European Journal of Agricultural and Rural Education (EJARE)
Available Online at: https://www.scholarzest.com
Vol. 2 No. 4, April 2021,
ISSN: 2660-5643

LOW-COST LIQUID ANIONIC SURFACTANT IN MASTITIS DETECTION FOR SMALL SCALE DAIRY BUFFALO RAISERS IN THE PHILIPPINES

Yancy O. Waminal Ph.D. [1]
[1]Department of Animal Science, College of Agriculture and Forestry, Tarlac Agricultural University, Camiling, Tarlac, Central Luzon, Philippines 2306
Email Address: Waminal0830@gmail.com
Telephone no: +63977-291-7552

Article history:

Received March 28th 2021
Accepted: April 10th 2021
Published: April 26th 2021

Abstract:

The study was conducted to evaluate the effect, consistency, as well as the economic viability of using different levels of liquid anionic surfactant in mastitis detection. A total of 42 fresh milk samples from dairy buffaloes in Central Luzon, Philippines were used in the study. The experiment was laid out following the Completely Randomized Design (CRD) with three replications. Test showed that there was no significant difference between the different levels of liquid anionic surfactant and the commercially available mastitis test reagent. Observation during the tabulation of the test results indicates that 30%, 40% and 50% dilution levels of liquid anionic surfactant to distilled water are generally similar to commercial reagent in detecting clinical and sub-clinical mastitis in dairy buffaloes. The result of the laboratory analysis for somatic cell count confirmed the tabulated results gathered during the macroscopic evaluation. It is concluded that different levels of liquid anionic surfactant register close results to commercial reagent in terms of detecting types and grades of mastitis infection. In terms of economic viability, the cost of raw materials used, preparation and cost of return analysis result was remarkable. The use of 30% liquid anionic surfactant is economical in terms of the cost of preparation compared to other mastitis detection reagents.

Keywords: Subclinical mastitis, milk, dairy buffalo, Liquid anionic surfactants

INTRODUCTION

Milk is the primary source of nutrition for young mammals before they are able to digest other types of food. Milk collection must be properly and aseptically done to ensure good quality milk with the absence of faint or bad odor, free from contamination and bad bacteria that is harmful to humans.

Mastitis is an economically significant communicable disease of dairy animals affected by reduced management practices or environmental pathogens like *S. aureus*, *S. dysgalactiae*, *S. agalactiae*, *E. coli* and *S. uberis*. Dairy industry paid more attention on sub clinical and clinical mastitis because of its adverse effect on the animal and the economic status of dairy farms worldwide. Through early detection of mastitis, treatments can be employed directly to reduce the infection and the transmission to other animals (Waminal et al, 2019).

Losses are due to decreased quantity and quality of milk heightened by medicine, veterinarian and labor costs. Significant losses are seen in subclinical forms of mastitis which do not show clinical symptoms and can be detected by somatic cell count or bacterial analysis. In many countries, they recently updated their breeding program to include mastitis resistance as a trait for selection of animals (Gulhane and Sangwan, 2012; Waminal et al. 2018). There are ways in detecting mastitis to dairy animals like the commercially available California Mastitis Test (CMT), the Wisconsin Mastitis Test (WMT) , the Electronic Somatic Cell Counts and the Whiteside Test but these tests are expensive and not accessible for some dairy farmers especially those who are in the small scale farmers in the country.

Given that the use of liquid anionic surfactant does not require expensive solutions, instruments and technical laboratory skill, it is also accessible hence, it is very practical to use in detecting mastitis infection the shortest possible time. Despite improvements made in animal production and health particularly in the field of prevention by early detection of contagious disease and therapeutic medicine, this study was conducted to develop and evaluate the efficacy of liquid anionic surfactant as a reagent for mastitis detection test. The significance of the study is for dairy

farmers in small scale and commercial operations, for easier and accessible use of the reagent in conducting mastitis tests.

