

Serum Protein Levels in Holstein Calves Fed Pasteurized-Frozen-Thawed or Unpasteurized First-milk Colostrum

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Abstract

This study compared serum protein (SP) concentrations in male and female Holstein calves fed pasteurized-frozen-thawed colostrum (PFTC) or unpasteurized colostrum (UPC) in a Mexican commercial dairy herd. To prepare PFTC, first-milk colostrum was collected and mixed to form 12 batches. All batches were pasteurized at 145°F (63°C) for 30 minutes and poured into 2.11 quarts (2 L) plastic bags for freezing for a minimum of 24 hours before use. Prior to feeding, frozen bags of colostrum were thawed in water at 122 to 131°F (50 to 55°C) for 15 minutes, and fed three times to each calf during the first 22.1 hours of life. Calves receiving UPC were hand-fed 1.74 quarts (1.65 L) of colostrum per feeding for three consecutive feedings with a suckling bottle in the first 22.9 hours of life. Blood from each calf was collected between 24 and 96 hours after birth. The SP level was 5.92 ± 0.15 g/100 mL for calves receiving PFTC and 6.53 ± 0.14 g/100 mL for those receiving UPC ($P=0.0048$). The percentage of calves with failure of passive transfer (FPT) of immunoglobulins with SP levels less than 5.2 g /100 mL was 24% for calves fed PFTC and 3.8% for calves fed UPC ($P=0.06$).

Keywords: bovine, calves, serum protein, colostrum, pasteurization, freezing

Résumé

Cette étude comparait la concentration sérique des protéines chez des veaux Holstein mâles et femelles nourris avec soit du colostrum pasteurisé congelé et puis dégelé ou soit du colostrum non-pasteurisé dans une ferme laitière commerciale mexicaine. Pour préparer le colostrum pasteurisé, le colostrum du premier lait a été recueilli et mélangé pour former 12 lots. Tous les lots ont été pasteurisés à 145°F (63°C) pendant 30 minutes

et versés dans des sacs en plastique de 2.11 pintes (2 L) pour congélation pendant au moins 24 heures avant leur utilisation. Avant l'abreuvement, les sacs congelés de colostrum ont été dégelés dans de l'eau entre 122 et 131°F (50 et 55°C) pendant 15 minutes et le colostrum a été donné trois fois à chaque veau pendant les premières 22.1 heures suivant la naissance. Les veaux qui recevaient le colostrum non-pasteurisé étaient nourris à la main avec 1.74 pintes (1.65 L) de colostrum par repas pendant trois repas consécutifs à l'aide d'un biberon pendant les premières 22.9 heures suivant la naissance. Un échantillon de sang a été prélevé de chaque veau entre 24 et 96 heures après la naissance. La concentration sérique des protéines était de 5.92 (±0.15) g/100 ml chez les veaux recevant le colostrum pasteurisé et de 6.53 (±0.14) g/100 ml chez ceux recevant le colostrum non-pasteurisé ($P=0.0048$). Le pourcentage de veaux pour lesquels le transfert passif n'a pas réussi pour les immunoglobulines avec des niveaux sériques de moins de 5.2 g /100 ml était de 24% chez les veaux recevant le colostrum pasteurisé et de 3.8% chez les veaux recevant le colostrum non-pasteurisé.

Introduction

Morbidity and mortality are much higher in calves with failure of passive transfer (FPT) when serum protein (SP) levels are less than 5.2 g/100 mL as compared to calves with SP levels above 5.5 g/100 mL.^{11,22,25} The two main factors associated with higher SP levels are volume of colostrum fed and the total immunoglobulin mass in the colostrum.⁷ First-milk colostrum has the highest immunoglobulin and protein concentrations compared to second and subsequent colostrum milkings.^{6,20}

