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Intramammary Infusion of Casein Hydrolysate for Involution of Single Mastitic Mammary Quarters Elevating Cow-Level Somatic Cell Count

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Abstract

Mastitis in a single quarter can cause high somatic cell counts (SCC), clinical mastitis, and death in dairy cows. Currently, management of these mastitic quarters presents a problem for the dairy industry. Casein hydrolysate (CH) is an intramammary (IMM) infusion treatment reported to induce mammary involution. The primary objectives of this study were to investigate whether IMM CH treatment of single high SCC quarters, followed by cessation of quarter milk production for the remainder of lactation, was effective in reducing cow–level SCC and whether that quarter resumed milk production following calving. Three treatment groups were used: CH, non-hydrolyzed casein (NHC), and cessation of milking only (negative; N). Treatments were assigned in a 2:2:1 ratio for 40 cows enrolled in the study; 27 cows completed the entire protocol. Following IMM infusion and involution of the single mastitic quarter, decreases in cow–level SCC (-966,000/ml) and milk production (-11 lb (5 kg), -14%) with 3 remaining lactating quarters were significant for all 28 cows combined. Cows treated with CH (n=17) had a significant decrease in cow–level SCC (-1,150,000/ml) during remaining lactation. All treated quarters returned to milk production after calving, and their proportion of total–cow milk production (24%) was not different than before treatment (28%). After calving, treated quarters' decrease in SCC was significant for CH (-2,763,000/ml; n=14) and N (–5,324,000/ml; n=5). Of 16 quarters with positive milk culture before treatment that completed the protocol, 88% (14/16) were cured (no isolation of the same bacteria for 3 weeks following calving). A new intramammary infection (IMI) was detected in 67% (18/27) of previously treated quarters post-calving. Infusing single mastitic quarters with casein hydrolysate to induce involution for the remainder of lactation may be a promising alternative to current methods.

Keywords: Dairy; Mastitis; Management; SCC; Milk quality

Abbreviations: SCC: Somatic Cell Count; IMM: Intramammary; CH: Casein Hydrolysate, NHC: Non-Hydrolyzed Casein, N: Cessation N: Cessation of Milking of Milking Only, Negative; IMI: Intramammary Infection; BTSCC: Bulk Tank Somatic Cell Count; DHIA: Dairy Herd Improvement Association; DIM: Days in Milk, CMT: California Mastitis Test

Introduction

Mastitis is the most expensive disease complex in the dairy industry. Costs include lost milk production, antibiotic treatment, discarded milk because of antibiotic therapy, and death loss. Reduced bulk tank milk quality and milk price also result from increased white blood cells, reported as somatic cell count (SCC), being shed into milk by affected quarters. The goal is to maintain a bulk tank SCC<200,000/ml [1-3]. A single mastitic quarter may have an individual SCC of millions per milliliter of milk, impacting the cow-level and bulk tank milk SCC. Previous studies have documented the negative impact that single quarters with extremely elevated SCC can have on the overall quality of bulk tank milk [4]. Most dairy producers receive economic benefit from maintaining a low bulk tank somatic cell count (BTSCC) and understand the advantage of diverting high SCC milk from chronically inflamed quarters from entering the bulk tank [5]. If cows with a single quarter causing high SCC in their composite milk are undesirable for other health or production reasons, the decision may be made to remove them from the herd altogether. However, many times these animals are pregnant and/or otherwise productive dairy cows, so producers simply cease milk production in the high SCC quarter. In modern high–producing dairy cows this can sometimes be difficult to achieve without causing permanent damage to the quarter or other adverse effects on the animal [6,7]. High–yielding cows with high SCC milk from one quarter or recurring mastitis episodes in a single quarter, commonly lead producers to the management practice of attempting to remove the affected quarter by intramammary infusion (IMM) of caustic substances such as strong iodine (e.g. 2% to 7% iodine) or 2% chlorhexidine [8]. Previous studies have shown efficacy of these methods for cessation of milk production in a single mammary quarter but both iodine and chlorhexidine were reported to induce undesired consequences [9]. Use of strong iodine was associated with no return to production, essentially creating a permanently "three–quartered" cow. Intramammary chlorhexidine resulted in some cows regaining full use of the treated quarter in the subsequent lactation but antimicrobial residue was detected 35 to 42 days post infusion by Delvotest® [10] validating the concern that accidental milking of an infused "dry" quarter could lead to antimicrobial residue violations in bulk milk. Therefore, the off–label IMM of chlorhexidine is not recommended as a method for ceasing milk production in a quarter.

