly and consistently to provide continued success toward controlling contagious mastitis (Fox and Gay, 1993; Harmon, R.J, 1994).

Response of the udder to infection

Mastitis occurs when the udder becomes inflamed because leukocytes are released into the mammary gland in response to invasion via the teat canal, usually by bacteria. These bacteria multiply and produce toxins that cause injury to milk secreting tissue and various ducts throughout the mammary gland. Elevated leukocytes, or somatic cells, cause a reduction in milk production and alter milk composition. These changes in turn adversely affect quality and quantity of dairy products (Jones G.M. 1998). Infections of the mammary gland by pathogenic bacteria results in decrease in milk production and compositional changes that vary with the intensity and duration of the infection. Compositional changes include decrease in lactose, fat, casein, and calcium and increase in sodium, chloride, and blood proteins in milk.

Sub clinical infections are those in which no visible changes occur in the appearance of the milk or the udder, but milk production decreases, bacteria are present in the milk, and composition is altered. It causes the greatest financial loss to dairy owners through lowered milk production. For every clinical case of mastitis, there will be 15 to 40 sub-clinical cases that means it is more prevalent than the clinical forms, it is of long duration and adversely affect milk quality (Harmon, R.J. 1990; Philpot and Nickerson, 1991; Schroeder, J. W. 1997).

Clinical mastitis is characterized by abnormal milk and swelling or pain of the udder; it may be accompanied by systemic signs such as elevated rectal temperature, depression, or decreased feed intake. As in sub clinical mastitis, milk production declines, bacteria are present in the milk, and dramatic changes in milk composition are usually present. Chronic mastitis is an infection that is long duration and may show periodic clinical symptoms (Harmon, R.J. 1990; Schroeder, J. W. 1997).

Strict milking time hygiene is a high priority to reduce spread of contagious bacteria from one cow to another and to reduce bacterial contamination of the bulk tank milk. Many acceptable practices may be used to prepare the teats for milking. However, teats should be clean and dry before applying the milking unit. The use of a germicidal udder wash is recommended, but rinsing a contaminated cloth or sponge in a germicide solution will not kill all the bacteria in the cloth. Therefore, do not use the same cloth or sponge to wash or dry all cows, because this

practice will spread bacteria from one cow to another. Use individual cloth or paper towels to clean or dry each cow (Oliver, *et al*., 1989).

Effect on milk composition

Mastitis resulting from major pathogens causes considerable compositional changes in milk (Table 5), including increases in SCC. The types of proteins present change dramatically. Casein, the major milk protein of high nutritional quality, declines and lower quality whey proteins increase which adversely impacts dairy product quality, such as cheese yield, flavor and quality. Serum albumin, immunoglobulins, transferrin, and other serum proteins pass into milk because vascular permeability changes. Lactoferrin, the major antibacterial iron-binding protein in mammary secretions, increases in concentration, probably because of increased output by the mammary tissue. Milk protein breakdown can occur in milk from cows with clinical or sub clinical mastitis due to post-secretory degradation of casein by proteolytic enzymes (proteinases) (Auldist and Hubble, 1998; Jones, G.M. 1998). Plasmin increases proteolytic activity by more than 2-fold during mastitis. Plasmin and enzymes derived from somatic cells can cause extensive damage to casein in the udder before milk removal. Deterioration of milk protein as a result of mastitis may continue during processing and storage. Mastitis decrease milk fat and increases the conductivity of milk and sodium; chloride concentrations are elevated. Potassium, normally the predominant mineral in milk, declines. Because most calcium in milk is associated with casein, the disruption of casein synthesis contributes to lowered calcium in milk (Harmon, R. J. 1994**;** Jones *et al*., 1984).

Impaired, synthetic and secretory activity of udder epithelial cells results in decreased casein, lactose, and fat content of the milk (Ashworth *et al*., 1967) and decreased milk yield. (Hortet *et al*., 1999; Koldeweij *et al*., 1999) and have recently assessed milk yield losses in relation to increasing SCC. As the change in the concentration of a milk component is strongly related to the actual milk secreted by the affected gland, it will be sensitive to dilution or up-concentration depending on directions, speeds, and sizes of concomitant changes in both milk yield and the milk component itself (Schultz, 1977). Dealing with the composition of cow composite milk samples, concentration of a milk component will furthermore be influenced by cow-level number of diseased quarters as investigated for SCC in milk investigated by (Natzke *et al*., 1972).

Table 4: Changes in milk constituents associated with high SCC

Constituent	Normal Milk	Milk with high SCC	Proportion of normal
Fat	3.5	3.2	91
Lactose	4.9	4.4	90
Total protein	3.61	3.56	99
Total casein	2.8	2.3	82
Whey protein	.8	1.3	162
Serum albumin	.02	.07	350
Lactoferrin	.02	.10	500
Immunoglobulins	.10	.60	600
Sodium	.057	.105	184
Chloride	.091	.147	161
Potassium	.173	.157	91
Calcium	.12	.04	33

Source: (Harmon, R. J. 1994)

Casein and fat was expected to decrease with mastitis. A possible explanation could be that, to some extent, milk yield decreased more rapidly than protein and fat syntheses and, furthermore, that increases in serum protein partly compensated for decreases in casein (Schultz, 1977; Kitchen, 1981).Mastitis reduces milk yield and alters milk composition. The magnitude of these changes in individual cows varies with the severity and duration of the infection and the causative microorganisms. Mastitis is almost always caused by bacteria. These microorganisms produce toxins that can directly damage milk producing tissue of the mammary gland, and the presence of bacteria initiates inflammation within the mammary tissue in an attempt to eliminate the invading microorganisms. The inflammation contributes to decreased milk production and is primarily responsible for the compositional changes observed in milk from infected quarters and cows. In general, compositional changes involve an increase in blood components present in milk and a decrease in normal milk constituents (Schroeder, J. W, 1997).

Changes in milk composition accompany the increase in SCC following infection of the mammary gland. Table 1 compares the composition of normal (low SCC) milk with milk having a high SCC. These comparisons frequently are made between high and low SCC milk from opposite quarters of the same cow to reduce cow to cow variation. Elevated SCC is associated with a decrease in the content of lactose and fat in milk because of a reduced ability of the mammary gland to produce these components. Although there may be little change in the total protein content as a result of sub clinical mastitis, there are marked and significant changes in the types of proteins present. The major milk protein is casein. This protein has high nutritional qualities and is very important in cheese manufacturing. Casein content of milk with a high SCC is reduced, but lower quality whey proteins increase in concentration, resulting in similar total protein content. The lower quality whey proteins are blood serum proteins such as serum albumin, immunoglobulins, and transferrin, which increase in milk as a result of the destruction of membranes that normally prevent blood serum proteins from entering milk (Schroeder, J. W. 1997).

Table 5: Interpretations and scoring of the CMT test

Score	Meaning	Description of visible reaction
N	Negative	Mixture remains liquid, homogeneous, with no evidence of thickening.
T	Trace	The slight thickening that forms is seen best by tipping the paddle back and forth and observing the mixture as it flows over the bottom of the cup. Trace reactions tend to disappear with continued rotation of the paddle. Read at 10 seconds.
1	Weak Positive	A distinct thickening of the liquid forms, but there is no tendency toward a gel formation. With some milk, the thickening may disappear after prolonged rotation of the paddle (20 seconds or more). Read at 10 seconds.
2	Distinct Positive	Mixture thickens immediately, and a gel formation is suggested. As the mixture is swirled, it tends to move in toward the center, exposing the bottom of the outer edge of the cup. When the motion is stopped, the mixture level out and covers the bottom of the cup. Read at 10 seconds.
3	Strong Positive	A gel is formed, which causes the surface of the mixture to become elevated like a partially fried egg. There is usually a central peak that remains projecting above the main mass, even after the rotation of the paddle is stopped.

