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National Dairy Development Board

For Efficient Dairy Plant Operation

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SOMATIC CELLS IN MILK

This bulletin includes technical information based on latest developments on products, systems, techniques etc. reported in journals, companies' leaflets and books and based on studies and experience. The technical information in different issues is on different areas of plant operation. It is hoped that the information contained herein will be useful to readers.

The theme of information in this issue is **Somatic Cells in Milk.** It may be understood that the information given here is by no means complete.

In this issue:

1. INTRODUCTION

Milk naturally contains somatic cells which originate from the body of milch animal. Milk somatic cell counts (SCC) have health, milk quality, legal and productivity implications and hence an important matter of discussion within the dairy industry. The issue is complex, yet it is important that dairy processors and producers understand the significance of somatic cell counts in dairy production and trade.

2. MILK SAFETY AND QUALITY CONCERNS [1-5]

2.1 Somatic cells and their function

Somatic cells are white blood cells (macrophages, lymphocytes and polymorphnuclear leucocytes), the basic function of which in the mammary gland is defense against bacterial invasion. Polymorphonuclear leucocytes (predominantly neutrophils) are phagocytic cells that engulf bacteria and destroy them with an arsenal of enzymes and chemical agents. Macrophages are also phagocytes and are involved in immune recognition. Lymphocytes are responsible for immune memory (retaining a memory of the information pertaining to previously encountered pathogenic infections) and antibody production.

Milch animals have evolved a complex internal body system, involving somatic cells, that enables them to defend and rid themselves of threats such as those posed by invading bacteria. Whenever infection develops there is inevitably some tissue

damage which releases a variety of different chemical compounds into the system. The body detects and interprets the presence of such materials as a call to action and very rapidly, large quantities of somatic cells are directed to the infection site.

Once at the infection site, they locate the invading bacteria and tend to lock on and envelope or internalize these invading microorganisms. This process of internalization is referred to as phagocytosis. Once bacteria are internalized the somatic cells proceed to destroy them by using various mechanisms including a number of enzymes capable of digesting bacterial components. When successful, they can stop an infection before it becomes severe enough to cause major problems and that is the desired result.

2.2 Relationship between the udder health and milk somatic cell counts

Somatic cells are invariably present in milk although their numbers depend on several factors. From various studies done world-over, it is generally concluded that the concentration of somatic cells in milk is directly related to the infection status of the udder. Research has demonstrated that an uninfected cow can have a milk SCC well below 1 lakh/ml and herds with low infection levels may have overall herd cell counts below 1 lakh/ml. Such levels are an indication of low infection rates and a general indication that many things are being done correctly. When the overall SCC reaches approximately 2 lakh/ml it is an indication that a certain low number of cows are infected. **A**s the cell count elevates it is directly related to more quarters of more cows being infected. This may result in cell levels of 4-8 lakh cells/ml and such levels are a concern for reasons of deteriorating

udder health and resultant economic consequences (treatment costs, production losses etc.).

Stated another way, if the cell count is high the cow has mastitis or inflammation of the mammary gland. No other factor(s) influences the milk somatic cell count to the degree that bacterial infections do. Somatic cell count (SCC) is the most widely accepted criteria for measuring udder health and milk quality in all major milk-producing countries throughout the world. Researchers reported a linear relationship between bulk tank somatic cell count (BTSCC) and the percentage of quarters infected with major pathogens. Using this relationship, the percentages of quarters infected in a dairy herd at different BTSCC levels are as under:

BTSCC Levels (cells / ml)	**% of quarters infected in a dairy herd**
2 lakh	6
4 lakh	13
5 lakh	16
7.5 lakh	24
10 lakh	32
15 lakh	48

2.3 Relationship between the milk producing capability and milk somatic cell counts

Infected cows with elevated somatic cell counts in milk have diminished milk production due to tissue damage. It is reported in the available literature, based on various studies in the developed countries, that in second and later lactation cows each time the SCC doubles (above 2 lakh cells/ml) the level of milk production

drops approximately 600 g/cow/day. In first calf heifers the reduction is considered to be approximately half this amount. Loss of production potential can be significant as the cell count rises to such levels.

