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Indicators of dehydration in healthy 4- to 5-day-old dairy calves deprived of feed and water for 24 hours

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ABSTRACT

Our objective was to identify practical indicators of calf dehydration that could be used in an industry context. Eleven healthy 4-d-old commercial dairy calves were fed 2 L of mixed colostrum, then deprived of food and water for 24 h. Total body water was determined in the fed state using the deuterium dilution method. Body weight, along with a range of behavioral and physiological variables, was recorded 1 h after feeding, then at 90-min intervals through to 24 h. Blood samples were collected at every second sampling to assess changes in plasma hemoglobin, hematocrit, and osmolality. Linear mixed-effects models were used to explore associations between hydration status (% body water) and outcome variables. All calves remained bright and alert with good suckling reflexes throughout the 24-h period. After 24 h, total body water had decreased by an average of 8.4% (standard error 1.18), consistent with mild to moderate dehydration. Skin tent return time, capillary refill time, and detectable enophthalmos were associated with hydration status. Calves with skin tent return times of 3 s or longer were 4.4 percentage points less hydrated than those with return times of less than 3 s. Similarly, a capillary refill time of 3 s or longer was associated with a 4.3 percentage point reduction in hydration compared with refill times of less than 3 s. Calves with detectable enophthalmos (≥1 mm) were 3.5 percentage points less hydrated than those without enophthalmos. The skin tent, capillary refill, and enophthalmos tests are all relatively simple to perform and, although requiring the calf to be briefly restrained, can easily be performed by a single operator. The outcome of these tests was relatively consistent, in that calves above the threshold in any test were 3.5 to 4.5% less hydrated than calves below the threshold. As such, these tests may be of practical utility to identify calves with mild to moderate dehydration in an industry setting.

Key words: bobby calf, dehydration, skin tent, capillary refill, enophthalmos

INTRODUCTION

The welfare of surplus dairy calves, known as bobby calves, in New Zealand has been the focus of recent attention. Bobby calves are typically transported to slaughter when they are between 4 and 7 d old, meaning they may be more susceptible to welfare compromise before slaughter than older animals. Although bobby calf mortality is low (0.06% in the 2017 spring calving season) (Anonymous, 2018), this still equates to more than 1,000 calves dying or being condemned before the point of slaughter each spring. This figure may be further reduced through early identification of calves that are at risk of death or welfare compromise. Although mortality is likely to be an objective indicator of calf welfare, its use alone is insufficient because death reflects the fact that welfare compromise was not recognized or appropriate steps were not taken to intervene before death, or both. Moreover, mortality data do not capture any potential welfare compromise among calves that survive to the point of slaughter. Thus, there is a need for validated indicators of calf health and welfare status that are practical for use across the supply chain.

Research undertaken in the 2016 New Zealand spring calving season found that dehydration was often cited among the reasons for calf condemnation before slaughter (Boulton et al., 2020). Furthermore, 63% of calves in lairage were identified as being at least mildly dehydrated (as assessed using the skin tent test) and 44% had evidence of fecal soiling, and postmortem examination of calves that died or were condemned before slaughter suggested that most had diarrhea, enteritis, or both (Boulton et al., 2020). Together, these suggest that dehydration, possibly associated with diarrhea, was a significant risk factor for bobby calf welfare compromise and death.

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Current New Zealand regulations require that bobby calves be slaughtered within 24 h of their last feed [Animal Welfare (Calves) Regulations, 2016]. Thus, there is the potential for calves to go up to 24 h without food before slaughter. In addition, calves have no access to water during transport, which may last up to 12 h [Animal Welfare (Calves) Regulations, 2016]. Although water is available to calves in lairage, it is not known whether any or all calves drink free water. As such, there is the potential for some calves to go for long periods without fluid intake before slaughter, which may lead to dehydration.

Moderate-to-severe dehydration may be accompanied by unpleasant experiences such as intense thirst, dizziness, weakness, or lethargy, thus representing a welfare concern. The development of severe dehydration in calves (characterized as >12% BW loss) is often fatal (Groutides and Michell, 1990; Smith 2009). Identifying the early signs of dehydration in calves may therefore improve calf welfare or reduce mortality (or both) through timely intervention.

Various methods have been used to evaluate hydration status, including direct measurements such as plasma osmolality, packed cell volume, and total plasma protein, and indirect assessments such as enophthalmos, color and feel of mucous membranes, and degree of skin elasticity evaluated by the skin tent test (Naylor, 1989; Constable et al., 1998; Smith, 2009). While several studies have examined indicators of dehydration in calves (e.g., Constable et al., 1998; Walker et al., 1998; Knowles et al., 1999), most of these have either involved older animals, have used measures of dehydration impractical for industry monitoring, or have evaluated dehydration induced by infectious or nutritional/osmotic diarrhea. The latter may influence the results, in terms of understanding the contribution of fluid deprivation alone on the development of dehydration. For example, infectious diarrhea also impairs acid/base and electrolyte balance, which can alter calf demeanor, behavior, and risk of death (Walker et al., 1998).

Our objective was to evaluate a range of physiological and behavioral indices, alongside BW and clinicopathological indicators of dehydration, in healthy calves over a 24-h period of fluid deprivation, to identify practical measures that could be used in an industry context to identify calves at risk of dehydration and thus potential welfare compromise.

MATERIALS AND METHODS

This study was undertaken with prior approval from the Massey University Animal Ethics Committee (protocol # 17/39). The study protocol supplied to the ethics committee is available as Supplemental File S1 (https://doi.org/10.3168/jds.2020-18743).

Animals

Eleven healthy calves, weighing 33.3 (SE 1.41) kg, sourced from a single commercial dairy farm were used in the study. These were primarily Jersey or Jersey/Friesian cross and comprised 9 females and 2 males. The calves were fed pooled colostrum (volume not recorded) twice daily before enrolment in the study. All calves were between 4 and 5 d of age and in good health (bright and alert with no evidence of diarrhea) at the start of the study. A sample size of 10 was selected based on data from an earlier study that examined associations between changes in packed cell volume and plasma volume with % change in BW over 48 h in 3- to 10-d-old calves with experimentally induced osmotic diarrhea (Constable et al., 1998). The authors reported correlation coefficients of 0.77 and 0.75, respectively. Based on a power of 80% and type 1 error rate of 5%, the required sample size was calculated as 8. Given that the present study was evaluating dehydration over a shorter time frame and in calves without diarrhea, a sample size of 10 was selected, with one additional animal to account for any dropouts.

