ABSTRACT

The objective of this study was to investigate the effects of feeding surplus dairy calves a milk replacer (MR) or one of 2 different oral rehydration solutions (ORS) during a mid-transportation rest period on metabolic and clinical health indicators, growth, and behavioral outcomes after arrival to a calf-raising facility. Surplus dairy calves (n = 128) were transported in 4 cohorts from February to July 2022 for 12 h to a holding facility, rested for 8 h, then transported for an additional 6 h to a calf-raising facility. Upon arrival to the holding facility, calves were randomly assigned to 1 of 3 treatments: MR (n = 43), a high sodium ORS developed for diarrhea (ORS-D; n = 43), or a high potassium ORS developed for transportation (ORS-T; n = 42). The exact age of calves at transportation was unknown, however all calves were under 14 d of age. Calf body weight at enrollment was 43.9 ± 5.9 kg, 43.7 ± 6.5 kg, and 45.0 ± 4.5 kg for calves fed MR, ORS-D, and ORS-T, respectively. Calves were fed 2.0 L of their treatment twice, once upon arrival and once before leaving the holding facility. Upon arrival to the holding facility, calves were weighed and blood sampled. Calves were also health scored at unloading at the holding facility. After arrival at the calf-raising facility, calves were weighed, health scored, and blood sampled. Blood samples were collected at 24 and 48 h and body weight (BW) was recorded at 24 h, 48 h, 72 h, 5 d, 7 d, 14 d, and at 8 wks after arrival to the calf-raising facility. Calves were also health scored daily for 14 d, which included fecal consistency scoring and evaluating the presence or absence of respiratory disease. Lying time, lying bouts, and activity index were measured during transportation and from 3 d relative to transportation using accelerometers. At arrival to the calf-raiser, calves fed ORS-D had higher concentrations of NEFA and BHB than calves fed MR. Furthermore, calves fed ORS-T had higher concentrations of BHB at arrival to the calf raiser compared with calves fed MR. In the 14 d after arrival to the calf-raiser, there was evidence that calves fed ORS-T had a higher proportion of days with diarrhea and respiratory disease compared with those fed MR. During transportation, calves fed ORS-T had a lower activity index than calves fed MR, suggesting that ORS-T calves had lower overall activity. Additionally, on the day of transportation (d 0), ORS-T and ORS-D calves had a lower activity index than calves fed MR. There were no treatment effects on growth outcomes. The results of this study suggest that feeding MR rather than an ORS during a mid-transportation rest period could minimize fat mobilization and can potentially improve diarrhea and respiratory disease but does not affect growth outcomes after arrival to calf-raisers.

Key words: calf, transportation, electrolytes, behavior

INTRODUCTION

Surplus dairy calves are commonly transported long distances from the dairy farm of origin to livestock auctions, veal farms, and calf-raisers at a young age (Wilson et al., 2020). Calves experience multiple challenges throughout transportation, such as handling, loading, and unloading, varying weather conditions, comingling with unfamiliar calves, and long periods of feed and water withdrawal (Trunkfield and Broom, 1990; reviewed by Creutzinger et al., 2021) – all of which may exacerbate morbidities.

Young, transported dairy calves commonly experience dehydration (Pemppek et al., 2017), diarrhea (Bähler et al., 2012), and respiratory disease (Pardon et al., 2012) after arrival to calf-raising facilities. Specifically, in the
weeks after arrival to a Canadian calf-raising facility, up to 90% of calves were treated for diarrhea or bovine respiratory disease at least once (Scott et al., 2019). It is likely that this compromised condition at arrival contributes to mortality in surplus calves (Renaud et al., 2018). High mortality is an additional issue for surplus calves, with 42% of mortality occurring within the first 21 d after arrival to calf-raising facilities (Renaud et al., 2018).

