Review Article

A review of bovine colostrum preservation techniques

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Abstract

Preservation of colostrum for neonatal dairy calves has been seldom studied in recent years with much of the peer reviewed literature published in the 1970s and 80s. First milking colostrum is high in bioactive immune enhancers such as immunoglobulins, lactoferrins, lysozymes and cytokines and is vital to confer passive immunity to newborn dairy calves to promote their health, welfare and productivity. Bovine colostrum is advisedly limited from bulk milk supply for the first 8 milkings post calving due to high somatic cell counts and the risk of antimicrobial residues. As such, many producers refer to 'colostrum' as not only the first milking post calving, but also the aformentioned 'transition' milk. Colostrum is preserved in order to protect supply for feeding when production may be poor or where there is a glut of colostrum such as in seasonal calving systems. There are multiple reasons for newborn calves not to have access to their dam's colostrum, including: multiple births, acute mastitis or maladapted maternal behaviour, especially in first lactation heifers. Shortages in colostrum may also be precipitated by purposive discarding of colostrum from cows infected with *Mycobacterium avium subsp paratuberculosis* and *Mycoplasma bovis*. Broadly, colostrum may be preserved using low temperature (refrigeration or freezing) or chemical preservatives. The aim of this scoping review article was to identify options for preservation and gaps in research and to propose best practice for colostrum preservation.

Keywords: preservation, colostrum, bovine, review

Introduction

Calves are born agammaglobulinaemic, and are dependent on the timely consumption of maternal colostrum in sufficient volume and quality to confer immunity in the first few weeks of life through passive transfer (Godden *et al.* 2019).

There are multiple reasons for newborn calves not to have access to their dam's colostrum, including: multiple births, acute mastitis or maladapted maternal behaviour, especially in first lactation heifers (Wereme *et al.* 2001). Shortages in colostrum may also be precipitated by purposive discarding of colostrum from cows infected with *Mycobacterium avium subsp paratuberculosis* and *Mycoplasma bovis* (McGuirk and Collins, 2004).

According to published literature, 90% of Irish dairy producers store colostrum, while colostrum is routinely stored on 89% of large dairy farms in North America (Cummins *et al.* 2017). Data on colostrum storage in the UK is limited, but recent survey data from Scottish farms found that 24/35 (68.6%) of farms stored colostrum and 22/24 (91.7%) of these used freezers to store colostrum (Haggerty *et al.* 2021).

In the UK, colostrum is often harvested and fed to calves later, most usually left in uncovered buckets at room temperature for extended periods (Haggerty *et al.* 2021). Bacterial species double in numbers every 30 minutes at room temperature (21◦C) and as such unpreserved colostrum feeding to neonatal calves should not be delayed (Stewart *et al.* 2005).

A high proportion (36-42%) of individual colostrum samples exceeded TBC thresholds (>100,000 CFU/ml) in international literature (Fecteau *et al*. 2002; Morrill *et al.* 2012; Phipps *et al.* 2016), while approximately 90% of pooled colostrum samples were

highly contaminated (Denholm *et al.* 2017b). McAloon *et al.* (2016) demonstrated that 56% of colostrum samples collected from Irish dairy farms were above the standard TBC and TCC thresholds; while in Scottish samples 31% and 27% failed to meet TBC and TCC thresholds respectively (Haggerty *et al.* 2021). This is comparable to estimates from Canadian dairy herds where 36% of samples exceeded TBC thresholds (Fecteau *et al.* 2002).

Bacterial contamination comes from the cows' udder, milking equipment, storage and feeding equipment (Donahue *et al.* 2012; Godden *et al.* 2019). As such, every effort should be made by producers to minimise bacterial contamination of colostrum through scrupulous hygiene practices, including: cleaning of cows' teats; thorough scrubbing of buckets and feeders with hot water and use of a detergent to break down the fatty residues deposited by colostrum. Some farmers also use sterile bags to collect and store colostrum and these may also be pasteurised (https://dairytechinc.com/perfect-udder).

Coliform species in particular have been shown to impair IgG absorption (Gelsinger *et al.* 2015), through a number of mechanisms (Johnson *et al.* 2007). Firstly, physical binding of the IgG by microbes within the gastrointestinal lumen blocks their uptake across the enterocytes. Secondly, pathogenic bacteria may attach and damage intestinal cells meaning their permeability is reduced. Thirdly, when these pathogenic bacteria damage intestinal cells there is accelerated gut closure. Fourthly, bacteria physically block absorption channels of the immunoglobulin molecules *(Corley et al.* 1977; James *et al.* 1981; Staley and Bush, 1985). Bacterial contamination could also include specific disease-causing calf pathogens such as *E.coli*, Salmonella species, Mycoplasma species or *Mycobacterium avium paratuberculosis* (Stewart *et al.* 2005; McAloon *et al.* 2016).

