

Evaluation of Experimental Novel Germicide Post Milking Teat Dips vs. a Commercial Iodine Barrier Post Milking Teat Dip on Teat Skin Coloration and Teat End / Teat Skin Health

A.S. Leaflet R2712

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Summary and Implications

The primary objective of this study was to evaluate a prototype novel germicide (proprietary formulation of citrate + methylene blue + parabens) based post milking teat dip with varying methylene blue and emollient concentrations versus a control commercial iodine barrier post milking teat dip on overall teat end and teat skin condition and health. A secondary objective was to assess teat skin coloration or staining due to the dip at the subsequent milking. Novel germicide prototype dips showed some teat coloration at the next milking with the highest % seen when .25% MB was added or 1% MB with a thixotropic agent. Novel germicide prototype dips showed excellent teat skin (TS) health (same as control dip). Novel germicide prototype dips showed very good teat end health integrity and maintained teat end health integrity through cold weather changes (significantly better than controls). Dips with 5% emollient were more consistent in TE health compared to 11% emollients. Novel germicide prototype dips showed softer, more pliable hyperkeratotic TE tissue. Overall, these novel germicide prototype dips performed very well and better than controls.

Introduction

Maintaining good teat end / skin health is recognized as an essential element in mastitis prevention and animal welfare. In addition to excellent germicidal activity, all teat dips should have both teat end and teat skin health data evaluation, and show excellent teat health prior to use and commercialization. The primary objective of this study was to evaluate a prototype novel germicide (proprietary formulation of citrate + methylene blue + parabens) based post milking teat dip with varying methylene blue and emollient concentrations versus a control commercial iodine barrier post milking teat dip on overall teat end and teat skin condition and health. A secondary objective was to assess teat skin coloration or staining due to the dip at the subsequent milking. A split udder design study was performed to minimize risk of experimental bias and maximize chances of seeing teat dip effects.

Materials and Methods

- Dips used:** Control dip was Bovi-Kote (1% iodine with 10% multiple emollient system (low drip): Boumatic, Inc.) and treatment dips were a prototype novel germicide dip with varying methylene blue (MB) (.1, .25, and .4 %) and emollient (5, 10, and 11%) concentrations (Zurex Pharma, Inc.)
- Cows:** All protocols were approved by ISU Committee on Animal Care (IACUC # 10-06-6228-B). 24 early-mid lactation Jersey cows at the ISU dairy were used for the study.
- Trial design:** Trial used a split pen and split udder design. 24 early- mid lactation Jersey cows in a single pen were used with 12 cows having a blue leg band (BLB group) applied to designate them as one trial group while the other 12 had no leg band (NLB group). Left teats (controls) of all 24 cows were post dipped with Bovi-Kote (control) while right side teats were dipped with a prototype novel germicide dip (treatment-see table below). Both treatment groups were initially dipped with a .1% methylene blue dip (1/29 pm) but differed in emollient % (5 v 11%). Dips were changed to a .25% MB (2/10 am) to enhance teat skin coloration. Teats of the NLB group were switched to a .4% MB dip with 11% emollients (2/19 pm) to potentially further enhance coloration. Teats in the BLB group were switched to a higher emollient (10 v 5 %) no drip teat dip (2/21) for the last week of the trial. The trial was 4.5 weeks in duration (1/ 30 – 2/ 28, 2011). All other farm and milking practices were similar across all 4.5 weeks.

Date	Blue Leg bands (BLB)			No Leg Bands		
	Dip	Emol.	MB %	Dip	Emol.	MB %
1/29/11 pm	3004	5%	/.1%	3007	11%	/.1%
2/10/11 am	4004	5%	/.25%	4007	11%	/.25%
2/21/11 pm	3008	10%	/.1% no drip	5007	11%	/.40%

- Milking practices:** Cows were milked twice a day in a double 12 parallel parlor. Cows were forestripped (3 strips/teat) and pre-dipped (6 cow sequence), then dried with terry cloth towels prior to milker unit attachment. Automatic detachers were set at 1.8 lb. flow rate and 1 second delay. Cows were housed in a single pen in a free stall barn with mattresses and sawdust bedding.
- Teat skin coloration and teat skin / teat end health evaluations:** Data collection was initiated on January 30, 2011 and continued until February 28. Test products were applied starting 1/29 at the pm milking with subsequent first data observations the following

am milking on 1/30. Teat coloration (blue coloration due to MB) was evaluated before teat sanitation / preparation at the subsequent milking after dipping (BP), after premilking teat sanitation (AP), and after milker unit removal (postmilking – AM) using a 0-2 scale (0 = no color; 1 = light blue; 2 = dark blue). Teat skin and teat end scoring was performed using a variation of the Goldberg and Timms methods, respectively, by trained graders (Tables 1 and 2). Scoring was performed at both milkings for the first 4 trial days, then three times per week. Data was entered into an Excel database. Results were compiled and analyzed using SAS.

6. **Statistical models:** SAS was used in all data analysis. Mixed procedure of SAS with repeated measured (mixed model with quarter within cow as a repeated measure) were used to analyze teat skin and teat end data, with $p < .05$ considered significant. GENMOD procedures of SAS with repeated measures (generalized linear model with quarter within cow as repeated measure) was used to analyze % cracked/rough teat ends and % dry/chapped teat skin data.