Source :(Schroeder, J. W. 1997)

2.2.2. Nutritional factors

Pasture species influence milk yield and composition, the use of species associated with improved pasture quality results in increased milk, fat and protein yields (Schroeder, 1997).
Increasing the intake of roughage such as grass and sorghum silage usually reduces SNF and milk production. The decrease is largely due to reduced energy or dry matter intake. Increasing energy or dry matter intake usually restores the SNF to normal. Good quality hay tends to increase SNF, but poor quality hay may reduce both intake and SNF. Adding more roughage to the ration has little to no effect on SNF. However, a minimum amount of roughage is needed for normal milk fat percent and health maintenance of the cow.

Fat concentration is the most sensitive to dietary changes and can be altered over a range of nearly 3.0 percent units. Milk protein concentration can also be altered by dietary manipulation. However, compared to the alterations possible in fat concentration the range is much smaller at approximately 0.60 percentage units. The concentrations of lactose and mineral, the other solids constituents of milk do not respond predictably to dietary alterations. Before attempting to alter and improve milk fat and protein production however, it is important to evaluate the potential of a herd to respond to feed management changes (Harris and Bachman, 1985; Rippel, 1997**).**

Nutrition of the cow has also a marked influence on milk composition, particularly, the fat content and SNF. Addition of whole cottonseed to dairy cattle rations may reduce the SNF content of milk. SNF content is relatively high in the first month, drops to a low level in the second, and then rises as lactation progresses. Cotton seed is fat rich seed increased milk fat by 0, 42% (Belibasakis and Tsirgogianni, 1995).Cows grazing on pasture during the spring season produce milk with high SNF content (Rai *et al*., 1980; Barbano and Lynch, 2001). Bovine somatotropin (BST) seems to have an effect on milk constituents. Treatment of cows with Bovine somatotropin for short period of intervals (less than 14 days) resulted in increased fat percentage, but reduced protein content. However, application of somatotropin over the whole lactation periods had no influence on protein, fat, lactose, total solids and solids not fat constituents of milk (Baer *et al*., 1988; Barbano and Lynch, 2001).

Supplementing minerals

The most common method of providing supplemental minerals to cattle is through a protein/energy supplement or through a free-choice mineral supplement. Animal to animal variation in intake is greatest with free-choice mineral supplements. Some cattle consume no supplement, while others may consume as much as four or five times the intended daily amount (Kincaid, 1999).This variation is reduced considerably when minerals are incorporated into protein/energy supplements that are provided on a regular basis. It is important to monitor and record average daily intake of free choice supplements so that the supplement formula can be adjusted if necessary to increase or reduce intake. Cattle will consume salt in excess. This is why salt is used as the base ingredient in free-choice supplements. Phosphorus and magnesium sources are unpalatable and may reduce mineral supplement consumption. When providing a complete free choice mineral supplement, all other sources of salt should be removed from the pasture (Greene, 1999).

2.2.3. Non-nutritional factors affecting milk composition

Breed.

Breeding is of considerable importance, since fat and protein levels in the milk are heritable characteristics. Gains in milk composition made from breeding are permanent and accumulate from year to year. Benefits of sire and cow selection, and of mating decisions made today, will continue to be realized in all future descendants of the herd. In this respect, selection is a very productive means of improving milk composition (Ali and Schaeffer, 1987.Reents *et al*., 1995,1998). The use of breeding to improve milk composition must be clearly understood, since selection to improve one production trait may lead to a decline in another. Selection based on milk yield will result in an increase in milk fat and protein yields, but will reduce fat and protein percentages (Norman; *et al*., 1983; Nielsen *et al*., 2003).

The major milk constituents of different types of lactating animals and different cow breeds are shown in table 1 and 2, respectively (Goff and Griffithis, 2006, Carlos *et al*., 2000; Barbano and Lynch, 2001).

Table 6: Average composition of cow's milk of different breeds

Breed	Fat (%)	Protein (%)	Lactose (%)	Ash (%)	SNF (%)
Ayrshire	3.9	3.4	4.81	0.68	8.89
Brown Swiss	3.30	3.00	5.08	0.72	8.80
Guernsey	3.60	3.20	4.96	0.74	8.90
Holstein	3.40	3.20	4.87	0.68	8.75
Jersey	4.40	3.60	5	0.70	9.30

Source: (Goff and Griffithis, 2006)

Lactation stage

The composition of milk varies with the stage of lactation. Cows that calve in good condition produce milk with a high fat and protein content during early lactation. The percentages of both fat and protein decline during the first six to eight weeks of lactation, and then progressively rise after the cow becomes pregnant to reach their highest levels in late lactation (Reents *et al*., 1995; 1998).

Seasonal effects

Some research results have shown an increase in SNF percent when cows go on spring pasture. Generally, it is believed that the increase was due to an increased energy intake. However, cows receiving adequate to excess energy prior to exposure to pasture will usually show a drop in SNF when shifted to pasture (Reents *et al*., 1995; 1998).

Environmental factors that affect feed intake can be associated with pronounced variations in milk yield and composition. Temperatures consistently above 30^0C will reduce milk yield as well as the percentage of milk protein, because of a reduction in energy intake. Cows in early to mid-lactation and receiving little or no supplementation (that is, relying on high pasture intakes) will be the most affected by heat stress.

Management is one of the factors which affect the quality of milk. Any change in production is the result of feed or management modifications (Meyer *et al*., 1989; Everett *et al*., 1994),

2.3. Milk quality tests

2.3.1. Specific gravity

Specific gravity is the relation between the mass of a given volume of any substance and that of an equal volume of water at the same temperature. Since 1 ml of water at 4 degree centigrade weighs 1 g, the mass of any material expressed in g / ml and its specific gravity will have the same numerical value. The average specific gravity of milk is about 1.032. That means 1 ml of milk weighs 1.032 g at 4 degree Centigrade (Connon, 1993).

Addition of water to milk can be a big problem where we have unfaithful farm workers, milk transporters and greedy. A few farmers may also fall victim of this illegal practice. Any buyer of milk should therefore assure himself/herself that the milk he/she purchases is wholesome and has not been adulterated. Milk has a known specific gravity. When it is adulterated with water or other materials are added or both misdeeds are committed, the density of milk changes from its normal value to abnormal. The lactometer test is designed to detect the change in density of such adulterated milk. Carried out together with the Gerber butterfat test, it enables the milk processor to calculate the milk total solids (TS) and solids not fat (SNF), (Rai *et al*, 1980).

2.3.2. pH

pH is the measure of hydrogen ion concentration. The pH of fresh milk is about 6.8.This pH level gradually decreases to 4.0 due to the production of lactic acid. The acid produced by lactic bacteria will prevent the development of certain putrefying bacteria and actually preserve the milk, although it becomes sour. Lactic acid bacteria themselves can only tolerate a certain degree of acidity. That means, during acidification of milk, various species of lactic acid bacteria may survive the acidity. Normally, acid production stops in milk at pH 4.2 (Eckles *et al*., 1982).

Low pH value can be obtained in milk and milk products by fermentation or addition of acids (inorganic and organic). Paper strips with bromocresol purple and bromothymol blue are sometimes used at milk reception as a rejection test for milk. Bromocresol purple indicator strips change from yellow to purple between pH 5.2 and 6.0, while Bromthymol blue indictor papers change from straw yellow to blue green between pH 6.0 and 6.9.

2.3.3. Titratable acidity

The production of acid in milk is normally termed as "souring" and the sour taste of such milk is due to lactic acid production. The percentage of acid present in milk is a rough indication of its age and the manner in which it has been handled. The acidity of milk is determined by titrataing 10 ml of milk in an equal volume of 0.1N NaOH solution using 0.5 ml of phenolphthalein as an indicator (Connon, 1993).

2.3.4. Water activity

Milk has high moisture content and as the result of this milk spoils very easily within a short period of time. On the water content or water activity of milk is very crucial for bacterial growth. Water activity is index of the availability of water (Connon, 1993).

