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Assessment of mastitic infection in bovine milk using ATP bioluminescence

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ASSESSMENT OF MASTITIC INFECTION IN BOVINE MILK USING ATP BIOLUMINESCENCE

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Summary

Few choices exist for a mobile, rapid, and nonsubjective assessment of mastitic infection in bovine milk. This project evaluated the effectiveness of using the Biotrace® raw milk quality ATP bioluminescence assay to serve this role. Milk samples with various somatic cell counts (13,000 - 2,500,000) and signs of mastitic infection were obtained from the Kansas State University Dairy Teaching and Research Center. Within 24 hr, raw milk samples were evaluated for microbial numbers and relative light units (RLU). The printed test procedure was modified to evaluate accurately clinical mastitic milk samples. As somatic cell count increased in raw milk, the RLU value increased. In addition, RLU values differentiated among milk samples with various levels of mastitic infection (none, subclinical, and clinical). Repeatability of the ATP bio-luminescence method was very good (CV = 4.76%). These results suggest that the Biotrace® raw milk quality test kit can serve effectively as a nonsubjective, rapid assay to determine the degree of mastitic infection in bovine milk.

(Key Words: Mastitis, Somatic Cell Count, ATP Bioluminescence.)

Introduction

Problems with raw milk quality and mammary gland health are among the most costly health concerns on dairy farms. Poor mammary gland health adversely affects a dairy's profitability in two distinct areas. First, milk price premiums and deductions are determined in part by milk quality as indicated

by somatic cell count (SCC). Secondly, a lactating cow with a mastitis problem (SCC > 300,000) can show in a 10 to 15% reduction in milk production compared to herd mates without a mastitis problem.

Mastitis is an inflammation of the mammary gland and can be caused by physical trauma or more commonly by microbial infestation of the mammary gland. There are two categories of mastitis: subclinical and clinical. Cows with subclinical mastitis produce milk without physical abnormalities apparent to the naked eye. Subclinical mastitis accounts for 90 to 95% of all mastitis cases. Cows with clinical mastitis produce milk with obvious physical abnormalities, namely, the presence of scar tissue.

To combat the inflammation, the animal's immune system floods the affected area with white blood cells or leukocytes (which make up the majority of the cells in an SCC). As a result, the leukocyte concentration in the milk increases. The degree of inflammation is directly proportional to the leukocyte concentration. This relationship allows the health status of a lactating mammary gland to be determined by enumerating somatic cells in the milk. Unfortunately, nonsubjective rapid assessment of milk SCCs requires the use of large, nonmobile, computer-driven equipment.

The physical environment inside the mammary gland serves as an ideal growth medium for a host of microorganisms, allowing them to flourish and resulting in the immune response previously described. This rapid microbial growth was confirmed recently;

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bacteria had an increased multiplication rate in mastitic milk. Increases in microbial adenosine triphosphate (ATP) seem to correlate well with indicators for mastitis inflammation. The fact that the most common cause of mastitis is microbial infestation of the mammary gland presents the opportunity to evaluate mammary gland health by rapid microbial enumeration procedures, such as ATP bioluminescence.

Our objectives were to determine whether: 1) the Biotrace™ raw milk quality test kit procedure was correlated with raw milk SCC and 2) the Biotrace™ raw milk quality test kit procedure could distinguish among raw bovine milk samples with various degrees of mastitic infection.

Procedures

Milk samples were collected from the complete milking of each cow, stored at 2.8EC, and assayed within 24 hr after collection. With each milk sample, triplicate microbial ATP assays were performed, and duplicate SCC values were obtained.

The Somacount 500 Analyzer (Bentley Industries Inc.) at the Heart of America DHIA Lab, Manhattan, KS, was used to perform the SCC assays. Microbial ATP concentration values were monitored using the Biotrace Multi-Lite Milk Bacterial Kit (Biotrace, Bregend, Wales). 1000µL of prewarmed (37EC) Somex-A was added to 1 mL of raw milk, rotamixed for 5 seconds, incubated in a water bath at 37EC for 4 minutes, and then rotamixed again for 5 seconds. Samples were filtered through a 13 mm sterile filter. The filter was rinsed with 5mL of sterile rinse solution. The filter containing the microorganisms then was removed with sterile forceps and placed horizontally into a vial containing 500FL of the microbial ATP extracting enzyme M-Bactex. Contents of the vial were gently mixed and allowed to stand for 1 minute. Then 200 FL of the solution was placed into a sterile cuvette, to which 100 FL of the luciferin/luciferase reagent (Enzyme-MLX) was added.

