Biochemistry and hematology reference intervals for neonatal dairy calves aged 5-12 days

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Abstract

Background
Comprehensive hematology and biochemistry reference intervals (RIs) are currently lacking in the literature for young dairy calves based on sample sizes more than 120. Young dairy calves are at a relatively high risk of poor health and welfare outcomes. They have a high risk of morbidity and mortality in the first 2 weeks of life, and many are transported and fasted during this time. For example, non-replacement calves in Australia and New Zealand are usually 5-12 days old when transported to abattoirs, meaning that calves of this age group are potentially at risk of both health and welfare compromise. Given these factors, sound, comprehensive, age-specific biochemical and hematologic RIs are needed for both veterinary clinical practice and to inform research on calf health and welfare.

Objectives
The aim of this study was to generate age-specific hematology and biochemistry RIs for dairy calves aged 5-12 days.

Methods
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We collected blood samples from 141 fasted, healthy dairy calves on 10 Australian farms. Reference Value Advisor software was used to calculate nonparametric RIs for multiple biochemistry and hematology variables.

Results
RIs for a panel of hematology and biochemistry variables in dairy calves aged 5-12 days old were derived.

Conclusions
These RIs will be useful for clinical veterinary practice, as well as for research on dairy calf health and welfare.

Keywords
blood, heifer, non-replacement calf, Reference Value Advisor, plasma, serum

Introduction
Young dairy calves are at relatively high risk for health and welfare challenges due to inadequate quantity, quality, and timing of colostrum ingestion, the poor hygiene of calf housing, restricted milk feeding, and low body fat reserves. High rates of morbidity and mortality in calves can occur during the first 2 weeks of life\(^1,2\). Consequently, young calves might be the focus of individual and herd health investigations by veterinarians. Additionally, non-replacement calves that are sold for slaughter, and replacement heifers that are transferred to calf rearing facilities, experience the added stressors of fasting and transport. Numerous studies have investigated the welfare impact of transporting and fasting young dairy calves. These studies often include the use of hematologic and biochemical measurements\(^3-5\). It is important to establish reference intervals (RIs) that are age-specific because many biochemistry and hematology variables vary with age in cattle\(^6-10\).

Despite the numerous studies on the changes in cattle hematology and biochemistry variables with age, there have been few published studies on hematology and biochemistry RIs for young calves\(^10-13\). Of the studies that have been published, one had a relatively low sample size (41 for the relevant age group) and did not specify whether calves had been fasted or not\(^11\), another reported hematologic values only\(^12\) or select biochemistry values only with minimal (4 hours) fasting\(^10\), while another had a smaller than ideal sample size (53)\(^13\).

Additionally, young calf RIs for beta-hydroxybutyrate (BHB) have not, to our knowledge, been previously reported. Although BHB is not commonly used diagnostically in calves, it can be a useful research tool, as it indicates a negative energy balance and metabolism of fat (where...
sufficient body fat is available)\textsuperscript{14}. For this reason, BHB has been used in a number of studies on young calf welfare, particularly in relation to fasting and transport\textsuperscript{3-5,15}. This study aims to address these knowledge deficits by establishing RIs for fasted 5-12-day-old dairy calves using a sample size appropriate for nonparametric statistical methods\textsuperscript{16-18}. This allows for more comprehensive and specific RIs for use both in clinical veterinary work and for research on young calf health and welfare. We expected that the RIs for most variables would be reasonably well aligned with previously reported intervals for young calves, with some discrepancies due to factors such as differences in sample sizes, fasting periods prior to sample collection, genetics, environment, management factors, analytical methods, and location. We anticipated that the upper reference limit for BHB in calves would be lower than that for adult cattle because BHB has been shown to increase with age\textsuperscript{9}.

Materials and methods

Ethics

This research was approved by the University of Melbourne Faculty of Veterinary and Agricultural Sciences Animal Ethics Committee (animal ethics identification number 1814448.2). Research was carried out in compliance with the Prevention of Cruelty to Animals Act 1986 and the Australian Code for the Care and Use of Animals for Scientific Purposes 2013. Farmers/farm managers gave written, informed consent for animals to take part in the research.