If leaving colostrum or milk out for prolonged periods at ambient temperatures or if bacterial counts are high (as they have been shown to be) then there is a place for some sort of colostrum preservative. Colostrum preservatives may also act to minimise decline in IgG concentration in colostrum with time (Denholm et al. 2017a), but the mechanism by which this occurs has not been established.

The aim of this scoping review article was to identify options for preservation and gaps in research and to propose best practice for colostrum preservation.

Measures of preserved colostrum quality

Measures of performance for colostrum preservation include: colostrum composition (including fat and protein), immunoglobulin concentration (IgG >50g/L); bacterial counts (<100,000 CFU/ml TBC and <10,000 CFU/ml coliforms); pH; serum IgG concentrations in calves (IgG >10g/L); calf morbidity (<10%) and mortality (<2%); palatability and average daily gains (>0.9kg/calf/day).

Colostrum pH and acidification

Normal pH of colostrum is 5.59- 6.42 (Stewart *et al.* 2005; Cummins *et al*. 2017; Hyrslova *et al.* 2020). Lowering the pH of colostrum is thought to inhibit microbial proliferation, however most of the work on manual acidification by chemical additives has used milk or milk replacer, rather than colostrum.

Early work by Wheeler *et al.* (1980) showed that the palatability of colostrum was negatively influenced by increasing concentration of acid preservative. Calves refuse more milk replacer preserved at pH 4.2 than 5.2, since low pH colostrum and milk is unpalatable

100 (Hill *et al.* 2013). Collings *et al.* (2011) demonstrated rejection of milk replacer acidified to
101 pH 4.3-4.4, however calves still seemed motivated to suck acidified milk (Todd *et al.* 2018).

102 Todd et al. (2016) also showed that milk replacer acidification tended to be
103 associated with earlier solid feed consumption (presumably due to a palatability issue with
104 the acidified liquid feed); while Coelho *et al.* 2020 showed no effect on feed intake when
105 acidified milk, milk replacer and whole milk were compared. It is worth noting that in the
106 same study, feeding acidified milk negatively affected calf weight gain compared with whole
107 milk, however in other work, calves fed acidified milk and non-acidified milk did not show
108 any differences in average daily gain (Ribeiro *et al.* 2009, Hill *et al.* 2013). Acidified milk has
109 also been reported to increase the incidence of alopecia and diarrhoea in calves (Campos *et*
110 *al.* 1986).

111 Previous research documented a reduction in immunoglobulin absorption in calves
112 fed colostrum of low pH (pH = 4.65; Foley and Otterby 1978), but a more recent study
113 suggested that a pH as low as 5.0 did not affect the absorption of IgG in calves (Quigley *et al.*
114 2000). It has also been shown that colostral total bacteria counts (TBC) were negatively
115 correlated with pH (Pearson r = -0.87), indicating that a greater TBC was associated with a
116 lower pH (Cummins *et al.* 2017).

117 Separation of milk and colostrum occurs as pH is lowered to 4.2 and gentle agitation
118 is needed to re-homogenise milk. There is little evidence that acidification affects nutrients
119 in milk or milk replacer or utilisation of these by the calf. A balance must be struck as if pH
120 is too low calves will not drink and if pH is too high, the milk will not be preserved leading to
121 spoilage.

122

123 **What is colostrum preserved with?**

Colostrum may be preserved by the addition of chemical preservatives; low temperatures (freezing and refrigeration) or by addition of bacterial cultures. Colostrum may also be preserved by 'natural' aerobic or anaerobic fermentation.

Low temperatures and low pH have been shown to slow bacterial growth (Stewart et al. 2005). *Mycoplasma* species can survive at pH in excess of 5 and *Salmonella* and *Mycobacterium avium paratuberculosis* (MAP) at pH in excess of 6 and 7 respectively. Optimal pH for growth of various pathogenic bacterial species (including *Escherichia coli*, *Clostridia sp*. and *Salmonella sp*.) range from 6-7.5 (Anderson 2008).

Preserving colostrum using chemical additives

Acid preservatives present a number of safety concerns. Some acids are available in powdered form making them easier to handle than caustic liquids. However, dust can irritate the eyes, nose and throat. Dry products will also absorb moisture so need to be kept in an airtight container, which has practical implications for on farm storage. Gloves, protective goggles and long sleeves are recommended as well as careful handling and immediate hand washing.