An earlier study found IMM of casein hydrolysate effective in inducing involution of the mammary gland without systemic disease or causing permanent quarter damage [11]. Casein hydrolysates are milk–borne factors believed to be part of the biological pathway which causes involution in the bovine mammary gland, 40-70 days prior to

expected parturition. Intramammary infusion of casein hydrolysates has been demonstrated to locally induce involution within a single quarter, as a management strategy for cessation of lactation in quarters with a persistently elevated SCC or repeated episodes of mastitis, without the consequences of antimicrobial residues and/or permanent mammary gland destruction.

The basis for this study was to address the need for managing mastitic quarters mid-lactation in otherwise healthy dairy cows. Currently, there is no widely accepted method available for producers to use on these animals. This shortfall has resulted in unsatisfactory outcomes. The primary objective was to evaluate cessation of milking in individual mastitic quarters using intramammary infusion of CH, in comparison with intramammary infusion of a placebo or no infusion.

Material and Methods

Selection of study cows

Study cows were selected from 6 commercial Idaho dairy farms. All cows were housed in outdoor, shaded dry lot pens typical of dairy farms in that region. Participating farms were on a twice per day milking schedule and following a regular monthly dairy herd improvement association (DHIA) testing schedule [12]. Lactation number, SCC, pregnancy status, days until expected dry off date, days until expected calving and daily milk production data were obtained from DHIA records. To be eligible, cow–level SCC ≥ 500,000/ml, confirmed pregnant, ≥ 35 days before scheduled dry–off date, estimated 95–220 days until expected calving, and daily milk production ≥ 50 lb (22.7 kg) were required.

Cows meeting eligibility requirements were then screened for quarter–level IMI using the California Mastitis Test (CMT) [13]. For trial inclusion, CMT scores of 2-plus in a single quarter and Negative or Trace in the other 3 quarters were required. Using aseptic sampling technique, an individual milk sample was collected from the quarter with an elevated CMT score, along with a pooled milk sample of the other three quarters. An aliquot of the individual quarter sample was used for microbial culture, while the remainder of the quarter sample and the pooled sample of the other quarters were both tested for SCC. Somatic cell count was measured by use of the FossomaticTM automatic cell counter [14]. An SCC of ≥ 1,000,000/ml in the single mastitic quarter, SCC ≤ 400,000/ml in the 3 non–mastitic quarters, and *Mycoplasma spp.*–negative culture results finalized enrollment in the study.

Using a completely randomized block design, cows were blocked by lactation number (1st, 2nd–plus) and mastitic quarter culture result (growth, no growth), for a total of 4 blocks. There were 3 treatment groups: casein hydrolysate (CH), non–hydrolyzed casein (NHC), and cessation of milking only (negative; N). Cows were randomly assigned to treatment groups within each block, in a 2:2:1 proportion due to the challenges of obtaining a large sample size and in a purposeful determination to allot most of the animals to the CH and NHC treatment groups.

Milk microbiology

Milk sample bacterial cultures were completed according to standard methods [15,16]. In brief, an inoculum of 10 µl of milk was plated on washed cow blood agar and placed in a standard, non–CO_2 incubator at 37°C for 48 h. Plates were examined by laboratory technicians at 24 and 48 h for bacterial growth; organism identification was determined by colony morphology and biochemical secondary tests on any isolates found.

Preparation of casein hydrolysate

Two batches of casein hydrolysate were prepared using aseptic technique. Each batch was prepared using 100 g of commercially purchased bovine casein powder dissolved in 1 L of autoclaved deionized water containing 3 g of TRIS buffer and enzymatically digested with Trypsin. After digestion, remaining particulate material was removed *via* two centrifugation cycles of 15 minutes at 3000 xg. Solution was boiled for 15 minutes between centrifugation cycles to denature any remaining enzyme and kill possible environmental contaminant bacteria, followed by sterilization using vacuum membrane filtration. The final product was dispensed (15 ml) into sterile syringes and stored frozen at –20°C. Two batches of NHC solution were produced following the same methods as above but omitting enzymatic digestion, thus preventing hydrolysis.

