# PhD THESIS


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# SOME ASPECTS OF UDDER HEALTH RELATED TO MILK QUALITY AND GRAVITY OF MASTITIS

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# SOME ASPECTS OF UDDER HEALTH RELATED TO MILK QUALITY AND GRAVITY OF MASTITIS

értekezés doktori (PhD) fokozat elnyerése érdekében

Írta:

**PONGRÁCZ LÁSZLÓ**

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a Szigorlati Bizottság elnöke

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## ABSTRACT

Somatic cell count (SCC) and milk yield from the Livestock Performance Testing Ltd., Gödöllő (Hungary) of Hungarian Spotted and red Holstein Friesian cows were used for statistical analyses. Means of 305 days milk production and SCC of lactations were calculated. Herd-year-season-calving age at first calving effects were not taken into consideration. Biometric calculation were processed by Microsoft Excel 97, BIO MATE and Statistica softwares. The maximum milk yield can be obtained at $n_{lact}$=3.88. It reflects the importance of longevity and lifetime performance. Correlation of milk yield and number of lactation was $r_f$=0.88, correlation of transformed somatic cell count (called SCS) and number of lactation was $r_f$=0.93 while correlation of milk yield and somatic cell count was $r_f$=-0.12.

Then the bacteriological status and some screening methods were studied. Flow cytometric technique may develop as a good alternative or supplementary tool to evaluate udder health. Further studies are required to establish discrimination limits.

Research increasingly demonstrates that selection for mastitis resistance is simultaneously necessary and potentially effective.


## A TŐGYEGÉSZSÉGÜGY NÉHÁNY VETÜLETE, KÜLÖNÖS TEKINTETTEL A TEJMINŐSÉGRE ÉS A TŐGYGYULLADÁS MÉRTÉKÉRE

## (KIVONAT)


A szerző dolgozatában az Állattenyésztési Teljesítményvizsgáló Kft. (Gödöllő) adatai alapján értékeli magyar tarka és vöröstarka holstein-fríz tehenek 305 napos tejtermelését valamint a termelt tej (logaritmikus transzformációval nyert) szomatikus sejt(pont)számát. Az állomány-év-évszak-első ellési kor hatásait figyelmen kívül hagyták. A biometriai számítások a Microsoft Excel 97, BIO MATE és Statistica programok használatával történtek. Maximális tejtermelést átlagosan a 3,88. laktáció körül várhatunk, ami a hosszú hasznos élettartam fontosságát hangsúlyozza. A 305 napos tejtermelés és a laktációk száma között $r_f$=0,88, míg a szomatikus sejtszám (illetve a logaritmikus transzformációval nyert sejtpontszám) és a laktációk száma között $r_f$=0,93 volt a korreláció. A tejtermelés és szomatikus sejtszám között ugyanez $r_f$=-0,12.

A továbbiakban a bakteriológiai állapotot és néhány gyakorlati vizsgálati módszert tanulmányoztak. Az áramlásos sejtanalízis alkalmazása kiegészítő eljárás lehet és a tőgyegészségügyi helyzet javításához vezethet. E téren még további vizsgálatokra van szükség.

Habár a tőgygyulladás visszaszorítására történő szelekció csekély mértékű, de mindenképpen szükséges, mert a tőgyegészségügyi állapot javításának területén vannak még teendőink.

# LIST OF SYMBOLS AND ABBREVIATIONS

| Symbol or abbreviation | Quantity or term | Unit |
|---|---|---|
| AMS | automatic milking system | |
| BLUP | best linear unbiased prediction | |
| BTSCC | bulk tank somatic cell count | cells/ml |
| CMT | California Mastitis Test | |
| CNS | Coagulase Negative Staphylococci | |
| CV% | coefficient of variation | |
| DCC | differential cell count | |
| DCS | differential cell stain | |
| DMSCC | direct microscopic somatic cell count | |
| ESCC | electronic somatic cell count | |
| $F_1$ | first generation | |
| FCM | flow cytometry | |
| g | gravitation | |
| HF | Holstein Friesian | |
| IDF | International Dairy Federation | |
| IMI | intramammary infection | |
| FL (HL) | front (hind) left (quarter) | |
| FR (HR) | front (hind) right (quarter) | |
| LSCS | lactation somatic cell score | |
| log | logarithm | |
| LP | lactoperoxidase | |
| M. | Mycoplasma | |
| NMC | National Mastitis Council | |

| | | |
|---|---|---|
| NS | not significant | |
| P | level of significance | % |
| PTA | predicted transmitting ability | |
| PTASCS | predicted transmitting ability for somatic cell score | |
| $R_1$ , $R_2$ ... | first, second ... re-cross generation | |
| $R^2$ | correlation coefficient | |
| REL | reliability | |
| $r_f$ | phenotypic correlation | |
| rpm | rotation per minute | |
| S. | Staphylococcus | |
| SCC | somatic cell count of milk | cells/ml |
| SCS | somatic cell score | |
| SD | standard deviation | |
| Str. | Streptococcus spp. | |
| TPC | total plate count | |
| x | mean | |
| | | |
| ~ | approximately | |
| = | is equal to | |
| > (<) | more than (less than) | |
| ≥ (≤) | is equal or more (less) then | |

# 1. CONTENTS

# 2. INTRODUCTION

Despite considerable research on bovine mastitis the disease still remains a relevant problem to the dairy industry. Losses are estimated due to reduced production, increased replacement costs, discarded milk, drug costs, veterinary fees and labour costs (DeGraves and Fetrow, 1993). Additional costs that are seldom mentioned are incurred by the processing industry in terms of reduced cheese yields and consumer acceptance (Heeschen et al., 1985; Barbano et al., 1991; Barbano, 1999).

While it has done a great job in mastitis control in producer herds the need to control mastitis is increasingly driven by the consumers of milk and milk products. Large numbers of consumers from all countries of the world are demanding dairy products that are wholesome, nutritious, safe and produced from healthy cows. The international trade in dairy products will likely continue to increase and governments will need to know that the quality and safety of imported products meets or exceeds their internal requirements. These quality and safety demands of the international trade also continue to apply pressure on producers in many countries to control mastitis (Rasmussen, 1999; Smith and Hogan, 1999).

Furthermore, the concept that mastitic animals are diseased people believe that mastitis is an animal welfare issue, too (Rasmussen, 1999; Smith and Hogan, 1999).

Dairy products possess a wide range of beneficial nutritional and sensory properties but this changes due to the composition of milk. Because correlation was found between some properties of milk and prevalence/gravity of mastitis a number of tests and methods have been developed for detecting the disease. Most tests estimate the somatic cell count (SCC) of a milk sample (Kitchen, 1981).

Milk somatic cells are primarily leukocytes coming from the blood. Some epithelial cells are shed from the lining of the mammary gland. Leukocytes accumulate at the inflamed site to combat invading bacteria. This indigenous antibacterial effect of the healthy udder is activated by the inflammatory and immune system. The defence system of the udder consists of not only the

chemical-cellular but also the anatomical-mechanical mechanisms (Korhonen and Sandholm, 1985).

According to an internationally accepted standard the cell count for "normal" milk is nearly always less than 200,000 cells/ml. Higher counts are considered "abnormal" and indicate probable infection, and are also associated with decreased production. Factors such as late lactation, age, environmental stress, milking technique and hygiene or genetical disposition may cause elevations of SCC. However, such increases are inconsequential when compared to the elevation that results from infection (Harmon, 1994).

SCC is widely used to predict the mammary health status of quarters and cows as a measure of the prevalence of mastitis in a dairy herd, safety and suitability of raw milk for human consumption, and is also used by regulatory agencies as an indicator of the wholesomeness and monetary losses to producers due to mastitis (DeGraves and Fetrow, 1993; Heeschen, 1996; Barkema et al., 1999; Hillerton, 1999; Smith and Hogan, 1999). Nearly all developed countries have adopted upper regulatory limits for SCC in milk. Hungary, the EU and Nordic countries, Switzerland, New Zealand, Australia etc. all accepted 400,000 cells/ml as the upper limit but already discussing lowering it to 300,000 or perhaps even 250,000 cells/ml. Canada has agreed on 500,000 cells/ml throughout all of the provinces and is discussing the possibility of going to 400,000 cells/ml. The limit for SCC in the USA is 750,000 cells/ml but this will be also officially reduced.

Many countries are able to determine a national average SCC based on all registered/evaluated producers in the country. These averages have been in general declining over the past 10 years and indicate considerable progress in control of subclinical mastitis or increased ability to control/manage the SCC of the herd bulk milk. In countries it is less than 200,000 cells/ml clearly indicates that producers can control the technical, environmental and hygienical, biological and genetical effects caused subclinical or clinical mastitis (Dohy, 1984; Iváncsics et al, 1996; Dohy, 1999).

The demand of basic and advanced research on mastitis shows an upward tendency worldwide. The National Mastitis Council (NMC) in the USA established a National Mastitis Research Foundation (NMRF) because of:

- a lack of funds for needed mastitis and udder health research,
- few research groups in the USA and the world focusing on mastitis,

- scientists being lost to other research areas, and mastitis not attracting enough new, capable people due to inadequate funding,
- a rapidly changing dairy industry being adversely affected because current efforts do not produce answers to problems which arise under modern management methods,
- consumer and consumer advocate concerns about what is in food.

The goal is to provide consumers with consistently high-quality, good tasting dairy products free of chemical and pathogenic contaminants (www.nmconline.org/nrmf/nmrfinfo.htm, 2001). This phenomenon can be accounted for people working in different sectors of the Hungarian dairy industry, too.

As a result of the facts discussed above, this Dissertation meets a long-felt want since reports on similar experiments were carried out in areas affecting udder and cow health and milk quality in order to protect the image of milk as one of nature's most complete foods.

## Aims

This work aims to study the genetic and bacteriological aspects of udder health and to carry out applied research related to milk quality.

Special attention follows:

- the role of some factors (environment and sire) on milk production and quality of milk (somatic cell count) in different stocks (Holstein Friesian and Hungarian Spotted),
- evaluation the effect of genotype – resistance (within and across breeds),
- examination of the bacteriological status,
- the comparison of some screening methods,
- the applicability of flow cytometry.

Thereafter, experiments will be conduct to identify the substances responsible for the observed effects according to regular and advanced technology of cattle breeding and milk production.

# 3. LITERATURE REVIEW

## 3.1. FUNDAMENTAL PRINCIPLES OF MASTITIS

### 3.1.1. Definition of mastitis

As it is known, mastitis is an inflammation of the mammary gland caused by injuries or microorganisms. These are usually bacteria that invade the udder, multiply and produce toxins. Based on duration the four different forms of mastitis are generally defined as peracute, acute, subacute and chronic. According to severity it can be clinical, subclinical and latent.

*Acute* mastitis is easily distinguished by its generalised, often life-threatening effect upon the individual. *Chronic* mastitis is characterised by continued clinical signs over a period of weeks or months, sometimes separated by apparently normal periods. *Clinical* mastitis is distinguished by visual abnormalities. A cow with *subclinical* mastitis does not have a swollen, painful udder or abnormal looking milk. The presence of subclinical mastitis only becomes obvious when milk is closely examined. Unfortunately, the apparently healthy cow can harbor subclinical mastitis, which creates tremendous loss in milk production.

The dairyman is generally aware of clinical mastitis because it can be seen as changes in the milk, swollen udder and other signs exhibited by the cow. Compared with subclinical mastitis, clinical mastitis is much less costly, tends to be an individual cow problem, and is detected without special tests (Schalm et al., 1971; Kitchen, 1981; Horváth, 1982; Giesecke, 1983; Klastrup, 1985; IDF, 1987a; Shuster et al., 1991; IDF, 1999a; IDF, 1999c).

### 3.1.2. Impact of mastitis

Mastitis is one of the most costly diseases of dairy cattle reported DeGraves and Fetrow (1993), Báder (1996), Fleischer et al. (2001) and many others. It has been estimated that mastitis costs about 150 to 300 dollars per cow per year, totally 1.5 to 3.0 billion dollars annually in the USA (Smith and Hogan, 1999). In herds without an effective mastitis control program, approximately 40% of cows are infected in an average of two quarters. Ózsvári

et al. (2001) reported that because of high somatic cell count losses only in production cost ~8,500 HUF and 25,000 HUF in Hungary by first lactation and older cows respectively.

The prevalence of mastitis ranges from 18 to 80%, in general about 40% in Hungary (Markus, 1994; Unger, 1996; Markus, 1999 personal communication; Baltay and Jánosi, 2001). This means that udder health is worth than it presented by the quality of milk processed in milk plants (Iváncsics et al., 1996). Based on the National Mastitis Council (NMC, 1987), Smith and Hogan (1999) and Ózsvári et al. (2001) practically it results approximately 20% decrease in production (*Table 1*). This may increase unless dairy producers can achieve a reduction in prevalence of the disease.

**Table 1.** Losses in milk production associated with elevated BTSCC (NMC, 1987)

| **BTSCC** (1,000 cells/ml) | **Infected quarters** (%) | **Production loss** (%) |
|---|---|---|
| 200 | 6 | 0 |
| 500 | 16 | 6 |
| 1,000 | 32 | 18 |
| 1,500 | 48 | 29 |

The most substantial losses due to mastitis result from decreased milk production (approximately 70% of the total losses), changes in the composition (reduced milk quality), discarded milk, increased treatment and management cost (drugs, veterinarian, labour), risk of contamination if antibiotics are used, culling or death, and decreased genetic advancement. Subclinical mastitis is the primary cause of these losses because it is quite difficult to recognise that less milk was produced (Afifi, 1968; Hansen et al., 1979; Eberhart et al., 1982; Craven, 1987; Hogan et al., 1989a; Browning et al., 1990; Emanuelson and Funke, 1991; Cullor, 1992; DeGraves and Fetrow, 1993; Ózsvári et al., 2001).

Genetic studies have found that single-trait selection of dairy cattle for higher milk production brings with it slightly higher rates of reproductive failures, mastitis and other diseases (Brochart et al., 1985 (cit.: Janke and

Funke, 1989); Oltenacu et al., 1990; Jánosa et al., 1999; Fleischer et al., 2001) (*Figure 1*).

However, not all high-production bulls sire high rates of mastitis (Dunklee, 1991; Dohy 1999).

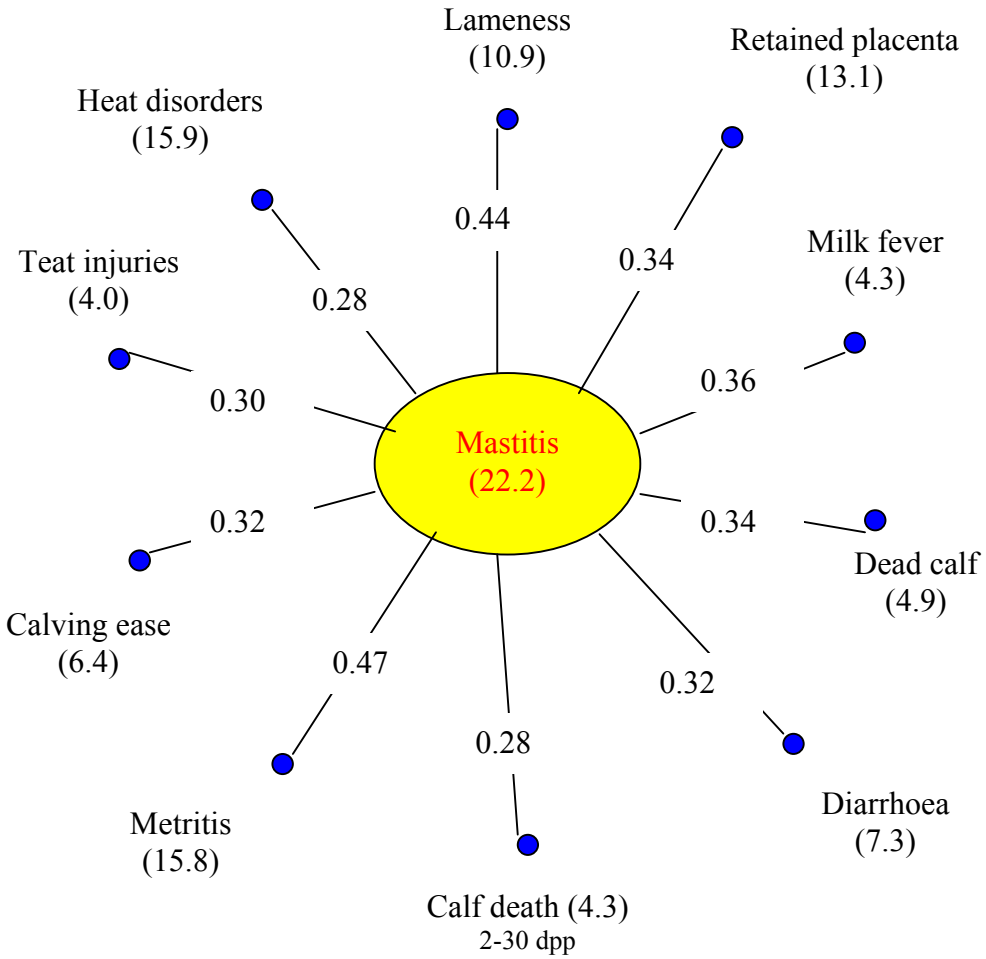



**Figure 1.** Prevalence of some diseases and its correlations
(n=3216 cow in 90 heard, Brochart et al., 1985; cit.: Janke and Funke, 1989)

## 3.2. COMPLEXITY OF MASTITIS

Many authors reported that numerous factors influence the prevalence and increase of mastitis (*Figure 2*) (Saloniemi, 1980; Horváth, 1982; Dohy, 1985;. Cassell, 1988; Janke and Funke, 1989; Dunklee, 1991; Erskine, 1993; Süpek, 1994; Süpek, 1995; Gulyás and Iváncsics, 1999; Iváncsics and Gulyás, 1999; Baltay and Bedő, 2000; Baltay et al., 2000; Busato et al., 2000).

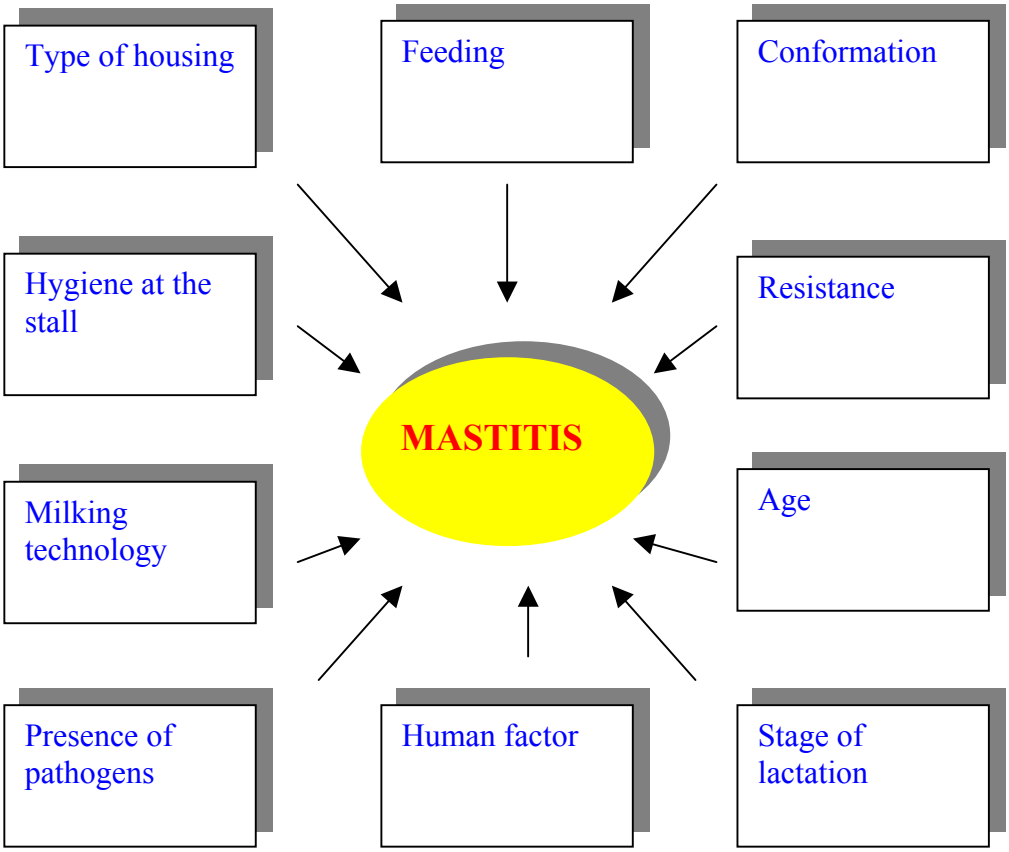



**Figure 2.** Complexity of mastitis (Iváncsics and Gulyás, 1999)

### 3.2.1. Environment: keeping system and technology

Several studies on the impact of the production environment on udder health have been published in the last years reported Woolford et al. (1998). In

the most recent studies epidemiological methods were employed and the numerous factors influencing the increase in mastitis accurately measured. Thus, the knowledge on the impact of the production environment on udder health is considerable. Identification and elimination of individual environmental factors that threaten udder health reduces the overall stress on the cow, and so udder health improves. However, it is important to remember that elimination of a single and obvious predisposing factor is not always sufficient to maintain the herd below the disease threshold (Neave et al., 1969; Saloniemi, 1980; Radostits and Blood, 1985; Rasmussen, 1999).

In many comparison of ***cow house types*** fewer cases of mastitis were treated in loose houses than in tie-stall housing because:

- movements of cows getting up and down are less restricted,
- the lying area is usually covered and therefore soft,
- cows are milked at a milking parlour.

However, health differences among the herds was large with both types of housing (Oltenacu et al., 1990; Báder, 1996; Kertész et al., 2001).

A concrete ***floor*** gives poor support to the hoof as the cow rises. A stall without a ***bedding*** and messy with dung can be very slippery and teat tramp is frequent. Injuries to the skin of the hock are common both in slippery and hard stalls. Damaged skin is a favourable environment for some pathogenic microbes of the udder. Using sufficient straw lessens the incidence of such injuries considerably and also protects the udder from cold and from rubbing on the concrete.

However, population of coliform bacteria increase rapidly in straw and sawdust bedding dirtied with faeces. In dirty bedding streptococci (and some staphylococci) are abundant therefore the risk of *(environmental)* mastitis increases. Cases of coliform mastitis are associated with dirty loose houses (Golodez, 1985; Hogan et al., 1989b; Oltenacu et al., 1990).

The effect of a night ***light*** in reducing the frequency of teats tramp is based on the natural instinct of cows. Cows, in common with other artiodactyls are lived in open areas. In a dark barn they do not equate strange noises with harmless situations and will invariable get up to escape the presumed danger, often tramping their teats as they do so (Seabrook, 1984).

High ***humidity*** in the cow house and a draught on the udder increase susceptibility to mastitis. Wetness of the udder due to moist stall floor or due to

frequent washing of the udder increases the deleterious effect of draughts by increasing heat loss from its skin (Seabrook, 1984; Barkema et al., 1999).

In North America, the practice of ***tail docking*** dairy cattle is on the increase. Producers cite a number of reasons for docking cows, including improved ease of milking, cow cleanliness and reduced SCC because reducing contact with bacteria via the tail.

Tucker et al. (2001) monitored milking cows after half of the animals in a herd were docked to determine whether tail docking would influence cow cleanliness and udder health in a free-stall system. No treatment differences were found in four measures of cow cleanliness, two measures of udder cleanliness, or udder health. However, analysis of a subsample of cows illustrated individual differences in cleanliness.

The correlation between mastitis and environment is often difficult to interpret as it is difficult to estimate the impact of the ***herdsman***. The incidence of mastitis in the herd may be much less then would be expected in the presence of several potentially deleterious factors if the herdsman is professional and manages milking, feeding, cleaning and other duties with care. When the potential etiological component of the production environment is estimated during investigation of a mastitis herd, both the range of causes of mastitis and the important role of the herdsman must be kept in mind.

It has been estimated that a large part of the variability in occurence of mastitis between herds is explained by differences between the care herdsman manage their herds (Neave et al., 1969; Miller, 1984; Seabrook, 1984; Radostits and Blood, 1985; IDF, 1987b; NMC, 1987; Lawstuen et al., 1988; Markus, 1994; Iváncsics et al., 1996; Barkema et al., 1999; Rasmussen, 1999; Hogeveen et al., 2001).

### 3.2.2. Milking: technique and hygiene

Milking machine (and nowadays milking robot) was developed to ease the heavy work of manual milking (Moxley et al., 1978; Mein and Thompson, 1993; Justesen and Rasmussen, 2000; Hogeveen et al., 2001). However, Weaver (1982) reported that machine milking can be so rough that the natural defence system of the udder fails to prevent the ingress of bacteria, significantly increasing the risk of mastitis (teat injuries because of vacuum fluctuation and incorrect pulsation). On the other hand, milking machines can transport

pathogens both passively from the skin of one cow to another and actively by dispersing them inside the udder (*contagious mastitis*) reported O'Shea and Meaney (1979), Grindal and Hillerton (1991), IDF (1994a) etc.

The cow and the machine are linked by the milker. No matter how good the machine is. A careless operator can cause serious teat injuries and active dispersal of pathogens inside the udder. Making the upper part of the udder wet is not recommended because the dripping water carries bacteria down to the teats (IDF, 1994a; Smith and Hogan, 1999). Recovery of the teat from machine milking takes more than half an hour whereas suckling by the calf or with manual milking the teat becomes slightly thinner and returns to normal within half an hour. For this reason it is suggested to provide food directly after milking. The more the teat swells during machine milking the more sensitive the quarter is to infection wrote Nickerson in 1992.