Numerous acids have been tested in colostrum and in cheese making to limit microbial growth. Acids can be short chain organic acids including citric, acetic formic, propionic and lactic acids. This approach may be complemented by the addition of low concentrations of specific lipid-soluble weak acids, for example, benzoic and sorbic acids. The combined effect of a low pH plus a high weak-acid concentration leads to acidification of the cytoplasm, which is usually sufficient to restrict microbial growth, but may also have other specific effects on cell activity (Booth and Stratford 2003). Acidification of colostrum may be problematic due to the decomposition of lactose, which reduces digestibility. Puppel *et al.*

147 (2019) showed that the absorbability of all colostral elements of acidified colostrum is
148 reduced (in comparison with fresh colostrum). IgG absorption is also depressed in
149 an acidic environment as the mechanism of non-selective pinocytosis by which IgG is
150 transported across the intestinal epithelium is pH-dependent (Heinrichs and Elizondo-
151 Salazar, 2009). Acid tolerant yeasts and moulds may contribute to poor palatability of
152 colostrum and degradation of nutrients (Drevjany *et al.* 1980).

153 Many of the trials conducted in the 1970s and 1980s advocated dilution of acidified
154 colostrum with water, which adversely affects calf growth rates by diluting the nutrients in
155 the feed. The efficiency of feeding pasteurized and acidified waste milk were comparable in
156 some work, and the acidification of waste milk was deemed an acceptable labour-saving and
157 diarrhoea-preventing feed for young calves (Zho *et al.* 2017).

158 *Citric acid*

159 Although citric acid is a well-recognised preservative in food, the effectiveness of citric acid
160 as a preservative in feeding stuffs and water for drinking has not been sufficiently
161 demonstrated (Matsuda *et al.* 1994). Inhibition of a wide range of bacteria and fungi
162 occurred only at concentrations above 25 000 mg citric acid/L, which are greater than the
163 recommended use concentration of citric acid in feed and corresponding concentration in
164 water for drinking (European Food Safety authority (EFSA). Citric acid is safe (according to
165 USFDA (United States Food and Drug Administration) and EFSA) and can be used legally
166 without restriction in the USA at rates of 15000mg/kg in feed and 5000mg/L in water in
167 Europe.

168 Canning *et al.* 2009 added citric acid to whole milk and pH was maintained at 4.5 for
169 about 4 days. In addition to the antimicrobial effect of citric acid (by lowering pH), studies

have indicated that the chelating effect of citric acid also inhibits bacteria. By chelating or binding metal ions, the substrate for bacterial growth is diminished in the food, thus influencing growth (Søltoft-Jensen and Hansen 2005).

The New Zealand livestock industry has been concerned with the eradication of *Mycoplasma* species (sp.), first identified in New Zealand in 2017. There are a number of practical guidelines developed by New Zealand industry bodies (Beef and Lamb NZ and DairyNZ) on the acidification of milk using citric acid to mitigate *Mycoplasma sp*. (see Figure 1).

Propionic acid

Using propionic acid (available in liquid form) to acidify milk at a concentration of 1% and a rate of 35-40ml/gallon resulted in a variation in pH of milk from 4.1 to 5. Milk acidified with propionic acid was not well accepted by calves as it has a pungent, rancid odour. There are safety concerns for liquid propionic acid, including burning of the skin and irritation of mucous membranes. The acid is also corrosive to most metals. Despite this, propionic acid is safe (according to USFDA and EFSA) and can be used legally without restriction in the USA and at rates of 10-30g/kg in feed.

Muller and Syhre (1975) found that propionic acid maintained pH after 23 days of fermentation, in comparison with lactic acid and 3 bacterial cultures (*Streptococcus lactis, Streptococcus tberrnopbilus,* and *Lactobacillus bulgaricus,* 1%).

Jenny *et al.* (1984) compared sodium benzoate, propionic acid, and formaldehyde as preservatives for colostrum and found that titratable acidity was highest for propionic acid preserved colostrum, with potential detrimental effects on palatability. In addition, first milking colostrum preserved with 1% propionic acid or 0.3% formic acid and stored for 4

