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On-Farm Pasteurized Milk Fed to Dairy Calves – Association of Bacteria Counts Following Pasteurization with Season, Temperature and Time until Feeding

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Abstract

On-farm pasteurization of waste milk fed to calves has become increasingly common over the last 15 years. This study investigated bacteria counts in milk before pasteurization and for 24 h following pasteurization at varying storage temperatures associated with seasons. Standard plate counts on Aerobic Count Plate Petrifilm™ to enumerate bacterial colony concentration were measured on raw and post-pasteurized milk after pasteurization at 63°C (145°F) for 30 min using commercial pasteurizers on 3 different commercial dairy farms. Each farm was sampled in each of 4 seasons of the year. All 12 batches of milk were divided into 2 aliquots. One aliquot was incubated at controlled temperature to mimic the season (refrigerated, room temperature, or incubated at 37 °C) and the other was incubated at ambient outdoor temperatures. The SPC was determined at pre-pasteurization, 0 (immediately post-pasteurization), 1, 2, 3, 4, 5, 6, 7, 8, 10, 12 and 24 h post-pasteurization. The final general linear model testing for factors associated with LogSPC was highly explanatory (R^2=0.71), and significant (P<0.0001). This final model for LogSPC included time since pasteurization, season, farm, the interaction of time and season, and the interaction of season and farm. The model showed that passage of time was associated with increased LogSPC, especially during summer. In a northern temperate climate under the conditions observed during this study, milk - could safely be fed-if defined as SPC<20,000 cfu/ml-for at least 8 h post-pasteurization during fall and spring, and for 24 h during winter, but only for 3 h if milk was stored outside during the summer. These results suggest that for milk kept outdoors during summer, any milk remaining after first feeding following pasteurization should not be fed to calves at subsequent feedings, but instead should be re-pasteurized or discarded.

Keywords: Pasteurization; Milk; Bacteria Count; Calves

Introduction

On-farm pasteurization of waste milk fed to calves has become increasingly common over the last 15 years [1-4], and has also been demonstrated to be profitable and of benefit to calf health [2,3]. However, there is inconsistency in bacterial reduction in pasteurized milk on dairy farms, due to mechanical and performance differences among farms. Bacteria counts are often greatly reduced from the levels in raw milk, but can rapidly recover and even increase to greater levels than those in raw milk even after few hours [2,3,5].

Pasteurized milk is often stored at ambient temperature for 4 to 8 h or more on farms before it is fed. The primary reason for this is that when an afternoon or night milking is completed, discard milk, such as from treated or recently calved cows is usually pasteurized soon afterward. However, the next feeding time for the dairy calves is often many hours later. Dairy producers do not usually wait to pasteurize milk because pasteurization can usually be started by some farm personnel while others clean the milking parlor, and the milk has to cool before it can be handled and then subsequently fed to calves.

There is a need for standardization of recommendations for specific pasteurization procedures and subsequent feeding of milk to calves on dairy farms [5]. There are no published studies regarding the effects of time until feeding of pasteurized milk on bacterial numbers in the milk, or the effects of variations in storage temperatures on the increase in bacteria before the milk is fed. This study investigated bacteria counts in raw milk, immediately post-pasteurized milk, and milk at time intervals up to 24 hr following pasteurization at varying storage temperatures. The principal objectives were to evaluate the change in milk bacteria counts as time passed following pasteurization and to investigate potential differences in how bacteria counts increased over time between seasons and their associated ranges of ambient temperature.

Some suggested standards state that only milk with bacteria counts less than 20,000 cfu/ml should be fed to calves, although associations with health effects on calves have not been studied [5]. Therefore a secondary objective was to evaluate the time following pasteurization that passed before bacteria counts increased to levels greater than 20,000 cfu/ml, including potential time differences between seasons and their associated ranges of ambient temperature.

Materials and Methods

Study herds

Three commercial dairy farms in Colorado milking approximately 800, 2400 and 1800 Holstein cows participated in the study (Farms A, B, and C). Commercially made batch pasteurizers were used within old bulk milk tanks to pasteurize discard milk at 63°C (145°F) for 30

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min, and the milk was later fed to replacement heifer calves. Cows that had recently calved or were treated with antibiotics were milked into the pasteurizer tanks, and pasteurization was begun after each such milking ended. There was no change made during the study to the pasteurization methods already employed on the farms.

