

DAIRY VETERINARY NEWSLETTER

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Colostrum - New Heat Treatment Data. How Can We Measure Colostral Quality on Farms?

Heat treatment and colostrum quality, a new study

Colostrum and milk fed to dairy calves become remarkably high in total bacteria count (cfu/ml) during summer weather unless they are refrigerated and/or fed very soon after collection. A paper that we published in 2012 examines the seasonal effect of milk kept at ambient temperature following pasteurization; the bacteria count rises to very high levels again in just a few hours during typical summer weather unless frozen or refrigerated.

<http://omicsonline.org/veterinary-science-technology.php> (search for “pasteurization”)

A new study reported in the March 2015 issue of J Dairy Sci by A. Kryzer et al. looks at heat treatment of bovine colostrum. This University of Minnesota study evaluated a particular commercial colostrum treatment system, but the paper and this newsletter article are not intended as a commercial endorsement. The randomized clinical trial took place on a commercial 6000 cow Jersey farm; all cows studied were Jerseys. (Presumably the results can be applied to Holsteins and other breeds.)

Within 2 h of calving, first-milking colostrum was collected and stored for 8 to 10 h refrigerated until a minimum batch of 22.8 L (6 gallons) was obtained. Each of 31 batches of harvested colostrum was pooled and mixed thoroughly. Duplicate 10 ml samples of fresh, raw colostrum were aseptically collected from each batch, labeled, and frozen at -20°C (initial batch). The batch was then divided, with 3.8 L (or 11.4 L for one treatment) allocated to each of 4 colostrum treatment groups:

Perfect Udder® system (bag heat treated): Colostrum put into a 3.8 L (1 gal) PU bag, heat-treated by floating in water in the Dairy Tech® (DT) 10 gal (38 L) batch pasteurizer at 60°C for 60 min. Following cooling to $\leq 30^{\circ}\text{C}$, duplicate 10 ml samples of heat-treated colostrum were aseptically collected, labeled, and frozen at -20°C . The bag of colostrum was then frozen at -20°C for at least 2 d before feeding. For feeding the bag was thawed in a hot water bath with water held at $48 - 51^{\circ}\text{C}$. After colostrum was warmed to feeding temperature ($37 - 40^{\circ}\text{C}$), the colostrum was thoroughly agitated and duplicate 10 ml samples of thawed

colostrum (prefeeding) were aseptically collected, labeled, and frozen at -20°C .

Dairy Tech® batch pasteurizer system (batch pasteurized): 11.4 L (3 gal) of colostrum, the batch pasteurizer's minimum capacity, was transferred into the DT 10 gal batch pasteurizer system and heat-treated at 60°C for 60 min. Following cooling to $\leq 30^{\circ}\text{C}$, duplicate 10 ml samples of heat-treated colostrum were aseptically collected, labeled, and frozen at -20°C . Colostrum (3.8 L) was then transferred to a PU bag and frozen at -20°C for at least 2 d before feeding. Thawing, warming ($37 - 40^{\circ}\text{C}$), and freezing (-20°C) of 10 ml prefeeding samples was the same as above.

Fresh frozen colostrum: Colostrum put into a 3.8 L PU bag. Duplicate 10 ml samples of fresh colostrum were aseptically collected, labeled, and frozen at -20°C . The bag of colostrum was then frozen at -20°C for at least 2 d before feeding. Thawing, warming ($37 - 40^{\circ}\text{C}$), and freezing (-20°C) of 10 ml prefeeding samples was the same as above.

Fresh refrigerated colostrum: Colostrum put into a 3.8 L PU bag. Duplicate 10 ml samples of fresh colostrum were aseptically collected, labeled, and frozen at -20°C . The bag of colostrum was then refrigerated at 4°C for no more than 2 d before feeding. No thawing was needed; warming ($37 - 40^{\circ}\text{C}$), and freezing (-20°C) of 10 ml prefeeding samples was the same as above.

Newborn Jersey female single birth calves that weighed at least 22.7 kg (50 lb), had no dystocia by calving ease score, had no obvious congenital abnormalities, and that were removed from the maternity pen within 30 min of birth before suckling were enrolled. Calves ($n = 124$, 31 calves per treatment group) were randomly assigned to the 4 treatment groups. A venous blood sample was collected immediately before colostrum feeding. After first colostrum feeding, calves were fed a commercial 22% protein:20% fat milk protein based milk replacer using a nipple bottle, 2 L twice at 12 h and 24 h. At 24 ± 1 h old, a second postfeeding blood sample was collected.