Each batch of casein hydrolysate or NHC was screened for bacterial contamination by inoculating tryptic soy broth with 1 ml of solution, incubating for 24 h and inoculating blood agar. Blood agar plates were incubated for a total of 48 h at 37°C and read at 24 and 48 h for bacterial growth. A separate blood agar plate was plated directly with 100 µl of non–enriched solution and then incubated and examined in the same way. The definition of an uncontaminated batch was no growth of any bacterial colonies on either direct or enriched cultures. Protein concentration of each batch was quantified using a bicinchoninic acid (BCA) assay, according to the manufacturer's instructions. The BCA assay is commonly used for protein quantification [17]. Final protein concentration of CH solutions was 1.5 mg/ml, which resulted in 22.5 mg per 15 ml dose. Final concentration of NHC was 0.2 mg/ml, which resulted in 3 mg/15 ml dose. Each batch was also assessed for purity by running a sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) gel.

Milk weight collection

Mastitic quarter contribution to total-cow milk production was measured by bucket milking on Day 1 of the 48 h treatment protocol. Animals were milked into a clear, graduated, 80 lb (36.3 kg) capacity bucket in two steps: the three healthy quarters were milked together first, followed by the single mastitic quarter. This allowed measurement of milk production from the total cow for that milking and the proportional contribution of the mastitic quarter. This bucket milking process was repeated at a single milking, between 10-21 days in milk (DIM) in the subsequent lactation, to evaluate the milk production and proportion of total–cow milk from the mastitic quarter.

Treatment administration

All mastitic quarters were milked once per day for the 3-day treatment period. Each animal had all 4 quarters milked in the morning, followed by infusion of the mastitic quarter with the assigned treatment (CH or NHC) or no infusion (N), followed by skipping the evening milking of that mastitic quarter. This process was repeated at 24 h (day 2) and again at 48 h (day 3) for the third and final treatment. This gradual cessation of milking was intended to cease milk production in the target quarter with minimal discomfort and risk of adverse effects to the animal. The mastitic quarter was not milked again for the remainder of the lactation. Somatic cell count data from

the 3 remaining quarters was obtained from the next DHIA test following treatment.

Evaluation of intramammary infections following dry period

Bacteriological cure of previous IMI and new infection rates were not primary objects of this study. However, involution is a mechanism of clearing existing IMI during the dry period [18,19] and this is an important outcome for any treatment during the dry period in dairy cattle. After calving the previously treated quarters were resampled 3 times, once per week at 1-7 DIM, 8-14 DIM and 15-21 DIM. Resulting case definitions were: Cure=all 3 post–calving cultures negative for any bacteria isolated from the pre–treatment sample; Chronic IMI=any bacteria isolated from the pre–treatment sample, followed by isolation of the same bacteria from at least one post–calving culture. New IMI=one or more bacteria not isolated from the pre–treatment sample, followed by isolation from at least one post–calving culture sample (multiple bacterial species only count as one IMI).

Statistical analysis

Statistical analyses were performed using GraphPad Prism version 7.01 and SAS Studio. Descriptive statistics were calculated. To evaluate for possible confounding, pre–treatment variables were compared among the 3 treatment groups. The continuous variables DIM, days until expected dry–off, cow–level SCC, mastitic quarter SCC, total–cow milk production, and proportion of treated quarter contribution to total–cow milk weight were evaluated for possible differences between treatment groups using analysis of variance (ANOVA). The grand means of cow–level SCC, mastitic quarter SCC, total–cow milk production, and mastitic quarter milk production (including comparison between all front and rear quarters) were compared pre– and post–treatment using a t–test. Means of the continuous variables cow–level SCC, mastitic quarter SCC, total–cow milk production and quarter proportion of total–cow milk were compared pre– and post–treatment between treatment groups using ANOVA.

The change in each of the above outcome variables from pre– to post–treatment was tested for significance within each group, also using ANOVA. For the continuous variables DIM, DCC, pre–treatment SCC of total cow and individual quarter, pre–treatment milk weight of total cow and individual quarter and contribution of the individual quarter to the total cow production, association with post–treatment SCC in the remaining three quarters was evaluated using multiple regression (PROC REG). Input variables that might logically be associated with the outcome variable–log of post–treatment SCC in the remaining 3 lactating quarters–were evaluated in both a linear mixed model (PROC GLIMMIX, SAS Studio) and a general linear model (PROC GLM, SAS Studio). Initial models included all logical potential input variables followed by backward elimination until the final model included only input variables with P<alpha. The categorical outcomes of Cure, Chronic IMI, and New IMI were compared for significant differences between the categorical variable treatment groups using Chi–square. Alpha was 0.05 for all statistical analyses.

