A comparison of thawing methods on IgG1 concentration in colostrum of dairy cows

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SUMMARY

Colostrum should be frozen with a good immune value based on IgG1 concentration. Previous research has examined pre-storage colostrum selection methods but paid little attention to thawing methods. Here we tested the effect of thawing by bain-marie at 40, 50, 60 and 70°C or microwaving at 200 and 350 W on 6 colostrum samples taken from specimens with either high (H; n=2), medium (M; n=2) or low (L; n=2) IgG1 concentration before freezing on final IgG1 concentration. Refractometry (using Brix measurements) and radial immuno-diffusion techniques were used to perform the IgG1 analyses. Bain-marie at 40°C proved to be the most conservative method in terms of IgG1 concentration but was also the longest method for preparing colostrum from thawing to distribution (39°C). Microwaving for 30 minutes at 200 W power could be recommended as an alternative method for samples with good immune value before freezing, as IgG1 concentration in thawed colostrum was still over 50 g/L. A bain-marie temperature higher than 60°C enabled faster thawing but created coagulation issues. IgG1 concentration losses after thawing averaged 20%, regardless of method. We recommend that colostrum should have a high IgG1 concentration (at least 60 g/L), i.e. a 25% Brix value before being frozen for storage, and should be thawed when needed in a bain-marie at 40°C.

Keywords: Colostrum, thawing, microwave; bain-marie, IgG1

RESUME

Comparaison de méthodes de décongélation sur la concentration en IgG1 du colostrum de vaches laitières

Le colostrum conservé sous forme congelée devrait avoir une bonne qualité immune, celle-ci étant basée sur sa concentration en IgG1 avant congélation. De nombreux travaux de recherche se sont focalisés par le passé sur les méthodes de sélection des colostrums avant congélation, mais les méthodes de décongélation ont été moins étudiées. Dans cet essai, les effets des températures du bain marie à 40, 50, 60 et 70 °C et de puissance au micro-onde de 200 et 380 W ont été comparés sur 6 échantillons, ayant une concentration en IgG1 élevée (E; n=2), moyenne (M; n=2) ou basse (B; n=2). Les mesures par réfractométrie et par immunodiffusion radiale ont été utilisées pour déterminer la concentration en IgG1. Le bain marie à 40 °C préserve le plus efficacement la concentration initiale en IgG1, mais c'est aussi la méthode la plus longue pour préparer le colostrum pour une distribution à 39 °C aux veaux. L'utilisation du micro-onde à 200 W pendant 30 minutes peut aussi être recommandée. Une température au bain-marie à 60°C permet une décongélation plus rapide, mais comme pour le micro-onde à des puissances élevées, provoque des phénomènes de coagulation. Quelle que soit la méthode, les pertes en IgG1 avoisinent les 20 %. Il est recommandé au final de conserver un colostrum ayant une teneur élevée en IgG1 (au moins 60 g/L) avant congélation, soit environ une valeur Brix obtenue au réfractomètre d'environ 25 %.

Mots clés : Colostrum, réchauffement, micro-onde, bain-marie, IgG1

Introduction

Colostrum is the first mammary secretion after birth [6]. The newborn calf needs to quickly ingest a sufficient amount of colostrum containing a minimum IgG1 concentration of 50 g/L or more [8]. According to Conneely et al. [4], a young calf needs to receive 8.5% of its body weight of colostrum within two hours of birth. There is often too much colostrum for one calf, so many farms store the surplus ready to feed a calf if its mother produces too little colostrum or colostrum with too little IgG1, or if she presents symptoms of mastitis. Colostrum IgG1 concentration does not decrease during the 3 days after milking when stored at ambient temperature, and it can be kept without change for 8 to 10 days if pasteurized or even longer if frozen (up to one year; [14, 16, 19]). Commercial farms tend to opt to freeze, as it facilitates colostrum management. A recent survey found that almost two in three farmers banked colostrum for later use, selecting it mainly based on visual considerations ([12]).

Farmers typically select colostrum for storage by estimating its immune quality via methods based on density measurements (Brix percentage using a refractometer). Brix percentage is a measure of sucrose concentration in liquids such as fruit juice, molasses and wine. When used in non-sucrose-containing liquids, Brix percentage approximates total solids percentage ([19]). Brix percentage usually ranges from 0 to 32%, and according to Bielmann et al. ([3]), a 22% value corresponds to a IgG1 concentration in colostrum of 50 g/L, which is considered a good-quality colostrum from an immune point of view.

The selected colostrum is generally batch-stored in bottles or small bags of 1.5 or 2 L, which is the minimum amount required per calf per meal ([2, 10, 11, 19]). As substandard thawing can alter IgG1 concentration, recommended practice is to heat frozen colostrum in a bain-marie at 45 to 50°C ([11], [18]). However, the process typically takes at least 45 to 60 minutes, inciting farmers either to use higher temperatures (55°C or more) or to opt for microwaving (at 200 or 350 W power; [9]), which cuts thawing time to around 30 minutes.