2.4 Potential human health risks

Consuming milk with a high SCC does not pose direct, specific health risks to people. However, relationship of high SCC milk with poor udder health and farm hygiene resulting in the possible presence of pathogenic organisms and their toxins in milk; and also presence of antibiotics residues in milk on account of mastitis treatments, offers insight into the potential increase in safety risk factors to consumers when high SCC milk is marketed.

2.4.1 Transfer of pathogens and their toxins: The primary cause of a high SCC in milk is intra-mammary infections. The vast majority of bovine intra-mammary infections are caused by bacteria. Many of these bacteria are also the causative agents of human diseases (e.g. *Escherichia coli, Staphylococcus aureus, Streptococcus agalactiae*). Fortunately, pasteurization of milk kills the most common mastitis causing bacteria. Proper pasteurized of milk is very effective in preventing the transfer of viable pathogens from milk of infected mammary glands to humans. However, emerging information about potential transfer of infectious agents from cow to man are emerging despite pasteurization of milk. Evidence has been reported that *Mycobacterium avium* subsp. *paratuberculosis* associated with Johne's disease in cattle and isolated from human patients with Crohn's disease, may survive some accepted milk pasteurization procedures.

Further, pasteurization, largely capable of reducing the number of viable microorganisms, often does not destroy toxins produced by bacterial pathogens. The transfer of heat stable toxins produced by mastitis-causing pathogens in milk is a potential concern. Specifically, enterotoxin produced by *S. aureus* in milk of infected cows has been implicated in cases of food poisoning. As *S. aureus* continues to be a major cause of mastitis in many parts of the world, the frequency of enterotoxin production among strains of this species causing mastitis is a potential concern.

2.4.2 Direct effects of neutrophils: The review of literature in general does not reveal a known, direct health concern as a result of consuming milk with an elevated SCC. As the SCC increases, the percentage of neutrophils (a type of white blood cell) increases. Although the ingestion of large numbers of bovine neutrophils in milk may not be acceptable from consumer point of view, direct negative effects on the safety of humans have not been reported as a result of consuming dairy products made with milk having high SCC.

2.4.3 Secondary relationships: The established ancillary relationships between SCC and human health concerns are possibly more problematic than the direct health concerns of consuming high SCC milk. Investigators have consistently reported a positive relationship between SCC of bulk milk and antibiotic residue violations. Consumption of milk products adulterated with antibiotic residues poses a potential risk to people hyper-sensitive to the antibiotic.

2.5 Effect on milk and milk product quality

In addition to the associated health concerns, milk with high somatic cell counts has altered compositional characteristics that

affect the quality of milk and milk products. The table below shows the compositional changes in milk from normal to high somatic cell count:

Milk components	**%**		
	in normal milk	**in milk with high SCC**	**of normal milk content**
Solids-non-fat	8.9	8.8	99
Fat	3.5	3.2	91
Lactose	4.9	4.4	90
Total protein	3.6	3.56	99
Total casein	2.8	2.3	82
Whey protein	0.8	1.3	162
Sodium	0.057	0.11	184
Chloride	0.091	0.15	161
Calcium	0.12	0.04	33

The consequent effects of such changes in milk components due to increased somatic cell count are summarized in the following table:

Milk components	**Nature of change**	**Consequences**
Fat	↓	Less butter yield
Total casein	↓	Less cheese yield (up to 1% / 1 lakh cells)
Lactose	↓	Economy
Calcium	↓	Altered heat stability
Sodium	↑	Salty taste
Chloride	↑	Salty taste

Milk components	Nature of change	Consequences
Free fatty acids	↑	Rancid taste
Lipase	↑	Increase in free fatty acids

↑ - Increase ↓ - Decrease

Some specific quality problems associated with milk and milk products prepared from high SCC milk are briefly detailed below:

Liquid milk: When somatic cells destroy bacteria, left behind are enzymes that have been involved in the process. These enzymes (such as plasmin) are often resistant to pasteurization procedures and can cause milk fat and protein breakdown. This can result in serious off-flavours in milk that consumers find objectionable. It also may reduce shelf life significantly, even when milk is kept properly refrigerated. In general, rancid off-flavors due to increased lipase activity, bitter flavours due to proteolytic enzyme activity, and salty flavours due to a change in milk mineral balance will all gradually appear as SCC increases.

