



Milk nutrient composition in relationship to somatic cells

Arun Singh Dalchand¹, Abirami Preethi Madhavan², Chitradevi Kaniraja³

¹⁻³ Kalasalingam Academy of Research and Education, Department of Food Technology, Krishnankoil, Tamil Nadu, India

Abstract

Somatic cells are leukocytes and epithelial cells from blood and gland. The somatic cell count increases when the cow has mastitis infection as the leukocytes in the blood increases to fight against the infection. So in this research we have concluded that, which quality parameter increases and decreases. We have studied increasing parameters such as pH, conductivity, MBRT time, and decreasing parameters such as protein%, fats%, lactose%, caesin%, snf%, and detection of antibiotics was done with the help of Delvotest SP NT DA kit. Mastitis in cow can alter the physical, chemical and bacteriological properties of milk which leads to low quality milk and according to animals this disease, decreases the life of animal. This article highlights the change in nutrient composition of milk with respect to increasing somatic cell count.

Keywords: mastitis, SCC, milk composition, milk quality

1. Introduction

Mastitis in cows is an inflammatory disease affection the udder tissues that reflects on loss in milk quality and nutrient composition. The two different types of mastitis: Clinical (visually observed infection) and subclinical (invisible infection). Infection occur due to bacteria and its toxins. Common mastitis causing bacteria are considered pathogenic like *Streptococcus dysgalacticae*, *Streptococcus agalactiae*, *Streptococcus uberis* and gram negative bacteria. The leukocytes (somatic cells) increase to fight against this infection, when the cow is infected by mastitis. This may cause an elevation of SCC to be discharged into milk. The cow is infected by mastitis is predictor by high SCC which act as an indicator. So milk producers should be aware of subclinical mastitis and associated milk nutrient loss. Many indicating test such as California Mastitis test (CMT), Sodium Lauryl test (SLST), White side test (WST), Surf field Mastitis test (SFMT) can be performed on farm level to avoid bulk tank somatic cell count. Therefore the current work views on the loss in milk composition in relationship to elevated SCC.

A nutrient rich white opaque fluid secreted by the mammary gland mainly served for nourishment. Standard milk nutrient composition of Holstein breed cow contains: Water(87.74%), Lactose(4.87%), Fat(3.4%), Protein(3.22%), Ash(0.68%)^[45]. and any deviation or changes in milk nutrients lead to low quality of milk. The Somatic cells or body cells (leukocytes) that enter into milk from blood are attracted by the chemical substances released from the injured mammary tissue. The leukocytes digest and engulf the bacteria causing mastitis. That is when the leukocytes increase, mastitis causing bacteria is reduced. The count can range up to a certain limit in milk that is <1, 00,000 cells/ml is acceptable and if >5, 00,000 cells/ml needs more attention. On positive result to mastitis there is an increase in SCC that may directly affect the milk composition by reducing the synthesis of milk components in the udder and change in permeability of membrane that cause leakage of materials from blood to milk. A decrease in Fat, Caesin and Lactose was observed. Also visible changes in protein,

MBRT time, SNF, pH, Conductivity was observed.

2. Materials and Methods

2.1 Sample Collection

100 milk samples (5 samples/day) was collected from a milk industry and was tested for nutrient composition by using Fourier transform machine (FT1), somatic cell count by using Lactoscan SCC machine and Methylene blue reduction test (MBRT) with methylene blue dye in water bath. The data were collected and recorded regularly.