193 weeks had lower IgG concentrations than aerobically fermented or frozen (-4◦C) colostrum
194 (Schipper *et al.* 1981).
195 Rindsig and Bodoh (1977) observed more refusals of liquid diets by calves fed
196 colostrum treated with propionic acid than when calves were fed whole milk, naturally
197 aerobically fermented colostrum or colostrum treated with formaldehyde. Refusals were
198 attributed to a combination of odour, taste and low pH. Conversely, Polzin *et al.* (1977)
199 observed no refusals of colostrum containing propionic or formic acids.
200 *Formic acid*
201 Formic acid is not currently approved by the USFDA due to skin and eye contact irritation
202 and serious eye damage. Formic acid is volatile, and exposure via inhalation for those
203 handling the additive is considered to present a risk to unprotected workers. Turnover of
204 formic acid is, however, rapid with no evidence of accumulation in body tissues and use in
205 animal nutrition is not expected to contribute to human exposure.
206 Formic acid is used as a preservative and antibacterial agent in livestock feed in the
207 UK at a rate of 10,000mg/kg complete feed. According to Canadian experience,
208 preservation with formic acid (based on a Finnish model) could facilitate storage of milk or
209 colostrum at room temperature. However, during warm seasons, refrigeration will ensure
210 optimal preservation for up to 20 days (Anderson, 2008). There is some dispute as to
211 necessary contact time for formic acid with some producers acidifying and feeding
212 immediately, and others leaving milk for 6-12 hours before feeding. Formic acid quickly kills
213 coliforms in 1-2 hours contact time. (Anderson 2008). Formic acid also kills about 90% of
214 MAP in 8 hours contact time at pH 4.0 and 100% of MAP at 48 hours (Mutharia *et al.* 2007).
215 Other acids (including hydrochloric and an orthophosphoric acid mix) vary in their effects on

MAP with better results at 48 hours contact time than 8 hours contact time (Anderson, 2008).

It has been demonstrated that calves fed acidified waste milk (using formic acid) consumed more starter grain (potentially due to poor milk palatability) than calves fed untreated waste milk (Zho *et al.* 2017), but these animals did not have as high serum IgG concentrations and did not grow well.

Acidification with formic acid (0.5% and 0.1 %) did not lead to significant changes in crude protein or total solids in colostrum from Sahiwal cows after 28 days at ambient temperatures (Mbuthia *et al.* 2002)

Finlanders stress the importance of using skim milk powder (rather than whey source milk powder) in their free-access formic acid acidified milk feeding systems, however these are expensive in the UK and the amount of skim milk powder in the product is difficult to determine from product labelling. Anecdotally, feeding acidified milk preserved with formic acid reported fewer clinical cases of diarrhoea and fewer treatment interventions were observed in calves fed acidified milk (Anderson et al., 2008), however palatability and safety issues have led some researchers to declare that formic acid is not a practical preservation agent for colostrum (Collings *et al.* 2011).

Formaldehyde and hydrogen peroxide

Formaldehyde has been used historically as a preservative (Mbuthia *et al.* 1997), but its carcinogenic properties mean it is no longer approved by the USFDA and while it may still be used in Europe (at concentrations of between 200 and 1000mg/kg feed) its use is not encouraged. Hydrogen peroxide is similarly problematic.

238 Early research by Muller and Smallcomb (1977) showed that 0.25% formaldehyde
239 maintained original colostral pH for 18 days. Bush et al., (1980) applied formalin and
240 fermentation to extend the shelf life of colostrum and reported a slower reduction in pH
241 (from 6.2 to 5.6) for 24 days (at ambient conditions of 20-26◦C) at 0.1% formalin than
242 untreated colostrum. Literature pertaining to the effects of this type of chemical
243 preservative on colostrum immunoglobulins is not available (Borad and Singh, 2018)

244 *Potassium sorbate*

245 Potassium sorbate has been used extensively as a 'stabiliser' in wine production. Unlike acid
246 agents potassium sorbate only limits bacterial growth in colostrum. Bey *et al.* (2007) found
247 that in refrigerated colostrum, preservation with potassium sorbate (0.5% final solution)
248 reduced bacteria counts initially (1 log difference *versus* raw non-preserved colostrum),
249 then delayed growth rate. Potassium sorbate is more effective at prohibiting growth of
250 moulds and yeasts than acids. Potassium sorbate preserved colostrum may last up to 7
251 days, preferably at refrigeration temperatures (4◦C) (Stewart *et al.* 2005); although some
252 work in seasonal calving systems demonstrated its effectiveness to maintain IgG
253 concentration and minimise bacterial proliferation even at ambient temperatures (Denholm
254 *et al.* 2017a).

255 Potassium sorbate is available in powdered form and is generally recognised as safe
256 by USFDA and the EFSA. It is added at a rate of 1% by volume of a 50% solution (EFSA safe
257 concentration 11mg/kg body weight).

258 Potassium sorbate can also be used in conjunction with heat treatment but needs to
259 be added afterwards to avoid curd formation during the heat treatment process. According

to DairyNZ potassium sorbate is not effective at elimination *Mycoplasma sp.* in colostrum in the 'required time frame', although proper referencing is not provided.