The ATP bioluminescence assay employs an enzymatic reaction between luciferase and microbial ATP. The light emitted via the

luciferase reaction is measured by a Biotrace Uni-Lite luminometer and quantified as relative light units (RLU). The RLU values then can be related to the microbial population of the sample. In order to assay strictly microbial ATP, somatic cell ATP and native milk ATP are removed. Somatic cells are less resistant to physical stress elicited by the Biotrace extractant Somex-A that causes the somatic cells to rupture. The somatic cell ATP and native milk ATP then are flushed away from the intact microbial cells in a filtration step. The extractant and filtration steps finalize the selective removal of all nonmicrobial cells.

Five milk samples (labeled 1 through 5) were prepared from raw milk samples collected from three different cows, each with different mammary health status. These cows were designated 1, 3, and 5. Cow #1 (n = 4) produced milk that showed no physical signs of clinical mastitis and consistently maintained a low SCC (<50,000). This sample represented milk from a healthy mammary gland. Cow #3 (n = 4) produced milk that showed no physical signs of clinical mastitis and consistently maintained SCCs of >300,000 and <1,000,000. This sample represented milk from a cow with subclinical mastitis. Cow #5 (n = 3) produced milk that showed physical signs of clinical mastitis (scar tissue in the milk) and consistently maintained SCCs >2,000,000. This sample represented milk from a cow with clinical mastitis. Sample #2 (n = 4) was prepared by a 50:50 volumetric mixture of milk samples from cows #1 and #3. This sample represented milk produced by a cow with mild subclinical mastitis relative to #3. Sample #4 (n = 3) was prepared by a 50:50 volumetric mixture of milk samples from cows #1 and #5. This sample represented milk produced by a cow with mild clinical mastitis relative to #5.

Experiments were replicated five times. All data were transformed into log₁₀ values before statistical analyses were compared. To determine if SCCs differed among the different milk treatments, SCCs were subjected to analysis of variance.

Results and Discussion

Figure 1 shows the strong correlation ($r^2 = .95$; $P < .05$) among the five milk treatments and the SCCs. This assay proved to be highly repeatable across all data points ($CV = 4\%$). Thus, the Somacount 500 SCC assay served as a standard for our investigation.

Figure 2 represents the relationship between the five milk treatments and the ATP assay results (expressed as log RLU). A strong correlation ($r^2 = .90$; $P < .05$) existed between the ATP assay and the samples with various levels of mastitic infection. The ATP assay also exhibited high repeatability ($CV = 4.8\%$).

Average values for the SCC assay and the ATP assay are shown in Tables 1 and 2, respectively. Both assays exhibited similar abilities to distinguish among clinical mastitis (treatment 4 and 5), subclinical mastitis (treatments 2 and 3), and no mastitic infection (treatment 1). The original milk samples (treatments 1, 3, and 5) were distinguished easily from each other. However, we found that both assays lack the ability to statistically differentiate between mild clinical (treatment 4) and clinical (treatment 5) mastitis and between mild subclinical (treatment 2) and subclinical (treatment 3) milk samples. Our results indicate that both assays were equally capable of repeatedly distinguishing among various degrees of mastitic infection.

As a final test, the Pearson correlation coefficient was calculated for the dependent variables, SCC and ATP value. The relationship between the two variables is represented

in Figure 3 by a scatter plot of all RLU and SCC data points. A strong correlation (Pearson correlation = $.91$; $P = .001$) existed between the microbial load of the milk samples (determined by the ATP assay) and the SCCs.