Inclusion and exclusion criteria

Farm selection

Participating farms in Northern and Western Victoria, Australia, were recruited through local veterinarians or contacts provided by other farmers in the area. Farms were not randomly selected but based on a convenience sample of farms that had calves available at the required time and whose contact details were provided. Ten farms were visited to sample calves, 9 of which were commercial dairy farms, and one of which was a research dairy farm. Some farms were visited on multiple days.

Northern Victorian farms (n = 7) were in an inland irrigated area, with monthly temperatures ranging from a mean low of 3°C (38°F) to a mean high of 32°C (90°F)\textsuperscript{19}. Western Victorian farms (n = 3) were in a coastal area, with monthly temperatures ranging from a mean low of 6°C (43°F) to a mean high of 25°C (77°F)\textsuperscript{19}.

Calf selection

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Blood was collected from 141 calves in August 2018 and January/February 2019. We aimed to achieve a sample size of 120 calves minimum, in line with recommendations for nonparametric analytical methods\textsuperscript{16}. We collected samples from 141 calves to ensure this while allowing for the fact that some samples might need to be excluded due to post-analytical factors. Criteria for inclusion in the study were that calves were of a dairy breed, aged 5-12 days old at the time of sampling, fasted from milk for at least 12 hours, assessed as clinically healthy on physical examination, and had not been given any medication. All calves present on the farms at the time of the visit that fulfilled the inclusion criteria were sampled. Both female (n=128) and male (n=12) calves were sampled (sex was not recorded for one calf). It was not possible to get even numbers of samples from males and females, as most dairy farmers in Australia do not keep male calves for much longer than 5 days, which is the legal minimum age for calves to travel to sale yards or abattoirs\textsuperscript{20}. Calves that were found to have abnormal heart sounds, severe ocular discharge, current diarrhea, or pyrexia (rectal temperature $>103.1^\circ\text{F} (39.5^\circ\text{C})$) were excluded from the study – see Figure 1 for details on excluded calves.

Background information about the calves was collected verbally in a standardized questionnaire format from farm owners/managers and recorded in written form by the researcher. Prior to sample collection, calves were examined either by a veterinarian or by a final-year veterinary student under veterinary supervision. Physical examinations included cardiac and pulmonary auscultations, rectal temperature, mucous membrane color, the condition of the umbilicus, the presence of a suckle reflex, calf demeanor, ocular or nasal discharge, and the presence/absence of diarrhea.

Direct and a priori sampling was used.\textsuperscript{16} Samples were collected prior to morning feed, between approximately 6:30 and 11:30 am. Apart from a slightly delayed morning feed on the day of sampling, calves were fed, housed, and cared for as per normal farm practice. Calves had previously been fed a milk or milk replacer diet once (n=41) or twice (n=100) daily, with total daily volume fed ranging between 3.5 and 6 liters. Based on farmer reported feeding times, calves had been fasted for approximately 14-15 hours (n=89), 18-19 hours (n=11), or 24-25 hours (n=41) at the time of sampling. All calves were kept under shelter in bedded pens; most calves (n=107) were housed in group pens within larger 3-sided sheds, while the remainder of the calves (n=34) were housed in individual pens under a roof with solid half-height walls on three sides.

**Sample collection and handling**

Samples were collected from calves that were manually restrained in either sternal recumbency or a standing position. Blood was collected by jugular venipuncture using a 20-gauge, 1-inch needle.
Blood was collected into a Vacuette (Greiner Bio-One, USA) 2 mL sodium citrate tube, a Vacuette 4 mL serum separator tube, a Vacuette 2 mL lithium heparin tube, and a 1 mL K3 EDTA tube, after preparing the venipuncture site with a gauze swab soaked in 70% alcohol. Not all tubes were collected/used for every calf due to animal ethical reasons (eg, a calf was resisting restraint and sampling was terminated early) or for sample quality reasons (eg, hemolysis). All tubes were rotated gently at least six times before being placed in a container cooled by ice packs. Serum separator tubes were centrifuged on the farm at 3461g for a minimum of 5 minutes on a Hettich EBA 20 (Sigma-Aldrich, USA) centrifuge within 4 hours of sample collection (most samples within 1-3 hours of collection). Blood smears were prepared on the farm. Samples were then transported chilled directly to a veterinary clinical pathology laboratory that was approximately a 3-4 hour drive away, and were refrigerated on arrival. All samples were analyzed within 10 hours of collection.