Milk collection and handling

One batch of milk was collected from each of the 3 study farms during each of the following 4 seasons: summer (June 21-Sept. 20), fall (Sept. 21-Dec. 20), winter (Dec. 21-March 20), and spring (March 21-June 20). During the milking of cows whose milk was discarded, the milk was periodically agitated in the pasteurizer tank but not cooled. The pre-pasteurized milk sample from each batch was aseptically collected into an 11 ml sterile vial by farm personnel just before the raw discard milk was pasteurized, following agitation of the milk, and immediately placed in a refrigerator on the farm at approximately 4°C. Personnel from The Dairy Authority (TDA) in Greeley, CO arrived on the farm shortly before pasteurization ended. After pasteurization, as soon as milk cooled to 49°C (120°F), they aseptically collected one milk sample –at 0 h, representing the time at which pasteurization ended and milk was cooled enough to be handled - into an 11 ml sterile vial. Personnel from TDA also inoculated all pre-pasteurization milk samples (that had been refrigerated by farm personnel) and all 0 h milk samples onto Petrifilm™ (Aerobic Count Plate, 3M Microbiology, St. Paul, MN) before leaving the farm. Microbiological methods will be described further. The inoculated films were placed into a portable incubator at 37°C. Next each batch of milk was divided into 2 aliquots of 6 L each by study personnel; these 6 L aliquots were transported at ambient temperature until arrival at the laboratory. All milk samples were transported to the laboratory of TDA within approximately 30 min, where the rest of the microbiological procedures were done.

One aliquot of each batch of milk was incubated indoors at TDA as follows: Fall (Sept. 21-Dec. 20), room temperature (target temperature 22°C); Winter (Dec. 21-March 20), refrigerated (target temperature 4°C); Spring (March 21-June 20), room temperature (target temperature 22°C); Summer (June 21-Sept. 20), incubated (target temperature 37°C). The other aliquot of each batch of milk was incubated outdoors at TDA in a plastic tub with a clear top with a thermometer placed in the milk and therefore exposed to seasonal ambient temperature. Subsequent milk samples were aseptically collected at 1, 2, 3, 4, 5, 6, 7, 8, and 24 h post-pasteurization. Temperature was recorded at each time point when milk was sampled. For some batches, additional samples were collected at 10 and 12 h post-pasteurization.

Bacteria counts

Bacterial concentrations in milk samples were enumerated using Aerobic Count Plate Petrifilm to determine SPC according to Standard Methods [6]. Each milk sample (1 ml inoculum) was pipetted onto Petrifilm at different dilutions – this had also been done with the pre-pasteurization and 0 h samples inoculated on the farm as described earlier - in Butterfield's Buffer (3M Microbiology, St. Paul, MN) according to logical expectations of the range of bacteria counts expected: pre-pasteurized samples - undiluted, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}; 0 to 12 h post-pasteurization - undiluted, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}; 24 h post-pasteurization - undiluted, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}. However, during the summer, as SPC were increasing to higher levels, as time progressed since pasteurization, subsequent dilutions of up to 10^{-10} were made. There were no samples where the highest dilution resulted in colonies too numerous to count.

Statistical analysis

Systat 13 was used for statistical testing. The SPC were transformed to the log of SPC (LogSPC) for analyses, and then reverse-transformed to raw SPC for some descriptive statistics. Analysis of variance (ANOVA), and if significant differences were indicated, Tukey's multiple comparison test were used to evaluate differences in means of continuous variables (LogSPC, temperature) among categorical variables (Season, Farm, indoor or outdoor incubation) at each different time relative to pasteurization. General linear models (GLM) evaluated the association of potential explanatory variables with the continuous outcome variable of interest, LogSPC. The initial general linear model (PROC GLM) testing for factors associated with LogSPC was:

$$Y=Time+Season+Farm+Incubation+Time\times Season+Time\times Farm+Time\times Incubation+Season\times Farm+Season\times Incubation+Farm\times Incubation + Time\times Season\times Farm + Season\times Farm\times Incubation + Time\times Season\times Farm\times Incubation+e$$

Where Y=LogSPC; Time=hr relative to pasteurization (where 0=immediately post-pasteurization, 1 to 24 hr=post-pasteurization); Season=Fall, Winter, Spring or Summer; Farm=farm A, B or C; Incubation=Indoor or Outdoor; Time×Season, etc.=interaction terms, whether one factors' effect on Y is associated with another factor e.g. whether LogSPC increases faster during Summer than during other seasons, and *e*=unexplained variation.

Non-significant variables were sequentially removed until all significantly associated explanatory variables remained in a final model. Level of statistical significance used was $\alpha=0.05$.