In addition, proper preparation increases milk yield by approximately 10% in comparison with poor preparation. In the design and use of milking machines the starting points must be the demands set by the cow and the milk, techniques must be compatible with the conditions set by biology (Pankey et al., 1984; Hamann, 1990; Hamann and Stanitzke, 1990; Hogan et al., 1990; Erskine and Eberhart, 1991; Pankey and Drechsler, 1993; Myllys et al., 1994).

Rasmussen et al. (2001) reported that robotic milking (AMS) had a negative influence on udder health measured by an increase in acutely elevated cell counts during the first year compared with the previous year with conventional milking. The number of cows with elevated SCC decreased slowly after 3 months. They did not have a conclusive reason for the increase but similar to IDF (2000), Justesen and Rasmussen (2000) and Hogeveen et al. (2001) suggested that more focus is needed on the introductory period.

Attention should pay on farms try milk production through organic ways, too (Chamings, 1984; Murdough and Pankey, 1993; Vaarst and Enevoldsen, 1997; Busato et al., 2000; Vorst and Hogeveen, 2000).

### 3.2.3. Conformation

The conformation of the cow significantly influences predisposition to mastitis reported Hámori (1974), Horváth (1982), Dohy (1985), Seykora and McDaniel (1985) etc. Because of the favourable relationship between some udder traits and the SCC of milk, screening on udder characteristics may have

slowed the genetic increase in LSCS (Rogers et al. 1991; Boettcher et al. 1998). Rogers et al., (1991) reported genetic correlation of LSCS with udder depth, fore udder attachment and front teat placement to be –0.35, -0.32 and –0.22 respectively. Gulyás et al. (1998) and Gulyás and Iváncsics (1999) also reported the importance of udder morphology and the role of pigmented teat ends. Dohy (1985), Seykora and McDaniel (1986), Schutz et al. (1993) and Dohy (1999) suggested that sire analysts from AI organizations should screen perspective bull-dams for udder conformation traits and should eliminate cows with deep udders or wide front teats from consideration.

The structure of legs and hoofs is an also important predisposing factor. Movements involved getting feed and lying down are naturally necessary. Leg injuries in a herd are indicative of likely increase in udder diseases. However, long and untreated hoofs are often a question of mismanagement rather than a conformation fault of the cow (Markus, 1999 personal communication).

Culling cows with poor udder shape and leg injuries results in improved udder health.

### 3.2.4. Genetic background

Several authors pointed out that prevalence of mastitis is genetically determinated (*Table 2*). Depending on the level of environment it ranges $h^2$=0.02-0.2. The overall mean is about $h^2$=0.1 (Shook and Schutz, 1994).

Breed also has an impact on mastitis. Ayrshire cows have a significantly lower LSCS (Schutz et al., 1994).

Many studies have reported genetic correlation of LSCS and ***milk yield*** ranged from –0.2 to 0.48 but most values were closer to the mean of 0.12 (Kennedy, 1982; Kennedy et al., 1982; Ruabertas and Shook, 1982; Monardes and Hayes, 1985; Emanuelson et al., 1988; Banos and Shook, 1990; Boettcheret al., 1992). Kennedy et al. (1982) and so Monardes and Hayes (1985) reported a mean correlation of 0.28 between SCS and milk yield for first lactation, -0.15 for second lactation and 0.05 for third and later lactations. Schutz et al. (1990) suggested that mastitis as indicated by LSCS is more common during first lactations of cows with sires that transmit higher milk yield, perhaps because of the stress from producing more milk.

**Table 2.** Heritability of mastitis (Pongrácz and Iváncsics, 2001)

| Author | Year | Heritability ($h^2$) | Remarks |
|---|---|---|---|
| Ward (cit.: Schutz, 1994) | 1938 | ? | susceptibility |
| Lush | 1950 | | |
| Miller | 1984 | 0.12 (0 – 0.5) | |
| Emanuelson et al. | 1988 | 0.01-0.02 | cases of treated cows |
| Simianer et al. | 1991 | 0.05 | |
| Weller et al. | 1992 | 0.01 (field study) | |
| O'Bleness et al. | 1960 | 0-0.07 | marks of resistance |
| Norman and Van Vleck | 1972 | 0-0.07 | |
| Lawstuen et al. | 1988 | 0.03 | |
| Hansen et al. | 1979 | 0.18 | bacteriological status |
| Miller | 1984 | 0.11 | |
| Weller et al. | 1992 | 0.04 | |
| Shook and Schutz | 1994 | 0.1 (0.02-0.2) | |

Mean correlation of LSCS and ***fat yield*** is about 0.2 while the genetic relationship of LSCS and ***protein yield*** found estimates of correlations ranged from –0.14 to 0.54 and had a mean of 0.17 (Kennedy et al., 1982; MacMillin et al., 1983; Monardes and Hayes, 1985; Weller et al., 1992). It means that genetic gain can be achieved in selection for lower SCS only by decreasing selection emphasis on milk and protein yields.

### 3.2.5. Other factors

Influences of ***age*** and ***season of calving*** on LSCS are documented (Norman and Van Vleck, 1972;. Coffey et al., 1986; Miller et al., 1991; Schutz et al., 1994). Solutions for LSCS increases with age and rates of increases are steeper after about 36 months of age (Laevens et al., 1997). Effects of ***month of calving*** are smaller than age at calving but LSCS is higher for cows calving during midsummer.

Smith et al. (1984), Thomas et al. (1990), Tucker et al. (1992), Erskine (1993), Block (1994) and Hogan et al. (1996) reported the role of ***feeding,*** mainly through the minerals and vitamin supply.

## 3.3. PHYSIOLOGY OF MASTITIS

### 3.3.1. Infection

When bacteriological examination of single quarter milk samples and careful analysis of the inflammation are carried out in parallel bacteria are found in approximately 80% of the inflamed quarter milk samples. Some 20% of the inflammations are such that the irritant is something other than bacteria sequestered at the moment in the udder, or the number of bacteria in milk might be too low to be detected, or the host has already eliminated the infection (IDF, 1975a; Piddock, 1990; Huszenicza and Albert, 2000; Egyházi and Hargitai, 2001).

Incorrect milking causes small traumas in the teat ends and they become predisposed to bacterial colonization, easily followed by infection of the quarter. The milker may also act as an infection source.

According to Fox and Gay (1993) ***contagious mastitis*** appears as high cell counts in milk. In a herd infected with contagious mastitis the causal bacteria are usually S. aureus. In some herds the Coagulase Negative Staphylococci (CNS) predominate. Furthermore, Str. agalactiae and Str. dysgalactiae can cause contagious mastitis, too (Anderson, 1982; Harmon and Langlois, 1989; Devriese, 1990; IDF, 1994b).

A characteristic of staphylococcal mastitis is that the number of bacteria in the milk varies greatly between repeated samples. The somatic cell count closely paralells the number of bacteria in milk (Tolle, 1982; Sanchez, 1988). S. aureus infections often turn chronic (Schukken et al., 1993). S. aureus (and Str. agalactiae) can survive on the mucous membrane. Therefore, milk from infected cows not allowed/suggested to (heifer) calves (Saperstein et al., 1988; Sandholm et al., 1990; Nickerson et al., 1993). Vaccination against staphylococci is not a practical tool nowadays (Watson, 1992; Markus, 2000 personal communication).

***Environmental mastitis*** is associated with a relatively high incidence of clinical (related to subclinical) mastitis in the herd that may fluctuate according to season (Smith et al., 1985a; Smith et al., 1985b; IDF, 1987b; Smith and Hogan, 1993). The bacteria isolated from the clinical cases are usually gram

negative or Str. uberis (Hogan et al., 1988). Cases of E. coli mastitis seldom become chronic (Golodez, 1985; Hogan et al., 1989a; Hogan et al., 1992).

With herds suffering from Str. uberis infections that often occur during the dry period the bacteria can be isolated from nearly all infected quarters. The sources of infection are generally the cow and its immediate environment, particularly the bedding (Golodez, 1985; Hogan et al., 1989b; Oltenacu et al., 1990;Williamson et al., 1995).

Klebsiella infections are typically very difficult to treat, and the infected animals usually have to be culled. Before the mastitis problem is found to be caused by Klebsiella, a few cows may already have been lost. Humid sawdust and chippings favour the growth of Klebsiella. The problem is often solved when sawdust storage is improved (dry storage) or preferably sawdust is replaced with some other bedding material.

With Pseudomonas infections, contaminated water is the most likely infection source. The detergents used for cleaning the milking equipment are potential sources as Pseudomonas can grow in the detergents.

***/Summer mastitis*** was first described at the beginning of the twentieth century. However, a precise definition of summer mastitis, which all researchers agree upon, has not yet been made. This mastitis type also goes under other names: heifer mastitis, dry cow mastitis, fly mastitis and pyogenes mastitis.

Summer mastitis occurs mainly in heifers and dry cows, and is typically associated with strong clinical symptoms with a suppurative and foul-smelling secretion from the quarter. Despite treatment the affected quarter is usually lost from milk production. Summer mastitis is most common towards the end of summer when *Hydrotea irritans*, the fly considered to be the main vector of the disease, is abundant. The causing microbe is *Actinobacillus (Corynebacteria) pyogenes*. The distribution of typical summer mastitis correlates with that of the fly; the disease is found in northern Europe and Japan, but never in Hungary.

A syndrome resembling summer mastitis is also found during the indoors season, affecting dry cows in particular, but also lactating cows. The disease develops following a teat injury. Summer mastitis is a mixed infection caused by aerobic and anaerobic bacteria and thus a comprehensive name for it would be anaerobe-aerobe-mastitis.

***Mycoplasmas*** are pleomorphic bacteria which lack a cell wall. They are smaller and structurally more simple than other bacteria. Approximately 70 species of

mycoplasmas are known, of which at least five cause bovine mastitis, the most important being *Mycoplasma bovis* and *M. bovigenitalium* (Erno and Perreau, 1985).

A mycoplasmal mastitis infection in the herd typically results in a fast spreading epidemic. The infection easily spreads during milking and is often widespread before the first symptoms are noticed. Morbidity in the herd depends on how fast the disease is recognized (and milking hygiene). Cows of all ages and at all stages of lactation are sensitive to mycoplasmal infections. Mycoplasmal mastitis may affect 50-60% of the cows.

This type of mastitis is characterized by a sharp drop in milk production and extremely swollen udders that are not painful. In the early stages the milk secretion becomes watery in appearance and contains fibrin flakes. Characteristically the milk, on standing e.g. in a test tube, rapidly separates into a flaky deposit and a clear supernatant. Later the milk becomes purulent. The quantity of leukocytes is usually high ($10^6$-$10^3$/ml) and the milk contains high numbers of mycoplasmas (up to $10^9$/ml).

A definitive diagnosis of mycoplasmal mastitis requires culture of the organism and species identification. Mycoplasmas require complex media containing sterols for growth. Therefore, they are not detected in routine mastitis culture of milk specimens. If mycoplasmal mastitis is suspected on the basis of clinical symptoms, it must be mentioned when the milk sample is sent to the laboratory (Rickhard et al., 1980; Horváth, 1982; Bushnell, 1984; Huszenicza and Albert, 2000)./

### 3.3.2. Defence mechanisms

The ***teat canal*** represents a physical barrier to the penetration of bacteria. It remains open after milking for approximately two hours. The cow should be prevented lying down during this critical period (the role and importance of feeding). The idea of post-milking teat dipping is to disinfect the teat canal and reduce the risk of ascending infection (Guidry, 1985; Nickerson, 1985; Nickerson, 1992).

Epithelial desquamation and milk flow are mechanisms of the host to decrease local bacterial colonization. The keratin layer contains basic antibacterial proteins and antibacterial fatty acids (Dohy, 2000). Specific immunological factors also play a role in the defence of the teat canal. Lymphocytes and plasma cells accumulate beneath and between the epithelium of the teat canal wall, particularly around the Fürstenberg's rosette. This indicates local immunological activity. Neutrophil phagocytes directly penetrate the teat wall to the infected and inflamed teat canal (Nickerson, 1985; Korhonen and Sandholm, 1995).

Besides the anatomical-physical defence mechanism of the teat canal, the ***immune system*** of the mammary gland consists of both humoral and cellular components (Davidson et al., 1982; Sears, 1984; Craven and Williams, 1985; Guidry, 1985; Sheldrake and Husband, 1985; IDF, 1991a; IDF, 1991b; Knight, 1991; Tyler et al., 1993; Sandholm and Korhonen, 1995). *Immunoglobulins* have specific antibody activity against antigenic stimuli form the humoral component. The *cellular components* consist of several different cell groups.

The total amount of ***immunoglobulin*** varies during the stage of lactation, as does the relative proportion of the different Ig-subclasses (isotypes). In colostrum the immunoglobulin content is as high as ~100 mg/ml but falls within the first week of lactation to less than l mg/ml. This low level is hardly of any importance in defence of the udder. This concentration is very low in comparison with that in human or sow milk. However, the antibacterial factors in milk have a concerted action and it is difficult to judge the relative importance of an individual factor like lactoferrin, transferrin, lysosyme, lactoperoxidase or complement (Reiter, 1978; IDF; 1985; Saeman et al., 1987; Sandholm and Korhonen, 1995).

The most important feature of ***antibodies*** in milk is their opsonizing ability: alien antigens become labelled and the phagocyting granulocytes and macrophages are directed to their targets. In addition, the antibodies neutralise toxins and are occasionally directly bactericidal (Andrews, 1983). An antibody can kill directly via the combined effect of the antibody and the complement, or via the concerted action of complement and lysozyme.

Bacterial elimination, which takes place through ***phagocytosis***, is however considered as the most important antibacterial mechanism of the udder (Sandholm and Korhonen, 1995).

**Lymphocytes**

During lactation normal milk contains a small number of lymphocytes. Approximately 50% of the lymphocytes in normal milk belong to various T-cell subsets and 20% to B-cells. The remaining cells belong to so-called null-cells.

The milk lymphocytes have been found to respond to antigen and mitogen stimulation in vitro. It can be presumed that most of the interaction between the lymphocytes and the macrophages occurs locally in the supramammary lymph nodes. Some lymphocyte activity can be observed in the Fürsterberg's rosette and beneath the mammary gland epithelium. Antigens stimulate the subepithelial B-cells to multiply and differentiate into plasma cells producing secretory antibodies

*Phagocytes*

**Macrophages**

Macrophages are phagocytic cells that serve to remove tissue debris and bacteria. Milk from a healthy udder contains approximately $10^5$ cells/ml, most of which are macrophages. Macrophages are the first cells to encounter bacteria and process information to other cells involved in host resistance. These cells function as recogniser and alarm cells in initiating an inflammatory reaction and immunity. They phagocyte and destroy bacteria, process antigens for the immune system, regulate the function at the lymphocytes and regulate the inflammatory cascade by secreting various cytokines and other mediators. Macrophages accumulate during involution of the mammary gland phagocyting residual milk, tissue debris as well as bacteria (Sanchez, 1988).

**Polymorphonuclear neutrophils (PMN)**

During inflammation the SCC of the lactating gland can increase to more than $10^6$ cells/ml. Most of this increase is due to neutrophils that move from blood into milk and milk gets a pus-like appearance (Kehrli et al., 1989).

The function of the granulocytes is based on their ability to adhere to endothelium at the site of inflammation and to find their way from the blood to the site of the inflammatory focus to phagocyte and destroy bacteria and to remove damaged tissue. The reaction of the neutrophils is considered to be the most important defence and cleaning mechanism of the udder. If the cells of the granulocyte series are destroyed, the cow is incapable of clearing an infection within the mammary gland.

Intramammary inoculation with graded doses of bacteria has shown that the udder is significantly protected against ascending infections when the somatic cell count of milk is $10^6$/ml or higher. In this case > 90% of cells belong to PMN. There has been a lot of interest in increasing the number of

PMN in milk in the hope that the udder would clear infections without the need for antimicrobials. In order to increase the number of PMN in milk, mechanically irritating intramammary devices were developed, e.g. a plastic loop to be inserted in the milk cistern. Another approach is to enhance production and stimulate transfer of PMN to the site of inflammation. This can be done by colony-stimulating factors and those cytokines which enhance the adhesion and transfer process.

The phagocytes kill bacteria by activating oxygen (oxygen burst) through the NADPH-oxidase – myeloperoxidase system. Therefore, the antibacterial mechanism is analogue to LP. Several lysosomal enzymes are involved in the lysis of the bacterial cell wall. The defensins produced by the phagocytes have recently aroused interest. They are small proteins (MW < 4000) that have an antibiotic-like effect on bacteria.

Phagocytes do not function effectively in the milk compartment of the udder. The neutrophils of milk phagocyte and destroy less effectively than the corresponding cells in blood. The phagocytic effect of the granulocytes is relatively poor in milk due to low energy reserves (the glucose content of milk is low) and low opsonin content (antibodies, complement). In the milk compartment, the granulocytes waste their capacity by phagocyting casein and fat globules. A steady transfer of fresh neutrophils from blood into milk is required for local defence against new infections.

### 3.3.3. Inflammation

Inflammation is defined as the response of the body to an alien substance (e.g. bacteria, bacterial metabolite or toxin), or tissue injury. Once activated, the body's inflammation mechanisms can combat microbial infections and pave the way for resolution and restoration of normal function (Guidry, 1985; Gallin et al., 1988; Knight, 1991; Sandholm, 1995; Sandholm and Korhonen, 1995; IDF, 1996b).

The microcirculation plays a crucial role in the initial events of the inflammatory reaction. The inflammation response consists of three stages:

1) Inflammation begins with endothelial reactions at the responding tissue site. During this acute, transient phase the local capillary circulation increases and the permeability of the capillaries increases as the endothelial cells contract. Inter-endothelial gaps are formed through which plasma and its

proteins leak in the interstitium causing oedema. Blood leukocytes begin to adhere to endothelium.
2) During the subacute phase, phagocyting cells migrate from the circulation to the infection site.
3) Tissue degeneration, regeneration and formation of fibrotic tissue is characteristic to the chronic proliferative phase.

Sometimes the inflammation fails to eliminate the causal microbe; in subclinical mastitis the udder maintains the inflammation without being able to eradicate the bacteria sequestered in the milk compartment.

*3.3.3.1. Physiological inflammation*

As milk production ceases towards drying-off the udder involutes and the body initiates a sterile inflammation in the udder which prepares it for repair and cleaning. Oestrogens play an essential role in the drying-off of the udder and in creating a sterile inflammation. The alveolar epithelial cells disappear by an apoptotic process. Udder macrophages are particularly aggressive during this period when they clean milk ducts of milk and other debris. The udder efficiently clears latent infections during involution. As a consequence of the inflammation milk plasmin is activated which leads to degradation of casein and absorption of the products. The fibrotic tissue developing in the udder during involution is considered as part of the inflammatory reaction (Afifi, 1968; Ali and Shook, 1980; Andrews, 1983; Guidry, 1985; Gallin et al., 1988; Browning et al., 1990; Knight, 1991; Sandholm, 1995; Sandholm and Korhonen, 1995; IDF, 1996b; IDF, 1996c).

*3.3.3.2. Invasion of leukocytes to the mammary gland*

According to Afifi (1968), Ali and Shook (1980), Andrews (1983), Guidry (1985), Gallin et al. (1988), Browning et al. (1990), Knight (1991), Sandholm (1995), Sandholm and Korhonen (1995), IDF (1996b) and IDF (1996c) leukocytes migrate extensively throughout the body to mediate immune surveillance and to mount inflammatory responses to foreign antigens. Neutrophils are rapidly recruited in large numbers from blood to the site of inflammation. Other circulating cells such as lymphocytes, platelets and eosinophils may also retained at the inflammatory area. Lymphoid T-cells are

recruited later and more selectively than neutrophils to sites of inflammation where they have antigen-restricted functions .

Normal milk contains some $10^5$ somatic cells/ml, most of which are macrophages. These function as recogniser and alarm cells in the udder. During an emergency, these cells begin to secrete substances which attract neutrophils from blood to the inflammation site. Most of the somatic cells in mastitic milk are neutrophil granulocytes. The purpose of the invading granulocytes is to clear the inflammation area of foreign matter including bacteria and tissue debris (Nickerson, 1985; Sandholm, 1995).

To reach the inflammation site from the blood compartment the leukocytes have to evade the circulatory system. The leukocyte migration into tissue is regulated by adhesion to endothelium. The endothelium of the postcapillary venules at the inflammatory site become adhesive to various leukocytes; the leukocytes within the blood flow initially come into brief contact with the vessel wall, slowing their movement, and roll on the endothelium. Over the next few minutes the cells undergo diapedesis and migrate between endothelial cells into tissue.

Endothelial cells also express immunoglobulin superfamily adhesion proteins. The selectivity of various endothelial adhesion proteins towards specific leukocyte subsets and chronological expression of various adhesion proteins results in selective granulocyte infiltration in the acute phase and mononuclear cell (lymphocytes, monocytes) infiltration in the chronic phase of inflammation.

#### *3.3.3.3. Infection - inflammation*

It is widely accepted that the predominant cause of mastitis is intramammary infection by a microorganism, usually bacteria. The infection, which results from bacteria entering the udder through the teat has traditionally been considered the primary process in mastitis (Afifi, 1968; Ali and Shook, 1980; Andrews, 1983; Guidry, 1985; Gallin et al., 1988; Browning et al., 1990; Grindal and Hillerton, 1991; Knight, 1991; Sandholm, 1995; Sandholm and Korhonen, 1995; IDF, 1996b; IDF, 1996c).

Whatever is the reason for inflammation, the change in the composition of milk makes it favourable for the growth of certain bacteria. Mastitis pathogens grow faster in milk from inflamed quarters than in normal milk,

although the levels of the endogenous antibacterial factors of milk (phagocytes, antibodies, complement factors, lysozyme, lactoferrin etc.) are elevated in mastitic milk. When foremilk from hand-milked and machine-milked quarters within cows are compared, machine milking induce a change in foremilk to support growth of pathogens. This means that current machine milking technique is traumatizing and induces faint inflammation within the teat.

Infection and inflammation are dynamic processes (*Figure 3*). The cow's response to the infection is inflammation. Clinical mastitis is, in most cases, short-lived and becomes subclinical, latent mastitis, and the inflammation response is suppressed subsequent. Analysis of samples taken from consecutive milking shows that short term subclinical infections are surprisingly common. The bacteria are eliminated quickly in most cases, but the inflammation takes longer to disappear (Guidry, 1985; Sandholm, 1995; IDF, 1996b; IDF, 1996c).

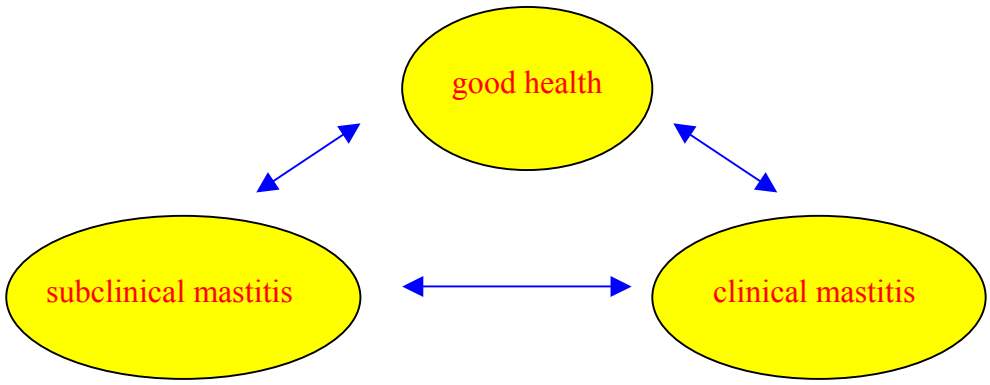



**Figure 3.** Dynamic of mastitis (Sandholm, 1995)

### 3.3.4. Changes in the composition of milk

Inflammatory reactions change the composition of milk in terms of quantity and quality reported many authors like Kitchen (1981), Harmon (1994), IDF (1996a) etc.

Several phenomena connected with inflammation occur simultaneously:

1) the permeability of the blood vessels increases resulting in the passage of ions and proteins from blood to milk,

2) inflammatory cells move from blood to milk,
3) the epithelial cells, which produce milk, become less efficient; cells break down and enzymes are released.

*3.3.4.1. The effect of mastitis on milk yield*

Reduction in milk yield is one of the clearest symptoms of mastitis. Reduction in yields depends on the degree of inflammation. This can be estimated from the somatic cell count in milk (*Table 1*). As the somatic cell count exceeds 100,000/ml, milk quantity begins to decrease linearly in relation to the logarithmic value of the cell count reported Eberhart et al. (1982), NMC (1987), Cullor (1992), DeGraves and Fetrow (1993), Smith and Hogan (1999) and Ózsvári et al. (2001).

Somatic cell count also reflects the changes that occur in the composition of milk (Griffin et al., 1977; Ali and Shook, 1980; Kitchen, 1981; Munro et al., 1984; Monardes and Hayes, 1985; Mattila, 1985; Mattila and Sandholm, 1986;.McFadden et al., 1988; Harmon, 1994; Korhonen and Kaartinen, 1995; IDF, 1996a etc.).

*3.3.4.2. Physical changes*

Along with the yield changes (and the chemical and bacteriological properties), the physical traits of mastitic milk change, too (Szakály, 1982; Politis and Ng-Kwai-Hang, 1988a; Korhonen and Kaartinen, 1995; IDF, 1997; Woolford et al., 1998; Szakály, 2001). Conductivity, pH and viscosity increases while density decreases and buffering capacity and titratable acidity shows no changes. The reduction ability increases.

*3.3.4.3. Changes in the chemical composition*

Mastitis affects both the quantity and the quality of milk. As the degree of inflammation increases, the chemical composition of milk approaches more and more that of blood because the components filter from the blood circulation into the mammary gland (Munro et al., 1984; Monardes and Hayes, 1985; McFadden et al., 1988; Jensen and Knudsen, 1991; Korhonen and Kaartinen, 1995). The changes in quantities of individual components vary.