Calves were tested in 2 cohorts: the first (n = 6) on April 3 and the second (n = 5) on April 9, 2018. On each date, all available calves of the appropriate age that met the inclusion criteria (healthy with no indication of diarrhea) were enrolled in the study. On the morning of testing, selected calves were transported approximately 50 km in a covered calf trailer from the farm of origin to Massey University where the experiment took place. On arrival, calves were transferred to a shared indoor pen approximately 3 m long and 2 m wide with solid walls and rubber flooring. The pen was partially exposed to the outdoor environment along one wall, mimicking typical on-farm conditions where calves are reared in dedicated sheds that are open on one side. No water was available in the pen, as this same pen was used throughout the study period. The left and right jugular furrow of each calf was shaved to facilitate catheter placement, after which the calves were left undisturbed for a minimum of 30 min to allow recovery from transport.

Experimental Procedure

Calves were removed from the group pen to an adjacent pen one at a time, weighed (Tru-Test MP600 load bars with XR3000 indicator, resolution 100 g; Tru-Test

Table 1. Summary of protocol used for sampling calves over a 24-h period of food and water deprivation[1]

	Time of day (h), sampling period, and time since feed (h)																
	1100	1200	1330	1500	1630	1800	1930	2100	2230	0000	0130	0300	0430	0600	0730	0900	1100
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Item	0	1	2.5	4	5.5	7	8.5	10	11.5	13	14.5	16	17.5	19	20.5	22	24
BW	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Behavior		√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Physical		√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Blood sampling		√[2]	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√

[1]Time 0 BW was recorded immediately after calves were fed.

[2]Blood sampled for deuterium analysis only.

Group Limited, Auckland, New Zealand), then gently restrained while local anesthetic (Xylocaine 2%, AstraZeneca, Cambridge, UK) was injected around the catheter placement site using an 18-gauge needle. A 14-gauge central venous catheter was inserted into the right jugular vein for repeat blood sampling and a 100-mm 10-gauge catheter placed in the left jugular vein for deuterium oxide administration. Approximately 4 mL of blood was removed via the left catheter for evaluation of background deuterium concentration, after which 0.4 mL/kg deuterium oxide (D_2O) was administered. The calf was then hand fed 2 L of pooled colostrum (as per standard practice, provided by the source farm), and BW was recorded at the conclusion of feeding to determine baseline hydrated weight (time 0). The calf was returned to the group pen without access to food or water for the following 24 h. Instrumentation, feeding, and baseline measures were performed on each calf sequentially. Subsequent measures on individual calves in each cohort were performed in the same order to ensure consistent between-sampling intervals.

One hour after feeding, a 5-min period of undisturbed behavior was video recorded in the group pen, after which calves' responses to the entrance of an experimenter were observed and recorded (see below for details). Following this, each calf was removed to an adjacent pen for assessment of physical measures and blood sampling, before being returned to the group pen. This was repeated at 90-min intervals until the conclusion of the 24-h test period. Blood sampling for analysis of deuterium dilution was performed once, in period 1, whereas blood sampling for hematology/biochemistry was performed in every second 90-min sampling period, beginning in period 2 (Table 1).

Due to the importance of BW measurement, each calf was weighed twice at each sampling point. If the weights agreed, this value was recorded. If the 2 initial weights did not agree, a third weight was obtained and the average of the 3 measures was recorded.

Body Water Assessment

To objectively quantify the degree of dehydration (body water loss) over time, baseline total body water content was determined for each calf using the isotope dilution method (Speakman 2001). Ninety minutes after injection of a known volume of D_2O, a 4-mL blood sample was taken and analyzed for plasma deuterium content. Plasma was separated by centrifugation (1,400 × g, 10 min at room temperature) within 10 min of sampling and stored at −20°C for up to 2 wk before being sent to the testing laboratory for analysis.

Baseline (1 h postfeeding) total body water mass was calculated for each calf as follows:

$$\text{mass } H_2O \text{ (kg)} = (\text{moles } H_2O \times 1.117)/\text{BW (kg)},$$

where 1.117 = correction factor required to account for overestimation of body water due to fractionation (Speakman, 2001).

$$\text{moles } H_2O = [\text{moles } D_2O \times (\text{Ein} - \text{Ep})]/(\text{Ep} - \text{Eb}),$$

where moles D_2O = moles D_2O in delivered dose (mass divided by molar mass), Ein = % D_2O in delivered dose (99.8%), Ep = % D_2O after equilibration, and Eb = % D_2O before dose administration (background).

The change in BW over time was subsequently used to determine water loss, based on the assumption that 100 g of weight loss = 100 mL of water loss. It is acknowledged there will have been a margin of error due to solids being excreted in feces. Given that calves were group housed, it was not possible to collect individual feces and correct for fecal solid content. However, as calf feces were semi-solid, the proportion of weight lost as solids is likely to have been relatively low.

Calf hydration status in each sampling period was expressed as a percentage of baseline body water content (100%; period 1) according to the following equation:

Table 2. Description of behaviors recorded in calves for 5 min at 90-min intervals over a 24-h period of food and water deprivation

Behavior	Description
Posture	Proportion of time spent standing
	– steady
	– unsteady
	Proportion of time spent walking
	– steady
	– unsteady
	Proportion of time spent lying in sternal recumbency
	– awake
	– asleep[1]
	Proportion of time spent lying in lateral recumbency
	– awake
	– asleep[1]
	Number of transitions (standing to lying or vice versa)
	Proportion of time spent awake
	Proportion of time spent asleep[1]
Oral behavior	Number of oral events (licking/sucking/chewing)
	– self-directed
	– directed toward pen mates (cross-sucking)
	– directed toward objects (pen walls or floor)
Vocalization	Number of vocalizations
Interaction with pen mates	Number of interactions (excluding cross-sucking)
Shivering	Present/absent
Coughing	Number of coughs

[1]Calves were deemed to be sleeping when motionless with the head turned against the flank (in sternal recumbency) or resting on the floor (in lateral recumbency; Ruckebusch, 1975).

$$\% \text{ Hydration} = [\text{BW (period 1)} - \text{BW in period X}]/[\text{mass } H_2O \text{ (period 1)} \times 100].$$

These values were later used to model predictors of hydration status.