Specific pathogens can be transferred from cow to calf through colostrums, such as *Campylobacter* spp,¹³ *Listeria monocytogenes*,³ *Salmonella* spp,⁸ *Mycobacterium avium* subsp *paratuberculosis* (MAP),^{21,23} *Mycobacterium*

bovis,^{10,24} other *Mycoplasma* spp, *Staphylococcus* spp, *Streptococcus uberis* and *Escherichia coli*.⁴ Butler *et al*¹ demonstrated killing of *Mycoplasma californicum*, *Mycoplasma bovis* and *Mycoplasma canadense* by pasteurization of discarded milk at 149°F (65°C) for 60 minutes. Pasteurization also destroys a portion of the immunoglobulins present in colostrum. Meylan *et al*¹⁵ reported a mean loss of 12.3% of immunoglobulin content after heating small volumes of colostrum to 145°F (63°C) for 30 minutes. When the initial immunoglobulin levels are determined using a colostrometer, these authors suggested adjusting the volume of colostrum fed to calves to ensure successful passive transfer of immunity. The partial destruction of immunoglobulins during pasteurization in colostrum influences the SP levels in calves. Godden *et al*,⁹ using on-farm commercial batch pasteurization of colostrum at 145°F (63°C) for 30 minutes and controlling for the time interval between feedings, reported significantly lower serum IgG concentrations in calves fed pasteurized colostrum (9.7 mg/mL) than for calves fed fresh colostrum (19.1 mg/mL). However, the use of high-temperature short-time (HTST) pasteurization at 161°F (72°C) for 15 seconds¹² resulted in similar mean serum IgG concentrations in treatment (1,476 mg/100 mL) and control (1,435 mg/100 mL) calves without differences in the proportion of calves with FPT between the treatment (16.22%) and control groups (19.55%).

Frequently, colostrum is frozen and thawed as a tool to feed high levels of immunoglobulins on many dairies without evidence of detrimental effects upon immunoglobulins or nutrients.^{2,17} According to Foley *et al*,⁵ there was no loss of vitamin A and only a small reduction in carotene content (6%) in colostrum stored at -5°F (-20.5°C) for six months. When Holloway *et al*¹¹ fed calves at three hours of age with 1.05 gallon (4 L) aliquots of frozen-thawed or fresh colostrum using oroesophageal intubations, no significant differences in serum IgG concentrations were found at 48 hours of age (2,097.0 ± 681.7 mg/100 mL vs 1,734.3 ± 958.6 mg/100 mL, respectively). When feeding the dam's colostrum is not possible, strategies to prevent transmission of infectious agents through the colostrum include feeding pasteurized colostrum or colostrum replacers.⁹ To our knowledge there are no studies of feeding pasteurized-frozen-thawed colostrum (PFTC) to calves to evaluate the effect upon SP levels.

The objective of this study was to determine and compare the SP concentrations in calves fed either PFTC or UPC.

Materials and Methods

Colostrum Collection

This study was conducted on a commercial dairy in the state of Queretaro, Mexico. The milking herd con-

sisted of 1,250 cows, and cows calved on a farm located three miles from the milking herd. Calvings occurred in individual maternity pens located within the close-up corrals. All cows that calved during the night were milked-out by machine and colostrums were kept and stored in individually identified buckets at room temperature (41-50°F; 5-10°C) before transport to the dairy herd, where pasteurization was carried out the next morning. As a general rule, colostrums from overnight calvings were pasteurized and colostrums from daytime calvings were fed fresh to the calves. Three days after calving, the cows were transported to the main dairy and placed in the milking herd.

Management of Pasteurized Frozen-thawed Colostrums (PFTC) and Unpasteurized Colostrum (UPC)

First-milk colostrum obtained from each cow calving during nighttime was visually inspected and smelled prior to pasteurization. Bloody, mastitic or soured colostrum was discarded. For all viable colostrums, a colostrometry test^a was performed at a range in temperature of 68-75°F (20-24°C), and only colostrums with high specific gravity (≥1.054) and high immunoglobulin contents (≥70 g/L) were included in the study. These colostrums were filtered through a mesh and mixed to form a batch per day, for a total of 12 batches during the experiment. For every batch, volume was recorded and the colostrometry test was performed again at a temperature of 68-75°F (20-24°C). Pasteurization was carried out using an automated commercial batch-type pasteurizer^b that heated the contents to 145°F (63°C) for 30 minutes, and then cooled to 39.2°F (4°C). Once pasteurization was completed, consistency of the colostrum was assessed as normal, slightly thickened or moderately thickened, and then was poured into 2.11 quart (2 L), zip-lock type plastic bags identified with the date and batch number. Bags were immediately placed in a freezer at 20°F (-7°C). The following day, the frozen colostrums were transported to the calf farm where they were stored in another freezer at -4°F (-20°C). These colostrums were kept frozen for a minimum of 24 hours before thawing to feed calves.