Milk synthesis diminishes when the udder tissue is inflamed. Consequently the quantities of the major components of milk decrease and the total dry matter drops by 5-15%. There is a significant negative correlation

between the somatic cell count and the *dry matter* content of milk (Kitchen, 1981; Mattila, 1985; Mattila and Sandholm, 1986).

The results of changes in the *fat* content of milk caused by mastitis are diverse. According to the results of most investigations, the fat content decreases by less than 10%. The fat composition, however, changes considerably, lowering the quality of milk products. The total amount of fatty acids remains unchanged, but the quantity of free fatty acids increases. On the other hand, the amount of phospholipids diminishes due to the decrease in the amount and size of fat globules. The membrane matter of fat globules decreases by approximately 10% and its composition changes in comparison with that of a healthy cow's milk. The composition of fatty acids changes so that the amount of short-chained fatty acids (C4-C12) increases slightly and the amount of the long-chained fatty acids (C16-C18) drops correspondingly. The quantity of unsaturated long-chained fatty acids is, however, higher in mastitic milk than in normal milk. The changes in the lipid phase increase the lipolytic sensitivity in mastitic milk. This is intensified by the increased lipase activity.

The total quantity of milk *proteins* does not decrease clearly until the SCC exceeds 1,000,000/ml. The ratios between the different proteins, however, change at a much lower SCC.

A highly significant negative correlation exists between *lactose* content and SCC (Seelemann, 1964).

The changes of *mineral* and *trace element* contents of milk have considerable importance both for processing properties and its nutritive value (Szakály, 2001).

The quantity of water soluble *vitamins* fall by 10-50%. The changes affect bacteriological fermentation process and lower the quality of sour milk products reported Szakály (1982).

Mastitis generally increases the enzymatic and biochemical activity in milk. Some of these characteristics have for many years been used to detect mastitis. Increased *biochemical activity* in milk may in particular cause faulty fermentation of sour milk products and induce various quality problems (Szakály, 1982; Renner, 1983; Heeschen et al., 1985; Politis and Ng-Kwai-Hang 1988b; Ma et al., 2000; Szakály, 2001).

*3.3.4.4. Microbiological changes*

Normal milk contains several kind of bacteria, too. Based on the update regulations the legal limit of total plate count is less than 100,000 cells/ml at first class (highest quality, called "extra") milk in Hungary. Sometimes it can detect as much as $10^6$ cells/ml in infected milk samples. However, 79% of the milk that have been sold to dairy plants contained less than 50,000 cells/ml because of the strict monitoring system. This reduction is a very important point of quality requirements (Hargitai et al.,1989; Unger, 1996; Markus, 2000).

The majority of mastitic cases are caused by the so called *contagious* S. aureus, Str. agalactiae, sometimes Corynebacterium bovis and perhaps Mycoplasma bovis and other Mycoplasmas. The microbiological background of *environmental* mastitis is usually E. coli, Str. uberis and other Str., Bacillus and Nocardia spp., Pasteurella, Actinobacillus and Klebsiella spp. and some yeasts.

CNS spp. and Str. dysgalactiae are frequently isolated and sometimes Pseudomonas aeruginosa and Prototheca zopfii can be detected, too. These species can be *either* contagious *or* environmental based on the circumstances (IDF, 1975a; Horváth, 1982; Hargitai et al., 1989; Huszenicza and Albert, 2000; Egyházi and Hargitai, 2001).

## 3.4. DIAGNOSTICS OF MASTITIS

From the several possibilities of diagnosing mastitis we just deal with the few most important and practically used methods. However, many other tools were developed that vary in accuracy (IDF, 1975a; Kitchen, 1981; Brolund, 1985; Hoare et al., 1980; Klastrup, 1985; Mattila, 1985; Sandholm and Mattila, 1985; IDF, 1987a; Swets, 1988; Sears et al., 1993).

### 3.4.1. Examination

A *preliminary diagnosis* is based on the health record of the cow and its clinical signs. The focus is on the individual cow/quarter but the case must always be seen in a broader perspective, i.e. the herd.

It is important to check the individual health record and earlier treatments. The exact calving date should be available.

According to Markus (1996, personal communication) the *clinical examination* must proceed systematically. Starting from a carefully documented case history is useful. The general examination should include assessment of the posture, behaviour, body condition, general condition, respiratory rate, pulse frequency, rumen motility and body temperature. The general examination of the cow will indicate how much the general health of the cow is affected by the udder disease.

Then the udder itself is examined by inspection, palpation and examination of quarter milk secretion and milk appearance. Inspection takes account the size, shape and symmetry of the udder and teats by viewing it from behind and each side.

The anatomical/clinical unit of the udder is the quarter. Therefore, diagnostic methods must be applied to each quarter if mastitis is present. Inter-quarter comparison is helpful in recognizing abnormal quarters.

Asymmetry of the udder is usually due to atrophy of one quarter, or on the other hand, enlargement caused by oedema (Al-Ani and Vestweber, 1986; Nestor et al., 1988). Skin of the udder and teats is inspected for injuries, discoloration or other abnormalities. Special attention should be paid to the teat orifices. Palpation includes teat canal and cistern, udder cistern, glandular tissue and skin, and supramammary lymph nodes. The udder is best palpated immediately after milking.

Milk sample can be examined first physically, then chemically and microbiologically if necessary. The CMT is a practical cow-side test for detecting mastitis in milk. The milk should be inspected for clots, discoloration or wateriness before adding the CMT reagent.

### 3.4.2. Detection of inflammatory changes in milk

Obviously, a trait is allowed to substitute for another if it is genetically correlated with the other trait, if recording is less expansive or easier, if measurement is earlier in life or, if heritability is higher.

Because of a strong relationship between some of the inflammatory or compositional changes in milk and the presence of infection, the measurement of certain components is used to monitor udder health, and so milk quality (Griffin et al., 1977; Ali and Shook, 1980; Kitchen, 1981; Munro et al., 1984; Brolund, 1985; Monardes and Hayes, 1985; Mattila, 1985; Sandholm and

Mattila, 1985; Mattila and Sandholm, 1986; McFadden et al., 1988; Hutton et al., 1990; Harmon, 1994; Korhonen and Kaartinen, 1995; Sandholm et al., 1995; Hillerton, 1996; IDF, 1996a etc.). So, several tests are available to determine the presence or absence of clinical and subclinical (unseen) udder infection. These range in difficulty and sensitivity from the very simple strip test to sophisticated laboratory procedures which detect the presence of microorganisms or some changes of composition. All of these tests serve a need and can be useful if *conducted properly* and *interpreted correctly*.

*3.4.2.1. Compositional changes*

Some proposed screening tests for monitoring the course of infections include the measurement of catalase, NAGase, antitripsin, chloride, sodium, serum albumin, and SCC in milk (Mattila, 1985; Woolford et al., 1998). The majority of these tests primarily indicate inflammation in the udder. They do not measure infection or bacterial presence.

*Somatic cell count* (SCC) has been most widely used as a measure of milk quality, indicator of mastitis, and a management tool to control mastitis worldwide (IDF, 1975b; Griffin et al., 1977; Ali and Shook, 1980; Kitchen, 1981; Dahoo et al., 1981; Horváth, 1982; Bramley and Dodd, 1984; Munro et al., 1984; Brolund, 1985; Mattila, 1985; Monardes and Hayes, 1985; Sandholm and Mattila, 1985; Mattila and Sandholm, 1986; Harmon, 1994; Korhonen and Kaartinen, 1995; Sandholm et al., 1995; IDF, 1996a; Barkema et al., 1999; IDF, 1999b).

Genetic correlation between SCC and clinical mastitis or bacterial status is moderately high. Young et al. (1960) estimated the correlation of SCC and clinical mastitis to be 0.8 or 0.98 from two methods. Afifi (1968) reported a correlation of 0.83. Using more appropriate statistical techniques, Emanuelson et al. (1988) found a genetic correlation of 0.46 for Swedish Black and White cattle and 0.78 for Swedish Red and White cattle from a field study. Weller et al. (1992) found a smaller genetic correlation between SCC and clinical mastitis of 0.3 but the genetic correlation estimated to be near 1 of SCC with bacterial infections status. Thus, selection for lower SCC would apparently reduce subclinical as well as clinical mastitis (Afifi, 1968; McDaniel, 1984; Dohy, 1985; Emanuelson et al., 1988; Powell, 1992; Weller et al., 1992; McDanielet al., 1993; Vági, 1996; Vági, 1998; Dohy, 1999; Dohy, 2000; Iváncsics et al., 2001).

Nearly all developed countries have adopted upper regulatory limits for SCC in milk (Model, 1994; IDF, 1996c; Savelle et al., 2000; Hogeveen et al., 2001). Hungary, the EU and the Nordic countries, Switzerland, New Zealand, Australia etc. all accept 400,000 cells/ml as the legal limit for a high quality bulk tank milk sold in the commercial market place but further reductions likely will occur (EEC 92/46, amend 94/71). The EU is already discussing lowering the regulatory SCC limit to 300,000 or perhaps even 250,000 cells/ml reported Hillerton (2001) and Schukken (2001). Canada has now agreed on 500,000 cells/ml throughout all of the provinces and is discussing the possibility of going to 400,000 cells/ml. The limit for SCC in the USA is 750,000 cells/ml but also will be officially reduced (Pongrácz and Iváncsics, 2001).

The following discussion presents the most commonly used/known tests to indicate elevated SCC including the strip test, the California Mastitis Test (CMT), the direct microscopic somatic cell count (DMSCC), the electronic somatic cell count (ESCC), the differential cell count (DCC) and the differential cell stain (DCS) mainly according to relevant literature (IDF, 1981; Kitchen, 1981; Sandholm and Mattila, 1985).

### Strip Test

The strip cup or strip plate is indispensable in the milking parlour for determining the presence of clinical mastitis. The milking machine operator visually examines the foremilk for gross abnormalities by squirting a few streams of milk onto the strip cup. The test is rapid and can easily be adapted as a part of the normal milking routine.

### California Mastitis Test (CMT)

The California Mastitis Test (CMT) is a simple, inexpensive and rapid screening test that estimates the number of somatic cells present in milk.

It is conducted by mixing the test reagent with an equal quantity of milk. The reagent reacts with the DNA of the somatic cells in the milk to form a gel. The reaction is then visually scored as 0 (or N, negative), T (Trace), 1, 2, or 3, depending upon the consistence or amount of gel that forms (*Table 3*). The more viscous the gel, the higher the score. This indicates the presence of a higher number of somatic cells (Schalm et al., 1971; IDF, 1975b; IDF, 1981; NMC, 1987).

**Table 3.** Approximate ranges in SCC for CMT scores (NMC, 1987)

| CMT score | SCC range (1000 cells/ml) |
|---|---|
| N (0) | 0-200 |
| T | 200-400 |
| 1 | 400-1200 |
| 2 | 1200-5000 |
| 3 | 5000- |

A simplified method of scoring is: Negative (N), Suspect (S) and Positive (P). Negative corresponds to 0 on the traditional system while Suspect corresponds to Trace and 1. Scores of 2 and 3 on the traditional method are scored Positive in the simplified system.

This procedure can be used in several ways. Bulk tank, composite samples from individual cows and individual quarter samples can all be examined using the CMT procedure. Each is valuable in monitoring udder infection; however, the interpretation is different depending upon the type of sample.

**Direct microscopic somatic cell counting (DMSCC)**

The direct microscopic somatic cell counting (DMSCC) is the most accurate of the mastitis screening tests when conducted properly. For this reason, regulatory agencies generally use this test for confirmation of high somatic cell counts based on other tests. This test is also the standard by which all other tests are calibrated.

Studies identifying cell types in milk have shown that somatic cells in milk are primarily (75%) leukocytes which include macrophages, lymphocytes and polymorphonuclear neutrophil leukocyte (Sandholm et al., 1995). Leukocytes increase in milk in response to *infection* (or injury). They are the body's primary defence against microorganisms and disease.

Epithelial cells (25%) that are secretary and lining cells, on the other hand, increase as a result of *injury* (or infection). They indicate that damage to body tissue, particularly udder tissue, has occurred. They are in fact dead cells that have been sloughed from the alveoli and canals within the udder.

Even though the DMSCC is the most accurate, it is also the most time consuming. Stained milk films are microscopically examined and somatic cells are counted. As one can easily see this is a very tedious procedure and requires extensive training.

**Electronic somatic cell count (ESCC)**

The electronic somatic cell count (ESCC) test fulfills several needs which dairymen desire. The ESCC focuses attention on the individual cow. It does not pinpoint the quarter(s) affected but does monitor udder health of individuals. The ESCC also allows a herd average SCC to be calculated which serves as a monitor of the udder health of the herd.

## *Management purposes*

According to Lindström and Syväjärvi, 1978; Solbu, 1978; Vági, 1990; Süpek, 1994; Vági, 1996; Luttinen and Juga, 1997; Vági, 1998; Barkema et al., 1999; Dohy, 1999; Ózsvári et al., 2001; Pongrácz and Iváncsics, 2001) the results from the electronic cell counting procedure can be converted to a somatic cell score (SCS) that is shown in *Table 4*. The SCS (and SCC) allows the calculation of estimated milk losses due to subclinical mastitis (*Table 1*).

**Table 4.** Relationship between SCC and SCS (NMC, 1987)

| SCS | SCC (1000 cells/ml) | |
|---|---|---|
| | Midpoint | Range |
| 0 | 12.5 | 0-17 |
| 1 | 25 | 18-34 |
| 2 | 50 | 35-70 |
| 3 | 100 | 71-140 |
| 4 | 200 | 141-282 |
| 5 | 400 | 283-565 |
| 6 | 800 | 566-1130 |
| 7 | 1600 | 1131-2262 |
| 8 | 3200 | 2263-4525 |
| 9 | 6400 | 4526- |

*Genetic evaluation*

Furthermore, records could be used for genetic evaluations, too (Boettcher et al., 1992; Da et al., 1992; Schutz et al., 1994; Strandberg and Shook, 1989). The cost of using data for genetic evaluation would be small because records could accompany records for yield, which have already been used in evaluation systems. Small costs would be incurred for relatively minor changes in programming. The logarithmic calculation of test day milk SCC is an everyday routine in the Nordic countries. In the USA has been used since 1994 and in Israel since 1996 (Pongrácz, 2000).

Recent heritability estimates for LSCS range from 0.05 to 0.27 and the mean is about 0.12 (Emanuelson et al., 1988; Banos and Shook, 1990; Danuser, 1991; Boettcher et al., 1992; Da et al., 1992; Schutz et al., 1994).

Also, the LSCS fits criteria for inclusion in breeding programs as a relatively inexpensive substitute for clinical mastitis or bacterial infection status.

Genetic evaluation for LSCS use an *animal model* similar to that used for milk, fat and protein yields (Crist et al., 1982; Cassell, 1988; Emanuelson et al., 1988; Wiggans et al., 1988; Shook, 1989; Wiggans and VanRaden, 1990; Weller et al., 1992; Welper and Freeman, 1992; Shook, 1993).

It is a simultaneous genetic evaluation of males and females that uses the animal's own performance and the genetic merit of all relatives. The technique considers that cow's performance is based on her genetic ability and her environment. The model is:

$$y_{ijkl} = m_{ij} + a_{kl} + p_{kl} + c_{ik} + e_{ijkl}$$

*where:*

$y_{ijkl}$ = standardised LSCS record of cow kl (daughter l of sire k) in herd i and year-season, parity and registration group j,
m = fixed management group,
a = random animal breeding value,
p = permanent environment,
c = herd by sire interaction,
e = residual.

Evaluations use BLUP procedures in which the random effects of animal, permanent environment, herd by sire interaction and residual are assumed to be normally distributed with variances of 0.10 (heritability), 0.20 and 0.05 and 0.65 respectively, relative to a phenotypic variance of 1 (100%).

Breeding values from animal model procedures are divided by 2 and are reported as PTA. It is not directly applicable to a given herd but rankings of PTA and differences among PTA are relevant for all herds if bulls are assumed to have been mated to cows of equal genetic merit. The difference between PTA for two animals predicts the expected mean differences between their progeny.

With the sire rankings for PTASCS producers can select bulls on their ability to sire daughters with lower rates of mastitis (Griffin et al., 1977; Grootenhuis, 1978; Miller, 1984;Danuser, 1991; Sender, 1992).

However, some breeders might avoid using any bull with a PTA lower than 0, regardless how good the bull may be for other traits and effectively eliminate half of the available population of bulls. Although use of 0 as a natural selection treshold would allow progress in reducing somatic cells, the loss in overall merit would not be economically justifiable. This problem is circumvented by adding a constant to all evaluations so that all PTA for SCS will be positive.

High production and high rates of mastitis are genetically correlated! Therefore, if people select only those sires with low SCS (PTASCS), will also likely select for lower rates of improvement in milk yield. In fact, studies show that selection programs that optimise total economic merit will not stop the increase in mastitis incidence. Most studies suggest such programs will simply slow the rate of mastitis increase by 20% to 25%.

PTA for SCS has been recommended to use as part of an economic index weighted to other economically important traits. Standberg and Shook (1994) found that selection for an index of total economic merit that included somatic cells slowed the rate of gain in yield traits by about 1-2% and decreased the rate of increase in clinical mastitis by 20-25%. Because yield traits are more highly heritable and are more important economically, optimal breeding programs did not reduce LSCS or clinical mastitis but merely slowed the rate of increase. Rogers (1993) concluded that LSCS could be included in breeding programs along with yield, udder conformation, and feet and leg traits with an increase in net merit of 1-4%. He found that placing 5-8 % as much

emphasis on PTA for SCS as on PTA for yield traits optimised net merit and slowed the rate of increase in clinical mastitis by about 25%. All in all, the best option for including PTA for SCS in breeding programs is an index with other traits to improve overall economic merit (Janke and Funke, 1989; Iváncsics et al., 1996; Dohy, 1999). In this view, longevity (fitness) has a great importance.

For example, in the USA a net merit index includes production (67%), herd life (28%) and SCS (7%). The SCS portion includes the cost of clinical mastitis and premium deduction for high SCS (NMC, 2001). Dohy (1999) reported that evaluation of breeding value in the USA has a negative factor (-28.22) for SCS and according to Pongrácz (2000) it is as high as -300 in Israel.

The level of genetic control - known as heritability - for SCS is estimated to be only 10%. In contrast, the heritability for milk production is about 25% (Kennedy et al., 1982; Rensing, 2001).

As a consequence, *reliabilities* of PTA for SCS will be much lower and may only be high enough to warrant mating a bull to many females after the bull has a large number of second crop daughters that are lactating. So, genetic progress will be slower and evaluations for mastitis will be less accurate than for milk production for bulls with a given number of daughters (*Table 5*). By the time REL of PTA for SCS are high enough, most bulls are no longer in consideration for use as sires of sons and have been replaced by younger bulls because of the rapid genetic progress for yield traits. Therefore, effectiveness of using PTA for SCS selecting sires of sons may be limited.

For example, a reliability of 73% is produced by 30 daughters in 30 herds for milk production. To achieve this same 73% reliability for mastitis, 100 daughters in 100 herds are needed. As additional records and daughters enter a sire's progeny proof, the accuracy (reliability) of the proof will improve.

**Table 5.** Reliability of breeding value based on heritability and time of evaluation (Rensing, 2001)

| **Trait** | **$h^2$** | **n** | **REL %** ($1^{st}$ calving) | **REL %** ($3^{rd}$ lactation) |
|---|---|---|---|---|
| Production | 0.35 | 100 | 80 | 90 |
| Conformation | 0.22 | 40 | 70 | 75 |
| Somatic cell count | 0.12 | 100 | 70 | 85 |
| Calving ease | 0.05 | 350 | 80 | 98 |

The REL of cow PTA for SCS is also low. Perhaps only cows with several sons or with many daughters from embryo transfer will attain high REL. Because the sire pathway is most important for genetic progress, probably the most progress in selection for lower LSCS will come through screening sires of prospective bull-dams. Many of those sires will have attained reasonably high REL of PTA for SCS (Dohy, 1999).

**Differential cell stain (DCS)**

Differential cell stain (DCS) like panoptic staining with Pappenheim, Giemsa, Wright, May-Grünwald or Leishman stains is a standard technique in haematological diagnostic procedures and, based on these, the direct smear method used for observing somatic cells in milk should be similar to blood smear technique (May and Grünwald 1902, Hoare et al., 1980, Dahoo et al., 1981).

Application of this simple and cheap procedure provides additional information about cell types for understanding the udder health status, treating mastitic cases and several characteristics of cells in milk can be evaluated.

This test is also the standard by which the differential cell count test is calibrated (Lee et al., 1980; Hageltorn and Saad, 1986; Wever and Emanuelson, 1989; Saad and Östensson, 1990).

**Differential cell counting (DCC)**

Differential cell counting (DCC) of milk somatic cells can be a useful diagnostic tool in bovine mastitis research because each cell type has its own more or less specific function in the immune response. Cell numbers (and types) in milk can be counted by microscopy or by particle counters, such as the Coulter counter and Fossomatic (Lee et al., 1980; Hoare et al., 1980; Dahoo et al., 1981). Pulse and flow cytometric techniques have also been used for counting milk cells (Hageltorn and Saad, 1986; Wever and Emanuelson, 1989; Saad and Östensson, 1990).

Quantification of the different physiologic and biochemical properties of individual cells or cell compartments in a cell population can be determined rapidly with flow cytometry (FCM). FCM is a multiparametric analysis of each cell as it passes through a light beam.

Elimination of microorganisms from the mammary gland depends mainly on the combination of humoral components and phagocytosis.

Phagocytosis is the function of the cellular defence mechanism of the udder. The flow cytometric method allows a rapid and accurate simultaneous determination of different parameters of phagocytosis, and so the response of the immune system (resistance) of individuals (Saad, 1987).

*3.4.2.2. Bacteriological changes*

Usually, the reason for mastitis is some kind of intramammary infection (IMI). The total plate count (TPC) is of particular interest to the dairy farmer and processor. It is a frequent factor of the price producers receive for their milk. Raw milk with a TPC value greater than 100,000 cells/ml may not be sold as "Extra" milk in Hungary.

From a different perspective, the TPC serves as a rough gauge of herd health, farm sanitation efficacy, and proper milk handling and storage temperatures (Mattila et al., 1986; Markus, 1996 personal communication).

The source of bacteria has a great importance, too. The "type" of mastitis and its clinical signs change across a wide range (Chamings, (1984). Therefore, it is good to know the properties of the different species because of the possibilities of treatment (IDF, 1975a; Erskine et al., 1993; Markus, 1994; Egyházi and Hargitai, 2001).

### 3.4.3. Other substitute markers

Detilleux (1993, cit. Schutz, 1994) and Detilleux et al., (1995) have suggested other traits as substitute or markers for mastitis.

Several allels of the gene complex of bovine lymphocyte antigen have been associated with clinical mastitis (Weigel et al., 1990). Intensive gene mapping research was done on the multifactorial background of mastitis in Germany (Kalm, 1997).

At present however, measurement of such markers and cell function remains too difficult and too expensive for routine evaluation of cattle.

# 4. MATERIALS AND METHODS

## 4.1. BREED AND GENOTYPE DIFFERENCES

Although microbiological testing of milk is the gold standard for measuring mastitis, electronic SCC has been widely used as an indirect measure of mastitis (IDF, 1981; Kitchen, 1981; Sandholm and Mattila, 1985).

Schutz et al. (1994) reported that breed has an impact on mastitis.

The number of red Holstein Friesian cattle is very small according to the size of the black population. It means that selection is not so efficient. Thus, red cows have generally disadvantages of about 5-30% or 2-4 years in many production traits. It is than suggested remember some special excellent traits when choosing bulls (Prange et al., 1990). Furthermore, some "old-fashioned" breeds like Hungarian Spotted has not really been studied so systematically.

Hence, the objectives of this part of the dissertation were:

- to study the environmental effect on production and milk quality,
- to determine the differences among the value of some sires in respect of milk yield and SCC in selected stocks (Hungarian Spotted and red Holstein Friesian cows),
- to calculate correlations between mastitis resistance (presented by SCC and SCS) and milk yield.

### 4.1.1. Quantity – quality: Simmental and crossbreed cows

*4.1.1.1. Farm selection*

To study the value of sires in respect of milk yield and SCC and correlations between mastitis resistance (presented by SCC and SCS) and milk yield farms were selected from an initial group of 9. Information from a background questionnaire was used to select farms with similar management practices and operational procedures. At last, 2 stocks keeping ***Simmental*** (*Hungarian Spotted*) cattle were chosen. To preserve anonymity, stocks were assigned a reference letter like A and B (*Table 6*).

**Table 6.** Parameters of the selected farms (A and B)

| ***Parameter*** | **Stock** | |
|---|---|---|
| | *A* | **B** |
| Heard size | 140 | 100 |
| Investigated cows | 66 | 32 |
| Breed | Hungarian Spotted (HF $F_1$) | |
| Milk production (kg/lactation) | ~ 4000 | |
| SCC (cells/ml) | ~ 400,000-900,000 | |
| Collection | daily | |
| Keeping system | tied-up | |
| Milking | at stand | |
| Feeding | half-monodietary | |

Among the stocks that were selected, herd size ranged 140 cows at A and 100 cows at B farm in 1996. Average milk production per cow was about ~4000 kg/lactation. Feeding was half-monodietary at both places. Cows were kept in a tied-up system at farm A and B and for dry cows there was a small fence. Average SCC ranged from ~250,000 to 900,000 cells/ml as estimated by farm managers. Farms operated with two or three bulk tanks. Collection frequency was daily. Milking was carried out twice a day at every farm. At stock A and B jars were used for milking (Elfa Impulsa).

*4.1.1.2. Statistical analysis*

Milk yield and SCC of Hungarian Spotted and crossed with red Holstein Friesian $F_1$ cows were used for the evaluation.

