

Colostrum source and passive immunity transfer in dairy bull calves

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SUPPLEMENTARY FILE S1

Supplementary Methods

* ***Bull calf selection and birth measurements.*** At birth, all birth information was recorded including calving difficulty score (Supplementary Table 1), calf ID, cow ID, and time of birth. Calves were taken from dam after licked clean (about 30 min after birth), but not allowed to suckle and scored for calf vigour (1 = weak, 2 = lazy, 3 = vigorous, 4 = very vigorous) and health (Supplementary Figure 1). The calves were randomly allocated to 3 groups in 6 blocks. The first calf was allocated in Group A and tagged as A1 and the first calves allocated in Groups B and C were tagged as B1 and C1, respectively. Three calves (A1, B1 and C1) were placed in the same pen. Only the Group A cows were tagged with necklace as number 1 to 12, sprayed with paint to be distinguished from other cows on the dairy, and kept for three days postpartum for milking. Newborn calves were weighed by using portable animal weight scales and crate (kg), and measured for girth by using tape measure (cm). Blood samples were taken and the calves given their first feeding of colostrum within 4 hours of birth.

****Calf feeding.*** Pairs of Group A and B calves were fed identical colostrum from the same milking of the dam of the Group A calf. The Group A dams were first milked within 2 hours postpartum and then milked again within 10 hours postpartum. The Group A and B calves were fed 5% of their birth weight within 4 hours after birth and another 5% of their birth weight within 12 hours of birth. Thereafter, the pairs of Group A and B calves were fed identical

colostrum from the same milking of the dam of the Group A calf twice per day (5% of their birth weight/feeding) for 3 days, and then were fed bulk tank milk twice per day (2 L/calf/feeding) for 4 days. The Group C calves were only fed 1 bottle of “pooled” colostrum for their first feeding (2 L) and then were fed bulk tank milk twice per day (2 L/calf/feeding) for 7 days. The “pooled” colostrum was the colostrum and transition milk collected from the other cows on the dairy 0-4 days postpartum. All calves were fed by nipple bottles until 3 days after birth and nipple buckets from day 4 to 7 after birth. All calves were given *ad lib* access to calf concentrate, lucerne or medic, and water from the beginning.

***Colostrum, pooled colostrum and bulk tank milk samples.** The Group A dams were first milked using a portable milking machine and then twice daily in the dairy. The colostrum and transition milk was collected for 3 days postpartum and the volume was measured. Dam colostrum was dispensed into bottles for 2 calves (Group A and B calves) based on calf birth weight (5% birth weight per feed) and each bottle clearly marked for the Group A and Group B calves with tags. Pooled colostrum was collected from the other dairy cows 0-4 days postpartum once per day for the first feeding of the Group C calves. The bulk tank milk was collected from dairy cows more than 5 days postpartum twice daily for feeding the Group C calves from their second feeding until day 7 after birth and for feeding the Group A and B calves from day 4 to 7 after birth. The samples of dam colostrum, pooled colostrum and bulk tank milk were collected into sterile 50 mL-tubes and total solids were measured by using Brix refractometer immediately after milking. The samples were frozen at -20°C , transported on dry ice and stored at -80°C for remaining analyses.

Calf blood sample samples. Calf blood was collected at day 0 (within 4 hours after birth, before any feeding) and day 1, 2, 3 and 7 after birth. The blood samples were taken into 2 red cap

vacutainer tubes (no coagulant) for serum and 2 green vacutainer tubes (heparin) for plasma. The glucose concentration was measured in whole blood immediately after blood collection. Total protein was measured by refractometry immediately after serum collection. The remaining serum and plasma were frozen at -20°C, transported on dry ice and stored at -80°C for further analyses.

Calf health measurements. Calf health was monitored from birth until 7 days after birth by Dr Rebel Skirving from Gambier Vets, Mount Gambier, SA 5290, Australia. All illnesses were recorded including no or slow drinking, coughing, diarrhea, fever, and respiratory problems. If symptoms appeared, then these symptoms and temperatures were monitored twice daily, and treated as required.

Supplementary Tables

Supplementary Table S1. Calving ease scoring system (6 point scale).