Drevjany *et al.* 1980 showed that potassium sorbate treated colostrum (applied at day 4 to fermented colostrum) resulted in increased calf starter consumption and greater weight gains in warm temperatures. Colostrum also retained palatability through 21 days of storage with little surface mould growth compared with untreated colostrum. Effective antimicrobial threshold for potassium sorbate is pH 6.5 (Drevjany et al. 1980).

Sodium benzoate

Sodium benzoate (benzoic acid) may be added to milk but at a maximum limit of 0.1%. Jenny *et al.* (1984) added sodium benzoate at 0.5% with acceptable preservative results (milk pH held at 5.1 for 10 days and 5.5 at 20◦C or higher). The same study demonstrated that colostrum treated with sodium benzoate was slightly higher in fat and pH (due to buffering capacity) and lower in protein than other colostrum treatments (propionic acid and formaldehyde).

In 1977 Muller and Smallcomb studied a number of chemicals: sodium benzoate (0.5%), sodium propionate, sodium formate, sodium acetate, benzoic acid, sorbitol, and gluconic acid lactone. Additions of sodium benzoate and benzoic acid resulted in a slower decrease in pH and maintenance of a more constant pH for 21 days than the control and colostrum with other additives. However, preservation with sodium benzoate altered physicochemical properties and destroyed nutritional components of colostrum (Borad and Singh 2018).

Preserving colostrum using low temperatures

According to some literature: 'Chemical preservatives cannot preserve colostrum satisfactorily; chilling and freezing are the most preferred methods' (Borad and Singh 2018). Warmer temperatures lead to proliferation of bacteria and highly contaminated colostrum resulted in lower serum IgG concentrations in calves (Elizondo-Salazar and Heinrichs 2009).

Morrill *et al.* (2012) recommended that colostrum should be fed fresh from the dam or frozen immediately. Frozen colostrum (-20◦C) may be stored for up to 1 year without affecting IgG concentration (Stewart et al., 2005). Proper labelling is recommended with cow identification number and date of collection; and storage in containers of no more than 2 litre capacity to aid thawing (Robbers *et al.* 2021).

Fresh or frozen first milking colostrum can be used to feed dairy calves, without the latter affecting the diversity in the colonization of the intestinal tract. No significant differences in serum IgG concentration between calves fed frozen and thawed colostrum and calves fed fresh colostrum (Holloway *et al.* 2001; Donovan *et al.* 2007).

Colostrum should be thawed in a hot water bath heated to 40◦C (Robbers *et al.* 2021). Avoid microwaving frozen colostrum as this will create hot 'pockets' (>60◦C) which may denature IgG molecules. A higher power of microwave has been associated with a loss of IgG; and heating above 60◦C in a hot water bath resulted in a significant (26%) reduction in IgG1 (Balthazar *et al.* 2015). Repeated freeze thaw cycles will cause denaturation of colostrum IgG molecules, so a single thaw is advised. Compared with fresh colostrum, repeated freeze/thawing resulted in a significant decrease in IgG concentration of 7.8 and 7.7% for two and three freeze/thaw cycles respectively (Robbers et al. 2021). A log reduction in *Mycoplasma sp.* through freezing has also been demonstrated (Gillie et al. 2018).

Refrigeration (at 4◦C) may be employed for short term storage of colostrum, but colostrum stored in this way should be fed within 2 days of harvest (Cummins *et al.* 2017). In this work colostrum stored at ambient temperatures (i.e., 22°C) had more than 42 times more bacteria present; a pH 0.85 units lower and serum IgG concentration 2 times lower than colostrum stored at 4°C for 2 days (Cummins *et al.* 2017). While colostrum stored at 4°C for 2 days had more bacteria present than pasteurized and fresh colostrum, this did not result in reduced calf serum IgG concentrations in this study. Langel *et al.* (2015) noted that refrigeration (4◦C) up to 8 h did not affect cell viability, but effects of refrigeration for a longer period are yet unclear.

The main disadvantage to using refrigeration or freezing facilities to preserve colostrum is the associated capital cost and the space required. Furthermore, many farmers don't have or don't check thermometers on refrigerators and freezers or have broken equipment (poorly maintained, dirty) (Haggerty *et al.* 2021).

Lactobacillus and yoghurt culture innoculations

Ellinger *et al.* (1980) inoculated whole milk with *Lactobacillus acidophilus* and demonstrated a linear decrease in coliforms suggesting an antagonistic action towards coliforms. A similar effect has also been demonstrated in pigs (Muralidhara *et al.*1977). *Lactobacillus acidophilus* may be fed as viable cultures or a dried preparation and has been shown to decrease the incidence of diarrhoeal disease in calves in some work, but not in others (Ellinger *et al.* 1980).