305 days of lactations were calculated from the database of Livestock Performance Testing Ltd., Gödöllő (Hungary) between January 1996 and September 1997 contained 66 and 32 cows, respectively. Sires were assigned with their registration number. Herd-year-season-calving age at first calving effects were not taken into consideration. Estimations based on SCC but data were transformed to SCS and LSCS, too. The existing differences among progeny groups of some sires were studied, too. Biometric calculations were processed by Microsoft Excel 97 and BIO MATE softwares. Student's t-test was used for comparison of means. A P value of 0.1 (***), 1 (**), 5 (*) and 10 (+) (and NS=not significant) was considered as significant.

## 4.1.2. Quantity – quality: red Holstein Friesian cows

*4.1.2.1. Farm selection*

To study the value of sires in the respect of milk yield and SCC and correlations between mastitis resistance (presented by SCC and SCS) and milk yield farms were selected from an initial group of 9. Information from a background questionnaire was used to select farms with similar management practices and operational procedures. At last, 1 stock keeping ***red Holstein Friesian*** cattle were chosen. To preserve anonymity, the stock was assigned a reference letter of C (*Table 7*).

**Table 7.** Parameters of the selected stock (C)

| ***Parameter*** | **Stock C** |
|---|---|
| Heard size | 650 |
| Investigated cows | 331 |
| Breed | HF |
| Milk production (kg/lactation) | ~ 7000 |
| SCC (cells/ml) | ~ 350,000 |
| Collection | daily |
| Keeping system | loose-housing |
| Milking | herring-bone |
| Feeding | half-monodietary |

Herd size consists of 650 cows in 1996. Average milk production per cow ranged about ~7000 kg/lactation. Feeding was half-monodietary. Cows were kept in a loose-housing system with a small fence connected to the barn. Average SCC was ~350,000 cells/ml as estimated by the farm manager. The farm operated with three bulk tanks. Collection frequency was daily. Milking was carried out twice a day in a "herring-bone" milking system (2 x 8 x 2 Alfa Laval, was built in 1996) supplied with an automatic "takeoff" that removes cluster when milk flow rate decreases below 0.2 kg/min.

*4.1.2.2. Statistical analysis*

Milk yield and SCC of red Holstein Friesian cows were used for the evaluation.

305 day of lactations were calculated from the database from the Livestock Performance Testing Ltd., Gödöllő (Hungary) between April 1995 and January 2002 contained 4614 lactations. Herd-year-season-calving age at first calving effects were not taken into consideration. Estimations based on SCC but data were transformed to SCS and LSCS, too. Correlations were estimated for means of SCS (LSCS) and milk yield in different lactations. The existing differences among progeny groups of some sires were studied, too. Biometric calculations were processed by Microsoft Excel 97, BIO MATE and Statistica softwares. Student's t-test was used for comparison of means. A P value of 0.1 (***), 1 (**), 5 (*) and 10 (+) (and NS=not significant) was considered as significant.

## 4.2. GENOTYPE AND ENVIRONMENT

### 4.2.1. Hygiene

As one of the main sources of prevalence of mastitis the bacteriological background and milking hygiene (technique) were studied at stock C and D.

Hence, the objectives of this part of the dissertation were:

- to study the environmental effect on milk quality,
- to determine the bacterial background.

*4.2.1.1. Farm selection*

To answer the questions mentioned above background information questionnaire was used to select farms. To preserve anonymity, stocks were assigned a reference letter. At last, experiments (combined with everyday routine) were carried out at farm C and D keeping (***red) Holstein Friesian*** cattle from November 1999 till the end of 2001.

Herd size consists of ~800 and 500 cows respectively in the studied period. Average milk production per cow ranged about ~7500 and 6900 kg/lactation at farm C and D. Feeding was half-monodietary at both places. Cows were kept in a loose-housing system with a small fence connected to the

barn. Average SCC was ~350,000 and 400,000 cells/ml respectively as estimated by farm managers. Farms operated with three bulk tanks. Collection frequency was daily at both places and milking was carried out twice a day in a "herring-bone" milking system. At farm C the 2 x 8 x 2 Alfa Laval milking parlour was built in 1996 supplied with an automatic "takeoff" that removes cluster when milk flow rate decreases below 0.2 kg/min (4.1.2.1.). Parameters can be seen in *Table 8*.

The so called environmental and contagious microorganisms were studied. Date of examinations can be seen in *Table 9*.

**Table 8.** Parameters of the selected stocks (C and D)

| ***Parameter*** | **Stock** | |
|---|---|---|
| | **C** | **D** |
| Heard size | 800 | 500 |
| Breed | HF | HF |
| Milk production (kg/lactation) | ~ 7500 | ~ 6900 |
| SCC (cells/ml) | ~ 350,000 | ~ 400,000 |
| Collection | daily | daily |
| Keeping system | loose-housing | loose-housing |
| Milking | herring-bone | herring-bone |
| Feeding | half-monodietary | half-monodietary |

**Table 9.** Date of examinations in stock C and D

| **1999** | | **2000** | | **2001** | |
|---|---|---|---|---|---|
| **C** | **D** | **C** | **D** | **C** | **D** |
| Nov. 12 | Nov. 13 | | March 13 | Febr. 19 | March 14 |
| | | | June 19 | April 03 | June 13 |
| | | | Oct. 19 | Mai 14 | August 22 |
| | | | Dec. 22 | June 12 | Oct. 17 |
| | | | | July 16 | Dec. 10 |
| | | | | August 21 | |
| | | | | Sept. 19 | |
| | | | | Nov. 28 | |

*4.2.1.2. Sample analysis*

Samples were obtained from quarters graded 3 and K (clinical) in both stocks and were transported on ice (cool box) to the laboratory (private veterinary laboratory run by DVM Gabriella Markus – udder health specialist). Quarter milk samples were cultured for presence of bacteria and antibiotic resistance. Bulk milk samples were taken at the same time to evaluate the hygienic and udder health status of the given heard. Bulk milk samples were analysed for SCC, TPC, coliform count, S. aureus count and antibiotic residuals (detergent) and the tanks and different sites of the milking machine (teat cup, collector, milk tube etc.) also were cultured

Somatic cell counts were performed by MT 01 (Agro-Legato Kft. Budapest) milk-tester according to the manufacturer's recommended procedures (KCMT01 reagent diluted with double amount of water).

Microbiological analyses were performed after a freezing (within ~6 h of pick-up at the farm). Total bacterial counts (TPC) were determined according to standard methods. Specific organisms or groups of organisms were detected and enumerated by serial dilution of milk samples and spread-plating of 0.1 ml of milk.

Test media were prepared from a dehydrated base in accordance with the manufacturer's recommendations. The autoclaved agar was cooled in a water bath and poured into Petri dishes. Inoculations were made at room temperature (in duplicate) by a sterilised loop onto bovine blood agar (bioMérieux 51039), purple lactose agar for presumptive coliforms (bioMérieux 51035) and salty agar (for presumptive Staphylococcus ssp). Plates were incubated for 24 h at 37°C. Then antimicrobial susceptibility was tested (Mueller-Hinton agar bioMérieux 51075, OXOID test discs) and plates were incubated 24 h more. Coagulase-test (rabbit plasma bioMérieux 55181: jelly - S. aureus, no changes - CNS S. argus), Japanese Gram-test (KOH or NaOH: jelly +, no changes -, CAMP-test (Str. agalactiae and dysgalactiae with test strains) and catalase-test (30% $H_2O_2$: fizz - Pasteurella and Staphylococcus spp., no changes – Streptococcus spp.) were made, too.

Delvotest®SP (Labomark Kft. Mosonmagyaróvár) according to manufacturer's prescription was used to detect residues. The agar medium contains a standardised number of Bacillus stearothermophilus var. calidolactis

and is coloured purple by indicator bromocresol purple. Water-bath with a well controlled temperature of 64°C is necessary. This test is extremely sensitive to antibiotics and other antibacterial substances (disinfectants, detergents etc).

### 4.2.2. Resistance

It is widely accepted that the predominant cause of mastitis is intramammary infection by microorganisms, usually bacteria. The cow's response to the infection is inflammation. Infection and inflammation are dynamic processes (*Figure 3*).

Hence, the objective of this part of the study was:

- to evaluate the differences of incidence risk (defence mechanism) of black and red Holstein Friesian cows.

#### *4.2.2.1. Farm selection*

To answer the questions mentioned above experiments (combined with everyday routine) were carried out in stock C and D from November 1999 till the end of 2001. Parameters of the farms can be seen in *Table 8*.

#### *4.2.2.2. Examination*

Combined with a heard mastitis control program cows with clinical signs of mastitis were selected and examined visually. Then milk samples were obtained from quarters graded 3 and K (clinical) in both stocks (and were transported for bacteriological examination). Basic data of cows were taken from the register of the stocks.

Date and numbers of examined cows were 1503 and 898, respectively (*Table 10*).

#### *4.2.2.3. Statistical analysis*

The number and ration of total and sampled black and red cows were calculated and comparisons were made.

**Table 10.** Date of examination and number of sampled cows at stock C and D

| Date | | Stock | |
|---|---|---|---|
| | | C | D |
| 1999 | Nov. 12 | 127 | |
| | Nov. 13 | | 73 |
| 2000 | March 13 | | 48 |
| | April 18 | | 5* |
| | June 19 | | 101 |
| | July 23 | | 5* |
| | August 24 | | 12* |
| | Sept. 15 | | 2* |
| | Oct. 19 | | 105 |
| | Dec. 22 | | 82 |
| 2001 | Febr. 19 | 125 | |
| | March 14 | | 92 |
| | April 03 | 219 | |
| | Mai 14 | 192 | |
| | June 12 | 151 | |
| | June 13 | | 76 |
| | July 16 | 144 | |
| | August 21 | 162 | |
| | August 22 | | 93 |
| | Sept. 19 | 179 | 30* |
| | Oct. 17 | | 95 |
| | Oct. 31 | 12* | |
| | Nov. 28 | 192 | |
| | Dec. 10 | | 79 |
| Total | | 1491+12= 1503 | 844+54= 898 |

* extra sample without a heard control

### 4.2.3. Herd mastitis control program

Clinical mastitis is, in most cases, short-lived and becomes subclinical, latent mastitis, and the inflammation response is suppressed subsequent. Analysis of samples taken during consecutive milking shows that short term subclinical infections are surprisingly common. The bacteria are eliminated quickly in most cases, but the inflammation takes longer to disappear (Guidry, 1985; Sandholm, 1995; IDF, 1996b; IDF, 1996c).

Hence, the objective of this part of the dissertation was:

- to study and compare some practical screening methods.

#### *4.2.3.1. Examination*

The focus is on the individual cow/quarter but the case must always be seen in a broader perspective, i.e. the herd.

A preliminary diagnosis was based on the health record (and calving date) of the cow and its clinical signs (if any).

**Table 11.** Points to consider during examination

| **Target** | *Findings and conclusions* |
|---|---|
| Udder | |
| • form | Poor form predispose cows to mastitis |
| • depth | Teats hanging close to the ground are susceptible to infections |
| • symmetry | Asymmetry may indicate mastitis |
| • supramammary area | Lymph nodes might react during mastitis |
| Teats | |
| • position | Outward projecting teats predispose the teats to injuries |
| • condition of skin and apices | Prolapsed teat canal epithelium indicates overmilking |
| **Legs and hooves** | Lameness and poor hoof trimming predispose the cow to mastitis |

*Source: Sandholm, 1995*

The general examination included assessment of the posture, behaviour, body condition and general condition (respiratory rate, pulse frequency, rumen motility and body temperature). Then the udder itself was examined during milking at the milking parlour by inspection, palpation and examination of quarter milk secretion and milk appearance. Inspection includes the size, shape and symmetry of the udder and teats by viewing it from behind and each side (*Table 11* and *12*).

**Table 12.** Points to be considered during palpation

| Target | Findings |
|---|---|
| ***Udder*** | Consistenty (after milking)<br>Swelling<br>Fibrosis<br>Comparison of quarters |
| Supramammary area | Lymph node |
| Teats | Fibrotic masses in the teat apex |

*Source: Sandholm, 1995*

Then the milk was examined and sample was taken if needed (*Table 13*).

**Strip Test**

Foremilk was visually examined for the gross abnormalities by squirting a few streams of milk onto the strip cup.

**California Mastitis Test (CMT)**

Testing individual quarter samples requires the use of a plastic paddle having four shallow cups (marked FL, FR, HR and HL for easy identification of the individual quarter from which the milk was obtained).

Approximately 1/2 teaspoon (2 cc) of milk was tested (the amount usually left in the cups when the paddle is held in an angle of nearly 45°). An equal amount of CMT reagent (Dosyl test, Diversey Ltd. Budaörs) was added to the milk. The paddle was then rotated in a circular motion to mix the contents thoroughly. It is important to "read" the test quickly as the reaction tends to

disintegrate after about 20 seconds. The reaction was visually scored as -, 1, 2, 3, "clinical" and "empty quarter" depending upon the amount of gel that forms (*Table 14*). The thicker the gel, the higher the score. At the end, rinsing the paddle thoroughly with water makes it ready for the next test (Schalm et al., 1971; IDF, 1975b; IDF, 1981; NMC, 1987).

**Table 13.** Inspection of milk and points to be considered

| **Method** | **Points to focus on** |
|---|---|
| ***Milking*** | Poor or dispersed milk flow indicates a chronic teat injury. |
| Inspection | Clots, flakes and serous or pus-like appearance indicate mastitis. (Dry secretion is serous and honey-like!) |
| Odour | Foul odour of the secretion indicates presence of anaerobic bacteria. |
| CMT | Lactation stage and age influence the results. Abnormal quarters can be found by quarter comparison. |
| pH | Increased pH indicates mastitis. The pH of colostrum is lower than in normal milk. |

*Source: Sandholm, 1995*

*4.2.3.2. Sample analysis*

Samples were obtained from quarters graded 3 and K (clinical) in both stocks (according to *Table 14*) and were transported on ice (cool box) to the laboratory (private veterinary laboratory run by DVM Gabriella Markus – udder health specialist). Quarter milk samples were cultured for presence of bacteria and antibiotic resistance. Bulk milk samples were taken at the same time to evaluate the hygienic and udder health status of the given heard. Bulk milk samples were analysed for SCC, TPC, coliform count, S. aureus count and antibiotic residuals (4.2.1.2.).

**Table 14.** Interpretation and scoring of the CMT test

| Symbol | Description of visible reaction |
|---|---|
| - (negative) | Mixture remains homogeneous liquid with no evidence of thickening. |
| 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). |
| 2 (distinct positive) | Mixture thickens immediately and a gel formation is suggested. As the mixture is swirled it tends to move in toward the centre, exposing the bottom of the outer edge of the cup. When the motion is stopped the mixture levels out and covers the bottom of the cup. |
| 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. |
| K (clinical) | Obvious clinical signs |
| S (empty quarter) | "Out of order" |

## 4.3. TOTAL AND DIFFERENCIAL CELL COUNT

The SCC measures all types of cells in milk, including lymphocytes, eosinophils, basophiles, neutrophils, macrophages, and epithelial cells. However, SCC cannot distinguish between the type of cells present in milk. SCC varies with time and frequency of milking, stage of lactation, and season.

Direct microscopic counting of milk somatic cells is the standard by which all other tests are calibrated. Differential counting of cells in milk can be a useful diagnostic tool in bovine mastitis research because each cell type has its own more or less specific function in the immune response.

Cell numbers (and types) in milk can be counted by microscopy or by particle counters, such as the Coulter counter and Fossomatic (Lee et al., 1980;

Hoare et al., 1980; Dahoo et al., 1981). Pulse and flow cytometric techniques have also been used for counting milk cells (Hageltorn and Saad, 1986; Wever and Emanuelson, 1989; Saad and Östensson, 1990).

### 4.3.1. Differential cell stain (DCS)

Panoptic staining with Pappenheim, Giemsa, Wright, May-Grünwald or Leishman stains is a standard technique in haematological diagnostic procedures and, based on these, the direct smear method used for observing somatic cells in milk should be similar to blood smear technique (May and Grünwald 1902, Hoare et al., 1980, Dahoo et al., 1981). This test is also the standard by which the differential cell count test is calibrated (Lee et al., 1980; Hageltorn and Saad, 1986; Wever and Emanuelson, 1989; Saad and Östensson, 1990).

#### *4.3.1.1. Farm selection*

Milk samples were obtained from one stock of farm E which consists of ~ 600 Holstein Friesian cows aged 2 to 10 years with most animals being 4-5 years of age. Samples (n=16 x 5) were collected weekly during January – February in 2000 from individual quarters in mid lactation cows by hand stripping and were examined within 1-2 hours.

#### *4.3.1.2. Sample preparation*

Smears of raw milk from *"healthy"* cows were air-dried, fixed and stained according to May-Grünwald (Reanal R6-R3).

Alternatively, 5 ml of milk was added to a centrifuge tube containing 3 ml of ice-cold isotonic salt solution. Aliquots were centrifuged at room temperature for 10 min at 2000 rpm in order to multiply cells. The supernatant, including the butterfat layer, was removed from the walls of the tube by cotton-tipped applicators and the pellet was resuspended in 0.5 ml isotonic salt solution. Smears were air-dried, fixed and stained as described before.

On each slide 100 (or 200) cells were counted at magnification and identified as lymphocytes, granulocytes (neutrophils, eosinophils and basophils) or monocytes.

*4.3.1.3. Sample analysis*

Approximately 10 ml of each sample were transported on ice (cool box) to the laboratory (Hungarian Dairy Research Institute at Mosonmagyaróvár). Samples were analysed for SCC, fat, protein and lactose content, and TPC.

Somatic cell counts were performed by a Fossomatic 5000 (Hillerød, DK) and ingredients by a MilkoScan FT 120 milk-tester according to the manufacturer's recommended procedures.

Microbiological analyses were performed within 36 h of pick-up at the farm. Total bacterial counts (TPC) were determined according to standard methods.

*4.3.1.4. Statistical analysis*

Means of somatic cell count and the number of different cells identified as lymphocytes, granulocytes (neutrophils, eosinophils and basophils) or monocytes were registered. Analysis was done to determine correlations between SCC, lymphocytes, granulocytes and monocytes.

### 4.3.2. Differential cell count (DCC)

Quantification of the different physiologic and biochemical properties of individual cells or cell compartments in a cell population can be determined rapidly with flow cytometry (FCM). FCM is a multiparametric analysis of each cell as it passes through a light beam. Differential cell count (DCC) is a flow cytometric technique that uses a combination of (DNA-binding) fluorescent dyes to identify the types of inflammatory cells present in milk (Hageltorn and Saad, 1986; Wever and Emanuelson, 1989; Saad and Östensson, 1990).

Elimination of microorganisms from the mammary gland depends mainly on the combination of humoral components and phagocytosis. Phagocytosis is the function of the cellular defence mechanism of the udder. The flow cytometric method allows a rapid and accurate simultaneous determination of different cells (phagocytosis), and so the response of immune system (resistance) of individuals (Saad, 1987).

Therefore, the objectives of this part of the study were:

- to test and adapt the use of this advanced method in the scientific and the routine animal breeding practice,
- to evaluate DCC as a tool to monitor udder health,
- to compare some different methods of sample preparation.

*4.3.2.1. Farm selection*

Individual quarter milk samples were obtained aseptically in mid lactation by hand stripping from one stock of farm E which consists of ~ 600 Holstein Friesian cows aged 2 to 10 years with most animals being 4-5 years of age.

*4.3.2.2. Sample preparation*

5 ml of raw milk sample (n=3 x 8 in duplicate, Σ 48) from so called "*sic*" cows (high somatic cell count) was added to a centrifuge tube containing

- 3 ml of ice-cold isotonic salt solution,
- 2 ml of ice-cold 1% formaldehyde,
- Bromopol pills.

Aliquots were centrifuged at room temperature for 10 min at 2000 rpm in order to multiply cells. The supernatant, including the butterfat layer, was removed from the walls of the tube by cotton-tipped applicators. Followed by two more centrifugations the pellets were resuspended in 5 ml isotonic salt solution.

Samples were then sent to flow cytometric analysis using a Becton-Dickinson FACS-III flow cytometer (Becton-Dickinson Inc., USA). To establish flow cytometric analysis regions, check beads (4.3-6.8-10.8 μm) were run individually. After a centrifugation and resuspension in 1 ml of PBS pellets ($\sim 10^6$/ml) were stained with PI (propidium jodide, Sigma Chemical Co.) and according to the manufacturers recommended procedures linear forward scatter (size) and log side scatter (cellular complexity) were collected simultaneously for 1 min. For excitation the argon ion laser was tuned to 488 nm. Cells were determined as lymphocytes, granulocytes or monocytes.

*4.3.2.3. Microbiological analysis*

Approximately 10 millilitres of milk from cows identified as "*sic*" (infected, high SCC) was obtained for bacteriological evaluation (mastitis pathogens) and somatic cell count. Milk samples from the same quarters were sent and transported on ice (cool box) simultaneously to a private veterinary laboratory in Budapest (4.2.1.2.) and to the laboratory of Hungarian Dairy Research Institute in Mosonmagyaróvár (4.3.1.3.). The used materials and methods were described before.

*4.3.2.4. Statistical analysis*

DCC data were digitalised and cell counts were analysed statistically using a spreadsheet program (Microsoft Excel for Windows).

# 5. RESULTS AND DISCUSSION

## 5.1. MILK PRODUCTION: YIELD AND CELL COUNT

### 5.1.1. Milk yield and somatic cell count of Simmental and Holstein Friesian $F_1$ cows in different lactations

Milk yield and somatic cell count of milk produced by the progeny groups of the same sires in two stocks were used for the analysis. Means of 305 days of lactations were calculated. Herd size ranged 140 at A and 100 at B stock. There were 66 and 32 cows respectively that had 11 data of test day milk yield and SCC in the selected period (calving interval is ~420 days). Some cows had shorter production period because of the relative "early" pregnancy and therefore had just 9-10 test day results.

Two out of five and six sires respectively were found in the studied stocks that had reasonable progeny group. Daughters of the Simmental sire No. 8958 produced in the 1., 2., and 3. (5.) lactations. Semen of the red Holstein Friesian sire No. 9117 was used earlier and therefore his daughters produced in the (2.), 3., 4., and 5. lactations.

Significant differences in fat and protein content were not found (*Table 15*). However, milk production and somatic cell count present large deviations. 305 days milk production, means of SCC and lactation No. of progeny groups of two proven sires at stock A and B can be seen in *Table 16*. Milk production and somatic cell count data were calculated according to stocks, progeny groups and lactations, too (*Table 16*, *Figure 4-11*).