Results and Discussion

1. **Teat skin coloration:** Teat skin coloration results are shown in Figure 1 (BLB group) and Figure 2 (NLB group). Overall general summary and comments regarding coloration across both groups are below followed by figures and summaries of each individual group (BLB and NLB).
 - a) **Overall teat skin coloration summary across both groups (BLB and NLB)**
 - Teats dipped with the initial dips containing .1% methylene blue (MB) showed on average 10-15% of teats with light blue coloration on teat skin prior to teat sanitation at the subsequent milking (BP). Teats dipped with the lower emollient (5 v 11%) showed a slightly higher average % of teats colored BP and always had some teats colored BP, while the 11% emollient dip showed no teats colored at 50 % of the milkings. Very few or most times did teats still have coloration following premilking teat sanitation (AP) or after milking (AM) for both dips.
 - Dips containing .25% MB resulted in enhanced teat skin coloration with 40-50% of teats (range 28-70) colored light blue BP. Very few teats were colored AP and AM with the 5% emollient dip, while 25% and 10% were colored AP and AM, respectively with the 11% emollient dip, possibly indicating an interaction between emollient % and MB. In contrast, the initial .1% MB dips showed higher consistent coloration with the 5% dip
 - Teats dipped with .4% MB dips had similar results to .25% MB, showing no advantage in coloration to higher MB concentration.
 - b) **Blue Leg Band Group Teat Coloration:** (Figure 1)
 - The initial dip in this group contained 5% emollients and .1 % methylene blue (MB). On average ~15% of teats (range 5-32%) showed a light blue coloration on teat skin prior to teat sanitation at the subsequent milking (BP). Very few or most times did teats still have coloration following premilking teat sanitation (AP) or after milking (AM).
 - The second dip was similar to initial dip but contained .25% MB. Teat dip coloration was enhanced with an average ~40% (range 28-70%) of teats showing a light blue coloration BP. Very few if any teats showed coloration at AP and AM.
 - Using a no drip dip with .1% MB resulted in similar teat skin coloration results BP as the .25% MB dip with 40-50% of teats colored BP. A higher but still small % of teats were colored AP (10-20%) and AM (5-10%). This data (although limited) may implicate the no drip formula enhances skin coloration.
 - c) **No Leg Band Group Teat Coloration** (Figure 2)
 - The initial dip in this group contained 11% emollients and .1 % methylene blue (MB). On average ~10% of teats (range 0-20%) showed a light blue coloration on teat skin prior to teat sanitation at the subsequent milking (BP). Very few or most times did teats still have coloration following premilking teat sanitation (AP) or after milking (AM). On 3 milkings, BP coloration was 20% but there was zero coloration for at least 5 milkings.
 - The second dip was similar to initial dip but contained .25% MB. Teat dip coloration was enhanced with an average ~50% of teats showing a light blue coloration BP. Approximately 25% of teats showed coloration AP and 10% showed coloration after milking.
 - The final dip was similar to the first 2 dips except MB was .4%. Results were similar to .25% MB with 50% of teats colored BP, 20% AP, and 10% AM
2. **Trial temperatures:** (Figure 3) Pre-trial and trial daily temperatures (minimum, average, maximum; National Weather Service, Ames, IA station) are shown in Figure 3. Temperatures 5-10 days prior to trial were very cold (0°F average). Temperatures for the next

- Teats with a no drip .1% MB, 10% emollient showed similar results to .25% MB. The thixotropic agent (no drip) may enhance coloration as this was not seen when dip was .1% MB, 11% emollients with no thixotropic agent.
- Abnormally high % of colored teats occurred across both dips and groups and all scoring times (BP, AP, and AM) at the 2/21 PM milking. Reasons for this are unknown and unexplained.

b) Blue Leg Band Group Teat Coloration: (Figure 1)

- The initial dip in this group contained 5% emollients and .1 % methylene blue (MB). On average ~15% of teats (range 5-32%) showed a light blue coloration on teat skin prior to teat sanitation at the subsequent milking (BP). Very few or most times did teats still have coloration following premilking teat sanitation (AP) or after milking (AM).
- The second dip was similar to initial dip but contained .25% MB. Teat dip coloration was enhanced with an average ~40% (range 28-70%) of teats showing a light blue coloration BP. Very few if any teats showed coloration at AP and AM.
- Using a no drip dip with .1% MB resulted in similar teat skin coloration results BP as the .25% MB dip with 40-50% of teats colored BP. A higher but still small % of teats were colored AP (10-20%) and AM (5-10%). This data (although limited) may implicate the no drip formula enhances skin coloration.

c) No Leg Band Group Teat Coloration (Figure 2)

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2. **Trial temperatures:** (Figure 3) Pre-trial and trial daily temperatures (minimum, average, maximum; National Weather Service, Ames, IA station) are shown in Figure 3. Temperatures 5-10 days prior to trial were very cold (0°F average). Temperatures for the next

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week (5 days pre – 3 days into trial) averaged 20⁰F. A 2 day drop in temperatures (0-5⁰F) was followed by 3 days of 20⁰F to end trial week 1. Trial week 2 was associated with a drop in temperatures (from 22 to < 10⁰F) early in that week and subsequently 4 continuous days of average temperatures < 10⁰F (minimum

temperatures were – 10⁰F). Temperatures increased trial weeks 3-4 and averaged 40 and 25⁰F during these weeks, respectively. These are seasonally very high for this time (winter).

