New Zealand Veterinary Journal

ISSN: (Print) (Online) Journal homepage: www.tandfonline.com/journals/tnzv20

The effect of three different preservatives on the numbers and types of bacteria, Brix percentage, pH and nutritional composition of bovine colostrum sourced from New Zealand dairy farms

EL Cuttance, WA Mason, S Cranefield & RA Laven

To cite this article: EL Cuttance, WA Mason, S Cranefield & RA Laven (02 Sep 2024): The effect of three different preservatives on the numbers and types of bacteria, Brix percentage, pH and nutritional composition of bovine colostrum sourced from New Zealand dairy farms, New Zealand Veterinary Journal, DOI: 10.1080/00480169.2024.2392686

To link to this article: https://doi.org/10.1080/00480169.2024.2392686

Published online: 02 Sep 2024.

NEW ZEALAND VETERINARY JOURNAL
https://doi.org/10.1080/00480169.2024.2392686

Taylor & Francis
Taylor & Francis Group

RESEARCH ARTICLE

OPEN ACCESS Check for updates

The effect of three different preservatives on the numbers and types of bacteria, Brix percentage, pH and nutritional composition of bovine colostrum sourced from New Zealand dairy farms

EL Cuttance [a], WA Mason [a], S Cranefield[b] and RA Laven [c]

[a]EpiVets, Te Awamutu, New Zealand; [b]AgriHealth, Grafton, New Zealand; [c]Tāwharau Ora – School of Veterinary Science, Massey University, Palmerston North, New Zealand

ABSTRACT

Aims: To investigate the effect of preservation by addition of yoghurt starter, potassium sorbate and citric acid on counts of aerobic bacteria, *Lactobacillus* spp., *Streptococcus thermophilus* and coliforms, Brix percentage, pH, protein, fat and anhydrous lactose concentrations at 0, 7 and 14 days after collection for colostrum stored at ambient temperature.

Method: Approximately 2 L of first milking colostrum was collected from 10 farms in the Waikato region. Following mixing, it was split into five 400-mL sub-samples and allocated randomly to a control (two sub-samples), or treatment with yoghurt, potassium sorbate, or citric acid preservative. Throughout the trial samples remained in the laboratory at ambient temperature with the lids slightly ajar, and were stirred daily for 15–30 seconds using a sterile spatula. Sub-samples were tested on Days 0, 7 and 14. On Days 0 and 14 aerobic bacteria (by aerobic plate count (APC)), *Lactobacillus* spp., coliforms and *Streptococcus thermophilus* counts, pH, Brix percentage, protein, fat and anhydrous lactose were measured. On Day 7 only bacterial counts were completed.

The data were analysed using non-parametric clustered bootstrap sampling to estimate the effect of treatment, time, and their interaction on the outcome variables.

Results: Compared to control samples, on Day 7 the APC for potassium sorbate (1.0 (90% CI = 0.6–1.6) × 10^8 cfu/mL) was approximately seven-fold lower than for yoghurt (7.3 (90% CI = 4.1–11) × 10^8 cfu/mL), and approximately three-fold lower than citric acid (3.2 (90% CI = 0.2–4.3) × 10^8 cfu/mL) remaining low to Day 14. All preservatives reduced coliform growth compared to control samples at Day 7 but growth was lower for potassium sorbate than the other preservatives. For *Lactobacillus* spp., at Day 7, samples with yoghurt preservative had greater counts than the other two preservatives. Potassium sorbate reduced growth of *S. thermophilus* compared to the other treatments, especially at Day 7, with 7–10 times fewer *S. thermophilus* per mL compared to the other three groups. All groups showed an obvious acidification over time, with very little variation within days and treatment groups. There was no evidence for change in fat or protein percentage over time regardless of treatment.

Conclusion and clinical relevance: Aerobic and coliform bacteria proliferate extensively in unpreserved colostrum. All preservatives decreased coliform counts compared to un-preserved colostrum, but potassium sorbate was more effective at decreasing both coliforms and aerobic bacteria than either yoghurt or citric acid.

Abbreviations: APC: Aerobic plate count; MPCA: Milk plate count agar.

ARTICLE HISTORY
Received 8 March 2024
Accepted 31 July 2024

KEYWORDS
Colostrum; preservation; bacteria; pH; Brix percentage; protein; fat; anhydrous lactose

Introduction

Colostrum is the milk from the first milking of mammals following birth (Davis *et al.* 2007; Christiansen *et al.* 2010). Colostrum has a unique nutritive profile, and in addition to protective concentrations of Ig, contains a higher concentration of fats, proteins and more vitamins than normal milk (Godden *et al.* 2019). Colostrum is thus a high-energy feed source for calves and, because of the seasonal nature of calving on New Zealand dairy farms (with 50% of all dairy cows calving in August; Burggraaf *et al.* 2022), it is often in surplus. It is, therefore, regularly collected, pooled and stored for variable lengths of time to feed to neonatal calves (Cuttance *et al.* 2018).

In the authors' experience, the most common storage method used on New Zealand dairy farms is the collection of surplus colostrum from multiple dams (pooled colostrum) into stainless steel vats of varying sizes, although some farmers prefer plastic 200-L drums or 1000-L containers. Colostrum is usually stored at ambient temperature with a loose-fitting lid and stirred daily before being fed to neonatal

CONTACT R. A. Laven r.laven@massey.ac.nz

calves. This method of storage can lead to bacterial proliferation (Stewart *et al.* 2005) and a decrease in Ig content (as estimated using a Brix refractometer) (Denholm *et al.* 2017a).

Bacterial contamination is potentially problematic for two reasons: bacterial pathogens (e.g. *Escherichia coli* or *Salmonella* spp.) may act directly to cause diseases such as enteritis or septicaemia (Godden *et al.* 2012); or the presence of bacteria in the small intestine at the time of colostrum administration may interfere with systemic absorption of IgG molecules (James and Polan 1978; James *et al.* 1981; Staley and Bush 1985). Although field research on this topic is limited, the impact of bacteria on the absorption of Ig in calves has been demonstrated in several studies on heat treatment of colostrum (Godden *et al.* 2012; Gelsinger *et al.* 2014, 2015). Those studies showed that heat treatment of colostrum successfully decreased all types of bacteria and, in turn, increased plasma IgG concentration and apparent efficiency of absorption. In addition, Gelsinger *et al.* (2015) reported that, irrespective of whether the colostrum had been heat-treated or not, greater colostrum bacterial content was associated with lower total protein and plasma IgG concentrations, and reduced efficiency of IgG absorption. These results suggest that reducing the bacterial load and, thereby, its impact on colostrum IgG availability and IgG absorption is a critical part of an effective colostrum storage plan.

