

A review of bovine colostrum preservation techniques

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Review Article

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Abstract

Preservation of colostrum for neonatal dairy calves has seldom been done in recent years, much of the peer reviewed literature having been published in the 1970s and 1980s. 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 future productivity. Bovine colostrum is advisedly restricted from the 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 aforementioned ‘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 purposeful 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.

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). Unfortunately newborn calves do not always have access to their dam’s colostrum, either because of 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 deliberate 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, often being left in uncovered buckets at room temperature for extended periods (Haggerty *et al.*, 2021). Bacterial species double in number every 30 min 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 udder, milking equipment, storage and feeding equipment (Donahue *et al.*, 2012; Godden *et al.*, 2019). 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 (Gelsing *et al.*, 2015), acting 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 that 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 there is an absolute need to leave 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 the 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 (focusing on fat and protein), immunoglobulin concentration (IgG >50 g/l), bacterial counts (<100 000 CFU/ml TBC and <10 000 CFU/ml coliforms), pH, serum IgG concentrations in calves (IgG >10 g/l), calf morbidity (<10%) and mortality (<2%), palatability and average daily gains (>0.9 kg/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 at 5.2, since low pH colostrum and milk is unpalatable (Hill *et al.*, 2013). Collings *et al.* (2011) demonstrated rejection of milk replacer acidified to pH 4.3–4.4, however calves still seemed motivated to suck acidified milk (Todd *et al.*, 2018).

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

Previous research documented a reduction in immunoglobulin absorption in calves fed colostrum of low pH (pH = 4.65; Foley and Otterby, 1978), but a more recent study suggested that a pH as low as 5.0 did not affect the absorption of IgG in calves (Quigley *et al.*, 2000). It has also been shown that colostrum total

bacteria counts (TBC) were negatively correlated with pH (Pearson $r = -0.87$), indicating that a greater TBC was associated with a lower pH (Cummins *et al.*, 2017).

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

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 to 7.5 (Anderson, 2008).

Preserving colostrum using chemical additives: general

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

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

Preserving colostrum using chemical additives: citric acid

Although citric acid is a well-recognised preservative in food, the effectiveness of citric acid as a preservative in feeding stuffs and water for drinking has not been sufficiently demonstrated (Matsuda *et al.*, 1994). Inhibition of a wide range of bacteria and fungi occurred only at concentrations above 25 000 mg citric acid/L, which are greater than the recommended-use concentration of citric acid in feed and corresponding concentration in water for drinking (European Food Safety authority: EFSA). Citric acid is safe according to USFDA (United States Food and Drug Administration) and EFSA (EFSA Feedap Panel, 2015) and can be used legally without restriction in the USA at rates of 15 000 mg/kg in feed and 5000 mg/l in water.

Canning *et al.*, 2009 added citric acid to whole milk and pH was maintained at 4.5 for about 4 d. 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 online Supplementary File Table S1).

Preserving colostrum using chemical additives: propionic acid

Using propionic acid (available in liquid form) to acidify milk at a concentration of 1% and a rate of 35–40 ml/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 10–30 g/kg in feed.

Muller and Syhre (1975) found that propionic acid maintained pH after 23 d of fermentation, in comparison with lactic acid and 3 bacterial cultures (*Streptococcus lactis*, *Streptococcus tbernophilus*, 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 weeks had lower IgG concentrations than aerobically fermented or frozen (−4°C) colostrum (Schipper *et al.*, 1981).

Rindsig and Bodoh (1977) observed more refusals of liquid diets by calves fed colostrum treated with propionic acid than when calves were fed whole milk, naturally aerobically fermented colostrum or colostrum treated with formaldehyde. Refusals were attributed to a combination of odour, taste and low pH. Conversely, Polzin *et al.* (1977) observed no refusals of colostrum containing propionic or formic acids.

Preserving colostrum using chemical additives: formic acid

Formic acid is not currently approved by the USFDA due to skin and eye contact irritation and serious eye damage. Formic acid is

volatile, and exposure *via* inhalation for those handling the additive is considered to present a risk to unprotected workers. Turnover of formic acid is, however, rapid with no evidence of accumulation in body tissues and use in animal nutrition is not expected to contribute to human exposure.