2.4. Raw milk quality control tests

Accurate sampling is the first pre-requisite for fair and just quality control system. Liquid milk in cans and bulk tanks should be thoroughly mixed to disperse the milk fat before a milk sample is taken for any chemical control tests. Representative samples of packed products must be taken for any investigation on quality (Barbano and Santos, 2006).

2.4.1. Sampling for bacteriological examination

Sampling milk for bacteriological tests require a lot of care. Dippers used must have been sterilized in an autoclave or pressure cooker for at least 15 minutes at 121° C before hand in order not to contaminate the sample. On the spot sterilization may be employed using 70% alcohol.

2. 4. 2. Sampling for chemical tests

Milk samples for butterfat testing may be preserved with chemicals like potassium dichromate (1 tablet or ½ ml 14% solution in a ¼ liter sample bottle is adequate.) Milk samples that have been in a refrigerator or icebox must first be warmed in water bath at 40 ºC, cooled to 20ºC, mixed and the sample then taken for butterfat determination. Other preservative chemicals include Sodium-acid at the rate of 0.08% and Bromopol (2-bromo-2-nitro-1, 3-propanodiol) used at the rate of 0.02%. If the laboratory cannot start work on a sample immediately after sampling, the sample must be cooled to near freezing point quickly and be kept cool till the work can be started. If samples are to be taken in the field e.g. at a milk cooling center, iceboxes with ice packs are useful (Barbano and Santos, 2006).

2. 4. 3. Labeling and record keeping

Samples must be clearly labeled with name of farmer or code number, dates, and places be recorded in standard data sheets. Good records must be kept neat and in a dry place. It is desirable that milk producers should see their milk being tested, and the records should be made available to them if they so require (Fedele, 2001).

2. 4. 4. Organoleptic tests

The organoleptic test permits rapid segregation of poor quality milk at the milk-receiving platform. No equipment is required, but the milk grader must have good sense of sight, smell and taste. The result of the test is obtained instantly, and the cost of the test is low. Milk, which cannot be adequately judged organoleptically, must be subjected to other more sensitive and objective tests.

Procedure: Open a can of milk. Immediately smell the milk, observe the color and consistency of the milk. If still unable to make a clear judgment, taste the milk, but do not swallow it. Spit the milk sample into a bucket provided for that purpose or into a drain basin, flush with water. Look at the can lid and the milk can to check cleanliness.

2.4. 5. Clot on boiling (C.O.B) test

The test is quick and simple. It is one of the old tests for too acid milk (pH<5.8) or abnormal milk (e.g. colostral or mastitic milk). If a milk sample fails in the test, the milk must contain much acid or rennet producing microorganisms or the milk has an abnormal by high percentage of proteins like colostral milk. Such milk cannot stand the heat treatment in milk processing and must therefore be rejected (Connon, 1993, O'Mahony; 1988).

Procedure: Boil a small amount of milk in a spoon, test tube or other suitable container. If there is clotting, coagulation or precipitation, the milk has failed the test. Heavy contamination in freshly drawn milk cannot be detected, when the acidity is below 0.20-0.26% lactic acid.

2. 4. 6. The alcohol test

The test is quick and simple. It is based on instability of the proteins when the levels of acid and/or rennet are increased and acted upon by the alcohol. Also increased levels of albumen (colostral milk) and salt concentrate (mastitis) results in a positive test.

Procedure: The test is done by mixing equal amounts of milk and 68% of ethanol solution in a small bottle or test tube. (68 % Ethanol solution is prepared from 68 ml of 96% (absolute) alcohol and 28 ml distilled water). If the tested milk is of good quality, there will be no coagulation, clotting or precipitation, but it is necessary to look for small lumps. The first clotting due to acid development can first be seen at 0.21- 0.23% lactic acid. For routine testing 2 ml milk is mixed with 2 ml 68% alcohol (Eckles *et al*., 1982; Connon, 1993).

2. 4. 7. Alcohol-Alizarin test

The procedure for carrying out the test is the same as for alcohol test, but this test is more informative. Alizarin is a color indicator. The color changes according to acidity (Connon, 1993).

2. 4.8. Resazurin test

Resazurin test is the most widely used test for hygiene and the potential keeping quality of raw milk. Resazurin is a dye indicator. Under specified conditions resazurin is dissolved in distilled boiled water. The resazurin solution can later be used to test the microbial activity in given milk sample. Resazurin can be carried out as 10 min test, 1 hr test and 3 hr test. The 10 min Resazurin test is useful and rapid screening test used at the milk platform. The 1 hr test and 3 hr tests provide more accurate information about the milk quality, but after a fairy long time. They are usually carried out in the laboratory. Resazurin impacts blue color to milk which when reduced to resorufin changes to pink and finally to white when reduced for complete decolorization, A comparator disc reading a start for value of 4 and above for 10 minutes resazurin test indicate

good quality but value less than 4 poor quality of milk (Connon, 1993; Ombui *et al*., 1995, Teka, 1997, Alehegne, 2004).

2. 4. 9. The methylene blue reduction test

The length of time milk takes to decolorize methylene blue is a good measure of its bacterial content and hence of its hygienic quality. Depending on the duration of time required to decolorize the methylene blue, raw milk can be categorized into different qualities. The shorter time is needed to decolorize the methylene blue the lowest is the quality of the milk tested (Barbano and Santos, 2006).

2. 4. 10. Freezing point determination

The freezing point of milk is regarded to be the most constant of all measurable properties of milk (Specter and Setser, 1994). A small adulteration of milk with water will cause a detectable elevation of the freezing point of milk from its normal values of -0.54ºC. Since the test is accurate and sensitive to added water in milk, it is used to detect whether milk is of normal composition or adulterated. Addition 1% water on milk the freezing point is raised approximately by 0.0055^{o}C (Alehegn, 2004).

2. 4. 11. Inhibitor test

Milk collected from producers may contain drugs and/or pesticides residues. These when present in significant amounts in milk may inhibit the growth of lactic acid bacteria used in the manufacture of fermented milk such as yogurt, besides being a health hazard.

Principle of the method: The suspected milk sample is subjected to a fermentation test with starter culture and the acidity checked after three hours. The values of the titratable acidity obtained are compared with titratable acidity of a similarly treated sample, which is free from any inhibitory substances.

2.5. Public health significance

Milk is one of the important balanced foods for human beings especially for children and also an ideal medium for bacteria growth. Bacteria finding accidental access to milk may give rise to consumer health problem and produce enzymes which affect milk composition synthesis regard all those factors hygienic quality of milk at the point of public health make important.

Raw processed milk is important vehicle of a various pathogens of human. Milk and milk products have significant risk to the consumers because it may is contaminated by any pathogen that at a favorable temperature can multiply and produce toxin (Radiostits *et al*., 1994). Brucellosis is an important disease for human health and causes Malta fever in man. The infection can to occur by consumption of unpasteurized milk (Alehegn, 2004).

Milk and milk products can become contaminated unless good hygiene and adequately pasteurized milk and prevention to control growth of bacteria such as staphylococci because the pathogencity of staphylococcus aureus has been recognized for many years to cause mastitis and skin disease and food born intoxication in milk and milk products (Asperger, 1994).There are so many pathogens that produce toxin. The public health standard for milk ordinance provides chemical, bacteria and temperature standard as well as sanitation requirement for production and processing safe raw pasteurized milk and milk products (Hagstad and Hubbert, 1986).

2.6. Status of bacteriological quality of milk in Ethiopia

Erick, (2001), reported prevalence rate of 46.6% for sub clinical mastitis at cow and 27.8% at quarter level and clinical mastitis of 6.6% at cow and 2.8% at quarter level. Gizat, (2004) recorded prevalence rate of 34.4% and 17.9% for sub clinical mastitis in crossbred and indigenous respectively.

