ORIGINAL ARTICLE

Effect of quality of colostrum on health, growth and immunoglobulin G concentration in Holstein calves in a hot environment

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ABSTRACT

The aim of this study was to determine the effect of ingestion of pasteurized and subsequently frozen–thawed pooled colostrum (≥50 mg Ig/mL) with different bacterial counts and immunoglobulin concentration (IgC) on the occurrence of diarrhea and pneumonia in 306 neonatal Holstein calves in a hot environment. Calves were assigned to be fed colostrum with total bacterial counts (TBC) lower or greater than 100 000 colony-forming units (cfu)/mL, total coliform counts (TCC) greater or lower than 10 000 cfu/mL, and IgC lower or higher than 85 mg Ig/mL. Calves fed colostrum with TBC ≥100 000 cfu/mL were more likely (risk ratio 1.34, confidence interval 1.05–1.71; $P < 0.05$) to present pneumonia than calves receiving colostrum with lower TBC (incidence 53.2 vs. 39.8%). Calves fed colostrum with high TCC had increased chances of suffering pneumonia (51.4 vs. 42.1%; $P < 0.05$) than calves fed colostrum with lower TCC. Calves fed colostrum with ≥85 mg Ig/mL tended to present higher daily weight gain (505 ± 113 vs. 484 ± 126 g; $P = 0.09$). TBC and TCC in colostrum did not influence the incidence rate of diarrhea. It was concluded that under the conditions of the present study, heavy contamination of on-farm pasteurized frozen–thawed colostrum is seemingly unavoidable and this contamination poses a threat for pneumonia, but not for diarrhea.

Key words: *colostrum pasteurization, dairy calves, diarrhea, immunoglobulins, pneumonia.*

INTRODUCTION

An adequate intake of high-quality colostrum constitutes one of the most important management practices in determining good health, growth rate and pre-weaning survival of neonatal dairy calves (McGuirk & Collins 2004; Godden 2008). Both morbidity and mortality of calves are not exclusively correlated with colostral immunoglobulin passive transfer in calves, but numerous studies have reported an increased risk of morbidity and mortality in calves with low levels of circulating immunoglobulins (Dewell *et al.* 2006; Rea *et al.* 1996). Dairy calves with satisfactory passive transfer of immunity have lower pre-weaning morbidity and mortality as well as fewer antibiotic treatments compared with calves with failure of passive transfer (Berge *et al.* 2005; Williams *et al.* 2014).

Other long-term benefits derived from adequate colostral immunoglobulin transfer shortly after birth include improved rate of weight gain (Dewell *et al.* 2006; Berge *et al.* 2009), reduced mortality in the post-weaning period, increased feed efficiency, reduced age at first calving, improved first and second lactation milk production (Faber *et al.* 2005), and younger age to first insemination (Furman-Fratczak *et al.* 2011).

Despite the health and nutritional benefits for the dairy calf, colostrum is a potential early source of exposure to microbial pathogens such as *Escherichia coli*, *Salmonella* or *Mycobacterium avium paratuberculosis*, the bacterial species responsible for Johne's disease. These pathogens can cause diseases such as septicemia and scours, and they may interfere with passive absorption of the antibodies from the gut into the circulation system (Gelsinger *et al.* 2015). Thus, clean milking and calf-feeding equipment and udders before harvesting, storing and feeding colostrum are necessary to prevent infectious diseases.

Microorganisms present in colostrum come from multiple sources, including secretion from the mammary gland, contamination during milking, storage

or feeding, or bacterial proliferation in stored colostrum (McGuirk & Collins 2004; Stewart *et al.* 2005). Insufficient data exist presently to describe the effect of quality of colostrum, particularly with a high bacterial load on animal health and growth under extreme thermal stress.

Therefore, the objective of this study was to determine the effects of feeding colostrum with different bacterial and immunoglobulin concentrations (based on colostrometer/hydrometer readings) on serum immunoglobulin G (IgG) concentrations and the occurrence of diarrhea and pneumonia in preweaning dairy calves exposed to high ambient temperatures. Another objective was to compare weaning weight, average daily gain, wither height, days treated for diarrhea, and days treated for respiratory disease of calves offered colostrum with high or low bacterial loads and high or medium immunoglobulin content. An additional objective was to determine the association between serum IgG content and the occurrence of pneumonia and diarrhea in pre-weaning calves reared in a high-temperature environment.

MATERIAL AND METHODS
Study herd, housing and feeding
The study was conducted in summer 2014 (5 June– 28 August), on a large (2400 milking cows) commercial highly technified dairy farm in northern Mexico (26° N). Precipitation during the study period was 124 mm with a mean relative humidity of 40.2%. The maximum ambient temperature during the trial varied from 32.6°C to 42.2°C. The peak minimum ambient temperature was 25°C and the lowest minimum temperature was 19.2°C. The study population consisted of 306 Holstein-Friesian calves. The mean date of birth of the calves was 16 July, 2014 (SD = 41 days). The mean calf body weight (BW) at birth was 39.8 kg (SD = 4.7 kg). Protocols used for enrolling and treating calves within this study were approved by the Animal Care Committee of the Autonomous Agrarian University Antonio Narro, Saltillo, Mexico.