Score	Code	Description
1	Unassisted	Cow calved unassisted/no difficulty
2	Easy pull	One person without mechanical assistance
3	Hard pull	Two people without mechanical assistance One person with mechanical assistance
4	Surgical assistance	Veterinary intervention required
5	Mal-presentation	E.g. breech
6	Elective surgical	Surgical removal of calf before cow had opportunity to calve

Supplementary Table S2. Descriptive statistics and comparison of the components in calf blood within 7 days after birth (Least Squares Means with Standard Errors (LSM \pm SE), letter superscripts indicate significant differences of $P < 0.05$).

Component ¹	Time point ²	Group A (n=12)	Group B (n=12)	Group C (n=11)	All calves ³ (n = 35)
Glucose (mmol/L)	Day 0	3.8 \pm 0.3	4.1 \pm 0.3	3.6 \pm 0.3	3.8 ^x \pm 0.2
	Day 1	6.8 \pm 0.3	6.6 \pm 0.3	7.3 \pm 0.3	6.9 ^z \pm 0.2
	Day 2	6.5 \pm 0.3	6.4 \pm 0.3	6.8 \pm 0.3	6.6 ^z \pm 0.2
	Day 3	6.2 \pm 0.3	6.4 \pm 0.3	6.6 \pm 0.3	6.5 ^{yz} \pm 0.2
	Day 7	5.7 \pm 0.3	6.0 \pm 0.3	6.2 \pm 0.3	6.0 ^y \pm 0.2
GGT (U/L)	Day 0	10.8 \pm 155.8	11.0 \pm 155.8	13.3 \pm 162.8	11.7 ^x \pm 94.8
	Day 1	1511.0 ^a \pm 155.8	1580.1 ^a \pm 155.8	783.1 ^b \pm 162.8	1271.4 ^y \pm 94.8
	Day 7	234.8 \pm 155.8	231.0 \pm 155.8	146.4 \pm 162.8	200.8 ^x \pm 94.8
TP-R (g/L)	Day 0	40.5 \pm 1.9	39.6 \pm 1.9	41.1 \pm 1.9	40.4 ^x \pm 1.2
	Day 1	60.9 ^a \pm 1.9	59.3 ^a \pm 1.9	52.4 ^b \pm 1.9	57.1 ^y \pm 1.2
	Day 2	60.0 ^a \pm 1.9	58.6 ^{ab} \pm 1.9	54.4 ^b \pm 1.9	57.3 ^y \pm 1.2
	Day 3	60.2 ^a \pm 1.9	57.8 ^{ab} \pm 1.9	53.5 ^b \pm 1.9	56.8 ^y \pm 1.2
	Day 7	57.8 ^a \pm 1.9	55.5 ^{ab} \pm 1.9	51.8 ^b \pm 1.9	54.7 ^y \pm 1.2
TP-B (g/L)	Day 0	55.0 \pm 3.3	52.1 \pm 3.3	50.1 \pm 3.4	53.2 ^x \pm 1.9
	Day 1	78.6 \pm 3.3	74.5 \pm 3.3	76.6 \pm 3.4	75.8 ^z \pm 1.9
	Day 2	64.1 \pm 3.3	57.7 \pm 3.3	63.3 \pm 3.4	61.2 ^y \pm 1.9
	Day 3	76.8 \pm 3.3	70.9 \pm 3.4	68.4 \pm 3.4	71.7 ^z \pm 2.0
	Day 7	62.1 \pm 3.3	64.0 \pm 3.3	65.1 \pm 3.4	63.1 ^y \pm 1.9
IgG (g/L)	Day 0	0.3 \pm 2.9	0.3 \pm 2.9	0.2 \pm 3.0	0.3 ^x \pm 2.0
	Day 1	27.9 ^a \pm 2.9	26.4 ^a \pm 2.9	15.5 ^b \pm 3.0	23.4 ^z \pm 2.0
	Day 2	35.0 ^a \pm 2.9	25.7 ^b \pm 2.9	15.5 ^c \pm 3.0	25.1 ^z \pm 2.0
	Day 3	23.6 ^a \pm 2.9	19.5 ^{ab} \pm 3.0	11.8 ^b \pm 3.0	18.1 ^y \pm 1.5
	Day 7	36.7 ^a \pm 2.9	29.0 ^a \pm 2.9	14.7 ^b \pm 3.0	26.5 ^z \pm 2.0

^{a,b,c} LSM within each time point with a different superscript letter are significantly different ($P < 0.05$)

^{x,y,z} LSM within an "All calves" column with a different superscript letter are significantly different ($P < 0.05$)

¹ GGT = gamma-glutamyl transferase; TP-R = Total protein by refractometer; TP-B = Total protein by Bradford assay; IgG = immunoglobulin G;

² Day 3, Group B had 11 samples instead of 12 samples.