Refrigeration, freezing, additives such as potassium sorbate, fermentation and acidification have all been used to prevent the proliferation of potentially harmful bacteria in milk/colostrum (Anderson 2005; Stewart *et al.* 2005; Denholm *et al.* 2017a). However, not all methods of colostrum preservation will necessarily reduce all bacterial types. In particular, preservation of milk and colostrum via fermentation relies on proliferation of some types of bacteria (either naturally occurring or inoculated), to alter and preserve the colostrum (Foley and Otterby 1978), and thereby reduce the development of other groups of bacteria.

Denholm *et al.* (2017a) compared preservation using a yoghurt culture containing *Lactobacillus bulgaricus, Streptococcus thermophilus* and *L. acidophilus* to preservation with potassium sorbate. They concluded that, in contrast to fermentation using a yoghurt culture, the use of potassium sorbate resulted in a lower total bacterial count whether colostrum was stored at 4°C or at ambient temperature, and lower total coliform counts (but only in colostrum stored at 4°C). However, in their analysis of total bacterial counts, Denholm *et al.* (2017a) did not identify whether the difference in total bacterial counts between sorbate- and yoghurt-treated colostrum were related to increases in counts of the bacterial species that were originally present in the yoghurt culture. Simply measuring total bacterial counts without accounting for the growth of the added bacteria present in the yoghurt culture may have biased the analysis towards assuming the non-bacterial method of preservation (i.e. sorbate) was better.

Adding acids as preservatives to milk replacer and colostrum has long been common practice overseas (Jenny *et al.* 1980; Woodford *et al.* 1987). However, it wasn't until the identification of *Mycoplasma bovis* in New Zealand in July 2017 (Laven 2019) and the recognition that spread via colostrum/waste milk was important, that acids (specifically citric acid) were widely recommended as additives for colostrum/waste milk as a means of preventing the spread of *M. bovis* (Anonymous 2018). One crucial factor when using acids as preservatives for colostrum (or stimulating fermentation of colostrum) is pH. The pH is likely to influence taste and therefore consumption of colostrum (Jenny *et al.* 1980), and is an interesting point of comparison between different preservatives.

Therefore, the aims of this study were to investigate the effect of the common colostrum preservation methods used on New Zealand dairy farms via a commercial yoghurt culture, potassium sorbate, and citric acid, on counts of aerobic bacteria, *Lactobacillus* spp., *S. thermophilus* and coliforms as well as Brix percentage (as a measure of IgG), pH, percentage of protein and fat and lactose (measured as anhydrous lactose) concentration, at 0, 7 and 14 days after collection for colostrum stored at ambient temperature.

Materials and methods

The study was undertaken in a convenience selection of 10 spring-calving herds in the Waikato region of the North Island of New Zealand. On each farm, at their first milking, approximately 2 L of colostrum was collected from cows that had calved within the previous 24 hours and to which no preservative had been added. This is colloquially referred to as "gold colostrum" and is fed to new-born calves. The colostrum was stirred with a spatula for 2 minutes before the sample was placed into a sterile 2-L container labelled with the farm identification number and date of collection. Technicians picked up the sample from the farm within approximately 1–8 hours of collection and then sent it via an overnight courier to Massey University (Palmerston North, NZ). The sample was not refrigerated on-farm.

Once at the Massey University laboratory, the ~ 2 L colostrum sample was mixed thoroughly for 2 minutes using a spatula and then separated into 5 × 400-mL sub-samples, which were placed into new 900-mL plastic (factory clean) containers (Sistema, Auckland, NZ), which were labelled with herd

Table 1. Description of the preservation treatments for colostrum sub-samples from 10 spring calving herds in Waikato, New Zealand where on each farm colostrum was collected and pooled from cows within 24-hours of calving in a study investigating the effect of different additives on components of colostrum quality.

Sub-sample	Treatment	Description
1	No preservative added	
2	No preservative added	
3	Yoghurt preservative	One sachet (170 g) of natural yoghurt culture (Easiyo, Hokitika, NZ) was mixed with 1 L of warm water (30°C) and then allowed to stand at ambient temperature for 24 hours. 5 mL of this solution was added to the 400 mL sub-sample of colostrum.
4	Potassium sorbate preservative	A 50% solution was made by adding 50 g of potassium sorbate powder (VetPak, Te Awamutu, NZ) to 100 mL of water and stirring until it was all mixed. 4 mL of the 50% potassium sorbate solution was added to the 400 mL sub-sample of colostrum.
5	Citric acid preservative	3 g of citric acid powder (Hansells Baking, Alderley, Australia) was added to the 400 mL sub-sample of colostrum.

identification, date and time of sample collection (from farm), and date and time of sample analysis at the laboratory. Sub-samples were randomly allocated as control 1, control 2, or treated with a yoghurt preservative (EasiYo, Hokitika, NZ), potassium sorbate preservative (VetPak, Te Awamutu, NZ) or citric acid preservative (Hansells Baking, Alderley, Australia) as described in Table 1.

To mimic on-farm practice, samples remained in the laboratory for 14 days at ambient temperature (measured at 22 ± 2°C using a digital thermometer on the wall of a temperature-controlled room). The lids were kept slightly ajar throughout the trial to replicate a real-life scenario where colostrum is not kept in airtight containers, and the samples were stirred daily for 15–30 seconds to try and replicate on-farm practice. However, a new, sterile spatula was used each day to reduce the introduction of environmental bacterial contaminants.

Samples were analysed on Days 0, 7 and 14. On these days, a sterile pipette was used to withdraw 5 mL from the colostrum for testing. On Day 0, this sample was taken from the submitted 2-L sample prior to it being split into sub-samples. On Days 7 and 14 the required amount of colostrum was extracted from the sub-samples. On Days 0 and 14 samples were tested for bacterial count and colostrum quality, while on Day 7, only bacterial tests were undertaken.

Bacterial counts (Days 0, 7 and 14)

Aerobic plate count

Aerobic plate count (APC) was undertaken using milk plate count agar (MPCA; Oxoid, Auckland, NZ). Colostrum samples were diluted (1:10) in 0.1% buffered peptone water and aliquots (1 mL) were transferred to petri dishes and mixed with MPCA. Four plates (one aliquot per plate) were incubated per sub-sample: two were incubated at 30°C for 72 hours, and two at 55°C for 72 hours (to identify thermophiles). All colonies on plates containing 30–300 colonies were counted. Further serial dilution was undertaken when there were too many colonies to count. The mean count for each pair of duplicate samples was then used to calculate the cfu/mL, producing two means, one for APC (30°C) and one for the thermophiles (55°C).