Calves were systematically enrolled, depending on alternating days of birth, into one of the treatment groups receiving either colostrum with high or low total bacterial counts or with low or medium levels of immunoglobulin. Calves born after difficult labor or coming from twin gestations were not included in the study. Holstein female calves were separated from their dams approximately 25 min after birth before suckling occurred. Navels in all newborns were sprayed with diluted iodine (7%) and were housed in individual 2.0 × 1.5 wooden pens with a metallic roof, without bedding. A fine net placed 4 m above the ground covered all pens in order to attenuate solar radiation. Calves were fed 2.8 L of colostrum using a nipple bottle within the first 90 min after birth, and again 2 L, 12 h later. Therefore,

intake of colostrum was 4.8 L for all calves within 24 h post-calving.

From days 2 to 30, calves were fed 2 L of milk replacer containing 20% protein (all milk protein), 20% fat and neomycin sulfate 270 g/ton, twice a day. From day 31 to weaning calves received 4 L of milk replacer in the morning and 4 L in the afternoon. Calf starter concentrate (22% crude protein) and water were offered free-choice starting on day 4 post-partum and given until weaning at approximately 60 to 65 days of age.

Colostrum management
Colostrum was collected immediately after calving, following fore-stripping and predipping with a 0.5% iodine-based teat dip, and drying the teat ends with a hygienic paper towel. The milking unit was then attached and cows were milked. First milking colostrums from various cows containing ≥50 mg Ig/mL (based on colostrometer/hydrometer; Biogenics, Mapleton, OR, USA) was pooled each day when several cows calved. Colostrums with <50 mg/mL of Ig were discarded for newborn calves. Batches of high-quality colostrum were pasteurized using a commercial pasteurizer (Dairy Tech Inc., Windsor, CO, USA) equipped with an agitator that heats evenly the contents to 60°C, holds it for 30 min, and then automatically cools it to ambient temperature. Then, colostrum was placed in 1.89 L calf nursing plastic bottles and these were frozen at −20°C until needed for feeding. Colostrum was thawed in a refrigerator at 3°C and then in hot water at approximately 42°C for 1 h, before offering it to calves (38°C).

Colostrum sample collection and analyses
Shortly before offering colostrum to calves, a 20 mL aliquot of colostrum was collected and placed in sterile vials, which were then placed in ice and taken immediately to the laboratory. Bacteriological analyses were carried out at the Central Laboratory for milk quality of the enterprise LALA, Torreon, Mexico, where they underwent microbiological culture procedures to determine total bacteria count (TBC) and total coliform count (TCC). Colostrum samples were thoroughly mixed, and serial 10-fold dilutions of the colostrum were made in sterile brain-heart infusion broth for TBC determination. Two hundred microliters of each dilution were placed on the surface of plate count agar plates. The plates were incubated for 48 h at 32°C and all colonies were counted.

Two hundred microliters of colostrum from each dilution were pipetted onto MacConkey agar and spread over the entire surface. The plates were incubated at 37°C for 48 h, and all pink colonies, as a result of lactose fermentation, were counted. Colonies were confirmed as coliforms by means of the API 20E coliform identification test (BioMerieux, Inc., Hazelwood, MO, USA).

Health monitoring

Each calf was monitored twice daily by dairy staff and at least once daily by the herd veterinarian for signs of illness. All health events and medical treatments prescribed by the herd veterinarian were recorded on waterproof cards hanging on each calf pen. Data on the health events and treatments for each calf were subsequently transferred daily to a standard database by herd personnel until the end of follow-up for each calf at approximately 62 ($\pm$ 3) days of age. All personnel monitoring the calves were blinded to the treatment.

The preweaning morbidity events of interest in this study were diarrhea and pneumonia. Diarrhea was defined as a calf passing abnormal, watery feces (voiding of feces that splashed when hitting the ground) with a foul odor. Pneumonia was defined as a calf displaying signs of spontaneous cough and increased respiratory rate, with or without ocular and/or nasal discharge or otitis. A health event that occurred more than 7 days following recovery from a previous health event was considered a new incident. Health events occurring within 7 days of a previous health event with similar clinical signs were considered the same incident. Any animal that became ill received the appropriate care and veterinary treatment as required. Time to recover from pneumonia was defined as the end of a 24 h period without any clinical signs of severe pneumonia. Time to recover from diarrhea was defined as first stools with normal consistency.

Variable measurements

The weight of calves immediately after parturition and at weaning was estimated using a single heart girth measurement with a weight tape. The height of calves at calving and weaning was recorded with a measuring stick. Blood samples from all study calves (5 mL in Vacutainer tubes containing a clot activator) were collected at 48 h and 5 days of age, using jugular vein venipuncture. Blood samples were refrigerated for 24 h before serum was separated by centrifugation at 3500 $\times$ g for 15 min at 4°C and frozen at -20°C before IgG and metabolites concentration determination. A drop of serum obtained 48 h after calving was placed in a digital-dairy refractometer (MISCO Digital-Dairy™, Solon, OH, USA), in order to determine serum total protein (TP) levels. This instrument gives direct TP readings and serum TP is highly correlated with °Bx ($r = 1.00$) and IgG ($r = 0.93$) in 36-day-old Holstein calves (Deelen *et al.* 2014). Before testing of each sample, the refractometer reading window was cleaned, and the refractometer was calibrated with distilled water. All readings were made at room temperature (approximately 30°C).

Serum IgG concentrations in blood collected at 5 days of age were estimated using the technique for determination of IgG in 1-day-old calf serum described by Morrill *et al.* (2013). One milliliter of calf serum was added to a tube containing 0.5 mL 0.6 mol/L acetic acid and 45 µL of caprylic acid and mixed for 10 sec and allowed to incubate for 60 sec. Samples were then centrifuged for 20 min prior to analysis of the IgG-rich supernatant. Refractive indexes were obtained with an automatic digital refractometer (Reichter Technologies, model AR200, Depew, NY, USA) and these values were applied to the following equation: y = 5919.1 x − 7949.1, where y = serum IgG concentration (mg/mL) and x = refractive index of the fractionated supernatant. However, these results are somehow biased by the fact that the above-mentioned equation was derived from the association between serum IgG concentration determined by radial immunodiffusion (RID) and the refractive index of whole calf serum ($r = 0.86$; Morrill *et al.* 2013), but in the present study, the refractive index of the fractionated supernatant was used instead of the whole calf serum, which results in a lower correlation coefficient between these variables ($r = 0.77$; Morrill *et al.* 2013). Samples were assayed in duplicate, with an interassay coefficient of variation for IgG concentration ($n = 300$) of 0.19. Serum TP concentrations at 48 h after birth were determined to reveal the successful passive transfer of immunoglobulin to calves, whereas IgG at 5 days of age was used to reveal the association between concentration of IgG in colostrum and serum IgG content.

Serum metabolites at 5 days of age were determined using spectrophotometric methods (Coleman Junior II). Glucose was assayed with kit 115-A, based on glucose oxidase, and urea was determined using kit 640-A, based on urease (Sigma-Aldrich Co., St. Louis, MO, USA). Serum total protein levels were determined with a kit based on the bicinchoninic acid reagent, with bovine serum albumin as a protein standard (Pierce Chemical, Rockford, IL, USA). Creatinine was assayed using the QuantiChromTM Creatinine Assay Kit (DICT-500; BioAssay Systems, Hayward, CA, USA). Serum cholesterol was determined using the EnzyChrom™ cholesterol assay kit (ECCH-100; BioAssay Systems). Respiration rates were taken by counting the breathing movements of the sides and flanks. Respiration condition was classified with a 1–3 scale, where 1 was normal respiratory sign, 2 encompassed rhinitis and coughing, and 3 was assigned to animals with the previous signs plus heavy thoracic and abdominal breathing. Physiological variables and feces score were registered at 5 days of age. Feces were scored on the basis of: 1 firm and 4 scours (severe diarrhea). Days to recovery were calculated as time in days from the occurrence of pneumonia or diarrhea to complete recovery; this variable was measured during the whole pre-weaning period.

Statistical analyses

Data were analyzed in a prospective cohort study design framework. Descriptive statistics (proc UNIVARIATE of SAS; SAS Institute Inc., Cary, NC, USA) were produced

for total bacterial counts and coliform bacterial counts in colostrum offered to calves. In order to reveal the degree to which the independent variables TBC and TCC were explained by each other, multicollinearity was tested using the variance inflation factor (PROC REG with the VIF TOL and COLLIN options; Marquardt method for VIF and eigenvalue analysis of the model factors). Variance inflation factor was 1.0 and the largest condition index was 0.75, which indicates an inexistence co-dependence between TBC and TCC; that is, the actual number of colony-forming units (cfu) counted in either group had no effect on the occurrence of the other. Additionally, it was revealed if immunoglobulin concentration and TBC, and immunoglobulin concentration and TCC in colostrum were collinear with which other. These analyses showed no evidence of collinearity between these variables.

The degree of contamination of colostrum was dichotomized at 100 000 cfu/mL, which was defined as the cut-off point for colostrum contamination considering TBC. TCC were dichotomized by using a level of 10 000 cfu/mL. Colostrometer/hydrometer readings were divided into two classes corresponding to <85 and ≥85 mg Ig/mL. Classes for serum total proteins 48 h postpartum were defined as <6.5 and ≥6.5 mg/dL. By using the Wilk–Shapiro/Rankin Plot procedure, it was found that data pertaining to feces consistency and respiration condition did not follow the normal distribution, and therefore, the KruskalWallis procedure (PROC NPAR1WAY in SAS) was used for the analysis of these variables.

The effects of TBC, TCC and colostrum immunoglobulin content on the occurrence of pneumonia and diarrhea were analyzed with a generalized lineal model with the Genmod procedure of SAS with a logit link function. The statistical model used was: $Y_{ijk} = \mu + B_i + C_j + G_k + (BC)_{ij} + (BG)_{ik} + (CG)_{jk} + e_{ijk}$, where Y_{ijk} = observation; μ = overall mean; B_i = fixed effect of total bacterial count (<100 000 or ≥100 000 cfu/mL); C_j = fixed effect of coliform count (<10 000 or ≥10 000 cfu/mL); G_k = colostrum immunoglobulin content effect (<85 or ≥85 mg/mL); $(BC)_{ij}$, $(BG)_{ik}$, $(CG)_{jk}$ = two-term interaction; e_{ijk} = error term. Variables and first-order interactions were removed from the model if found to be non-significant. Therefore, only significant ($P < 0.05$) variables or interactions were left in the reduced model. The relative risks for the development of diarrhea or pneumonia in association with colostrum characteristics were estimated along with the corresponding 95% confidence intervals (CIs), using the PROC NLMIXED procedure of SAS with the LOGISTIC function.

Analysis of variance (PROC MIXED in SAS) was used to describe the association between variables related to colostrum quality and weight traits, physiological variables and serum metabolite concentrations of calves. Birth weight of calves was included in the models as a covariate. The following model was used: physiological variables, weight traits, withers height and various serum metabolite concentrations $(Y_{ijk}) = \mu + V + B_i + C_j + G_k + (BC)_{ij} + (BG)_{ik} + (CG)_{jk} + e_{ijk}$, where: μ = overall mean, V = covariate (birth weight of calves), B_i = total bacterial count effect (<100 000 or ≥100 000 cfu/mL), C_j = coliform count effect (<10 000 or ≥10 000 , cfu/mL), G_k = colostrum immunoglobulin content effect (<85 or ≥85 mg/mL), $(BC)ij$, $(BG)_{ik}$, $(CG)_{jk}$ = two term interaction; e_{ijk} = random residual error term. Variables and first-order interactions were removed from the model if found to be non-significant. Final significance was declared at $P < 0.05$ for all models. $P = 0.07$ or 0.09 was considered a significant trend.

RESULTS

Six calves (2%) died during the first 60 days of life and were not replaced. During the preweaning period, diarrhea (at least one episode) occurred in 43% of calves, whereas respiratory tract disease occurred in 45% of calves. The TBC for 300 colostrum samples ranged from 20 to 400 000 cfu/mL. The average TBC was 152 970 cfu/mL (95% CI: 132 214 to 173 725 cfu/mL). Mean colostrum TCC was 14 248 cfu/mL (95% CI: 11 504 to 16 992 cfu/mL). Considering the serum total protein obtained with a refractometer, and the fact that serum IgG and total protein concentrations are moderately correlated ($r = 0.67$ to 0.79; Jersey or Holstein heifer calves between 1 and 30 days of age; Villarroel *et al.* 2013; Elsohaby *et al.* 2015), the proportion of calves having failure of passive transfer 48 h after birth, where the serum IgG concentration was less than 1000 mg/dL (calves with serum total protein concentrations ≥5.2 g/dL would have serum IgG concentrations >1000 mg/dL, Foster *et al.* 2006; Priestley *et al.* 2013), was only 2%.

There were no significant differences in serum total protein concentrations 48 h postpartum (6.52 ± 0.49 g/dL for high TBC and 6.48 ± 0.54 g/dL for low TBC; mean ± SD) among calves fed colostrum with high or low TBC. Likewise, there were no differences in serum proteins (6.53 ± 0.52 g/dL for high TCC and 6.48 ± 0.52 g/dL for low TCC; mean ± SD) in calves fed colostrum with contrasting levels of TCC. There was no significant difference in serum total proteins at 48 h of life (6.58 ± 0.56 g/dL vs. 6.61 ± 0.54 g/dL) between calves ingesting colostrum with <85 or ≥85 mg Ig/mL (based on colostrometer/hydrometer readings). Compared to calves that ingested colostrum with TBC <100 000 cfu/mL, calves fed colostrum with TBC ≥100 000 cfu/mL were more likely to present pneumonia (Table 1). Highly contaminated colostrum (≥100 000 cfu/mL) did not affect the risk of diarrhea. Likewise, TCC did not represent a risk for the occurrence of diarrhea, but calves fed colostrum with TCC ≥10 000 cfu/mL were more likely to present pneumonia (Table 1). There was a TBC by colostrum

Table 1 Relative risk of pneumonia in relation to total bacteria counts and coliform bacteria counts in pasteurized frozen–thawed colostrum ingested by Holstein calves ($n = 300$) in a hot environment. Values in parenthesis are incidences (%)

Bacteria counts	Immunoglobulin content, mg/mL			Relative risk (95% CI)	P
	≥85	<85	Combined		
TBC, cfu/mL†					
≥100 000	31/58 (53.5)[a]	35/66 (53.0)	66/124 (53.2)	1.34 (1.05–1.71)	0.02
<100 000	31/84 (36.9)[b]	39/92 (42.4)	70/176 (39.8)		
TCC, cfu/mL†					
≥10 000	20/45 (44.4)	34/60 (56.7)[a]	54/105 (51.4)	1.24 (0.97–1.55)	0.05
<10 000	42/97 (43.3)	40/98 (40.8)[b]	82/195 (42.1)		

[a,b]Values in the same column followed by different superscript letters differ significantly ($P < 0.05$). †Bacteria counts × immunoglobulin content interaction ($P < 0.05$). cfu, colony-forming units; CI, confidence interval; TBC, total bacteria counts; TCC, total coliform counts.

immunoglobulin concentrations interaction ($P < 0.05$), with a lower ($P < 0.05$) incidence of pneumonia in calves offered colostrum low in TBC and rich in immunoglobulin than calves receiving colostrum rich in immunoglobulin but highly contaminated (Table 1). Likewise, there was a TCC × immunoglobulin concentration interaction ($P < 0.05$), with the highest ($P < 0.05$) incidence of pneumonia in calves ingesting colostrum with high TCC and low immunoglobulin concentrations as compared with a lower incidence of this disease with high TCC but high immunoglobulin concentrations (Table 1).

Calves that received first-milking pooled colostrum with TBC ≥100 000 cfu/mL had higher ($P < 0.01$) scores for fecal consistency than calves with lower bacterial counts; however, no difference was noted between the calves fed high or low TBC colostrum for respiration traits and rectal temperature (Table 2). Calves fed colostrum with TBC ≥100 000 cfu/mL tended ($P = 0.09$) to present a longer recovery period from pneumonia or diarrhea. Analysis of variance showed a significant effect of both TCC and colostrum immunoglobulin content on fecal consistency, with more watery feces in calves

ingesting colostrum with ≥10 000 cfu/mL and <85 mg Ig/mL. Colostrometer/hydrometer readings had no significant effect on the occurrence of pneumonia (46.8 vs. 43.7) incidence rate for colostrum immunoglobulin concentration readings lower or greater than 85 mg/mL) and diarrhea (45.8 vs. 39.4 incidence rate for colostrum immunoglobulin concentration readings lower or greater than 85 mg/mL), but calves with <6.5 mg/dL serum total protein levels at 48 h post-calving presented more ($P < 0.05$) rapid breathing (65.5 breaths/min), which is a typical sign of pneumonia, than calves with ≥6.5 mg/dL total proteins in blood (60.1 breaths/min). Average daily gain tended ($P = 0.09$) to be higher in calves receiving the colostrum with higher immunoglobulin concentration than calves with <85 mg Ig/mL (Table 3). Both TBC and TCC did not influence growth traits of calves. There was no effect of TBC and TCC in first-milking pooled colostrum on the majority of serum metabolites indicative of nutritional status (Table 4), except that serum cholesterol concentrations were higher ($P < 0.05$) in calves fed colostrum with ≥100 000 TBC than calves receiving colostrum with <100 000 TBC. Calves receiving the colostrum with

Table 2 Physical examination findings at 5 days of age and fecal characteristics in preweaning Holstein calves ($n = 300$) fed colostrums with different bacterial counts and IgG levels in a hot environment. Values are means ± standard deviations

Variables of colostrum	Respiration rate (breaths/min)	Respiration condition†	Rectal temperature (°C)	Fecal consistency score‡	Days to recovery¶
Total bacterial counts, cfu/mL					
≥100 000 ($n = 124$)	61.1 ± 21.1	1.48 ± 0.9	39.2 ± 0.3	1.49 ± 0.9[b]	4.1 ± 3.7[x]
<100 000 ($n = 176$)	64.6 ± 20.7	1.42 ± 0.8	39.2 ± 0.4	1.23 ± 0.6[a]	3.4 ± 3.6[y]
Coliform bacteria counts, cfu/mL					
≥10 000 ($n = 105$)	64.1 ± 20.4	1.5 ± 0.8	39.2 ± 0.3	1.6 ± 0.9[a]	3.6 ± 3.2
<10 000 ($n = 195$)	61.5 ± 21.9	1.5 ± 0.9	39.2 ± 0.4	1.2 ± 0.6[b]	3.8 ± 4.0
Ig content, mg/mL§					
≥85 ($n = 142$)	61.1 ± 20.2[x]	1.4 ± 0.9	39.2 ± 0.3	1.2 ± 0.6[b]	3.6 ± 3.6
<85 ($n = 158$)	65.0 ± 21.1[y]	1.5 ± 0.8	39.2 ± 0.3	1.5 ± 0.9[a]	4.3 ± 3.9

[a,b]Each variable means within a column followed by different superscripts differ ($P < 0.01$). [x,y]Each variable means within a column followed by different superscripts tend to differ $P = 0.09$). †Scale 13; 1 = normal respiratory signs, healthy; 3 = severe respiratory signs. ‡Fecal consistency score; scale from 1 to 4, with 1 being normal feces to 4 being severe diarrhea. §Colostrometer/hydrometer readings at room temperature. ¶Time to recover from pneumonia (end of a 24 h period without any clinical signs of this disease) or diarrhea (first stools with normal consistency) during the preweaning period. CFU, colony-forming units; IgG, immunoglobulin G.

Table 3 Growth traits in preweaning Holstein calves (*n* = 300) fed colostrums with different bacterial counts and IgG levels in a hot environment. Values are means ± standard deviations

Colostrum variables	Weaning weight (kg)	Withers height (cm)	Average daily gain (g)
Total bacterial counts, cfu/mL			
≥100 000 (*n* = 124)	64.7 ± 6.5	85.4 ± 2.9	495 ± 113
<100 000 (*n* = 176)	65.0 ± 7.1	85.7 ± 2.6	493 ± 123
Coliform bacteria counts, cfu/mL			
≥10 000 (*n* = 105)	64.0 ± 6.2	85.6 ± 2.9	486 ± 118
<10 000 (*n* = 195)	65.3 ± 7.1	85.5 ± 2.7	498 ± 123
Ig content, mg/mL†			
≥85 (*n* = 142)	65.8 ± 6.2	85.8 ± 2.8	505 ± 113[a]
<85 (*n* = 158)	63.9 ± 7.2	85.4 ± 2.7	484 ± 126[b]

For each variable, means within a column followed by different superscripts tend to differ (*P* = 0.09). †Colostrometer/hydrometer readings at room temperature. CFU, colony-forming units; IgG, immunoglobulin G.

Table 4 Serum IgG 5 days postpartum and serum metabolites indicative of nutritional status, in preweaning Holstein calves (*n* = 300) fed colostrums with different bacterial counts and IgG levels in a hot environment. Values are means ± standard deviations

Colostrum variables	IgG (mg/mL)†	BUN[**] (mg/dL)	Glucose (mg/dL)	Creatinine (mg/dL)	Cholesterol (mg/dL)	Serum total protein (mg/dL)
Total bacterial counts, cfu/mL						
≥100 000 (*n* = 124)	29.8 ± 9.6	13.8 ± 2.1	111 ± 9	2.3 ± 0.2	58.4 ± 16.7[a]	7.8 ± 2.0
<100 000 (*n* = 176)	30.3 ± 9.0	14.2 ± 2.3	111 ± 10	2.3 ± 0.3	55.5 ± 17.4[b]	7.7 ± 1.8
Total coliform counts, cfu/mL						
≥10 000 (*n* = 105)	30.7 ± 9.3	14.0 ± 2.1	111 ± 10	2.3 ± 0.2	56.5 ± 16.7	7.7 ± 2.1
<10 000 (*n* = 195)	29.8 ± 9.2	13.9 ± 2.3	112 ± 9	2.3 ± 0.3	56.8 ± 17.4	7.8 ± 1.8
Ig content, mg/mL‡						
≥85 (*n* = 142)	31.1 ± 8.9[a]	14.2 ± 2.2[x]	112 ± 8	2.3 ± 0.2	57.8 ± 18.1	7.9 ± 1.7
<85 (*n* = 158)	28.9 ± 9.4[b]	13.8 ± 2.3[y]	110 ± 8	2.3 ± 0.3	55.7 ± 16.1	7.6 ± 2.0

[a,b]Each variable means within a column followed by different superscripts differ (*P* < 0.05). [x,y]Each variable means within a column followed by different superscripts tend to differ (*P* = 0.07). †Serum IgG, 5 days post-calving. ‡Colostrometer readings at room temperature. BUN, blood urea nitrogen; CFU, colony-forming units; IgG, immunoglobulin G.

≥85 mg Ig/mL presented higher (*P* < 0.05) serum IgG 5 days postpartum. Likewise, calves fed with the colostrum with the higher levels of immunoglobulin tended (*P* = 0.07) to present higher blood urea nitrogen (BUN) compared with calves fed colostrum with lower immunoglobulin concentrations (Table 4). No interaction effect between colostrum variables for physiological variables, serum metabolites concentration and growth traits was found to be significant.

DISCUSSION
Colostrum contamination and morbidity

Previous studies using commercial batch pasteurizers at 60°C for 60 min have reported success in reducing total bacterial count below the current industry recommended upper limit of 100 000 cfu/mL for colostrum (McGuirk & Collins 2004; Kryzer *et al.* 2015), while preserving colostral IgG concentrations (McMartin *et al.* 2006; Johnson *et al.* 2007). In the present study, pasteurization of colostrum and subsequent freezing and thawing failed to reduce both TBC and TCC, which indicates that the magnitude of bacterial contamination of pasteurized frozen–thawed colostrum is important, with

42% of samples showing counts above 100 000 cfu/mL. Thus, the colostral management practice used in this study was unsuitable for reducing bacterial load. One possible factor influencing the rapid colonization of colostrum by bacteria was the hot weather prevailing during this trial, because colostrum fed during warm months is contaminated more often than is colostrum fed during cold months (Fecteau *et al.* 2002). A possible alternative to reduce contamination of pasteurized frozen-thawed colostrum is thawing it in a microwave oven for short periods on low power (Pfeiffer *et al.* 2010).

It is common to have 23 to 29% herd level incidence of preweaning diarrhea and 22 to 28% for pneumonia in dairy calves in intensive dairy herds in temperate zones (Virtala *et al.* 1996; Teixeira *et al.* 2013; Windeyer *et al.* 2014). The total morbidity found for these diseases in the present study is twice the level as that previously reported from temperate areas. The higher incidence of these diseases in the present study might be due to a compromised immune function of calves due to heat stress (both in the uterus during late gestation; Strong *et al.* 2015, and in the field; Peli *et al.* 2013; Yun *et al.* 2014). Calves held under stressful conditions, such as the severe heat experienced in the present study, are

more prone to diseases (Cobb *et al.* 2014). In addition, the large size of this dairy herd may have contributed to the high prevalence of diseases in calves, because the workload on large farms is associated with less time being available for calf care. The lack of bedding material for calves could be another reason for high morbidity in this herd because the use of bedding decreases the risk of calf pen bacterial counts (Lago *et al.* 2006).

Calves health

Six calves died during the course of the experiment, but these incidences of death were not different between groups fed colostrum with high or low TBC or TCC. TP 48 h after calving did not differ between calves fed colostrum with high or low bacterial loads, which coincides with earlier studies by Godden *et al.* (2003) and Teixeira *et al.* (2013), where calves fed pasteurized colostrum had no differences in serum IgG concentrations in comparison with calves fed untreated colostrum.

An important finding in this study was the increased risk of developing pneumonia in those calves ingesting colostrum with high TBC or TCC. Also, evidence is provided of a positive interaction between colostrum TBC levels with the lowest pneumonia incidence rate in calves fed colostrum with low TBC and high immunoglobulin concentrations. Likewise, colostrum high in immunoglobulin concentration ameliorated the pneumonia incidence rate in calves with colostrum with high TCC. Thus, feeding colostrum with high immunoglobulin concentration is an essential management practice for minimizing the incidence rate of pneumonia in dairy calves.

The occurrence of pneumonia depends on complex interactions between different infectious agents, environmental factors and the immunological status of the calf. It is widely accepted that viruses are the first pathogens to intervene, whereas bacteria act as secondary invaders which worsen the already deteriorated animal's condition (Solis-Calderon *et al.* 2007; Taylor *et al.* 2010). Contaminated colostrum did not seem to be the source of the lung infection, but may have caused the immune system to stop functioning at optimal levels, which apparently triggered the outbreaks of pneumonia; a challenged immune system may have facilitated the conversion of long contamination into long infection.

A previous observational study on a high number of calves indicates that calves fed colostrum with a high bacteria count experienced lower levels of passive transfer compared with calves fed colostrum with a low bacteria count (Poulson *et al.* 2002). Thus, these data offer evidence toward a preventative strategy for pneumonia that would take advantage of low-contaminated colostrum in combination with high concentrations of immunoglobulin in colostrum, which would reduce this lung infection.

Surprisingly, there were no consistent differences in the occurrence of diarrhea between calves fed colostrum high in TBC as well as specific pathogens such as coliforms, or less contaminated colostrum. Similar results were observed by Teixeira *et al.* (2013). Also, Araujo *et al.* (2015) observed that colostrum with ≥100 000 cfu/mL did not cause diarrhea in dairy calves. We speculated that colostrum with high total bacterial counts would provide a more substantial source of bacteria for gut colonization and subsequently be reflected in a greater incidence of diarrhea. Also, it was expected that high TCC in colostrum could lead to high endotoxin levels, which might cause harm to neonatal calves, as has been the case in other studies (Moore *et al.* 2009). However, this was not observed and indicates that contamination of colostrum following pasteurization and freeze–thawing may provide a less pathogenic source of bacteria for gut colonization. Also, the lack of negative effect of high TBC on the occurrence of diarrhea was likely due to the presence of colostral or blood plasma immunoglobulins, which provide local immunity against several pathogens associated with calf diarrheal disease such as *Escherichia coli*, coronavirus and rotavirus (Crouch *et al.* 2000; Arthington *et al.* 2002). Another plausible reason for the absence of effect of highly contaminated colostrum on the occurrence of diarrhea apparently was the inclusion of an antibiotic in the milk replacer. Thus non-therapeutic levels of antibiotic could have had a positive effect on the calf gut microbiota. Other researchers have documented that provision of antibiotics in milk replacer improves weight gain and reduces mortality of dairy calves (Quigley & Drew 2000; Sarker *et al.* 2010).

Regarding the high incidence of diarrhea, these results are at odds with observations of other authors, in the sense that calves exposed to reduced pathogen exposure (TBC and TCC) and with improved serum IgG concentrations are at lower risk for illness (Godden *et al.* 2012). However, the health of a calf is dependent on many variables (Windeyer *et al.* 2014); therefore, it is not surprising that the present data do not agree regarding the influence of colostrum contamination on the health of calves.

Serum IgG concentration is well documented as an important factor in calf health (McGuirk & Collins 2004; Dewell *et al.* 2006). An important finding in this study was the lack of association between serum IgG concentration and health complications in calves. This response seems to be explained by the fact that all calves received colostrum containing ≥50 mg/mL of immunoglobulin, and therefore, practically all calves did not show failure of passive transfer 48 h after birth (serum IgG ≥ 1000 mg/dL; Priestley *et al.* 2013).

Calf performance

Overall, there was no benefit in weaning weight and daily weight gain from feeding colostrum with low

bacterial counts. We conjectured that differences in weight gain might become evident with highly contaminated colostrum because decreased food intake is a characteristic in sick or immune-challenged animals as a response to cytokines released by activated leukocytes (Johnson 1998). Given the large volume of colostrum fed during the first 12 h of life (4.8 L) and the high serum total protein levels 48 h after colostrum feeding in most calves, presumably, calves challenged with high bacterial counts during their first feeding were able to neutralize them, so that weight gain was unaffected. Increased growth rates in preweaning calves are consistent with increased intake of nutrients (Quigley *et al.* 2006), thus, it seems that calves fed the highly contaminated colostrum were equally affected in their health as calves fed colostrum with low bacterial counts, and consequently they were not affected in their growth rate.

The calves receiving the colostrum with higher immunoglobulin concentration tended to present higher daily weight gain during the preweaning period, compared with calves fed colostrum with <85 mg Ig/mL. The daily weight gain difference for the calves may be due to the combination of greater energy intake from the colostrum rich in immunoglobulin (Godden 2008) through the liquid feed, increased grain intake (Berge *et al.* 2009), and lower fecal scores (Quigley *et al.* 1997), indicative of fewer or less severe diarrhea.

Physiological variables and fecal scores

No differences were detected between calves receiving colostrum with high or low bacterial load with respect to mean heart rate, respiratory rate or rectal temperature, but fecal scores improved with feeding of colostrum containing high concentrations of immunoglobulin. Thus, excluding fecal scores, physiological variables used in the physical examination of calves did not provide any guidance in predicting enteric or respiratory illness early in life. This finding does agree with information from Fecteau *et al.* (1997), who found these variables useless to predict bacteremia (bacteriological culture of blood) from dairy calves. In the current study, feeding colostrum with <85 mg Ig/mL resulted in a decrease in fecal consistency. This response has been observed in calves challenged with coronavirus (Arthington *et al.* 2002) and *Escherichia coli* (Quigley & Drew 2000) infections. Thus, fecal scores are one of several measures of enteric health, but it is important to note that this variable also varies with nutrition, with more loose feces or greater fecal scores with better plane of nutrition (Bartlett *et al.* 2006; Ballou *et al.* 2015).

Blood serum metabolites

Calves receiving the colostrum with higher immunoglobulin concentrations tended to have higher BUN concentrations than calves fed colostrum with less than 85 mg Ig/mL. It is likely that greater absorption of protein from initial feedings of colostrum rich in proteins could lead to increased BUN concentrations, as the excess protein is metabolized and cleared from the body. Also, it could be that a portion of the greater crude protein in colostrum consumed by calves was used for energy with subsequent deamination and increased urea N concentration (Hadorn *et al.* 1997). Serum cholesterol levels were higher in calves fed the colostrum with ≥100 000 TBC. Low serum concentration of this metabolite seems to negatively affect the immune system (Nyman *et al.* 2008), but in the present study, this was not the case as calves offered the colostrum with the highest TBC and higher serum cholesterol levels were more prone to pneumonia.

Glucose levels were not affected by colostrum bacterial load and immunoglobulin concentration. Due to the reflexive closure of the reticular groove in neonatal calves, the primary source of energy substrate is glucose derived from intestinal absorption. Thus, these data suggest that neither colostrum contamination nor immunoglobulin concentration alters serum glucose in neonatal calves. The same was true for TP and creatinine, which indicates that calves in the different groups were ingesting the same dry matter, energy and protein (Khan *et al.* 2007).

Conclusions

Bacterial colonization of on-farm pasteurized frozen–thawed colostrum occurred rapidly in this hot environment; therefore, under the conditions of the present study, this colostrum management should not be incorporated into any calf-rearing program. Total bacterial count in pasteurized frozen–thawed colostrum, measured shortly before feeding, may provide important prognostic information on neonatal pneumonia in Holstein calves, which may be useful in reducing the risk of respiratory diseases in a hot environment. However, high bacterial load in colostrum does not represent a risk factor for neonatal diarrhea in calves fed medicated milk replacer.

This study reinforced the importance of high immunoglobulin concentration in first colostrum by illustrating the interconnectedness of high immunoglobulin concentration with lower respiration rate, increased fecal consistency and higher preweaning daily weight gain. Additionally, an interaction between TBC and TCC in colostrum with colostrum immunoglobulin content was evident since calves fed colostrum with high amounts of immunoglobulin had lower rates of pneumonia, but not if colostrum with high amounts of TBC was provided. Finally, our results show that the morbidity risk due to preweaning calf diarrhea and pneumonia is very high in an international perspective, which indicates that colostrum with high immunoglobulin concentration does not decrease the incidence of calf health problems in a hot environment.

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