2.2 Methods of Sample Analysis

2.2.1 Milkscan Fourier Transform Machine (Ft1)

Fourier transform infrared (FT-IR) spectroscopy is a rapid biochemical fingerprinting technique that could be used to reduce this sample analysis period significantly. 8ml of milk at temperature 5 to 55°C is analyzed for 30 seconds to estimate fat, protein, lactose, total solids, SNF, total acidity, density, FFA, FPD, total acidity, density, citric acids, urea, casein, glucose, galactose. The MilkoScan FT1 analyses the main product components in milk as well as screening for milk abnormalities. The analysis results can be used for payment, securing the safety of raw material, calculating the mass balance for standardizations purposes and verifying end products.^[41]

2.2.2 Delvo test

Delvotest SP NT DA is a test system that is designed to test milk for the presence of antibacterial substances such as antibiotics. The test is incubated in the Delvotest Accelerator. The test contains a solid and buffered agar medium, including all required nutrients, a standardized number of spores of test organism *Bacillus stearothermophilus* var. *calidolactis*, and a purple-colored pH indicator bromocresol purple[4,4'-(1,1-Dioxido-3H-2,1-benzoxathiole-3,3-diyl)-bis(2bromo-6-methyl phenol)]. The principle of the test is based on the diffusion of possible inhibitory substances that may be present in the milk sample into agar. This reduces growth and acid production by the test organism, and delays or prevents the agar from

changing color from purple to yellow [25].

2.2.3 Methylene Blue Reduction Test (Mbrt)

The methylene blue reduction test is based on the fact that the color imparted to milk by the addition of a dye such as methylene blue will disappear more or less quickly. The removal of the oxygen from milk and the formation of reducing substances during bacterial metabolism cause the color to disappear at 32°C in water bath. It is a quick method to assess the microbiological quality of raw and pasteurized milk [22].

2.2.4 Lactoscan SCC Machine

LACTOSCAN SCC is a unique, 3D, multi-image, patent application protected, sequential scanning process, based on a precise fluorescent optics and low magnification, images analysis software. It is fast, precise and reliable counter of somatic cells based on fluorescent image cytometry for counting cells in milk. It offers visual control for excluding accidental errors. In order the somatic cell to be counted by with LACTOSCAN SCC, the sample is mixed with Sofia green dye. It is only 8 µL that is needed to be pipette onto the single lactochip. After that, the chip is loaded in the device. The analysis is being conducted during a period between 10 seconds and 2 minutes and the duration depends

on the number of filmed fields. The system of LACTOSCAN SCC is focusing automatically on the CHIP and the dyed cells are filmed by the sensitive CMOS camera. This algorithm of analysis of digital images determines the number of the fluorescent cells and counts their concentration and size. The somatic cell count result is automatically displayed [24].

2.2.5 California Mastitis Test Kit

The California mastitis test (CMT) is a simple cow-side indicator of the somatic cell count of milk. It operates by disrupting the cell membrane of any cells present in the milk sample, allowing the DNA in those cells to react with the test reagent, forming a gel [26].

3. Results and Discussion

On comparison of Somatic cell count with MBRT time, conductivity and nutrient composition it was found that the milk sample with high SCC count (>1,00,000 cells/ml) showed more MBRT time, increase in conductivity, delvo positive(test for antibiotics) and reduce in nutrient composition. The same test was repeated for 2 months and 100 milk samples were obtained from same loctaion as stated in table no 1.

Table 1: nutritional changes in milk due to scc.

Scs Cells/ml	0-100000	100000-200000	200000-300000	300000-400000	400000-500000
Antibiotic test parametres	Negative	Positive	positive	Positive	positive
Protein (%)	3.327	3.292	3.29	3.28	3.287
Fats (%)	4.541	4.5	4.388	4.42	4.434
Snf (%)	8.659	8.604	8.613	8.628	8.597
ph	6.612	6.625	6.617	6.61	6.62
conductivity	5.174	5.162	5.194	5.151	5.22
Mbrt(hrs)	3.9	3.718	4.126	4.857	4.327
Lactose (%)	4.629	4.63	4.599	4.607	4.587
Casein (%)	2.492	2.473	2.463	2.467	2.463

3.1 Nutritional changes

3.1.1 Protein changes

Mastitis is caused by bacterial invasion into the udder,so bacteria and leukocytes in the infected quarters release chemo-attractive products for leukocytes, especially neutrophils is produced due to this the neutrophil

polymorphonuclear (PMN) leukocytes are generated for defence mechanism by the animal body which consumes milk protein such as [3-casein, [3-lactoglobulin, a-lactalbumin) by putrefaction method and reduced synthesis process so the decrease in protein was observed in the figure no 1 [43, 15].