Calves are typically fasted for long periods of time during transportation (Roadknight et al., 2021). Limited access to feed and water during transportation has been shown to cause marked effects on fat mobilization, and as a result, feed deprivation during transportation is associated with a lighter body weight upon arrival to calf-raising facilities (Goetz et al., 2023b; Rot et al., 2021). For example, at arrival to a calf-raising facility, calves that were transported and feed deprived for 12 h and 16 h had greater concentrations of nonesterified fatty acids and β-hydroxy-butyrate (Goetz et al., 2023b) and lighter body weights (Rot et al., 2021) compared with calves transported for 6 h. Furthermore, extensive periods of feed deprivation and dehydration around transportation in calves results in changes in strong ion concentrations in the blood, which can lead to blood acid-base imbalances causing a mild metabolic acidosis (Schaefer et al., 1998, 1990, 1992; Wilms et al., 2023). Regulations regarding feed deprivation in transported calves vary by country. For example, in Europe, calves may be transported for up to 9 h before requiring a rest stop but can be fasted for up to 19 h (Council of the European Union, 2004). Comparatively, in the United States, calves can be transported without feed, water, and rest for up to 28 h (United States Government, 2011). Regulations in Canada state that calves can be transported for up to 12 h until a rest period is required where they must be provided with feed, water, and rest (Government of Canada, 2022). Currently, there is little research evaluating feeding practices during rest periods in young dairy calves.

Dehydration is a common challenge for calves transported long-distances. Previous research has found that 35% of calves are clinically dehydrated upon arrival to calf-raising facilities after long-distance transportation (Pempek et al., 2017). Feeding an ORS to dairy calves before or in the middle of transportation has been explored to minimize dehydration. For example, Knowles et al. (1997) found that one month old calves fed an ORS during the middle of a 16 h transportation bout resulted in fewer dehydrated calves than those fed MR. However, Marcato et al. (2020a) found that a single feeding of 1.5 L of MR to male Holstein-Friesian and dairy-beef cross calves before 6 h of transportation resulted in less fat mobilization but more dehydration than calves fed ORS. However, after 18 h of transportation, there were no differences in fat mobilization between treatment groups, although calves given MR were less dehydrated (Marcato et al., 2020a). It is currently unclear what the best feeding practices and ORS compositions are for transported calves to mitigate negative outcomes after arrival to calf-raising facilities. Commercially used ORS are generally high in sodium to replenish electrolyte losses from diarrhea and dehydration in calves (Wilms et al., 2020). However, transportation impacts mineral balances in calves differently than diarrhea (Schaefer et al., 1997; Steinhardt and Hans-Herma, 1998; Goetz et al., 2023a,b) likely due to feed deprivation (Wilms et al., 2023). For example, transported calves may experience potassium depletion (Trefz et al., 2015; Parker et al., 2003). Wilms et al. (2023) found that feeding calves a high potassium ORS to feed deprived Holstein bulls resulted in lower body weight loss and maintenance of acid-base balance. Based on this evidence, providing transported calves with a high potassium ORS may mitigate negative health and growth outcomes after arrival to calf-raising facilities. Therefore, the present study aimed to investigate the use of a high potassium ORS in transported calves to address the different electrolyte imbalances of transported calves compared with diarrheic calves.

The objective of this study was to investigate the effect of feeding calves either MR (26% crude protein and 20% fat), a high sodium ORS developed for diarrhea (ORS-D), or a high potassium ORS developed for transported calves (ORS-T) during a rest period between 2 legs of transportation on growth, activity behaviors, and metabolic and clinical indicators of health. The study also aimed to examine how the treatments impacted morbidity and mortality in the 14-d following arrival to the calf-raising facility. We hypothesized that calves fed MR during a rest period would experience less energy mobilization and would lose less body weight during transportation than calves fed an ORS. We also hypothesized that calves fed MR during a rest period would have a lower prevalence of factors associated with morbidity and mortality, such as a sunken flank and dehydration, and therefore have a lower risk of morbidity and mortality in the weeks after arrival to the calf-raising facility.