While it has been suggested that fermentation of bovine colostrum by suitable strains might be helpful in the prevention of diarrhoea in calves or to increase colostrum quality by inhibition of pathogenic and spoilage microbiota, a comparison of 'Easiyo'

328 yoghurt cultures and untreated colostrum showed no difference in bacterial growth in
329 pooled colostrum samples form seasonal calving herds (Denholm *et al.* 2017a).
330 Bush *et al.* (1980) found that 0.1% formalin was more effective in preserving
331 colostrum than either *Streptococcus lactis* or yoghurt culture. Drevjany *et al.* (1975)
332 reported that colostrum inoculated with *Lactobacillus acidophilus* was unacceptable to
333 calves due to a pH of less than 4.0.

334 **Fermentation**

335 Fermentation may be an alternative to low temperature or chemical storage and may be
336 aerobic or anaerobic. Fermentation causes the development of beneficial microorganisms,
337 such as lactic acid bacteria, and the concomitant pH reduction preserves colostrum at room
338 temperature (Otterby *et al.* 1980).

339 *Aerobic fermentation*

340 Much of the work from the late 1970s and early 1980s found that fermenting
341 colostrum under aerobic conditions resulted in a rapid drop in pH particularly when
342 colostrum was stored at higher temperatures (Muller and Syhre 1977; Bush *et al.* 1980).
343 Jenny *et al.* (1977) also reported a putrid odour and mould development when colostrum
344 was stored at 27°C or at higher temperatures. This was corroborated by Rindsig and Bodoh
345 (1977), when colostrum was stored at temperatures between 32 and 39°C. The authors
346 suggested discarding colostrum under these conditions since its voluntary intake by calves
347 was also low.
348 Carlson and Muller (1977) showed that naturally fermented colostrum had more
349 nutrient breakdown during storage than did propionic acid (1%) treated, with formaldehyde
350 (0.05%) treated colostrum intermediate. Aerobic bacteria counts (particularly coliform

351 counts) were still high after 21 days of storage in some work (Thompson and Marth 1975),
352 discounting the theory that the fermentation process produces sufficient lactic acid to
353 eliminate *E. coli* from colostrum so the calf does not ingest these organisms in large
354 numbers and hence does not develop scours. (Thompson and Marth 1975). Furthermore, it
355 has been suggested in much of the published work that aerobically fermented colostrum
356 should be fed diluted with water such as not to induce scouring (Thompson and Marth
357 1975), which is inadvisable as previously mentioned.

358 Foley *et al.* (1978) went on to assert that aerobically fermented colostrum is a
359 potential source of antibodies for newborn calves when maternal colostrum is not available,
360 but it is difficult to form colostrum banks since storage periods are short. Feed costs were
361 estimated to be reduced by 90% with a fermented colostrum feeding program compared
362 with a whole milk feeding program (Yu *et al.* 1976).

363 *Anaerobic fermentation*

364 Ferreira *et al.* (2013) experimented with anaerobic fermentation, making 'colostrum
365 silage' and found that the pH quickly decreased when ensiled colostrum was stored at
366 higher temperatures (32.5°C). Their results indicated that the temperature at which
367 colostrum was fermented directly influenced the speed and intensity of microbial
368 population development and degradation of the main nutritional parameters, such as casein
369 and lactose; although Saalfield *et al.* (2013) did not find such detrimental effects of higher
370 temperatures.

371 Saalfield *et al.* (2013) stored colostrum in sealed bags at room temperature for 21
372 days. Physicochemical evaluation of colostrum silage revealed a tendency to maintain
373 protein, dry matter and fat values, but lactose percentage decreased. pH of anaerobically
374 fermented colostrum fell after 7 days of fermentation with a concurrent increase in lactic

375 acid percentage, but 'colostrum silage' fed calves gained more weight than the control milk
376 fed calves indicating that the drop in lactose in the anaerobically fermented colostrum was
377 not detrimental to calf growth (0.7kg/day versus 0.6kg/day) . The presence of the bacteria
378 *Lactobacillus*, *Staphylococcus, Escherichia, Klebsiella*, *Bacillus* and *Candida* yeast species.
379 was observed in 'colostrum silage' for up to 14 days, but from 21 days of fermentation only
380 bacteria of the genus *Lactobacillus* spp was isolated. This indicated that the pH of the
381 colostrum fermented anaerobically does not support the proliferation of pathogenic
382 organisms which may otherwise have been transmitted via colostrum to calves (Stewart *et*
383 *al.* 2005). Further work by Saalfield *et al.* (2014) showed that colostrum immunoglobulin
384 concentration was not compromised by anaerobic fermentation (compared with frozen
385 colostrum) stored for 12 months and passive immunity was adequately transferred to
386 newborn calves.