Coliforms

Samples were plated as described for APC, except that MacConkey molten agar (Oxoid) was used instead of MPCA. Once set, plates were incubated aerobically for 24 hours at 35°C. Colonies were counted as described for APC.

Lactobacillus *spp.*

Samples were plated as described for APC, except that Rogosa agar (Oxoid) was used instead of MPCA. Once set, plates were incubated anaerobically for 3 days at 37°C. Colonies were counted as described for APC.

Streptococcus thermophilus

Samples were plated as described for APC, except that M17 agar (Oxoid) was used instead of MPCA. Once set, plates were incubated aerobically for 48 hours at 35°C. Colonies were counted as described for APC.

pH (Days 0 and 14)

The pH was measured as per the standard approach detailed in Downes and Ito (2001) using a pH meter (Seven Compact S220; Mettler Toledo, Hamilton, NZ).

Brix percentage (Days 0 and 14)

The Brix percentage was determined using a hand-held optical temperature-compensating Brix refractometer (Reichert Technologies, Depew, NY, USA). The Brix refractometer was calibrated prior to each day of measurements using a single drop of distilled water.

Nutritional composition (Days 0 and 14)

Fat was measured using a modified Mojonnier ether extraction method (AOAC Official Method 989.05; Anonymous 1992). In brief, fat was extracted from colostrum using a mixture of ethers from a known weight of colostrum (3–5 g). The ether extract was then decanted onto the weighing dish and the ether evaporated. Extracted fat was then dried to a constant weight, with the result expressed as percentage fat by weight. Crude protein was measured using the total Dumas combustion method (AOAC Official Method 968.06; Anonymous 1969). In brief, nitrogen was freed using a combination of pyrolysis and combustion from a known weight of colostrum (0.5–2.0 g). Nitrogen was then carried by a CO_2 carrier into a nitrometer, where CO_2 was absorbed by KOH and the residual volume of nitrogen measured and converted to % crude protein by weight. Lactose was measured using the Lactose/D-galactose UV-method kit (R-Biopharm, Darmstadt, Germany), with light absorbance measured at 340 nm using a TriStar^{2}S LB 942 Plate Reader (Berthold Technologies, Bad Wildbad, Germany). Results are expressed as g anhydrous lactose per 100 mL of colostrum.

Statistical analysis

All data manipulation and statistical modelling were carried out using R Version 4.1.0 (R Foundation for Statistical Computing, Vienna, Austria). Two separate groups of outcome variables describing colostrum quality were analysed: bacterial counts (count of APC, coliforms, *Lactobacillus* spp. and *S. thermophilus)*, and other colostrum measurements (pH, Brix percentage, and nutritional content: crude protein, fat and anhydrous lactose). The median and range of the 10 samples for all outcomes were reported on the Day 0 samples to ensure that there were not large differences between the sub-samples at the start of the study.

For all the outcomes there were repeated measurements for each colostrum sample collected; that is each bacterial count outcome for each sample had a result on Days 0, 7 and 14, and colostrum quality and nutritional content had a result on Days 0 and 14. This type of data is often analysed using generalised linear mixed models; however, the number of colostrum samples collected was small (n = 10) and it was thought unlikely that all outcomes, especially bacterial count, would approximate any distribution with this sample size (Feng *et al.* 1996). The data were therefore analysed using a non-parametric clustered bootstrap sampling technique (Deen and de Rooij 2020). This was implemented using the ClusterBootstrap package within R. Briefly, this method involves calculating a statistic (in this case a regression coefficient without assuming a particular distribution for the link function) by resampling, with replacement, from the data collected. Thus, a statistic is calculated that is an estimate of the study population, which in itself is an estimate of the total population. This bootstrap sample is then repeated *x* times calculating the same statistic each time, and a resulting distribution of said statistic is produced. This enables inferences such as CI to be calculated. For clustered data such as repeated measures from a colostrum sample, the sampled unit becomes the colostrum sample. That is, if a colostrum sample is randomly selected from within the sample population, all of the values (e.g. Day 0, 7 and 14) for that particular colostrum sample within the study population are selected. How this method accounts for clustering of data and temporal correlations of repeated measures is described in detail in Deen and de Rooij (2020).

Predictors of interest for each outcome were day of sample and treatment group. As one of the major research questions was how colostrum quality changes over time for each treatment group, an interaction term between day and treatment group was forced into all models. In the current dataset, 1,000 clustered bootstrap samples, with colostrum sample as the cluster, were produced for each outcome variable. The bootstrap statistics calculated were the coefficients for the predictor variables and the interaction terms from a linear regression model. A bias-corrected, accelerated CI was estimated for each coefficient. This adjusts for both bias and skewness in the bootstrap distribution (Deen and de Rooij 2020), and forms the non-parametric component of the inferences. Data are presented in tabular and graphical format as the estimated marginal means from each outcome.

For this study, we chose a 90% CI (see McElreath (2018) for a discussion on the choice of the percentile interval) to illustrate where, in this case, 90% of the estimators of our distribution calculated from the 1,000 random bootstrap samples lie. For example, the 90% CI of the mean coliform counts is the interval that covers 90% of the mean coliform counts calculated from the bootstrap samples. The inference from this is that we can be 90% confident that the estimated CI contains the true population mean. This is a more intuitive and biologically relevant measure than a p-value, which only states the probability of obtaining results at least as extreme as those shown by the data collected, given that the null hypothesis is correct.