**Table 15.** Overall means of 305 days milk production, fat and protein content, somatic cell count and average lactation No. at stock A and B ($n_A$=66, $n_B$=32)

| | Milk kg | Fat % | Protein % | SCC | Lactation |
|---|---|---|---|---|---|
| Stock A | 3998 *** | 3.78 NS | 3.28 NS | 479,000 *** | 3.3 * |
| Stock B | 3027 *** | 3.75 NS | 3.30 NS | 946,000 *** | 3.7 * |

(level of significance: ***: P=0.1 %, **: P=1 %, *: P=5 %, +: P=10 %, NS=not significant)

**Table 16.** 305 days milk production, means of SCC and lactation No. of progeny groups of two proven sires at stock A and B

| Stock | Sire | Milk kg (Σ) | SCC (mean) | Lactation (mean) |
|---|---|---|---|---|
| A | 9117 | 4334 ** | 737,000 *** | 3.5 *** |
| | 8958 | 4043 ** | 455,000 *** | 1.9 *** |
| B | 9117 | 3433 *** | 547,000 *** | 4.1 *** |
| | 8958 | 2894 *** | 1,121,000 *** | 2.1 *** |

$n_{9117A}$=23, $n_{8958A}$=24, $n_{9117B}$=10, $n_{8958B}$=13
(level of significance: ***: P=0.1 %, **: P=1 %, *: P=5 %, +: P=10 %, NS=not significant)

**Figure 4.** Milk production of progeny groups and means at stock A ($n_{mean}$=66, $n_{8958}$=24, $n_{9117}$=23)

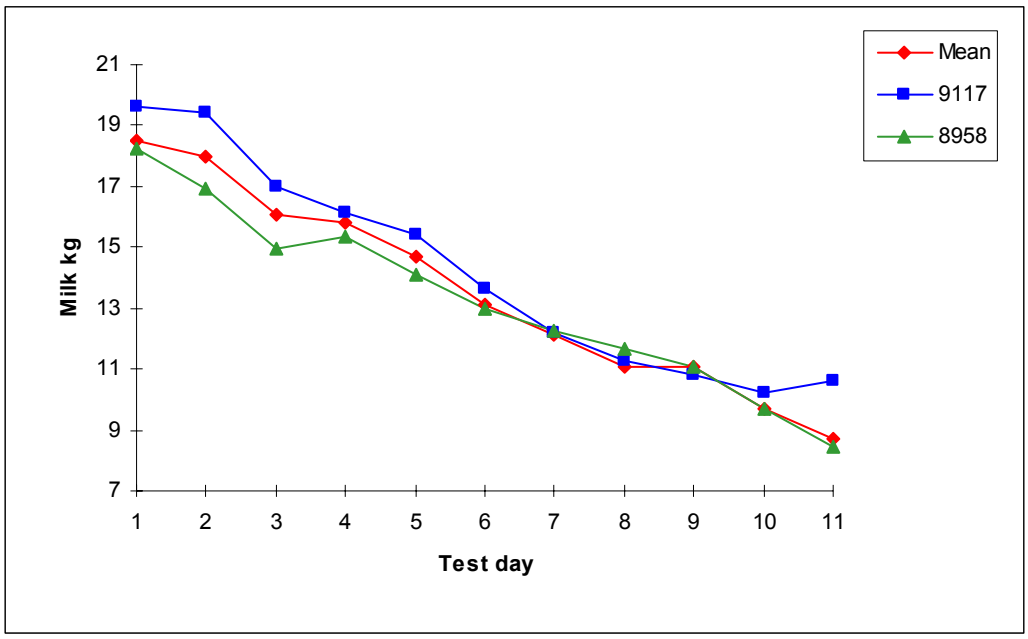


**Figure 5.** Milk production of progeny groups and means at stock B
($n_{mean}$=32, $n_{8958}$=13, $n_{9117}$=10)

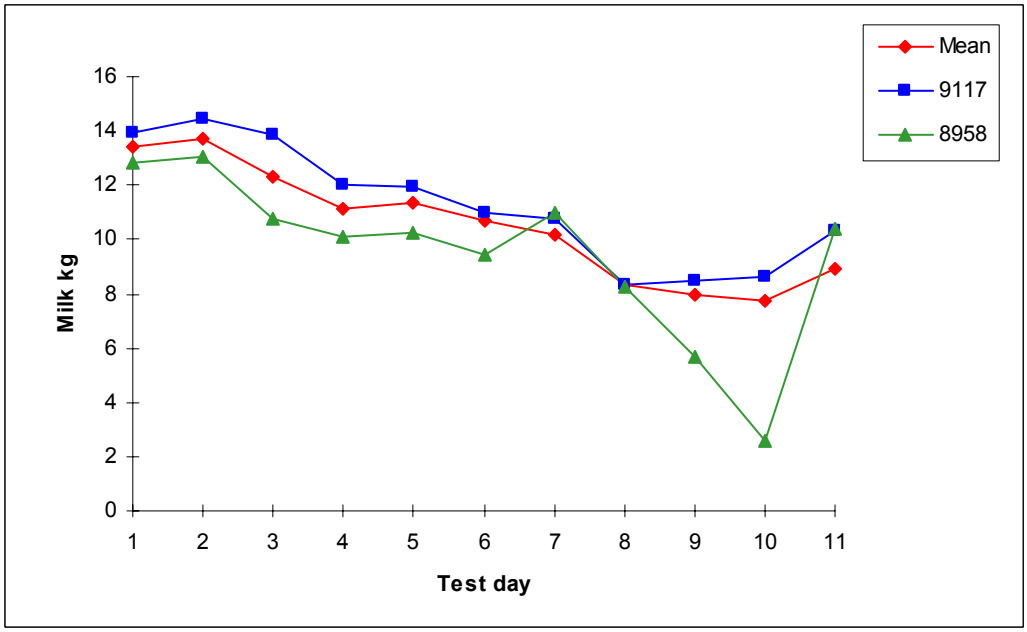



**Figure 6.** Somatic cell count of progeny groups and means at stock A
($n_{mean}$=66, $n_{8958}$=24, $n_{9117}$=23)

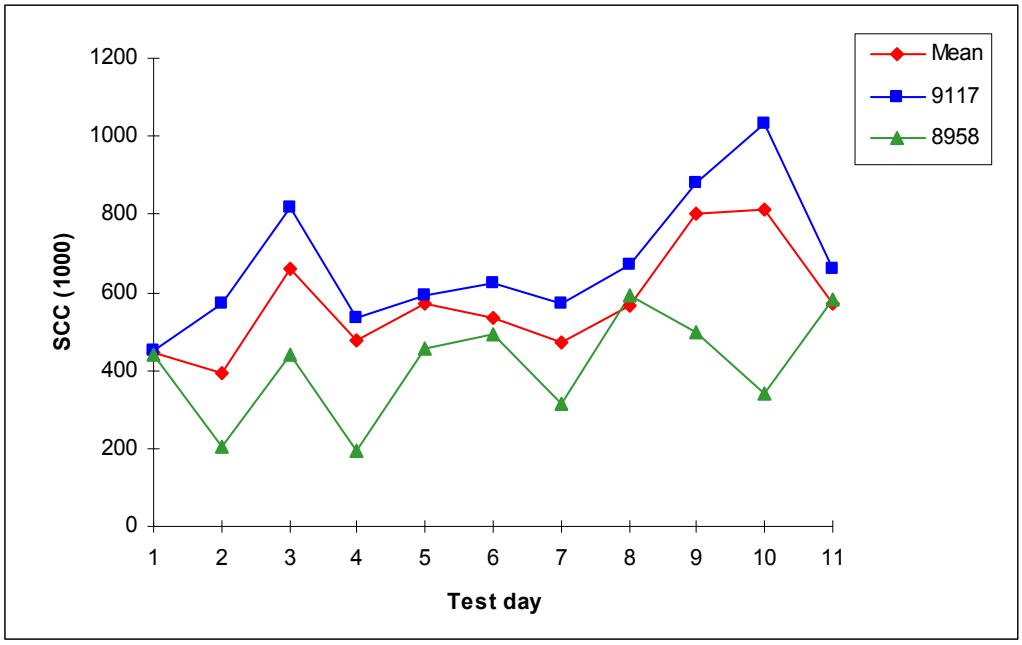


**Figure 7.** Somatic cell count of progeny groups and means at stock B
($n_{mean}$=32, $n_{8958}$=13, $n_{9117}$=10)

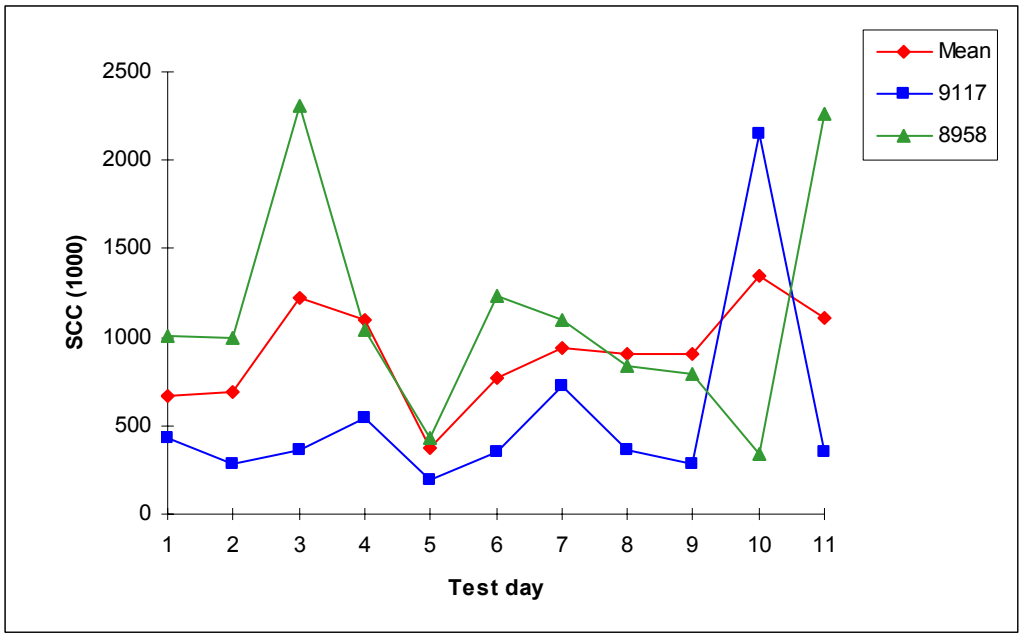



**Figure 8.** Milk production of progeny group of sire No. 8958 according to lactations and stocks based on test day data
($n_{1A}$=6, $n_{2A}$=13, $n_{3A}$=4; $n_{1B}$=4, $n_{2B}$=4, $n_{3B}$=5)

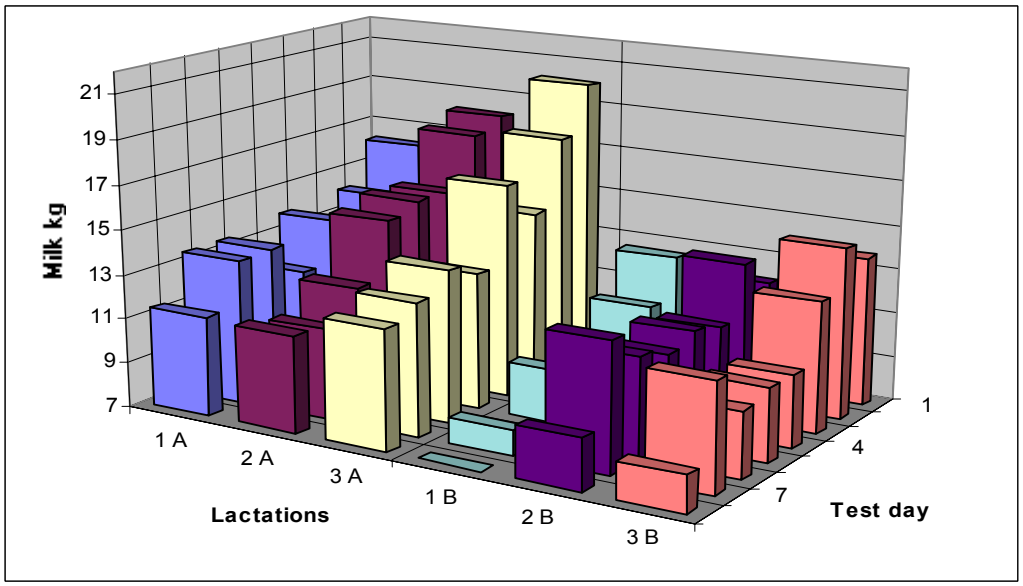


**Figure 9.** Milk production of progeny group of sire No. 9117 according to lactations and stocks based on test day data
($n_{3A}$=13, $n_{4A}$=6, $n_{5A}$=3; $n_{3B}$=3, $n_{4B}$=3, $n_{5B}$=4)

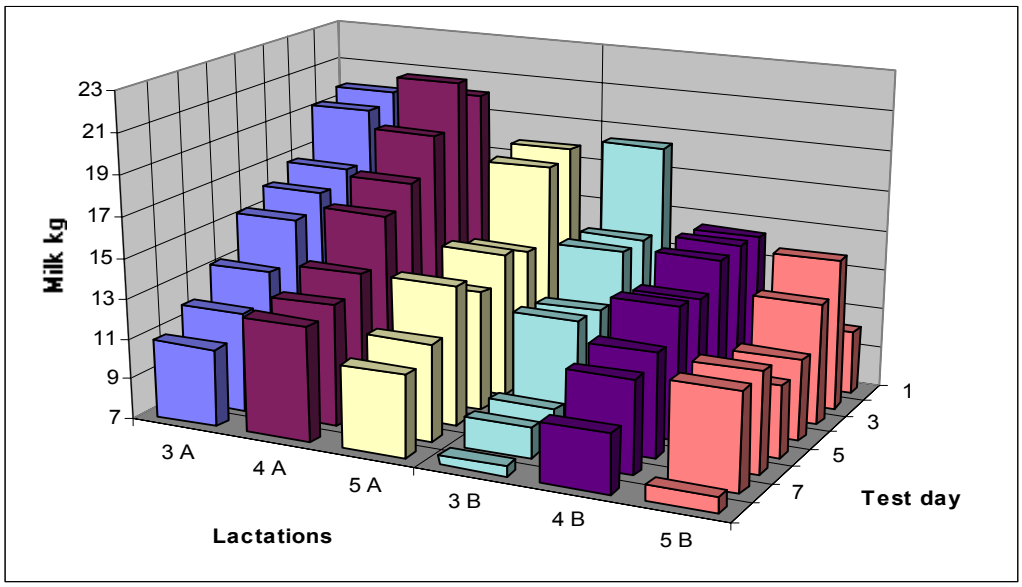



**Figure 10.** Somatic cell count of progeny group of sire No. 8958 according to lactations and stocks based on test day data
($n_{1A}$=6, $n_{2A}$=13, $n_{3A}$=4; $n_{1B}$=4, $n_{2B}$=4, $n_{3B}$=5)

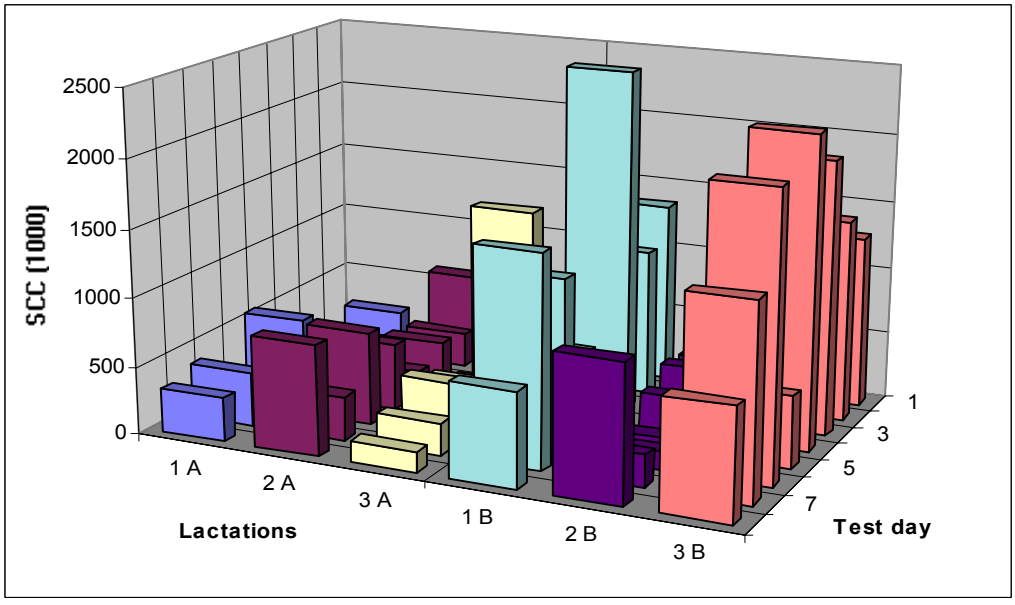
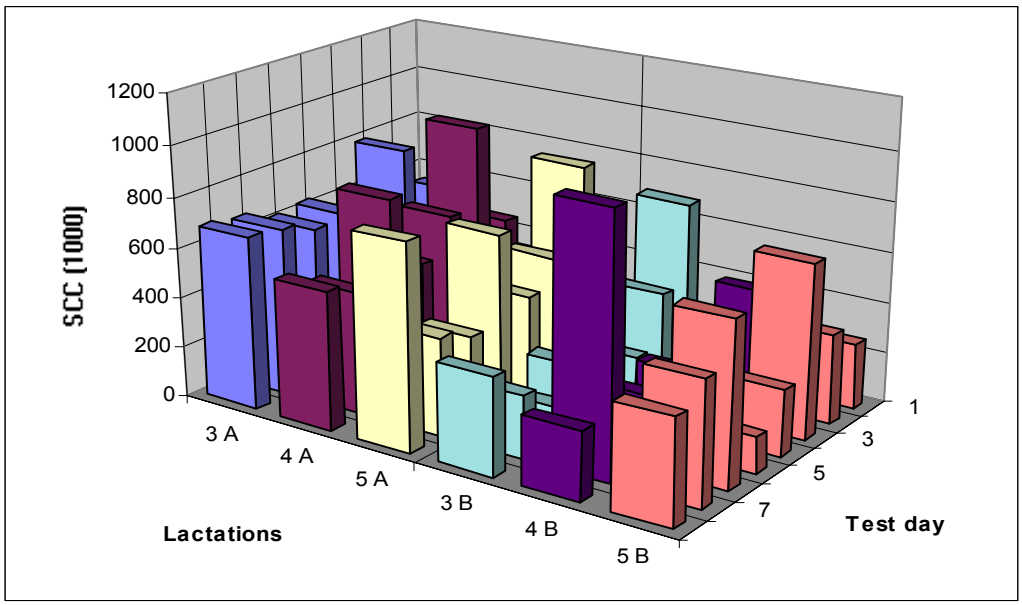


**Figure 11.** Somatic cell count of progeny group of sire No. 9117 according to lactations and stocks based on test day data
($n_{3A}$=13, $n_{4A}$=6, $n_{5A}$=3; $n_{3B}$=3, $n_{4B}$=3, $n_{5B}$=4)




Environmental factors (keeping and milking hygiene, feeding etc.) and moreover, number of lactations and stage of lactation should be known for correct evaluation of the udder health status of a stock. Test day milk production and somatic cell count data are very important nowadays to ensure a reasonable production level. Because of the insufficient circumstance trends well known in the literature could not be seen. However, selection for mastitis resistance is necessary and could be efficient.

Conclusions are as fallows:

- milk yield was higher at stock A,
- daughters of sire No. 9117 produced more milk at both stocks than daughters of sire No. 8958,
- somatic cell counts are especially high and show extreme differences,
- evaluations show that there is a lot to be desired and a lot to be done for improving udder health,
- test day somatic cell count is just a very basic step in udder health work but even the first most important.

### 5.1.2. Milk yield and somatic cell count of red Holstein Friesian cows in different lactations

Relationship between genetic improvement for milk yield and somatic cell count as the main tool to detect incidence of mastitis were studied. Means of 305 days milk production and somatic cell count ($log_2$ SCC, SCS) of offspring of sires in different lactations were used for the analysis (*Table 17*).

Table 17. **Means of 305 days milk production, somatic cell count, $log_2$ SCC and its standard deviations according to number of lactations**

| Lactation | n | $n_{sire}$ | 305 days milk (kg) | | SCC x 1000 | | |
|---|---|---|---|---|---|---|---|
| | | | yield | SD | mean | SD | $log_2$ |
| 1 | 1230 | 40 | 6223 | 1482 | 195 | 214 | 7.74 |
| 2 | 1124 | 52 | 7098 | 2311 | 267 | 252 | 7.98 |
| 3 | 854 | 58 | 7169 | 2321 | 344 | 395 | 8.63 |
| 4 | 630 | 48 | 7183 | 2315 | 380 | 397 | 8.63 |
| 5 | 397 | 38 | 7111 | 2408 | 498 | 528 | 9.04 |
| 6 | 206 | 29 | 6778 | 2638 | 554 | 573 | 9.16 |
| 7 | 103 | 20 | 5960 | 2647 | 755 | 788 | 9.62 |
| 8 | 48 | 17 | 6361 | 2158 | 675 | 683 | 9.42 |
| 9 | 18 | 6 | 5449 | 2241 | 908 | 661 | 9.37 |
| 10 | 5 | 4 | 5557 | 1025 | 658 | 434 | 8.76 |
| Mean/Σ 2.84 | 4614 | 83 | 6835 | 2200 | 328 | 393 | 8.62 |

Correlation of milk yield and number of lactation is presented in *Figure 12* ($r_f$=0.88). The maximum milk yield can be obtained at $n_{lact}$=3.88. It reflects the importance of longevity and lifetime performance.

Many hypothesis tests and estimation procedures assume a *normal distribution* of the variable of interest. A transformation is a mathematical manipulation applied to each data point. The aim of the transformation is to produce a data set, which satisfies the requirements of the procedure. Logarithmic transformation makes the distribution of *x* more nearly normal if it is skewed to the right, in which case *x* is said to have a *lognormal distribution*.

Because of the lognormal distribution of somatic cell count data were transformed by $log_2$. *Figure 13* shows the correlation of transformed somatic cell count (called SCS) and number of lactation ($r_f$=0.93). *Figure 14* presents

the correlation of the transformed somatic cell count (SCS) and milk yield according to the number of lactation (age of cow) and *Figure 15* shows the same but separately the younger and older group of cows.

**Figure 12.** Correlation of milk yield and number of lactation ($n_{lact.}$=4614)

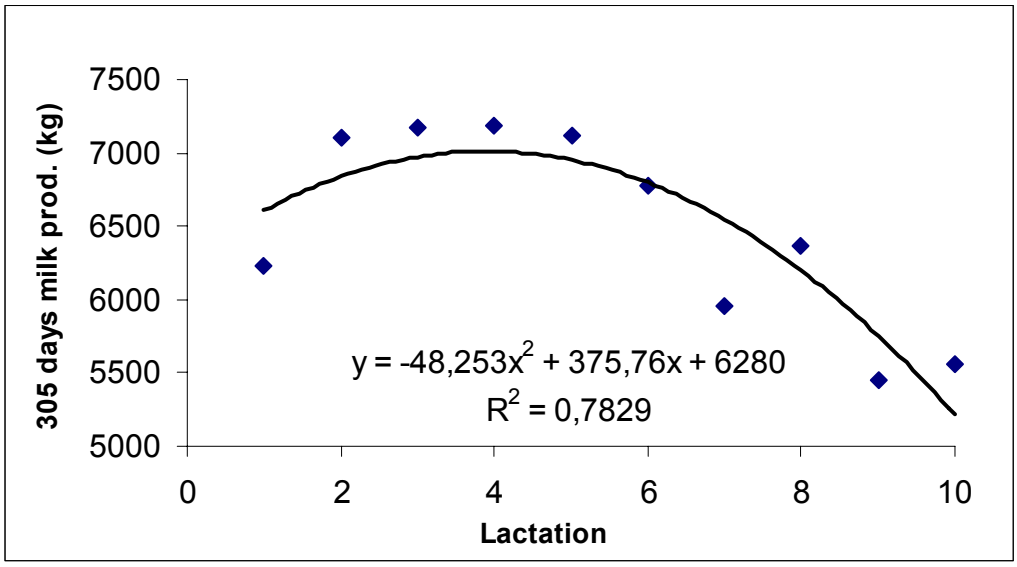



**Figure 13.** Correlation of transformed somatic cell count (SCS) and number of lactation ($n_{lact.}$=4614)

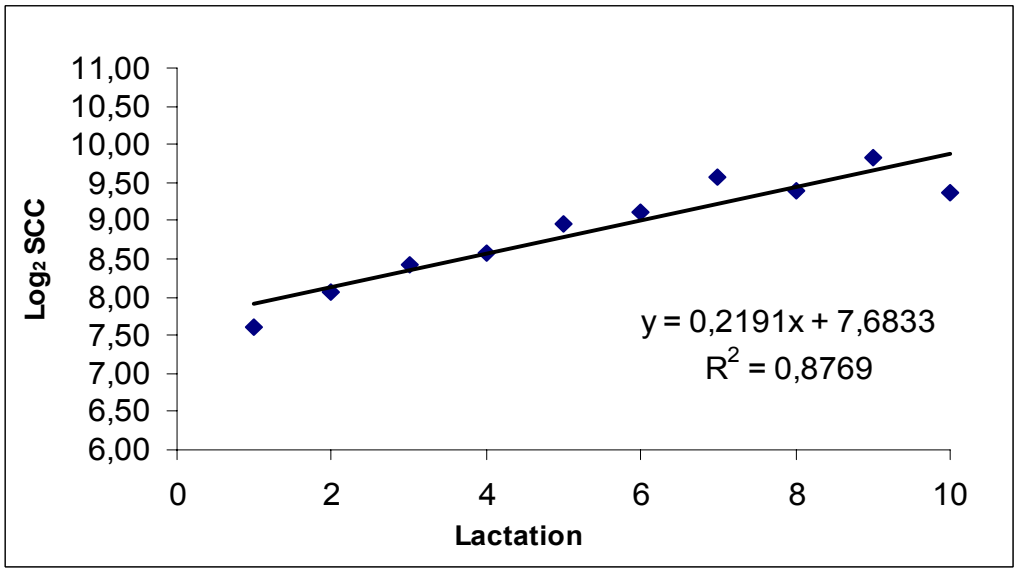


**Figure 14.** Correlations of transformed somatic cell count and milk yield ($n_{lact.}$=4614)

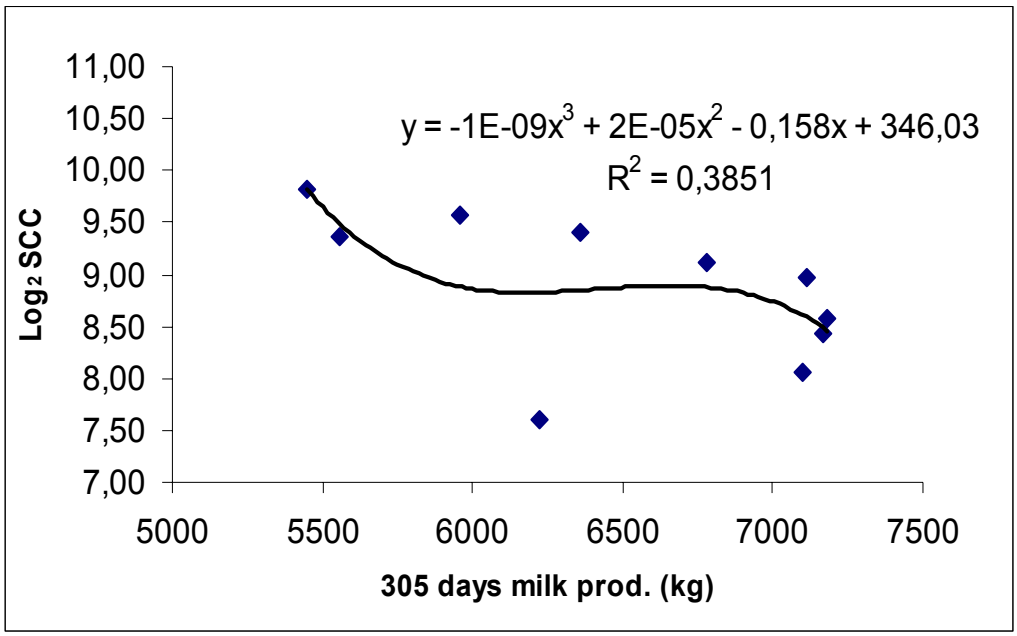



**Figure 15.** Correlation of transformed somatic cell count and milk yield according to the number of lactation ($n_{lact.}$=4614)

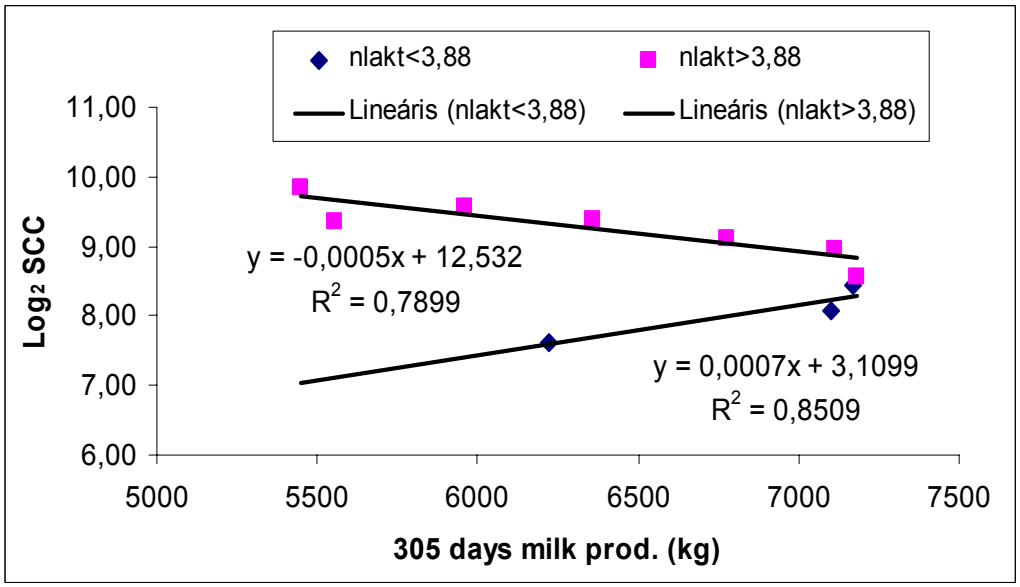


*Figure 16* shows the 305 days milk production and the somatic cell count of it according to the number of lactation. Correlations (based on transformed data) can be seen in *Table 18* and *Table 19* presents the level of significance.