Formic acid is used as a preservative and antibacterial agent in livestock feed in the UK at a rate of 10 000 mg/kg complete feed following evaluation by the European Food Safety Authority (EFSA Feedap Panel, 2014). According to Canadian experience, preservation with formic acid (based on a Finnish model) could facilitate storage of milk or colostrum at room temperature. However, during warm seasons, refrigeration will ensure optimal preservation for up to 20 d (Anderson, 2008). There is some dispute as to necessary contact time for formic acid with some producers acidifying and feeding immediately, and others leaving milk for 6–12 h before feeding. Formic acid quickly kills coliforms in 1–2 h contact time. (Anderson, 2008). Formic acid also kills about 90% of MAP in 8 h contact time at pH 4.0 and 100% of MAP at 48 h (Mutharia and Raymond, 2007). Other acids (including hydrochloric and an ortho-phosphoric acid mix) vary in their effects on MAP with better results at 48 h contact time than 8 h 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 (Zou *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 d 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 resulted in fewer clinical cases of diarrhoea and fewer treatment interventions in milk fed calves (Anderson, 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).

Preserving colostrum using chemical additives: 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 1000 mg/kg feed) its use is not encouraged. Hydrogen peroxide is similarly problematic.

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

Preserving colostrum using chemical additives: potassium sorbate

Potassium sorbate has been used extensively as a 'stabiliser' in wine production. Unlike acid agents, potassium sorbate only

limits bacterial growth in colostrum. Bey *et al.* (2007) found that in refrigerated colostrum, preservation with potassium sorbate (0.5% final solution) reduced bacteria counts initially (1 log difference vs. raw non-preserved colostrum), then delayed growth rate. Potassium sorbate is more effective at prohibiting growth of moulds and yeasts than acids. Potassium sorbate-preserved colostrum may last up to 7 d, preferably at refrigeration temperatures (4°C) (Stewart *et al.*, 2005); although some work in seasonal calving systems demonstrated its effectiveness to maintain IgG concentration and minimise bacterial proliferation even at ambient temperatures (Denholm *et al.*, 2017a).

Potassium sorbate is available in powdered form and is generally recognised as safe by USFDA and the EFSA. It is added at a rate of 1% by volume of a 50% solution (EFSA safe concentration 11 mg/kg body weight). Potassium sorbate can also be used in conjunction with heat treatment but needs to 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 d 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).

Preserving colostrum using chemical additives: 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 d 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 d 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, as well as storage in containers of no more than 2 l 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 colonisation of the intestinal tract. No significant differences in serum IgG concentration were observed 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). One should 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 (Gille *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 d 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 as well as pH 0.85 units lower and serum IgG concentration 2 times lower than colostrum stored at 4°C for 2 d (Cummins *et al.*, 2017). While colostrum stored at 4°C for 2 d had more bacteria present than pasteurised 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 inoculations

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 as 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' yoghurt cultures and untreated colostrum showed no difference in bacterial growth in pooled colostrum samples from seasonal calving herds (Denholm *et al.*, 2017a).

Bush *et al.* (1980) found that 0.1% formalin was more effective in preserving colostrum than either *Streptococcus lactis* or yoghurt culture. Drevjany *et al.* (1975) reported that colostrum inoculated with *Lactobacillus acidophilus* was unacceptable to calves due to a pH of less than 4.0.

Fermentation: general

Fermentation may be an alternative to low temperature or chemical storage and may be aerobic or anaerobic. Fermentation causes

the development of beneficial microorganisms, such as lactic acid bacteria, and the concomitant pH reduction preserves colostrum at room temperature (Otterby *et al.*, 1980).

Fermentation: aerobic fermentation

Much of the work from the late 1970s and early 1980s found that fermenting colostrum under aerobic conditions resulted in a rapid drop in pH particularly when colostrum was stored at higher temperatures (Muller and Syhre, 1975; Bush *et al.*, 1980). Jenny *et al.* (1977) also reported a putrid odour and mould development when colostrum was stored at 27°C or at higher temperatures. This was corroborated by Rindsig and Bodoh (1977), when colostrum was stored at temperatures between 32 and 39°C. The authors suggested discarding colostrum under these conditions since its voluntary intake by calves was also low.