Citrate tubes noted to be under-filled or over-filled by more than 3mm (n = 11) were excluded from the fibrinogen (modified Clauss method) analysis. Total protein (refractometer) results were excluded if plasma was more than slightly hemolyzed (n = 4). Biochemistry results, including electrolytes, were also excluded if serum was noted to be more than slightly hemolyzed (n = 12). Hematology and fibrinogen results were excluded if there was evidence of inflammation, based on the presence of bands (more than 0.1 x 10^9/L [adult RI for the laboratory]), toxic change in neutrophils (basophilic or foamy cytoplasm, Döhle bodies), or a Millar fibrinogen concentration greater than 7.5g/L (adult RI for the laboratory, n = 3). Samples with platelet clumping noted on the blood smear were excluded from the platelet index determinations, including PCT, MPV, and PDW (n = 10). Six EDTA tubes were clotted; for these samples, hematology was analyzed on blood from citrate tubes (dilution corrected, n = 5) or a lithium heparin tube (n = 1). Figure 1 summarizes excluded samples.

**Laboratory analytical methods**

Blood smears were stained with a Wright-Giemsa stain using Siemens Hematek (Siemens, Germany). All blood smears were examined, and manual differential counts were performed. Hematology was analyzed on a Sysmex XT-2000i analyzer (Sysmex, Japan), fibrinogen on the Stago Compact Max (Stago, France), and manually by the Millar method for a subset of samples (n = 50). Serum biochemistry was analyzed using a Cobas Integra 400 Plus biochemistry analyzer (Roche, Switzerland). Electrolytes were measured on an IDEXX VetLyte Na+K+Cl- analyzer (IDEXX, USA). A subset of samples was also analyzed for total protein using a hand-held refractometer (REF312ATCbp, Bacto Laboratories, Australia) (n = 54). See Tables 1 and 2 for details on the reagents.
used for analysis. The biochemistry assays in this study have been commonly used in commercial laboratories and had undergone general validation in the laboratory.

**Statistical analysis**

The number of calves enrolled in this study was based on guidelines from the American Society for Veterinary Clinical Pathology\(^\text{18}\). RIs were calculated using Reference Value Advisor Software\(^\text{22}\).

Nonparametric methods were used for all variables with more than 120 samples; therefore, normality and distribution tests were not required for these variables\(^\text{18}\), though histograms for all variables were visually inspected for evidence of any unusual/unexpected distributions or data points. Outliers were retained unless there were pre-analytic factors or post-analytic factors that could account for the results. This resulted in one calf being excluded, with a high outlier CK result as it was known to have had increased handling compared with the other calves.

For variables with less than 120 samples, data distribution was assessed with a combination of visual assessment using histograms and Q-Q plots, the Anderson-Darling test for normality, and the symmetry test for the Robust method, both before and after Box-Cox transformation. For total protein (refractometer, \(n=54\)), data were assessed as non-Gaussian and non-symmetrical before and after transformation using the above methods. Therefore, the nonparametric method was used for this variable despite the smaller sample size. For Millar fibrinogen (\(N=50\)) and plateletcrit (\(N = 105\)) measurements, the data were assessed visually as non-Gaussian and non-symmetrical before and after transformation, and therefore, the nonparametric RI was also used for these variables. The platelet distribution width, platelet large cell ratio, and mean platelet volume (all \(n = 105\)), were also assessed using nonparametric methods, as the sample sizes were large enough, though their data were assessed visually as approaching Gaussian distribution.