Calf Behavior

Undisturbed calf behavior was video recorded in the group pen for 5 min at the start of each sampling period using 2 video recorders covering the pen from different angles. Behavior was later scored as described in Table 2. All scoring was carried out by a single experimenter, who was blinded with regard to the period being scored.

Analysis of subtle behaviors, such as changes in ear position, was not feasible because this would have required the calf to be in the same orientation toward the camera in each sampling period (Guesgen et al., 2016). As calves were free to move within the pen, their position relative to the cameras changed throughout the observation period.

Response to Experimenter

At the end of the 5-min video recording period, the experimenter entered the group pen and approached each calf individually. Calves that did not stand on experimenter entry were approached head on until the experimenter was directly in front. Calves that still did not rise were touched on the flank or nudged gently if required. Those that failed to rise were then assisted to their feet by reaching under the calf between the front and rear legs and lifting. Responses were scored as described in Table 3 below.

Physical Measures

The physical measures described in Table 4 below were assessed in each calf in each sampling period.

Blood Parameters

After recording of physical measures, approximately 10 mL of blood was drawn from the right jugular catheter using a sterile syringe and needle and allocated to pre-labeled heparin (4 mL) and EDTA (2 mL) vials for laboratory analysis of hematocrit, hemoglobin, total protein, and osmolality. Sample vials were stored at 4°C before delivery to the testing laboratory (within 26 h of collection).

Humane Endpoint

Throughout the study calves were routinely assessed by a veterinarian, using New Zealand's Ministry for Primary Industries verification services inspection criteria (i.e., equivalent of industry condemnation criteria; Ministry for Primary Industries 2015). Before under-

Table 3. Description of scores used to describe the responsiveness of calves to the presence of an experimenter in the pen

Score	Description
1	Responds to experimenter entry to pen (visual surveillance, ears oriented toward entry point), stands when experimenter >1 m away (includes calves that were standing before experimenter entry)
2	Responds to experimenter entry, stands when approached <1 m
3	Responds to entry, does not rise until touched or nudged, stands or walks unaided
4	Responds to entry, rises with assistance only, stands or walks unaided
5	No response to entry, stands when experimenter >1 m away
6	No response to entry, stands when approached <1 m
7	No response to entry, does not rise until touched or nudged, stands or walks unaided
8	No response to entry, rises with assistance only, stands or walks unaided
9	No response to entry, rises with assistance only, unable to stand unaided (equivalent of industry condemnation)

taking the study, it was determined that any calves identified as reaching these criteria would be removed from the study and provided with milk replacer, i.v. fluids and electrolytes (or both), based on veterinary recommendation. Any calves failing to respond to fluid therapy would be euthanized via an intravenous overdose of sodium pentobarbital.

Fate of Calves

At the conclusion of the 24-h data collection period, all calves were given 2 L of oral electrolyte solution (Vytrate Duo Sachet; Jurox Animal Health, NSW, Australia) followed immediately by 2 L of colostrum from the source farm or milk replacer (Ngahiwi Premium Calf Milk Replacer; Ngahiwi Farms, Auckland, New Zealand) when sufficient colostrum was unavailable. Once deemed fit by a veterinarian, calves were rehomed to private farms later the same day.

Statistical Analyses

The changes in all outcome variables, relative to baseline, over time were explored using mixed-effects models, with the precise model used dependent on whether the outcome was a binary, count, or linear variable. Each model included sampling period as a covariate and a random effect to account for repeat measures in calves. For binary or categorical outcomes (shivering, response to experimenter, sucking reflex, nasal and ocular discharge, fecal soiling), the effect of sampling period was assessed using a mixed-effects logistic regression model. Outcomes that could be treated as a count (oral behaviors, vocalization, interaction with pen mates, coughing) were analyzed using a mixed-effects Poisson model. Linear mixed-effects models were used to explore the relationship between sampling period and the linear outcomes hydration status, respiratory rate, skin tent, eyeball recession, capillary refill, body temperature, hematocrit, hemoglobin, total protein, and osmolality. Associations between calf hydration status (% hydration) and other outcome variables were explored using linear mixed-effects models. In the models constructed to assess the association between calf hydration and other outcome variables, sampling period was also included as a repeated measure.

All statistical analyses were performed in R version 3.4.2 (2016, R Foundation for Statistical Computing,

Table 4. Description of physical measures recorded in calves over a 24-h period of food and water deprivation

Measure	Description
Respiratory rate	Breaths per minute, counted over 15 s and multiplied by 4
Body weight	Weight in kg (resolution 0.1 kg)
Skin tent test	Time (s) for skin at nape of neck to return to normal after 1-s pinch and twist. Average of 3 tests per sampling period calculated.
Eyeball recession	Extent of eyeball recession (mm), estimated by gently everting the lower eyelid and estimating the recession of the globe into the orbit
Capillary refill	Time (s) for color to return to gums after blanching out by applying firm thumb pressure for 2 s. Average of 3 tests per sampling period calculated.
Suckling reflex	Suckling response to fingers placed in the mouth: 3 = strong (normal); 2 = diminished; 1 = markedly diminished; 0 = absent
Nasal discharge	0 = normal/none; 1 = small amount of unilateral cloudy discharge; 2 = bilateral, cloudy, or excessive mucus discharge; 3 = copious bilateral mucopurulent discharge
Ocular discharge	0 = none/normal; 1 = small amount; 2 = moderate amount of bilateral discharge; 3 = heavy ocular discharge
Fecal soiling	0 = none, 1 = mild (≤20% area of rump/hind legs), 2 = moderate (soiling over >20% of rump/hind legs), 3 = severe (profuse watery diarrhea)
Core body temperature	Rectal temperature (°C)

Vienna, Austria). Statistical significance was set at $P < 0.05$ and all reported means are based on model estimates. Results were not adjusted for multiple comparisons.

RESULTS

Body Water Assessment

Body weight measures, and therefore hydration status data, for 3 calves were excluded from analysis due to technical issues with the scales. Otherwise, data from all 11 calves were included in the analyses.

Based on deuterium dilution, mean (±SE) baseline (1 h after feeding) body water content in calves was 68.8 ± 1.7% (range 66.6–70.5%). After 24 h without food or water, calf BW had decreased by 6.23 (±0.91)%, corresponding to an average water loss of 8.4 ± 1.18% (minimum 5.6, maximum 11.3%).