Cows calving during the day were immediately milked out (first-milk colostrums) and colostrum were filtered through an extended gauze. This UPC was not pooled, but instead colostrum from each cow was fed to her own calf without performing the colostrometry test.

Calf Allocation and Treatment, Colostrum Thawing, Sample Collection, Tests and Record Keeping

All calves (40 males and 11 females) born from October 1 to October 30, 2004 and included in the study were immediately placed in clean, individual hutches after birth. Calves were enrolled alternatively according to the order of birth into either the group receiving PFTC

or the control group receiving UPC. Bags containing 2.11 quarts (2 L) of PFTC were thawed by immersion in warm water at 122-131°F (50-55°C) for 10 to 15 minutes. Once close to or at body temperature, 2.11 quarts of PFTC was fed to calves in the experimental group, using a suckling bottle, for three consecutive feedings during the first 24 hours of life. Control calves were fed 1.74 quarts (1.65 L) of UPC using a suckling bottle for three consecutive feedings during the first 24 hours of life.

Between 24 and 96 hours after birth, blood was obtained from every calf by jugular venipuncture for serum collection and a handheld refractometer^c with temperature compensation was used to determine SP¹⁴ values.

Information recorded for every calf included calf identification number, date and time of birth, gender, treatment allocation, volume fed in the first, second and third feedings and time of each feeding. Age (hours) and total volume fed in the first 24 hours of life were calculated.

From October 1 to 15, 2004, colostrum pasteurization, freezing, blood sample collection and record keeping were done by the first author, and from October 16 to 30, 2004 by the second author. Throughout the study at least one of the authors provided daily supervision of colostrum collection, calf allocation, colostrum thawing and feeding, and record keeping at the farm.

Statistical Analysis

Of the 51 calves born during the study period, 25 calves were fed PFTC and 26 were fed UPC. The statistical analysis was performed with linear models (ANOVA) using the General Model Procedure.¹⁹ The analysis examined the associations between colostrum assignment and SP levels. Final model for analysis of the dependent variable SP (g/100 mL) included only the treatment effect. The effects of time to first feeding (in hours), calf gender, calf age at sampling (hours) as linear and quadratic covariates and all first-order interactions were excluded from the final model because they were not found significant ($P>0.05$).

Results

Colostrum

The average volume of the 12 batches of first-milk colostrum was 5.7 ± 2.2 gallons (21.6 ± 8.5 L), and average immunoglobulin content was 85.2 ± 18.9 g/L. Consistency after pasteurization remained normal in six samples, slightly thickened in four and moderately thickened in two. Colostrum ingestion occurred at 1.3, 12.2 and 22.1 hours of life in calves fed PFTC, and at 1.1, 12.4 and 22.9 hours for calves fed UPC. There was no significant difference in colostrum ingestion time between treatment groups ($P>0.05$). Average volume consumed per feeding in the first day of life was 2.11 quarts (2 L) for calves fed PFTC and 1.74 quarts (1.65 L) for those fed UPC, for a total of 1.58 gallons (6 L) and 1.30 gallons (4.95 L), respectively.

Serum Protein

Average SP level was lower in calves consuming PFTC (5.92 ± 0.15 g/100 mL) compared to those fed UPC (6.53 ± 0.14 g/100 mL), as shown in Table 1 ($P=0.0048$). The percentage of calves with FPT (SP level <5.2 g/100 mL) tended to be higher for the calves fed PFTC (24%) compared to calves fed UPC (3.8%) (Table 2; $P=0.06$).