One of the duplicate frozen colostrum samples of initial batch, post-processing, and prefeeding samples from each batch was sent to a laboratory for culture to determine total bacteria plate count (TPC) and total coliform count (cfu/ml). The other sample went to a laboratory for IgG (g/L) measurement using radial immunodiffusion analysis. Sera were also sent to the second lab for serum IgG (mg/ml) and serum total protein (STP, g/dL) levels by radial immunodiffusion (RID) analysis.

Mixed linear regression analysis evaluated factors to see whether each was significantly associated (accounting for the influence of other factors as well) with several outcome variables (different model for each outcome variable): colostrum IgG, TPC, and TCC; and calf serum IgG and STP. Besides colostrum treatment method, other factors tested for association with those outcomes included dam parity, calf birth weight, calving ease score (1–3; as noted previously no dystocia cases with scores of 4 or 5 were included), time (min) at colostrum feeding, and batch number (1–31). Contrast analysis compared each of the 4 treatment colostrum groups to each other. Significance level (α) was whether $P < 0.05$.

Results

One colostrum batch had one missing sample collection and was omitted. The other 30 batches were analyzed. Dam, calf and calving ease measures were not different among treatment groups, and presumably batch number did not affect colostrum results because it was not mentioned again.

Initial batch, post-treatment, and prefeeding IgG (mean 78 g/L) were not different among the 4 colostrum

treatment methods; heat treatment did not affect IgG. Initial bacterial TPC (mean=199,526 cfu/ml) and coliform TCC counts (6,309 cfu/ml) were not different among milks prior to the 4 treatment methods.

Post-treatment TPC was significantly lower for batch pasteurized colostrum (3,981 cfu/ml), next lowest for bag heat treated (15,849) but was significantly higher and unchanged from initial TPC of 199,526 for fresh frozen and fresh refrigerated colostrum. Prefeeding TPC was also significantly lower for batch pasteurized colostrum (3,981 cfu/ml), next lowest for bag heat treated (15,849), but had increased for fresh frozen (398,107) and even more so for refrigerated colostrum (3.2 million). Coliform TCC had a similar pattern, except that the lowest prefeeding levels were for bag heat treated and next lowest for batch pasteurized colostrum. Higher prefeeding TCC was again found in frozen colostrum and the highest concentration was in refrigerated colostrum.

Despite observation of calves not having nursed, 11 calves were omitted because they had prefeeding serum IgG >1.0 mg/mL; 3, 3, 2 and 3 calves were excluded from analysis from the treatment groups. (Data was also analyzed with these 11 calves included, and did not affect results so there was no selection bias.) Before colostrum feeding, serum IgG and STP concentrations were not different among the 4 treatment groups' calves.

Because IgG concentration and volume (3.8 L; 1 gal) of colostrum fed to each calf was not different, the total mass of IgG fed (mean 296.3 g) was not significantly associated with colostrum heat treatment. However, the 24 h postfeeding serum IgG and STP were significantly higher in calves fed bag heat treated (41.0 mg/ml, 6.96 g/dL) and batch pasteurized colostrum (40.6, 6.80) than for calves fed fresh frozen (31.9, 6.46) or refrigerated colostrum (32.3, 6.51). IgG absorption was apparently enhanced with the heated colostrum.

The authors made the following practical points: It took 60 min in a water bath at 48 to 51°C to thaw 3.8 L (1 gal) of frozen colostrum in the PU bag to a feeding temperature of 37 to 40°C, but warming refrigerated colostrum in the PU bag to feeding temperature took approximately 20 min. As the authors say, this is important because underheating or overheating cause problems. Damage to the oral, esophageal, or gastric mucosa results when colostrum is too hot when fed. "Because heating to above 61°C is known to damage colostrum IgG, we recommend using a hot water bath set at 48 to 51°C to create a large margin of safety."

The authors concluded, "Heat-treating colostrum at 60°C for 60 min, using either the Perfect Udder bag or batch pasteurizer system, resulted in a significant reduction of colostrum bacterial counts while maintaining colostrum IgG concentrations compared with fresh frozen or fresh refrigerated colostrum. - - When fresh colostrum was stored in a refrigerator, significant bacterial growth occurred even when the average storage time was less than 24 h. Storing colostrum in the freezer prevented significant bacterial growth and did not negatively affect colostrum IgG concentrations."