Undisturbed Calf Behavior

The following behaviors were never observed: standing unsteady, walking unsteady, lying in lateral recumbency (awake or asleep), shivering, or coughing. In addition, vocalizations were extremely rare (6 events over all observations), and therefore were excluded from further analysis. Oral behaviors directed toward pen mates or objects were rarely observed; therefore, these were combined, along with self-directed oral behavior, into a single category "oral." Calves were rarely observed standing (23/176 observations) or walking (17/176 observations); therefore, these were treated as binary variables coded 1 if the behavior occurred or 0 if it did not. Conversely, many calves were observed lying down for the entirety of each observation period (145/176 observations). The proportion of time lying was therefore also modeled as a binary variable coded 1 if the calf lay for the whole 5-min observation period (proportion = 1) or 0 if some time was spent in another posture (proportion <1).

Response to Experimenter

A 9-point categorical scale was used to score calf responsiveness (Table 3). Seventy-three percent of observations were scored either 3 (responds to entry, does not stand until touched, stands/walks unaided) or 4 (responds to entry, does not stand until assisted, stands/walks unaided), with a further 9% scored as 1 (responds to entry, already standing, or stands when experimenter >1 m away). A score of 8 (no response to entry, does not stand until touched, stands/walks unaided) was assigned to 12% of observations. No scores of 5, 6, or 9 were assigned. Responsiveness to an experimenter was subsequently modeled as a binary variable (1 = responds to entry, 0 = no response to entry).

Physical Measures

Only 2 of 4 possible categories were scored for fecal soiling (none/mild), nasal discharge (none/small amount), ocular discharge (none/small amount), and suckling reflex (normal/diminished). These were subsequently modeled as binary variables (0 = absent, 1 = present for fecal, nasal, and ocular score; 0 = normal, 1 = diminished for suckling reflex).

Skin tent and capillary refill times, along with rectal temperature, were modeled as both continuous and binary variables. Data for capillary refill were coded 0 for values <3 s (within the accepted normal range; Aldrich, 2005) or 1 for values ≥3 s (prolonged). Likewise, skin tent time was coded 0 for values <3 s (normal) or 1 for values ≥3 s (prolonged), based on values considered indicative of moderate dehydration in calves (Walker et al., 1998). Rectal temperature was coded 0 (normal) if it was between 38 and 39.5°C or 1 (cool) if it was <38°C, based on the normal range reported for calves (Blood et al., 2007).

Enophthalmos was only noted in 44/176 observations and was scored as either 1 mm (95%) or 2 mm (5%). This was subsequently modeled as a binary variable (0 = absent, 1 = present).

Effect of Time on Outcome Variables

Calf hydration, assessed by change in BW, decreased over time ($P < 0.001$; Table 5; Figure 1). After 24 h without food or water, mean hydration had reduced to 91.6 (SE 1.2)% of baseline value (1 h after feeding 2 L of colostrum). Body water percentage was lower than baseline from period 3 (4 h after feeding) onward.

No effect was observed of time on calves' responses to an experimenter ($P = 0.29$), presence of ocular discharge ($P = 0.13$), plasma hemoglobin ($P = 0.98$), hematocrit ($P = 0.89$), plasma total protein ($P = 0.22$), plasma osmolality ($P = 0.72$), or whether calves stood ($P = 0.71$) or walked ($P = 0.36$) during the observation period. Statistical outcomes for variables for which there was an effect of period are provided in Table 5, with summary statistics by time shown in Figures 2 to 6. Changes in skin tent time, capillary refill time, and oral events are plotted against time (h) since last feed, whereas respiration rate and rectal temperature are plotted against time of day, as these may be subject to diurnal variations (Mortola, 2004).

Although an overall effect of period was identified for presence of fecal soiling, presence of nasal discharge,

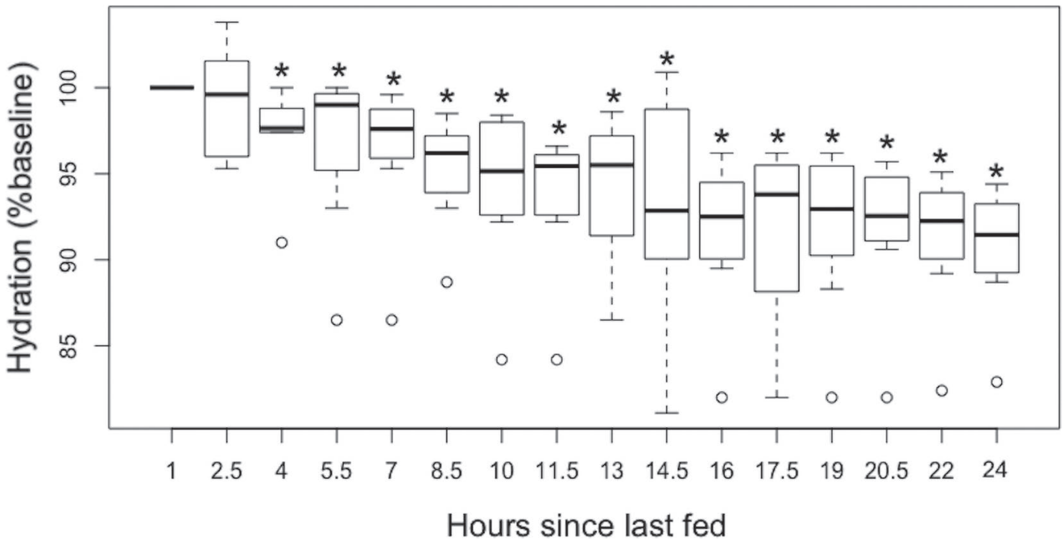

Figure 1. Boxplot depicting the change in hydration status, relative to baseline (1 h after feeding), in 4-d-old calves over a 24-h period of food and water deprivation. Asterisks indicate sampling periods that differed from baseline (1 h after feeding; $P < 0.05$). The box extends from the 25th to the 75th percentile, with a line at the median. Whiskers extend from the box a distance of 1.5 times the interquartile range, and the circle symbol (○) denotes outliers or extreme values that fall outside of the range of the whiskers.

lying time, and number of postural transitions, no differences were identified between period 1 and subsequent periods (i.e., no changes from baseline over the 24-h sampling period). Suckling reflex (normal or diminished) differed from baseline in period 2 only ($P = 0.04$), where 6/11 calves had a diminished suckling reflex compared with 2/11 in period 1. Calves spent more time sleeping in period 9 (0000 h) compared with period 1 ($P = 0.03$), whereas proportion of time sleeping did not differ in any other period.