MATERIALS AND METHODS

Experimental design

The study was laid out following the completely randomized design (CRD) and the treatments with a corresponding dilution levels was replicated three (3) times. The 3-day sample collection represented the three (3) replications. Treatments were as follows: Treatment 1 (10% commercial mastitis reagent + 90% distilled water), Treatment 2 (30% LAS + 70% distilled water), Treatment 3 (40% LAS + 60% distilled water), and Treatment 4 (50% LAS + 50% distilled water)

Sample collection

A total of 42 milk samples from lactating water buffaloes on their two months of lactation onwards, regardless of age, breeds and weight were selected from previously identified farms in Central Luzon, Philippines. Housing, feeding, and overall management of the animals in the farm were not changed for the conduct of this study. Moreover, the farms included in the study housed their animals in concrete housing with coral, and a monitor type roof. It was during summer season in the Philippines (March to June) during the collection the samples. All necessary equipment were thoroughly cleaned, disinfected, and air dried to avoid further infestation/infection. The experimental animals were manually milked from the udder and pooled to collect 30 mL of milk sample.

Mastitis Test Solution Preparation

Commercial mastitis reagent was mixed with distilled water and was stirred gently in a liter of beaker to avoid formation of bubbles. The mixture was transferred in the empty bottle and was labelled appropriately. For a liter of liquid anionic surfactant solution preparation, the different dilution levels and distilled water was computed to the desired volume of test solution. The distilled water/s computed amount volume was measured for the different dilution levels using a beaker, then measured the computed amount volume of liquid anionic surfactant and added into the prepared water solution. The mixture was stirred gently to avoid the excessive formation of bubbles. The solution was carefully transferred into a clean empty container bottles and was labeled. The same procedure was done following treatments specified in the experiment.

Mastitis Testing Using Different Test Solutions

Mastitis testing on commercially available mastitis reagent and liquid anionic surfactant at different levels had the same procedure. Raw milk was collected and placed in a conical tube. Five (5) ml of milk from each quarter was placed into the paddle with four compartments labeled as A, B, C, and D. An equal amount of commercially available reagent and liquid anionic surfactant solution with dilution levels was added to each quarter in the paddle. The paddle was rotated in a circular motion to thoroughly mix the milk and the solution. Visible reaction disintegrated after about 20 seconds. The reaction was scored visually according to the gel formation. The more gel formation, the higher the score. Results were recorded accurately per milk samples of the animal.

Table 1. Guide to macroscopic evaluation

RESULT SYMBOL	INTERPRETATION	DESCRIPTION	REMARKS
-	Negative	Free from gelatinous formation	Negative of Mastitis
-+	Trace	Slight thickening of the mixture. Trace reaction seems to disappear with continued rotation of the paddle. If all four quarters read trace there is no infection. If one or two quarters read trace, infections are possible.	Possible Mastitis Infection
+	Weak Positive	A distinct precipitate formed and milk become slightly viscous but no tendency of gel formation	Sub-clinical Infection
++	Distinct Positive	The mixture thickens immediately with some gelatinous formation formed	Serious Mastitis Infection

+++	Strong Positive	A gelatinous formation will be formed and causes the surface of the mixture to become convex and a thick mass is form in the middle of the paddle while doing swirling and titling and when you pour out the mixture the whole mass formation will drop without leaving any remains or if there is presence of little sticky mixture will be left but will drop also	Serious Mastitis Infection

Note: *Formation of gel involves binding of leukocytes due to the anionic of the liquid antibacterial detergent on the milk. Gelatinous formation usually indicates the quarter of the cow's udder is positive or infected with mastitis.*

Source: McCurnin's Clinical Textbook for Veterinary Technicians

Collection of Milk Samples for Somatic Cell Count (SSC)

Procedure of collection was done in accordance with the protocol of the PCC dairy laboratory. Thirty (30) ml of milk was drawn from four quarters of the udder into the conical tube. The milk collected was placed in a cooler with ice transported to the laboratory for somatic cell count (SSC). The tubes were labelled with the buffalo number and teat location and immediately store in the ice chest. The somatic cell count of each milk samples including the buffalo number, caracalving date, milk yield per day, and the SSC result of commercial reagent and liquid anionic surfactant dilution levels for each quarter were recorded.

Table 2. Somatic cell range and interpretation

TEST RESULT CMT CODE	**EQUIVALENT SOMATIC CELL RANGE (cells/ml)**	**INTERPRETATION**
(-) Negative	0-200,000	Healthy Udder
(-+) Trace	> 200,000 – 400 , 000	Sub-clinical Infection
(+) Grade 1 weak positive	400,000 – 1,200,000	Sub-clinical Infection
(++) Grade 2 distinct positive	1,200,000 -5,000,000	Serious Mastitis Infection
(+++) Grade 3 strong positive	Over 5,000,000	Serious Mastitis Infection

Source: Philippine Carabao Center, National Gene Pool Headquarters – Dairy Laboratory, Science City of Muñoz, Nueva Ecija, Philippines.