In UHT milk, plasmin may cause milk to change from a liquid to a gel.

Cheese: There is also a significant relationship between the milk SCC and the cheese yield - the higher the cell count the lower the cheese yield and vice versa. This is partly because of a decrease in casein, as a percentage of total protein, in milk from mastitis infected animals. High SCC milk may also produce a cheese that is of inferior quality because of high moisture retention. The increase in cheese moisture is caused by a slow, weak coagulation

largely due to alteration in milk protein composition and mineral balance, combined with increased milk pH. These changes in milk composition interfere with the expulsion of water from the curd during syneresis.

Other milk products: It is reported that an elevated SCC in milk can cause increase in coagulation time of butter and yoghurt cultures. The organoleptic quality of sweet cream butter made from high SCC milk is also reduced due to milkfat breakdown by increased lipase activity and the product deteriorates more rapidly during storage than butter made from low SCC milk.

A high SCC can also be associated with poor heat stability of whole milk powder. The functional characteristics (foam stability, gel strength, heat stability, etc.) of the milk proteins in condensed and dried milk products can also change as a result of protein breakdown by residual enzymes and decrease the value of these products.

A summary of reported product defects associated with use of elevated SSC in milk is given in the table below:

Product	**Effect**
Pasteurized fluid milk	Reduced shelf life Organoleptic defects
UHT milk	Accelerated age gelation
Cream	Reduced whipping properties
Cultured products	Increased coagulation time Inferior organoleptic properties
Butter	Extended churning time Reduced shelf life Inferior organoleptic properties

Product	Effect
Milk powder	Altered heat stability Reduced shelf-life
Cheese	Reduced yields and yield efficiencies Elevated moisture content Increased rennet clotting time Soft cheese and textural defects Higher loss of solids in whey Inferior organoleptic properties

Some researchers have reported that SCC begins to affect product quality as it increases above 1 lakh cells/ml of milk while others have suggested that the threshold is closer to 5 lakh cells/ml of milk.

3. STANDARDS FOR SOMATIC CELL COUNTS IN MILK [1 and 6]

3.1 Country regulations on somatic cell counts in milk:

Limits on the maximum SCC in bulk milk collected from farms are applied in many countries with a common maximum limit of 400,000 cells / ml. Some specific national standards applicable in several major developed countries are given in the table below:

Country	SCC Limit (cells / ml)
Switzerland	3.5 lakh
European Union	4 lakh
New Zealand	4 lakh
Australia	4 lakh

Country	SCC Limit (cells / ml)
Canada	5 lakh
United States of America	7.5 lakh

The US limit for SCC is currently the highest specified by any country.

3.2 Need for an international standard

Milk and milk products are valuable food items traded amongst various parts of the world. Inevitably, any food item that is traded internationally becomes subject to strict quality standards and milk is no exception. The issue regarding somatic cell count in milk is also important for international trade considerations. The standards specified by Codex Alimentarius Commission are considered as benchmark / reference standards for quality and safety of food products in international trade. However, currently no Codex standard exists regarding an acceptable upper limit for SCC in milk intended for trade in the international market. In the absence of an international standard, there is lack of consensus at international level on the SCC above which bulk milk should be considered unacceptable.

4. MEASURES FOR CONTROL OF SOMATIC CELL COUNTS IN MILK [1 and 7- 11]

As explained in previous sections, milk somatic cell counts are largely influenced by the udder health (mastitis condition) of animals. Consequently, the milk SCC can be controlled by employing various on-farm handling measures that improve the

udder health condition and the general environment of milking animals. Some important measures for comprehensive mastitis management include:

Maintaining clean and dry environment: A dirty environment will lead to a build up of environmental bacteria and high levels of environmental bacteria will increase chances of opportunistic udder infections. Dry cows and heifers need special attention as udder is particularly susceptible to new infection in early and late dry period.

Since some of the mastitis-causing bacteria (such as *coliforms* and *Streptococcus* species other than *Streptococcus agalactiae*) are found in the environment, it is of paramount importance to reduce the animal exposure to these pathogens by providing milking animals with a clean and dry environment. Animal faeces should be removed at the earliest and bedding in the animal holding area should be clean, suitable, plentiful and changed frequently.