Different studies conducted in Ethiopia on the bacteriological quality of milk taken from cow, goats and camels indicate that various species of were involved in contamination of milk. Abiot (2004) reported that *Staphylococcus* species were the major causes of milk contamination,

accounting for 30.37% of the isolates followed by Enterobateriaceae (23.7%) and *Micrococcus* species (13.33%). According to Mekonnen, (1989), he rate of contamination of raw milk with *Staphylococcus pyogenes*, *Streptococcus agalactiae* and *Corynebacteriun* species were (16%), (13%) and (4%), respectively.

A study conducted on camel milk indicated that similar types of organisms were involved in the contamination of camel milk. Mebratenesh, (2003) reported that *Staphylococcus* species, *Streptococcus* species and coliforms were the prominent bacterial isolates accounting for 43.5%, 19.6% and 17.4%, respectively. *Actinomyces pyogens* and *Bacillus* species have also been isolated at the rate of 6.5%. Similar types of microorganisms were also isolated from goat's milk. Area (2005), reported that *Staphylococcus* species were the major isolates accounting for 22.5%, followed by *Escherichia coli* (*7.9*%), *Pseudomonas aeuroginosa* (7.9%), *Micrococcus species (4.7%), Streptococcus species (3.5%), Bacillus* cereus (3.5%) and *Actinomyces pyogenes* (3.2%).

3. MATERIAL AND METHOD

The present study was conducted in Gondar Zuria and Gondar town, North Gondar Zone of the Amhara National Regional State, from October 2006 to April 2007.

3.1. Description of the study area

Gondar Zuria and Gondar town districts are found in North Gondar Zone of the Amhara Regional State. The districts are located at 12^{o} North and 27.25^{o} East with an altitude ranging from 1800 to 2200 meters above sea level (masl). For both districts, the average annual temperature ranges from 12.3 to 30 ^{o}C with an average annual rainfall of 1000 mm. The rainfall, like in most other parts of the country, has a bimodal pattern (Bureau of Agriculture North Gondar Zone).

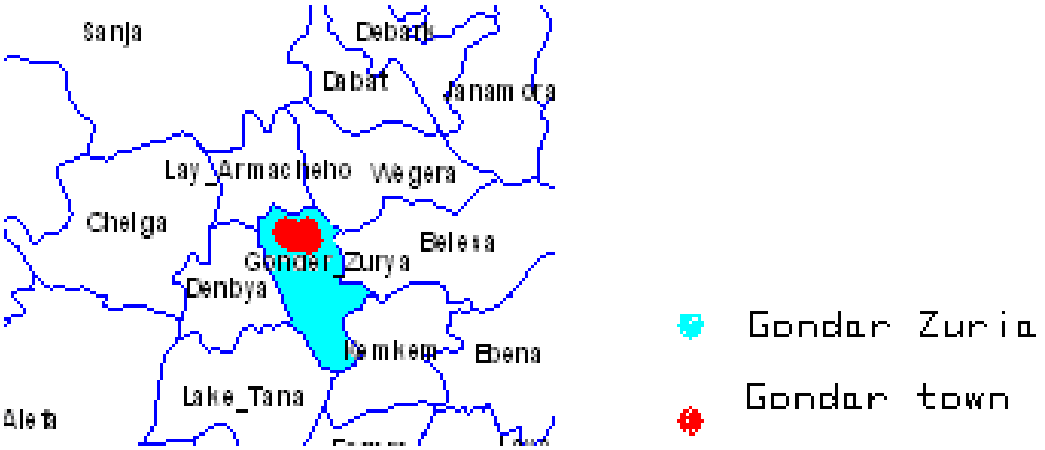

Figure 1: Maps of the study area

Source: Amhara National Regional State Bureau of Finance and Economic Development, 2006.

North Gondar is the home of the Fogera breed and cross breeds. Moreover the dairy sector is relatively well developed in Gondar zuria and Gondar town districts; for this reasons why the districts were considered for the study.

3.2. Sample size calculation

The target populations were milking cows, be it local or cross breeds. Sample size was determined at 10% percent confidence interval and 95% confidence level by using a mathematical formula indicated here below (Thrusfield, 1995). Since there was no previous study on the prevalence of mastitis in the study area, 20% prevalence was used for the sample size determination.

$$N=\frac{1.96^2 \text{ X Pexp X } (1- \text{Pexp})}{d^2}$$

Where

N	=	required sample size
P_{exp}	=	expected prevalence
d	=	desired absolute precision

$$N = \frac{1.96^2 \text{ X } 0.2 \text{ } (1- 0.2)}{0.05^2} = \frac{3.842 \text{ X } 0.2 \text{ X } 0.8}{0.0025} = 245$$

3.3. Study methods

The sampling unit of interest was households. Samples were drawn randomly from the list of farmers owing dairy cows in the two districts. The list was obtained from the respective district's agricultural bureaus.

Structured questionnaire was administered to collect data on husbandry practices, feeding stuffs, breed and blood level of crosses, etc. The questionnaire, which was closed type for its major part was pre tested before administering it. Besides the questionnaire administered by the same interviewer, a one time farm inspection and clinical examination of all cows were conducted at the same time as the interview. Variables inspected and/or measured were breed of the cow, housing condition, feeding practices, general health condition and CMT test. Dairy cows were

identified in to three classes' namely local breed (type1), 50% crosses (type 2), and 75% (type3) or above exotic blood level.

The husbandry system was broadly divided in two based upon housing condition handling facilities general hygienic condition in the farm yard, feeding and watering troughs and practices.

- Satisfactory management practice: refers to management category under which dairy cows were kept in clean barn, had access to adequate roughage and water supply.

- Poor management practice was attributed to conditions where cows were kept under poor hygienic conditions, inadequate water and feed trough space, no or inadequate feed, water and/or concentrate supply.

The feeding systems were grouped in to two.

- Feeding system 1 involved cows grazing on pasture, but which were provided with minor feed supplements (roughage, and brewery residues).

- Feeding system 2: housed cows with adequate supply of roughage supplemented with wheat barn *nuge* cake, cotton seeds and brewery residues.

3.4. Sample Collection for laboratory analysis

Composite milk samples were collected from all lactating cows aseptically, using sterile disposable plastic containers. The milk samples, at the cow side, were tested for evidence of sub clinical mastitis by using California Mastitis Test (CMT). The results of CMT were recorded according to established procedures (Schroeder, 1997). Following this, collected milk sample were transported in ice pack immediately to the Bahir-Dar Food and Bio-chemistry Technology Laboratory.

3.5. Laboratory Analysis

All milk samples were subjected to laboratory analysis at Bahir-dar University Food and Biochemistry Technology laboratory for determination of fat and protein percentage, using Gerber and formol titration methods, respectively.

Determination of fat percentage

Preparation of sample

Fresh milk samples were warmed to approx. 20^{o}C and made homogeneous by pouring back and forth into another vessel. Samples that showed evidence of slight churning by the presence of white flakes were slowly warmed to 34 to 40^{o}C. Samples that appeared not to be homogeneous after the back and forth pouring were rejected.

Milk fat percentage was determined by using the Gerber method, the standard procedure as described by Connon, (1993). 10 ml concentrated sulphuric acid (H_2SO_4) was transferred into the Gerber butyrometer using 10 ml pipette, taking care not to wet the neck of the butyrometer. Following this, 10 ml milk sample was drained slowly into the Gerber butyrometer in such a way, that the vertical axis of the pipette makes an angle of 45^{o} with the vertical axis of the butyrometer. After adding 1ml amyl alcohol, the butyromter was firmly closed with a rubber stopper and turned up and down several times by holding it with both hands using a towel, until no white precipitates or curdles were seen in the mixture. The butyrometer was then put into water bath at 65^{o}C for 5 minutes in such a way that water covers up to neck part of the butyrometer. Following this, the butyrometer was taken out of the water bath and centrifuged at 2000 r p m for 2 minutes. The centrifuge was then brought to stop gradually and the butyrometer was put once again in water bath, at 65 for 5 min.