³ All calves = Group A + Group B + Group C calves; Group A = calves fed own dam colostrum, Group B = calves fed foster cow colostrum, Group C = calves fed one bottle of pooled colostrum

Supplementary Table S3. Frequency and proportion of calves in each Group categorized by serum IgG levels on day 2 after birth (Lombard et al., 2020).^{1,2}

Category ¹	Frequency		Proportion (%)				P-values of pairwise comparisons		
	Group A (n = 12)	Group B (n = 12)	Group A (n = 12)	Group B (n = 12)	Group C (n = 11)	Group C (n = 11)	Groups A & B	Groups A & C	Groups B & C
Excellent	9	8	75.0 ^a	66.7 ^a	9.1 ^b	1	1.000	0.003	0.009
Good	1	0	8.3	0.0	18.2	2	1.000	0.590	0.217
Fair	2	3	16.7 ^b	25.0 ^{ab}	63.6 ^a	7	1.000	0.036	0.099
Poor	0	1	0.0	8.3	9.1	1	1.000	0.478	1.000

^{a,b} Means within a row of a category proportion with different superscripts differ ($P < 0.01$), comparing pairwise by using 2 x 2 tables with Fisher's exact test.

¹ Categories based on Lombard et al. (2020) where calf serum immunoglobulin G (IgG) levels of ≥ 25.0 , 18.0–24.9, 10.0–17.9, and < 10 g/L of IgG at 24-48 hours are classified as excellent, good, fair, and poor, respectively.

² Group A = calves fed own dam colostrum; Group B = calves fed foster cow colostrum; Group C = calves fed one bottle of pooled colostrum.

Supplementary Figures

Table 1 University of Guelph: Calf vitality score sheet

Visual Appearance				
1. Meconium Staining	Normal: no staining	Slight: around anal/tail head area	Moderate: extending over body	Severe: completely covered
	0	1	2	3
2. Tongue/Head	Normal (no swelling, tongue not protruding)	Tongue protruding but not swollen	Tongue protruding and swollen	Head and tongue swollen, tongue protruding
	0	1	2	3
Initiation of Movement				
3. Calf movement	Taken within:			
	0 - 30 min	30 min - 1.5 hr	1.5 hr - 3 hr	> 3 hr
	Standing/walking	Attempts to stand	Sternal	On side, no efforts to rise
	0	1	2	3
General Responsiveness				
4. Head shake in response to straw in nasal cavity	Shakes head vigorously	Moves head away	Twitches or flinches	Does not respond
	0	1	2	3
5. Tongue Pinch	Actively withdraws tongue	Attempts to withdraw	Twitches tongue	Does not respond
	0	1	2	3
6. Eye Reflex (in response to touching eyeball)	Actively blinks and closes eye	Slow to blink	Does not respond	
	0	1	2	
Oxygenation				
7. Mucous Membrane Colour	Bright Pink	Light Pink	Brick Red	White/blue
	0	1	2	3
8. Length of Tongue*	 < 50 mm	 50 - 61 mm	 > 62 mm	
	2	0	1	
Rates				
9. Heart Rate**	< 80 bpm	80 - 100 bpm	> 100 bpm	
	2	0	1	
10. Respiration (approximate)	SLOW (~ 24 rrpm)	NORMAL (~ 24 - 36 rrpm)	FAST (~ > 36 rrpm)	
	1	0	2	

*Length of Tongue: Measure from lips. Record this measurement only if within 5 minutes of calving.

**Heart Rate: Put your hand on the calf's chest. Take pulse for 15 seconds then multiply by 4.

Supplementary Figure S1. Scoring system to assess calf health at birth. (Based on the Calf Vigor Scoring System from the University of Guelph, Canada; https://www.progressivedairycanada.com/downloads/2012/09/0912ca_lee_vigor.pdf, accessed February 2018).