Maintaining cleanliness during calving: A cow's immune function is at its lowest around the time of calving. Further during calving, both her udder and reproductive tract are open to the environment. Thus, a calving animal is more prone to udder and uterine infections as a result of dirty environment. Therefore, the milking animal should calve in a clean and freshly bedded area.

Disinfecting calving area is even better. The best way to disinfect a calving area is to clean it and leave it unbedded and unoccupied for a day or so. Most disease causing bacteria aren't able to tolerate exposure to light, air and drying conditions. Alternatively, disinfectants or even hydrated lime can also be used. Organic

matter (manure & bedding) reduces the efficacy of disinfectant, so higher disinfectant concentrations would be required for effective disinfection.

Increasing cleanliness of milking process: By maintaining the highest possible levels of cleanliness, the bacterial load in the milking area can be minimized. This will reduce the chances of infection occurring during the milking process.

Milking personnel can not be overlooked as a possible source of bacterial contamination that could lead to mastitis. Anyone who works on livestock operations should change clothing and wash arms and hands thoroughly before milking animals. Milking personnel should be encouraged to wear clean gloves, if possible, while milking as the practice has also been shown to reduce exposure of udder to bacteria.

Care in machine milking: An improperly functioning and/or unhygienic milking machine is potentially a major factor in the spread of contagious pathogens among milking animals. However, such infections can be avoided by use of milking machines fitted with appropriate liners that do not slip during milking, short milk tubes of adequate diameter, non-return valves etc. and ensuring proper hygiene of the equipment.

Post-milking teat dip use: A consistent post-milking teat dip program is very useful in mastitis control. More than 50% of new udder infections can be prevented by disinfecting teats with an effective product immediately after every milking. Post-milking teat disinfection is especially effective against the contagious pathogens – namely, *Staphylococcus aureus* and *Streptococcus*

agalactiae. Producers should use only those teat dip products that are permitted and of proven effectiveness. Some post dip formulations that are reported to be effective under field conditions include[8]:

- chlorhexidine (0.5 percent)
- iodophor (0.5 to 1.0 percent available iodine)
- hypochlorite (4 percent)
- chlorous acid-chlorine dioxide
- linear dodecyl benzene sulfonic acid (1.94%) etc.

It is important to follow standard operating procedures describing: how to prepare animals for milking; procedure for applying post milking teat dip; the need to wear gloves and when to clean and sanitize the hands etc. This approach helps everyone understand what is expected and helps remove inconsistencies associated with different people handling milking animals. Some important aspects of handling of teat disinfectants are given below:

- Store teat disinfectants in cool, dry area.
- Do not allow disinfectants to freeze.
- Keep containers closed to prevent contamination.
- Do not use after the expiration date.
- Follow label instructions for use. Be sure to use teat disinfectants at recommended concentration. Do not dilute unless indicated on the label. If dilution is necessary, be sure that water quality standards (bacteria, pH, hardness, etc.) are met. Use a clean container for diluting and thoroughly mix the final product.
- Disinfectant cups should be emptied and cleaned as part of the routine wash-up after each milking
- Never pour used disinfectant back into the original container.

Using dry cow therapy: Teat disinfection does not affect existing infections. Existing infections are best eliminated by dry cow treatment. Dry cow therapy is much more effective than antibiotic therapy during lactation. Dry cow antibiotic treatments are one of the most effective ways to eliminate existing infections and preventing new infections. Treatment during the dry period is 80-90% effective in eliminating infections, while treatment during lactation is only 30-40% effective. This is because antibiotics designed for dry cows can be formulated to provide higher levels of antibiotic activity over longer periods of time without the usual worry about presence of antibiotic residues in milk.

When administering dry cow treatments, make sure that teat ends are thoroughly scrubbed with cotton-alcohol pads. Otherwise, dry cow treatments can end up introducing large numbers of bacteria into the udder and causing more harm than good.

Teat sealant should also be considered as part of dry off treatment procedures. After the teats have been infused with dry cow antibiotics teat sealant should be applied to help seal off the teat end to prevent environmental bacteria from entering during the early dry period.