Results

The mean, median and range of incubation temperatures for the post-pasteurized milk samples during the seasons were as follows: Fall, indoor (n=27) 23°C, 23°C, (21 to 26°C), ambient outdoors (n=27) 8°C, 8°C (-5 to 17°C); Winter, indoor refrigerated (n=33) 6°C, 6°C (2 to 10°C), ambient outdoors (n=33) 8°C, 9°C (-5 to 25°C); Spring, indoor (n=33) 18°C, 17°C (14 to 19°C), ambient outdoors (n=33) 10°C, 9°C (5 to 25°C); Summer, indoor in incubator (n=33) 37°C, 37 °C (35 to 39°C), ambient outdoors (n=33) 30°C, 28°C (18 to 43°C). The mean indoor (room temperature during Fall and Spring, refrigerated during Winter, incubated during Summer) temperatures were all significantly different among the 4 seasons ($P<0.0001$, ANOVA, Tukey's). The mean ambient outdoor temperatures were not significantly different during Fall, Winter or Spring, but Summer had significantly higher outdoor temperatures (mean 30°C) than each of the other 3 seasons ($P<0.0001$, ANOVA, Tukey's, Tables 3-6).

The LogSPC (in cfu/ml) (n=12; 3 farms, 4 seasons) immediately before pasteurization were compared by farm and season; there were no significant differences in pre-pasteurized LogSPC between farms ($P=0.99$, ANOVA) or seasons ($P=0.12$, ANOVA). The mean pre-pasteurized LogSPC during the 4 seasons were as follows: Fall 7.8, Winter 12.7, Spring 14.4, and Summer 12.6.

The change in LogSPC (n=24; 3 farms, 2 time points, 4 seasons) from pre-pasteurization (-1 hr) to post-pasteurization (0 hr) was analyzed. Mean LogSPC decreased significantly after pasteurization ($P<0.0001$, ANOVA). The most significant decreases in (reverse transformed) raw SPC following pasteurization were during Winter from 439,000 cfu/ml to 809 cfu/ml (Table 4) and during Spring from 1,749,167 cfu/ml to 1194 cfu/ml (Table 5) ($P<0.05$, Tukey's).

The final GLM to test for factors associated with LogSPC (n=285)

Table 1: General linear model estimated effects of significant factors on log of bacteria count (LogSPC in cfu/ml) in milk following pasteurization.

Parameter	Estimated Effect§
Intercept	5.82
Time ¥	0.13
Fall†	-2.95
Summer	2.83
Farm A‡	-0.54
Time*Summer£	0.24
Farm B*Spring€	1.39
Farm A*Summer	1.15

§ Estimated effect on log of SPC bacteria count (log of cfu/ml) in milk
¥ Effect of each increase in 1 hr of time since pasteurization
† Effect of season (Winter is baseline season)
‡ Effect of farm (Farm C is baseline farm)
£ Interaction - Effect of each increase in 1 hr of time since pasteurization during each season; SPC increased more with each hr since pasteurization during Summer (Winter is baseline)
€ Interaction – effect of season on a specific farm (Farm C is baseline farm)
R^2=0.71, P<0.0001

Table 2: General linear model tests of significance for effects on log of bacteria count (LogSPC in cfu/ml) in milk following pasteurization (see Table 1 for explanation of Time, Season and Farm).

Source	Type III SS	degrees of freedom	Mean Squares	F-ratio	P-value
Time	203.53	1	203.53	39.0	<0.0001
Season	586.81	3	195.60	37.5	<0.0001
Farm	65.43	2	32.72	6.3	0.002
Time*Season£	303.67	3	101.22	19.4	<0.0001
Season*Farm€	524.50	6	87.42	16.7	<0.0001
Error	1410.24	270	5.22		

£ Interaction - effect of each increase in 1 hr of time since pasteurization within each season; see Table 1 for estimated effects
€ Interaction – effect of season on a specific farm; see Table 1 for estimated effects
R^2=0.71, P<0.0001

was highly explanatory for variation in LogSPC (R^2=0.71), and highly significant (P<0.0001). This final GLM for LogSPC included Time since pasteurization, Season, Farm, the interaction of Time and Season, and the interaction of Season and Farm. The model showed that passage of Time was associated with increased LogSPC, especially during Summer, that Summer alone was associated with higher log of bacteria count, and that these effects were especially observed on Farm A. Also, Spring was associated with relatively higher log of bacteria count on Farm B than on other farms, despite the overall highest increase in LogSPC during Summer (Tables 1 and 2). Incubation, whether aliquots of samples were incubated indoors or outdoors, was not significantly associated with LogSPC.