387 Anaerobically fermented colostrum may potentially be stored for much longer
388 periods (up to 12 months) than aerobically fermented colostrum. Natural aerobic
389 acidification, with and without preservatives, makes colostrum preservation feasible for
390 only between 28 (Gonzales *et al*. 1978) and 90 (Thompson and Marth 1975) days.

391 **Pasteurisation**

392 While pasteurisation is not strictly speaking a method of preservation, it is a useful tool in
393 storage and managing the shelf life of colostrum.

394 As early as 1981, James *et al.* suggested that a greater bacterial concentration in the
395 calf's gut may adversely affect the passive transfer of IgG. Numerous studies have
396 demonstrated that heat treatment and consequent decreased bacterial counts in colostrum
397 lead to improved immunity and weight gain in dairy calves (Johnson *et al.* 2007; Elizondo-
398 Salazar and Heinrichs, 2009; Gelsinger *et al.* 2015). However, IgG molecules may be

399 destroyed if colostrum is heated to greater than 60◦C. This is because immunoglobulins are
400 mono- or polymeric proteins, formed by two light and two heavy polypeptide chains which
401 are connected by disulfide bonds into a Y-shaped particle (Puppel *et al.* 2019) and excessive
402 heating leads to an initially reversible unfolding of this native structure, with loss of globular
403 configuration, which can proceed further to irreversible denaturation and aggregation via
404 hydrophobic and disulphide interactions (Indyk et al., 2008).

405 Cummins *et al.* 2017 investigated the effects of colostrum, stored under various
406 conditions, fed to Irish spring born calves and found that pasteurised colostrum resulted in
407 serum IgG concentrations two times higher than colostrum stored in warm conditions
408 (22◦C). Pasteurisation also effectively destroys MAP, *Salmonella* and *Mycoplasma* species.
409 in milk deliberately spiked with these organisms (Stabel et al., 2004). Pasteurisation units
410 are not commonplace on UK dairy farms due to the high capital cost involved.

411 **Goat colostrum preservation**

412 In some countries, dairy goats are prevalent and international research has focussed on
413 colostrum additives for preservation. Spanish researchers found no difference in aerobic
414 mesophilic bacteria counts between either 10 or 14% glycerol and propylene glycol
415 additives. These additions reduced bacterial count to a greater extent than untreated
416 colostrum, and 2 or 6% additions of these compounds. They concluded that glycerol
417 addition to goat colostrum before heat treatment is suitable to enhance bacterial reduction
418 (Morales-delaNuez *et al.* 2020).

419 Sodium dodecyl sulfate (1%) was found to be an efficient colostrum biocide that,
420 unlike pasteurization, does not affect immune passive transfer or goat kid health. (Morales-
421 delaNuez *et al.* 2011). Neither of these compounds have been tested in bovine colostrum
422 and this could be an are for further research.

New technologies for colostrum preservation for human consumption or for neonatal calves

Many of the following colostrum processing treatments would be difficult to practically perform on farm and are more suited to the processing of colostrum in a laboratory or controlled setting. They are included here for completeness and may be the future of on-farm colostrum preservation with advances in technology.

UV light radiation

Texeira *et al.* (2013) found that IgG and lactoferrin concentrations were significantly lower in UV light treated colostrum than in raw colostrum, however there were no significant differences in serum IgG concentrations among calves fed heat or UV treated or untreated colostrum. It is important to note that UV light treatment may not work as well in thick colostrum as in milk (Texeira *et al.* 2013) and that the presence of dissolved and suspended solids can scatter UV light and provide a site for bacterial aggregation, attenuating the bactericidal activity of this form of radiation (Koutchma *et al.* 2004; Ye *et al.* 2007). UV light radiation did not reduce bacterial counts as effectively as heat treatment (63◦C for 6 minutes) and resulted in a greater reduction in colostrum IgG concentrations (for unknown reasons) (Texeira *et al.* 2013). UV irradiation of milk spiked with MAP also did not result in an adequate reduction in infectivity (Donaghy *et al.* 2009).

Pereira *et al.* (2014) also studied the effect of UV light on colostrum IgG and bacterial contaminants and observed a negative linear relationship between duration UV treatment and IgG concentration.