Results

Bacterial counts

Total aerobic plate count (APC)

On Day 0 there was a median APC of 8.1×10^6 cfu/mL with a range between farms of $2,000–1.7 \times 10^8$ cfu/mL. The control group increased from 2.8×10^7 to 5.7 (90% CI = 3.7–7.6) $\times 10^8$ cfu/mL by Day 7, then decreased by Day 14 to 3.2 (90% CI = 1.1–5.7) $\times 10^8$ cfu/mL. Compared to all other groups, preservation with potassium sorbate was associated with a reduced APC (Figure 1(a)). On Day 7 potassium sorbate decreased APC by a factor of approximately seven compared to yoghurt (1.0×10^8 (90% CI =

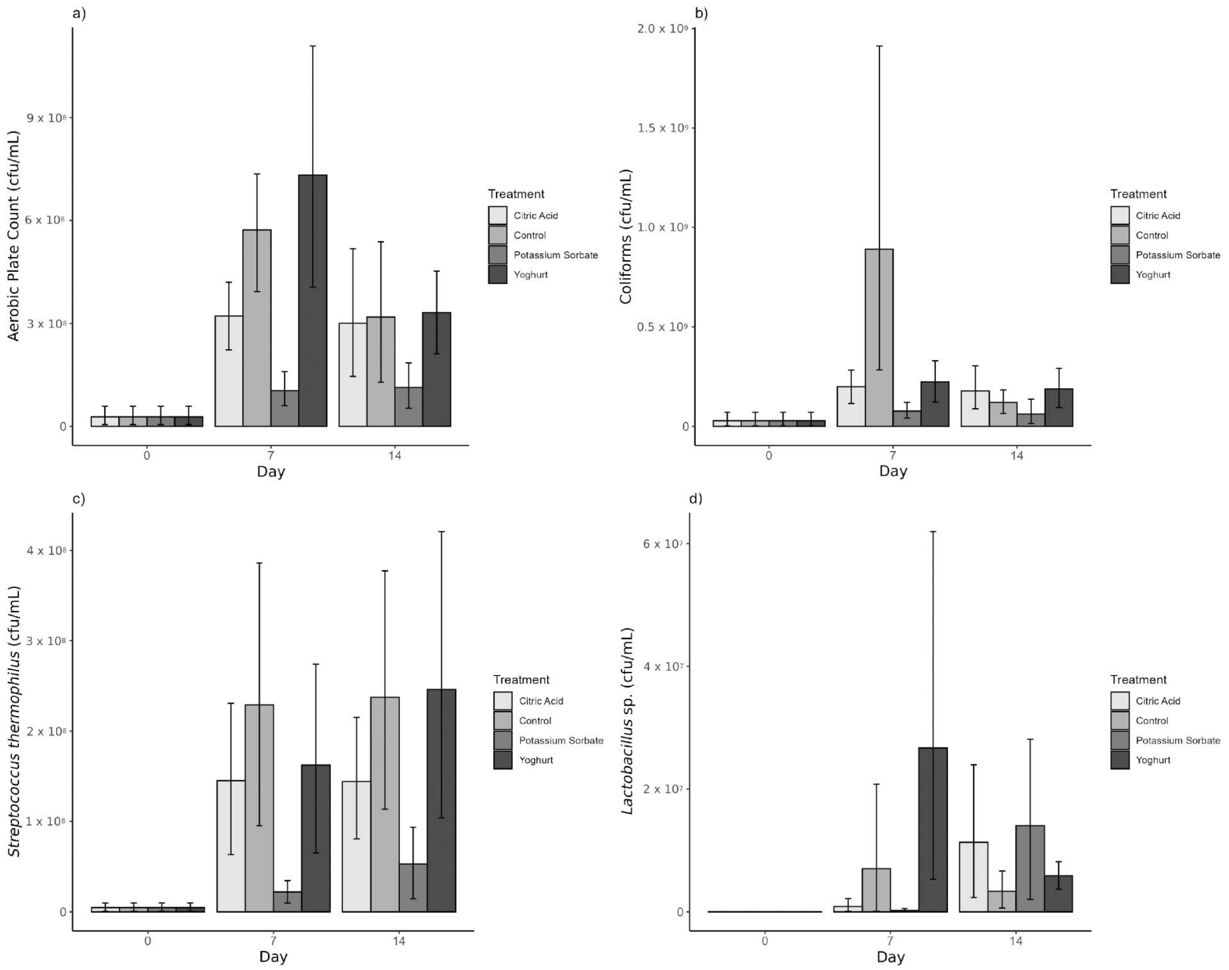

Figure 1. Mean (90% CI) estimated farm value for (a) aerobic plate count; (b) coliform count; (c) *Streptococcus thermophilus* count; and (d) *Lactobacillus* spp. count from colostrum collected and pooled within 24 hours of calving from spring-calving herds (n = 10) in Waikato, New Zealand, and treated with different preservation treatments (citric acid, yoghurt and potassium sorbate) or with no preservative (control) on Days 0, 7 and 14.

5.9×10^7–1.6×10^8) cfu/mL) *vs.* 7.3×10^8 (90% CI = 4.1×10^8–1.1×10^9) cfu/mL, and by a factor of approximately three compared to citric acid (3.2×10^8 (90% CI = 1.9×10^7–4.3×10^8) cfu/mL) and maintained low APC count up to Day 14. There was no clear difference in APC between yoghurt preservative and control at either Day 7 or 14 (Figure 1(a)).

Coliform count

The median coliform count at Day 0 was 4.3×10^7 with a range between farms of 460–2.2×10^8 cfu/mL. The number of coliforms in the control sample increased markedly between Days 0 and 7 from a predicted Day 0 count of 2.8×10^7 (90% CI = 3.1×10^6–6.8×10^7) cfu/mL to 8.9×10^8 (90% CI = 2.8×10^8–1.9×10^9) cfu/mL on Day 7. All preservative treatments reduced coliform growth compared to the control samples at Day 7. Mean coliform counts were lower on Day 7 for potassium sorbate than for the other two preservative groups; i.e. 7.6×10^7 (90% CI = 4.2×10^7–1.2×10^8) cfu/mL compared to 2.2×10^8 (90% CI = 1.2×10^8–3.3×10^8) cfu/mL for yoghurt and 2.0×10^8 (90% CI = 1.1×10^8–2.8×10^8) cfu/mL for citric acid (Figure 1(b)). However, by Day 14, this difference between the groups had reduced (Figure 1(b)).

Lactobacillus spp.

The median *Lactobacillus* spp. count was 125 cells/mL at Day 0, with a range between farms of 10–5,300 cells/mL. On Day 7, colostrum samples with a yoghurt preservative had greater counts than either of the other two preservatives. The yoghurt *Lactobacillus* count at Day 7 was 2.7×10^7 (90% CI = 5.3×10^6–6.2×10^7) cfu/mL compared with 8.6×10^5 (90% CI = 8.1×10^4–2.1×10^6) cfu/mL for citric acid, and 2.2×10^5 (90% CI = 1.6×10^4–5.3×10^5) cfu/mL for potassium sorbate. No evidence of a difference was observed between yoghurt preservative and control colostrum in *Lactobacillus* spp. counts. By Day 14, there was no clear evidence of a difference between any of the groups in *Lactobacillus* counts (Figure 1(c)).