The changes in raw SPC after pasteurization during each season are shown in Tables 3-6. In Fall, for the samples incubated indoors (at room temperatures centered around 23°C), SPC (mean of 3 farms) did not increase above 20,000 cfu/ml until after the 8 h sample (7,796 cfu/ml), increasing to 2,121,818 cfu/ml with a median of 206,364 cfu/ml by the time of the next sample at 24 h. The Fall outdoor samples were incubated at ambient temperatures centered around 8°C and never had SPC greater than 37 cfu/ml for 24 h (Table 3).

In Winter, all samples both refrigerated indoors and incubated outdoors were at cool temperatures (centered around 7°C). After a mean raw SPC of 809 cfu/ml 1 hr post-pasteurization, no subsequent SPC were greater than 228 cfu/ml for 24 h (Table 4).

In Spring, for the samples incubated indoors (at room temperatures

Table 3: Standard plate counts for bacteria in pasteurized milk samples over time post pasteurization by incubation method and hourly temperature for Fall season. Each value is the mean for 3 samples, one from each study farm.

Time (h) relative to pasteurization	Standard Plate Count (cfu/ml)	Incubation	Temperature (°C)
-1§	6861	Farm	NR
0£	40	Farm	13
1	59	Indoor¥	24
2	25	Indoor	24
3	23	Indoor	24
4	30	Indoor	23
5	38	Indoor	23
6	91	Indoor	23
7	6068	Indoor	23
8	7796	Indoor	23
24	2,121,818†	Indoor	22
1	28	Outdoor	5
2	20	Outdoor	6
3	24	Outdoor	8
4	35	Outdoor	9
5	32	Outdoor	9
6	31	Outdoor	11
7	29	Outdoor	11
8	37	Outdoor	11
24	25	Outdoor	1

§-1 indicates immediately pre-pasteurization; raw milk on the farm
£0 indicates immediately post-pasteurization
NR=Not recorded
¥-Indoor incubation was at room temperature during Fall
†-Median=206,364 cfu/ml

Table 4: Standard plate counts for bacteria in pasteurized milk samples over time post-pasteurization by incubation method and hourly temperature for Winter season. Each value is the mean for 3 samples, one from each study farm.

Time (h) relative to pasteurization	Standard Plate Count (cfu/ml)	Incubation	Temperature (°C)
-1§	439,000	Farm	8
0£	809	Farm	43
1	103	Indoor¥	7
2	123	Indoor	6
3	137	Indoor	6
4	163	Indoor	6
5	157	Indoor	6
6	172	Indoor	5
7	185	Indoor	5
8	177	Indoor	5
10	181	Indoor	5
12	163	Indoor	5
24	173	Indoor	5
1	150	Outdoor	2
2	121	Outdoor	5
3	132	Outdoor	7
4	147	Outdoor	9
5	173	Outdoor	10
6	180	Outdoor	13
7	168	Outdoor	14
8	150	Outdoor	13
10	163	Outdoor	13
12	228	Outdoor	7
24	148	Outdoor	2

§-1 indicates immediately pre-pasteurization; raw milk on the farm
£0 indicates immediately post-pasteurization
¥ Indoor incubation was refrigeration during Winter

Table 5: Standard plate counts for bacteria in pasteurized milk samples over time post-pasteurization by incubation method and hourly temperature for Spring season. Each value is the mean for 3 samples, one from each study farm.

Time (hr) relative to pasteurization	Standard Plate Count (cfu/ml)	Incubation	Temperature (°C)
-1§	1,749,167	Farm	NR
0£	1194	Farm	48
1	1323	Indoor¥	17
2	1780	Indoor	18
3	1434	Indoor	18
4	1747	Indoor	18
5	1609	Indoor	18
6	1340	Indoor	17
7	1854	Indoor	17
8	1210	Indoor	17
10	1699	Indoor	17
12	1904	Indoor	17
24	9,234,633	Indoor	18
1	1349	Outdoor	7
2	1353	Outdoor	7
3	1435	Outdoor	11
4	1212	Outdoor	12
5	1405	Outdoor	10
6	1291	Outdoor	10
7	1255	Outdoor	10
8	1096	Outdoor	10
10	1127	Outdoor	15
12	1299	Outdoor	10
24	992	Outdoor	8

§ -1 indicates immediately pre-pasteurization; raw milk on the farm
£0 indicates immediately post-pasteurization
NR=Not recorded
¥-Indoor incubation was at room temperature during Spring

centered around 18°C), raw SPC mean did not increase above 20,000 cfu/ml until after the 12 h sample (1,904 cfu/ml), increasing to 9,234,633 cfu/ml with a median of 8,900,000 cfu/ml by the time of the next sample at 24 h. The Spring outdoor samples were incubated at ambient temperatures centered around 10°C and never had SPC greater than 1435 cfu/ml for 24 h (Table 5).