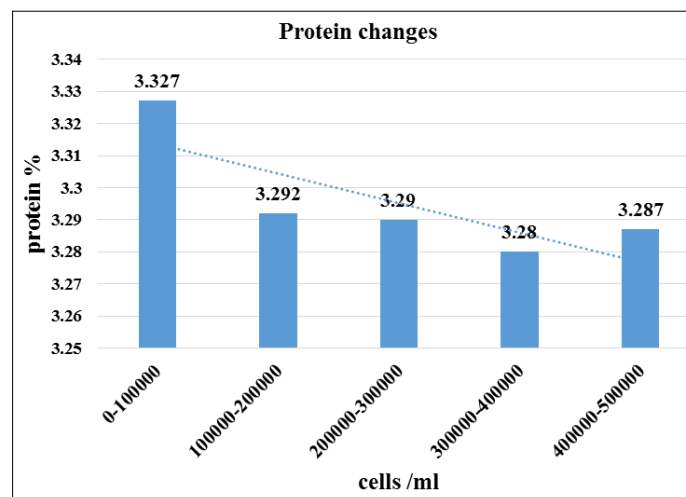


Fig 1: decrease in protein % w.r.t. increase in scc

3.1.2 Fats

Due to injury to the secretory cells reduce fat synthesis takes place, Phospholipids appear to be synthesized in the gland so due to inflammation in the cells Phospholipid content of the fat globule membrane is reduced due to decrease in phospholipid level the lipase activity increases due to production of leucocytes which can be responsible for decrease in fat content which was observed in the figure no 2 [15].

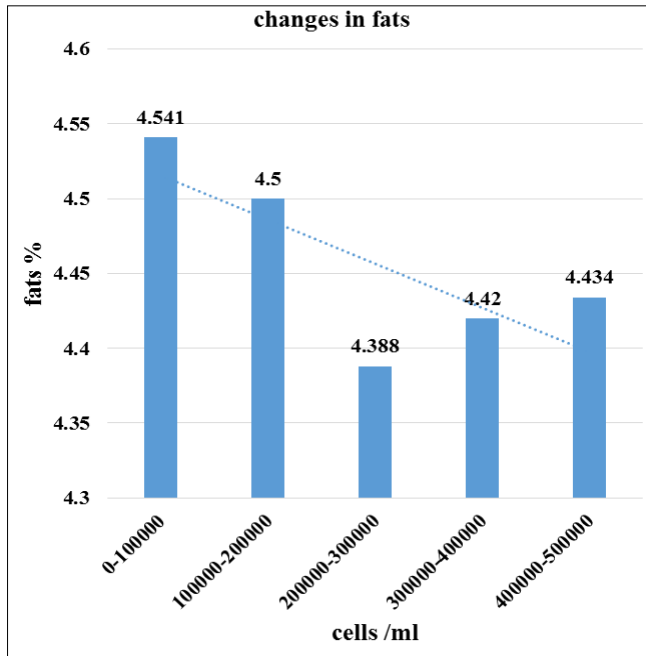


Fig 2: decrease in fat % w.r.t. increase in scc.

3.1.3 Lactose:

Decrease in lactose was observed due to decrease synthesis. The generation of lactose in milk is from glucose removed from the blood streams. The milk protein a-lactalbumin is responsible for synthesis of lactose so the level of a-lactalbumin decreases and this results in decrease in lactose percentage as somatic cells increase in milk as shown in figure no3 [15].