**MATERIALS AND METHODS**

**Data collection, animals, and housing**

Calves were sourced from a single dairy farm and ear tagged before transportation. Calves were loaded between 8:30 a.m. and 9:00 a.m. onto a single 20.9 m² (9.1 m × 2.3 m) gooseneck trailer that was disinfected.
before transportation and deep bedded with clean, chopped straw in the winter and spring (cohorts 1 and 2; February and April) and sawdust in the summer (cohorts 3 and 4; June and July). A total of 4 cohorts were transported from February until July 2022. The first, second, and fourth cohort transported 35 calves and the third cohort transported 24 calves. Calves had a space allowance of 0.43 m² in cohorts 1, 2, and 4, and 0.60 m² per calf in cohort 3. Different drivers were used for each cohort; however, the same cattle transportation company and truck and trailer were used throughout.

At the holding facility, calves were housed indoors in pens separated by corral gates on saw-dust bedding. Calves were housed in 3 separate pens according to the color of livestock marker to ensure that researchers fed the calves the correct treatment assignments. However, the treatment assignments were randomized to each pen by cohort to ensure that each pen was represented by each treatment group throughout the study. The number of calves per pen was dependent on the number of calves per treatment during the transportation cohort (cohort 1, 2, and 4 = 12 calves per pen; cohort 3 = 8 calves per pen). Calves were not given free-choice access to water or solid feed during the rest period as the holding facility was not equipped to do so. They were rested for 8 h at the holding facility before the second leg of transportation.

Calves were transported for 6 h from the holding facility to the calf-raising facility. After arrival to the calf-raising facility, they were housed in individual hutches outdoors and had outdoor access by tether. The hutches were deep bedded on chopped straw for cohort 1 in February and cohort 2 in April and sawdust bedding in the summer for cohort 3 in June and cohort 4 in July. Calves were fed 3.0 L of MR (22% crude protein and 17% fat) twice daily by bucket until weaning at 56 d after arrival. During the preweaning period, calves had ad libitum access to solid feed and water. Weaning occurred gradually over a 2-wk period and, after weaning, were fed only calf starter, which was blended on farm and contained 18% protein. Water was provided every day. Calves that experienced diarrhea were provided with meloxicam (2.5 mL/100 kg subcutaneously once; Metacam, Boeringher Ingelheim) and, if they continued to have diarrhea, they received trimethoprim sulfadoxine (3 mL/45 kg intramuscularly once per day for 3 consecutive days; Borgal, Merck). Mortality and disease treatment records were kept by the producer and sent to researchers.

**Experimental Design**

This randomized controlled study was conducted at a livestock holding facility and a commercial calf-raising facility within 100 km from the University of Guelph in southern Ontario, Canada from February to July 2022. Animal use was approved by the University of Guelph Animal Care Committee (Animal Use Protocol #4430). Calves (n = 129) were sourced from a single dairy farm and transported in 4 cohorts by road continuously for 12 h to a livestock holding facility. Both Holstein (n = 70) and dairy-beef cross (n = 59) calves were included in the study. Calves were assigned to treatments using a random allocation sequence generated in Microsoft Excel version 16.66 (Microsoft Corp., Redmond, WA), which was applied in the order that calves were unloaded from the trailer at the holding facility. To indicate the treatment assigned to the calves, they were marked on their back with a chalk-based colored livestock marker. For each cohort, the color representing the treatments changed so that a different color represented a different treatment each time. Additionally, as the livestock marker was chalk-based and the calves were housed outdoors at the calf-raising facility, it did not remain on them after 24 h. The 3 treatments included: milk replacer (MR; n = 43; 130 g/L; Mapleview Agri LTD, Palmerston, Canada; Table 1), high sodium ORS developed for calves with diarrhea (ORS-D; n = 43; 57.5 g/L; Mapleview Agri LTD, Palmerston, Canada), and high potassium ORS developed for transported calves (ORS-T; n = 42; 43 g/L; Trouw Nutrition, Amstersfoot, Netherlands). Both ORS treatments were designed to have a high strong ion difference (SID, > 60 mEq/L) to maintain or restore blood acid–base balance (Smith and Berchtold, 2014). In ORS-D, the high SID was driven by high sodium concentration, whereas in ORS-T, the high SID was driven by high potassium concentration, to align with the strong ion theory, which states that ORS should deliver an excess of strong cations (sodium and potassium) relative to the concentration of strong anions (chloride; Constable, 2014). The MR fed at the holding facility was a skim milk powder base with a blend of 80% palm and 20% coconut as the fat source. The MR and ORS treatments were mixed at the holding facility in their respective buckets using a drill mixer until the mixture was visibly homogeneous. Calves were fed 2 L of their treatment by bottle twice: within 1 h after arrival and within 1 h before reloading at the holding facility. Refusals were measured by volume (mL) and recorded. Blood samples and body weights were taken at arrival to and before departure from the holding facility.