The effects of sex on blood variables were not analyzed due to the lack of male calves in the sample (only 9% of the calves were male). Similarly, the effects of breed on the blood variables were not analyzed, as approximately half of the calves were Holstein-Friesian, with the other half were almost exclusively Holstein-Friesian crossbreds, making a comparative analysis of limited use.

For BHB, results for 51 calves were left-censored due to being less than the limit of detection for the analyzer (0.1 mmol/L). Reference Value Advisor was not used for this variable, and instead, nonparametric bootstrap RIs, confidence intervals, and median were calculated using the MASS and boot packages in R statistical software\(^\text{23}\). The accelerated bias-corrected percentile limits method was used, using 10,000 bootstrap samples.\(^\text{24, 25}\) Maximum likelihood estimation was used to calculate the mean and standard deviation for BHB, using the R package, fitdistrplus. The log-normal distribution was chosen as the best fit for the data, based on visual inspection of plotted predicted
Values for the mean and standard deviation generated in the log-normal scale were then transformed to the linear scale using the R package, tsiMisc.

Results

Calf breeds were reported by owners/managers as Holstein-Friesian (n = 63), Holstein-Friesian/Jersey cross (n = 42), Holstein-Friesian/Jersey/Australian Red cross (n = 34), Jersey (n = 1), and dairy cross breeds (n = 1). RIs for hematology, biochemistry, and electrolyte variables for dairy calves are presented in Tables 3 and 4, including 90% confidence intervals for the upper and lower limits and select descriptive statistics.

Discussion

The RIs presented in this paper will be useful both as a diagnostic tool for veterinarians treating sick dairy calves and as a research tool. Dairy calves are often fasted and transported at a young age, either prior to slaughter or for transfer to a rearing facility. In countries such as Australia and New Zealand, non-replacement calves are fasted and transported to abattoirs as young as 5 days old, but this is likely to range from between 5-12 days old due to the pick-up schedules of calf transporters. Previous research on the effect of transport or fasting on calf welfare has used hematology and biochemistry, either comparing these values over time or between different groups of calves. The RIs presented here for the 5- to 12-day-old calves are useful references for future studies in this area.

The RIs in this study are in broad agreement with previous studies. Relatively minor differences between studies are seen for all analytes, probably reflecting differences in sample sizes, genetics, environmental factors, management factors, and/or analytical methods. The upper reference limit for BHB was, as expected, lower than that reported for adult cattle (0.59 mmol/L for calves compared to 1.00 mmol/L for adult cattle). We believe that our study represents the most comprehensive source of young calf biochemistry and hematology RIs and the only young calf reference interval for BHB.

Some potential limitations of this study relate to calf management on the farms. While the RIs presented in this paper are likely to reflect expected values in healthy calves on typical Australian dairy farms, this does not necessarily mean that calves had been managed to the best standards possible. For example, the reference interval reported in this paper for total protein is 44-82 g/L, but values for total protein below 50-52 g/L in young calves are regarded as being indicative of the...
failure of passive transfer of immunity\textsuperscript{28,29}. In the current study, 7% of included calves had total protein results that indicated failure of passive transfer (<52 g/L), which means that these calves are at a higher risk of morbidity and mortality\textsuperscript{30,31}. However, this rate of passive transfer failure is still much lower than previously reported in Victoria\textsuperscript{28}, and the first author (NR) considered the participating dairy farms to be representative of conditions that would be found on average Australian dairy farms.

Despite being assessed as reflecting normal industry standards, a relatively large number of calves (35 calves, or 15% of available calves) were not sampled due to evidence of illness on physical examination (Figure 1). Of these, most were excluded due to severe ocular discharge (12 calves) or diarrhea (11 calves); other reasons including pyrexia, cardiac murmurs, or prior illness. The rates of illness in the calves, and the proportion of calves sampled that had a failure of passive transfer, indicate that, although these calves were considered to be managed in a way that was broadly consistent with standard industry practices, this might not reflect the gold standard of calf management, and, therefore, calf health.