Table 1. Teat skin scoring scale.

Score	Description
0	Teat skin has been subjected to physical injury (stepped on/ frost bite)
1	Teat skin is smooth, soft and free of any scales, cracks, or chapping.
2	Teat skin shows some evidence of scaling especially when feeling (areas of dryness by feeling drag when sliding a gloved hand along the teat barrel &/or seeing areas of lower reflective sheen to the surface of the skin).
3	Teat skin is chapped. Chapping is where visible bits of skin are visibly peeling.
4	Teat skin is chapped and cracked. Redness, indicating inflammation, is evident.
5	Teat skin is severely damaged / ulcerated / open lesions.

Table 2. Teat end scoring scale (0*- 5).

Teat End Scoring system	Degree of hyperkeratosis or callousing				
Cracking	none	minor	mild	moderate	severe
No cracking	1	1.5	2	2.5	3
Cracked	---	3.5	4	4.5	5

0* zero score – physical injury of teat not associated with trial

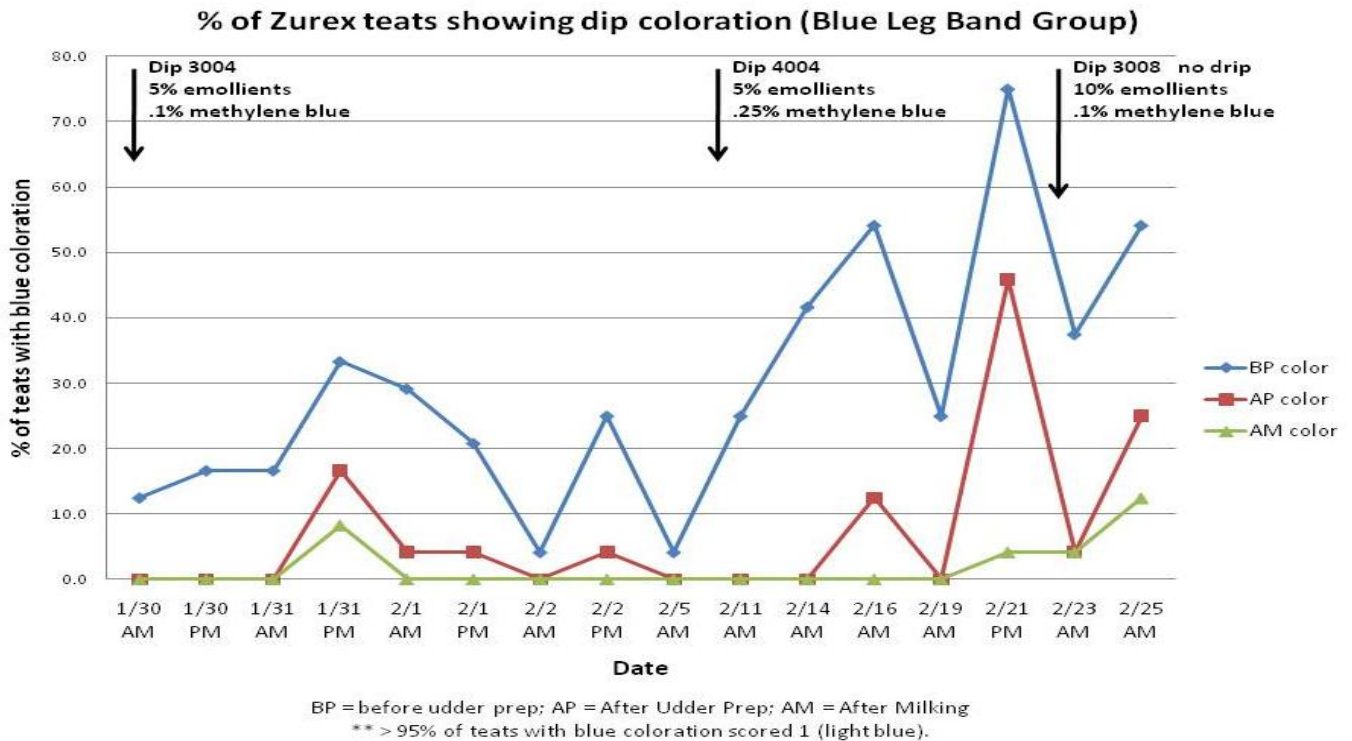


Figure 1. Percentage of teats showing skin coloration or staining before premilking teat sanitation at the subsequent milking (BP), after teat sanitation but prior to milking machine attachment (AP), and after milking or following milking machine removal (AM) when dips with different methylene blue concentrations and / or emollients/thixotropic agents were used (BLB group. Teat coloration scores were: 0 no color; 1 light blue; 2 dark blue).