Reading was taken at the lowest point of the fat meniscus and surface of separation between the fat and the acid after the lower end of the fat column was brought on to the main graduation mark by slightly withdrawing the stopper.

Protein percentage determination

In determining protein percentage, milk sample was first put into water bath at 37 ^{0}C for some minutes.10 ml of this warmed milk was transferred into a transparent glass beaker to which 0.4 ml of saturated aqueous potassium oxalate and 0.5ml of 0.5% phenolphthalein solution were added. The glass beaker was allowed to stand for 2 minutes. 0.1m of NaOH was titrated to the solution, until a weak pink color was observed. Following this, 2 ml of neutral 40% formaldehyde was added. Titration was again done with 0.1m NaOH until a weak pink color of equal intensity was obtained. Protein percentage was then derived by multiplying the number of ml of NaOH used after the addition of the formalin by 1.74.

Protein % = Formol titer X 1.74

3.6. Data storage and statistical analysis

Microsoft Excel and SPSS 11.0 were employed for data entry for windows computation.

Regression linear was used to any liner relationships between outcome and explanatory variables Descriptive statistics by explain proportion and percentage.

4. REULTS

4.1. Results of questionnaire survey, farm inspection and animal examination

A total of 105 smallholder dairy farmers participated in the study and the total number of milking cows examined were 236. All farms included in this study were having 1 to 8 indigenous and crossbreed lactating cows.

The smallholder farms headed by females represented 20%. Age range varied from 35 to 70 years with average age of 45. The large majority (95%) of the smallholders were married. Almost all smallholders were also engaged in other income generating activities.

Management practices were qualified as poor in 63.8% of the smallholder farms, while in the remaining 36.2% of the farms it was judged as satisfactory. Feedstuffs were, primarily, constituted of grazing; supplemented with *nuge cake*, wheat barn and brewery by products. Mastitic cows managed under feeding system 1 and 2 categories were respectively 62 (26.3 %) and 174 (73.7%).

The milkers wash their hands with cold water and without the use of boiled water only at the beginning of milking and they do not wash their hand between milking and did not dry their hand. The result of present study show dairy cow owners cleaned their cows' udder only with cold water and did not perform the cleaning sufficiently and properly using towels correctly.

The total number of cows that were CMT positive was 72 in sub clinical mastitis representing 39 numbers of smallholders. This shows that mastitis prevalence at cow and heard levels were respectively 30.5% and 37.1 % where clinical mastitis was at animal based 2.6% which were not included by laboratory determination of fat and protein.

4.2 Results of laboratory analysis

Table 7 illustrates the average values of protein and fat in normal and mastitic milk samples. Cows which tested positive for CMT had reduced values of milk fat and protein. Fat and protein were found reduced by 15.4% and 8.2% respectively. There were statistically significant differences between CMT- and CMT+ animals for both fat (P=0.019) and protein (P=0.043).

Table 7: Change in milk protein and fat concentration due to sub clinical mastitis

Units	Normal milk (n = 164)		Milk with CMT + (n = 72)		
	Average	SD	Average	SD	
Protein	3.7	0.49	3.4	0.57	91.8%
Fat	3.9	0.93	3.3	0.75	84.6%

Table 8: Show the fat and protein percentage, average values of healthy and mastitic cows managed under two different feeding statuses. The combination of poor nutritional status with mastitic conditions appeared to have more negative impact on the fat and protein percentages of milk. Good level of nutrition on the other hand had some positive role in reducing the level of fat and protein in mastitic cows. The differences were statistically significant (P=0.008) and (P=0.05).

Table 8: Milk protein and fat concentration in healthy and mastitic cows of different feeds status

Feed	CMT+ cows Protein %	CMT –cows Protein %	CMT + cows Fat%	CMT – cows Fat%
Feed 1 (n = 62)	3.4	3.5	3.2	3.3
Feed 2 (n =174)	3.5	3.7	3.5	3.9

Figure 2: shows the effect of mastitis on cow milk fat and protein concentrations in animals with different feeding regimen.

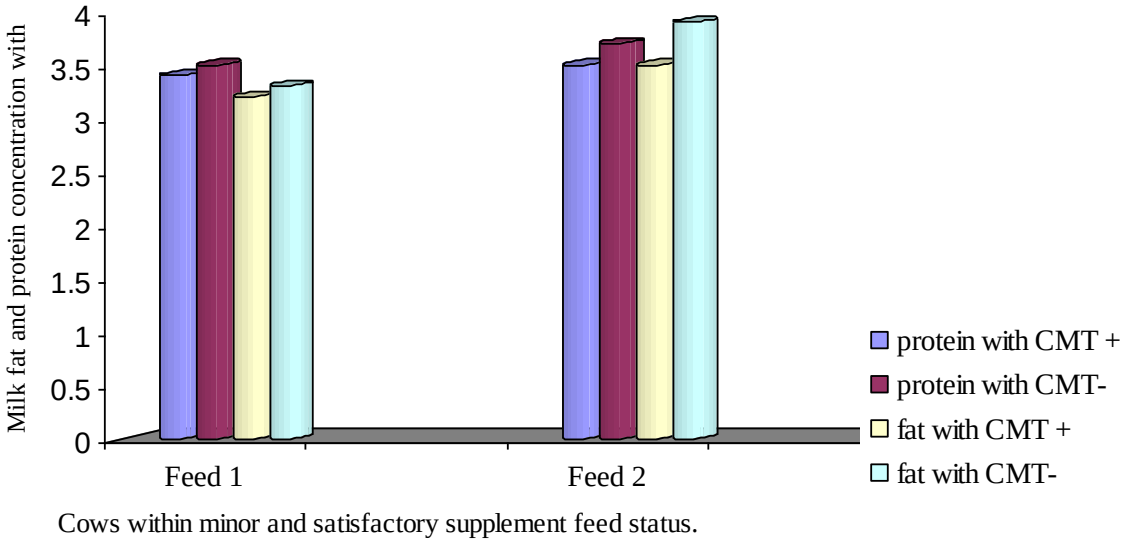

Figure 2: Change in milk fat and protein concentrations in healthy and mastitic cows of different feeding status

Table 9: illustrates the combined effects of breed and sub clinical mastitis on fat and protein concentration of milk. Milk concentration of mastitic cows, for both fat and protein, were found lowest in breed 2 groups (50% crossbreeds) as compared to the two other breed categories.

Table 9: Average of milk fat and protein concentration in healthy and mastitic cows of different breeds

Milk composition	type 1 (n = 49)	type 2 (n = 51)	type 3 (n = 136)
CMT + cows Protein %	3.5	3.4	3.5
CMT - cows Protein %	3.8	3.6	3.7
CMT + cows Fat %	3.4	3.0	3.4
CMT – cows Fat %	4.0	3.9	3.8

The bar graph shown in Figure 3 illustrate the variability in milk fat and protein concentration between breeds and udder health where (P=0.031) fat concentration differ significantly and protein (P=0.024).