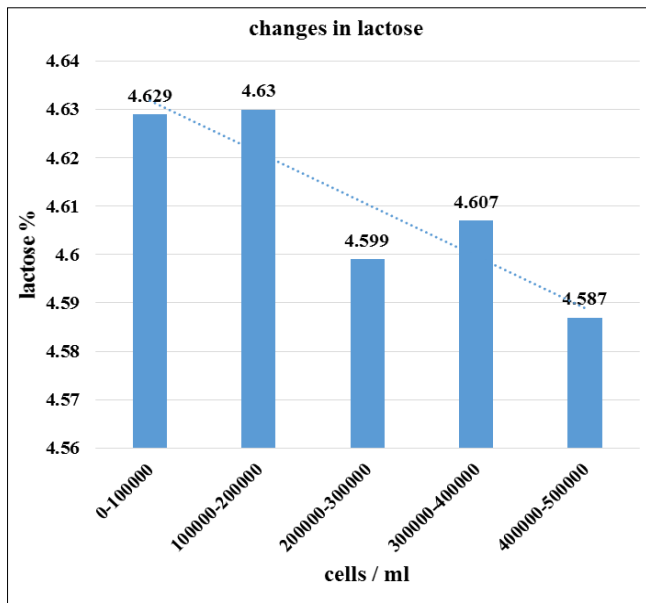


Fig 3: decrease in lactose% w.r.t. increase in scc.

3.1.4 Caesin

Caesin percentage decreases due to post secretory degradation of caesin by proteinases originating from mastitis causing organisms, Caesin percentage also decreases due to physical damage to the mammary epithelial cells by microbial toxins during mastitis.[1], and by the reduce synthesis process also caesin % decreases as shown in the figure no 4.

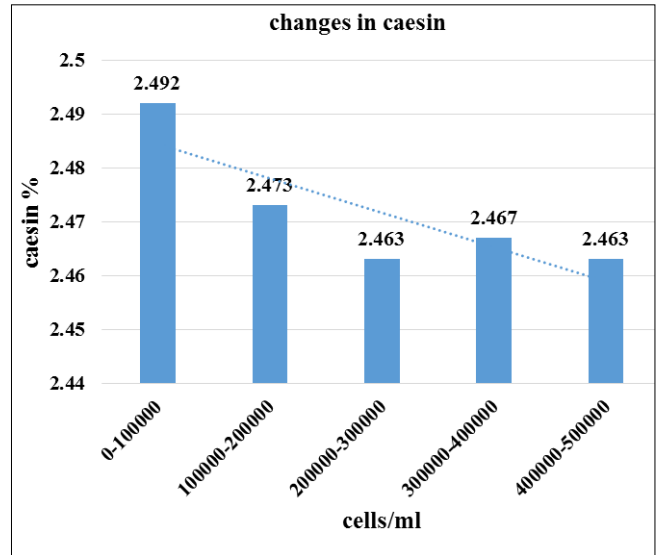


Fig 4: decrease in lactose% w.r.t. increase in scc.

3.1.5 Ph

Two major bacteria that causes mastitis (strptococcus agalactiae and staphylococcus aureus) [51]. will damage the mammary gland cell walls allowing blood serum to leak into the milk. Due to this increases in sodium and chloride and a decrease in potassium minerals takes place and mixes with the milk. Increase in somatic cells increases chloride content and decreases casein content due to which the alkaline minerals from blood increases ph of milk. [15].

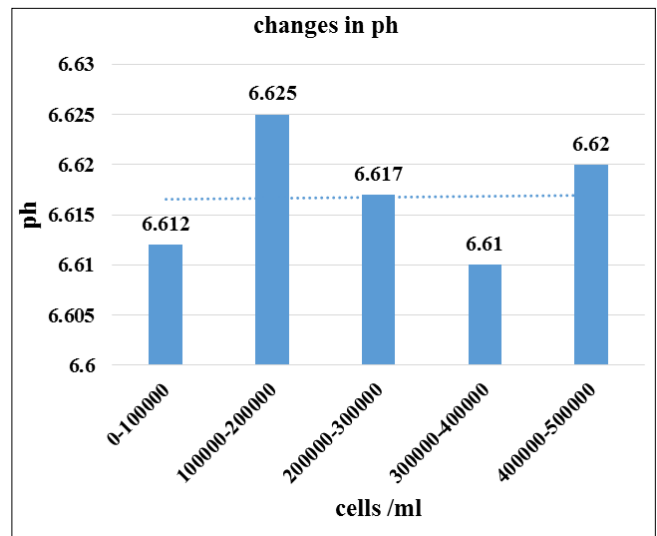


Fig 5: increase in lactose% w.r.t. increase in scc.