Another limitation of the study relates to calf feeding practices on farms. While individual animal intakes were not recorded, calves were fed an average of 4.3L per day each, with a range of 3.5-6L. The lower to middle end of this range represents approximately 10% of body weight per day. There is now a large body of scientific evidence that supports the feeding of higher volumes (generally around 15-20% of body weight) for improved calf growth, health, and future milk production (reviewed in Khan et al 2011 and Kertz et al 2017)\textsuperscript{32-34}. In addition, 3 of the 10 farms, participating in this study, fed calves once daily, despite Dairy Australia guidelines recommending that calves less than 2 weeks old be fed a minimum of twice daily.\textsuperscript{34} These factors mean that some of the RIs reported here could be different if calves were fed more often and with greater volumes, in line with current recommendations. Variables that are likely to be most affected by feeding regimes and fasting times are plasma glucose and serum BHB, with a higher glucose RI and a lower BHB upper limit expected with increased feed volumes and feeding frequencies.

Another point to note is that the assays that we used in this study had not been specifically validated for cattle, as they form a minority of our laboratory caseload, and species validation of these assays was beyond the scope of this study. This would be a worthwhile future study.

Conclusions

Young dairy calves are particularly vulnerable to poor health and welfare outcomes; high morbidity and mortality rates have been reported for the first 2 weeks of life, and calves often undergo stressful management practices, such as transport and fasting. This has made young calves a focus of
research on calf health and welfare, which often uses biochemistry and hematology measurements. Our results support the hypothesis that our RIs would be similar to those published previously, with some small differences likely due to variations in sample size, genetics, animal management and location, and methodology. Our results also support our hypothesis that the upper reference limit of BHB for calves would be lower than that for adult cattle. The comprehensive biochemistry and hematology RIs presented here for fasted calves aged 5-12 days old can be used both for clinical veterinary practice and for research purposes.

Acknowledgments

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References


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### Table 1. Reagents used for the hematology analyses

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<th>Reagent</th>
<th>Use</th>
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<tr>
<td>Cellpack</td>
<td>Red blood cell, platelet, and hemoglobin diluent; rinsing of instrument; hydrodynamic focusing (Sysmex, Japan)</td>
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<td>Stromatolyser-4DL</td>
<td>Differential lysing reagent (Sysmex, Japan)</td>
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<tr>
<td>Stromatolyser-4DS</td>
<td>Differential stain (Sysmex, Japan)</td>
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<tr>
<td>Stromatolyser-FB</td>
<td>Diluent for white blood cell count and differential lysing agent (Sysmex, Japan)</td>
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<tr>
<td>Sulfolyser</td>
<td>Sodium lauryl sulfate, a non-cyanide hemoglobin lysing agent (Sysmex, Japan)</td>
</tr>
<tr>
<td>Ret-Search (II) Diluent</td>
<td>Dilutes sample for reticulocyte analysis (Sysmex, Japan)</td>
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<tr>
<td>Ret-Search (II) Dye</td>
<td>Stains reticulocytes and platelets for analysis (Sysmex, Japan)</td>
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### Table 2. Reagents used for biochemistry analysis

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<th>Reagent</th>
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<th>SD</th>
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<th>Max</th>
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<th>URL (90%CI)</th>
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<td>WBC</td>
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<td>21.2</td>
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<td>(14.2-)</td>
<td>(4.0-)</td>
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</table>