In Summer, for samples incubated indoors (at temperatures centered around 37°C), raw SPC was greater than 20,000 cfu/ml by 4 hr (186,800 cfu/ml) and increased markedly after that, reaching a mean of 200,005,443,333 cfu/ml and median of 14,500,000 cfu/ml by 24 hr. Outdoor samples were incubated at ambient temperatures centered around 30 °C. Standard plate count was greater than 20,000 cfu/ml by 3 hr (34,200 cfu/ml) and increased markedly after that, reaching a mean of 66,075,516,061 cfu/ml with a median of 44,000,000 cfu/ml by 24 hr (Table 6).

Discussion

While pasteurized milk is increasingly fed to calves on dairy farms or dairy heifer raising operations, there is currently no agreement upon "safety threshold" for bacteria counts above which milk should not be fed to calves [5]. Others have speculated that this number is between 20,000 and 100,000 cfu/ml, with no published direct evidence regarding health effects on calves. Some recommendations have been made previously that only milk with bacteria counts less than 20,000 cfu/ml should be fed to calves, but it was observed that less than 2% of farms could meet this goal [5]. A primary reason for the suggested limit of 20,000 cfu/ml of bacteria in calf milk is extrapolation from the United States Pasteurized Milk Ordinance which states that it is the upper limit for safety of milk consumed by humans [7]. Another recent study reported that of 147 batches of pasteurized milk intended for feeding to calves on 6 dairy farms, 21% of the batches had SPC>20,000 cfu/ml after pasteurization [1].

Under conditions of the study reported here (except on days with unseasonably warm temperatures, which did not occur during the study period), milk could have been safely fed to calves - SPC<20,000 cfu/ml-during fall for at least 8 hr post-pasteurization, during spring for at least 12 h, and during winter for at least 24 hr. During summer, it was important whether pasteurized milk was stored outside, as is common on farms, or whether it could be stored completely indoors at room temperature between 18 and 23°C (or cooler). During summer, outdoor temperatures as would be expected in a northern temperate climate were markedly and statistically significantly higher than for the other seasons, and so was the growth of bacteria as time passed following pasteurization. Thus, in summer, milk stored outdoors could have been safely fed as defined by SPC<20,000 cfu/ml for only 3 h post-pasteurization, and by 4 h, feeding it would definitely not have been recommended. On most dairy farms, some milkings produce more discard milk than can be fed to calves at the next feeding, and therefore some milk is left over. Especially during summer, or in warm climates where temperatures exceed 30°C during other seasons of the

Table 6: Standard plate counts for bacteria in pasteurized milk samples over time post-pasteurization by incubation method and hourly temperature for Summer season. Each value is the mean for 3 samples, one from each study farm.

Time (h) relative to pasteurization	Standard Plate Count (cfu/ml)	Incubation	Temperature (°C)
-1§	56,342,000	Farm	NR
0£	8489	Farm	37
1	2370	Indoor¥	37
2	17,747	Indoor	37
3	17,140	Indoor	36
4	186,800	Indoor	36
5	1,054,900†	Indoor	36
6	1,739,333	Indoor	36
7	6,498,309‡	Indoor	37
8	43,400,600≠	Indoor	37
10	113,377,000	Indoor	38
12	141,300,000¶	Indoor	38
24	200,005,443,333±	Indoor	37
1	10,543	Outdoor	27
2	11,701	Outdoor	29
3	34,200	Outdoor	31
4	12,712	Outdoor	34
5	20,667	Outdoor	32
6	104,733	Outdoor	30
7	114,467	Outdoor	30
8	658,509	Outdoor	31
10	760,733	Outdoor	30
12	727,267	Outdoor	28
24	66,075,516,061€	Outdoor	23

§ -1 indicates immediately pre-pasteurization; raw milk on the farm
£0 indicates immediately post-pasteurization
NR=Not recorded
¥ Indoor incubation was at 37°C during Summer
† Median=2500 cfu/ml
‡ Median=220,000 cfu/ml
≠ Median=2,200,000 cfu/ml
¶ Median=3,400,000 cfu/ml
± Median =14,500,000 cfu/ml
€ Median =44,000,000 cfu/ml

year, for milk stored outdoors, any milk remaining after the first feeding following pasteurization should not be fed to calves at subsequent feedings; it should be re-pasteurized or discarded.

Further research is needed regarding health and growth effects on dairy calves of feeding milk at different levels of SPC. More precise guidelines regarding bacteria counts in post-pasteurized milk for calves can be developed when more is known about health effects of various levels of total bacteria in milk.

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