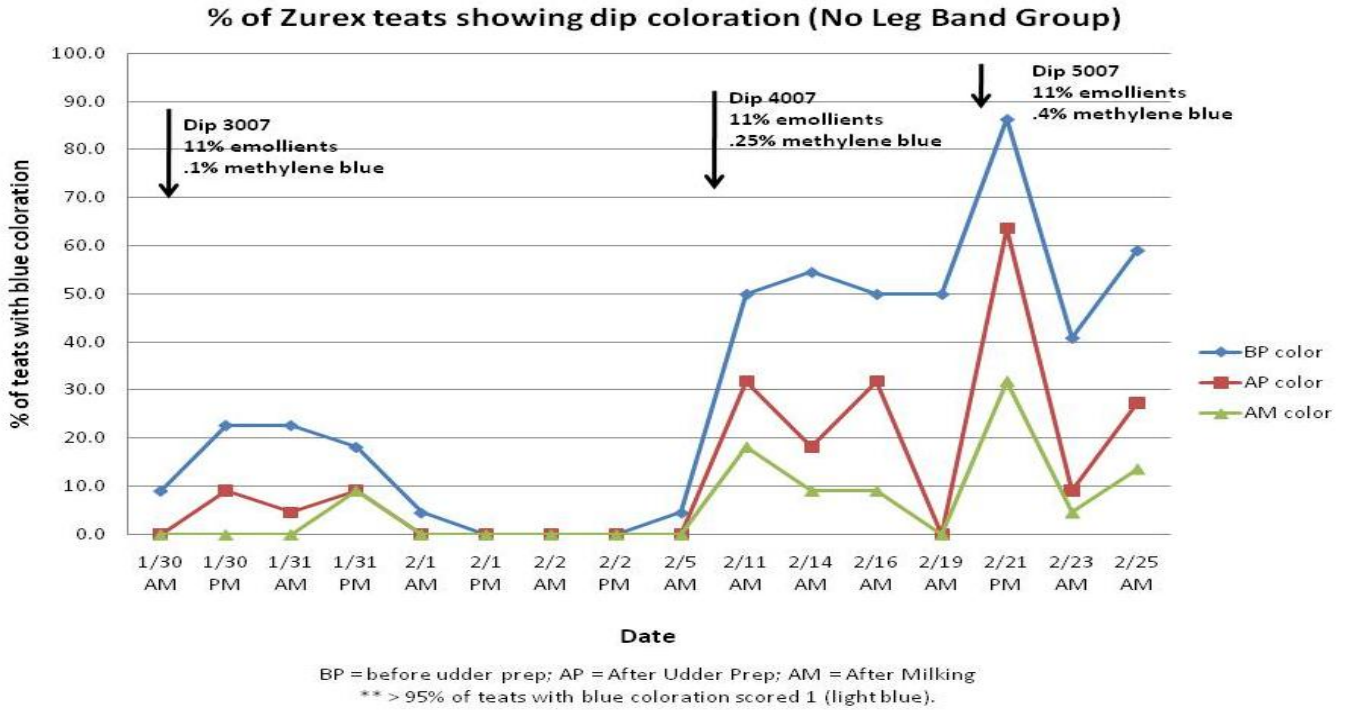


Figure 2. Percentage of teats showing skin coloration or staining before premilking teat sanitation at the subsequent milking (BP), after teat sanitation but prior to milking machine attachment (AP), and after milking or following milking machine removal (AM) when dips with different methylene blue concentrations were used (NLB group). Teat coloration scores were: 0 no color; 1 light blue; 2 dark blue.