After loading for the second leg of transportation, calves were continuously transported by road for 6 h to a commercial calf-raising facility where they were removed from the truck, weighed, placed into individual hutches by tether and health scored immediately after arrival. Additional blood samples were taken from the...
subset of calves immediately after offloading the truck at 0 h, and at 24 h, 48 h, and 72 h after arrival. Calves were weighed again at 24 h, 48 h, 72 h, 5 d, 7 d, 10 d, 14 d, and 8 wk after arrival. Calves were health scored daily by 2 researchers (e.g., further described in health exam section) for 14 d following arrival. The first author (AB), who completed health assessments at the holding and calf-raising facilities, was aware of which livestock marker color represented each treatment and was thus not blinded to the treatment allocation; however, other observers and researchers were blinded. The calf-raiser and staff responsible for disease treatment and feeding at the calf-raising facility were also blinded to the treatment groups.

**Blood metabolites.** A subset of calves was randomly selected to have blood samples taken (MR = 20, ORS-D = 22, ORS-T = 23) using a random allocation sequence generated in Microsoft Excel version 16.66 (Microsoft Corp., Redmond, WA) and were blocked by treatment group. Calves were marked on their back with a livestock marker to indicate their treatment group and if a blood sample was needed. Blood samples were marked on their back with a livestock marker to indicate their treatment group and if a blood sample was needed. Blood samples were taken via jugular venipuncture from calves within the blood sampling subset using a 20-gauge 1-inch needle into a 10 mL serum vacuum tube without anticoagulant (BD Vacutainer Serum Blood Collection Tubes; Becton, Dickinson and Co.). Blood was allowed to clot, then centrifuged at 1,500 x g for 15 min. Serum was separated into 2 samples and stored at −20°C until further analysis.

**Outcome assessment**

**Blood sampling.** Samples taken immediately after unloading at the holding facility (baseline) were sent to Saskatchewan Colostrum Company for IgG analysis by radial immunodiffusion analysis (Saskatoon, SK, CAN). Serum samples were processed by the Animal Health Laboratory at the University of Guelph for nonesterified fatty acids (NEFA), β-hydroxy-butyrate (BHB), creatine kinase (CK), lactose dehydrogenase (LDH), and cholesterol concentration. Randox NEFA and Randox BHB kits (Randox Laboratories Canada Ltd., Mississauga, ON) were used to assess NEFA and BHB concentrations. A CK assay (Roche, Mississauga, ON) was used to assess the CK concentrations. A Roche CHOL2 kit (Roche, Mississauga, ON) was used to assess the concentration of cholesterol and a Roche LDHI2 kit (Roche, Mississauga, ON) was used to assess the concentration of LDH. The intra-assay coefficients of variation (%) for BHB, NEFA, cholesterol, and CK were 1.20, 2.06, 0.97, and 0.80, respectively (Goetz et al., 2023b). The inter-assay coefficients (%) for BHB, NEFA, cholesterol, and CK were 2.96, 4.06, 3.62, and 1.12, respectively (Goetz et al., 2023b).