Carlson and Muller (1977) showed that naturally fermented colostrum had more nutrient breakdown during storage than did 1% propionic acid treated, with formaldehyde (0.05%) treated colostrum intermediate. Aerobic bacteria counts (particularly coliform counts) were still high after 21 d of storage in some work (Thompson and Marth, 1976), discounting the theory that the fermentation process produces sufficient lactic acid to eliminate *E. coli* from colostrum so that the calf does not ingest these organisms in large numbers and hence does not develop scours (Thompson and Marth, 1976). Furthermore, it has been suggested in much of the published work that aerobically fermented colostrum should be fed diluted with water such as not to induce scouring (Thompson and Marth, 1976), which is inadvisable as previously mentioned.

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

Fermentation: anaerobic fermentation

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

Saalfeld *et al.* (2013) stored colostrum in sealed bags at room temperature for 21 d. Physicochemical evaluation of colostrum silage revealed a tendency to maintain protein, dry matter and fat values, but lactose percentage decreased. pH of anaerobically fermented colostrum fell after 7 d of fermentation with a concurrent increase in lactic acid percentage, but 'colostrum silage' fed calves gained more weight than the control milk fed calves indicating that the drop in lactose in the anaerobically fermented colostrum was not detrimental to calf growth. The presence of the bacteria *Lactobacillus*, *Staphylococcus*, *Escherichia*, *Klebsiella*, *Bacillus* and *Candida* yeast species was observed in 'colostrum silage' for up to 14 d, but from 21 d of fermentation only bacteria of

the genus *Lactobacillus* species were isolated. This indicated that the pH of the colostrum fermented anaerobically does not support the proliferation of pathogenic organisms which may otherwise have been transmitted *via* colostrum to calves (Stewart *et al.*, 2005). Further work by Saalfeld *et al.* (2014) showed that colostrum immunoglobulin concentration was not compromised by anaerobic fermentation (compared with frozen colostrum) stored for 12 months and passive immunity was adequately transferred to newborn calves.

Anaerobically fermented colostrum may potentially be stored for much longer periods (up to 12 months) than aerobically fermented colostrum. Natural aerobic acidification, with and without preservatives, makes colostrum preservation feasible for only between 28 (González *et al.*, 1978) and 90 (Thompson and Marth, 1976) days.

Pasteurisation

While pasteurisation is not strictly speaking a method of preservation, it is a useful tool in storage and managing the shelf life of colostrum. As early as 1981, James *et al.* suggested that a greater bacterial concentration in the calf's gut may adversely affect the passive transfer of IgG. Numerous studies have demonstrated that heat treatment and consequent decreased bacterial counts in colostrum lead to improved immunity and weight gain in dairy calves (Johnson *et al.*, 2007; Elizondo-Salazar and Heinrichs, 2009; Gelsing *et al.*, 2015). However, IgG molecules may be destroyed if colostrum is heated to greater than 60°C. This is because immunoglobulins are mono- or polymeric proteins, formed by two light and two heavy polypeptide chains which are connected by disulphide bonds into a Y-shaped particle (Puppel *et al.*, 2019) and excessive heating leads to an initially reversible unfolding of this native structure, with loss of globular configuration, which can proceed further to irreversible denaturation and aggregation *via* hydrophobic and disulphide interactions (Indyk *et al.*, 2008).

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

Goat colostrum preservation

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

Sodium dodecyl sulphate (1%) was found to be an efficient colostrum biocide that, unlike pasteurisation, does not affect immune passive transfer or goat kid health (Morales-de la Nuez *et al.*, 2011). Neither of these compounds has been tested in bovine colostrum and this could be an area 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.

New technologies: UV light radiation

Teixeira *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 (Teixeira *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 min) and, for unknown reasons, resulted in a greater reduction in colostrum IgG concentrations (Teixeira *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. Puppel *et al.* (2019) cite that preserving colostrum using UV irradiation, membrane filtration, pulsating electric field (PEF) and concentrated microwave fields (CMF) resulted in a number of changes in the chemical composition of the colostrum.

New technologies: Lyophilisation, spray drying or freeze drying

Lyophilisation (drying in a lower temperature and vacuum) has been shown to negatively impact colostrum fat with consequent rapid spoilage. In addition, IgG absorption from lyophilised colostrum by the calf is 30% lower than fresh colostrum (Borad and Singh, 2018).