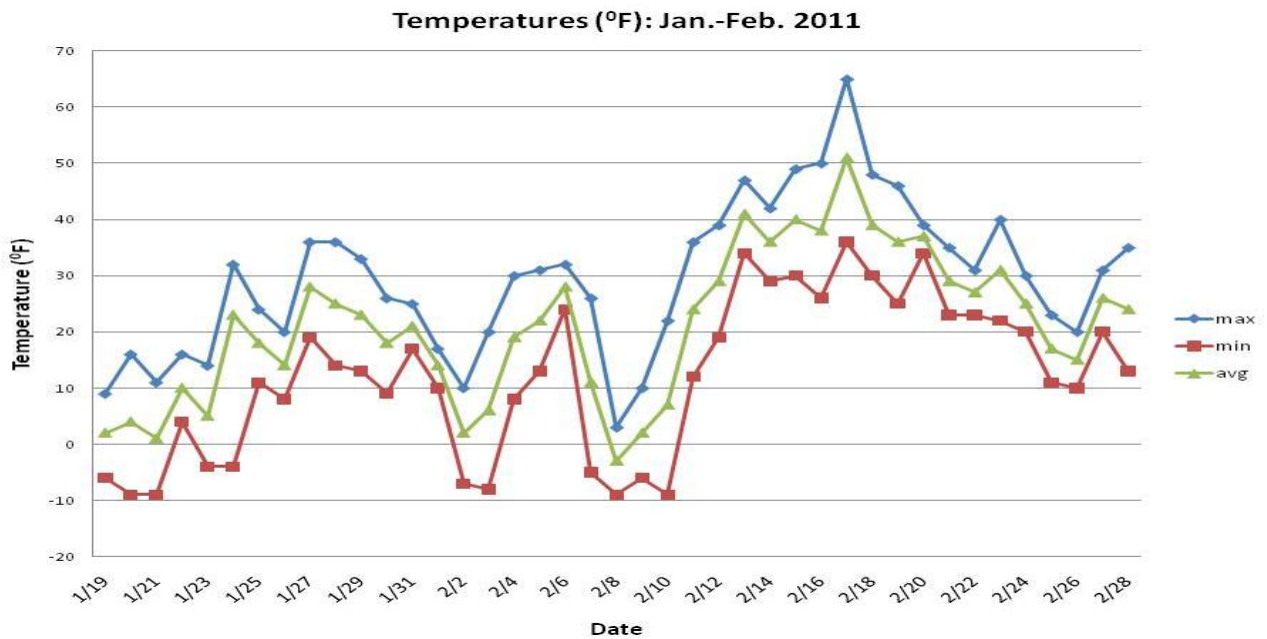


Figure 3. Average, minimum, and maximum average daily temperatures from 1/19 – 2/28, 2011 recorded at the National Weather Service station in Ames, Iowa.

3. **Teat skin (TS) and Teat end (TE) health and integrity:**

Average teat skin and teat end scores for control and treatment dipped teats for the BLB and NLB groups are shown in Figures 4 and 5. Although MB concentrations may have an effect on TS and TE scores, our data shows little effect while other factors contributing much higher variability and effect on TS and TE health. Therefore, any potential effects or comments on MB are precluded from this report.

a) **Blue Leg Band Group Teat skin (TS) and Teat end (TE) health and integrity: (Figure 4)**

i. **Teat skin health and integrity**

- There were no significant differences in teat skin health and integrity between control and treatments dip and across all milkings and dates. Teat skin health and integrity were excellent across all dips and time periods (average of 1 or all excellent). This was accomplished even though there were some very cold temperatures /temperature fluxes during the trial (Fig. 3.)

ii. **Teat end health and integrity**

- There were no significant differences between control and treated teats during the first week of the trial (1/30 – 2/5). Teat end scores showed some variation during the first 2 days (possibly related to teat scorer) and treated teats slightly but not significantly higher initially due to initial teat end integrity prior to dipping (not measured) or a slight early effect when dips were changed from control (Iodine barrier) to treatment (citrate / MB/ parabens dip).
- Average teat end scores for control cows were significantly higher (lower integrity) compared to treatment cow teats during weeks 2-3 of the trial. Week 2 was associated with a drop in temperatures (from 22 to < 10⁰F) early in that week and subsequently 4 continuous days of average temperatures < 10⁰F (minimum temperatures were – 10⁰F) (Figure 3).
- Average teat end scores for control cows were not significantly higher compared to treatment cow teats during week 4 of the trial and showed improvement compared to weeks 2-3. Temperatures increased weeks 3-4 and averaged 40 and 25⁰F during these weeks, respectively. Also, emollient % increased from 5 to 10% (no drip dip) in week 4. Despite these changes, teat end scores still remained elevated compared prior to cold temperatures.
- Average teat end scores were not significantly different across all milkings and time periods for treatment teat dips, respective of cold temperatures and rapid temperature fluxes.

Teat ends dipped with experimental dips showed consistent very good health and integrity across the trial period (changing temperatures), while control

teats showed decreased integrity (significantly higher TE scores) associated with cold, changing temperatures and these effects carried over into weeks of higher temperatures.

b) **No Leg Band Group Teat skin (TS) and Teat end (TE) health and integrity: (Figure 5)**

i. **Teat skin health and integrity**

- There were no significant differences in teat skin health and integrity between control and treatments dip and across all milkings and dates. Teat skin health and integrity were excellent across all dips and time periods (average of 1 or all excellent). This was accomplished even though there were some very cold temperatures /temperature fluxes during the trial (Fig. 3.)

ii. **Teat end health and integrity**

- There were no significant differences between control and treated teats during the first week of the trial (1/30 – 2/5). Teat end scores showed some variation during the first 2 days (possibly related to teat scorer) and treated teats slightly but not significantly higher initially due to initial teat end integrity prior to dipping (not measured) or a slight early effect when dips were changed from control (Iodine barrier) to treatment (citrate / MB/ parabens dip).
- Average teat end scores for control cows were significantly higher (lower integrity) compared to treatment cow teats during week 2 of the trial and significantly higher than their own week 1 TE scores. Week 2 was associated with a drop in temperatures (from 22 to < 10⁰F) early in that week and subsequently 4 continuous days of average temperatures < 10⁰F (minimum temperatures were – 10⁰F) (Figure 3).
- Average teat end scores for control cows were non- significantly higher compared to treatment cow teats during weeks 3-4 of the trial (except last day of trial) and showed improvement in week 4. Temperatures increased in weeks 3-4 and averaged 40 and 25⁰F during these weeks, respectively.
- Average teat end scores were not significantly different across all milkings and time periods for experimental treatment teat dips. Since TE scores of treated dipped teats and cows were slightly elevated at trial initiation, elevations in TE score in weeks 2-3 were not significant. However, some average TE scores for treatment dipped teats in week 2 were significantly elevated compared to 3 day average of these teats at end of week 1 (significance not shown or noted on figure), signifying some slight interactions with cold temperatures and changes.
- Teat ends dipped with experimental dips showed consistent good health / integrity across trial

periods, but did show some higher average TE scores associated with cold, changing temps. during weeks 2-3. Control teats showed decreased integrity (significantly higher TE scores) with cold, changing temperatures and effects carried over into weeks of higher temperatures.