At the time of the serum samples, a second 10 mL blood sample was taken via jugular venipuncture with a reduced heparin arterial blood gas sampler (Vyaire Medical, Mettawa, IL) and analyzed within an hour of collection with an i-STAT EC8+ cartridge in an i-STAT

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**Table 1. Comparison of Composition of Treatments**

<table>
<thead>
<tr>
<th></th>
<th>Milk Replacer (MR)</th>
<th>Diarrhea Electrolyte (ORS-D)</th>
<th>Transportation Electrolyte (ORS-T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dosage (g/L)</td>
<td>130</td>
<td>57.5</td>
<td>43</td>
</tr>
<tr>
<td>ME (MJ/kg DM product)</td>
<td>20.2</td>
<td>12.0</td>
<td>10.6</td>
</tr>
<tr>
<td>ME in 2.0 L solution (MJ)</td>
<td>5.45</td>
<td>1.07</td>
<td>0.63</td>
</tr>
<tr>
<td>Sugars</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose mmol/L</td>
<td>0</td>
<td>214</td>
<td>0</td>
</tr>
<tr>
<td>Lactose mmol/L</td>
<td>185</td>
<td>0</td>
<td>77.9</td>
</tr>
<tr>
<td>Minerals</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium mmol/L</td>
<td>31</td>
<td>125</td>
<td>34.6</td>
</tr>
<tr>
<td>Potassium mmol/L</td>
<td>21</td>
<td>27</td>
<td>104.8</td>
</tr>
<tr>
<td>Chloride mmol/L</td>
<td>37</td>
<td>72</td>
<td>27.8</td>
</tr>
<tr>
<td>Alkalizing Agents</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bicarbonate</td>
<td></td>
<td>35</td>
<td>19.5</td>
</tr>
<tr>
<td>Citrate</td>
<td></td>
<td>12</td>
<td>19.2</td>
</tr>
<tr>
<td>Propionate</td>
<td></td>
<td>0</td>
<td>34.6</td>
</tr>
<tr>
<td>Osmolality (mOsm/kg)</td>
<td>367</td>
<td>542</td>
<td>319</td>
</tr>
<tr>
<td>SID (mEq/L)</td>
<td>45</td>
<td>80</td>
<td>112</td>
</tr>
<tr>
<td>Glu:Na Ratio</td>
<td></td>
<td>1.7</td>
<td>4.5</td>
</tr>
</tbody>
</table>
Alinity V hand-held analyzer (Abaxis, Union City, CA) for concentrations of sodium, potassium, chloride, anion gap ([(Na + K) − (Cl + HCO₃)], glucose, blood urea nitrogen (BUN), hematocrit, hemoglobin, pH, pCO₂, TCO₂, HCO₃, and base excess (the amount of an acid or a base required to return the blood to a pH of 7.4 (Hopper, 2015); BE). The inter-assay coefficient of variation (%) for sodium, potassium, chloride, glucose, BUN, pH, and pCO₂ were 0.40, 1.30, 0.70, 1.60, 1.40, 0.08, and 2.50, respectively (Goetz et al., 2023b).

Selected blood disorders were classified as follows: respiratory acidosis as a pH <7.370, PCO₂ > 58.7 mmHg, and blood BE between >2.6 mmol/L <10.8 mmol/L; metabolic acidosis as a pH <7.370, PCO₂ > 43.3 mmHg but <58.7 mmHg and BE <2.6 mmol/L (Constable, 2000; Constable, 2014).

Health outcomes. Two researchers conducted daily health exams which included an assessment of fecal consistency (normal (0); semi formed, pasty (1); loose, stays on top of bedding (2); or watery, sifts through bedding (3); where a score of ≥2 was considered abnormal; McGuirk, 2008; Renaud et al., 2020), navel inflammation (normal, <1.3 cm (0), slightly enlarged, not warm or painful (1), slightly enlarged with slight pain or moisture (2), enlarged with pain, heat, or discharge (2); ≥2 was considered abnormal; Renaud et al., 2018), joint score (>0 was considered abnormal), respiratory scoring (presence or absence of a spontaneous cough (0 or 2), abnormal nasal discharge (0 or 2), ocular discharge (0 or 2), abnormal respiratory rate (0 or 2) and/or uni- or bi-lateral ear droop (0 or 4); ≥5 was considered abnormal; Love et al., 2014), sunken flank (flank not sunken = 0, flank sunken = 1), attitude (bright and alert = 0, slightly depressed = 1, depressed = 2; Goetz et al., 2023a), and rectal temperature (≥39.5°C was considered a fever).