Respiration rate was lower than baseline in periods 9 (0000 h) and 10 (0130 h), but did not differ from baseline in any other period (Figure 2). Rectal temperature was higher than baseline in period 5 (1800 h) and lower than baseline in periods 14 to 16 (0730–1100 h; Figure 3). Skin tent and capillary refill times increased over the 24-h period and were both higher than baseline from period 3 (4 h after last feed) onward (Figures 4 and 5). The number of oral behaviors observed was lower than baseline from 2.5 to 4 h and 14.5, 16, and 19 h after last feed, but was greater than baseline at 7 h (Figure 6). The number of calves with evidence of enophthalmos at each sampling point is shown in Table 6.

Associations Between Measured Variables and Calf Hydration

Associations were found between calf hydration (% body water) and the continuous variables respiration rate and rectal temperature. For every 1°C decrease in

Table 5. Results of linear or logistic regression analysis exploring the effect of sampling period on outcome variables in 4-d-old calves deprived of feed for a 24-h period

Variable	Likelihood ratio test statistic	Chi-squared test statistic	P-value
Hydration	148.36		<0.001
Respiration rate	106.26		<0.001
Rectal temperature	60.80		<0.001
Presence of fecal soiling		25.30	0.05
Presence of nasal discharge		51.24	<0.001
Skin tent time	253.52		<0.001
Capillary refill time	209.09		<0.001
Diminished suckling reflex		33.05	0.005
Presence of enophthalmos		67.11	<0.001
Lying time		25.47	0.04
Proportion of time sleeping	25.77		0.04
Number of oral events		127.19	<0.001
Number of postural transitions		29.43	0.01

rectal temperature, there was a 3.1 (95% CI 1.7–4.5; $P < 0.001$) percentage point decrease in hydration. When temperature was analyzed as a binary factor (normal/cool), there was no association with hydration ($P = 0.37$). For every 5 breaths-per-minute decrease in respiration rate, hydration decreased by 0.6% (95% CI 0.4–0.7%; $P < 0.001$). Associations were also identified between hydration and the categorical variables skin tent time, capillary refill time, and presence of enophthalmos (Table 7). Calves with skin tent return times of 3 s or more were 4.4% less hydrated than those with return times of <3 s. Similarly, a capillary refill time of 3 s or more was associated with a 4.3% reduction in hydration compared with refill times of less than 3 s. Calves with detectable enophthalmos were 3.5% less hydrated than those without evident enophthalmos.

DISCUSSION

Previous research has identified the need for practical means of assessing bobby calf welfare, to identify calves at risk of welfare compromise earlier in the supply chain. Based on prior evidence that dehydration was prevalent in calves in lairage and in those that died or were condemned before slaughter in the 2016 New Zealand spring calving season (Boulton et al., 2020), we sought to evaluate practical measures for assessing calf hydration status. Although serial measurement of BW is considered the best index of hydration status, particularly in cases of acute fluid loss (DiBartola and Bateman, 2012), this is not feasible in calves that are moving along the supply chain. We therefore evaluated a range of potential practically measurable indicators, alongside changes in BW and clinicopathological indicators of fluid imbalance, to determine which were most closely associated with hydration status.

In clinical terms, mild dehydration is typically defined as a 5% reduction in BW, with 7% equaling moderate dehydration and 12% equating to severe dehydration (Aldrich, 2005). Based on a mean body water content of 69%, these figures equate to 7.2, 10.1, and 17.4% water loss, respectively. In the present study average body water loss after 24 h of food and water deprivation was 8.4 ± 1.2% (range 5.6–11.9%), suggesting that the calves in this study had mild to moderate dehydration after 24 h. In support of this, no significant changes in hematocrit, hemoglobin, or plasma total protein were observed. These variables are typically elevated in cases of clinically significant dehydration (Carlson and Bruss, 2008). It should be noted that calves in the present study were not exposed to potential stressors such as transportation, mixing with unfamiliar calves, or holding in lairage, unlike calves moving along the supply chain. As such, the extent of dehydration observed over the 24 h may have differed from that which would occur in an industry setting.

While several indicators showed some variation, relative to baseline, over the 24-h observation period, only

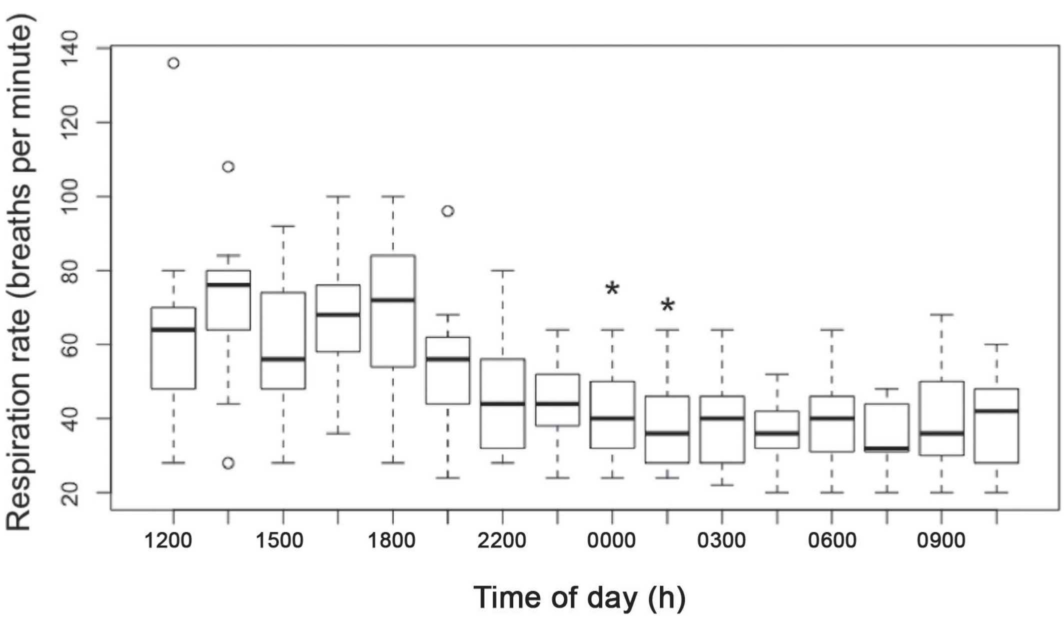

Figure 2. Boxplot depicting the change in respiration rate (breaths per minute) over time in eleven 4-d-old calves over a 24-h period of food and water deprivation. Asterisks indicate values that differed from baseline (1200 h; $P < 0.05$). The box extends from the 25th to the 75th percentile, with a line at the median. Whiskers extend from the box a distance of 1.5 times the interquartile range, and the circle symbol (○) denotes outliers or extreme values that fall outside of the range of the whiskers.