c) **Overall teat integrity summary (TE and TS) across both groups (BLB and NLB)**

- There were no significant differences in teat skin integrity and scores (TS) between treatment and control dipped teats across the trial.
- Teat skin integrity and scores (TS) across all dips, milkings, time periods, and temperatures were excellent (99+% score 1 or excellent). Teat skin integrity was excellent even when faced with environmental challenges (cold, fluctuating temperatures).
- Teat ends (TE) were not significantly different between treated and control teats during trial week 1 across both groups where temperatures were mild (mostly 20⁰F with slight 2 day dip).
- Control TE scores were significantly higher than treated TE scores during weeks 2-3 of trial (BLB group) and week 2 (non-significant increase week 3) (NLB group) associated with lower temperatures during week 2, and non-significantly higher during week 4.
- NLB treated teats (11% emollient) showed slightly elevated TE scores following week 2 cold snap while BLB (5% emollients) did not. Both treatment dips showed better TE than control during this period. Higher emollient % was not associated with better TE scores although comparisons were across cows (not within) so caution in interpretation should be used.
- ** Hyperkeratotic or thickened and/ or rough teat ends were always soft and pliable with treated dips, but often dry and harder with control dip (visual and tactile observations). **

4. **% Rough / Cracked Teat Ends (TE > 3.5):**

Percentages of rough / cracked teat ends (TE > 3.5) for control and treated groups for BLB and NLB groups are shown in Figures 6 and 7, respectively. Evaluating elevations in TE score (Figures 4 and 5) are important as they reflect overall TE integrity. However, it is equally important to assess if these TE score changes are associated with increased roughness and/ or cracking of teats. Increased TE scores related to increased roughness / cracking are associated with increased mastitis or mammary infection risks, while increased TE scores without increased roughness / cracking are not.

a) **Overall teat integrity summary % rough / cracked TE across both groups (BLB and NLB)**

- % rough TE were not significantly different between treated and control teats during trial week 1 across both groups where temperatures were mild (mostly 20⁰F with slight 2 day dip). % rough teats were higher in NLB treated group at trial initiation and 1st 2 trial days.
- Control % rough TE were significantly higher than treated TE scores during weeks 2-3 of trial (BLB group) and week 2 (non-significant increase week 3) (NLB group) associated with lower temperatures during week 2, and non-significantly higher during week 4 in NLB group only. % rough teats were a major reason for elevated group TE scores.
- Treatment teats dipped with 5% emollient had lower, more consistent % rough teats compared to 11% emollient dipped teats. Again, caution should be exercised as cows were different.
- Most TE roughness / cracking in both groups was mild (TE score 3.5-4) and associated with weather (cold temperature and temperature changes). Many changes were transient in nature.

b) **Blue Leg Band Group (BLB) % rough / cracked teat ends (TE > 3.5): Figure 6**

- % rough / cracked teat ends were not significantly different between control and treated dip teats during weeks 1 and 4 of the trial.
- Control teats had significantly higher % rough / cracked teats during weeks 2-3 of the trial, associated with a cold temperature shift (Figure 3). This also resulted in significantly higher TE scores (Figure 4).
- Treatment dipped teats showed minimal changes in % rough / cracked teats across the whole trial period.

c) **No Leg Band Group (NLB) % rough / cracked teat ends (TE > 3.5): Figure 7**

- % rough / cracked teat ends were not significantly different between control and treated dip teats during week 1. Teats of treated dipped teats had numerically higher % rough teats the first 3 milkings of the trial due to a) initially higher levels at trial start; or b) early reaction to dip but this cannot be delineated since no scores were taken prior to trial, but groups were equivocal by the 4th milking.
- Control teats had significantly higher % rough / cracked teats compared to treatment dipped teats during trial week 2, and non-significantly higher during weeks 3-4 (except last trial date).
- Treatment dipped teats had similar % rough teats across the trial (no significant differences) but more variation in weeks 2-3 compared to week 1.