Some farmers and advisors claim that if the stored colostrum contains a high IgG1 concentration (approximately 50 g/L or more), the thawed colostrum will contain a sufficiently high IgG1 concentration regardless of thawing method, although others assert that different thawing methods warrant careful management.

The aim of this experiment was to compare IgG1 concentrations after thawing with either microwave or bain-marie methods at different powers or temperatures using colostrum samples taken from specimens with high, medium or low IgG1 concentration.

Material and methods

The samples tested originated from a colostrum bank created to study within- and between-farm differences in IgG1 concentrations (unpublished). A total of 543 specimens were collected from 31 dairy farms in Normandy (western France) during winter and spring 2014. IgG1 concentration was estimated using refractometry (Brix measurement, [19]). A total of 6 specimens of colostrum from 6 different dairy cows were selected at calving regardless of geographic origin, breed or animal management. These 6 specimens had Brix values of 13, 14, 19, 21.5, 26 and 27%, and were thus defined as low (L: 13 & 14%), medium (M: 19 & 21.5%) and high (H: 26 & 27%)-colostrum specimens, respectively.

On top of Brix measurement, IgG1 concentration was also determined on fresh colostrum samples of these specimens using lab-based radial immunodiffusion (RID) analysis (see below) and this value was considered as the control value for each specimen. A total of 16 sub-samples of 5 mL per sample were prepared and frozen at -20°C until RID analysis. A semi-automated single RID technique was used to determine colostrum IgG1 concentration ([13, 15]. Commercial kits (ID Ring BOV IgG; ID Biotech, Issoire, France) were used for this test. Sample dilution was adapted to commercial recommendations (1,000 times). Between- and within-assay imprecision was 5% and 9%, respectively, according to ID Biotech, and 10% and 15%, respectively, according to in-lab tests run before performing the analyses.

All RID-based IgG1 analyses were performed on samples at 39°C. We then determined IgG1 concentration in L, M and H colostrum specimens according to the different thawing methods. Four bain-marie-based re-heating temperatures were tested, i.e. 40, 50, 60 and 70°C. The 40°C temperature was considered as representative of commercial farm practice for feeding newborn calves. Microwave thawing was tested at 200 and 350 W power on frozen fractions of the samples from each specimen. For each 200 and 350 W power, samples were either collected and stored at the end of thawing process (C samples) or collected every 3 to 5 minutes during the thawing process, then collected and mixed at the end of the process (NC). In all cases, each sample was tested in duplicate, and thawing time was recorded.

Table I reports the mean IgG1 values of the L, M and H-colostrum specimens. As the data showed non-normal distribution, we used a non-parametric Kruskal-Wallis test followed by a multiple post-hoc comparisons test. Values at $P<0.05$ were considered statistically significant.

Results

Results of the RID analyses on fresh specimens were consistent with the classification performed according to Brix measurements (Table I). IgG1 values were 13.1 and 16.4 g/L in L specimens and were 88.1 and 90.3 g/L in H specimens, with intermediate values (40.6 and 46.6 g/L) found in M specimens. There were no significant differences

Specimen ID	Fresh-sample values (1)		After thawing IgG1, g/L					
	Brix value, %	IgG1, g/L	Bain-marie temperature, °C				Micro-wave power, Watts	
			40	50	60	70	200	350
L1	13	13.1	10.4	10.6	9.3	6.8	8.9	6.5
L2	14	16.4	14.6	12.7	13.2	13.8	13.1	12.7
M1	19	40.6	41.8	37.9	37.4	28.3	34.4	31.0
M2	21.5	46.6	43.7	40.5	37.9	34.2	38.1	37.3
H1	26	88.1	80.7	79.3	80.2	71.1	70.4	53.1
H2	27	90.3	90.2	83.1	89.8	75.8	74.8	63.2
Mean loss, % (2)	-	49.1	8a	14ab	15ab	26b	20a	31b

[1]IgG1 concentrations were estimated using refractometry (Brix measurement, [15]) and semi-automated single radial immunodiffusion (RID) ([10, 12].) methods. In all cases, each sample was tested in duplicate.

[2]Mean: percent losses (process / RID values for fresh sample).

L1, L2, M1, M2, H1, H2: colostrum specimens were classified according to Brix value (Low, Medium and High immune quality) confirmed by RID analysis before storage.

For each heating method (bain-marie or microwaving), different lowercase letters indicate significant difference in mean values ($P<0.05$).