**Figure 16.** 305 days milk production and somatic cell count according to the number of lactation ($n_{lact.}$=4614)

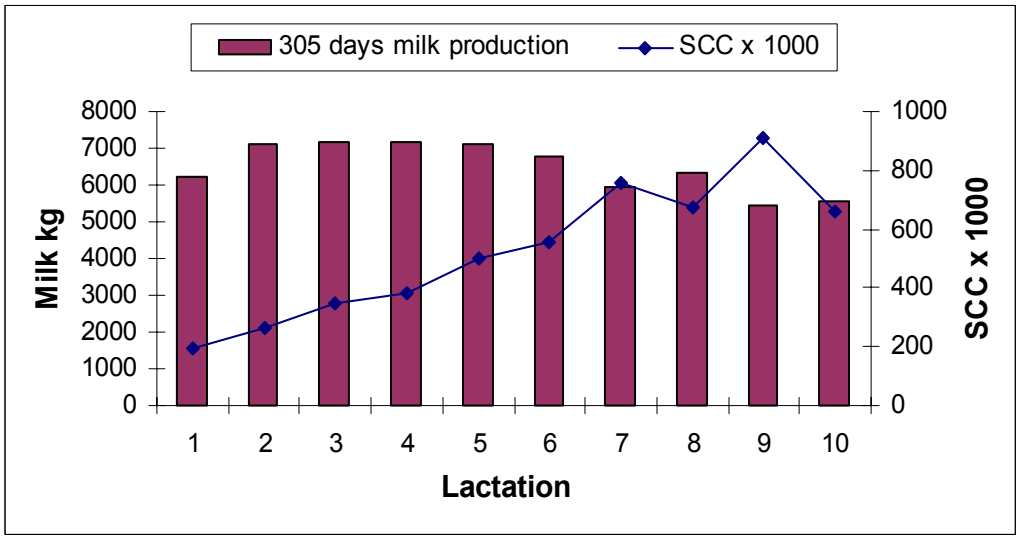



**Table 18.** Correlation of milk production and somatic cell count (transformed data) according to the number of lactation

| Lactation | n | *305 days milk (kg)* | SCC x 1000 | *$Log_2$ SCC* | $r_f$ |
|---|---|---|---|---|---|
| 1 | 1230 | *6223* | 195 | *7.74* | **-0.13** |
| 2 | 1124 | *7098* | 267 | *7.98* | **-0.12** |
| 3 | 854 | *7169* | 344 | *8.63* | **-0.21** |
| 4 | 630 | *7183* | 380 | *8.63* | **-0.09** |
| 5 | 397 | *7111* | 498 | *9.04* | **-0.16** |
| 6 | 206 | *6778* | 554 | *9.16* | **-0.16** |
| 7 | 103 | *5960* | 755 | *9.62* | **-0.14** |
| 8 | 48 | *6361* | 675 | *9.42* | -0.20 |
| 9 | 18 | *5449* | 908 | *9.37* | -0.50 |
| 10 | 5 | *5557* | 658 | *8.76* | -0.54 |
| Mean/Σ (2.84) | 4614 | *6835* | 328 | *8.62* | **-0.12** |

Correlation of LSCS and milk production was found as Kennedy (1982), Ruabertas and Shook (1982), Monardes and Hayes (1985), Emanuelson et al. (1988) Banos and Shook (1990), Boettcher et al. (1992) and many others have reported.

**Table 19.** Significance of 305 days milk production and transformed SCC according to number of lactations ($n_1$=1230, $n_2$=1124, $n_3$=854, $n_4$=630, $n_5$=397, $n_6$=206, $n_7$=103, $n_8$=48, $n_9$=18, $n_{10}$=5, $n_{mean}$=4614)

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | - | *** | *** | *** | *** | ** | NS | NS | * | NS | *** |
| | - | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** |
| 2 | | - | NS | NS | NS | + | *** | * | ** | *** | *** |
| | | - | *** | *** | *** | *** | *** | *** | *** | *** | *** |
| 3 | | | - | NS | NS | * | *** | * | ** | *** | *** |
| | | | - | + | *** | *** | *** | ** | *** | + | NS |
| 4 | | | | - | NS | * | *** | * | ** | *** | *** |
| | | | | - | *** | *** | *** | *** | *** | NS | ** |
| 5 | | | | | - | NS | *** | * | ** | ** | ** |
| | | | | | - | NS | *** | * | ** | NS | *** |
| 6 | | | | | | - | * | NS | + | * | NS |
| | | | | | | - | * | NS | * | NS | *** |
| 7 | | | | | | | - | NS | NS | NS | *** |
| | | | | | | | - | NS | NS | NS | *** |
| 8 | | | | | | | | - | NS | NS | NS |
| | | | | | | | | - | NS | NS | *** |
| 9 | | | | | | | | | - | NS | ** |
| | | | | | | | | | - | NS | *** |
| 10 | | | | | | | | | | - | ** |
| | | | | | | | | | | - | + |
| Mean | | | | | | | | | | | - |

(level of significance: ***: P=0.1 %, **: P=1 %, *: P=5 %, +: P=10 %, NS=not significant)

Test day data were processed according to the somatic cell count, too. Taking into account the SCC lower and higher than 400,000 cells/ml during 8 test day procedures, at last 1175 and 117 observations (lactations) have left, respectively. *Table 20* present the trend of decrease in both groups. *Figure 17* and *Figure 18* show the test day milk yield and the somatic cell count in

comparison with mean of the stock. The difference is clear but the level of significance and the ratio of 305 days production loss can be seen in *Table 21*.

**Table 20.** The trend of decrease in the "active" population after selection on SCC

| Test day | SCC<400,000 | | SCC > 400,000 | |
|---|---|---|---|---|
| | $n_{lactation}$ | % | $n_{lactation}$ | % |
| | Total: 4614 | 100 | Total: 4614 | 100 |
| 1 | 3164 | **68.6** | 1135 | **24.6** |
| 1-2 | 2677 | **58.0** | 540 | **11.7** |
| 1-3 | 2347 | **50.9** | 382 | **8.3** |
| 1-4 | 2085 | **45.2** | 284 | **6.2** |
| 1-5 | 1850 | **40.1** | 218 | **4.7** |
| 1-6 | 1615 | **35.0** | 179 | **3.9** |
| 1-7 | 1408 | **30.5** | 154 | **3.3** |
| 1-8 | 1175 | **25.5** | 117 | **2.5** |

**Figure 17.** Test day milk yield of low and high SCC groups in comparison with mean of the stock ($n_{mean}$=4614, $n_{<400}$=1175, $n_{>400}$=117)

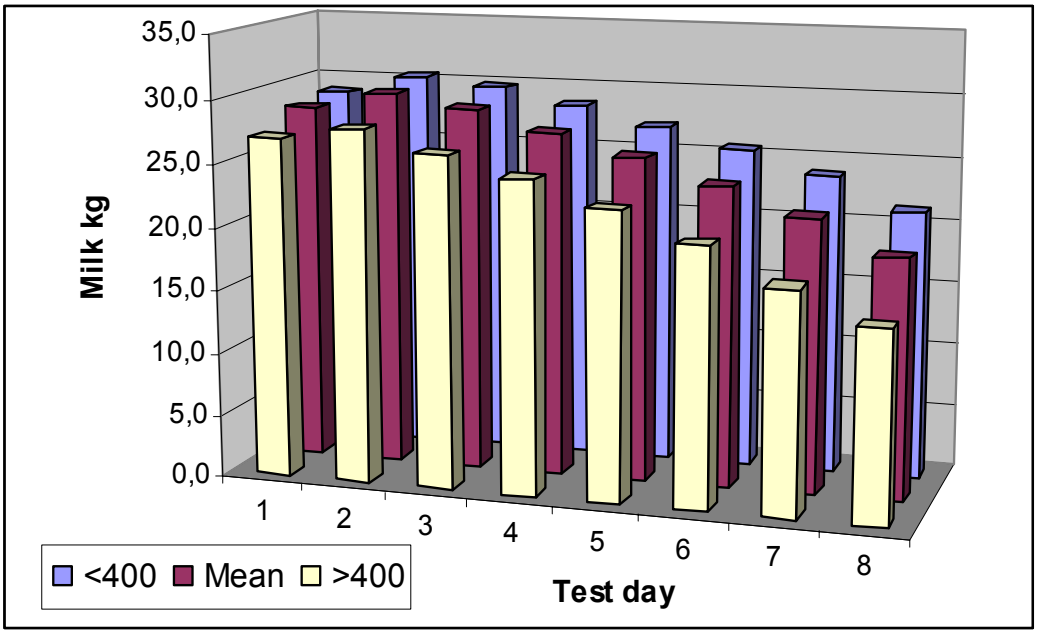


**Figure 18.** Test day somatic cell count of low and high SCC groups in comparison with mean of the stock ($n_{mean}$=4614, $n_{<400}$=1175, $n_{>400}$=117)

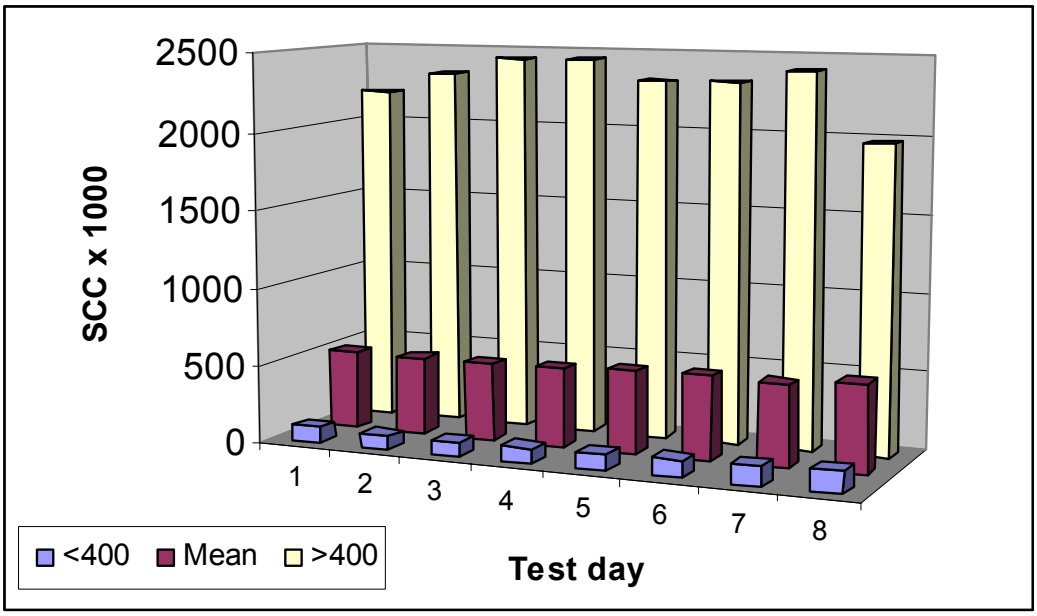



**Table 21.** 305 days milk yield (and ratio of losses), SCC and its phenotypic correlation in high and low SCC groups in comparison with mean of the stock ($n_{mean}$=4614, $n_{<400}$=1175, $n_{>400}$=117)

| | **SCC<400,000** | **Mean** | **SCC>400,000** |
|---|---|---|---|
| $n_{lact.}$ (SD) | 2.4 *** (1.5) | 2.8 (1.7) | 4.4 *** (2.0) |
| 305 days milk yield (kg) | 7351 ***<br>100% | 6835<br>92.9% | 6194 **<br>84.3% (90.6) |
| SD | 1998 | 2200 | 2020 |
| SCC x 1000 | 107 *** | 328 | 1650 *** |
| SD | 55 | 393 | 814 |
| $r_f$ | 0.06 | -0.12 | -0.25 |

(level of significance: ***: P=0.1 %, **: P=1 %, *: P=5 %, +: P=10 %, NS=not significant)

*Table 22* presents the results according to the number of lactations. 432 out of 1175 lactations/cows (%[a]=n/Σ=36.8%) complete their first lactation produced good quality of milk that is 35.1% of the whole population

($\%^b=n/n_{Total}$). Among older animals (in lactation 9 and 10) there were no cow producing only high quality milk during the 8 test day procedures. However, cows producing only high somatic cell count milk during 8 test day procedures represent 53.0% of the whole population in lactation 3-5.

**Table 22.** The trend of changes and ratio of groups producing low and high somatic cell count in milk
($n_{mean}$=4614, $n_{<400}$=1175, $n_{>400}$=117)

| Lactation | Total | **SCC<400,000** | | | **SCC > 400,000** | | |
|---|---|---|---|---|---|---|---|
| n | 4614 | Σ=1175 | $\%^a$ | $\%^b$ | Σ=117 | $\%^a$ | $\%^b$ |
| 1 | 1230 | 432 | 36.8 | 35.1 | 11 | 9.4 | 0.9 |
| 2 | 1124 | 312 | 26.5 | 27.8 | 9 | 7.7 | 0.8 |
| 3 | 854 | 192 | 16.3 | 22.5 | 24 | 20.5 | 2.8 |
| 4 | 630 | 113 | 9.6 | 17.9 | 18 | 15.4 | 2.9 |
| 5 | 397 | 79 | 6.7 | 19.9 | 20 | 17.1 | 5.0 |
| 6 | 206 | 29 | 2.5 | 14.1 | 14 | 12.0 | 6.8 |
| 7 | 103 | 13 | 1.1 | 12.6 | 15 | 12.8 | 14.6 |
| 8 | 48 | 7 | 0.6 | 14.6 | 3 | 2.6 | 6.2 |
| 9 | 18 | - | | | 2 | 1.7 | 11.1 |
| 10 | 5 | - | | | 1 | 0.8 | 20.0 |

$\%^a$: n/Σ, $\%^b$: $n/n_{Total}$

Conclusions are as follows:

- The maximum milk yield can be obtained at $n_{lact}$= 3.88. It reflects the importance of longevity and lifetime performance. Correlation of milk yield and number of lactation was $r_f$=0.88.
- Somatic cell counts are readily available to most dairy farmers today on a monthly basis through the Livestock Performance Testing Ltd., Gödöllő (Hungary). Because of the lognormal distribution of somatic cell count data were transformed by $\log_2$. (The logarithmic transformation may facilitate the international comparison of breeding value estimation of Hungarian dairy herds and therefore adaptation and home application of this method is also desirable and suggested.)
- The correlation of transformed somatic cell count (SCS) and number of lactation was $r_f$=0.93 and the correlation of the transformed somatic cell

count (SCS) and milk yield ranged from –0.5 to –0.09 in different lactations but most values were closer to the mean of –0.12 as many authors reported (Kennedy, 1982; Monardes and Hayes, 1985; Emanuelson et al., 1988; Banos and Shook, 1990; Boettcheret al., 1992). Remarkable that older cows, producing more milk, has lower somatic cell count in milk.

- The ESCC test fulfills several needs which dairymen desire. The ESCC focuses attention on the individual cow. It does not pinpoint the quarter(s) affected but does monitor udder health of individuals.
- The ESCC also allows a herd average SCC to be calculated which serves as a monitor of the udder health of the herd.
- Losses in milk production associated with elevated SCC can be estimated, too. Reasons are lower yields and worst persistence. The differences were statistically significant.
- 25% of the cows start their lactation with high somatic cell count. Till the second test day it drops to the half.
- The ratio of healthy cows during the whole lactation is approximately 25%.
- Clinical mastitis is an expensive, management-intensive problem. Selection to improve udder health is desirable for numerous reasons. Single-trait selection for increased milk yield should result in increased susceptibility to mastitis of dairy cows. However, direct selection for reduced mastitis is not possible because mastitis incidence is not consistently recorded in majority of the cow population. Indirect selection for lower mastitis incidence is an alternative to direct selection.
- Health professionalists and geneticists in the dairy industry have the responsibility to inform producers of the proper use of SCC evaluations. These evaluations will in no way displace improved environmental conditions as the key ingredient in mastitis control. However, long-term trend in incidence of mastitis will have major economic implications if genetic resistance to mastitis is ignored by breeding programs. Genetic evaluations for SCC enable producers to moderate such undesirable economic consequences. All in all, for producing high quality milk we should balance the importance of technological - environmental, biological - genetic and economic factors.

## 5.2. INFECTION, DEFENCE MECHANISMS AND DETECTION

Many authors reported that numerous factors influence the prevalence and increase of mastitis (*Figure 2*) (Saloniemi, 1980; Horváth, 1982; Dohy, 1985;. Cassell, 1988; Janke and Funke, 1989; Dunklee, 1991; Erskine, 1993; Süpek, 1994; Süpek, 1995; Gulyás and Iváncsics, 1999; Iváncsics and Gulyás, 1999; Baltay and Bedő, 2000; Baltay et al., 2000; Busato et al., 2000).

### 5.2.1. Bacteriological environment and milking

The luck of significant variation among bacterial counts from triplicate bulk tank milk samples and the high correlation among corresponding bacterial counts indicates that, with proper sampling technique, one sample can reliably present the microbial status of the entire bulk tank.

Some bacteriological properties of milk at farm C and D can be seen in *Table 23*.

**Table 23.** Some parameters of milk at farm C and D (12-13th November, 1999)

| Parameter | TPC (1000) | coliforms | S. aureus | SCC (1000/ml) | Det. |
|---|---|---|---|---|---|
| | ***Stock C*** | | | | |
| • group 2 | 10 | <1000 | <100 | 230 | - |
| • morning | 450 | <1000 | <100 | 330 | - |
| • afternoon | 40 | <2000 | <100 | 240 | - |
| | Stock D | | | | |
| • tank 1 | 27 | <1000 | <100 | 260 | - |
| • tank 2 | 10 | <1000 | <100 | 265 | - |
| • tank 3 | 37 | <1000 | <100 | 350 | - |

Somatic cell counts varied widely. Individual sample counts ranged ~300,000 (from 12,000 to 7,000,000) cells/ml, whereas individual farm averages ranged from 230,000 to 420,000 cells/ml during the studied period.

There are no magic cell counts at which a cow is free from mastitis. A level of 400,000 cells per ml of milk is commonly used as a starting point for closer observation. This would correspond to a SCC Score of 5 (*Table 4*). Over 50 percent of the cows with SCCs above this level will be infected to some

degree. Cows with counts below this level may be infected since the sample is a composite from all four quarters. Those cows whose SCC begins to increase should be more closely examined. This may be in the form of visual and CMT examination or bacteriological culture.

Microbial contamination of bulk milk originates from three main sources:

- within the udder,
- the teats and udder exterior, and
- milk handling and storage equipment.

TPC presented sometimes extremely high results (450,000 cells/ml) reflecting on the luck of proper washing!

#### *5.2.1.1. "Environmental" microorganisms*

Coliforms, which have been shown to cause environmental mastitis, are found in fecal and bedding material and on poorly cleaned milk handling and storage equipment. Gram-negative organisms are frequently found in water used for cleaning and on poorly cleaned milk handling and storage equipment. Gram negatives easily multiply in the milk residues left after improper cleaning of milking equipment. Subsequent milking can flush these residues into the bulk tank and, thus, greatly increase the TPC.

Examination of Gram-negative levels in bulk tank milk must consider the many psychrotrophs, pseudomonads in particular, present in this group of bacteria. Psychrotrophs survive and multiply at refrigeration temperatures. Longer holding times on the farm combined with the growth of psychrotrophs could result in total bacterial numbers being higher at the time of pick-up than when the milk was collected into the bulk tank.

The key to solving an E. coli mastitis problem is usually a dry environment – water-use is kept to a minimum in the barn. The traditional cornerstones in mastitis prevention – teat dipping and dry cow treatment – have significant influence on coliform mastitis. A vaccine against coliform mastitis is commercially available in some countries but according to Markus (1999, personal communication) the main question is: efficacy.

Fecal and bedding contamination from poorly cleaned udders could theoretically have raised the TPC. Some organisms typically found in fecal and

bedding matter are E. coli, Klebsiella spp. and Str. uberis and Pseudomonas spp.

Good hygiene is most essential in the prevention. The concentrations of the cleaning and disinfecting solutions must be checked to ensure their correct use.

*5.2.1.2. "Contagious" microorganisms*

The effect of antibiotic treatment is usually poor and culling of the infected cow is often the only means of preventing the spread of infections. The objective must be to prevent new cases of infection and cull old cases as soon as possible. S. aureus infections easily turn chronic. A general rule is that the infected animal is culled after one or no more than two unsuccessful treatments.

S. aureus (and Str. agalactiae) can survive on the mucous membrane reported Saperstein et al. (1988), Sandholm et al. (1990) and Nickerson et al. (1993). Therefore, milk from infected cows not allowed/suggested to (heifer) calves. According to Watson (1992) and Markus (1999, personal communication) vaccination against staphylococci is nowadays not a practical tool.

The most frequent mistake made with a herd infected with staphylococci is insufficient culling. In prevention of staphylococcal infections, milking hygiene is most important. In such a herd it is necessary to maintain a strict milking order, with infected cows being milked last (5.2.1.3.). The milkman may also act as infection source and this possibility should be kept in mind.

*5.2.1.3. Proper milking order*

In 1996 a new milking parlour was built and in 1999 a strict udder health control program was started at stock C. Results are increased milk production and decreased somatic cell count. *Figure 19* shows the 305 days milk production and somatic cell count according to the first test day (year). Means of number of lactation from 1995 till 2001 were 2.5, 2.7, 2.8, 2.9, 3.0, 2.8 and 3.2, respectively but considered its correlations with the milk production and the somatic cell count results are even more valuable. Reasons of differences are not (only) the increased age of the population but better environment (milking technique and hygiene) and of course, genetic improvement in production and conformation traits.

305 days milk production and somatic cell count data were extrapolated according to number of lactation, too (*Figure 12* and *Figure 13*). The used formula was $y=-48.253\ x^2 + 375.76\ x + 6280$ for milk production and $y=69.647\ x + 140.3$ for somatic cell count. *Table 24* and *Table 25* present the absolute differences and *Table 26* shows the level of significance.