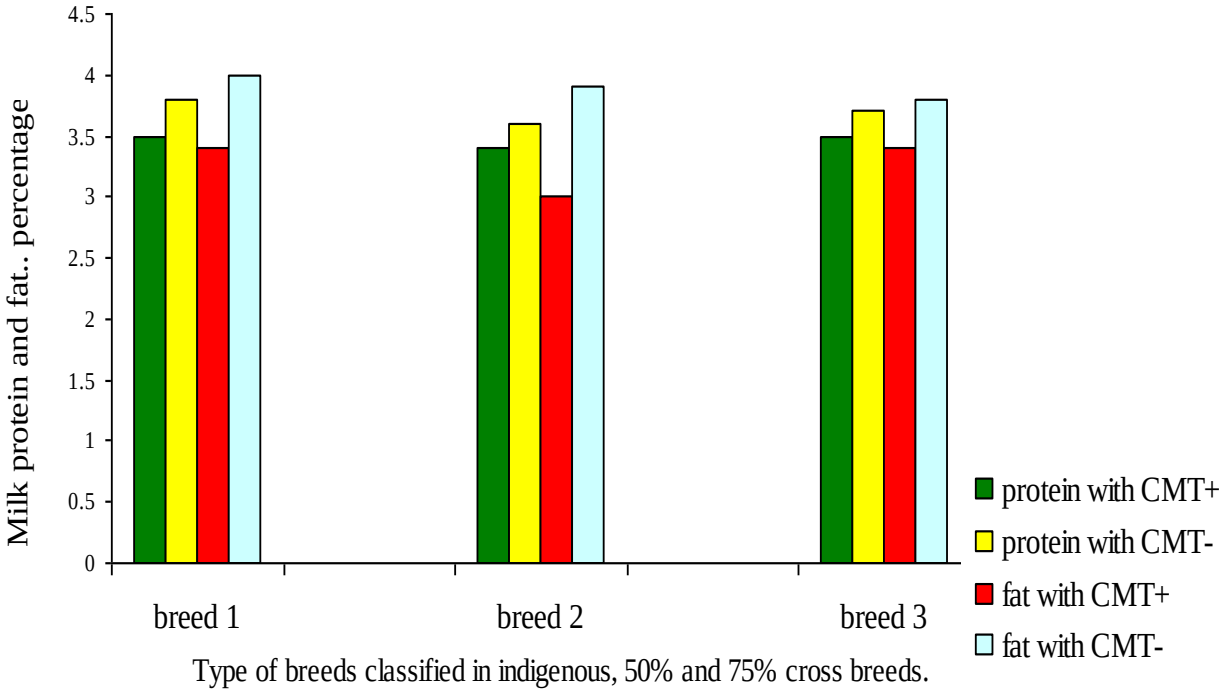

Figure 3: Average fat and protein concentration in healthy and mastitic cows of different breeds

Table 10 gives the combine effects of sub clinical mastitis and management level on milk fat and protein concentrations. Improved management appeared to raise the level of concentration of fat and protein in healthy udders. In mastitic cows, however, no further depression, in the level of concentration, was registered due to poor managements.

Table 10: Average concentration of milk protein and fat in mastitic and healthy cows of different management level

	CMT + cows Protein %	CMT - cows Protein %	CMT + cows Fat %	CMT – cows Fat %
Manage 1 n = 151	3.4	3.6	3.3	3.6
Manage 2 n = 85	3.5	3.8	3.5	4.1

Figure: 4. shows changes in milk fat and protein percentage associated with mastitis and management levels. Normal and mastitic milk fat (P=0.004) and protein differs significantly (P=0.01).

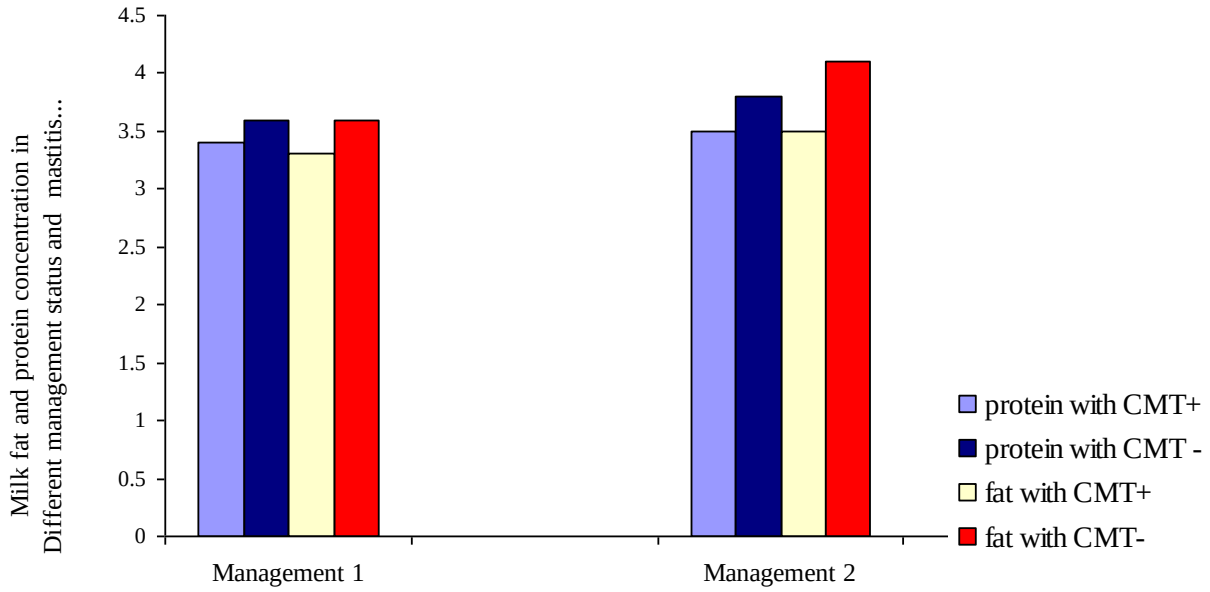

Figure 4: Milk fat and protein percentage in mastitic and healthy cows of different management levels

4.3. Mathematical equation for predicting fat and protein percentage in liquid milk

The regression analysis taking in to account different breeds allowed the fitting of linear model using the following linear regression model respectively Y= a + bx.

Table 11: simple regression equation and correlation analysis results

Breed	n	Equation	r	p-value	% Within±15% of measured value
Indigenous (type 1)					
Fat	49	Y = 3.663 + 0.057x1	0.058	P= 0.041	88.6
Protein	49	Y = 3.567 + 0.125x1	0.101	P= 0.022	94.2
50% crosses (type 2)					
Fat	51	Y = 3.663 - 0.003x2	-0.018	P= 0.19	88.6
Protein	51	Y = 3.567 + 0.033x2	-0.058	P= 0.57	97
75% (type 3)					
Fat	136	Y = 3.663 + 0.003x3	-0.033	P= 0.041	85.7
protein	136	Y = 3.567 + 0.039x3	-0.035	P= 0.08	88.6

Where Y are the estimated protein and fat percentage whereas X1, X2, X3 are their respective breeds and B are the beta value where A is the constant.

A single linear regression equation did not fit for the whole data taken singly. The equation was found to best estimate the actual fat and protein concentration within the corresponding breeds which is showed in figure 5.

A= Difference between estimated and observed value of protein within interval 1 to 15 %

B= Difference between estimated and observed value of protein above 15 %.

C= Difference between estimated and observed value of fat within interval of 1 to 15 %.

D = Difference between estimated and observed value of fat above 15 %.