Table I: Brix values and IgG1 concentrations of colostrum specimens before storage, and post-storage losses as a function of thawing process

between pre- and post-thawing Brix values regardless of thawing method applied (results not shown), whereas RID found a significant post-thawing loss (21%; P<0.001). All further discussion is thus based on RID-determined IgG1 concentration.

For the same specimen, analysis showed huge variations in IgG1 concentration after microwave-based thawing but no variation after bain-marie thawing (Table I). Regardless of initial IgG1 concentration, increasing bain-marie temperature resulted in a significant decrease in post-thawing colostrum IgG1 concentration. IgG1 losses averaged 8% of initial fresh value at a bain-marie temperature of 40°C but reached up to 20-25% at a bain-marie temperature of 70°C (P<0.05). At 50°C and 60°C, IgG1 losses reached 15% but were not significantly different to the losses at 40°C. There were no significant differences between losses at 50°C, 60°C and 70°C. Thawing time decreased from 65 min at 40°C to 37 min at 70°C. Losses in IgG1 concentration were always greater for L specimens than M and H specimens, except at 70°C (P<0.05). Results observed with microwaving followed a similar pattern, i.e. loss of initial IgG1 concentration increased (P<0.05) from 20 to 31% when power was increased from 200 to 350 W. Time needed to reach 39°C from the start of thawing was 14.7 minutes at 200 W *vs* 26.6 minutes at 350 W (P<0.05). Comparison of the methods based on either collecting thawing colostrum at regular intervals (C) or all at the end once totally thawed (NC) showed no significant difference in percentage of losses (around 20%) or thawing time needed (20 min).

Discussion

Like the colostrum selection process, the colostrum thawing process also warrants careful management. Many farmers and advisors have recently refocused on freezing surplus colostrum for storage (up to ⅔ of farmers in a survey by Le Cozler et al. [12]). The rationale comes from the fact that a number of studies have indicated that calves may not receive colostrum of good immune quality rapidly enough after birth. Vasseur et al. [20] concluded that 22% of Holstein calves aged 2 to 6 hours did not drink the recommended 2 L or more of colostrum, and Franklin et al. [6] claimed that a majority of calves who only ingest maternal colostrum by natural suckling are unable to get enough colostrum to receive adequate transfer of immunity.

This experiment confirmed that keeping low IgG1-concentration colostrum specimens is of limited interest, not only because they have little value in terms of immune transfer but also because thawing had more impact on their IgG1 concentration than for higher-IgG1-concentration colostrum specimens. Post-thawing Brix measurements, regardless of thawing method, provided the same values as before freezing. This is not surprising, since the Brix method is based on the measurement of protein refractometry but does not take into account the fact that IgG1 or other proteins may have been drastically modified or denatured [5]. It is therefore important for farmers to test colostrum quality before distributing it to newborn calves, which can easily be done by on-farm methods but should only be done on fresh samples.

High-temperature (>60°C) bain-marie thawing resulted in significant IgG1 losses, which is in line with the literature ([18]). This study confirmed that optimal bain-marie temperature is between 40 and 60°C, and while 40°C appears to be more efficient, it does require more time to bring the colostrum specimen to the right temperature ready for delivery to the newborn calf. However, IgG1 concentration in M and H samples was still above the recommended threshold after bain-marie thawing at 70°C. Here, heating was stopped the instant that thawing ended. In practice, however, if specimens are heated at 60°C or above for longer than one hour, this could not only denature the proteins (i.e. IgG1 concentration) but also require extra time to fall back to the right temperature for delivery to the calf (40°C). From both the labour time and IgG1 concentration standpoints, thawing by bain-marie at above 50°C is of limited interest if farmers do not strictly supervise the process.

Many farms are now opting for microwaving as a quickest thawing method. The results found here confirmed that microwaving not only more dramatically alters IgG1 than bain-marie thawing but probably also denatures other potentially important components of colostrum ([7]). Jones et al. [9] reported that microwaving at high-watt power can create undesirable coagulation. Nevertheless, despite several negative effects, microwaving also offers certain benefits, as the method is fast and does not require preliminary preparation. Even if it generates significant immune-concentration losses, it may be of interest on colostrum of good-enough IgG1 quality before freezing.

Conclusion

From a practical standpoint, bain-marie at 40°C is the safest method to keep a good IgG1 concentration in the colostrum, but is also the longest method in terms of time invested to prepare samples ready for delivery to newborn calf when required. Bain-maries at temperature up to 60°C or microwaving at 200 W power for 30 minutes are potential options, resulting in faster thawing, but with increasing risks of coagulation issues in some cases.

Regardless of thawing method, the stored colostrum should already have a high immune concentration (at least 60 g/L) before freezing, since thawing procedures result in an average 20% loss of IgG1. This threshold for good immune concentration thus corresponds to a Brix value of at least 25%.

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