Data Gathered

The following data were gathered:

1. ***Macroscopic Evaluation Results*** are tabulations in mastitis detection using commercially available mastitis test reagent and different brands of liquid anionic surfactants.
2. ***Somatic Cell Count Results*** are results of the milk samples collected from the different quarters of the udder of experimental animals that was conducted by PCC.
3. ***Cost of Producing Liquid Anionic Surfactant Agent*** is the computed expenses of producing test solutions.

Data Analysis

All the data gathered were statistically analyzed using the Analysis of Variance (ANOVA) for Completely Randomized Design (CRD). Mean of results were compared employing the Least Significant Difference test at 0.05 level of confidence.

RESULTS AND DISCUSSION

Mastitis Detection by Macroscopic Evaluation

A total of 42 samples from lactating buffaloes in the same breed, two months of lactation, regardless of age, weight present in the various cooperative assisted by the Philippine Carabao Center was used in this study. Milk testing in milking parlor infrastructure was done early in the morning.

Table 3. Mean grade for mastitis detection using the CMT 5-point Hedonic Scale

TREATMENTS	TREATMENT ID	MEANS
Treatment 1	Commercial Brand	1.40 a
Treatment 2	30 % Liquid anionic surfactant and 70% distilled water	1.38 a
Treatment 3	40 % Liquid anionic surfactant and 60% distilled water	1.48 a
Treatment 4	50 % Liquid anionic surfactant and 50% distilled water	1.45 a

Note: Means followed by the same letter are not significantly different at 5% level of significance. P(>F) = 0.0005

Analysis of variance showed that commercially available mastitis test reagent and different dilution levels of liquid anionic surfactant have no significant differences and no statistical evidence to declare that commercial reagent and different levels of liquid anionic surfactants are significantly different in their Mastitis detection capability. Both Liquid anionic surfactant at 30%, 40% and 50% dilution levels and commercially available mastitis test reagent dissolves or disrupts the outer cell wall and the nuclear cell wall of any leukocyte, which are primarily fat (detergent dissolves fat). DNA is released from the nuclei. DNA will gel together to form a stringy mass. As the number of leukocytes increase, the amount of gel formation will increase in a linear fashion. This is parallel to the study conducted by Gangan, 2018 that liquid anionic surfactant can detect sub clinical and clinical mastitis in dairy buffaloes.

Economic Viability

Presented in the Table 4 is the economic viability of using commercially available mastitis detection reagent and liquid anionic surfactants at different dilution levels in detecting sub clinical and clinical mastitis in water buffaloes.

Table 5. Cost efficiency of using commercial reagent and liquid anionic surfactants (LAS) at different dilution levels.

	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Cost per gallon(₱)	1,136	304.00	392.00	480.00
Cost of commercial reagent/LAS solution per sample @ 5ml (₱)	1.42	0.38	0.49	0.60
Savings from the use of LAS solution (₱)	-	1.04	0.93	0.82
Percentage of savings from the use of LAS solution (%)	-	273.68	189.80	136.67

The cost efficiency of producing a gallon and sample of solution table showed that liquid anionic surfactant ant at 30% dilution level obtain the lowest cost per gallon (₱ 304) and cost per sample (₱ 0.38) among all treatments including Commercial reagent. In terms of amount saved using LAS and percentage savings from using commercial brand, LAS at 30% dilution level acquire the highest savings and percentage savings among all treatments with ₱ 1.04 and 273.68%.

This is similar to the result of the study conducted by Waminal *et al.* (2018), stated that using liquid anionic surfactants in detecting mastitis for ruminants was viable because of its availability in nationwide market and has cheaper cost compared to Commercial brand

It is also conformed to the study of Gangan (2018) that mastitis detection using antibacterial liquid anionic surfactant is an easy, cheap and a helpful tool in controlling the spread of mastitis by early detection and treatment of the disease. Preparation of the solution is very simple, the component of the mixtures are readily available and affordable. The use of the solution (liquid anionic surfactant) will prevent waste of milk through early detection of the infection that will lead to increased milk production among small and commercial scale of dairy industries.

SUMMARY

The study was conducted to evaluate the effect, consistency, as well as the economic viability of using different levels of liquid anionic surfactant in mastitis detection. A total of 42 milk samples from dairy buffalo farms in Luzon, Philippines were used in the study. The experiment was laid out following the Completely Randomized Design with three replications.