Streptococcus thermophilus

There was a median 1.1×10^6 cfu/mL of *S. thermophilus* bacteria on Day 0 with a range from 110–3.0×10^7 cfu/mL between farms. Potassium sorbate reduced the

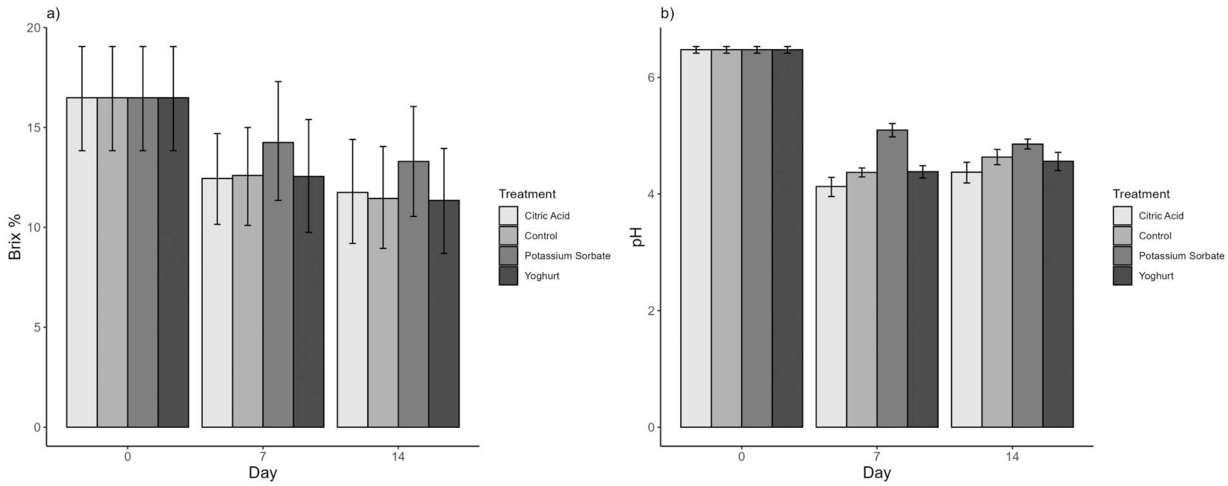

Figure 2. Mean (90% CI) estimated farm value for (a) Brix percentage; and (b) pH in colostrum collected and pooled within 24-hours of calving from spring-calving herds (n = 10) in Waikato, New Zealand and treated with different preservation treatments (citric acid, yoghurt and potassium sorbate) or with no preservative (control) on Days 0, 7 and 14.

growth of S. *thermophilus* compared to the other treatments, particularly at Day 7, where there were between seven and 10 times fewer *S. thermophilus* cells per mL compared to the other three groups (Figure 1(d)). The potassium sorbate S. *thermophilus* count at Day 7 was 2.2×10^7 (90% CI = 9.7×10^6–3.4×10^7) cfu/mL compared with 1.5×10^8 (90% CI = 6.3×10^7–2.3×10^8) cfu/mL for citric acid, 1.6×10^8 (90% CI = 6.5×10^7–2.7×10^8) cfu/mL for yoghurt, and 2.3×10^8 (90% CI = 9.5×10^7–3.9×10^8) cfu/mL for the control samples. No clear evidence of a difference was observed in the number of *S. thermophilus* counted in the colostrum preserved by yoghurt, citric acid or control.

Brix percentage

The mean Brix percentage at Day 0 was 16%, with a range between samples of 12–23.5%. Across all treatments, numerically Brix percentage decreased over time, with little observable difference between the treatment groups (Figure 2(a)). After accounting for treatment group, compared to Day 0 samples, Brix percentage reduced by 3.5% (90% CI = 2.9–4.3%) and 4.5% (90% CI = 3.8–5.3%) for Day 7 and Day 14 samples, respectively. There was substantial variation in Brix percentage between colostrum samples as illustrated by large CI for each treatment group at each sample day.

pH

The pH of the colostrum at Day 0 was 6.48 (90% CI = 6.41–6.54). There was an obvious acidification over time for all four treatment groups, including the control group, with very small variation within days and treatment groups. Potassium sorbate reduced the pH to a lesser extent than the other groups (Figure 2(a)). At Day 7, potassium sorbate colostrum samples had a pH of 5.1 (90% CI = 5.0–5.2), compared to 4.4 (90% CI = 4.3–4.4) for control samples, 4.1 (90% CI = 4.0–4.3) for citric acid samples and 4.4 (90% CI = 4.3–4.5) for yoghurt samples.

Nutritional analysis

There was no biologically meaningful change in fat or protein percentage over time regardless of treatment (Figure 3(a and b)). Anhydrous lactose decreased over the 14 days in all groups (Figure 3(c)). The decrease in anhydrous lactose content was lower in potassium sorbate- and citric acid-preserved colostrum than the yoghurt-preserved and control colostrum samples.

Discussion

The objective of this study was to understand how different preservatives perform when added to colostrum. The focus of the study was on total bacterial counts, the counts of individual groups of bacteria, Brix percentage, pH and the nutritional composition of colostrum.

Compared to the other three treatments, colostrum treated with potassium sorbate had a reduced APC that was maintained across the 14 days of the study. In contrast, neither citric acid nor yoghurt preservation was clearly differentiated from unpreserved colostrum. These results are consistent with those reported by Denholm *et al.* (2017a) in their comparison of yoghurt and sorbate as colostrum preservatives, and further support their conclusion that potassium sorbate is an effective preservative for colostrum.

Potassium sorbate was also the most effective preservative in regard to reducing coliform counts. However, in contrast to APC, there were clear effects, at least on Day 7, of both yoghurt and citric acid in