Spray-drying produced a dried colostrum in which immunoglobulin quantity and function were preserved and was the most cost-effective at preserving the therapeutic potential of colostrum for human consumption (Chelack *et al.*, 1993). Earlier investigations also showed that freeze-drying did not alter the concentration of immunoglobulins in colostrum (Klobasa *et al.*, 1998).

Spray drying is the most commonly applied technology for the manufacture of dairy powders and other ingredients, but concerns about heat-induced damage to colostrum proteins limited the adoption of spray drying for colostrum powder preparation since much of the IgG activity is destroyed.

Freeze-drying is the most preferred dehydration method for heat-sensitive biological material, as the low processing temperature and rapid local transition of frozen material from hydrated to dehydrated state minimises nutrient and immunoglobulin losses. Chelack *et al.* (1993) reported a 10% loss in biological activity of immunoglobulins upon freeze-drying of colostrum, whereas Elfstrand *et al.* (2002) reported 34 and 25% losses in total

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.* (2020) 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.

New technologies: 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 d at 4°C after treating colostrum at 300 and 400 megapascals (MPa) for 10 min. 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 colostrum IgG to be stable at treatments up to 400 MPa, as long as duration was limited to 30 min. 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, whereas potassium sorbate additives could be useful where colostrum is left at ambient temperatures for more than 6 h 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

Table 1. Summary table of options for preservation of bovine colostrum detailing suitability for on-farm and laboratory use and advantages and disadvantages of each method

Preservation type	Method	Suitable for on farm use	Suitable for laboratory use	Advantages	Disadvantages
Chemical	Citric acid	Yes, in use	Yes	Efficacious against <i>Mycoplasma</i> sp.	High concentrations needed, efficacy not adequately demonstrated against all bacteria
	Propionic acid	Yes, not in popular use due to safety concerns	Yes	Available in liquid form	Pungent rancid odour affects palatability. Corrosive to most metals. Highly irritant. Does not preserve IgG concentration.
	Formic acid	Yes, not in popular use due to safety concerns	Yes	Efficacious against <i>Mycobacterium avium paratuberculosis</i> (MAP)	Safety concerns. Highly irritant. 8 h contact time required. May affect IgG absorption and calf growth rates. Poor palatability
	Formaldehyde and hydrogen peroxide	No, carcinogenic	No		Safety concerns
	Potassium sorbate	Yes, in popular use	Yes	More effective at prohibiting growth of moulds and yeasts than acids. Safe and palatable.	Only limits bacterial growth does not destroy bacteria. Ineffective against <i>Mycoplasma</i> sp.
	Sodium benzoate	Yes, not in popular use	Yes	Consistently low pH maintained.	Alters nutritional composition of colostrum.
	Low temperature	Freezing	Yes, in popular use	Yes	Storage up to 1 year. IgG concentration maintained.
Refrigeration		Yes, in popular use	Yes	Effective short-term storage	Capital cost and space required. Temperature needs to be monitored at 4°C
Bacterial cultures	<i>Lactobacillus</i> and yoghurt cultures	Yes, although inefficacious and not in popular use	Yes	Safe and readily available.	Inefficacious against all bacterial species. Palatability issues.
Fermentation	Aerobic	Yes, although inefficacious and not in popular use	Yes	May be ok for short term storage at low temperatures	Putrid odour and mould at high temperatures. Nutrient breakdown. High bacterial counts.
	Anaerobic	Yes, may become more popular with more research		Most nutrients maintained. Efficacious at reducing pathogenic bacteria and maintaining IgG concentration. Longer term storage than aerobic fermentation. No need for low temperature storage	Lactose breakdown
Modern technology	UV light radiation	No, impractical	Yes, although inefficacious		May damage IgG molecules. Inefficacious against bacterial species
	Lyophilisation	No, impractical	Yes		Rapid spoilage, poor IgG absorption by calf.
	Spray drying	No, impractical	Yes		May destroy IgG molecules
	Freeze drying	No, impractical	Yes	Preferred dehydration method for heat sensitive biological material	May destroy IgG molecules
	High-pressure processing	No, impractical More research needed	Yes		Better preservation of IgG than dehydration methods.

and freezers) and allow colostrum to be stored at room temperature. With more local focused 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 preservation where colostrum is produced in abundance or low temperature storage options are in short supply.

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