Discussion

At the beginning of this study, three batches of pooled colostrums were discarded due to extreme thickening after pasteurization. In the 12 batches included in the study, there was moderate thickening in two batches, which resulted in some difficulty in consumption by the calves, although they consumed all that was offered. The first batch included in the study had a volume of 11.88 gallons (45 L) and an immunoglobulin content of 42 g/L. However, if we separate these data, the average volume for the remaining 11 batches was of 5.0 ± 1.3 gallons (19.7 ± 5 L), with a minimum of 2.38 and a maximum of 7.4 gallons (9-28 L). The average immunoglobulin content was of 88.8 ± 14.5 g/L, with a minimum of 70 g/L and a

Table 1. Serum protein levels in calves fed pasteurized-frozen-thawed colostrum or unpasteurized colostrum for the first three feedings within the first 24 hours of life.

Group	Calves		Serum protein (g/100 mL)	
	n	x	Std error	
Pasteurized-frozen-thawed colostrum	25	5.92 ^a	0.15	
Unpasteurized colostrum	26	6.53 ^b	0.14	

^{ab} Different superscripts indicate statistically significant differences ($P=0.0048$)

Table 2. Proportion of calves fed pasteurized frozen-thawed colostrums that had serum protein levels of <5.2, 5.2-5.5 or >5.5 g/100 mL.*

Group	Calves	Serum protein (g/100 mL)					
		< 5.2		5.2- 5.5		> 5.5	
		n	%	n	%	n	%
Pasteurized-frozen-thawed colostrum	25	6	24	2	8	17	68
Unpasteurized colostrum	26	1	3.8	1	3.8	24	92.4

* Independence chi-square test, $P=0.06$

maximum 110 g/L. The sensitivity of the colostrometry test increases in colostrums with higher specific gravity.¹⁶ In this study, and based only on the colostrometry test, we purposely selected only colostrums with a specific gravity equal or greater than 1.054. This resulted in a high immunoglobulin content (>70 g/L), with the exception of the first batch.

The use of small-sized batches in our study was due to either a low number of calvings per night, insufficient production of colostrums of high specific gravity or discarded colostrums due to blood or mastitis. According to Godden *et al.*,⁹ smaller-volume batches are associated with less loss in immunoglobulin content in pasteurized colostrums.

The differences in hours at first, second and third feedings between the two treatment groups were not significant ($P>0.05$). In the group fed PFTC, three plastic bags containing 2.11 quarts of frozen colostrum were thawed and fed during the first day of life to each calf. Calves fed UPC received colostrums from their own dams. Calves in the UPC group were fed 1.74 quarts (1.65 L) three times in the first day of life, resulting in a total volume of 1.30 gallons (4.95 L). In spite of the smaller volume of UPC fed to the control calves, the SP levels attained by this group were higher than those in calves receiving PFTC, suggesting that the immunoglobulin concentrations of the UPC were higher than those in PFTC.

Godden *et al.*,⁹ found significantly higher IgG concentrations in calves fed unpasteurized colostrum (19.1 mg/mL) than in those fed pasteurized (9.7 mg/mL) colostrum. In our study, calves fed UPC were handled and fed according to the farm's management protocol, which explains why the colostrum given to these animals was not evaluated with a colostrometer. In spite of this, calves that received UPC from their own dams had higher SP ($P=0.0048$) levels (6.53 ± 0.14 g/100 mL) than calves fed PFTC (5.92 ± 0.15 g/100 mL). Results from this study suggest that calves fed at least 1.30 gallons (4.95 L) of first-milk UPC within the first 23 hours of

life achieved a satisfactory SP level. Calves fed PFTC also developed satisfactory SP concentrations and had successful passive transfer of immunoglobulins. The levels of SP in both groups in this study are higher than those recommended by Tyler *et al.*,²² who suggested that 5.5 g/100 mL are indicative of an efficient passive transfer in the calf.

PFTC was subjected to three processes: pasteurization, freezing for a minimum of 24 hours and thawing. The two latter processes apparently did not destroy a significant level of immunoglobulins in the colostrums, as has been suggested by Holloway *et al.*¹¹ and Quigley.¹⁸ In our study, frozen-thawed colostrums were an adequate source of IgG for Holstein calves. Jamaluddin *et al.*¹² pasteurized colostrum at 161°F (72°C) for 15 seconds, and reported no significant differences in percentage of calves experiencing FPT (<10 mg/mL of IgG measured 48 to 96 hours after colostrum intake) between treatment (16.2%) and control groups (19.5%). In our study we found a higher percentage ($P=0.06$) of FPT in calves fed PFTC; 24% for calves fed PFTC and 3.8% for calves fed UPC. Differences reported in studies may be due to variations in pasteurization protocols.