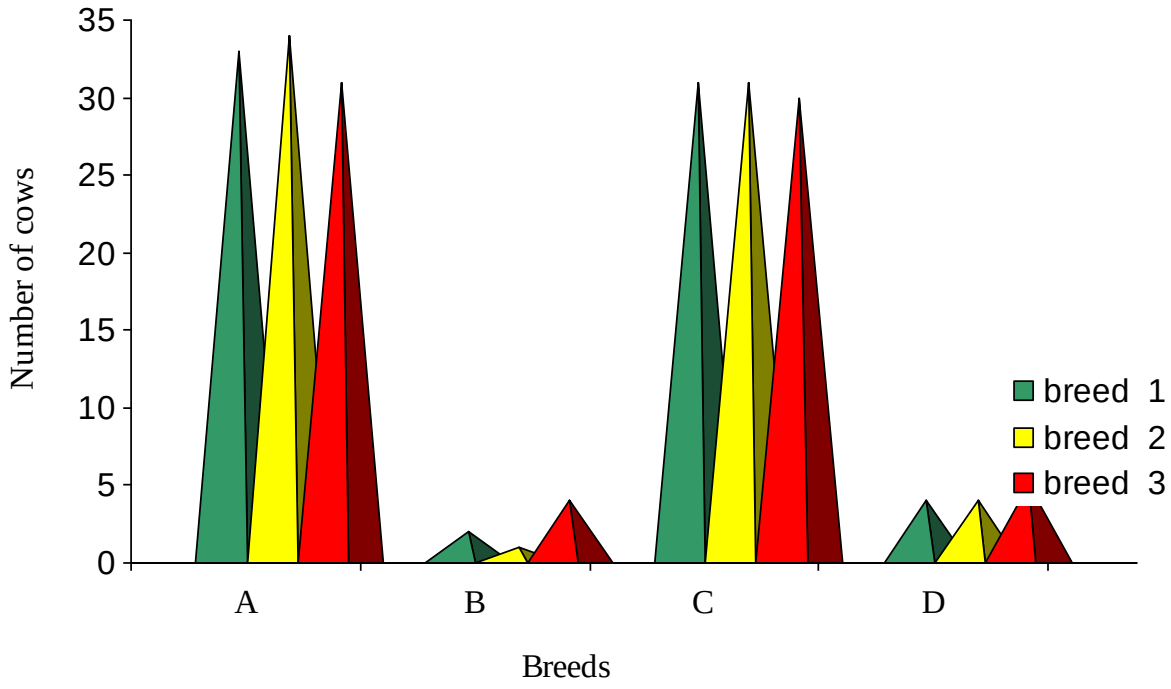

Figure 5: Difference between estimated and observed value of protein and fat percentage

5. DSCUSSION

Mastitis prevalence at heard (37.7%) and cow level (30.5%) by were quite high. This high mastitis prevalence could be associated with milking poor hygienic practices witnessed during farm inspections.

Dairy cow owners were cleaning their cow's udder only with cold water and not perform the cleaning sufficiently and properly using towel Galton *et al*., (1986) report pre-milking udder preparation plays an important role in the prevention of contamination of milk during milking.

The result of the present study shows that the fat percentage of milk drawn from mastitic and non mastic cows varied significantly (P=0.019). Likewise, the fat percentage between cows kept on pasture and those which were provided with supplemented feed differed significantly (P=0.008). Significant difference (P=0.031) was also recorded between breed type and between management status (P=0.004).

Protein concentration differed significantly (P=0.043) between infected and uninfected cows; between breeds (P=0.024); between poor and satisfactory management status (P=0.01) and also between cows grazing on pasture and those provided with supplement feed items (P=0.05).

Our result is in agreement with the findings of Harmon, (1994) and Schroeder, J. W. (997) who reported that cows manifesting mastitis with high somatic cell count (SCC) revealed reduced fat and protein concentration than uninfected cows. The significant reduction of cow milk fat and protein, more likely reflects the effect of mastitis and the degree of alteration of milk constituents due to mastitis is influenced by severity of the udder infection.

Laboratory result indicate that out of the 236 milk samples tested, the proportion of cows with mastitis which revealed an average of 3.3 fat percentage was 15.4 %, while those of non mastitic cows with 3.9 fat percentage was 84. 61 %. Likewise, the proportion of mastitic cows with 3.4 % protein percentage was 8.2 %, while those of none infected cows with protein percentage of 3.7 was 91.9 %.

Harmon, (1994) and Schroeder, J. W. (1997) indicated that plasmin increases the proteolytic activity by more than two fold during mastitis. Plasmin and enzyme derived from somatic cells cause extensive disintegration to casein. As the result of this, blood protein in milk increases, resulting in the destruction of membranes that normally prevent blood serum proteins from entering in to the milk (Harmon, 1994). Auldist and Hubble (1998) also report on decrease in milk fat during mastitis, but Bruckmaier and Blum, (2004) report on an increase in milk fat and this is totally different from the result of the present study as well as the findings of other investigators (Auldist and Hubble, 1998; Jones, 1998; Harmon, 1994) yet total fat production declines with the decrease in milk production. Probably, the decrease in milk constituents is further complicated by the effect of factors such as feed, management and breeds. Beside Bruckmaier and Blum seed change in the total protein content as a result of mastitis, there are marked and significant changes in the types of proteins present. The major milk protein is casein which is decrease with high SCC this report of Bruckmaier and Blum, (2004) is an agreement of the present study

As per the results of the present study milk fat and protein percentages of cows kept under satisfactory management with supplement was higher than cows kept under poor management with minor feed supplement. This is in agreement with the reports of Meyer *et al*., (1989) and Everett *et al*., (1994).these authors indicated that poor management and lack of feed cause not only reduced milk production but also decreased concentration of milk constituents.

Management, which includes provision of adequate feeding and watering trough space, the hygienic status of the barn, including the cow and its health status plays decisive role in influencing milk composition.

Present study shows that poor management predisposes dairy cows to sub clinical mastitis, which in turn results in reduced milk fat and protein percentage.

Even though mastitis was also present satisfactory management system the reduction in milk fat and protein percentage was not as high as in the case of poor management system.

Healthy cows kept under satisfactory management system had higher milk fat and protein percentage than those cows affected with mastitis under the same managements system.

Rai *et al*., (1980), Barbano and Lynch, (2001) reported, that cows grazing in the field with little supplemental feed had low milk fat and protein percentages, when compared with cows provided with supplements. Our study also showed that mastitis positive cows kept on pasture had the lowest milk fat and protein percentage than cows which were given supplements.

The result of this study show indigenous breeds have higher protein and fat concentration than 75% and 50% cross breeds. On the other hand 50% cross breeds showed lower fat concentration cows than 75% cross breeds. The latter might be due to the fact that dairy owners were gives more feed to 75% cross breeds in order to obtain more milk for consumption and to sell the surplus to cover family expenditures. The reduced milk protein and fat concentration in mastitis positive 50% cross breeds could thus be attributed to poor management. Goff and Griffithis, (2006) also reported considerable variations in milk composition due to breed effects.

Body condition and udder hygiene were assessed during milk sample collection Jackwood, (1994). Body condition scoring (BCS) is a useful management tool for distinguishing differences in nutritional needs of beef cows in the herd. This system uses a numeric score to estimate body energy reserves. Monitoring body condition using the BCS system is an important managerial tool for assessing production efficiency Beverly, (1985). The majority members of the BCS level lies on average scoring between 2 and 3 cover 78% ; 74% of the cows had 2-5 calves and 85% of the cow give 2-6 litters of milk.

Annexe 2 in place of regression indicate as improve cross breed level and in high SCC decrease protein and fat concentration where breed and mastitis are independent variable and protein; fat are dependent variables respectively that are to mean the value of dependent variable depends on the value of independent variable. Whereas in improved daily feed intake and livestock management status there is increase milk protein and fat concentration.

The result of linear modeling analysis indicate that in breed 1(indigenous) milk protein increase by 0.154 compared to 50% cross breeds. Cows kept on grazing pasture with minor supplement

had protein content decrease by 0.129. There was also decrease in milk protein in CMT positive cows by 0.142. Fat percentage increase by 0.133 in indigenous breeds than 50% and 75% cross breeds; decreased by 0.192, 0.199 and 0.151 in cows grazing on pasture, in cows kept under poor management and in mastitic cows, respectively.

Among risk factors, sub clinical mastitis very significantly affected the fat and protein concentration of the milk tested. Dairy owners were not much aware of sub-clinical mastitis, but focused more on the treatment of the clinical mastitis.

6. CONCLUSION AND RECOMMENDATION

Based on the finding of this study sub clinical mastitis and others production factors such as breed, feed and management resulting in changes in milk constituents. Sub clinical Mastitis was found to decrease fat and protein percentages of milk by 15.4% and 8.2%, respectively.

Regarding breed effect, indigenous breeds had higher milk fat and protein percentage changes than crosses and/or high grade cattle. The higher the quality of management the higher the quality of milk will be. The study also shows that milk composition is strongly related with breed, management and feeding system accompanied with sub clinical mastitis.

Feeding status affects milk fat and protein percentage. Where improved daily feed intake was found to increase milk fat and protein percentages However; the positive effect of improved nutrition was partly nullified under mastitic conditions.