3.1.6 Snf

Composition of milk snf is (lactose, protein such as caesin, lactalbumin, lactoglobulin, proteose peptone, minerals, etc) so from figure no 6 it was observed that snf of milk reduces

when the scc count increases in the milk. So from figure no 1 shows in decrease in protein which leads to decrease in snf content, figure no shows the decrease in lactose due to decreased synthesis. Lactose in milk is synthesized only in the udder from glucose removed from the bloodstream so the due to mastitis the glucose level in blood decreases and the lactose reduces in the milk, from fig no we observed that there was decrease in caesin level, mineral content of milk was also observed as increases in sodium and chloride and a decrease in potassium due to this changes we can conclude that snf of milk reduces as the somatic cells increases in the milk, [45, 43, 15].

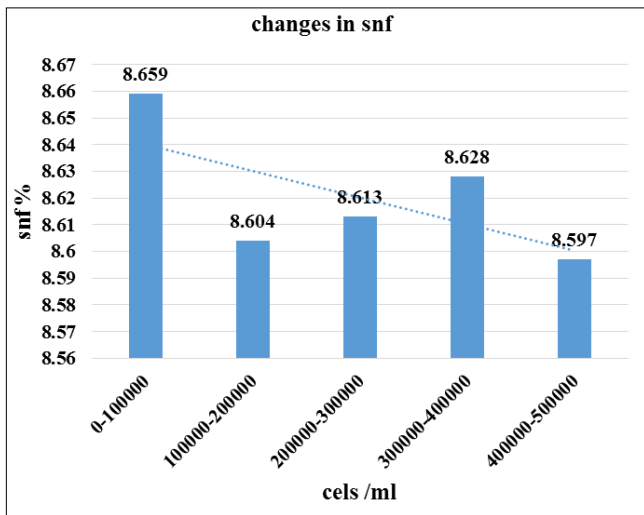


Fig 6: decrease in snf % w.r.t. increase in scc.

3.1.7 Conductivity

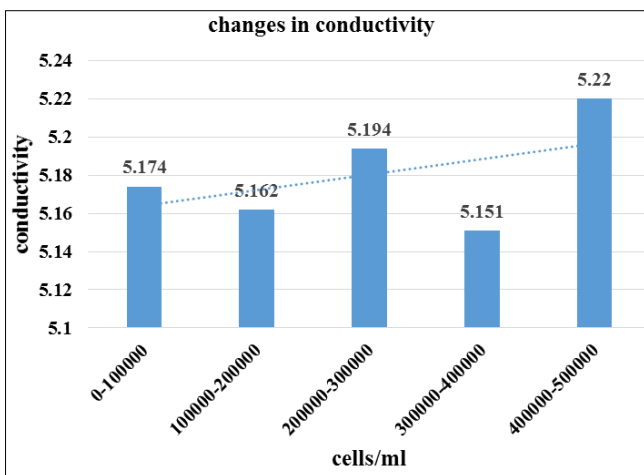


Fig 7: increase in conductivity w.r.t. increase in scc.

Streptococcus and staphylococcus bacterial species is responsible for the inflammation of udder which damages tissue and the defence mechanism of animal experiences in the change of ions such as increase in concentration of sodium, chlorine ions and decrease the concentration of potassium ions, concentration of lactose [30], which results in increase level of conductivity as shown in the figure no 7.