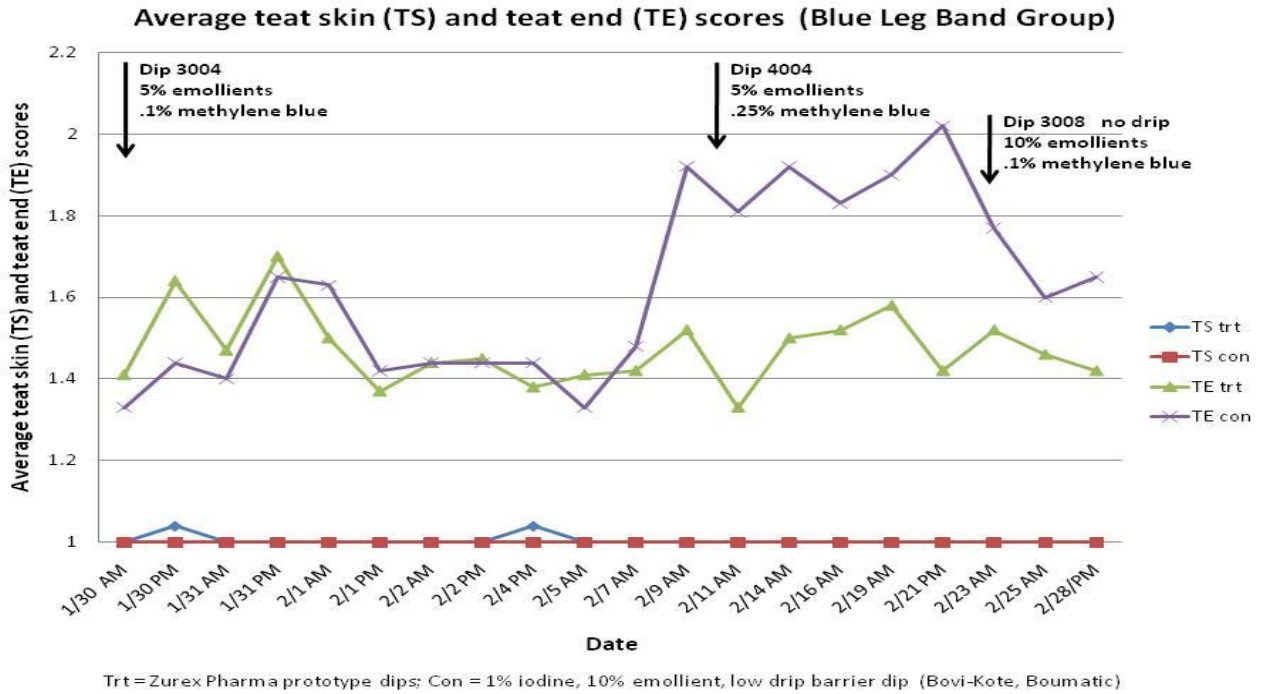


Figure 4. Average teat skin (TS) and teat end scores for control teats (left side teats – Iodine barrier dip) and treated teats (right side teats – citrate / methylene blue / parabens dips) in BLB group.

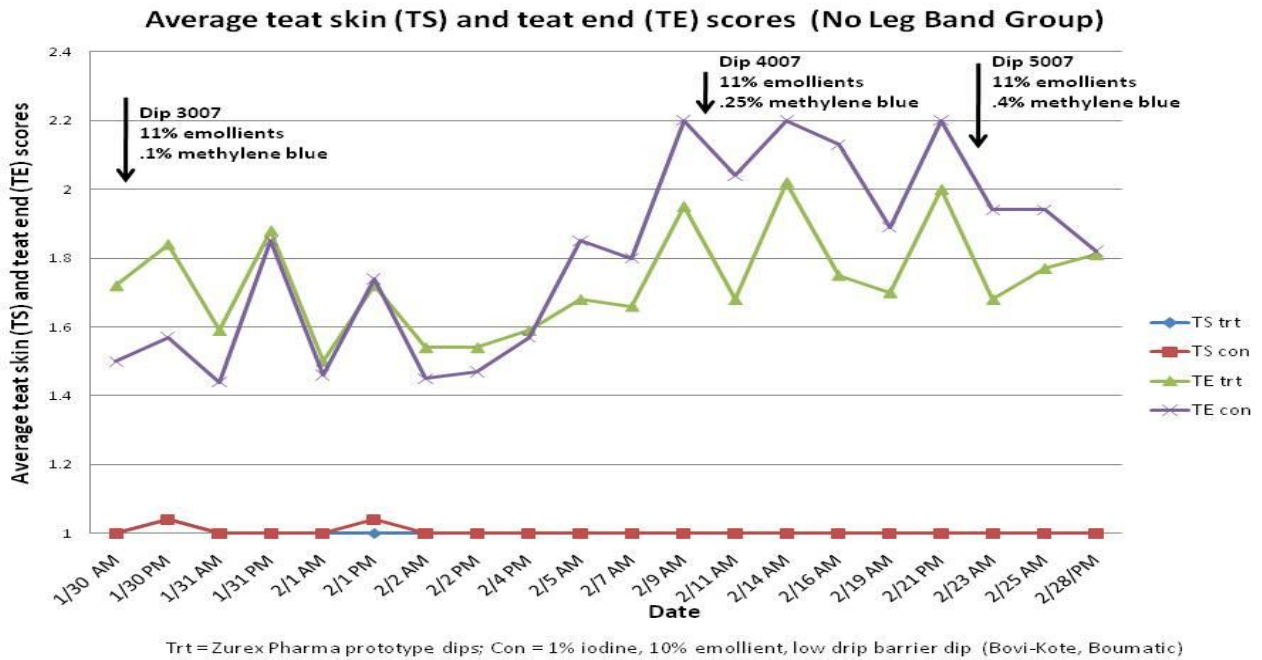


Figure 5. Average teat skin (TS) and teat end scores for control teats (left side teats – Iodine barrier dip) and treated teats (right side teats – citrate / methylene blue / parabens dips) in NLB group.