Enhancing immune system of milch animals: Stressed animals have higher levels of hormones that suppress immune function in their blood. Good comfort, appropriate handling procedures, and sufficient feed and water can go a long way towards reducing the stress levels of the herd. A herd with lower stress levels will have better immune function. A proper nutrition programme, with special attention to vitamin and mineral nutrition, will improve the overall function of the immune system of the milch animal.

Withholding fresh milk from bulk tank for longer periods: Milk from freshly-calved animals is generally higher in SCC. Withholding this milk from bulk tank can reduce tank SCC, but may be economically non-viable to hold out milk from fresh cows beyond the standard 72-96 hours post calving.

5. METHODS FOR ESTIMATING SOMATIC CELL COUNTS IN MILK [1]

The somatic cell count (SCC) is important to dairy producers as high SCC is an important indicator of the udder health (mastitis condition) and associated poor milk quality. Hence SCC is regularly used to monitor mastitis incidence in the herd. Various methods of estimating somatic cell numbers in milk are as under:

5.1 Reference method

Direct Microscopic Somatic Cell Count (DMSCC): Somatic cell count by direct optical microscopy is the reference method for SCC evaluation, but it is extremely time consuming. A test portion of milk to be examined is spread over a slide to form a smear. The smear is dried and during the process the cells are stained with a dye. Subsequently, the stained cells are counted using a microscope. The number of cells counted in a defined area on the slide is multiplied by a working factor, to give the number of cells per milliliter of milk.

Details of the method are given in the joint IDF-ISO International Standard 'Milk - Enumeration of somatic cells – Part 1: Microscopic method (Reference method) (ISO 13366-1 / IDF 148-1)'.

5.2 Routine methods

Internationally, dairy laboratories use the following instrumental systems that provide, when calibrated against direct microscopic counts, accurate, rapid and précised measurement of SCC. However, these systems are complex and expensive for routine application in field.

- **Electronic somatic cell count:** These instruments use electric-counting of formalin-fixed somatic cells passing through an aperture. These instruments are based on the *Coulter* principle (Electrical Sensing Zone Method) for counting cells or particles.
- **Opto-electronic somatic cell count:** These systems label the nuclei of somatic cells using ethidium bromide and then count the stained nuclei as they pass in a thin film under high-energy light source.

  Guidance on enumeration of somatic cells by fluoro-opto-electronic counters is given in the joint IDF-ISO International Standard 'Milk - Enumeration of somatic cells – Part 2: Guidance on operation of fluoro-opto-electronic counters (ISO 13366-2 / IDF 148-2)'
- **Flow cytometry:** These systems also label the nuclei of somatic cells using ethidium bromide and then count the stained nuclei through application of laser-based flow cytometry.

Additionally, a number of rapid tests have been developed for 'on-farm' estimation of SCC such as the California Mastitis Test (CMT) and the Wisconsin Mastitis Test (WMT).

- **California Mastitis Test (CMT):** CMT is an indirect measure of somatic cell concentration in milk. The test is based on detergent interaction with DNA of somatic cells present in milk. In the CMT, a small sample of milk (from individual milking animal or quarter) is mixed with an alkaline reagent in a special mixing device, and the extent of subsequent formation of a gel is ranked on a simple scale which relates gel formation to milk SCC.
- **Wisconsin Mastitis Test (WMT):** The CMT, a slightly more complex variation of the CMT and a rapid milk quality test, involves the gel formation being read as the volume of sediment in a calibrated tube.

6. IMPORTANT TERMINOLOGY [12]

The International Dairy Federation suggested the following interpretations of some common terminology related to somatic cells and somatic cell counts:

- **Somatic cell count:** The concentration of somatic cells in milk, generally expressed as cells / milliliter of milk.
- **Bulk milk:** Bulk milk is co-mingled milk produced by all the milking animals in a herd or co-mingled milk produced by several herds.
- **Bulk tank:** Bulk tank is an on-farm sanitary storage vessel or vat for the milk produced by milking animals on that farm.
- **Bulk milk somatic cell count:** Bulk milk somatic count is a cell count value calculated as an average for the whole herd.

The count is estimated by calculating the product of the cell count for each individual milking animal and the weight of milk sample over a 24-hour period divided by the total weight of the milk produced by all milking animals. This value is an indirect measure of the prevalence of intra-mammary infection in the herd.