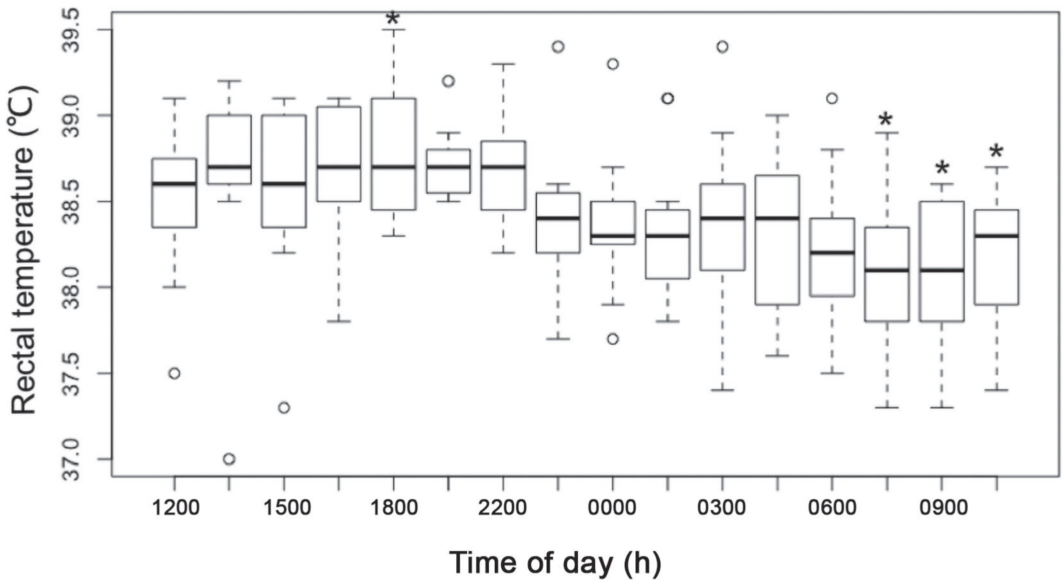

Figure 3. Boxplot depicting the change in rectal temperature (ºC) over time in eleven 4-d-old calves over a 24-h period of food and water deprivation. Asterisks indicate values that differed from baseline (1200 h; $P < 0.05$). The box extends from the 25th to the 75th percentile, with a line at the median. Whiskers extend from the box a distance of 1.5 times the interquartile range, and the circle symbol (○) denotes outliers or extreme values that fall outside of the range of the whiskers.

5 were associated with hydration status. These were cervical skin tent return time, capillary refill time, presence of enophthalmos, respiration rate, and rectal temperature. Rectal temperature and respiration rate were positively related to hydration status (i.e., a decrease in either was associated with a decrease in hydration, whereas skin tent time, capillary refill time, and enophthalmos were inversely related to hydration status).

When modeled as binary variables, prolonged (>3 s) skin tent and capillary refill times, and the presence of enophthalmos, were associated with a 3.5 to 4.5 percentage point reduction in total body water. Previ-

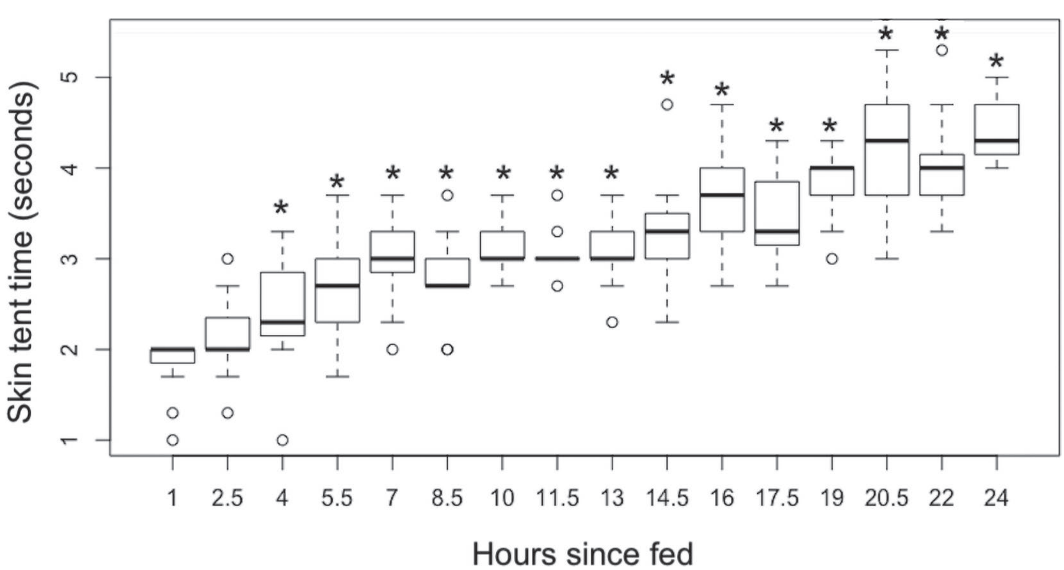

Figure 4. Boxplot depicting the change in skin tent return time (s) over time in eleven 4-d-old calves over a 24-h period of food and water deprivation. Asterisks indicate values that differed from baseline (1 h after feeding; $P < 0.05$). The box extends from the 25th to the 75th percentile, with a line at the median. Whiskers extend from the box a distance of 1.5 times the interquartile range, and the circle symbol (○) denotes outliers or extreme values that fall outside of the range of the whiskers.