Finally, freezing colostrum requires a freezer, extra handling, daily thawing of required colostrum and is less cost-effective than refrigeration.^{2,5,18} Use of PFTC, however, can provide a source of immunoglobulins when fresh colostrum is in short supply.

Conclusions

We acknowledge some aspects of our study might limit the value of the results, such as the use of a colostrometer for ruling out colostrums with low immunoglobulins content, differences in handling the colostrums between cows calving during night hours and in daylight, feeding a smaller volume to calves fed UPC and variation in batch sizes of PFTC which were determined by the number of suitable colostrums available from the night before.

The selection of first-milk colostrums of high immunoglobulin content for pasteurization at 145°F (63°C) for 30 minutes, followed by freezing, thawing and then feeding to just-born calves, provided SP levels 0.42 g/100 mL or 10.3% higher than the minimum levels recommended to avoid FPT. These levels, however, were lower than those of the calves fed UPC. Likewise, a strong trend for a higher percentage of calves with FPT was found in the group fed PF²TC, but the difference was not significant ($P=0.06$).

Endnotes

^aColostrometer, Biogenics, Mapleton, OR 97453

^bDT Silver pasteurizer, Dairy Tech. Inc. Windsor, CO 80550

^cRefractometer, TS 400, PO Box 123, Buffalo, NY 14240

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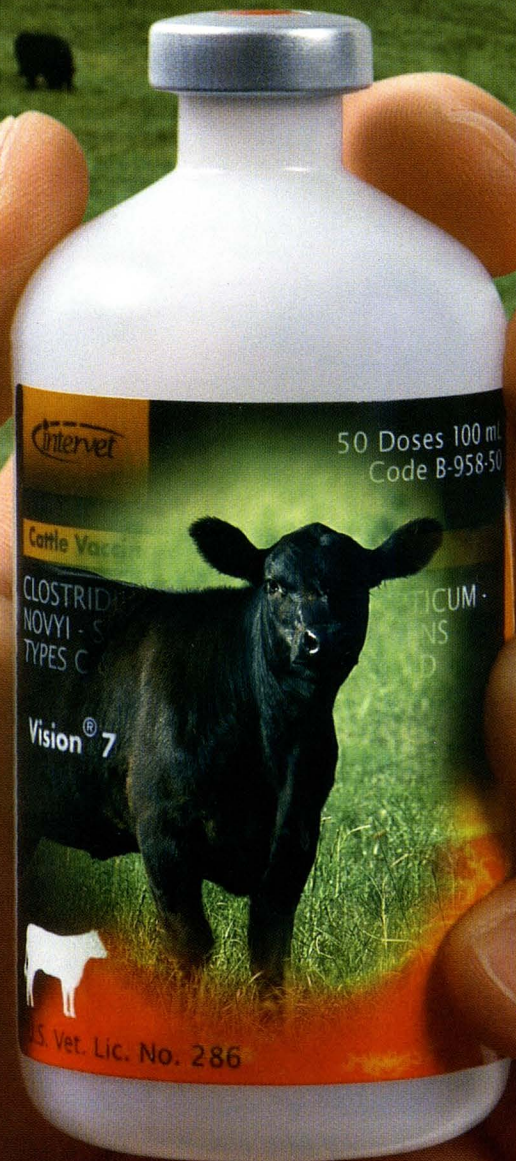
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As with all drugs, the use of EXCEDE Sterile Suspension is contraindicated in animals previously found to be hypersensitive to the drug. Though safe in cattle when properly administered, inadvertent intra-arterial injection in the ear is possible and is fatal. EXCEDE has a pre-slaughter withdrawal time of 13 days. As with all drugs, EXCENEL RTU EZ should not be used in animals found to be hypersensitive to the product. EXCENEL RTU EZ has a pre-slaughter withdrawal period of 3 days.

¹ Based on the results of a blinded well-controlled study. Data on file, Study 0788-7922-2003-001, Pfizer Inc.

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Full product information can be found on page 197