**Figure 19.** 305 days milk production and somatic cell count according to the first test day (year) in stock C
($n_{1995}$=675, $n_{1996}$=673, $n_{1997}$=694, $n_{1998}$=697, $n_{1999}$=642, $n_{2000}$=728, $n_{2001}$=463)

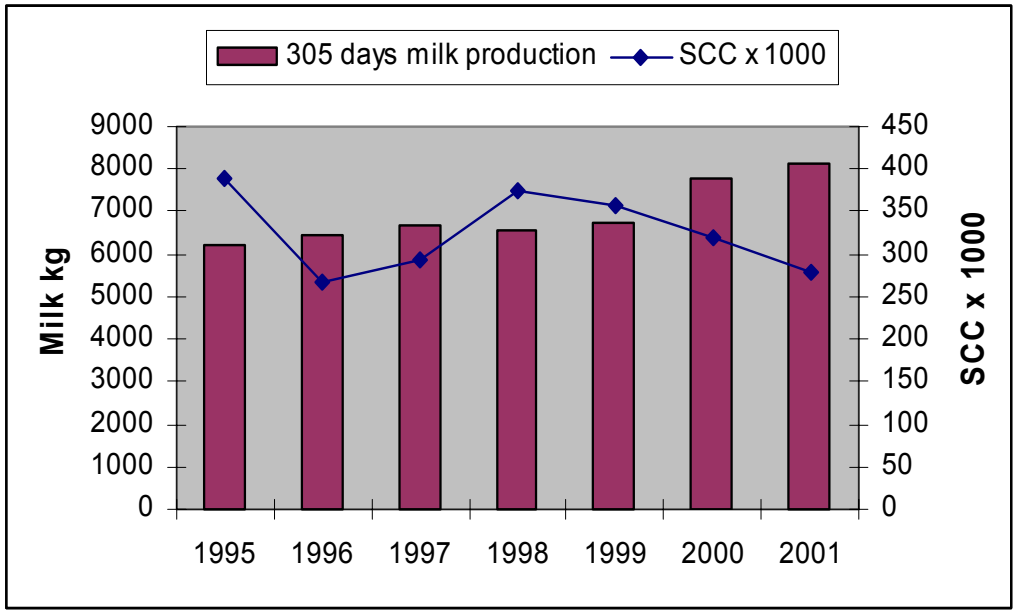



**Table 24.** Means and SD of 305 days milk production according to years in comparison with calculated milk production and the absolute difference
**($n_{1995}$=675, $n_{1996}$=673, $n_{1997}$=694, $n_{1998}$=697, $n_{1999}$=642, $n_{2000}$=728, $n_{2001}$=463)**

| Year | Lactation | | 305 days milk production | | | |
|---|---|---|---|---|---|---|
| | mean | SD | mean | SD | *calculated* | difference |
| 1995 | 2.46 | 1.6 | 6239 | 1467 | *6910* | **-671** |
| 1996 | 2.67 | 1.6 | 6461 | 1605 | *6937* | **-476** |
| 1997 | 2.81 | 1.6 | 6685 | 1705 | *6952* | **-267** |
| 1998 | 2.91 | 1.8 | 6579 | 2002 | *6962* | **-384** |
| 1999 | 3.04 | 1.7 | 6733 | 2130 | *6974* | **-241** |
| 2000 | 2.81 | 1.9 | 7801 | 1959 | *6952* | **849** |
| 2001 | 3.21 | 1.8 | 8103 | 3231 | *6986* | **1117** |

**Table 25.** Means and SD of somatic cell count according to years in comparison with calculated SCC and the absolute difference
**($n_{1995}$=675, $n_{1996}$=673, $n_{1997}$=694, $n_{1998}$=697, $n_{1999}$=642, $n_{2000}$=728, $n_{2001}$=463)**

| Year | Lactation | | SCC x 1000 | | | |
|---|---|---|---|---|---|---|
| | mean | SD | mean | SD | *calculated* | difference |
| 1995 | 2.46 | 1.6 | 389 | 411 | *312* | **78** |
| 1996 | 2.67 | 1.6 | 267 | 280 | *326* | **-59** |
| 1997 | 2.81 | 1.6 | 293 | 346 | *336* | **-43** |
| 1998 | 2.91 | 1.8 | 374 | 471 | *343* | **31** |
| 1999 | 3.04 | 1.7 | 357 | 431 | *352* | **4** |
| 2000 | 2.81 | 1.9 | 319 | 412 | *336* | **-17** |
| 2001 | 3.21 | 1.8 | 279 | 316 | *364* | **-85** |

**Table 26.** Significance of 305 days milk production and SCC according to year ($n_{1995}$=675, $n_{1996}$=673, $n_{1997}$=694, $n_{1998}$=697, $n_{1999}$=642, $n_{2000}$=728, $n_{2001}$=463)

| | 1995 | 1996 | 1997 | 1998 | 1999 | 2000 | 2001 |
|---|---|---|---|---|---|---|---|
| 1995 | - | ** | *** | *** | *** | *** | *** |
| | - | *** | *** | NS | NS | ** | *** |
| 1996 | | - | * | NS | ** | *** | *** |
| | | - | NS | *** | *** | ** | NS |
| 1997 | | | - | NS | NS | *** | *** |
| | | | - | *** | ** | NS | NS |
| 1998 | | | | - | NS | *** | *** |
| | | | | - | NS | * | *** |
| 1999 | | | | | - | *** | *** |
| | | | | | - | + | + |
| 2000 | | | | | | - | *** |
| | | | | | | - | + |
| 2001 | | | | | | | - |
| | | | | | | | - |

(level of significance: ***: P=0.1 %, **: P=1 %, *: P=5 %, +: P=10 %, NS=not significant)

In addition to a correctly adjusted milking machine, good milking technique is essential. Incorrect milking causes small traumas in the teat ends and they become predisposed to bacterial colonization, easily followed by

infection of the quarter. Careless preparation of the udder may transfer bacteria from the skin to the teats thus increasing the risk of mastitis.

Making the upper part of the udder wet is not recommended, because the dipping water carries bacteria down to the teats. According to results of a Danish study (Rasmussen 1991), it would be more appropriate to speak about cleaning the teats than cleaning the udder. The cleaning towel should be divided into four parts, one for each teat, to prevent transferring bacteria from one teat to another. Separate cleaning towels must naturally be used for different individuals. A cotton cloth is the most efficient for removing bacteria and it remains warm and is therefore pleasant for the cow. Cotton cloths should be washed with care. The teats and teat ends should be cleaned particularly carefully.

After careful preparation forestrips are taken from each quarter. Milk quality is visually assessed. With the correct technique milk with the highest bacterial contamination can be removed before milking. An impact may transfer bacteria from one teat to another. In addition, bacteria may move from one cow to another on the teatcup liners. To avoid transfer of bacteria from cows with mastitis to healthy ones, the milking order must be designed according to CMT-test results, so that cows with mastitis are milked last. The milkman's hands may transfer bacteria between cows.

### 5.2.2. Defence system

*5.2.2.1. Genotype differences (red and black)*

From 1999 till 2001 observations were made to study the genotype differences of defence system and ability (resistance), if exist, between the red and black coloured Holstein Friesian cows. At that time there was a change in the breeding goal at both stocks of C and D. To reach higher production, black sires were used instead of keeping red colour.

*Table 27* presents that prevalence and detection of mastitis is much probable (45.3% and 100%) among black cows. It is because of the high production and therefore, lower resistance against environment. Red cows produce less milk but their power of resistance is higher. It is also worth to ponder over balancing the harmony of genotype and circumstances.

**Table 27.** Number of red and black cows sampled at stock C and D (12-13th November, 1999)

| Group | Stock C | | Stock D | |
|---|---|---|---|---|
| | n | % | n | % |
| Σ cows | 650 | | 400 | |
| Productive | 500 | 100 | 300 | 100 |
| *Σ red* | *447* | *89.4* | *295* | *98.3* |
| **Sampled** | **103** | **23.0** | **68** | **23.0** |
| *Σ black* | *53* | *10.6* | *5* | *1.6* |
| **Sampled** | **24** | **45.3** | **5** | **100** |
| Σ sampled | 127 | 25.4 | 73 | 24.3 |

The conformation of the cow significantly influences predisposition to mastitis reported Hámori (1974), Horváth (1982), Dohy (1985), Seykora and McDaniel (1985) etc. Because of the favourable relationship between some udder traits and SCC of milk, screening on udder characteristics may have slowed the genetic increase in susceptibility to mastitis (and so LSCS) (Rogers et al. 1991; Boettcher et al. 1998). Gulyás et al. (1998) and Gulyás and Iváncsics (1999) also reported the importance of udder morphology and the role of pigmented teat ends. Dohy (1985), Seykora and McDaniel (1986), Schutz et al. (1993) and Dohy (1999) suggested that sire analysts from AI organizations should screen perspective bull-dams for udder conformation traits and should eliminate cows with deep udders or wide front teats from consideration.

The structure of legs and hoofs is also important predisposing factor. Movements involved getting feed and lying down are naturally necessary. Leg injuries in a herd are indicative of likely increase in udder diseases. However, long and untreated hoofs are often a question of mismanagement rather than a conformation fault of the cow (Markus, 1999 personal communication).

Culling cows with poor udder shape and leg injuries results in improved udder health.

*5.2.2.2. Unusual correlations ("breakers")*

Schutz et al. (1990) suggested that mastitis as indicated by LSCS is more common during first lactations of cows with sires that transmit higher

milk yield, perhaps because of the stress from producing more milk. However, not all high-production bulls sire high rates of mastitis (Dunklee, 1991; Dohy 1999).

On the other hand, with the sire rankings for Predicted Transmitting Ability for Somatic Cell Score (PTASCS), producers can select bulls on their ability to sire daughters with lower rates of mastitis reported Emanuelson et al. (1988), Banos and Shook (1990), Boettcher et al. (1992), Shook (1993) and Schutz et al. (1994). The sire evaluations will be reported in terms of a bull's predicted transmitting ability for somatic cell score. If the difference of PTASCS of two bulls is 0.5 and daughters of these bulls are housed in the same herd at the same time, the SCS of the bull's daughters is expected to differ by 0.5. Furthermore, the rankings of sires by PTASCS hold up across herds, regardless of mastitis-control levels in those herds. So, both high and low SCS herds can benefit from selection of the same sires with low PTASCS.

**"Across herd" results**

*Figure 4, 5, 6* and *7* show overall means of milk production and somatic cell count of stock and progeny group of sire No. 9117 at farm A and B. *Table 28* presents means of 305 days milk production, SCC and number of lactations at stock A, B and C. The number of lactations should always be taken into account!

**Table 28.** Means of 305 days milk production, SCC and number of lactations at stock A, B and C

| Stock | Group | n | Milk kg | SCC x 1000 | Lactation |
|---|---|---|---|---|---|
| A | mean | 66 | 3998 *** | 479 *** | 3.3 NS |
| | 9117 | 23 | 4334 *** | 737 *** | 3.5 NS |
| B | mean | 32 | 3027 *** | 946 *** | 3.7 * |
| | 9117 | 10 | 3433 *** | 547 *** | 4.1 * |
| C | mean | 4614 | 6835 *** | 328 ** | 2.8 *** |
| | 9117 | 366 | 6025 *** | 439 ** | 4.8 *** |

(level of significance: ***: p=0,1 %, **: p=1 %, *: p=5 %, +: p=10 %, NS=not significant)

**"Within a herd" results**

In stock C, 83 progeny groups of different sires were found by studying 4614 lactations. Based on pedigrees of sires closer relations can be noticed. Sire No. 12152 & 11762 and so 10748 & 9786 are in closer relations (*Table 30-33*). Being extrapolated, all the selected progeny groups produced lower SCC of milk than calculated according to the number of lactation *(5.2.1.3.)*. However, 305 days milk productions show larger differences (*Table 29*). Progeny groups of sire No. 12152 & 11762 produced more milk than the overall mean of the stock while progeny group of 9786 produced like mean and offsprings of sire No. 10749 produced less milk than the average. It reflects the importance of maternal ancestry. Sire analysts from AI organizations should screen perspective bull-dams for milk production and SCC and should eliminate cows with lower production and higher SCC during only a few lactation from consideration as suggested Dohy (1985), Seykora and McDaniel (1986), Schutz et al. (1993) and Dohy (1999).

Daughters of sire No. 9117 produced less milk but somatic cell count was less, too.

**Table 29.** Production traits, correlations and its significance of progeny groups of some sires at stock C
($n_{12152}$=136, $n_{11762}$=101, $n_{10748}$=565, $n_{9786}$=292, $n_{9117}$=366, $n_{mean}$=4614)

| No. | $n_{lact}$ | 305 days milk kg | | SCC x 1000 | | $r_f$ |
|---|---|---|---|---|---|---|
| | (SD) | (SD) | calc. (dif.) | (SD) | calc. (dif.) | |
| 12152 | 2,51 * | 7558 *** | 6916 | 287 NS | 315 | -0,03 |
| | (1,43) | (2118) | **(642)** | (259) | **(-56)** | |
| 11762 | 2,81 NS | 7554 ** | 6952 | 303 NS | 336 | -0,10 |
| | (1,36) | (2069) | **(602)** | (250) | **(-33)** | |
| 10748 | 2,55 * | 6606 * | 6922 | 285 * | 318 | 0,04 |
| | (1,43) | (2131) | **(-316)** | (345) | **(-33)** | |
| 9786 | 2,16 *** | 6942 NS | 6865 | 245 *** | 291 | -0,05 |
| | (1,17) | (2253) | **(77)** | (295) | **(-46)** | |
| 9117 | 4,82 *** | 6025 *** | 6965 | 439 *** | 476 | -0,19 |
| | (1,57) | (1703) | (-940) | (469) | (-37) | |
| Mean | 2,84 | 6835 | 6955 | 328 | 338 | -0,12 |
| | (1,72) | (2200) | (-120) | (393) | (-10) | |

(level of significance: ***: p=0,1 %, **: p=1 %, *: p=5 %, +: p=10 %, NS=not significant)

**Table 30.** Pedigree of sire 12152

| | | | |
|---|---|---|---|
| S | | 5138 | |
| | 9212 | CAN | |
| | | | - USA |
| 12152 | CAN | | |
| | | 7668 | 4278 |
| | | USA | |
| USA | | | |
| | | | |

**Table 31.** Pedigree of sire 11762

| | | | |
|---|---|---|---|
| S | | 5138 | |
| | 9212 | CAN | |
| | | | - USA |
| 11762 | CAN | | |
| | | 7668 | 4278 |
| | | USA | |
| USA | | | |
| | | | |

**Table 32.** Pedigree of sire 10748

| | | | |
|---|---|---|---|
| HB | | 5138 | |
| | 9212 | CAN | |
| | | | - USA |
| 10748 | CAN | | |
| | | 5510 | - CAN |
| | | USA | USA |
| (D) | | | |
| | | | |

**Table 33.** Pedigree of sire 9786

| | | | |
|---|---|---|---|
| HB | | 5138 | |
| | 9212 | CAN | |
| | | | - USA |
| 9786 | CAN | | |
| | | 5510 | - CAN |
| | | USA | USA |
| (D) | | | |
| | | | |

Genetic studies of dairy cattle have found that single-trait selection for higher milk production brings with it slightly higher rates of mastitis and other diseases. Therefore, selection only for those bulls with low PTASCS will be the same as selection for lower rates of improvement in milk yield (Boettcher et al., 1992; Da et al., 1992; Schutz et al., 1994).

Conclusions are as fallows:

- Environmental factors (keeping and milking hygiene, feeding etc.) may differ but genetic trends are "constant". (However, because of the insufficient circumstances trends well known in the literature sometimes could not be seen.)
- The number of lactations should be known for correct evaluation of the udder health status of a cow/progeny group/stock.
- Younger cows (1$^{st}$ lactation) usually produce less milk and less somatic cells in it.
- Cows in their 2$^{nd}$ and 3$^{rd}$ lactation produce a lot of milk but sometimes older cows (4$^{th}$ lactation) are able to produce also higher yields than the overall mean of the stock. Remarkable that older cows, producing more milk, has lower somatic cell count in milk. It reflects the importance of the value of "correlation breaker" sires and longevity of cows.
- The ESCC also allows a SCC (and SCS) calculation, which serves as a monitor of the udder health of a progeny group average in the herd.
- Studying the pedigrees of sires closer relations can be noticed that reflect the importance of maternal ancestry.

All in all, test day milk production and somatic cell count data are very important nowadays to ensure a reasonable production level and selection for mastitis resistance is necessary and could be efficient.

### 5.2.3. The use of a few screening methods

*5.2.3.1. Clinical examination*

The clinical unit of the udder is the quarter. Therefore, diagnostic methods must be applied to each quarter if mastitis is present. Inter-quarter comparison is helpful in recognizing abnormal quarters.

Skin of the udder and teats is inspected for injuries, discoloration or other abnormalities. Special attention should be paid to the teat orifices.

Asymmetry of the udder is usually due to atrophy of one quarter, or from the other hand, enlargement caused by oedema (Al-Ani and Vestweber, 1986; Nestor et al., 1988). Palpation includes teat canal and cistern, udder cistern, glandular tissue and skin, and supramammary lymph nodes. The udder is best palpated immediately after milking.

*5.2.3.2. Strip test*

The strip test is rapid and can easily be adapted as a part of the normal milking routine. Foremilk is visually examined for the gross abnormalities by squirting a few streams of milk onto the strip cup. With the correct technique milk with the highest bacterial contamination can be removed before milking.

*5.2.3.3. The California Mastitis Test*

The CMT can be used in several ways. Bulk tank, composite samples from individual cows and individual quarter samples can all be examined using this procedure. Each is valuable in monitoring udder infection. However, the interpretation is different depending upon the type of sample. The test should be conducted at least monthly and the scores recorded to be of any value. Low test scores do not indicate absence of udder irritation but high scores do indicate that a herd problem exists and the milk supply is of poor quality. Correct interpretation of test results requires some experience and knowledge of the cow or herd. In very early or very late lactation there are many epithelial cells in milk but CMT scores stay lower.

Normal *bulk tank milk* will have a CMT score of negative or trace. Whenever the CMT score reaches 1 or greater (*Table 3*), one should closely examine the milking system, the milking procedures, the cows and their environment for problems.

*Composite milk* samples from individual cows can be valuable in the identification of those cows that need to be examined more closely. This test does not identify the individual quarter(s) involved, only the cow. Closely examine all cows scoring 1 or greater to determine problem quarters. Initiate corrective measures if the percentage of cows scoring 1 or greater increases from test to test. A good herd goal should be to have less than 15 percent of the herd scoring CMT 2 or CMT 3. For this reason a young herd should have a higher percentage of low CMT scores than an older herd. Conduct a close

examination of mastitis control methods if a high percentage of first lactation cows have elevated CMT scores (1 or greater).

Take special care when interpreting results of individual cows after treatment of a clinical case of mastitis. CMT scores may remain elevated for weeks or months depending upon the severity of the damage and the causative organism. Infections caused by Staphylococcus aureus in particular may cause CMT reactions to remain elevated for several months.

As a cow-side test, the CMT can be useful in indicating and controlling mastitis since it focuses attention on the *individual quarters* that are secreting milk with high numbers of leukocytes. It is particularly useful in indicating subclinical and chronic mastitis. This can be quite valuable since subclinical mastitis is the most costly form of mastitis. Also, most clinical cases begin as subclinical mastitis while the chronic cases serve as a constant reservoir of mastitis causing organisms. Whenever the percentage of quarters scoring CMT 1 or greater increases, begin an evaluation of control methods. Microbiological examination of quarters scoring CMT 1 or greater may also be conducted (Markus, 1999 personal communication).

Injury the udder tissue by malfunctioning or misused milking equipment can cause elevated CMT scores unrelated to bacterial infection. Always examine milking equipment and procedures when CMT scores become elevated on a herd basis. Correct any equipment failure or milking procedure that may cause irritation to udder tissue.

Chronically infected cows are identified by consistently elevated CMT readings over a number of tests. These cows should be subjected to a laboratory evaluation. They are also candidates for either early drying off and dry cow antibiotic therapy or culling from the herd. Culling is generally the better method of dealing with this type of cow, since therapy may be ineffective against some organisms.

**Advantages of the CMT**

- The CMT is fairly accurate in measuring somatic cell concentration in milk, correlating well with other tests.
- Primarily developed for sampling quarters, it can also be used on bulk tank milk samples.
- It is sensitive.

- It is inexpensive.
- The test is simple and little equipment is needed.
- Easy clean up after each test - simply rinse with water.
- Foreign material, such as hair or other matter, does not interfere with the test.
- Environmental temperature changes have little effect on the CMT as long as the milk has been refrigerated and is not over two days old.
- Herd mastitis levels can be estimated from tank CMTs. A CMT of 2 or 3 on tank milk indicates a probable high percent of infected cows.
- Evaluation of late lactation milk cell count is more reliable with CMT than with different electronic counters.

**Disadvantages of the CMT**

- Scoring the test may vary between individual testers. It is necessary to be as consistent as possible to insure uniform results.
- Scores represent a range of leucocyte content rather than an exact count.
- False positive reactions occur frequently on cows that have been fresh less than ten days, or on cows that are nearly dry.
- These animals should be tested closer to the middle of the lactation.
- Occasionally, acute clinical mastitis milk will not score positive due to the destruction of leucocytes by toxins (poisons) from the infecting organism.

Conclusions are as follows:

The use of the CMT on the entire herd at monthly intervals can be extremely useful as an aid in detecting herd mastitis problems. Individual and total quarter infections can be determined and, with proper records, the level of herd mastitis can be monitored. This test yields information that can aid in determining faulty milking procedures or equipment function, as well as the effectiveness of teat dips and dry cow treatment programs.

The CMT provides information on the individual cow and can provide insight into the total herd status. By recording the CMT results periodically, the herdsman can monitor herd levels and investigate the possible causes early when results show elevated CMT scores from one test to the next.

Regardless of CMT results, it is recommended to maintain a carefully planned mastitis control program consisting of:

- Proper machine function and milking procedure.
- Hygiene and use of effective teat dips.
- Culture and treatment of clinical cases as recommended by veterinarian.
- Dry cow treatment - all cows, all quarters - under the direction of veterinarian.
- Culling of problem cows with repeated clinical flare-ups and with a CMT score of 2 or more, in two or more quarters.

All in all, the first step is always inspection, while washing should include palpation, too. Milk sample can be examined first physically, then chemically and microbiologically, if necessary. The CMT is a practical cow-side test for detecting mastitis in milk. The milk should be inspected for clots, discoloration or wateriness before adding the CMT reagent.

*5.2.3.4. The Electronic Somatic Cell Count*

Electronic counting procedures have numerous ***advantages***:

- The procedure can be automated thus allowing centralisation of laboratory procedures.
- Preserved samples can be counted. This further allows centralisation.
- The procedure is more precise and more objective. Electronic SCC is more precise and objective than the CMT.
- The procedure is also more repeatable than the CMT.
- Transformed data can be used for genetic evaluations, too.

Primary ***disadvantages*** of electronic SCC procedures are as follows:

- The equipment is expensive, and
- Requires constant monitoring.
- Trained individuals are needed to conduct the test.
- Since the equipment is expensive, centralisation of the testing is necessary.
- This results in the loss of immediate access to results, which are available with the CMT.

*5.2.3.5. High SCC cows*

The SCC program pinpoints problem cows. Unfortunately, even after problem cows are identified, management options for these cows are limited.

(1) SELECTING COWS FOR CULTURE

The major reason for elevation in SCC (SCS) is intramammary infection. Monthly individual cow SCC/SCS are good indicators of infections caused by the major contagious pathogens (Streptococcus agalactiae and Staphylococcus aureus). This is because these infections are usually of long duration. Conversely, infections caused by the environmental pathogens (e.g., coliforms and environmental streptococci) are often of shorter duration. Thus, infections by environmental organisms may go unnoticed by a test administered only once each month. Although elevated SCC is an indicator of probable intramammary infection, a distinction between contagious and environmental mastitis cannot be made on the basis of SCC alone. This distinction must be made by microbiological culture of milk. Determining whether problems are contagious or environmental is necessary to enable a producer to make decisions regarding a herd's mastitis control program.

(2) LACTATION TREATMENT

Producers in danger of losing their market because of high BTSCC may find it necessary to treat cows with high SCC during lactation. The veterinarian and producer should consider last SCC, milk culture results, milk production, stage of lactation, and age when selecting cows for treatment. Early drying off and dry treatment should be used for cows in late lactation. Milk from cows with the highest SCC in early to mid-lactation can be withheld from the bulk tank. Using this method, withholding milk from only a few cows can lower the BTSCC by as much as 50% or more.

The number of times a cow is SCC 400,000/ml or greater in a lactation can indicate chronic problem cows requiring special attention. High SCC in early lactation, followed by a decrease later in lactation may indicate problems with dry cow management, maternity pens, or dry cow therapy (often poor treatment technique). SCC that generally rise throughout lactation are usually associated with cows infected by contagious pathogens and may indicate

problems with milking hygiene, milking equipment, milking practices, or housing of the milking herd.

(3) DRY COW TREATMENT

The general recommendation is that all quarters of all cows be treated with an intramammary antibiotic preparation at drying off. Dry treatment has a higher cure rate than lactation treatment, eliminating existing infections and preventing new infections during the early dry period.

If a producer decides to use selective dry cow therapy, SCC results can be used as a guide. However, quarters not treated at drying off are more likely than treated quarters to become infected during the early dry period.

(4) MILKING ORDER.

Cows with high SCC should be milked last to decrease the spread of infection to uninfected cows during the milking process. In some herds this may not be practical but an alternative method is to identify cows with high SCC by a leg band and milk these cows with a separate milking unit.

(5) CULLING

Culling is often the most practical means for eliminating chronically infected cows. These cows may be reservoirs of infection, which may spread to other cows during the milking process.

#### *5.2.3.6. Evaluation of the herd mastitis control program based on SCC (SCS)*

A monthly summary of SCC (SCS) on milk samples from each cow provides an evaluation of the effectiveness of the mastitis control procedures. SCC allows measuring the reduction in subclinical mastitis as management is improved.

A realistic goal is for more than 90% of cows to have a SCC of less than 400,000 cells/ml (SCS 5, *Table 4*). Producers with more than 25% of their cows with a SCC of 400,000 cells/ml or greater can improve their herd mastitis control procedures in the following ways:

- Correct milking, including milking time sanitation (emphasizing dipping all teats immediately after each milking with a product proven effective under controlled research conditions).
- Restore milking equipment to proper operating condition.
- Review other management practices such as the basis for culling, source of herd replacements, condition of lots and free stall bedding, etc.
- Evaluate dry cow treatment and management program. Comparing each cow's SCC before drying off and a month after calving will give an indication of the effectiveness of the dry cow treatment used and dry cow management program.

Improvements in mastitis control program will appear within a few months. Perhaps the best group of cows to monitor is first lactation cows. These cows should not have SCC above 100,000 cells/ml (SCS 3) since they are not affected as much as older cows by prior herd conditions. The percentage of cows in a herd with SCC greater than 100,000 cells/ml for the first time is a good indicator of the success or failure of a control program.

Arranging SCC (SCS) data by ***days in milk***, eg. 0 (10) to 39, 40 to 99, 100 to 199, and 200+ and by ***lactation***, eg. 1, 2, 3, and greater than 4.

SCC is very helpful in identifying those few cows that contribute the major portion of the total somatic cell count in the bulk tank. Often, withholding milk from this relatively small number of cows is enough to reduce the BTSCC enough to qualify for bonuses.

*5.2.3.7. Treatment - antibacterial mastitis therapy*

Antimicrobial drugs have been used by veterinarians since the 1940's. Wide use of antibacterials has resulted in selection of resistant species of bacteria and development of resistant bacterial strains among those bacterial populations, which were earlier susceptible. Furthermore, it is theoretically not feasible to sterilize the mammary gland selectively without considerable damage to the symbiotic microflora of the gastrointestinal tract and therefore the host.