Results

Forty cows (Holstein n=38, Jersey n=2) were initially enrolled in the study. Their single mastitic quarters (5 right front, 8 left front, 14 right rear, 13 left rear), were randomly assigned among the 3 treatment groups (2:2:1 ratio). There were 18, 15 and 7 cows in the CH, NHC and N treatment groups, respectively. Six cows were in 1st lactation and 34 were in 2nd–plus lactation, 23 treated quarters had bacteria isolated pre–treatment, while 17 quarters had no growth on culture. All 40 mastitic quarters were successfully dried off, without any reports of adverse effects to the animal (e.g., swelling, edema, milk leakage, etc.). Before they could calve again, 12 cows were sold (4 CH, 6 NHC, 2 N treated cows) because of mastitis (n=3), abortion (n=5), infertility (n=2) or died from displaced abomasum complications (n=2). One cow died before her third post–calving culture sample could be collected; cause of death was unknown. Therefore, 27 cows finished the entire study protocol. However, the last cow who died contributed data for all other outcome variables, so for most outcomes, there were 28 cows, with the final distribution of animals per treatment group: CH (n=14), NHC (n=9) and N (n= 5). All treated quarters of the cows that remained in the study resumed milk production following the next calving.

There were no statistically significant differences between cows assigned to treatment groups for the following parameters: DIM (CH=264, NHC=219, N=222), days carried calf (DCC) (CH=115, NHC=102, N=86), pre–treatment cow–level SCC (CH=1,792,000/ml, NHC=1,464,000/ml, N=1,590,000/ml), mastitic quarter SCC (CH=4,363,000/ml, NHC=3,745,000/ml, N=5,852,000/ml), total–cow milk production before treatment (CH=79 lb [36 kg], NHC=74 lb [34 kg], N=86 lb [39 kg]) and proportion of mastitic quarters' contribution to total–cow milk production (CH=26%, NHC=28%, N=32%) (all P>0.5 ANOVA, Table 1). The average length of the dry period for the treated quarters was 181, 177 and 151 days for CH, NHC and N, respectively (P>0.14, ANOVA).

Measurement/treatment group	Pre-	Post-	P-value ≠	Change£
Cow SCC-Casein (x 1000 cells/ml)	[a]1792 (n=18)	[e]642 (n=17)	0.003	[i]-1150
Cow SCC-Non-hydrolyzed casein (x 1000 cells/ml)	[a]1464 (n=15)	[e]755 (n=15)	0.08	[i]-709
Cow SCC-Negative (x 1000 cells/ml)	[a]1590 (n=7)	[e]546 (n=7)	0.08	[i]-1044
Qtr. SCC-Casein (x 1000 cells/ml)	[b]4363 (n=18)	[f]1600(n=14)	0.0002	[j]–2763
Qtr. SCC-Non–hydrolyzed casein (x 1000 cells/ml)	[b]3745 (n=15)	[f]1616 (n=9)	0.01	[j]-2129
Qtr. SCC-Negative (x 1000 cells/ml)	[b]5852 (n=7)	[f]528 (n=5)	<0.0001	[j]-5324
Total milk-Casein (kg)	[c]36 (n=18)	[g]32 (n=17)	0.2	[k]-4

Total milk-Non-hydrolyzed casein (kg)	[c]34 (n=15)	[g]27 (n=15)	0.04	[k]-7
Total milk-Negative (kg)	[c]39 (n=7)	[g]36 (n=7)	0.57	[k]-3
Qtr.% of total Cow-Casein	[d]26 (n=18)	[h]24.5 (n=14)	0.6	[l]-1.5
Qtr.% of total Cow-Non-hydrolyzed casein	[d]28 (n=15)	[h]22 (n=9)	0.17	[l]-6
Qtr. % of total Cow-Negative	[d]32 (n=7)	[h]27 (n=5)	0.41	[l]-5
Cow SCC (x 1000 cells/ml)-All cows	1634 (n=40)	668 (n=39)	0.0002	-966
Qtr. SCC (x 1000 cells/ml)-All cows	4392 (n=40)	1414 (n=28)	<0.0001	-2978
Cow Milk Yield (lb)-All cows	78 (n=40)	67 (n=39)	0.02	-11
≠ For test of significance of difference from pre– to post–treatment, within each treatment group				
£ Change in measurement (post minus pre), post–infusion				
Means with same letter were not significantly different between treatment groups (p>0.05, ANOVA)				

Table 1: Pre and post-treatment comparisons of cow–level SCC, quarter SCC, total–cow milk and quarter percentage of total–cow milk by treatment group and overall.