3.1.8 Mbrt

When antibiotic is present in milk it kills the harmful bacteria and pathogens present in milk results in increase mbrt

level in milk. As antibiotics in milk can retain till 72-hrs and even for 2-3 weeks [14], which depend on the characteristics of antibiotic given to animals for decreasing the inflammation caused due to mastitis. And in animals body also immunoglobulins are produced as a defence mechanism to prevent the animal from foreign bacteria entering in the animals body due to climatic condition or due to some other factors. Due to this mbrt level was seen increasing in figure no 8.

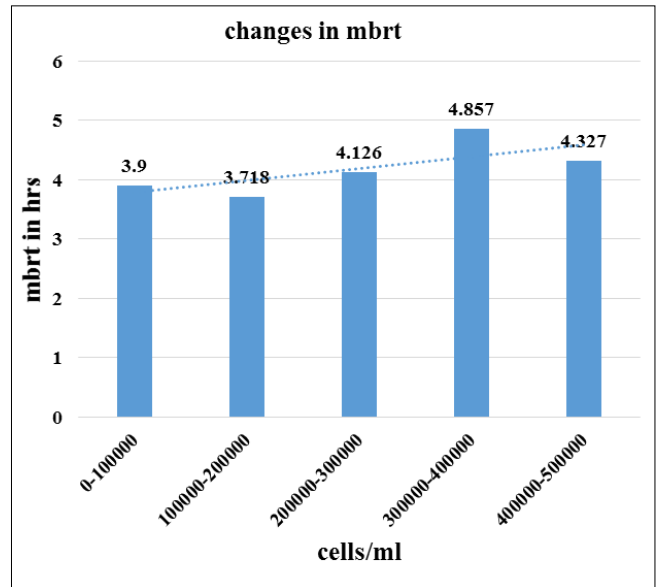


Fig 8: increase in mbrt w.r.t. increase in scc.

4. Conclusion

When somatic cell count is high (>100000 cells/ml) the nutrient composition of milk reduces due to decreased synthesis and leakage of blood from udder. Milk production suppresses and milk consistency changes. Decrease in protein is caused by bacterial invasion into the udder, so bacteria and leukocytes in the infected quarters release chemo-attractive products for leukocytes generation, fat is reduced due to lower volume of milk, the leakage of lactose from milk will take more water with it and the volume of secretion left in the gland will decrease, lactose % gets decline in milk due to the damage in the alveolar epithelial cells. The generation of lactose in milk is from glucose removed from the blood streams. The milk protein α -lactalbumin is responsible for synthesis of lactose so the level of α -lactalbumin decreases and this results in decrease in lactose percentage, Caesin percentage decreases due to post secretory degradation of caesin by proteinases originating from mastitis causing organisms, so there is decrease in lactose%, caesin%, protein%, so snf is mainly based on these components so if these parameters decrease so the snf of milk also decreases, apart from decrease in the parameters there are few parameters which increase such as Ph increases as the milk experiences increase in chloride content and decrease in caesin content in the milk, conductivity of milk increases as animal experiences change of ions such as increase in concentration of sodium, chlorine ions and decrease the concentration of potassium ion, concentration of lactose, and mbrt of milk can also be increased due to the antibiotics present in milk which may retard the pathogenic bacteria to grow in milk. This all parameter changes leads to low quality milk.