Another take home point is that this adds to the evidence that we can call colostrum pasteurization "heat treatment" because people have been concerned that "pasteurization" damages colostrum. Nevertheless, it is really the same thing except that pasteurization is not always done for as long as 60 min. Heating discard milk or colostrum to 60°C (140°F) for 60 min before feeding to calves should be adopted on dairy farms.

Measuring colostrum quality on the farm

Interest in refractometry vs. colostrometer measurement of colostrum quality is not new. Another paper in the March 2015 issue of J Dairy Sci by A.L. Bartier et al. reports on a study comparing colostrometer and refractometer measurements of IgG with those by radial immunodiffusion (RID) analysis. In Alberta,

Canada 13 dairy farms collected 569 individual cow samples of colostrum, frozen at -20°C on farms and then at -80°C at the University of Calgary.

After thawing, specific gravity was measured using a JorVet Bovine Colostrometer®. “The colostrometer was gently floated in the sample until it came to rest and IgG concentration was measured at the meniscus.” Total solids were also measured with a digital Brix® refractometer. “2 to 3 drops of colostrum were placed on the measurement prism and left for 1 min to allow for temperature adjustment before the reading was taken.” For IgG measurement, samples were sent to another laboratory for RID. Radial immunodiffusion analysis IgG concentration of 50 mg/ml was defined as the threshold for adequate quality colostrum as has been reported by others.

Statistical analysis included calculation of correlation between the continuous variables of IgG concentration by colostrometer and by RID and between those by refractometer and by RID. Also a threshold to define adequate colostrum IgG was sought for both colostrometer and refractometer IgG measurement to maximize sensitivity (defined as % of poor quality colostrum samples according to RID that were tested as poor by colostrometer or refractometer) and specificity. Specificity was defined as % of adequate colostrum samples according to RID that were tested as adequate by colostrometer or refractometer. (I would have considered adequate IgG as the target to be detected, so my definition of sensitivity (% of adequate samples correctly tested as adequate) and specificity (% of poor quality samples correctly tested as poor) would have been reversed. However, the authors explained their criteria and statistically they are just as correct. A multivariable model tested whether parity, month of colostrum production or farm were associated with IgG concentration.

Results

Because of reasons related to another study, only 460 of the 569 cows' colostrum samples were tested for IgG by RID; therefore the comparisons could only be done on 460 samples. Month of calving was not significantly associated with IgG and farm had little association. Cows in 3rd-plus lactation had higher colostrum IgG (69.5 mg/ml) than 1st lactation cows (62.2 mg/ml) or 2nd lactation cows (59.8 mg/ml) ($P < 0.02$). Of all colostrum samples, 29% were of poor quality (RID IgG < 50 mg/ml). The % of poor samples by parity was not shown.

Correlations between RID and the two on-farm methods were reported primarily as r values. However, r^2 - or R^2 from a model such as this study's model - explain what fraction of the variation in the outcome variable of interest (IgG) is explained by one or more other factors and are generally of more interest. I always want to know r^2 or R^2 . The colostrometer r^2 was 0.59 and the refractometer r^2 was 0.41 and its R^2 was 0.43 in the model (the only additional factor was apparently the weak influence of farm of origin).

The authors did a good job of presenting the effect of changing the cut-point (threshold) used to define adequate vs. poor colostrum using the colostrometer or the refractometer (% Brix). As with all continuous measurements, changing the threshold used to define a disease or condition affects specificity in one direction and sensitivity in the other direction; one increases while the other decreases. They did something that is very common today in evaluating diagnostic tests: “Sensitivity and specificity were summed, and the highest combined value was used to determine the appropriate cut-points for the colostrometer and Brix refractometer.” I don't think that is the best way to use most diagnostic tests for most purposes. I like to maximize specificity (avoidance of false positives) most of the time. (Sometimes I like to maximize sensitivity when false positives are less of a concern than missing true positive cases.) In this case, I would like to set the threshold so that if the colostrometer or refractometer says the colostrum is adequate, I would

like that to be almost certainly correct. I would have used a % Brix threshold of 25% instead of the authors' chosen level of 23%. That improves the probability that we don't mistakenly call inadequate colostrum samples adequate to 82% instead of 66%. I would also have used a colostrometer threshold of 90 mg/ml instead of the authors' chosen level of 80 mg/dl. That improves the probability that we don't mistakenly call inadequate colostrum samples adequate to 93% instead of 84%. You can see the details for yourself in the original paper. I want to be sure to state that I thought this was a good paper.