Test showed that there was no significant difference between the different levels of liquid anionic surfactant including the commercial reagent. Observation during the tabulation of the test results indicates that levels of liquid anionic surfactant are generally similar to commercially available reagent in detecting clinical and subclinical mastitis in dairy buffaloes. The result of the laboratory analysis for somatic cell count conducted by the Philippine Carabao Center Dairy Laboratory confirmed the tabulated results gathered during the macroscopic evaluation. As per results of the Somatic Cell Count, different levels of liquid anionic surfactant register very close results to commercially available mastitis test reagent in terms of detecting types and grades of mastitis infection.

In terms of economic viability, the cost of raw materials used as well as the preparation and cost of return analysis result was remarkable. The use of 30% liquid anionic surfactant is much cheaper in terms of the cost of preparation compared to commercially available mastitis test reagent. In addition, the cost and return analysis of the test solutions resulted to the higher ROI in the dairy farm operation using liquid anionic surfactant than using Commercially available mastitis test reagent in mastitis detection.

CONCLUSIONS

The use of liquid anionic surfactant at different inclusion levels (30%-50%) is very helpful in detecting sub clinical and clinical mastitis in dairy buffaloes and can be substitute to commercially available mastitis test reagent. Based on the economic viability, 30% liquid anionic surfactant solution is cheaper in terms of cost of preparation and it is more readily available in the market nationwide.

LITERATURE CITED

Journals

1. Andersen, H.J., L.H. Petersen, F.M. Aarestrup and M. Chriel. 2003. Evaluation of the surveillance program of Streptococcus agalactiae in Danish dairy herds. Journal of Dairy Science. 86 (4): 1233-1239.
2. Andersen, S., I.R. Dohoo, R.O. RIEKERINK, and H. STRYHN. 2011. Diagnosing intramammary infections: evaluation of definitions based on a single milk sample. Journal of Dairy Science. 94:250-291.Doi: 10:3168 / jds 2010-3559.
3. Breen, J.E., A.J. Bradley, and M.J. Green. 2009. Quarter and cow risk factor associated with somatic cell count greater than per milliliter in United Kingdom dairy cows. Journal of Dairy Science. 92 (7): 3106-3115.
4. Bytyqi, H., U. Zaugg, K. Sherifi, A. Hamidi, M. Gjonbalaj, S. Muji & H. Mehmeti. 2010. Influence of management and physiological factors on somatic cell count in raw milk in Kosova. Veterinarski Archiv, 80(2):173-183.
5. Cervinkowa, D., Vlokova, H., Borodacouva, I. and Makouvoca, J. 2013. Prevalence of mastitis pathogens in milk from clinically healthy cows. Veterinary Medicine Journal. 58 (11):567-575.
6. Cunha, R.P., Molina, L.R., Carvalho, A.M., and Ferreira, R.M. 2008. Sub-clinical mastitis and relationship between somatic cell count with number of Lactations, production, and chemical composition of milk. Vet Med. 60:19-24 doi: 10.1590 / 50102. 093520080010003.
7. Dingwell, R.T., K.E. Leslie, Y.H. Schukken, J.M. Sargeant and L.L. TImms. 2003. Evaluation of the california mastitis Test to detect an intramammary infection with a major pathogen in early lactation dairy cows. *Canadian Veterinary Journal.* 44: 413.
8. Dobranie, V., Njari, B. Miokovic, B., Resanova, R. 2008. The influence the season on chemical composition and somatic cell Count of bulk tank cow`s milk. Vet Archive. 78.235-242
9. El-attar A.A., M.E. Salama and M.M. Abd el-samie (2002). Incidence of mastitis in lactating cows and buffaloes kept under different management conditions in Ismailia province. Veterinary Medicine Journal. 2: 583.
10. Gonzales, R.N., and Wilson, D.J. 2003. Mycoplasmal mastitis in dairy herds. Vet Clinic North Food Animal Pract.19 (1) 199-221
11. Halasa, T., and Osterus, O. 2007. Economic effects of bovine mastitis and mastitis management. Vet Q. 29:18-31. doi: 101080 / 016521. 9695224
12. Kandasamy, S., Green, B.B., Benjamin, A.L., and Kerr D.E. 2011. Cow variation in dermal fibroblast response to lipopolysaccharide reflected in resolution of inflammation during E. coli mastitis. Journal of Dairy Science. (94(12):5963-75 doi: 103168 / jds 20111-4288.
13. Khan, M. Z., and Khan, A. 2006. Basic Factors of Mastitis in Dairy Animals. Pakistan Veterinary Journal. 26:204-8.
14. Kuang, Y., JIA, H., Miyanaga, K., & Tanji, Y. (2009). Effect of milk on antibacterial activity of tetracycline against Escherichia coli and Staphylococcus aureus isolated from bovine mastitis. Applied microbiology and biotechnology. 84. 135-42. 10.1007/s00253-009-2008-6.
15. Leslie, K. E., Dingwell, R. T., & Tiims, L. L. 2002. Evaluation of the California mastitis test to detect an intramammary infection with a major pathogen in early lactation dairy cows. The Canadian Veterinary Journal. 44(5): 413-416.
16. Le Roux, Y., and Moussaqui, F. 2003. Polymorphonuclear protolyse acting and milk composition change. Vet Res. 629-645 doi: 101051 Vet Res: 2003021