- **Bulk tank somatic cell count (BTSCC):** Bulk tank somatic cell count is the cell count measured on a small sample of the whole consignment of milk, taken after mixing of the whole tank contents, and obtained from a single bulk tank. The count is a measure of the quality of milk sold.

7. EPILOGUE

Somatic cells, originating from milking animal's body, are invariably present in milk. The cellular composition and concentration of cells change dramatically on account of the changes in the udder health. Milk somatic cell count is increasingly considered as an indicator of mastitic condition and general hygiene of the milk production environment, and the associated risks to human health. Absence of an international standard on maximum limit of SCC in milk and difference of opinion between the countries has important implication for international trade. Nevertheless, the producers need to effectively manage production conditions, with assistance and guidance of the processors, in such a way that helps in achieving low SCC in milk.

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NEWS SECTION

Indian Food Laws

- **Notification No. P.15014/9/2001-PH(F)Vol. IV of 27 February 2009 of the Ministry of Health and Family Welfare:** The notification is a corrigendum to its previous notification GSR 664 (E) of 19 September 2008 (Refer Technews Issue 76, September-October 2008) that is now scheduled to become effective from 18 March 2009 (Refer Technews Issue 77, November-December 2008).

  The notification GSR 664 (E) mandated declaration of the name of ingredients and their weight or volume used in the product at the time of its manufacture, in the list of ingredients. As per the corrigendum, the names of the ingredients shall be declared in descending order of their composition by weight or volume, as the case may be, at the time of the manufacture of the product. Therefore, quantity of all the ingredients need not be declared in the list of ingredients.

  The provisions relating to the quantitative declaration of the ingredients under the specified conditions, however, remain unaffected.

- **Notification GSR 117 (E) of 24 February 2008 of the Ministry of Health and Family Welfare:** The notification is a latest corrigendum to an earlier notification GSR 356(E) of 7 June 2005 (Refer Technews Issue 60, January–February 2006). The Corrigendum indicates that the provisions in the GSR 356 (E) on the definitions, food additive provisions and microbiological specifications for specified dairy products (cheese, processed cheese, processed cheese spread, ice cream/ *kulfi* / chocolate ice cream/softy ice cream, dried ice cream mix/dried frozen dessert/confection, frozen dessert/frozen confection, milk ice / milk lolly and yoghurt) shall come into force after 4 years from the date of publication of GSR 356(E). Therefore, these provisions are now likely to be applicable from 7 June 2009.

- **Notification GSR 43 (E) of 22 January 2009 of the Ministry of Health and Family Welfare:** This notification provides draft PFA Rules to amend the existing provisions, pertaining to the use of artificial sweeteners and bulking agents in a few dairy products, in the PFA Rules.

  Use of Aspartame is proposed to be permitted, among others, in ice-cream and frozen dessert (1000 ppm); flavoured milk (600 ppm); and yoghurt (600 ppm).

  Use of the polyols, as per GMP, is proposed to be permitted as follows:

  - Isomalt, among others, in traditional Indian milk products based sweets namely *khoya burfi*, *peda*, *gulabjamun* (also in *gulabjamun* mix), *rasogolla* and similar milk based sweets

sold by any name; edible ices; ice-cream and frozen desserts; and sweetened yoghurt.

- Erythritol, among others, in dairy drinks (chocolate and flavoured milk), ice-cream and yoghurt; and

Use of Polydextrose is proposed to be permitted as per GMP, among others, in ice-cream, frozen dessert, yoghurt and in traditional Indian milk products based sweets namely *khoya burfi*, *peda*, *gulabjamun* (also in *gulabjamun* mix), *rasogolla* and similar milk based sweets sold by any name.

The packages of food containing polyols or polydextrose shall make a label declaration indicating the laxative effect associated with the consumption of these additives.

Codex Alimentarius Commission (CAC)

- Meetings of the following Codex Committees are scheduled during May 2009:

  → Codex Committee on Food Labeling, 4-8 May 2009, Calgary, Canada.
  → Codex Committee on Residues of Veterinary Drugs in Foods, 11-15 May 2009, Natal (Rio Grande do Norte), Brazil.

SOMATIC CELLS IN MILK

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