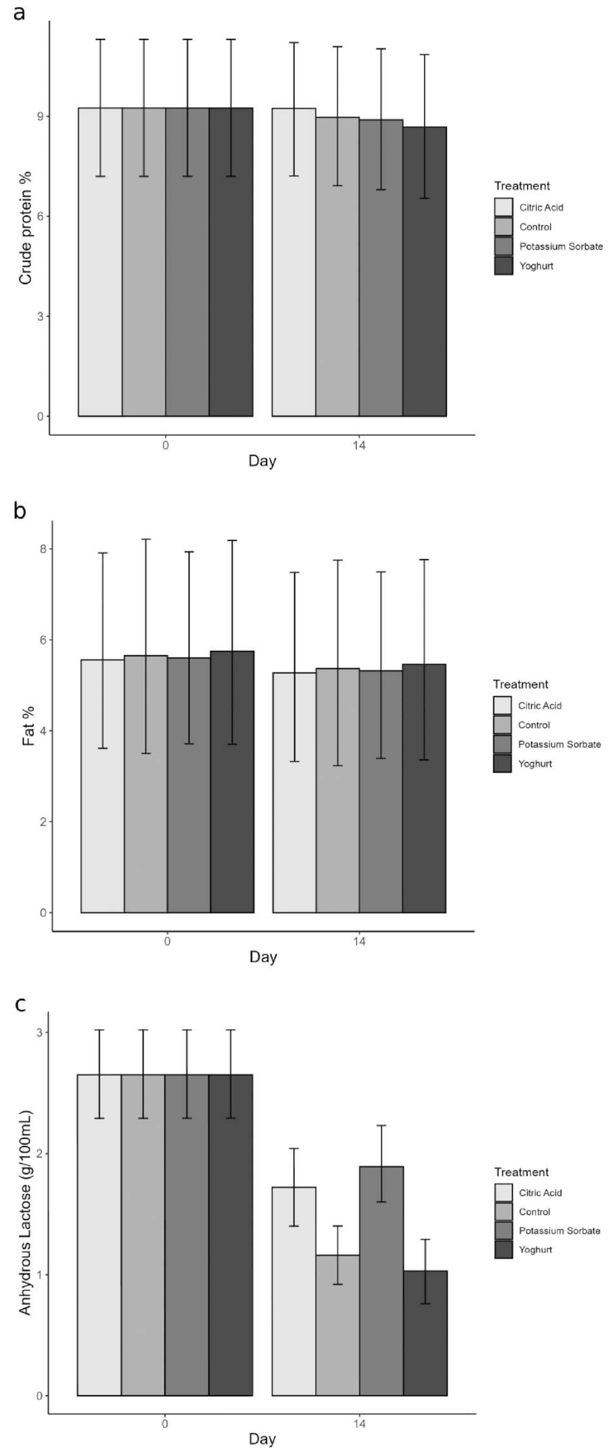

Figure 3. Mean (90% CI) estimated farm value for (a) crude protein percentage; (b) fat percentage; and (c) anhydrous lactose concentration in colostrum collected and pooled within 24 hours of calving from spring-calving herds (n = 10) in Waikato, New Zealand and treated with different preservation treatments (citric acid, yoghurt and potassium sorbate) or with no preservative (control) on Days 0, 7 and 14.

reducing coliform counts compared to the control. This effect was not reported by Denholm *et al.* (2017a), who stated that, in samples kept at ambient temperature for 7 days, yoghurt did not decrease coliform counts compared to the control; however, they also failed to find a difference between yoghurt and potassium sorbate (although potassium sorbate coliform counts were identified as being lower than the control). Thus the data reported by Denholm *et al.* (2017a) are consistent with our finding that both potassium sorbate and yoghurt reduced coliform counts. The coliform counts reported in this study on Day 7 were much higher than reported by Denholm *et al.* (2017a). This may be because the farm population was different, or it may be because the colostrum in this study was stored in large containers with a lid that was ajar compared to the small, tightly closed samples used by Denholm *et al.*

Lactobacilli counts were different between groups. On Day 7, yoghurt-fermented colostrum had higher lactobacilli numbers than both citric acid- and potassium sorbate-treated colostrum, both of which had lower lactobacilli numbers than the control group. By Day 14, differences between the treatment groups were no longer apparent. It is likely that these results reflect the naturally high level of lactobacilli that develop in unpasteurised or yoghurtised colostrum, and relatively limited impact of acid-based preservatives on their growth. By Day 14, the lactobacilli numbers likely decline as the available energy (lactose) is depleted. Lactobacilli are not considered to be pathogenic; indeed they are often supplemented as probiotics, and their use as probiotics has been associated with stimulating the development of the immune response against pathogenic bacteria and counteracting negative effects of illnesses (Frizzo *et al.* 2010). A meta-analysis on published studies on the use of probiotics on the growth and performance in young calves (Frizzo *et al.* 2011) concluded that supplementation with lactic acid bacteria improved growth rate and feed efficiency in calves fed a milk replacer but did not have an effect when calves were fed whole milk. Looking at the lactobacilli count on Day 7 in the present study, the intake of lactobacilli by calves if they were fed 4 L of 7-day-old, yoghurt-preserved, gold colostrum collected at first milking, would have been three times that reported in Frizzo *et al.* (2008). However, in that study, and other similar studies reported by Frizzo *et al.* (2011), the lactobacilli-based probiotics were fed for 35 days or longer; far longer than yoghurtised colostrum is fed for. Further research is required to establish whether supplementing lactobacilli via yoghurtised colostrum has benefits, over and above the value it has by reducing coliforms.

Streptococcus thermophilus, alongside *Lactobacillus* spp. were part of the yoghurt culture used in this study. In contrast to lactobacilli, there was no clear evidence that yoghurt-preserved colostrum had any more *S. thermophilus* than the control group, nor was there clear evidence that citric acid reduced *S. thermophilus* numbers. Overall, potassium sorbate reduced *S. thermophilus* compared to the other three treatments, consistent with it having the most potent anti-bacterial effect of any of the products being tested.

The difference in APC between potassium sorbate- and yoghurt-treated colostrum was not principally due to bacteria added to produce fermentation. On Day 7, APC for yoghurt and potassium sorbate were 7.3×10^8 and 1.0×10^8 cfu/mL respectively, a difference of 6.3×10^8 cfu/mL. However, the excess in lactobacilli seen on Day 7 in the yoghurt group compared to the potassium sorbate group was $\sim 2 \times 10^7$ cfu/mL, equivalent to only 3% of the difference in APC. Thus, the results of this study suggest that potassium sorbate controls bacterial growth more effectively (especially over the first 7 days after collection) than either yoghurt or citric acid.

The mean starting Brix percentage indicated that colostral IgG was below the ideal amount to feed to newborn calves (Godden 2008) but unsurprisingly there was a considerable range between farms that supplied the colostrum (Denholm *et al.* 2017b). All treatment groups showed a decline in Brix percentage over time with no clear differences noted between them. In contrast, Denholm *et al.* (2017a), whose study design was very similar to the present study (and had an almost identical starting average Brix percentage) reported that the addition of potassium sorbate to colostrum resulted in a minimal decline in Brix percentage compared to a control or yoghurtised colostrum. It is unclear what is driving this difference.

Mean pH and range on Day 0 were consistent with the results of McIntyre *et al.* (1952). In all four treatment groups, pH declined with time. This was expected, as all the preservatives tested and natural fermentation work, at least in part, by reducing pH. Potassium sorbate-treated colostrum had the highest pH of any treatment. Differences in pH can have important effects on the palatability of the feed and therefore consumption of colostrum; less is likely to be consumed the lower pH gets (Jenny *et al.* 1980; Hill *et al.* 2013). However, it is unclear whether the differences between preservatives were large enough to affect palatability.