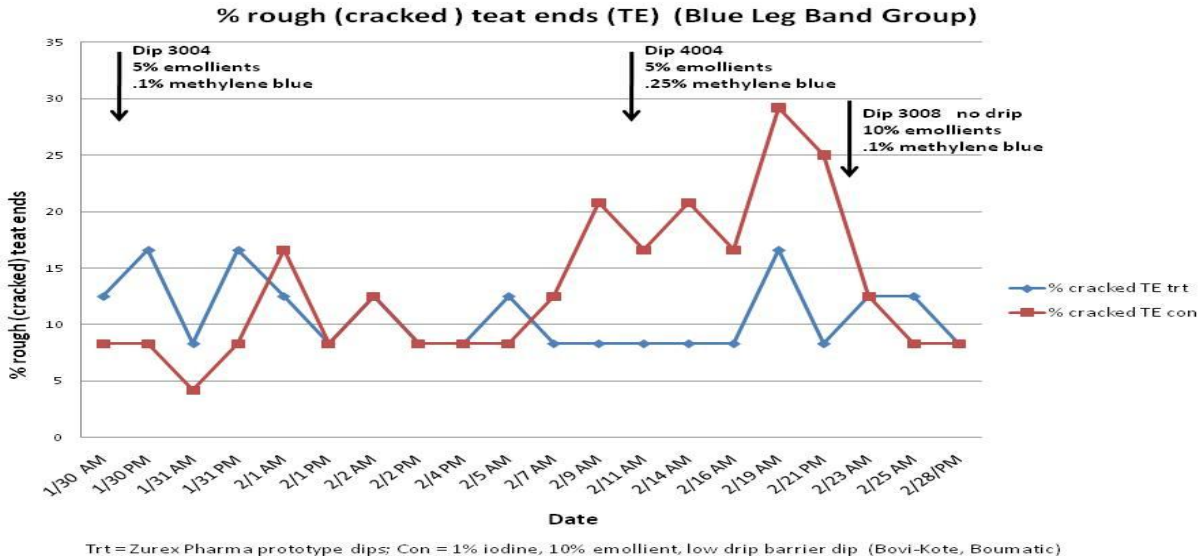


Figure 6. Percentages of cracked / rough teats for control teats (left side teats – Iodine barrier dip) and treated teats (right side teats – citrate / methylene blue / parabens dips) in BLB group.

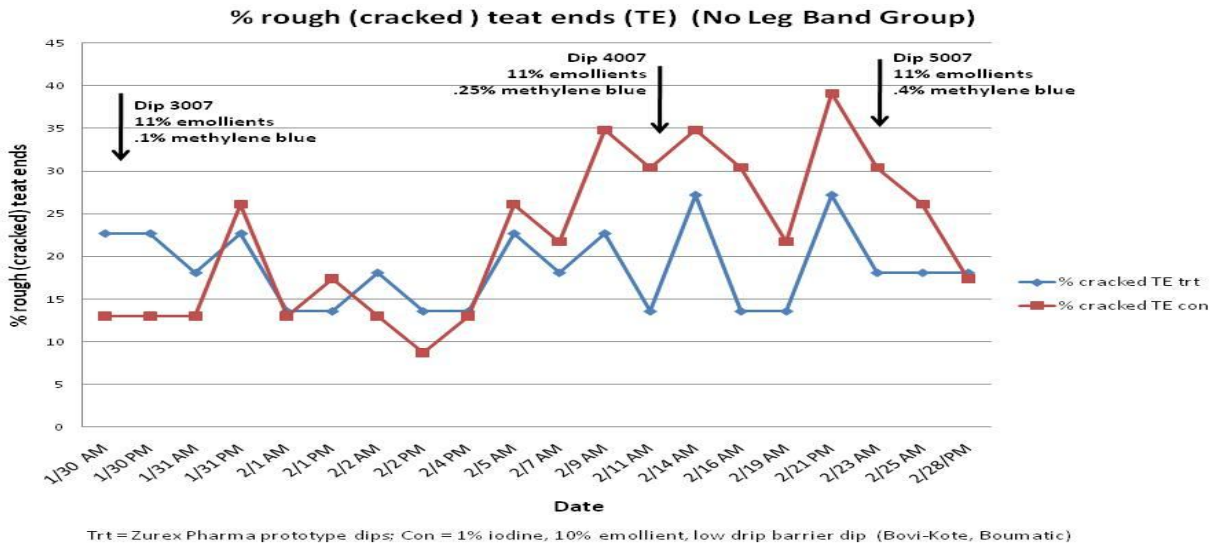


Figure 7. Percentages of cracked / rough teats for control teats (left side teats – Iodine barrier dip) and treated teats (right side teats – citrate / methylene blue / parabens dips) in NLB group.

Summary and Implications

The primary objective of this study was to evaluate a prototype novel germicide (proprietary formulation of citrate + methylene blue + parabens) based post milking teat dip with varying methylene blue and emollient concentrations versus a control commercial iodine barrier post milking teat dip on overall teat end and teat skin condition and health. A secondary objective was to assess teat skin coloration or staining due to the dip at the subsequent milking. Novel germicide prototype dips showed some teat coloration at the next milking with the

highest % seen when .25% MB was added or 1% MB with a thixotropic agent. Novel germicide prototype dips showed excellent teat skin (TS) health (same as control dip). Novel germicide prototype dips showed very good teat end health integrity and maintained teat end health integrity through cold weather changes (significantly better than controls). Dips with 5% emollients were more consistent in TE health compared to 11% emollients. Novel germicide prototype dips showed softer, more pliable hyperkeratotic TE tissue. Overall, these novel germicide prototype dips performed very well and better than controls.