17. Ma, Y., Ryan, C., Barbano, D. M., Gakton, D. M., Rudan, M. A. and Boor, K. J. 2000. Effect of somatic cell count on quality and shelf-life of pasteurized fluid milk. Journal of Dairy Science. 83:264-274. https://doi.org/10.1016/j.idairyj.2005.10.020
18. Mahmoud, A. A., 1988. Some studies on subclinical mastitis in dairy cattle. Assiut Veterinary Medicine Journal, 20: 150.
19. Ogola, H., and Shitandi, A. 2007. Effect of Mastitis on Raw Milk Composition Quality. Journal of Dairy Science. 8:237-242.
20. Rady, A.A., and M. Sayed. 2009. Epidemiological studies on subclinical mastitis in dairy cows in Assiut Governorate. Veterinary World, Vol. *2*(10):373-380.
21. Samad, M.A. 2008. Animal husbandry and veterinary science vol. 2.11th. Bangladesh Agricultural University campus, Mymensingh.
22. Santos, M.V., and Barbaro, D.M. 2003. Effect to somatic cell count on proteolyses and lipolysis in pasteurized fluid milk during shelf-life storage. Journal of Dairy Science. 86:2491-2503. doi 10.3168 / jds.5002-0303(03)73843-0.
23. Seegers, H., Flouricho, C., and Beaudu, F. (2003). Related to mastitis economics in dairy cattle herds. *Veterinary Research*. 34:475-91.
24. Schukken, Y.H., D.J. Wilson, F. Welcome, L.G. Tikofsky and R.N. Gonzalez. 2003. Monitoring udder health and milk quality using somatic cell counts. Veterinary Research. 34:579-596 doi: 101051 vetres: 200328.
25. Sharma, N., Singh, N. K., & Bhadwal, M. S. 2011. Relationship of somatic cell count and mastitis: an overview. Division of veterinary clinical medicine and jurisprudence, skuast-j, india. Asian-Australian Journal of Animal Science.
26. Viguier, C., Arora, S., Gilmartin, N., Welbeck, K., & O'kennedy, R. 2009. Mastitis detection: current trends and future perspectives. Trends in Biotechnology. 27. 486-493.
27. Waminal, Y.O., Tubalinal, G.A.S.P., & Mingala, C.N. 2019. Molecular characterization of lactoferrin gene as genetic marker to subclinical mastitis in water buffaloes (*Bubalus bubalis*). *Asian Journal of Agriculture and Biology.* 7(4):564-572.
28. Zdunczyk, S., H. Zerbe and M. Hoedemaker. 2003. Importance of oestrogen and oestrogen-active compounds for udder health in cattle: A review. Dtsch Tierarztl Wochenschr, 110: 461.

BOOKS

1. Radostits, O.M., and Hinchcliff, K.W. 2007. A textbook of the Disease of Cattle, Horses, Sheep, Pigs and Goats. London LTD.

THESIS/DISSERTATIONS

1. Gangan, J.A. 2018. Detection of Mastitis in Dairy Buffalo Using Different Brands and Dilution Levels of Liquid Anionic Surfactants. Thesis. Graduate School, Pangasinan State University, Pangasinan
2. Waminal, Y.O., Tubalinal, G.A.S.P., & Mingala, C.N. 2018. Molecular Characterization and Association of Lactoferrin Gene to Subclinical Mastitis Detection in Goats (*Capra hircus*). Thesis. Graduate School. Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines