

## Impact of volume, immunoglobulin G concentration, and feeding method of colostrum product on neonatal nursing behavior and transfer of passive immunity in beef calves



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### ABSTRACT

One-third of beef calves fail to achieve adequate transfer of passive immunity (**TPI**) through timely ingestion of colostrum, which substantially increases their risk of preweaning morbidity and mortality. Two randomized clinical trials were designed to assess the impact of volume, immunoglobulin G (**IgG**) concentration, and feeding method of colostrum product on neonatal nursing behavior and TPI. In Trial 1, 47 calves were randomly assigned to receive one of three colostrum interventions by oro-esophageal tube feeder (**OET**): 1 L with 100 g/L IgG, 1.4 L with 70 g/L IgG, or 2 L with 100 g/L IgG. In Trial 2, 29 calves were randomly assigned to be fed 1 L of colostrum product with 100 g/L IgG by either nipple bottle (**NB**) or OET. Colostrum intervention (i.e. feeding of colostrum product) occurred within 60 minutes of birth. Cow-calf pairs were monitored by video surveillance in individual stalls for 24 h. Dam colostrum was collected at 10 minutes and calf serum was collected at 24–36 h after birth to assess IgG concentration. Differences among colostrum intervention groups on latency to stand and nurse were analyzed using Kaplan-Meier survival curves and Cox proportional hazard models. The impact of colostrum intervention group on TPI was assessed using multivariable linear regression modeling. In Trial 1, calves fed 1.4 L with 70 g/L IgG by OET nursed from their dams statistically significantly earlier compared to calves fed 1 L with 100 g/L IgG ( $P = 0.003$ ) and calves fed 2 L with 100 g/L IgG ( $P = 0.008$ ). Six of the 15 calves in the NB group in Trial 2 refused to consume part of the colostrum feeding offered by bottle and required follow-up tube feeding of the remaining volume. These calves were analyzed as a separate group (NB + OET). Calves fed 1 L by NB stood and nursed statistically significantly earlier than calves fed by OET ( $P = 0.005$ ) or a combination of NB + OET ( $P = 0.003$ ). Calf serum IgG concentrations were not statistically significantly different among colostrum intervention groups ( $P > 0.1$ ). Overall, the colostrum interventions assessed in this study led to only one calf with failed TPI. While statistically significant differences in serum IgG concentrations were not detected in this study, subsequent nursing behavior did vary and was improved by feeding a moderate volume (1.4 L with 70 g/L IgG) of colostrum when using an OET, and by using the NB when feeding a smaller volume (1 L with 100 g/L IgG).

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### Implications

Colostrum intervention is necessary whenever calves fail to consume colostrum within the first hours of life. However, the impact of different colostrum intervention strategies on subsequent nursing behavior and transfer of passive immunity in beef calves is unclear. Nursing behavior was best when a moderate volume (1.4 L, 70 g/L IgG) of colostrum was fed using a tube feeder or

when a bottle was used to feed a small volume (1 L, 100 g/L IgG) of colostrum. Statistically significant differences in transfer of passive immunity among colostrum intervention groups were not detected. This study provides useful information to guide colostrum management in beef calves.

### Introduction

Ingestion of high-quality colostrum shortly after birth is crucial for successful transfer of passive immunity (**TPI**) in calves (Weaver et al., 2000; Lombard et al., 2020). The amount of immunoglobulins

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transferred to the calf depends on multiple factors including the total immunoglobulin G (IgG) mass consumed, timing of colostrum ingestion, method used to feed the colostrum, and apparent efficiency of absorption (Godden et al., 2019; McGee and Earley, 2019). Calves with serum IgG concentrations <10 g/L are defined as having failed TPI (FTPI) (Godden et al., 2019), and beef calves with serum IgG concentrations <24 g/L are considered to have inadequate TPI (ITPI) due to their increased risk of preweaning morbidity and mortality (Dewell et al., 2006; Waldner and Rosengren, 2009). Evidence-based colostrum management guidelines are available for dairy (Godden et al., 2019) but not for beef calves. This represents an important knowledge gap, given that 33–37% of beef calves experience ITPI (Waldner and Rosengren, 2009; Pearson et al., 2019a; 2019b; Bragg et al., 2020). Developing colostrum intervention strategies for beef calves based on dairy calf guidelines is neither advisable nor feasible because of substantial differences in management and breed types of these animals. Dairy calves are usually permanently separated from their dam shortly after birth, and colostrum is exclusively administered by human intervention. An initial and often only feeding of 3–4 L of colostrum or colostrum replacement product is currently recommended to achieve a total IgG intake of at least 200 g (Godden et al., 2019). This recommendation is likely not suitable for beef calves because beef cows yield lower colostrum volumes with higher colostrum IgG concentration (McGee and Earley, 2019) and because the volume of voluntary first colostrum consumption in beef calves is much smaller (McGee et al., 2006). Additionally, the cow-calf bond is of high importance in beef cattle (Von Keyserlingk and Weary, 2007), and beef calves continue to nurse over the first 24 h and beyond (McGee et al., 2006). Under optimal circumstances, beef calves will consume maternal colostrum by nursing their dam within 60–260 minutes of birth (McGee and Earley, 2019). If voluntary nursing does not occur, or if the dam does not produce adequate volume or IgG concentration of colostrum, human intervention is warranted. Colostrum replacement or supplementation products are widely available in North America. However, most colostrum products are derived from dairy colostrum, serum, or whey and are designed mainly for use in dairy calves with the current maximum IgG concentration available being just over 70 g/L (Calf's Choice Total®, Saskatoon Colostrum Company Ltd., Saskatoon, SK). Beef calf specific colostrum products containing concentrations of IgG more similar to beef cow colostrum IgG concentrations (McGee and Earley, 2019; Gamsjäger et al., 2020) are lacking, as are studies evaluating the use of colostrum products in beef calves. Over 90% of beef producers in western Canada provide colostrum intervention when deemed necessary, with 24% of producers using an oro-esophageal tube feeder (OET) and 19% of producers using a nipple bottle (NB) to administer colostrum (Pearson et al., 2019a). When dairy calves were fed a small volume (1.5 L) of colostrum, TPI was superior when a NB was used compared to OET (Godden et al., 2009b). However, beef calves that received any form of colostrum intervention (NB or OET) had significantly increased odds of FTPI or ITPI compared to sucking their dam (Bragg et al., 2020). The goal of colostrum intervention is to minimize ITPI and provide important nutritional factors, while avoiding a negative impact on nursing behavior as reported previously (Kaske et al., 2005). However, there is no peer-reviewed information available comparing the effectiveness of various colostrum intervention strategies for beef calves to guide recommended best practices.

Two clinical trials were conducted to assess the impact of different colostrum intervention strategies on neonatal nursing behavior and TPI in beef calves. The first objective was to assess the impact of volume (1 L, 1.4 L, or 2 L) and IgG concentration (70 or 100 g/L) of colostrum product on the latencies to stand and nurse, total time spent standing, nursing, and lying down over 24 h, and TPI in beef

calves. The second objective was to evaluate the impact of feeding method (NB or OET) of colostrum product on the same neonatal behaviors and TPI. We hypothesized that calves receiving a larger volume of colostrum product as their first feeding would have longer latencies to stand and nurse, while having similar serum IgG concentrations as calves receiving a smaller volume. Furthermore, we hypothesized that calves receiving their first colostrum feeding by NB would have shorter latencies to stand and nurse and show higher serum IgG concentrations when compared to calves receiving their first colostrum feeding by OET.

## Material and methods

This study was approved by the University of Calgary Veterinary Sciences Animal Care Committee (AC18-0204) and performed in accordance with guidelines established by the Canadian Council on Animal Care. The required sample size to detect a 25% difference in serum IgG concentration with a standard deviation of 7 g/L following colostrum administration by OET (Bonk et al., 2016; Godden et al., 2009a), alpha of 0.05, and 80% power was 11 calves per group.

### *Trial 1 – Volume and immunoglobulin G concentration of first colostrum feeding*

Calves were enrolled between February and April 2019 from two commercial cow-calf operations (Farm 1 and Farm 2) in Alberta, Canada. On both farms, dams were Angus and Angus-crossbred and were managed on extensive grazing pastures until approximately 4 weeks prior to calving when they were relocated into a smaller pasture to allow for closer monitoring. All calves, including singletons and twins, whose births were directly observed or assisted were enrolled in the study, given a study pen equipped with video cameras was vacant at the time of birth. In cases of twins, one twin remained with the dam and was enrolled in the study, while the other twin was separated from its dam as per farm protocol and not enrolled in the study. At birth, a calving ease score was assigned to each cow-calf pair, which was categorized as unassisted, easy assist, or difficult assist (see [Supplementary Material](#) for details). Calves delivered via Caesarian section were not enrolled due to their rare occurrence in this population. To account for maternal colostrum IgG concentration, a 10 mL sample of pooled colostrum from all quarters was collected from each dam in a small sterile plastic container (VWR International Ltd., Edmonton, AB, Canada) within 10 minutes of parturition by trained farm personnel. Samples were refrigerated on farm, transported to the University of Calgary on ice within 24–48 h, and subsequently frozen at –20 °C until analysis. Calves were weighed and vigor parameters (i.e. presence of meconium staining, mucous membrane color, suckle reflex, and tongue withdrawal) were assessed and recorded by farm personnel as described previously (Homerovsky et al., 2017). Calves were then randomly assigned to one of three intervention groups: a small volume of colostrum product with high IgG concentration (Group **SH**; 1 L, 100 g/L IgG, 60% crude protein, 19% crude fat), a medium volume of colostrum product with moderate IgG concentration (Group **MM**; 1.4 L, 70 g/L IgG, 55.7% crude protein, 17.8% crude fat), or a large volume of colostrum product with high IgG concentration (Group **LH**; 2 L, 100 g/L IgG, 60% crude protein, 19% crude fat). Randomization was determined using a random number generator (Microsoft Excel for Mac). All products used in this study were derived from dairy cow colostrum, prepared using the same methods, and provided by the Saskatoon Colostrum Company Ltd. The colostrum product used for group MM was commercially available (Bovine Dried Colostrum, Calf's choice Total®, Saskatoon Colostrum

Company Ltd., SK, Canada), while the product used for groups SH and LH was specifically designed for this study by selecting for higher IgG concentration. The products used throughout the study originated from the same manufacturing batches. The IgG concentrations of the reconstituted colostrum products were confirmed by performing radial immunodiffusion (RID) at the Saskatoon Colostrum Company Ltd. Quality Assurance Laboratory as described previously (Chelack et al., 1993; Shivley et al., 2018) on one bag of each product. The measured IgG concentrations of the reconstituted colostrum samples were 100.2 g/L for the product used for the SH and LH groups and 74.3 g/L for the product used in the MM group. Colostrum powder was mixed with warm water (43–49 °C) according to the manufacturer's instructions and fed via OET within 60 minutes of birth by trained farm personnel.

After calves received their assigned colostrum intervention, the cow-calf pairs were placed in one of three individual box stalls equipped with two video cameras (Lorex 4K DVR 8CH/4CAM P18, LHV 5814KXF, Markham, Ontario, Canada) for 24 h surveillance. Calves were also closely monitored by farm personnel and when a calf had not been observed nursing by 8 h postpartum, personnel were instructed to manually intervene in the nursing process (i.e. lead the calf to the udder of the dam either within the pen or while restrained in a chute). If this approach was unsuccessful, a second dose of colostrum product was administered by OET. The cow-calf pair continued to be monitored closely and if the calf had not nursed its dam by 20–24 h postpartum, the same intervention process was repeated. Calves in groups SH and MM that required a second dose received 2 L of 100 g/L IgG colostrum product (200 g IgG) as their second dose, while calves in group LH needing a second dose received 1 L of 100 g/L IgG colostrum product (100 g IgG). Any calves requiring a third dose received 1.4 L of 70 g/L IgG colostrum product (100 g IgG). This was to assure any calf requiring all three colostrum interventions would ingest a total of 400 g IgG. This relatively high total mass of IgG offered for calves requiring additional intervention was chosen based on the hypothesis that calves that refused to nurse by 8 h would likely have lower efficiency of IgG absorption (Bush and Staley, 1980) and therefore would be at higher risk for ITPI. Calves that nursed from their dams within the first 8 h received only the initial colostrum feeding and the additional IgG they consumed from the dam's colostrum. Our goal was to compare intervention strategies that should have each ensured adequate TPI for all calves while balancing the desire to assess practical strategies and not impede cow-calf bonding.

Serum samples of the calves were collected into serum separator vacutainer tubes (BD Vacutainer® SST™) by venipuncture of the jugular vein at 24–36 h after birth. Samples were refrigerated for up to 24 h until they were transported to the University of Calgary on ice where they were centrifuged at 3 000g (LWS M24 Combo Centrifuge, LW Scientific, Lawrenceville, Georgia) for 16 minutes, and aliquots were frozen at –20 °C until analysis. Data recorded included calf identification number, farm, birth date, time of birth, birthweight, singleton or twin, sex, calving ease score, calf vigor parameters, dam identification number, dam parity (categorized as heifers, second parity cows, and mature cows), colostrum intervention group, number of colostrum interventions administered, and time of calf blood collection.

#### *Trial 2: Method of first colostrum feeding*

Study calves for this trial were enrolled between February and April 2020 from one cow-calf operation (Farm 1). Enrollment criteria were identical to those in Trial 1. A sample of 10 ml of colostrum was collected from each dam as described in Trial 1. For this trial, teat and udder appearance were also recorded based on modified guidelines (Rasby, 2011) to account for the potential

impact of these covariates (see [Supplementary Material](#) for details). Vigor assessment was performed as described previously (Homerosky et al., 2017). For this trial, calves were randomly assigned to one of two colostrum intervention groups (NB or OET), stratified by birthweight (<45 and ≥45 kg). Calves allocated to the NB group were offered 1 L of the high IgG colostrum product used in Trial 1 (100 g/L IgG, 60% crude protein, 19% crude fat) using a 2 L plastic bottle equipped with a snap-on nipple (Mamalac bottle nipple, Model #115–784, United Farmers of Alberta Cooperative Ltd, Alberta, Canada) designed for calves. If the calf did not start nursing within 10 seconds or stopped nursing for >10 seconds, the sucking reflex was stimulated by putting a finger into the calf's mouth and then the nipple bottle was re-introduced. If calves refused to nurse for >10 minutes, the remainder of colostrum product was fed by OET and the volume fed by each method was recorded. Calves in the OET group were tube fed 1 L of the high IgG product by OET (Jorvet Oral Calf Feeder, Model J138, Jorgensen Laboratories, Inc., CO, US). The product with the smallest volume was selected for this trial for the following reasons: (1) to minimize the risk of calves not finishing their feeding by NB as reported in similar studies (Bonk et al., 2016; Godden et al., 2009b; Kaske et al., 2005), (2) preliminary results from Trial 1 did not show statistically significant differences in TPI among groups, and (3) economic considerations (e.g. cost of colostrum product and labor time for administering colostrum product) for producers. Colostrum intervention was completed within 60 minutes of birth. After calves had received their colostrum intervention, the cow-calf pairs were moved into their individual box stalls equipped with video cameras and monitored as described for Trial 1. Calves received a second (8 h), third (12–16 h), or fourth (20–24 h) colostrum feeding if they had not nursed their dam at these time points, at which point 1 L of the same colostrum product was fed by the same route as was assigned initially (i.e. NB or OET). Calves requiring all four colostrum interventions therefore again received a total of 400 g IgG. Serum samples of the calves were obtained and processed as described for Trial 1. Data recorded for this trial were also the same as for Trial 1, with the addition of teat and udder scores.

#### *Video surveillance*

Neonatal behaviors for both trials were recorded for the first 24 h after birth at one-minute intervals by one individual, who was blinded to colostrum intervention group assignments. Recorded behaviors of the calves were lateral recumbency, sternal recumbency, attempting to stand, standing, and nursing. Behavioral definitions were based on those described previously (Barrier et al., 2012) with modifications as defined in [Supplementary Table S1](#). All behaviors during which calves were unable to nurse (lateral and sternal recumbency, attempting to stand) were later grouped into one category (time lying down). The latencies to stand and nurse were recorded. A five-minute wash-out period was applied after the calves and dams first arrived in the pen and after any human intervention (e.g. ear tagging, feeding, removal of placenta from pen, placing new straw in pen) before resuming behavioral observations. During these episodes, the behavior was coded as unobserved. Whenever the dam was completely obstructing the view of the calf, behaviors were also recorded as unobserved. When nursing position could not be confirmed but the calf was confirmed to be standing, the calf was classified as standing. Inter-observer agreement between the individual evaluating the videos and the first author was evaluated yearly to assess potential drift by calculating the kappa coefficient based on a subset of videos (Trial 1:  $n = 3$ , 9% of total observed time; Trial 2:  $n = 2$ , 7% of total observed time). The total time budget in minutes was calculated as the sum of total time standing, total time nursing, total time lying down, and total time unobserved. The time spent

exhibiting specific behaviors was then calculated and analyzed as a proportion of the total observed time of behaviors. Calves with less than 90% of the 24 h study period observed (<1 296 of 1 440 minutes) were excluded from this part of the behavioral analysis.

### Sample analysis

Colostrum and serum samples were shipped on ice to the Saskatoon Colostrum Company Ltd. Quality Assurance Laboratory where IgG concentrations were determined by RID assays as described previously (Chelack et al., 1993; Shivley et al., 2018). Personnel performing the assays were blinded to colostrum intervention group assignments. Complete postmortem examinations were performed on calves that died during the 24 h study period under the supervision of a board-certified pathologist at the University of Calgary Diagnostic Service Unit.

### Statistical methods

Statistical analyses for both trials were performed using statistical software (STATA 16, StataCorp, College Station, TX, US; Prism 9 for MacOS, San Diego, CA, US). Data were assessed for normality by Shapiro-Wilk test and visual examination of histograms. Descriptive statistics were subsequently calculated as means  $\pm$  SD or median (range). Characteristics of study calves among groups were compared using Fisher's exact or  $\chi^2$ -test. Median dam colostrum IgG concentration and calf blood collection time among groups were compared by Kruskal-Wallis test or Mann-Whitney U test. Mean birthweight among groups was compared using ANOVA or Student's *t*-test. Calves that refused to consume part of the colostrum feeding offered by bottle and required follow-up tube feeding were analyzed as a separate group (NB + OET) in subsequent statistical analysis.

Kaplan-Meier survival curves were created to assess differences in latencies to stand and nurse among groups. The entry point into survival analysis was time of birth, and the exit point was 24 h, which was the end of the study period. Calves that did not exhibit the event of interest (standing or nursing) by 24 h were censored. Survival curves were compared using the log-rank test, and the Bonferroni correction was applied to account for multiple, pairwise comparisons. Multivariable Cox proportional hazard models were constructed to determine if colostrum intervention group was statistically associated with the event of standing or nursing while accounting for potential confounding variables. Models were built with the dependent outcomes of standing by 2 h and nursing by 4 and 8 h. These time points were chosen because beef calves should be standing by 2 h (McGee and Earley, 2019) and nursing by 4 h (Homerovsky et al., 2017; McGee and Earley, 2019), and in our study, calves received a second colostrum intervention if they had not nursed by 8 h. First, associations between the outcomes of interest and potential explanatory variables were screened by univariable analysis. Variables assessed included herd (Trial 1 only), udder and teat appearance (Trial 2 only), and dam parity, calving ease score, birthweight, sex, twinning, meconium staining, mucous membrane color, tongue withdrawal, suckle reflex, and colostrum intervention group (both Trials). Spearman correlation coefficients between explanatory variables were examined and if collinearity ( $r > |0.7|$ ) was detected, only the one deemed to have higher biological importance was included in the model. Screened variables with  $P < 0.1$  were offered to subsequent multivariable models, and colostrum intervention group was included in all models because it was the variable of interest. If there were more variables than available degrees of freedom, several different models were built, and the best one was chosen based on the Akaike information criterion. Variables were retained in the multivariable model if  $P < 0.05$  or if the effect of removing the variable resulted in

a change of  $\geq 20\%$  in the coefficient of the remaining variables in the model, indicating confounding. First-order interactions between independent variables were investigated. Schoenfeld residuals were analyzed to confirm the proportional hazard assumption. Martingale residuals were plotted against continuous covariates, when applicable, to assess linearity. The Harrell's C concordance statistic (see [Supplementary Material](#)) was calculated for each survival model to evaluate the overall predictive ability. Backward stepwise linear regression models predicting proportion of time standing, nursing, and lying down as well as serum IgG concentration were constructed using colostrum intervention group and other potential explanatory variables, with the addition of dam colostrum IgG concentration and total number of colostrum interventions for the TPI model. Residual and normality plots were examined for each model to confirm an approximately normal distribution of residuals, absence of outliers, and linearity. Homoscedasticity was confirmed using the Cook-Weisberg test. The proportion of calves with FTPI and ITPI among groups were compared by Fisher's exact test and multivariable logistic regression. Due to the small sample size in some of the groups in Trial 2, the risk for FTPI and ITPI of calves fed by NB alone was also compared to calves that received any OET intervention (i.e. OET and NB + OET).

## Results

### Trial 1

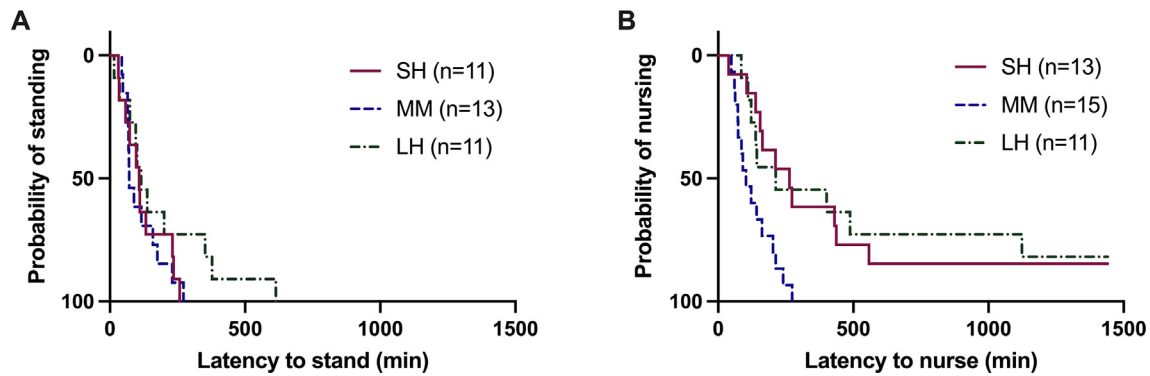
#### Study population

A total of 17, 18, and 12 calves were randomly allocated to groups SH, MM, and LH, respectively. Two calves died within the first 2 h (SH:  $n = 1$ ; MM:  $n = 1$ ) due to suspected perinatal hypoxia, and six calves had to be excluded due to non-adherence to the study protocol (SH:  $n = 3$ ; MM:  $n = 2$ ; LH:  $n = 1$ ). The final sample sizes for the respective analyses are reported in the tables and figures pertaining to this trial and the specific reasons for exclusion of calves are shown in [Supplementary Fig. S1](#). Calf characteristics at enrollment, including calf vigor parameters, were not detected to be statistically significantly different among colostrum intervention groups ([Supplementary Table S2](#)).

#### Neonatal nursing behavior

Inter-observer agreement was 97%. Median latency to stand for calves in Trial 1 was 100 min (range: 15–614 min) with a median latency to stand of 108, 71, and 115 minutes for calves in groups SH, MM, and LH, respectively. Statistically significant differences were not established in latencies to stand among groups ( $P = 0.3$ , [Fig. 1A](#)). Median latency to nurse was 162 min (range: 39–>1 440 min) with a median latency to nurse of 264, 103, and 213 minutes for calves in groups SH, MM, and LH, respectively. Latency to nurse was statistically significantly shorter for calves in the MM group compared to calves in the SH ( $P = 0.003$ ) and LH groups ( $P = 0.008$ ) as depicted in [Fig. 1B](#). Calves in the MM group were 3.7 times more likely to nurse by 4 h ( $P = 0.008$ ) than calves in the SH group and 2.9 times more likely to nurse by 4 h ( $P = 0.03$ ) than calves in the LH group ([Table 1](#)). The model was able to correctly order survival times (i.e. latency to nurse) 66% of the time, as indicated by the Harrell's C concordance statistic. Calves in the MM group were 3.5 and 3.9 times more likely ( $P = 0.005$ ) to nurse by 8 h than calves in the SH and LH groups, respectively, when adjusting for suckle reflex ([Table 1](#), Harrell's C concordance statistic = 0.67). Latency to nurse did not differ statistically between calves in the SH and LH groups ( $P = 0.9$ ) and there was no detectable statistically significant difference in hazard ratios when comparing calves in the SH and LH groups at the various time points





**Fig. 1.** Trial 1 – Kaplan-Meier survival curves demonstrating the differences in the (A) latency to stand and (B) latency to nurse among calves that received one of three colostrum interventions (SH = 1 L with 100 g/L IgG; MM = 1.4 L with 70 g/L IgG; LH = 2 L with 100 g/L IgG) by oro-esophageal tube within 60 minutes of birth. No statistically significant difference was detected in latency to stand ( $P = 0.3$ ). The latency to nurse was statistically significantly shorter for calves in the MM group compared to calves in the SH and LH groups ( $P = 0.003$  and  $P = 0.008$ , respectively). No statistically significant difference in latency to nurse was detected between calves in the SH and LH groups ( $P = 0.9$ ). Calves that did not nurse by 24 h were censored ( $n = 4$ ). Abbreviation: IgG = immunoglobulin G.

**Table 1**

Trial 1 – Multivariable Cox proportional hazard models describing statistically significant associations among colostrum intervention group and other statistically significant covariates and likelihood to nurse by 4 and 8 h. Calves were randomly allocated to receive one of the following colostrum interventions by oro-esophageal tube within 60 minutes of birth: SH = 1 L, IgG 100 g/L ( $n = 13$ ); MM = 1.4 L, IgG 70 g/L ( $n = 15$ ); LH = 2 L, IgG 100 g/L ( $n = 11$ ).

Variable	Hazard Ratio	SE	95% CI	P-value
<b>Nurse by 4 h</b>				
SH	0.27	0.13	0.10–0.71	0.008
MM	Referent			
LH	0.34	0.17	0.13–0.90	0.03
<b>Nurse by 8 h</b>				
SH	0.29	0.13	0.12–0.68	0.005
MM	Referent			
LH	0.26	0.13	0.10–0.67	0.005
Normal suckle reflex	Referent			
Abnormal suckle reflex	0.26	0.16	0.08–0.88	0.03

Abbreviations: CI = confidence interval; IgG = immunoglobulin G.

( $P > 0.1$ ). One calf required one additional colostrum feeding due to lack of nursing by 8 h (LH) and four calves required all three colostrum interventions because they did not nurse the dam (SH:  $n = 2$ ; LH:  $n = 2$ ) within the study timeframe. Proportions of time standing, nursing, and lying down did not differ statistically among groups (Table 2). As these results did not change when accounting for covariates and confounders in multivariable models, only unconditional associations are presented.

**Table 2**

Trials 1 and 2 – Proportion of time spent standing, nursing, and lying down of beef calves receiving one of the following colostrum interventions: Trial 1 – SH = 1 L, IgG 100 g/L; MM = 1.4 L, IgG 70 g/L; LH = 2 L, IgG 100 g/L. Calves were fed by oro-esophageal tube within 60 minutes of birth. Trial 2 – 1 L of colostrum product with 100 g/L IgG within 60 minutes after birth by nipple bottle (NB), oro-esophageal tube (OET), or a combination of the two (NB + OET). Data are presented as median and range because they followed a non-parametric distribution.

Proportion of time	Trial 1 – Colostrum volume and IgG concentration			P-value
	SH ( $n = 12$ )	MM ( $n = 12$ )	LH ( $n = 10$ )	
Standing (%)	11.6 (0.8–19.3)	13.7 (2.0–17.2)	11.9 (0.7–18.1)	0.6
Nursing (%)	4.3 (0–6.4)	4.0 (1.5–7.8)	2.9 (0–7.7)	0.9
Lying down (%)	83.4 (74.6–99.1)	81.8 (77.7–96.5)	82.9 (75.8–98.9)	0.7
Proportion of time	Trial 2 – Colostrum feeding method			P-value
	NB ( $n = 6$ )	OET ( $n = 14$ )	NB + OET ( $n = 14$ )	
Standing (%)	11.4 (8.9–14.6)	10.5 (0–24.5)	8.0 (6.0–13.5)	0.6
Nursing (%)	5.4 (2.5–10.3)	3.6 (0–7.6)	4.3 (1.5–7.5)	0.3
Lying down (%)	83.2 (76.6–88.8)	85.9 (71.9–99.9)	84.7 (81.7–92.5)	0.5

Abbreviation: IgG = immunoglobulin G.

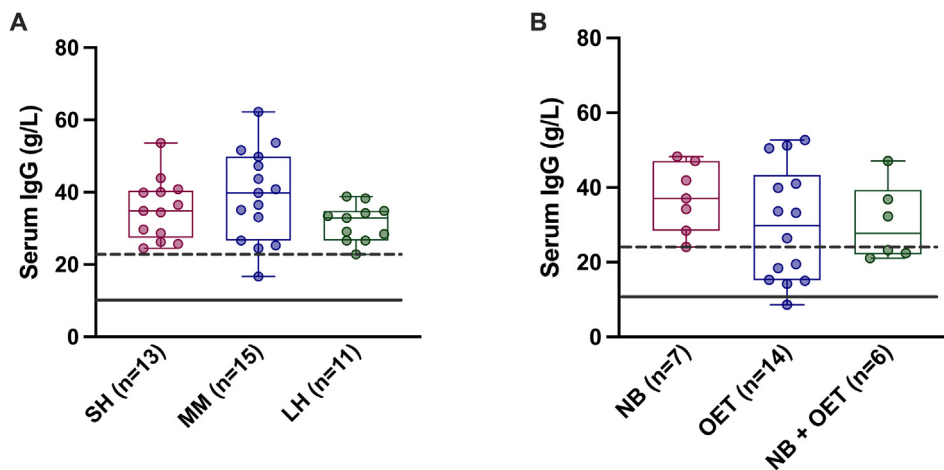
### Transfer of passive immunity

Calf serum samples were collected at a median of 25.5 h after birth (range: 24–36 h) with no statistically significant difference in time of blood collection among groups ( $P = 0.97$ ). Mean and SD serum IgG concentrations were  $35.3 \pm 8.4$ ,  $39.1 \pm 12.6$ , and  $32.6 \pm 5.3$  g/L for SH, MM, and LH group calves, respectively, and were not noted as statistically different ( $P = 0.2$ ) among groups (Fig. 2A). Colostrum intervention group, birthweight, and twinning were offered to the multivariable model predicting serum IgG, and only birthweight remained in the final model ( $P < 0.001$ ). None of the calves in this trial had FTPI, and only two calves had ITPI (MM:  $n = 1$ , IgG 22.8 g/L; LH:  $n = 1$ , IgG 16.7 g/L). These two calves nursed within 2 and 3.5 h, respectively. No statistically significant difference was detected among groups ( $P = 0.7$ ) in the proportion of calves with ITPI. Due to the small number of calves with ITPI and perfect prediction of serum IgG  $\geq 24$  g/L by group SH, multivariable logistic regression modeling could not be completed. None of the four calves that required all three colostrum interventions had ITPI (IgG range: 26.6–39.9 g/L).

### Trial 2

#### Study population

A total of 15 and 14 calves were randomly allocated to the colostrum intervention groups NB and OET, respectively. However, seven of the 15 NB calves did not consume their entire first colostrum feeding by bottle and were tube fed the remaining volume (median: 0.6 L, range 0.5–0.7 L; NB + OET). One calf (NB + OET) died



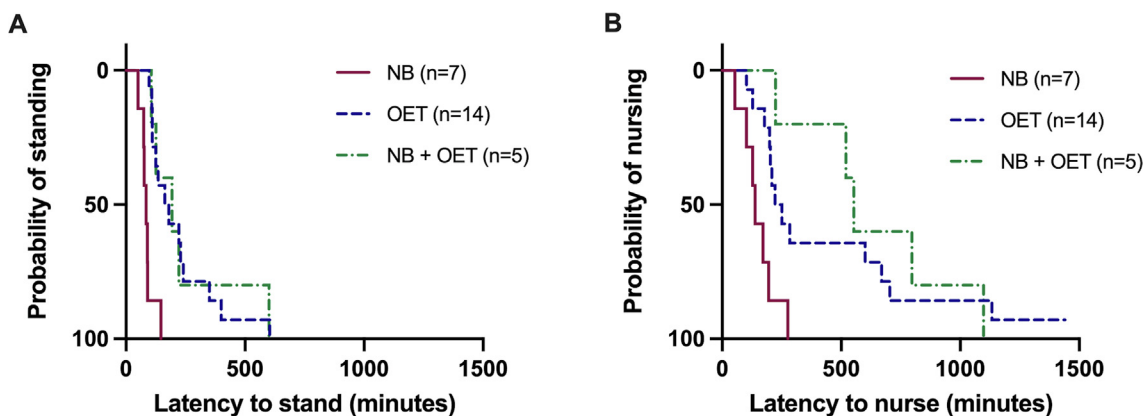
**Fig. 2.** Serum IgG concentration in (A) 39 1-day old beef calves enrolled in Trial 1 and (B) 27 1-day old beef calves enrolled in Trial 2. Calves in Trial 1 were randomly assigned to receive one of the following colostrum products by oro-esophageal tube within 60 minutes of birth: SH = 1 L with 100 g/L IgG; MM = 1.4 L with 70 g/L IgG; LH = 2 L with 100 g/L IgG. Calves in Trial 2 received 1 L with 100 g/L IgG by either nipple bottle (NB), oro-esophageal tube (OET), or a combination of the two (NB + OET). The boxes contain 50% of observations with the middle line demonstrating the median. The solid dark gray line indicates failed TPI (IgG < 10 g/L), and the dashed dark gray line indicates the target IgG concentration for adequate TPI ( $\geq 24$  g/L). Serum IgG concentrations were not deemed statistically significantly different among groups in Trial 1 (A,  $P = 0.2$ ) and Trial 2 (B,  $P = 0.08$ ). Abbreviations: IgG = immunoglobulin G; TPI = transfer of passive immunity.

8 h after birth due to intestinal torsion and another calf (NB) was excluded due to non-compliance with the study protocol. The final sample size for each analysis is reported in the tables and figures pertaining to this trial and the specific reasons for exclusion are shown in [Supplementary Fig. S2](#). Calf and dam characteristics were not statistically significantly different between the two initially assigned colostrum intervention groups (NB and OET; [Supplementary Table S3](#)) or among the three subsequently analyzed actual colostrum intervention groups (NB, OET, and NB + OET; [Supplementary Table S3](#)). Serum samples were collected at a median of 27 h (range: 24–36 h) after birth with no statistical difference detected in time of blood collection times among groups ( $P = 0.9$ ).

**Neonatal nursing behavior**

Inter-observer agreement was 98%. Overall, median latency to stand was 131 minutes for all calves in Trial 2. Calves that consumed their first colostrum feeding by NB alone had a statistically significantly shorter latency to stand (median: 84 minutes) compared to calves in the OET group (median: 171 minutes,  $P < 0.001$ ) and calves in the NB + OET group (median: 193 minutes;

$P = 0.008$ ; [Fig. 3A](#)). Calves in the NB group were 9.5 and 13.4 times more likely ( $P = 0.001$  and  $P = 0.02$ ) to stand by 2 h than calves in the OET and NB + OET groups, respectively ([Table 3](#); Harrel’s C concordance statistic = 0.76). The latency to stand did not differ statistically between calves in the OET and NB + OET groups and hazard ratios were also not detected to be statistically significantly different between OET and NB + OET groups at any of the time points examined ( $P > 0.1$ ). Median latency to nurse was 222 minutes for all calves enrolled in this trial. Calves that consumed their first colostrum feeding by NB nursed statistically significantly sooner (median: 138 minutes) than calves in the OET (median: 237 minutes,  $P = 0.005$ ) and NB + OET (median: 553 minutes,  $P = 0.003$ ) groups ([Fig. 3B](#)). Calves in the NB group were 3.8 and 12.5 times more likely to nurse by 4 h than calves in the OET ( $P = 0.02$ ) and NB + OET ( $P = 0.02$ ) groups, respectively ([Table 3](#); Harrel’s C concordance statistic = 0.73). Latencies to nurse did not differ statistically significantly between calves in the OET and NB + OET groups ( $P = 0.6$ ), and no statistically significant difference in hazard ratios was detected when comparing calves in the OET and NB + OET groups ( $P = 0.1$ ). The models predicting nursing by 8 h showed



**Fig. 3.** Trial 2 – Kaplan-Meier survival curves demonstrating the differences in the (A) latency to stand (LS) and (B) latency to nurse (LN) among calves that received 1 L of colostrum product with 100 g/L of IgG within 60 minutes after birth by nipple bottle (NB), oro-esophageal tube (OET), or a combination of the two (NB + OET). There were statistically significant differences in LS ( $P < 0.001$ ) and LN ( $P = 0.002$ ) among groups. The LS and LN were statistically significantly shorter for calves in the NB calves compared to calves in the OET and NB + OET groups ( $P < 0.001$  and  $0.008$ , respectively). No statistically significant difference was measured in latencies to nurse between calves that in the OET and NB + OET groups ( $P = 0.6$ ). Calves that did not nurse by 24 h were censored ( $n = 1$ ). Abbreviation: IgG = immunoglobulin G.

**Table 3**

Trial 2 – Multivariable Cox proportional hazard models describing statistically significant associations among colostrum intervention group and other statistically significant covariates and likelihood to stand by 2 h and nurse by 4 and 8 h. Calves received 1 L of colostrum product with 100 g/L IgG by either nipple bottle (NB,  $n = 7$ ), oro-esophageal tube (OET,  $n = 14$ ), or a combination of the two (NB + OET,  $n = 5$ ) within 60 minutes of birth.

Variable	Hazard Ratio	SE	95% CI	P-value
Stand by 2 h				
NB	Referent			
OET	0.10	0.07	0.03–0.40	0.001
NB + OET	0.08	0.08	0.01–0.65	0.02
Nurse by 4 h				
NB	Referent			
OET	0.27	9.15	0.09–0.82	0.02
NB + OET	0.08	0.09	0.01–0.69	0.02
Nurse by 8 h				
NB	Referent			
OET	0.32	0.18	0.11	0.04
NB + OET	0.08	0.09	0.01	0.03
Unassisted				
Easy assist	0.26	0.24	0.04	0.1
Difficult assist	0.16	0.16	0.02	0.07

Abbreviations: CI = confidence interval; IgG = immunoglobulin G.

similar results with the exception that calving ease score was identified as a confounding variable (Table 3). One calf (OET) required a second colostrum feeding and one calf (OET) never nursed over the 24 h study period and thus, required all four colostrum feedings. Proportions of time standing, nursing, and lying down did not differ statistically among groups (Table 2). As these results did not change when accounting for statistically significant covariates and confounders in multivariable models, only unconditional associations are presented.

#### Transfer of passive immunity

Overall, mean and SD serum IgG concentration was  $33.2 \pm 12.9$  g/L with mean serum IgG concentrations of  $37.3 \pm 9.1$ ,  $30.0 \pm 15.2$ , and  $30.5 \pm 10.3$  for NB, OET, and NB + OET group calves, respectively (Fig. 2B). No statistically significant difference ( $P > 0.09$ ) was detected among groups even when accounting for the covariates of dam colostrum IgG concentration ( $P = 0.006$ ) and tongue withdrawal ( $P = 0.007$ ; data not shown). Only one calf experienced FTPI (OET). None of the NB calves showed ITPI, whereas six (43%) of the OET and three (50%) of the NB + OET calves did, but there was no statistically significant difference measured among groups ( $P = 0.08$ ). Due to quasi separation (NB group perfectly predicted serum IgG  $\geq 24$  g/L), multivariable logistic regression modeling could not be completed. Calves that received any colostrum intervention by OET (i.e. OET and NB + OET) were at higher risk for ITPI ( $P = 0.04$ ) when compared to calves in the NB group.

#### Discussion

This study investigated the impact of volume, IgG concentration, and feeding method of the first colostrum feeding on neonatal nursing behavior and TPI in beef calves. The latency to nurse of beef calves in this study was influenced by the colostrum intervention strategy used at first feeding, with calves being fed a moderate volume (1.4 L with 70 g/L IgG) by OET or a small volume (1 L with 100 g/L IgG) by NB nursing their dams statistically significantly sooner than the other groups. The TPI was not statistically significantly different among groups.

Calves enrolled in Trials 1 and 2 stood within 100 and 131 minutes, respectively. This is consistent with the expected latency to

stand of 30–120 minutes in beef calves (McGee and Earley, 2019). The median latency to nurse of 162 and 222 minutes for calves enrolled in Trials 1 and 2, respectively, is consistent with the 60–260 minutes expected for unassisted beef calves (McGee and Earley, 2019). While typically longer latencies to nurse would be expected in assisted calves, we did not observe this in our study, potentially due to the early colostrum intervention. In addition to different protein concentrations, calves in groups SH, MM, and LH received a total of 543, 716, and 1 083 kilocalories, respectively, from fat in their first feeding. These differences in fat content resulted from the different volumes consumed and the different colostrum product compositions. Differences in fat and protein intake may have impacted subsequent calf behavior, as endogenous fat and energy reserves are very limited in newborn calves (Okamoto et al., 1986). It is possible that calves in the SH group were lacking energy to nurse the dam, while calves in the LH group may have experienced prolonged satiety or moderate depression, as has been previously reported in dairy calves fed 4 L of colostrum by OET (Kaske et al., 2005).

Anecdotal evidence by some suggests that calves fed by nipple bottle may adjust more easily to nursing from the udder, while others assume that calves may experience “nipple confusion” after being bottle fed and thus, take longer to nurse efficiently from their dam. Our study results are consistent with the first hypothesis. In addition to the potential benefit of getting accustomed to drinking from a nipple, there are two additional reasons that could explain why calves fed exclusively by NB nursed statistically significantly sooner. Firstly, NB calves that failed to consume their entire initial feeding by NB may have had underlying compromising conditions that were not detected using the at-birth vigor parameters assessed in this study. While poor suckle reflex has been associated with an increased risk of not consuming colostrum voluntarily by 4 h and subsequent lower TPI (Homerovsky et al., 2017), another study reported that the strength of the suckle reflex was not related to colostrum intake (Vasseur et al., 2009), which is consistent with our results. Secondly, the potential of esophageal irritation and subsequent differences in calf behavior following the use of an OET (Bonk et al., 2016) could explain why OET and NB + OET calves had longer latencies to stand and nurse when compared to NB calves. An alternative hypothesis is that calves fed by NB received more stimulation than those fed by OET with potential associated benefits to respiration and metabolism. The impact of colostrum feeding method on latency to nurse is especially important because producers may use nursing observations to decide when cow-calf pairs can be moved out of the calving barn. Hence, there may be a benefit of administering the first colostrum feeding by NB if the goal is to relocate cow-calf pairs as soon as possible.

The average IgG concentration in beef calves enrolled in this study was consistent with other recent Canadian (35.9 g/L, Pearson et al., 2019b) and international studies (30.9 g/L, Barry et al., 2019; 30.2–32.3 g/L, Reppert et al., 2019), but higher than recent reports from Poland and France (20 g/L, Wojtas et al., 2020; Martin et al., 2021). In contrast to a similar study in dairy calves (Godden et al., 2009b), we did not detect a statistically significant impact of volume, IgG concentration, or feeding method of colostrum on serum IgG concentration. Comparison of this type of study between dairy and beef calves is difficult because dairy calves often receive the entirety of required IgG in their first feeding, whereas beef calves mostly only receive supplementation and subsequently consume additional IgG through nursing. This was also the case for most of our study calves, and due to the high IgG concentrations calves subsequently nursed from their dams, the lack of detectable statistically significant differences in serum IgG concentration within this study population is not surprising. While the high average colostrum IgG concentration in this study

population is comparable to results from previous studies in our geographic area (150 g/L, Homerosky et al., 2017; Gamsjäger et al., 2020) and in Ireland (170–180 g/L, McGee et al., 2005; McGee et al., 2006), it could have masked the effect of our various colostrum product feeding regimes on TPI. Feeding a colostrum product with higher IgG concentration (100 g/L) than currently commercially available (70 g/L) did not result in statistically significantly different calf serum IgG concentrations in this study, but this may likely be different in situations in which beef calves do not return to nursing their dam after colostrum intervention or in situations with lower average dam colostrum IgG concentrations. Based on previous literature and ethical concerns, we did not have a control group receiving no colostrum intervention. We are therefore unable to compare calves that received colostrum intervention with calves that did not in this specific study population. However, 39–64% of beef calves born by assisted birth fail to nurse colostrum within 4 h, and such calves show significantly lower serum IgG concentrations and higher odds of being treated than those that do nurse within 4 h (Homerosky et al., 2017). Despite the high percentage of assisted calves in this study, only a single calf had FTPI (IgG = 8.6 g/L, Trial 2, OET). This is a much lower percentage than would be expected (Waldner and Rosengren, 2009; Bragg et al., 2020). A total of 2 (5%) and 9 (33%) calves had ITPI in Trials 1 and 2, respectively. While the low risk of ITPI among calves in the first trial is excellent, results of the second trial are comparable to the average of 33% reported for Alberta and Saskatchewan (Waldner and Rosengren, 2009). By including twins and mostly assisted births in our study, our calves were likely at higher risk for ITPI, regardless of the intervention. While the proportions of calves showing ITPI were not deemed statistically different ( $P = 0.08$ ), no calves in the NB group had ITPI while substantial proportions in the other groups (OET = 43% and NB + OET = 50%) did. As such, we believe that the observed differences may be biologically important and that additional studies with larger sample sizes are warranted. The higher frequency of ITPI in calves that were fed part or all of their first colostrum meal by OET (i.e. NB or NB + OET) was likely associated with the prolonged time to nurse their dams. Overall, our results suggest that regardless of the colostrum intervention strategy used, supplying colostrum within the first hour of life is beneficial for high-risk calves (e.g. calves born by dystocia, born to heifers, twins).

All calves in Trial 1 were fed by OET to assess the impact of volume and IgG concentration on calf behavior and TPI. This was to avoid calves having to be fed by OET following refusal of the NB and hence, the potential confounding factor of feeding method. The esophageal groove reflex is not stimulated when feeding by OET and subsequently, colostrum is deposited in the forestomachs, and transport to the small intestine can be delayed for several hours (Lateur-Rowet and Breukink, 1983). This has been discussed as a potential disadvantage of using the OET with smaller volumes, given the decline in absorptive capacity of IgG over time (Bush and Staley, 1980; Stott et al., 1979). When feeding larger volumes of  $\geq 3$  L, no statistical differences were reported between NB and OET regarding apparent efficiency of absorption and TPI in multiple dairy calf studies (Chigerwe et al., 2012; Desjardins-Morrisette et al., 2018; Godden et al., 2009b), likely due to the immediate overflow of such a large volume from the reticulorumen into the abomasum. Calves in the SH group might have encountered delayed transport of colostrum from the reticulorumen to the abomasum and small intestine, and subsequently delayed absorption of nutrients providing them with energy for the nursing process. In contrast, calves in the LH group took longer to nurse, which was consistent with our hypothesis and previous literature (Kaske et al., 2005).

Despite the relatively small volume of colostrum fed in Trial 2, 47% (7/15) of calves assigned to the NB group in our study did not

voluntarily consume the whole liter of colostrum product offered. This was unexpected given the reported voluntary colostrum consumption of beef calves at first feeding of 1.6–2.6 L (McGee and Earley, 2019) and the very low number of calves with poor suckle reflex in our study. The product used for the SH and LH groups was designed specifically for this study, and as such, palatability was not previously assessed. The failure to consume the entire NB feeding could not be predicted by the at-birth vigor parameters assessed. In contrast to our study, subgroups of calves that required follow-up tube feeding due to incomplete ingestion of the volume offered by NB in other studies were analyzed as part of their originally assigned groups, with (Godden et al., 2009b) or without (Kaske et al., 2005; Desjardins-Morrisette et al., 2018) evaluating any important differences in TPI. Because calves that failed to consume the entire first colostrum feeding via NB in our study consumed  $\geq 50\%$  of their meal by an alternate route (OET), it was deemed inappropriate to report their results as part of their initial randomization group. Statistically significant differences found in the present study between latencies to stand and nurse of the NB and the NB + OET groups support the decision to report these groups separately. The NB + OET feeding method was associated with the largest negative impact on nursing behavior, and therefore, refusal of the NB may be used as an indicator of compromised calves in the absence of poor vigor parameters.

The small sample size in Trial 2 after the formation of the third colostrum intervention group (NB + OET) is a limitation of this study and results of this trial must be interpreted with caution. However, our sample size calculation was based on prior knowledge of serum IgG concentrations and did not focus on the nursing behavior. To our knowledge, this is the first study assessing the impact of different colostrum intervention strategies on nursing behavior in beef calves, and hence, no data was available for sample size calculation for this outcome. The small sample size may predispose to type II error. Performing both trials on a small number of commercial cow-calf operations helped minimize variability of factors such as breed and general management but may not accurately reflect management practices of all herds in Alberta, Canada, or elsewhere. While more calves could have been enrolled in a research facility or by including more farms, we elected to perform these trials in a small number of commercial cow-calf operations to increase external validity while also maintaining sufficient quality control of the intensive study protocols. Additionally, our study population consisted of mostly assisted calves and subsequently a relatively high percentage of twins. While this may decrease external validity for unassisted calves, it is important to note that two of the main target populations of beef calves requiring colostrum intervention are assisted calves and twins, given their higher risk of failed TPI and preweaning mortality (Hickson et al., 2008; Homerosky et al., 2017). We therefore conclude that our results are applicable to most calves requiring colostrum intervention in beef herds. The participating farms were selected based on their proximity to our research institution, reliable record-keeping, and willingness to participate in these studies, which may have led to selection bias. Based on these limitations, caution is warranted when translating results of this study to calf populations that differ from the ones used in this study. Further, while Trial 2 demonstrated a benefit of the NB when feeding 1 L, more studies should be conducted to consider if there are additional benefits to NB consumption of the larger volume (1.4 L) that was found to be optimal in Trial 1 when fed by OET. Conversely, if calves fail to accept the NB, it might be useful to determine if feeding larger volumes (1.4 L) by OET would improve outcomes.

Results of the two trials in this study have practical implications for colostrum management strategies on cow-calf operations. When using an OET, feeding a moderate volume of 1.4 L of colostrum product within 60 minutes after birth is recommended for



high-risk calves, because calves nursed sooner than calves fed a smaller or larger volume of colostrum product, while serum IgG concentrations were not shown to be different among groups. When feeding a smaller volume (1 L), the NB should be chosen over the OET, based on statistically significantly shorter latency to stand and nurse compared to calves fed by OET or a combination of NB + OET. Failure to nurse the total volume offered by NB may be considered a sign of overall poor vigor, as these calves showed statistically significantly longer latencies to stand and nurse, and thus should be monitored closely.

### Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.animal.2021.100345>.

### Ethics approval

This study was approved by the University of Calgary Veterinary Sciences Animal Care Committee (AC18-0204) and performed in accordance with guidelines established by the Canadian Council on Animal Care.

### Data and model availability statement

None of the data were deposited in an official repository. Models and their associated diagnostics are reported within the supplementary material.

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### Declaration of interest

Dr. Deborah M. Haines is the Director for Research and Development at the Saskatoon Colostrum Company Ltd., which provided in-kind products and RID testing for this study. Dr. Deborah Haines was not involved in data collection and analysis.

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