445 Puppel *et al.* (2019) cite that preserving colostrum using UV irradiation, membrane
446 filtration, pulsating electric field (PEF) and concentrated microwave fields (CMF) resulted in
447 a number of changes in the chemical composition of the colostrum.
448
449 *Lyophilisation, spray drying or freeze drying*
450 Lyophilisation (drying in a lower temperature and vacuum) has been shown to negatively
451 impact colostral fat with consequent rapid spoilage. In addition, IgG absorption from
452 lyophilised colostrum by the calf is 30% lower than fresh colostrum (Borad and Singh 2018)
453 Spray-drying produced a dried colostrum in which immunoglobulin quantity and
454 function were preserved and was the most cost-effective at preserving the therapeutic
455 potential of colostrum for human consumption (Chelack *et al.* 1993). Earlier investigations
456 also showed that freeze-drying did not alter the concentration of immunoglobulins in
457 colostrum (Klobasa *et al.* 1998).
458 Spray drying is the most commonly applied technology for the manufacture of dairy
459 powders and other ingredients, but concerns about heat-induced damage to colostrum
460 proteins limited the adoption of spray drying for colostrum powder preparation since much
461 of the IgG activity is destroyed.
462 Freeze-drying is the most preferred dehydration method for heat-sensitive biological
463 material, as the low processing temperature and rapid local transition of frozen material
464 from hydrated to dehydrated state minimises nutrient and immunogloblubin losses. Chelack
465 *et al.* (1993) reported a 10% loss in biological activity of immunoglobulins upon freeze-
466 drying of colostrum, whereas Elfstrand *et al.* (2002) reported 34 and 25% losses in total
467 immunoglobulins during freeze-drying of colostrum.

Data from first milking postpartum colostrum samples from 18 Egyptian buffaloes and 36 Holstein cows showed that freeze dried colostrum stored at 7◦C for 3 months had significantly reduced IgG concentrations compared with frozen colostrum (Abd El-Fattah *et al.* 2014).

A study by Bartkiene *et al.* (2018) concluded that a combination of ultrasonication, fermentation, and dehydration could be used to reduce microbial contamination of bovine colostrum; however, more investigations are needed to evaluate the influence of these treatment methods on sensitive biologically active compounds in bovine colostrum.

High pressure processing

Among novel technologies, high pressure processing has been found to be a promising preservation method for colostrum immunoglobulins (Borad and Singh 2018). High pressure processing retained 20% more bovine IgG in soy milk than heat treatment (at 75-78◦C) (Li et al 2006), but IgA molecules in human breast milk were destroyed by high pressure processing (Permanyer *et al.* 2010)

Masuda *et al.* (2000) reported effective suppression of bacterial growth for 9 days at 4◦C after treating colostrum at 300 and 400 megapascals (MPa) for 10min. Up to 300 MPa, IgG remained intact, but application of 400 MPa resulted in altered viscosity of the colostrum and denaturation of IgG. Indyk *et al.* (2008) and Foster *et al*. (2016) found colostral IgG to be stable at treatments up to 400 MPa, as long as duration was limited to 30min. Increasing pressure (500 or 600 MPa) or duration resulted in increased denaturation and aggregation.

Conclusion

Which preservation method is best for on farm preservation of bovine colostrum?

Table 1 summarises each of the preservation options available. Limited work has been done on chemical acidification of colostrum, but work on milk replacer and milk would suggest that palatability and digestibility issues may prohibit its use. IgG absorption from acidified colostrum may also be impaired. *Lactobacillus* cultures added to colostrum are inefficacious. Controlled anaerobic fermentation of colostrum may provide an alternative to low temperature storage facilities where these are unavailable. Potassium sorbate additives could be useful where colostrum is left at ambient temperatures for more than 6 hours before feeding to newborn calves. Heat treatment of colostrum is useful to control pathogenic bacteria and reduce overall bacteria counts, but pasteurisation units are costly.

Opportunities for further research

Little recent work has been published on alternative chemical preservatives or explored new technologies to preserve bovine colostrum on farm. Currently, the most promising avenues for future work include exploring user friendly on-farm technology for high pressure processing as this preserves IgG molecules more effectively than UV light and dehydration methods. There is also plenty of scope for more research into practical, on farm colostrum preservation techniques which preclude the requirement for large low temperature storage devices (such as refrigerators and freezers) and allow colostrum to be stored at room temperature. With more local focussed research, industry bodies, veterinarians and other agricultural professionals could collaborate to create a 'joined up' approach to extension messaging of use of preservatives such as potassium sorbate to best effect. In addition, extension messaging of local research on anaerobic fermentation, including how to optimise and practically perform this type of preservation are currently lacking. Seasonal, tropical and low income production systems would most benefit from employing this type of

514 preservation where colostrum is produced in abundance or low temperature storage
515 options are in short supply.

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