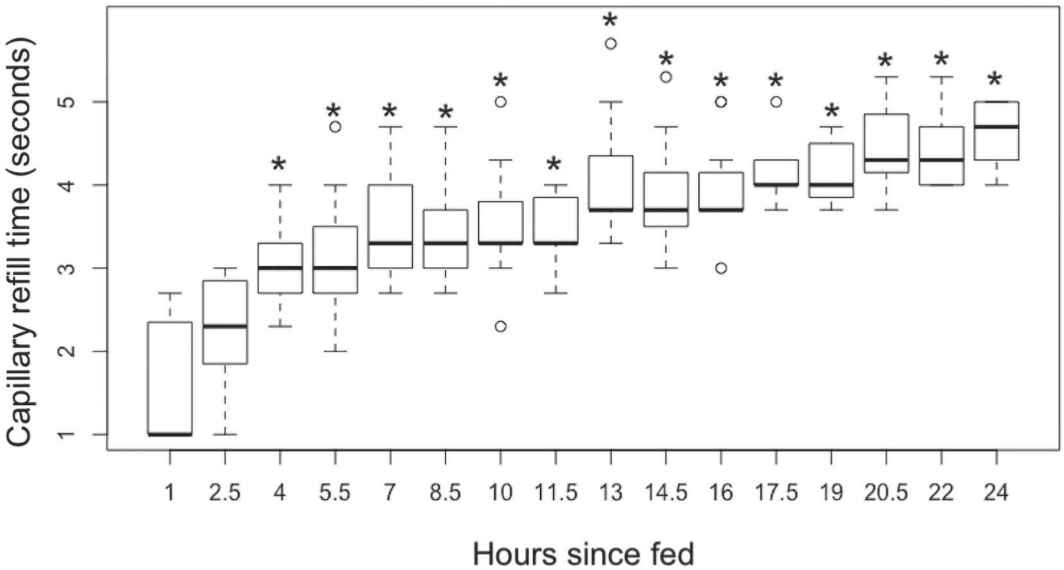

Figure 5. Boxplot depicting the change in capillary refill time (s) over time in eleven 4-d-old calves over a 24-h period of food and water deprivation. Asterisks indicate values that differed from baseline (1 h after feeding; $P < 0.05$). The box extends from the 25th to the 75th percentile, with a line at the median. Whiskers extend from the box a distance of 1.5 times the interquartile range, and the circle symbol (○) denotes outliers or extreme values that fall outside of the range of the whiskers.

ous studies have also identified significant associations between hydration status and skin tent duration, enophthalmos, and capillary refill time in calves with mild to moderate (Constable et al., 1998) or moderate to severe (Walker et al., 1998; Sentürk, 2003) dehydration. With regard to the accuracy of skin tent return time and extent of enophthalmos as measures of degree of dehydration, the results of the present study align well with those reported in a study of neonatal dairy calves with experimentally induced diarrhea (Constable et al., 1998). The authors reported that skin tent return times of 3 and 4 s were indicative of 2% and 4% dehydration, respectively, which is consistent with the present study, in which calves with return time of >3 s had

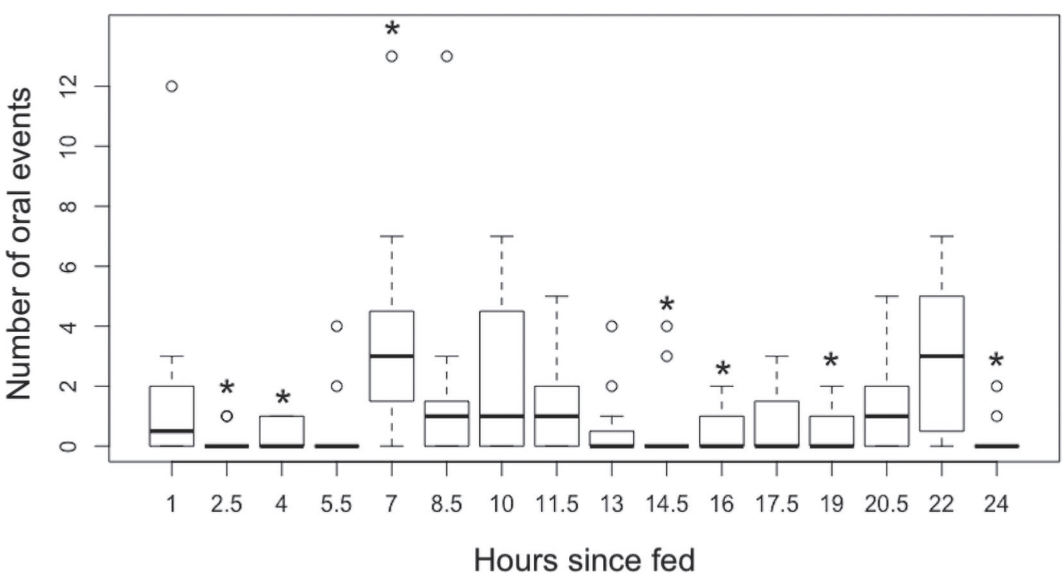

Figure 6. Boxplot depicting the change in count of oral behaviors observed at each sampling time in eleven 4-d-old calves over a 24-h period of food and water deprivation. Asterisks indicate values that differed from baseline (1 h after feeding; $P < 0.05$). The box extends from the 25th to the 75th percentile, with a line at the median. Whiskers extend from the box a distance of 1.5 times the interquartile range, and the circle symbol (○) denotes outliers or extreme values that fall outside of the range of the whiskers.

Table 6. Number of calves (from a total n = 11) with evidence of enophthalmos in each sampling period over a 24-h period of food and water deprivation

Sampling period	Time of day (h)	Enophthalmos present
1	1200	0
2	1330	0
3	1500	1
4	1630	0
5	1800	0
6	1930	2
7	2100	3
8	2230	1
9	0000	3
10	0130	1
11	0300	3
12	0430	4
13	0600	5
14	0730	7
15	0900	6
16	1100	8

4.4% lower body water than those with shorter return times. Similarly, our finding that calves with detectable enophthalmos (1 or 2 mm) had 3.5% lower body water content than those without detectable enophthalmos is consistent with the prior study in which enophthalmos of 1 and 2 mm were indicative of 2% and 4% dehydration, respectively (Constable et al., 1998).

In the present study, capillary refill time was associated with hydration status in mild to moderately dehydrated calves. However, the use of capillary refill time to evaluate hydration in young calves has been questioned on the basis that healthy young calves tend to have pale mucous membranes, making it more difficult to observe changes (Constable et al., 1998). Although calves in our study had pale gums, this did not prevent evaluation of capillary refill times. In a study of diarrhea-induced dehydration, it was noted that mucous membranes became paler with increasing dehydration, to the point where calves with severe dehydration (17% BW loss) had white-colored mucous membranes, preventing assessment of capillary refill time (Constable et al., 1998). Therefore, in cases of more severe dehydration, capillary refill time may not be feasible for assessing hydration status.