Referance

1. Auldlist MJ, Hubble IB. Effects of mastitis on raw milk and dairy products. the uustralian journal of dairy technology. 1998; 53:28-36.
2. Barbano DM, Rasmussen RR, Lynch JM. "Influence of milk somatic cell count and milk age on cheese yield" Journal of Dairy Science, 1991, 369-388.
3. Barkema HW. Management practices associated with low, medium, and high somatic cell counts in bulk milk Journal of dairy science, 1998.
4. Berglund I. Quarter milking for improved detection of increased SCC. *Reproduction in Domestic Animals*. 2007; 42(4):427-432.
5. Brahma CP, Ghosh SS, Kesh D Roy, Tudu NK. Somatic Cell Counts (SCC) in Milk, 2017.
6. Bruckmaier CE, Ontsouka JW Blum. Fractionized milk composition in dairy cows with subclinical mastitis, 2004.
7. Blowey Roger William. Peter Edmondson. Mastitis control in dairy herds. Cabi, 2010.
8. Canadian bovine mastitis research network. The california mastitis test (CMT), http://www.medvet.umontreal.ca/rcrmb/dynamiques/PDF_AN/Toolbox/Factsheets/CMTProENG.pdf
9. Cristina Castillo, Víctor Pereira, Ángel Abuelo, Joaquín Hernández, Effect of Supplementation with Antioxidants on the Quality of Bovine Milk and Meat Production, *The Scientific world Journal* Volume 2013, ArticleID616098, 8pages <http://dx.doi.org/10.1155/2013/616098>.
10. Dohoo Ian R, Meek AH. "Somatic cell counts in bovine milk" *The Canadian Veterinary Journal*, 1982, 119.
11. Dos Reis, Carolina Barbosa Malek. Effect of somatic cell count and mastitis pathogens on milk composition in Gyr cows. *BMC veterinary research*. 2013; 9(1):67.
12. Forsbäck L. Udder quarter milk composition at different levels of somatic cell count in cow composite milk. *Animal*. 2009; 3(5):710-717.
13. Gonzalo C. "Mammary pathogens and their relationship to somatic cell count and milk yield losses in dairy ewes" *Journal of dairy science*, 2002, 1460-1467.
14. Hanna Róžańska, Jacek Osek, Stability of antibiotics in milk samples during storage, *Bull Vet Inst Pulawy*. 2013; 57:347-349. DOI: 10.2478/bvip-2013-0060.
15. schultz H. Somatic Cells in Milk-Physiological Aspects and Relationship to Amount and Composition of Milk, 1977.
16. Haenlein George FW. Relationship of somatic cell counts in goat milk to mastitis and productivity, *Small ruminant research*, 2002, 163-178.
17. Hameed Karima, Galal Abdel, Grazyna Sender, Agnieszka Korwin-Kossakowska. Public health hazard due to mastitis in dairy cows. *Animal Science Papers and Reports*. 2007; 25(2):73-85.
18. Hamann J. "Relationships between somatic cell count and milk composition" *Bulletin of the International Dairy Federation*, 2002.
19. Hortet Philippe. Henri Seegers Calculated milk production losses associated with elevated somatic cell counts in dairy cows review and critical discussion, 1998.
20. Hutton CT, Fox LK, Hancock DD. Mastitis control practices: differences between herds with high and low milk somatic cell counts. *Journal of Dairy*
21. Hortet, Philippe, and Henri Seegers "Calculated milk production losses associated with elevated somatic cell counts in dairy cows: review and critical discussion, 1998. IS.1479.3.1977.
22. Hazlett J, Little PB, Maxie MG, Barnum DA, Fatal Mastitis of Dairy Cows: A Retrospective Study, *Can J. Comp Med*, 1984.
23. Latcoscan. <http://lactoscan.com/editor/ufo/manuals/COMBO/IMCOMBOEN.pdf>, 2017.
24. Mark mitchell, Validation of the Delvotest SP NT DA, *Journal of aoa C InternatIonal*. 2012; 95(1).
25. MC Gill, California Mastitis Test (CMT). Macdonald Campus Farm Cattle Complex Standard Operating Procedure DC-617.
26. Michael Looper Professor. Reducing Somatic Cell Count in Dairy Cattle, Issued in furtherance of Cooperative Extension work, Acts of May 8 of the Department of Animal Science at the University of Arkansas, and, 1914, 30.
27. Miller RH. The relationship of milk somatic cell count to milk yields for Holstein heifers after first calving. *Journal of Dairy Science*. 1993; 728-733.
28. National mastitis council, inc. board of directors report, human health risks associated with high somatic cell count milk, symposium summary, 2005.
29. Norberg H, Hogeveen IR, Korsgaard NC, Friggens KHMN, Sloth P. Løvendahl Electrical Conductivity of Milk: Ability to Predict Mastitis Status, *American Dairy Science Association*, 2004.
30. Nickerson Stephen C. Choosing the best teat dip for mastitis control and milk quality. *Natl. Mastitis Council. Prof. Dairy Prod. Wisconsin Milk Quality Conf., Madison, WI. Natl. Mastitis Council*, 2001.
31. O'Rourke D, Nutrition and udder health in dairy cows, *Irish Veterinary Journal*. 2009; 62(15):20.
32. Omore Amos O. Impact of mastitis control measures on milk production and mastitis indicators in smallholder dairy farms in Kiambu district, Kenya. *Tropical animal health and production*, 1999, 347-361.
33. Ogola Henry, Anakalo Shitandi, Jackin Nanua. Effect of mastitis on raw milk compositional quality. *Journal of Veterinary Science*. 2007; 8(3):237-242.
34. Pamela L. Ruegg, DVM, MPVM Douglas J. Reinemann, *Milk Quality and Mastitis Tests*, 2002.
35. Reneau Jeffrey K. "Effective use of dairy herd improvement somatic cell counts in mastitis control" *Journal of dairy science*, 1986, 1708-1720.
36. Ruegg PL, Pantoja JCF. Understanding and using somatic cell counts to improve milk quality. *Irish Journal of Agricultural and Food Research*, 2013, 101-117.
37. *Science*, 1990, 1135-1143.
38. Schukken Y, nte H. "Monitoring udder health and milk quality using somatic cell counts" *Veterinary research*, 2003, 579-596.
39. Sharif, Muhammad g, somatic cell count as an indicator of udder health status under modern dairy production: a Review, *Pakistan Vet. J*. 2008; 28(4):194-200.
40. Subir Kumar Nandy, Venkatesh KV. Application of methylene blue dye reduction test (MBRT) to determine growth and death rates of microorganisms, *African Journal of Microbiology Research*. 2010; 4(1):061-070