Following treatment (leaving 3 remaining lactating quarters), mean cow–level SCC was 668,000/ml and mean milk production was 67 lb (30 kg) for all 39 cows who survived to the next monthly DHIA test (Table 1). For those 39 cows, the decreases in cow–level SCC (–966,000/ml; P=0.0002) and in total–cow milk production, –11 lb (–5 kg, –14%; P=0.02) were statistically significant, as tested by students t–test (Table 1). No statistical significance was found for the effects of DIM, pre–treatment cow–level SCC, pre–treatment mastitic quarter SCC, total–cow milk production or individual quarter contribution on post–treatment SCC, as tested in the multiple regression models.

The final general linear model was significant ($P \leq 0.001$) with R2=0.65. The input (explanatory) variables pre–treatment cow–level SCC, bacterial agent, lactation number (1st, 2nd–plus), treatment, and interaction of treatment and lactation number were all significantly associated with the outcome variable, log of post–treatment SCC in the remaining 3 lactating quarters (all $P \leq 0.02$). The same variables with the same P values were also detected as significant in the final mixed model. Higher pre–treatment cow–level SCC was associated with lower post–treatment SCC in the 3 remaining quarters, and this was particularly evident among cows infected with several bacterial agents.

For the following agents, pre–treatment cow–level SCC and post–treatment SCC in the 3 remaining quarters, respectively were: *E. coli*, 1,780,000/ml, 1,436,000/ml; no growth, 1,731,000/ml, 841,000/ml; *Staphylococcus spp.*, 1,089,000/ml, 436,000/ml. The treatment effect and its interaction with lactation number were driven by cows in first lactation; their post–treatment means of SCC in the 3 remaining quarters by treatment were: CH=1,020,000/ml, NHC=90,000/ml, N=1,000/ml. In contrast, for cows in 2nd–plus lactation, the post–treatment means of SCC in the 3 remaining quarters were: CH=561,000/ml, NHC=857,000/ml, N=637,000/ml.

Within treatment groups, the decrease in total–cow milk production following involution of the mastitic quarter was not significant except for the decrease from 74 lb (34 kg) to 59 lb (27 kg) in the 15 NHC treated cows (P=0.04, ANOVA, Table 1). All 28 treated quarters in the cows who calved again returned to milk production after calving; mean SCC of the 28 quarters was 1,414,000/ml, significantly decreased (–2,978,000/ml; P=<0.0001, Table 1) and their contribution to total–cow milk production was 24%, not different from their 28% contribution during the previous lactation before treatment (P=0.46, student's t–test). Front or rear quarters did not differ significantly in outcomes (all P>0.6, ANOVA).

Cow–level SCC during the remainder of lactation decreased significantly following treatment with CH in the mastitic quarter (–1,150,000/ml [n=17]; P=0.003, Table 1). Following calving and resumption of milk production, significant decreases in SCC in the previously treated mastitic quarters were observed within all three treatment groups (CH: –2,763,000/ml [n=14], P=0.0002; NHC: –2,129,000/ml [n=9], P=0.01; N:–5,324,000/ml [n=5]; P<0.0001, all ANOVA, Table 1). All other pre–versus post–treatment comparisons by treatment group were not statistically significant.

Bacterial isolation, cures and new intramammary infections

The pre–enrollment milk culture screening found a pathogenic bacterial organism in 23/40 (58%) mastitic quarters. The predominant organisms isolated were *Streptococcus spp.* (n=10) and coagulase–negative *Staphylococcus spp.* (n=5). Also isolated were *Escherichia coli* (n=3), *Staphylococcus aureus* (n=3) and *Pseudomonas spp.* (n=1), unknown pathogen (n=1). Of the 23 cows whose mastitic quarters had bacteria isolated, 16 finished the study and were available for evaluation of bacterial cure or persistence; 14/16 (88%) quarters had a bacteriological Cure (Table 2). Of the 27 cows who calved again and survived to have all 3 post–calving milk cultures, 18/27 (67%) contracted a New IMI in the previously involuted quarter.