The udder has a certain capacity to cleanse itself. It is therefore difficult to judge whether an antibacterial was effective, whether the udder cleansed itself without antibacterial, or whether the inflammation was suppressed

without elimination of bacteria. The most that can be achieved is the temporary reduction or suppression of the bacterial population to give the host a chance to cleanse itself of the infection. The cleansing mechanisms are obviously poorly developed in the udder as relapses and reinfections commonly follow antimicrobial therapy. The statement, „the antibacterial did not work", should be rephrased, „the udder could not cleanse itself of the infection."

**Intramammary therapy**

Bacteriological cure rates from intramammary therapy during lactation are uncertain. Despite therapy, many of the infected and inflamed quarters remain latent carriers. Relapses are common. The reasons for these results from intramammary therapy include:

- poor distribution of antimicrobial in the tissue due to local oedema or poor penetration of the drug,
- milk flow from the alveoli washing out the antimicrobial from the ducts and udder tissue,
- bacterial colonisation as a consequence of pathological changes of tissue, fibrotic capsules or persistence of bacteria within phagocytes,
- antibacterial resistance,
- binding or inactivation of antimicrobials by host factors, and
- negative interactions of drugs with the body's own defence mechanisms.

All in all, effective and economic mastitis control has to rely on prevention rather than treatment by antibacterials. This recent change of the bacterial spectrum towards less virulent and less contagious organisms hint that the resistance of the cow has decreased. One should look for means to increase the *endogenous resistance of the cow*.

*5.2.3.8. Follow-up*

A mastitis control programme, even when successful, requires follow-up. Bulk milk somatic cell count is a basic means for permanent monitoring of udder health. Inflammation and infection percentages on a cow and quarter basis, in addition to various health reports, are useful tools. And, only a motivated herdsman can achieve a lasting result in mastitis control.

Grouping of cows in a herd with S. aureus mastitis problem has a great importance. The grouping represents the basis for milking order and the various measures focused on the different groups:

- ***Group of healthy cows.*** This group also includes the heifers, but their udder health must be checked before calving or immediately after it. The udder is frequently examined with CMT. The aim is to continuously increase the size of this group.
- ***Group of infected cows.*** Part of this group will be culled later when the situation in the herd allows (highest production is passed, the culled cows can be replaced etc.). One part waits for dry cow treatment (because a single, subclinically infected quarter can be left unmilked from mid-lactation). However, the cow remains in the infected group. The aim is to keep this group small!
- ***Group of treated cows.*** This group must be small as the prognosis for treatment is quite low. The group includes recently calved, high producing, previously healthy and young cows. The treatment should be as effective as possible and be based on the in vitro sensitivity of the bacterial strain. The treatment result is checked after 3-4 weeks with CMT and bacteriologically if needed. If the quarter still harbours infection, the cow is moved to the next groups.
- ***Group of cows dried off early.*** This group includes cows infected with S. aureus at the end of their lactation. They can be dried off 3 months before calving. After calving the status of the quarters is examined with CMT or bacteriologically if needed.
- ***Group to be culled immediately.*** This group includes chronically infected cows (treated once or twice without success or infection remains after dry cow treatment). This group should be small.

## 5.3. SOMATIC CELL COUNT AND DISTRIBUTION

### 5.3.1. Differential staining of milk somatic cells

Based on standard haematological diagnostic procedures, the direct May-Grünwald staining method was used to observe the distribution of somatic cells in milk (Materials and methods 4.3.1.2.)

Smears of weekly (5) raw milk samples (n=16, Σ80) from "healthy" cows were stained according to May-Grünwald. Because of milk fat, after centrifugation parallel staining was made, too. Then cells were counted and identified as lymphocytes, granulocytes (neutrophils, eosinophils and basophils) or monocytes. At last, correlations were calculated.

Fat, protein and lactose content presented "normal" samples. Microbiological analyses (and TPC) resulted negative (first class) samples ("healthy" cows), but two. In week 4 and 5 HL (hind left) quarter of cow No. 4 had E. coli infection. This could be the reason of the elevated somatic cell count.

Overall means of somatic cell count (SCC) and percentages of different cell types are given in *Table 34*. Correlations of SCC and ratio of cell types can be seen in *Table 35*.

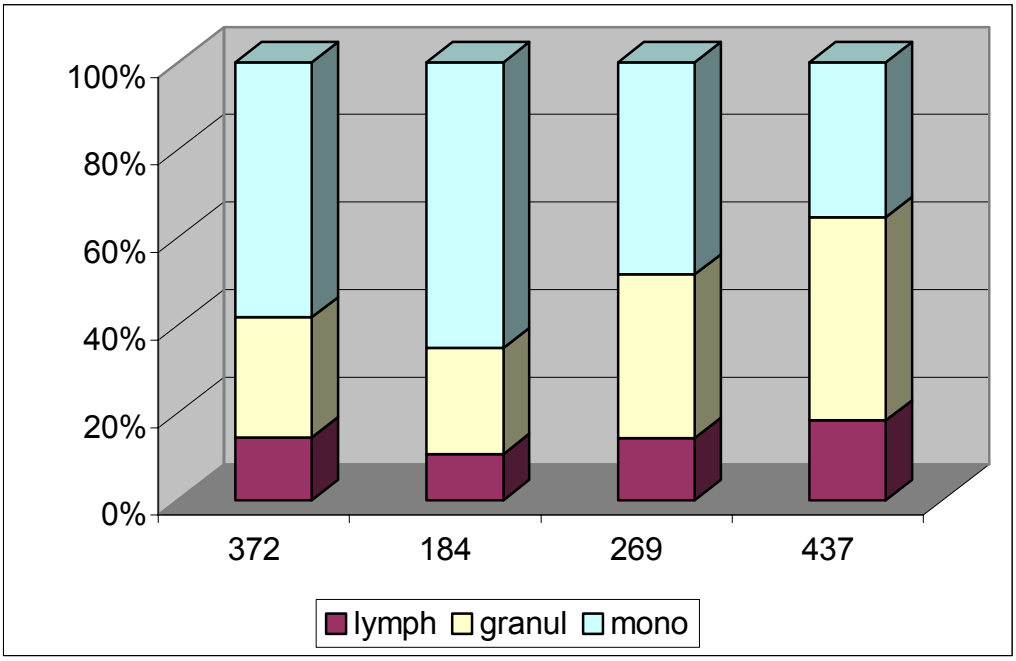
**Table 34.** SCC, ratio of cell types (lymphocytes, granulocytes and monocytes) and level of cignificance

| Cow №. | n | mean (SD) | | | |
|---|---|---|---|---|---|
| | | SCC x 1000 | lymph % | granul % | mono % |
| 1 | 4 x 5 | 372 (120) + | 13 (3) NS | 25 (7) ** | 53 (12) NS |
| 2 | 4 x 5 | 184 (54) *** | 10 (2) *** | 23 (5) *** | 62 (16) ** |
| 3 | 4 x 5 | 269 (61) NS | 14 (3) NS | 37 (5) + | 48 (15) NS |
| 4 | 4 x 5 | 437 (284) * | 18 (6) *** | 46 (18) *** | 35 (17) *** |
| Σ/mean | 16x5 | 315.5 (133) | 13.75 (4) | 32.75 (10) | 51 (14) |

(level of significance: ***: p=0,1 %, **: p=1 %, *: p=5 %, +: p=10 %, NS=not significant)

The ratio of cell types according to the somatic cell count can be seen in *Figure 20*. The lymphocyte (*Figure 21*) was the least frequent, granulocyte (*Figure 22*) the most variable and monocyte (*Figure 23*) the most common cell type in the samples.

**Figure 20.** The ratio of cell types according to the somatic cell count



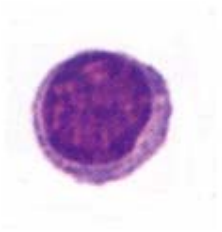



**Figure 21.**

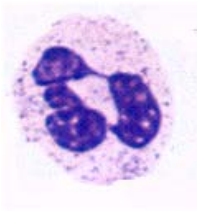


**Figure 22.**

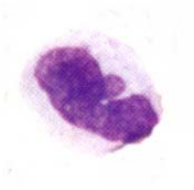


**Figure 23.**

**Table 35.** Correlations of SCC and ratio of cell types

| | SCC | Lymphocyte | Granulocyte | Monocyte |
|---|---|---|---|---|
| SCC | - | 0.83 | 0.62 | -0.83 |
| Lymphocyte | | - | 0.93 | -0.99 |
| Granulocyte | | | - | -0.95 |
| Monocyte | | | | - |

Conclusions are as follows:

- Each cell type has its own more or less specific function in the immune response.
- The number of lymphocytes and monocytes show higher correlation with SCC than granulocytes.
- Monocytes show a strong negative effect in comparison with SCC and the other type of cells.
- DCS is a tedious staining procedure and requires extensive training but sufficient to allow identification of cell populations in milk and appropriate for processing relatively "large" numbers of samples.

### 5.3.2. DCC: flow cytometric analysis of milk samples

SCC cannot distinguish between the type of cells present in milk but measures all types of cells, including lymphocytes, eosinophils, basophils, neutrophils, macrophages, and epithelial cells. However, SCC varies with time and frequency of milking, stage of lactation, and season (Miller et al., 1991; Schutz et al., 1994).

The types of cells present in milk must be known because uninfected milk consists mainly of macrophages (60%) and lymphocytes (28%) with few (polymorphonuclear) leukocytes (PMN, 5 to 12%). In mastitic milk, the percentage of PMN has been shown to increase considerably (up to 90%) (Saad, 1987; Kehrli et al., 1989; Saad et al., 1990). Therefore, it would be beneficial to know the types of inflammatory cells present in milk.

In the present study, flow cytometric analysis regions were established by check beads and milk cells with DNA binding dyes were accurately identified as lymphocytes, granulocytes or monocytes.

Differential cell count (DCC) is a quite new flow cytometric technique that uses a DNA-binding fluorescent dyes to identify the types of inflammatory cells present in milk. PI in detergent uniformly stains all cells with a red nuclear fluorescence. Based on linear forward scatter (size) and log side scatter (cellular complexity), cell type determination is available.

Hageltorn and Saad (1986) used flow cytometry in combination with fluorescent and light microscopy to differentiate lymphocytes, monocytes, macrophages, and neutrophils present in milk. Milk samples labelled with

carboxydimethylfluorescein diacetate resulted five populations of cells including intact and degenerating PMN, lymphocytes, monocytes, and macrophages. Saad and Ostensson (1990) used acridine-orange staining of milk cells for flow cytometric evaluation. This method enabled tracking of milk cell types up to 4 days after infusing mammary glands with endotoxin, but microscopic evaluation of sorted cells was necessary to make semiquantitative estimates of changes in milk cell types. Miller et al. (1993) used carboxydimethylfluorescein diacetate to stain milk neutrophils for flow cytometric evaluation. They were able to estimate the percentage of neutrophils in milk but observed variability in flow cytometric data.

Other flow cytometric studies have used monoclonal antibodies to leukocyte cell surface receptors to study inflammatory cells from milk. However, these studies mainly focused on evaluation of cell surface receptors to classify subtypes within a specific population of leukocytes and were qualitative in nature. Besides, these techniques may be more suited to research settings than to routine evaluation of udder health.

In this study, experiment 1 and 2 produced quite similar results (*Table 36*). Theoretically and practically almost all the samples were bacteriologically negative because samples were obtained from treated (but "originally" high SCC) quarters.

**Table 36.** SCC, means, SD and level of significance of the identified populations and the bacteriological results (Exp. 1-2, n=3 x 8 x 2)

| Sample | SCC (x $10^3$) | Pop. I. % | Pop. II. % | Pop. III. % | Bacter. |
|---|---|---|---|---|---|
| 1 | 2,600 (897) NS | 67 (6) NS | 17 (2) NS | 7 (3) NS | - |
| 2 | 740 (266) *** | 69 (5) NS | 17 (2) NS | 2 (1) NS | - |
| 3 | 1,470 (237) ** | 67 (4) NS | 18 (3) + | 3 (1) NS | - |
| 4 | 3,150 (1301) + | 63 (5) + | 17 (3) NS | 6 (3) NS | - |
| 5 | 830 (325) *** | 57 (3) *** | 15 (1) NS | 8 (2) * | - |
| 6 | 950 (316) *** | 66 (6) NS | 17 (2) NS | 9 (3) ** | - |
| 7 | 2,330 (612) NS | 82 (10) *** | 11 (2) *** | 1 (1) * | - |
| 8 | 4,860 (1563) *** | 74 (9) + | 18 (5) + | 1 (1) * | - |
| Mean | 2,116 | 68 | 16 | 4.6 | |

(level of significance: ***: p=0,1 %, **: p=1 %, *: p=5 %, +: p=10 %, NS=not significant)

Based on FSC and SSC dot plots three populations were identified. Both parameters are small at Pop. I. (lymphocytes). The average size is ~2.4-2.9 μm. The proportion of lymphocytes ranged from 57 to 82% (mean 68%). SSC is larger at Pop. II. (granulocytes) and the size of cells is ~3.5-4.5 μm. The proportion of granulocytes ranged from 11 to 18% (mean 16%). At last, SSC is approximately equal with Pop. II. but FSC is markedly larger. The average size of Pop. III. (monocytes) is ~17.6-21.2 μm and the portion ranges from 1 to 9 (mean 4.6). "Unfortunately" we couldn't realise bacterial infection but other studies have also reported that number of lymphocytes increases significantly when there is infection of the udder.

Conclusions are as follows:

- The electronic SCC is the standard method for monitoring udder health at the moment but it seems to be clear that flow cytometric assay is comparable, too. The findings of the present study suggest that DCC may develop as a good alternative or supplementary tool to SCC to evaluate udder health.
- PI in detergent may be used to stain milk cells, too.
- Formaldehyde increased the ratio of cell debris but there were no significant differences between samples treated with isotonic salt solution or Bromopol pills.
- The ratio of lymphocytes and monocytes show significant differences with SCC more often than granulocytes.
- Further studies are required to establish discrimination limits for intramammary infections.

# 6. CONCLUSIONS AND SUGGESTIONS

An effective mastitis control program combines several methods with proven control procedures. Health professionalists and geneticists in the dairy industry have the responsibility to inform producers of the proper use of SCC evaluations. These evaluations will in no way displace improved environmental conditions as the key ingredient in mastitis control.

For producing high quality milk the importance of technological - environmental, biological - genetic and economical factors should be balanced.

**Evaluation the susceptibility of mastitis**

The maximum milk yield can be obtained at $n_{lact}$= 3.88. It reflects the importance of longevity and lifetime performance. Correlation of milk yield and number of lactation was $r_f$=0.88.

Somatic cell counts are readily available to most dairy farmers today on a monthly basis through the Livestock Performance Testing Ltd., Gödöllő (Hungary). Because of the lognormal distribution of somatic cell count data were transformed by $\log_2$. The logarithmic transformation may facilitate the international comparison of breeding value estimation of Hungarian dairy herds and therefore adaptation and home application of this method is also desirable and suggested.

The correlation of transformed somatic cell count (SCS) and number of lactation was $r_f$=0.93 while the correlation of the transformed somatic cell count (SCS) and milk yield ranged from -0.54 to -0.09 in different lactations but most values were closer to the mean of $r_f$=-0.12. Remarkable that older cows, producing more milk, has lower somatic cell count in milk.

The number of lactations should be known for correct evaluation of the udder health status of a cow/progeny group/stock. Younger cows (1$^{st}$ lactation) usually produce less milk and less somatic cells in it. Cows in their 2$^{nd}$ and 3$^{rd}$ lactation produce a lot of milk but sometimes older cows (4$^{th}$ lactation) are able to produce also higher yields than the overall mean of the stock. Remarkable that older cows, producing more milk, has lower somatic cell count in milk. It reflects the importance of the value of “correlation breaker” sires and longevity of cows. Studying the pedigrees of sires closer relations can be noticed that reflect the importance of maternal ancestry.

Losses in milk production associated with elevated SCC can be estimated, too. Reasons are lower yields and worst persistence. The differences were statistically significant. 25% of the cows start their lactation with high somatic cell count. Till the second test day it drops to the half. The ratio of healthy cows during the whole lactation was approximately 25%.

Clinical mastitis is an expensive, management-intensive problem. Selection to improve udder health is desirable for numerous reasons. Single-trait selection for increased milk yield should result in increased susceptibility to mastitis of dairy cows. However, direct selection for reduced mastitis is not possible because mastitis incidence is not consistently recorded in majority of the cow population. Indirect selection for lower mastitis incidence is an alternative to direct selection.

Genetic evaluations for SCC enable producers to moderate such undesirable economic consequences. A long-term trend in incidence of mastitis will have major economic implications if genetic resistance to mastitis is ignored by breeding programs.

With the publication of the evaluations for PTASCS, sire analysts have a more direct measure of mastitis in a bull's daughters. Using these new data will help ensure that highly unfavourable bulls are not used to sire future generations of dairy cattle. The overall positive effects are that the use of antibiotics decreases, therefore the quality of products and so animal welfare improves and human health is ensured.

**Herd mastitis control program: screening methods**

During udder health work it is essential that the environmental factors, which predispose cows to mastitis, should be pointed out to the herdsman. Knowledge of the nature of the impact of the predisposive factors generally increase willingness on the farmer’s part to make improvements.

In addition to a correctly adjusted milking machine, good milking technique is essential. Incorrect milking causes small traumas in the teat ends and they become predisposed to bacterial colonization, easily followed by infection of the quarter. Careless preparation of the udder may transfer bacteria from the skin to the teats thus increasing the risk of mastitis.

Making the upper part of the udder wet is not recommended, because the dipping water carries bacteria down to the teats. It would be more

appropriate to speak about cleaning the teats than cleaning the udder. The cleaning towel should be divided into four parts, one for each teat, to prevent transferring bacteria from one teat to another. Separate cleaning towels must naturally be used for different individuals. A cotton cloth is the most efficient for removing bacteria and it remains warm and is therefore pleasant for the cow. Cotton cloths should be washed with care. The teats and teat ends should be cleaned particularly carefully. The milkman's hands may transfer bacteria between cows. To avoid transfer of bacteria from cows with mastitis to healthy ones, the milking order must be designed according to CMT-test results, so that cows with mastitis are milked last.

The use of the ***strip test*** has some benefits in addition to the identification of clinical mastitis. Stripping the first streams of milk stimulates milk let-down, resulting in faster milk out. This can result in a shorter milking time. Foremilk is higher in bacteria than subsequent milk. Removal of this milk may reduce bacterial contamination of the milking machine, reduce the probability of udder contamination and, thus enhances the quality of the milk produced.

The use of the ***CMT*** on the entire herd at monthly intervals can be extremely useful as an aid in detecting herd mastitis problems. Individual and total quarter infections can be determined and, with proper records, the level of herd mastitis can be monitored. This test yields information that can aid in determining faulty milking procedures or equipment function, as well as the effectiveness of teat dips and dry cow treatment programs.

The ***ESCC*** test fulfills several needs which dairymen desire. The ESCC focuses attention on the individual cow. It does not pinpoint the quarter(s) affected but does monitor udder health of individuals. The ESCC also allows a herd average SCC to be calculated which serves as a monitor of the udder health of the herd/progeny group.

Wide use of antibacterials has resulted in selection of resistant species of bacteria and development of resistant bacterial strains among those bacterial populations, which were earlier susceptible. In countries where antimicrobials have been used for a long time, the prevalence of infections by contragious streptococci has decreased and been replaced by staphylococcal infections.

Bacteriological cure rates from intramammary therapy during lactation are poor. Despite therapy, most of the infected and inflamed quarters remain latent carriers. Relapses are common.

In conclusion, an effective and economic mastitis control has to rely on prevention rather than treatment by antibacterials. The recent change of the bacterial spectrum towards less virulent and less contagious organisms hint that the resistance of the cow has decreased. The increase of endogenous resistance of the cow has a great importance and will be a valuable factor in the future.

A mastitis control programme, even when successful, requires follow-up. The above discussed different interpretations of milk somatic cell counts are a basic means for permanent monitoring of udder health. The aim is to continuously increase the size of healthy cows.

**Differential staining and counting**

The SCC measures all types of cells in milk but cannot distinguish between the type of cells present in milk. However, SCC varies with time and frequency of milking, stage of lactation, and season. Therefore, in addition to SCC, it would be beneficial to know the types of inflammatory cells present in milk because each cell type has its own more or less specific function in the immune response.

***DCS*** is a tedious staining procedure and requires extensive training but sufficient to allow identification of cell populations in milk and appropriate for processing relatively "large" numbers of samples. The number of lymphocytes and monocytes show higher correlation with SCC than granulocytes. Monocytes show a strong negative effect in comparison with SCC and the other type of cells.

Differential cell count (***DCC***) is a new flow cytometric technique that uses a combination of DNA-binding fluorescent dyes to identify the types of inflammatory cells present in milk. Formaldehyde increased the ratio of cell debris but there were no significant differences between samples treated with isotonic salt solution or Bromopol pills. DCC may develop as a good alternative or supplementary tool to SCC to evaluate udder health. Further studies are required to establish discrimination limits for intramammary infections.

# 7. SUMMARY

SCC is widely used to predict the mammary health status of quarters and cows as measure of the prevalence of mastitis in a dairy herd, safety and suitability of raw milk for human consumption, and also used by regulatory agencies as an indicator of the wholesomeness and monetary losses to producers due to mastitis.

Many countries are able to determine a national average SCC based on all registered/evaluated producers in the country. These averages have been in general declining over the past 10 years and indicate considerable progress in control of subclinical mastitis or increased ability to control/manage the SCC of the herd bulk milk. In countries it is less than 200,000 cells/ml clearly indicate that producers can control the technical, environmental and hygienic, biological and genetic effects caused subclinical or clinical mastitis.

In this work the genetic and bacteriological aspects of udder health were studied and applied research were carried out related to milk quality. The genetic disposition of mastitis was studied at different stocks. The existing differences and the use of SCS was examined among progeny groups of some sires under Hungarian circumstances between 1995 and 2001.

The maximum milk yield can be obtained at $n_{lact}$= 3.88. It reflects the importance of longevity and lifetime performance. Correlation of milk yield and number of lactation was $r_f$=0.88. The correlation of transformed somatic cell count (SCS) and number of lactation was $r_f$=0.93 and the correlation of the transformed somatic cell count (SCS) and milk yield ranged from -0.5 to -0.09 in different lactations but most values were closer to the mean of $r_f$=-0.12.

The number of lactations should be known for correct evaluation of the udder health status of a cow/progeny group/stock. Younger cows (1$^{st}$ lactation) usually produce less milk and less somatic cells in it. Cows in their 2$^{nd}$ and 3$^{rd}$ lactation produce a lot of milk but sometimes older cows (4$^{th}$ lactation) are able to produce also higher yields than the overall mean of the stock. Remarkable that older cows, producing more milk, has lower somatic cell count in milk. It reflects the importance of the value of “correlation breaker” sires and longevity of cows.

Losses in milk production associated with elevated SCC can be estimated, too. Reasons are lower yields and worst persistence. The differences were statistically significant. 25% of the cows start their lactation with high somatic cell count. Till the second test day it drops to the half. The ratio of healthy cows during the whole lactation is approximately 25%.

Clinical mastitis is an expensive, management-intensive problem. Selection to improve udder health is desirable for numerous reasons. Health professionalists and geneticists in the dairy industry have the responsibility to inform producers of the proper use of SCC evaluations. These evaluations will in no way displace improved environmental conditions as the key ingredient in mastitis control.

Furthermore, IMI and the related microorganisms also were identified. With proper use of some hygienic requirements and some screening methods milk quality and therefore udder health status will significantly improve.

The use of DCS and DCC were investigated from a practical point of view as tools to monitor udder health. DCS is a tedious staining procedure and requires extensive training but sufficient to allow identification of cell populations in milk and appropriate for processing relatively “large” numbers of samples. DCC may develop as a good alternative or supplementary tool to SCC to evaluate udder health. Further studies are required to establish discrimination limits for intramammary infections.

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# APPENDICES

# 1. List of farms involved into the investigations

| Code | Name of farm |
|---|---|
| ***A*** | Zöldmező Co., Öttevény (stock at Öttevény) |
| B | Zöldmező Co., Öttevény (stock at Börcs) |
| C | Kisalföld Inc., Nagyszentjános (stock at Nagyszentjános) |
| D | Kisalföld Inc., Nagyszentjános (stock at Balogtag) |
| E | Lajta-Hanság Inc., Mosonmagyaróvár (stock called Tangazdaság) |

## 2. Information to record the factors predisposing the cow and the herd to mastitis

| Information | **Factors to consider** |
|---|---|
| Owner | • Poor management predispose the cow to mastitis |
| Age of the cow | • Risk of mastitis increases with age |
| Stage of lactation | • Coliform mastitis is common around calving<br>• Streptococcal mastitis occurs in dry cows<br>• Acute infection during the first month often drive from the previous lactation of drying of |
| Time of the year | • During dry summer the environmental factors are better (but heat stress) |
| Incidence | • Number of clinical cases/100 cow/year |
| Prevalence of subclinical mastitis | • SCC of the bulk milk |
| Teat injuries | • Teat injuries are predisposing factors to staphylococcal mastitis |
| Husbandry conditions | • Dirt and humidity (environmental bacteria)<br>• Milking technique (vacuum, pulsation rate, attachment and removal of teat cups) |
| Use of antimicrobials | • Resistance of pathogens |
| Teat dipping | • Selective pressure on Gram-negative bacteria |

*Source: Sandholm, 1995*

### 3. Observations to be recorded during examination

General symptoms: .....

Is the cow recumbent? .....

Is it suffering from: .....

- Diarrhoea? .....
- Decreased rumen motility? .....
- Lack of appetite? .....
- Fever? .....
- Increased heart rate? .....
- Increased respiratory rate? .....
- Lameness? .....

*Source: Sandholm, 1995*