Yoghurt cultures preserve milk through bacterial production of, principally, lactic acid from milk sugars (Shah 2003); the lactic acid then gets taken up into the bacterial cell, decreasing cytoplasmic pH and interfering with bacterial growth by disrupting bacterial cell metabolism and regulation (Boomsma *et al.* 2015). The conversion of lactose to lactic acid is why yoghurt-treated colostrum had the lowest anhydrous lactose content. Citric acid acts on the bacterial cytoplasm in the same way as lactic acid but is reportedly less effective than lactic acid at inactivating bacteria (Virto *et al.* 2005). Potassium sorbate is the potassium salt of sorbic acid and is ionised to sorbic acid when added to water. In addition to decreasing pH (and thus acting like lactic and citric acids), sorbic acid also inhibits microbial growth by changing cell membrane morphology, integrity and function, and disrupting transport functions and metabolic activity (Lück *et al.* 2011). These differences may explain why even though the three preservatives studied ultimately all decreased pH, they performed differently over time in other measures of colostrum preservation such as bacterial counts and nutritional analysis.

The nutritional data collected suggested that all preservatives maintained the fat and protein content of colostrum. The difference in anhydrous lactose between the groups is likely a function of bacteria including lactobacilli using this as a feed source (Hickey *et al.* 2015).

This study only included 10 colostrum samples from 10 farms in the Waikato region. Although we believe that it is unlikely that the broad effect of the preservatives would vary markedly by farm or by colostrum sample, our study samples do not necessarily encompass all potential types and numbers of bacteria that might be encountered in New Zealand dairy farms. Further studies may be needed to definitively confirm our conclusions.

Practically, these results allow farmers a little more scope in their choice of preservatives. Although in most circumstances, potassium sorbate is probably the best choice to limit proliferation of any bacteria and had the smallest reduction in pH (which may improve palatability), it does beg the question of whether inhibiting growth of all bacteria is the most beneficial option for the calf. More research needs to be undertaken to determine if the potential benefits of adding lactobacilli when preserving colostrum with yoghurt outweigh the potential disadvantages of not reducing all aerobic bacteria (which may include pathogens such as *Salmonella, E. coli* or *Staphylococcus aureus*) to the same extent as preservatives such as potassium sorbate.

Conclusion

Aerobic bacteria and coliform bacteria proliferate extensively in unpreserved colostrum. All preservatives tested in this study decreased coliform counts in comparison to un-preserved colostrum, but potassium sorbate was more effective at reducing coliform and aerobic bacterial counts (especially over the first 7 days after collection) than either yoghurt or citric acid. All of the preservation methods maintained the nutritional quality of stored colostrum as measured by fat and protein percentage, but the impact on lactose varied.

Acknowledgements

We gratefully acknowledge the farmers who provided colostrum for this study. Thank you to the Don McLaren Trust Fund and AgriHealth Ltd. for funding this study.

Disclosure statement

Steve Cranefield is employed by AgriHealth who funded this research.

ORCID

EL Cuttance http://orcid.org/0000-0003-0354-5295
WA Mason http://orcid.org/0000-0002-0006-7323
RA Laven http://orcid.org/0000-0002-8938-8595

References

*__Anderson N.__ Plate loop count of acidified, raw, bulk tank milk. *CEPTOR Animal Health News* 13, 14–5, 2005

*__Anonymous__. *Protein (Crude) in Animal Feed. Dumas Method.* http://www.aoacofficialmethod.org/index.php?main_page=product_info&products_id=2149 (accessed 27 June 2024). AOAC International, Rockville, MD, USA, 1969

*__Anonymous__. *Fat in Milk – Modified Mojonnier Ether Extract.* http://www.aoacofficialmethod.org/index.php?main_page=product_info&products_id=175 (accessed 27 June 2024). AOAC International, Rockville, MD, USA, 1992

*__Anonymous__. *Key Facts About* Mycoplasma bovis *for Farmers.* https://www.mpi.govt.nz/dmsdocument/29039-Key-facts-about-M-bovis-for-farmers (accessed 13 August 2024). Ministry for Primary Industries, Wellington, NZ, 2018

Boomsma B, Bikker E, Lansdaal E, Stuut P. L-Lactic acid – a safe antimicrobial for home-and personal care formulations. *Sofw Journal* 141, 2–5, 2015

*__Burggraaf V, Benson C, Rollo M.__ *Dairy Cattle Population Model: Improving Estimates of Within-Year Population Changes Used in the Inventory*. Ministry for Primary Industries, Wellington, NZ, 2022

Christiansen S, Guo M, Kjelden D. Chemical composition and nutrient profile of low molecular weight fraction of bovine colostrum. *International Dairy Journal* 20, 630–6, 2010 https://doi.org/10.1016/j.idairyj.2009.12.005

Cuttance E, Mason W, Laven R, Denholm K, Yang D. Calf and colostrum management practices on New Zealand dairy farms and their associations with concentrations of total protein in calf serum. *New Zealand Veterinary Journal* 66, 126–31, 2018 https://doi.org/10.1080/00480169.2018.1431159

Davis PF, Greenhill NS, Rowan AM, Schollum LM. The safety of New Zealand bovine colostrum: nutritional and physiological evaluation in rats. *Food and Chemical Toxicology* 45, 229–36, 2007 https://doi.org/10.1016/j.fct.2006.07.034

Deen M, de Rooij M. ClusterBootstrap: an R package for the analysis of hierarchical data using generalized linear models with the cluster bootstrap. *Behavior Research Methods* 52, 572–90, 2020 https://doi.org/10.3758/s13428-019-01252-y

Denholm KS, Hunnam JC, Cuttance EL, McDougall S. Influence of preservation methods on the quality of colostrum sourced from New Zealand dairy farms. *New Zealand Veterinary Journal* 65, 264–9, 2017a https://doi.org/10.1080/00480169.2017.1342574

Denholm KS, Hunnam JC, Cuttance EL, McDougall S. Associations between management practices and colostrum quality on New Zealand dairy farms. *New Zealand Veterinary Journal* 65, 257–63, 2017b https://doi.org/10.1080/00480169.2017.1342575

*__Downes F, Ito K.__ *Compendium of Methods for the Microbiological Examination of Foods*. American Public Health Association, Washington, DC, USA, 2001