Conclusion

Statistical analyses of the results obtained during this investigation illustrated the high repeatability of the ATP assay and the high degree of correlation between milk treatments and ATP assay values. Further analysis showed a strong correlation between the ATP and SCC values. The ATP assay also demonstrated the ability to differentiate among milk samples with various levels of mastitic infection: none, subclinical, and clinical. These results suggest that the Biotrace ATP assay could serve as a highly repeatable and mobile alternative to the SCC assay in monitoring bovine mastitic infection. Use of the Biotrace ATP for rapid, on-the-farm, quantitative analyses of mastitic infection deserves further investigation.

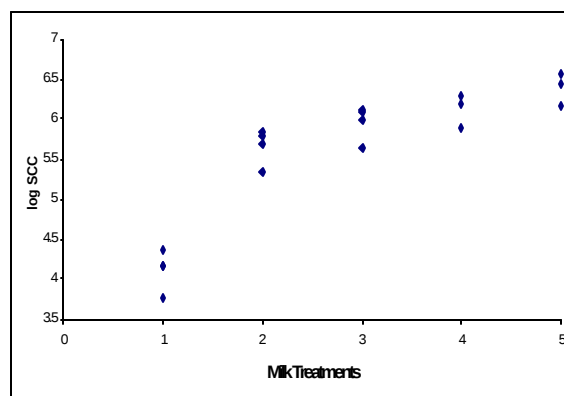


Figure 1. Scatter Plot of Somatic Cell Count (SCC) Values and Milk Treatments ($r^2 = .95$; $P < .05$; $CV = 4\%$).

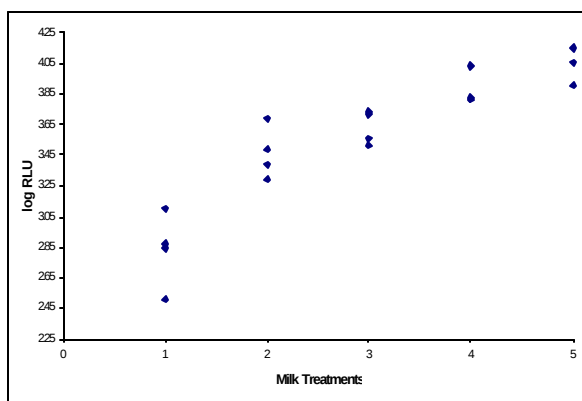


Figure 2. Scatter Plot of Microbial ATP(RLU) Values and Five Milk Treatments ($R^2 = .90$; $P < .05$; $CV = 4.8\%$).

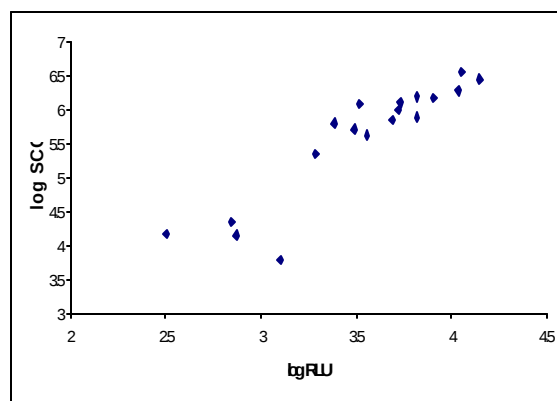


Figure 3. Scatter Plot of Microbial ATP (RLU) and Somatic Cell Count (SCC) Values from All Treatments ($r = .91$; $P = .001$).

Table 1. Mean Relative Light Unit (RLU) Values from ATP Bioluminescence Assay

Treatment	Mastitis	No.	RLU
1	None	3	4.0 ^a
2	Mild subclinical	3	3.9 ^{ab}
3	Subclinical	4	3.6 ^{bc}
4	Mild clinical	4	3.5 ^c
5	Clinical	4	2.8 ^d

^{a,b,c,d}RLU values with uncommon superscript letters differ ($P < .05$).

Table 2. Mean Somatic Cell Counts

Treatment	Mastitis	No.	Log SCC
1	None	3	6.4 ^a
2	Mild subclinical	3	6.1 ^{ab}
3	Subclinical	4	6.0 ^{bc}
4	Mild clinical	4	5.7 ^c
5	Clinical	4	4.1 ^d

^{a,b,c,d}Log SCC values with uncommon superscript letters differ ($P < .05$).