**Table 3.** Hematology reference values for 5- to 12-day-old Australian dairy calves

Abbreviations: ALP, alkaline phosphatase; AST, aspartate aminotransferase; BHB, beta-hydroxybutyrate; GGT, gamma-glutamyl transferase; GLDH, glutamate dehydrogenase; fib, fibrinogen.
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<th></th>
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<th>9.1</th>
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<th>12.3</th>
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<td><strong>RBC</strong></td>
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<tr>
<td>Hemoglobin</td>
<td>g/L</td>
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<td>Hematocrit</td>
<td>L/L</td>
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<td>MCV</td>
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<td>26.5</td>
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<td>MCHC</td>
<td>g/L</td>
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<td>9</td>
<td>297</td>
<td>348</td>
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<td>Reticulocytes</td>
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<td>6</td>
<td>13</td>
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<td>85</td>
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<td>51</td>
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<td>RCDW-SD</td>
<td>fL</td>
<td>35.9</td>
<td>3.3</td>
<td>26.8</td>
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<td>RCDW-CV</td>
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<td>29.3</td>
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<td>49.7</td>
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<tr>
<td>Platelets</td>
<td>x 10^9/L</td>
<td>769</td>
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<td>PDW</td>
<td>fL</td>
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<td>MPV</td>
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Table 4. Biochemistry, electrolyte, and fibrinogen reference values for 5- to 12-day-old Australian dairy calves.

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<td>Albumin</td>
<td>g/L</td>
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<td>27</td>
<td>37</td>
<td>27 (27-37)</td>
<td>28 (36-37)</td>
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<td>Globulin</td>
<td>g/L</td>
<td>31</td>
<td>10</td>
<td>11</td>
<td>54</td>
<td>14 (11-16)</td>
<td>53 (50-54)</td>
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<tr>
<td>Total protein</td>
<td>g/L</td>
<td>63</td>
<td>9</td>
<td>44</td>
<td>84</td>
<td>45 (44-48)</td>
<td>82 (80-84)</td>
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<tr>
<td>Total protein (refractometer)</td>
<td>g/L</td>
<td>65</td>
<td>67</td>
<td>8</td>
<td>50 (50-52)</td>
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<tr>
<td></td>
<td>U/L</td>
<td>293</td>
<td>105</td>
<td>123</td>
<td>738 (123-166)</td>
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<td>ALP</td>
<td>U/L</td>
<td>215</td>
<td>269</td>
<td>26</td>
<td>1379 (26-47)</td>
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<td></td>
<td>U/L</td>
<td>13</td>
<td>14</td>
<td>5</td>
<td>111 (5-6)</td>
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<tr>
<td>GGT</td>
<td>U/L</td>
<td>297</td>
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<td>GLDH</td>
<td>U/L</td>
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<tr>
<td>AST</td>
<td>U/L</td>
<td>35</td>
<td>6</td>
<td>24</td>
<td>58 (24-28)</td>
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<tr>
<td>Creatine kinase</td>
<td>mmol/L</td>
<td>5.4</td>
<td>5.6</td>
<td>2.3</td>
<td>2.8 (2.3-3.3)</td>
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<tr>
<td>Glucose</td>
<td>mmol/L</td>
<td>0.11</td>
<td>0.14</td>
<td>&lt;0.1</td>
<td>0.94 (0.44, 0.80)</td>
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<td>BHB</td>
<td>mmol/L</td>
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<tr>
<td>Cholesterol</td>
<td>mmol/L</td>
<td>2.0</td>
<td>0.7</td>
<td>0.5</td>
<td>4.2 (0.5-1.0)</td>
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<tr>
<td>Triglycerides</td>
<td>mmol/L</td>
<td>0.3</td>
<td>0.2</td>
<td>0.1</td>
<td>1.2 (0.1-0.1)</td>
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<td>Creatinine</td>
<td>mmol/L</td>
<td>74</td>
<td>14</td>
<td>45</td>
<td>118 (45-52)</td>
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<tr>
<td>Urea</td>
<td>mmol/L</td>
<td>4.1</td>
<td>1.3</td>
<td>1.1</td>
<td>8.4 (1.1-2.2)</td>
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<td>Calcium</td>
<td>mmol/L</td>
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<td>0.18</td>
<td>2.39</td>
<td>3.55 (2.39-2.53)</td>
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<td>Magnesium</td>
<td>mmol/L</td>
<td>9</td>
<td>0.1</td>
<td>0.8</td>
<td>1.2 (0.8-0.8)</td>
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<table>
<thead>
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<th>Substance</th>
<th>Unit</th>
<th>Values</th>
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<th>SD</th>
<th>Min</th>
<th>Max</th>
<th>CI</th>
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<tr>
<td>Phosphorous</td>
<td>mmol/L</td>
<td>2.7, 0.3, 2.3, 3.3, 2.3 (2.3-3.3)</td>
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<td>2.3</td>
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<td>Sodium</td>
<td>mmol/L</td>
<td>143, 4, 124, 152, 130 (124-148)</td>
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<td>Potassium</td>
<td>mmol/L</td>
<td>5.70, 0.48, 4.20, 7.20, 4.75 (4.20-6.75)</td>
<td>5.76</td>
<td>0.48</td>
<td>4.20</td>
<td>7.20</td>
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<td>Chloride</td>
<td>mmol/L</td>
<td>103, 3, 95, 108, 96 (95-108)</td>
<td>102</td>
<td>3</td>
<td>95</td>
<td>108</td>
<td>129</td>
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<tr>
<td>Millar fibrinogen</td>
<td>µ/L</td>
<td>4.7, 0.9, 3.0, 6.6, 3.1 (3.0-6.6)</td>
<td>4.8</td>
<td>0.9</td>
<td>3.0</td>
<td>6.6</td>
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<td>Modified Clauss</td>
<td>µ/L</td>
<td>4.01, 0.90, 2.00, 6.52, 2.32 (2.00-6.52)</td>
<td>4.00</td>
<td>0.90</td>
<td>2.00</td>
<td>6.52</td>
<td>127</td>
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Reference intervals determined with Reference Value Advisor macro for Excel, using the nonparametric method, except for BHB, calculated using a bootstrap bias-corrected and accelerated method. Abbreviations: SD, standard deviation; Min, minimum; Max, maximum; LRL, lower reference limit; URL, upper reference limit; CI, confidence interval; ALP, alkaline phosphatase; AST, aspartate aminotransferase; BHB, beta-hydroxybutyrate; GGT, gamma-glutamyl transferase; GLDH, glutamate dehydrogenase.