Feng Z, McLerran D, Grizzle J. A comparison of statistical methods for clustered data analysis with Gaussian error. *Statistics in Medicine* 15, 1793–806, 1996 https://doi.org/10.1002/(SICI)1097-0258(19960830)15:16<1793::AID-SIM332>3.0.CO;2-2

Foley JA, Otterby DE. Availability, storage, treatment, composition, and feeding value of surplus colostrum: a review. *Journal of Dairy Science* 61, 1033–60, 1978 https://doi.org/10.3168/jds.S0022-0302(78)83686-8

Frizzo L, Bertozzi E, Soto L, Zbrun M, Sequeira G, Santina RD, Armesto RR, Rosmini M. The effect of supplementation with three lactic acid bacteria from bovine origin on growth performance and health status of young calves. *Journal of Animal and Veterinary Advances* 7, 400–8, 2008

Frizzo L, Soto L, Zbrun M, Bertozzi E, Sequeira G, Armesto RR, Rosmini M. Lactic acid bacteria to improve growth performance in young calves fed milk replacer and spray-dried whey powder. *Animal Feed Science and Technology* 157, 159–67, 2010 https://doi.org/10.1016/j.anifeedsci.2010.03.005

Frizzo LS, Zbrun MV, Soto LP, Signorini ML. Effects of probiotics on growth performance in young calves: a meta-analysis of randomized controlled trials. *Animal Feed Science and Technology* 169, 147–56, 2011 https://doi.org/10.1016/j.anifeedsci.2011.06.009

Gelsinger SL, Gray SM, Jones CM, Heinrichs AJ. Heat treatment of colostrum increases immunoglobulin G absorption efficiency in high-, medium-, and low-quality colostrum. *Journal of Dairy Science* 97, 2355–60, 2014 https://doi.org/10.3168/jds.2013-7374

Gelsinger S, Jones C, Heinrichs A. Effect of colostrum heat treatment and bacterial population on immunoglobulin G absorption and health of neonatal calves. *Journal of Dairy Science* 98, 4640–5, 2015 https://doi.org/10.3168/jds.2014-8790

Godden S. Colostrum management for dairy calves. *Veterinary Clinics of North America: Food Animal Practice* 24, 19–39, 2008 https://doi.org/10.1016/j.cvfa.2007.10.005

Godden SM, Smolenski DJ, Donahue M, Oakes JM, Bey R, Wells S, Sreevatsan S, Stabel J, Fetrow J. Heat-treated colostrum and reduced morbidity in preweaned dairy calves: results of a randomized trial and examination of mechanisms of effectiveness. *Journal of Dairy Science* 95, 4029–40, 2012 https://doi.org/10.3168/jds.2011-5275

Godden SM, Lombard JE, Woolums AR. Colostrum management for dairy calves. *Veterinary Clinics of North America: Food Animal Practice* 35, 535–56, 2019 https://doi.org/10.1016/j.cvfa.2019.07.005

Hickey CD, Sheehan JJ, Wilkinson MG, Auty MA. Growth and location of bacterial colonies within dairy foods using microscopy techniques: a review. *Frontiers in Microbiology* 6, 99, 2015 https://doi.org/10.3389/fmicb.2015.00099

Hill T, Bateman H II, Aldrich J, Quigley J, Schlotterbeck R. Evaluation of *ad libitum* acidified milk replacer programs for dairy calves. *Journal of Dairy Science* 96, 3153–62, 2013 https://doi.org/10.3168/jds.2012-6132

James R, Polan C. Effect of orally administered duodenal fluid on serum proteins in neonatal calves. *Journal of Dairy Science* 61, 1444–9, 1978 https://doi.org/10.3168/jds.S0022-0302(78)83747-3

James RE, Polan CE, Cummins KA. Influence of administered indigenous microorganisms on uptake of [iodine-125] γ-globulin *in vivo* by intestinal segments of neonatal calves. *Journal of Dairy Science* 64, 52–61, 1981 https://doi.org/10.3168/jds.S0022-0302(81)82528-3

Jenny B, Costello B, Van Dijk H. Performance of calves fed colostrum treated with sodium benzoate or benzoic acid. *Journal of Dairy Science* 63, 959–63, 1980 https://doi.org/10.3168/jds.S0022-0302(80)83032-3

Laven R. *Mycoplasma bovis* in New Zealand: where have we been and where are we going? *Livestock* 24, 266–72, 2019 https://doi.org/10.12968/live.2019.24.6.266

***Lück E, Jager M, Raczek N.** Sorbic acid. In: Elvers B, Bellussi G (eds). *Ullmann's Encyclopedia of Industrial Chemistry*. Pp 715–23. Wiley-VCH, Weinheim, Germany, 2011

***McElreath R.** *Statistical Rethinking: A Bayesian Course with Examples in R and Stan*. Chapman and Hall/CRC, Boca Raton, FL, USA, 2018

McIntyre R, Parrish D, Fountaine F. Properties of the colostrum of the dairy cow. VII. pH, buffer capacity and osmotic pressure. *Journal of Dairy Science* 35, 356–62, 1952 https://doi.org/10.3168/jds.S0022-0302(52)93714-4

***Shah N.** Yogurt. The product and its manufacture. In: Caballero B (ed). *Encyclopedia of Food Sciences and Nutrition.* 2nd Edtn. Pp 6252–9. Academic Press, Oxford, UK, 2003

Staley T, Bush L. Receptor mechanisms of the neonatal intestine and their relationship to immunoglobulin absorption and disease. *Journal of Dairy Science* 68, 184–205, 1985 https://doi.org/10.3168/jds.S0022-0302(85)80812-2

Stewart S, Godden S, Bey R, Rapnicki P, Fetrow J, Farnsworth R, Scanlon M, Arnold Y, Clow L, Mueller K. Preventing bacterial contamination and proliferation during the harvest, storage, and feeding of fresh bovine colostrum. *Journal of Dairy Science* 88, 2571–8, 2005 https://doi.org/10.3168/jds.S0022-0302(05)72933-7

Virto R, Sanz D, Álvarez I, Condón, Raso J. Inactivation kinetics of *Yersinia enterocolitica* by citric and lactic acid at different temperatures. *International Journal of Food Microbiology* 103, 251–7, 2005 https://doi.org/10.1016/j.ijfoodmicro.2004.11.036

Woodford S, Whetstone H, Murphy M, Davis C. Abomasal pH, nutrient digestibility, and growth of Holstein bull calves fed acidified milk replacer. *Journal of Dairy Science* 70, 888–91, 1987 https://doi.org/10.3168/jds.S0022-0302(87)80088-7

*Non-peer-reviewed