Based on the above conclusion the following recommendations are forwarded:

1 Feeding status and management systems should be improved to increase the fat and protein content of milk.

2 Dairy owners should improve milk yield and composition through selective breeding and by applying good management practice, feeding system and control of mastitis.

3 Milkers must wash their hands between milking intervals to avoid contamination, clean appropriately the udder of the cow and also dry using clean towel.

4 Dairy owner should give supplement feed, especially during lactation period and keep 50% cross breeds indoors as well as 75% cross breeds.

5 Clean the barn adequately; construct feed and water trough with enough space based on the number of cows.

6 Dairy owners shall seek advice from veterinarians how to prevent and control mastitis in their farms.

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8. ANNEXES

ANNEXE 1

Farm Number ----------

Date ----------

I. **FARM IDENTIFICATION**

Owners Name ---------- Sex ---------- Age---------- Profession ----------

Level of Education Address Other Activities ----------

Illiterate ---------- Woreda ----------

Elementary ---------- Kebele ----------

High School ---------- House No. ----------

College ---------- Tel No. ----------

II. DISCUSSION WITH THE FARM OWNER

1. Date of farm started ---------- origin of animals ----------
2. Number of animal started ----------Number of animal at present ----------
   - Calf: Male ----------
   - Female ----------
   - Heifers: ----------
   - Breeding Bull: -------
   - Cow: ----------

3. Breeding System used: AI ☐ Natural ☐ Both ☐

If natural mating breeding bull is: own ☐ Hired ☐

4. Replacement animals are: Reared on the farm itself ☐ Purchased form outside ☐

5. Was there a practice of introducing new animal to your herd? Yes ☐ No ☐

If yes; source of animal: purchase form market ☐ Dairy farm ☐

6. Examination made before mixing the new animal: Clinical exam

Laboratory exam. For mastitis ☐ other lab exam ☐

7. Feeding system: Grazing ☐ Stall feeding ☐ Both ☐

a. Roughage: Farm produced ☐ Purchase ☐ Both ☐

b. Concentrate: Farm produced ☐ Purchase ☐ Both ☐

8. Commonly used feed staffs:

_____

9. Health service provided: Vet ☐ AHA ☐ Traditional healers ☐ Owner ☐

If by vet or AHA: How often: when called ☐ periodically ☐

10: Who takes care of the animal?

╬ Feeding Owen ☐ Family worker ☐ Laborer ☐

╬ Cleaning barn Owen ☐ Family worker ☐ Laborer ☐

╬ Milking Owen ☐ Family worker ☐ Laborer ☐

11. Is there any practice of record keeping: yes ☐ No ☐

If yes: Breeding recodes ☐ Health record ☐ Feeding record ☐

Production record ☐ Financial record ☐ Others ☐

12. Routine prophylactic and deworming practice yes ☐ No ☐

Deworming: as per established schedule ☐ as needed ☐

13. Culling practice: yes ☐ No ☐

If yes:

╬ Common causes of culling: Health problem ☐ Space shortage ☐ Feed ☐

Shortage ☐ Reproduction problem ☐ Low production level Others ☐

╬ Method of removal: Selling ☐ Death ☐ Slaughter ☐

III. FARM INSPECTION AND ANIMAL IDENTIFICATION

1. Housing

1.1. Housing: closed type ☐ Semi open ☐ Open ☐

1.2. Floors: concrete ☐ Stone ☐ Soil ☐

1.3. Roof: Metal Sheet ☐ Grass ☐ Other (specify)_____

1.4. Drainage (slope): Good ☐ Satisfactory ☐ Poor ☐

1.5. Maternity pens: Yes ☐ No ☐

If yes: Hygienic condition: excellent ☐ Satisfactory ☐ Poor ☐

2. Farm cleanness: Excellent ☐ Satisfactory ☐ poor ☐

3. Feeding trough space/Animal: Excellent ☐ Satisfactory ☐ poor ☐

4. Water trough space/ Animal: Excellent ☐ Satisfactory ☐ poor ☐

5. Feed & and water though cleanness: Excellent ☐ Satisfactory ☐ Poor ☐

6. Hay & concentrate storage condition: Excellent ☐ Satisfactory ☐ poor ☐

ANIMALS EXAMINATION										
	Date of birth	BCS	Parity	Exotic Blood Level	Last Calving Date	Milk Yield	Mammary gland	General health	Previous health problems	Sample collected from
COW1										
COW2										
COW3										
COW4										
COW5										
COW6										
COW7										

ANNEXE 2

Dumification, of variables where dependant variable is protein, content in sub clinical mastitis.

Predictors: (Constant), If 1, case health dum1, If 1, case breed1, If 1, case management dum1, If 1, case breed2 dum, If 1, case feed dum1

Model	Coefficients Unstandardized Coefficients B	Std. Error	Standardized Coefficients Beta	t	Sig.
Constant	3.786	.058		65.194	.000
If 1, case breed1	.199	.086	.154	2.309	.022
If 1, case breed2 dum	4.862E-02	.086	.038	.564	.573
If 1 case breed3	-.124	.070	-.117	-1.759	.080
If 1, case feed dum1	-.153	.087	-.129	-1.766	.079
If case 1, feed2	.155	.087	.130	1.782	.076
If 1, case management dum1	-.193	.077	-.176	-2.488	.014
If case 1, magt2	.196	.078	.179	2.528	.012
If1,casehealth dum1	-.162	.078	-.142	-2.084	.038
If case 1, Hlth0	.160	.078	.140	2.049	.042

Dependent Variable: protein content in sub clinical mastitis

Dependant variable is fat content in sub clinical mastitis.

Predictors: (Constant), If 1, case health dum1, If 1, case breed1, If 1, case management dum1, If 1, case breed2 dum, If 1, case feed dum1

Coefficients

	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
1 (Constant)	4.023	.099		40.837	.000
If 1, case breed1	.300	.146	.133	2.051	.041
If 1, case breed2 dum	.189	.146	.085	1.296	.196
If 1 case breed3	-.245	.119	-.132	-2.057	.041
If 1, case feed dum1	-.399	.147	-.192	-2.707	.007
If case 1, feed2	.401	.147	.193	2.720	.007
If 1, case managmentdum1	-.378	.131	-.199	-2.878	.004
If case 1, magt2	.381	.131	.200	2.903	.004
If 1, case healthdum1	-.300	.132	-.151	-2.274	.024
If case 1, Hlth0	.298	.132	.150	2.264	.024

Dependent Variable: fat content in sub clinical mastitis

9. CURRICULUM VITAE

1: Personal detail

Name: Yeshimebet Chanyalew Getahun Date of birth: 29-08-1969

Place of birth Addis Ababa Sex: Female

Marital status: Married Nationality: Ethiopian

Address Gondar Ethiopia Tele----------

2: Educational background

Elementary school: Jerusalem commercial school Addis Ababa- 1974-1976

Nigat kokeb (Morning star) commercial school Addis Ababa- 1977-1980

Cuba June 21 Secondary school- 1980-1982

Cuba Mengistu Hailemariam preparatory school- 1983-1988

3: professional level

Havana University in D.V.M 1986-1991

4: Special training

Veterinary public health for 1 month

Management and gender issue for 5 days

5: Work experience

As veterinarian 1992-2002

Animal and fish production team leader 2003-2005

6: Research work

Application of attenuated vaccine of Babesia bovis in calves of six months Havana Cuba.

7: Hobbies and interests

Reading books and literature

Visiting historical places, monastery of churches

Participate in social ceremonies

Helping disabled people.

10. SIGHNED DECLARATION SHEET

I, under signed, declare that this thesis is my original work and has not been presented for a degree in any university and that all sources of material used for the thesis have been duly acknowledged.

Name. Yeshimebet Chanyalew…….
Signature ……..
Date of submission ……..

This thesis has been submitted for examination with my approval as an academic advisor.

Dr Mekonen H/Mariam ……

Dr Girma Zewde……