**Figure 1.** Exclusion flowchart for the biochemical and hematologic samples of 5- to 12-day old calves. CK = creatine kinase.
194 calves available; 35 calves not sampled due to illness

Samples collected from 159 calves; 18 further calves excluded for illness identified after sampling

11 underfilled or overfilled citrate tubes excluded from fibrinogen analysis

Samples from 141 calves used for analysis

Samples from 141 calves used for analysis

Samples from 141 calves used for analysis

Samples from 135 calves analyzed for most hematology variables

Fibrinogen results for 3 calves removed from analysis due to evidence of inflammation/ill health

Samples from 129 calves analyzed for biochemistry and electrolytes

Hematology results for 3 calves removed from analysis due to evidence of inflammation/ill health

Samples from 129 calves analyzed for biochemistry and electrolytes

No EDTA sample collected for 3 calves

12 calves removed from biochemistry and electrolyte analysis due to serum hemolysis

Samples from 129 calves analyzed for biochemistry and electrolytes

127 samples analyzed for fibrinogen (modified Clauss)

127 samples analyzed for fibrinogen (modified Clauss)

11 platelet results excluded due to platelet clumping

127 samples analyzed for fibrinogen (modified Clauss)

Analyzer unable to measure platelet variables for 19 samples (platelet count excepted)

1 CK result removed due to increased animal handling

11 underfilled or overfilled citrate tubes excluded from fibrinogen analysis

1 CK and 1 urea value not reported due to lab error; 127 samples analyzed for CK and 128 for urea

127 samples analyzed for fibrinogen (modified Clauss)

105 samples analyzed for most platelet variables, 124 for platelet count

No EDTA sample collected for 3 calves

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Author/s:
Roadknight, NW; Courtman, NF; Mansell, PD; Jongman, EC; Loh, ZA; Fisher, AD

Title:
Biochemistry and hematology reference intervals for neonatal dairy calves aged 5-12 days

Date:
2021-06

Citation:

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