Rectal temperature and respiration rate, when modeled as continuous variables, were positively associated with hydration status (i.e., a decrease in either was associated with decreased hydration). Rectal temperature is considered to be a good proxy for core body temperature, even in hypovolemia (Mansel et al., 2008). In the present study, rectal temperature was lower than baseline from 20.5 to 24 h after calves were last fed, corresponding to the hours between 0730 and 1100 h. Additionally, rectal temperature was higher 7 h after feeding, at 1800 h. Body temperature in cattle follows a daily circadian rhythm, typically peaking at sundown then decreasing throughout the night to its lowest point at around 0500 h (Piccione et al., 2003). The observed temperature increases at 1800 h in the present study, along with the pattern of falling temperatures overnight, generally fits with this pattern. However, the persistence of lower temperatures after 0600 h does not fit with this cycle.

Severe dehydration may be accompanied by a decrease in peripheral body temperature as a consequence of reduced perfusion of peripheral tissues due to reduced plasma volume (Jones et al., 1984; Walker et al., 1998). In calves with experimentally induced severe dehydration, fetlock temperature (a measure of peripheral temperature) was reduced after 24 h; however, rectal temperature (an indicator of core body temperature), remained stable (Walker et al., 1998). Therefore, while peripheral temperature is flexible in response to changes in hydration, core temperature is not expected to change. When rectal temperature was modeled as a binary variable in the present study, using a cut point of ≤38.0°C to define low temperature (Blood et al., 2007), there was no longer an apparent association between rectal temperature and hydration (i.e., hydration status did not differ between calves with low versus normal rectal temperatures). The relative

Table 7. Results of simple linear regression analysis of categorical predictor variables for calf hydration (% body water) in calves deprived of food and water for a 24-h period

Variable	Beta	Lower 95% CI	Upper 95% CI	SE	*P*-value
Skin tent time					
<3 s	Referent				
≥3 s	−4.42	−5.5	−3.4	0.53	<0.001
Capillary refill time					
<3 s	Referent				
≥3 s	−4.33	−5.8	−2.9	0.73	<0.001
Enophthalmos					
Absent	Referent				
Present	−3.46	−4.9	−2.1	0.74	<0.001

stability of core temperature in response to dehydration, along with confounding due to circadian rhythm, mean that change in rectal temperature is likely to be of limited value in assessing mild to moderate changes in hydration status.

Although respiration rate tended to decline over the 24-h observation period, mean respiration rates were above the reported normal range for calves of 24 to 36 breaths per minute (Jackson and Cockcroft, 2007) during the first 8 to 10 sampling periods. This may have been due to physiological arousal in response to the novel research environment and handling, as calves were tested on the same day that they were collected from the source farm. The lower rates seen in the later observation periods may thus reflect habituation to the novel environment and handling, given that these were largely within the normal range. In light of this potential confounder, further research is necessary to understand the relationship between respiratory rate and hydration status.

The skin tent, capillary refill and enophthalmos tests are all relatively simple and rapid to perform and, although requiring the calf to be restrained, can easily be performed by a single operator. As such, any of these measures may be of practical utility in identifying bobby calves at risk of welfare compromise due to dehydration. In the present study, skin tent and capillary refill times were obtained by calculating the average of 3 successive measures per calf, whereas enophthalmos was tested only once per calf in each observation period. The decision to average 3 measures per calf was based on previously described methodology (Constable et al., 1998). However, other studies have reported using a single measure (Walker et al., 1998). Although there were small discrepancies among the 3 measures on some occasions in the present study, a single measure may be sufficient to assess hydration status, particularly where a threshold has been established.

In the present study, all measures were performed by a single person, ensuring consistency in sampling (e.g., site of skin tent test, pressure applied to gums). To ensure consistent application by multiple operators across the dairy supply chain, some degree of training would need to be provided. Additional research is required to evaluate the validity and reliability of these tests in an industry setting.

Given that calves in the present study were only mildly to moderately dehydrated, systematic validation of the skin tent, capillary refill, or enophthalmos tests as diagnostic indicators of calf dehydration would require their evaluation over a wider range of hydration states (i.e., including moderately to severely dehydrated calves). Nevertheless, the results of this study support the use of these measures as early indicators of reduced hydration in bobby calves.

The potential applicability of these results to the wider industry should also be acknowledged. For instance, dehydration was reportedly common among young calves on arrival at a Canadian veal calf facility and was further identified as being a significant risk factor for early mortality (Renaud et al., 2018). The development of simple, practical, and reliable clinical indicators of early dehydration could therefore benefit the welfare and survival of young calves in both the dairy and veal industries.

Several limitations in the study design that may have influenced the accuracy or generalizability of the results must be acknowledged. For instance, all calves enrolled in the study were obtained from a single commercial farm. Although this served to reduce variability in the test population, it must be acknowledged that differences in calf management between farms may reduce the extrapolation of results to the wider bobby calf population. Moreover, the ratio of heifers to bulls in the present study was 9:2, potentially reducing the generalization of results, given that bulls typically make up around two-thirds of bobby calves sent to slaughter (Boulton et al., 2020).

The calves in the present study were receiving pooled colostrum twice daily on farm before entering the study. Given that some farms practice once a day feeding of bobby calves (Boulton et al., 2020), the nutritional status of the study calves may not be representative of the wider population. The effects of 24-h feed deprivation on hydration status may differ in calves with different baseline nutritional states.

Finally, it was not possible for the researcher carrying out the clinical scoring of calves over the course of the study to be blinded as to time of day; therefore, subconscious measurement bias cannot be ruled out.

CONCLUSIONS

In young calves, body water loss of 8.4% was accompanied by an increase in skin tent and capillary refill times and the presence of enophthalmos. It may be cautiously inferred that skin tent time or capillary refill times of 3 s or more, or the presence of enophthalmos, are associated with mild to moderate dehydration in healthy 4-d-old calves. As such, these indices are of potential value in identifying calves at risk of welfare compromise related to dehydration before slaughter. However, the strength of these associations in moderate to severely dehydrated healthy calves, along with the validity and reliability of these tests in an industry setting, requires further investigation.

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