Treatment groups were not significantly different in their outcomes of Cure or Chronic IMI, (all $P \geq 0.35$, Fisher's exact test). There were significantly fewer new IMI among cows treated with CH (P=0.046, Fisher's exact test). For cows in the NHC and N treatment groups, the proportion of New IMI was not significantly different from that for all cows ($P \geq 0.19$).

	Pre-Treatment Culture (n=40)		Post-Treatment Culture (n=27)				
Treatment Group	**Culture-positive**	**Culture- negative**	**Previously positive**	**Previously negative**	**Chronic IMI[1]**	**IMI Cured[2]**	**New IMI[3]**
CH+	10	8	7	6	(0/7) 0%	(7/7) 100%	(6/13) 46%
NHC+	9	6	6	3	(1/6) 17%	(5/6) 83%	(8/9) 88%
N+	4	3	3	2	(1/3) 33%	(2/3) 67%	(4/5) 80%
Totals	23[£]	17	16[§]	11	(2/16) 12%	(14/16) 88%	(18/27) 67%
[1]Chronic IMI=any bacteria isolated from the pre–treatment sample, followed by isolation of the same bacteria from at least one post–calving culture							
[2]Cure=all 3 post–calving cultures negative for any bacteria isolated from the pre–treatment sample							
[3]New IMI=one or more bacteria not isolated from the pre–treatment sample, followed by isolation from at least one							
[+]CH=Casein hydrolysate; NHC=Non–hydrolyzed casein; N=cessation of milking only, Negative.							
[£]23/40 Cows had a mastitis organism present pre–treatment; 16 remained in the study long enough for 3 post–treatment sample collection							
[§]27/40 cows completed the study with 3 post–treatment cultures; 11 of these did not have a mastitis organism present pre–treatment, which left 16 cows eligible to be chronic IMI or cured							

Table 2: Bacteriological cures and new infections by treatment group.

Discussion

After involution of a single mastitic quarter with high SCC, cows produced a mean of 86% of their previous total production from the 3 remaining lactating quarters at the time of their next monthly DHIA test. Postpartum, all cows resumed milk production with the treated quarters contributing a mean of one–fourth of total–cow production. The average decrease of 1,100,000/ml in cow–level SCC following involution of a single mastitic quarter, and the reduction of SCC in mastitic quarters by over 2.5 million/ml when their milk production resumed after calving provides support for IMM casein hydrolysate as a management option to create three–quartered cows to enhance milk quality. Interestingly, our analysis identified that first lactation cows varied considerably in post–treatment SCC among the treatment groups, resulting in a highly significant interaction between treatment and lactation. Among older cows, which comprised majority of cows in the dataset, there were no significant differences in cow–level SCC between treatment groups. In many cases, mammary involution is believed to be a contributor to the spontaneous cure of previous IMIs from one lactation to the next [20]. By design, the mastitic quarters in our study were dry for longer than the typical dry period of approximately 60 days [21], with more than 200 days in some cases. Bacterial cure was observed in over 85% of the treated and involuted quarters in this study, while two–thirds contracted a new IMI while dry. Average IMI prevalence reported in early lactation cows ranges from 10%–29% [22–24]. A limitation of this study was a relatively small sample size, which was due to logistics and expense.

The results of this study indicate that IMM use of CH may be a promising alternative to traditional methods of treating mastitis. Some disadvantages of using conventional intramammary antibiotic treatment for mastitis include the potential for antibiotic residues, the milk lost due to required withhold times and the increasingly negative consumer perception of antibiotic use in food animal management. Additionally, many bacterial organisms that are responsible for causing mastitis are not susceptible to antibiotics and for that reason; do not warrant treatment with such. Milk cessation in individual quarters using IMM CH is a novel approach to mastitis management, which utilizes the natural process of tissue rebuilding that, occurs during mammary involution, without any use of intramammary antibiotics. This method could potentially extend the productive life of many dairy animals, without jeopardizing milk quality or causing adverse physical effects.

Conclusion

This study evaluated three methods for cessation of milking of a single mastitic quarter mid–lactation to attempt to improve cow–level milk quality. None of the animals showed any signs of pain or physical distress in response to drying off the quarter. All treated quarters returned to adequate milk production, with the favorable outcome of prolonged life of lactating cows. Cessation of production in the chronic mastitic quarters resulted in a decrease in cow–level SCC for all treated animals, regardless of treatment, which indicates this is an appropriate milk quality management tool.

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