The r^2 and R^2 values reported were not extremely high (this has been reported consistently by other studies), indicating that there is some inaccuracy in either colostrometer or refractometer IgG measurement. Nevertheless both are practical. In my experience, I think a colostrometer is easier to use and in this study it had a stronger relationship to actual colostrum IgG levels. Dairy farm personnel should use a colostrometer on all batches of colostrum (assuming they are not going to run RID for IgG which I have never heard of a farm doing) and should especially not assume that cows calving with their first calf have high quality colostrum.

Pasteurizing Waste Milk For Calves - Is There Yet Another Reason to Do It?

We have heard a lot regarding pasteurization of waste milk fed to dairy calves. I think it makes a lot of sense, particularly during warm weather. (Farms that pasteurize calf milk properly never want to go back to not doing it during any season.) However, my colleague Dr. Greg Goodell, who among his many professional activities is involved in heifer raising, and I have discussed what we think would be a difficult study that is needed. Feeding one group of dairy calves low bacteria count milk (consider the logistics and cost of trying to do that when it takes 2 days to get a bacteria count result from just before each batch was fed) and another group of similar calves high bacteria count milk (same challenge regarding getting the bacteria counts) for the entire preweaning period. What about just simply feeding pasteurized vs, raw milk? In my experience you can't be completely sure what kind of bacteria count differences you will have without measuring them, although you can be pretty sure that during summer, and many times during every season, they will be large. I think this study would need to monitor the bacteria count of each batch fed every day and in addition to time, it would be costly. Mortality, disease and growth data would be compared between the 2 calf groups. Would a comparison of the calves' subsequent first lactation milk production and disease be valid? I'm not sure. At any rate, we have no evidence that I have seen regarding the direct benefit of reducing bacteria counts in waste milk from hundreds of thousands to trillions of cfu/ml to less than 10,000 in summer and around 1,000 in other seasons. Also, what if one group of calves drinks milk with for example a mean and median bacteria count around 10,000 cfu/ml and another group drinks milk around 100,000 and another around 1,000,000? We don't really know. It seems inherently logical that lower bacterial load would be better and empirical observations on farms suggest that.

Now a new possible reason to pasteurize waste milk for calves has been advanced in an article in the May-June 2015 Bovine Veterinarian by R. Van Vleck Pereira, DVM. What about development of drug resistant bacteria in calves fed waste milk?

Waste milk samples in New York state were tested and the most common antibiotics found were not considered surprising by the article's author: ceftiofur, penicillin G, and ampicillin (number of samples and %'s positive not stated). Concentrations in milk were low, subtherapeutic. Calves fed 1 gal of milk twice daily until 6 weeks old were randomly assigned to be fed raw milk test-negative for drug residues or raw milk spiked with ceftiofur, penicillin, ampicillin and oxytetracycline mimicking on-farm waste milk. I do not think spiking milk with antibiotics is the same pharmacologically as finding those antibiotics in milk as metabolized by a treated cow, but this was of course a lot easier to control with precision. After 1 week of

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feeding, the percentage of weekly fecal samples containing *E. coli* that was resistant to ampicillin, ceftiofur, cefotoxin, streptomycin, tetracycline, and apparently tested in some calves, ceftriaxone, was significantly higher in the calves fed antibiotic-spiked milk. I found it interesting that the % of multiple resistant *E. coli* in calf feces went down from weeks 2 through 6 for both groups. With constant feeding of spiked milk, it seems strange to me that resistance % in fecal bacteria went down. Nevertheless, by week 6 multiple resistance remained at approximately 80% in the antibiotic fed group and 10% in the antibiotic-negative group. There was a good discussion of how low-level antibiotics may actually enhance the development of multiple antibiotic resistant bacteria. So the author asked, “What to do with waste milk?”

Pasteurization may actually degrade antibiotics in milk. I did a literature search on this subject and could not find anything. The article states that, “ β lactam drugs have been shown to biodegrade when exposed to high temperatures”. So the author speculates that pasteurization may not only reduce bacterial load in milk from treated cows fed to calves, but the antibiotics contributing to resistant bacterial selection may be reduced as well. The conclusion: “More studies are needed on options for biodegrading drug residues in [discard] milk to concentrations that will not result in selection of resistance [if fed to calves].”

Please let us know your comments and also suggestions for future topics. I can be reached at (435) 760-3731 (Cell), (435) 797-1899 M-Tues, (435) 797-7120 W-F or David.Wilson@usu.edu.



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