41. Shemaa Mohammed Abdelreda, Dr. Reyam Naji Ajmi. Basic configuration of determination of Fat, Protein, Lactose and TS, SNF in infant formula milk powder by using MilkoScan FT120, International Journal of Scientific & Engineering Research, 2016, 7(3).
42. Sharma NK Singh, Bhadwa MS. Relationship of Somatic Cell Count and Mastitis: An Overview, Asian-Aust. J. Anim. Sci. 2011; 24(3):429-438.
43. Schukken Y, nte H. Monitoring udder health and milk quality using somatic cell counts. Veterinary research, 2003, 579-596.
44. Sukumer de outlines of dairy technology 2016.
45. Sharif A. Effect of severity of sub-clinical mastitis on somatic cell count and lactose contents of buffalo milk. Pakistan Veterinary Journal. 2007; 27(3):142.
46. Technews, National Dairy Development Board For Efficient Dairy Plant Operation, 2002.
47. Tripaldi C. Effects of mastitis on buffalo milk quality. Asian-Australasian Journal of Animal Sciences. 2010; 23(10):1319-1324.
48. Wegner TN. Effect of Stress on Blood Leucocyte and Milk Somatic Cell Counts in Dairy Cows. Journal of Dairy Science, 1976, 949-956.
49. Wilson David J, Keith N. Stewart, and Philip M. Sears. Effects of stage of lactation, production, parity and season on somatic cell counts in infected and uninfected dairy goats. Small Ruminant Research. 1995; 16(2):165-169.
50. Park hc Yk, koo SH kim. the analysis of milk components and pathogenic bacteria isolated from bovine raw milk in korea, dec, 2007.
51. Zhang L. A proteomic perspective on the changes in milk proteins due to high somatic cell count. Journal of dairy science. 2015; 98(8):5339-5351.