Immediately after unloading at the holding facility, immediately after arrival to the calf raising facility, and 24 h, 48 h, and 72 h after arrival, a more thorough health exam was conducted including assessing calves for dehydration (presence of a skin tent ≥2 s and/or eye recession ≥2 mm; Wilson et al., 2000) and weakness (calf stands up by itself = not weak, calf needed assistance from the researchers to stand = weak). All health exam outcomes were recorded in a Qualtrics survey on a tablet and processed into CSV files in Microsoft Excel.

The observer agreement was calculated after one of the daily health exams, one researcher performed health exams on 100 calves that were housed at the facility for one week. The second researcher also performed health exams on these calves to calculate interobserver reliability. The calves used were not part of this study. Interobserver reliability for health examinations was calculated using SAS to assess the κ statistic and was 0.86 between researchers one and 2.

Growth outcomes. Body weight loss during transportation was assessed as a percentage from the time reloading at the holding facility until arrival at the calf-raising facility (Percent BW loss = [(body weight at arrival to the calf-raising facility - body weight at reloading at the holding facility) / body weight at reloading at the holding facility] x 100). Short-term ADG was assessed from the time of unloading at the rest period until 14 d after arrival to the calf-raising facility (short-term ADG = [(body weight at d 14 - body weight at unloading at the rest period) / 14 d]). Long-ADG was assessed from the time of unloading at the rest period until 8 wk after arrival to the calf-raising facility (long-term ADG = [(body weight at 8 wk - body weight at unloading at the rest period) / 56 d]).

Lying behavior. Ear tag numbers of each calf and the serial number of the IceQube were recorded to ensure that recorded lying behavior was associated with the correct calf. Activity was recorded at 4 Hz and a summary of lying bouts (no./15 min), lying time (min/15 min), and activity index was created for every calf in 15 min summaries. The activity index is a metric which measures total activeness in the calves, including total step counts and rate of acceleration (Cantor and Costa, 2022). The IceQubes were removed at d 14 after arrival to the calf raising facility, scanned using an RFID reader, and sent to the CowAlert data cloud. The data were exported as a Microsoft Excel CSV file to be summarized. Daily lying time (h/d), lying bouts (no./d), and activity index were summarized from 12:00 a.m. on the day of transportation until 11:59 p.m. on the third day after arrival to the calf-raiser in 24 h periods. Percentage of lying time (min/360 min of transportation), number of lying bouts, and activity index were summarized from the time calves were loaded onto the trailer at the holding facility (6:30 a.m.) until calves were unloaded at the calf-raising facility (12:30 p.m.). Over the course of the study, calves were loaded onto the trailer no earlier than 6:30 a.m. and arrived at the calf-raising facility no later than 12:30 p.m. on the same day.

Exclusion Criteria. Fitness for the first leg of transportation was assessed by the truck driver at the time of loading at the dairy farm before transportation. Per the Health of Animals Regulation, only calves that were not dehydrated, able to stand, and had a healed umbilicus were transported (Government of Canada, 2020). If calves showed signs of distress (e.g., open mouth breathing, lateral recumbency, depression) at any time before reloading for the second leg of transportation at the holding facility, they were removed from the study and treated by a veterinarian. One calf...
was excluded from the study due to lack of fitness for transportation after unloading at the holding facility. This calf was not assigned a study treatment, evaluated by a veterinarian, and humanely euthanized.

Sample size calculation

Transportation. The sample size calculation was performed a priori based on physiological outcomes outlined by Rot et al. (2021), based on average body weight in transported calves before and after transportation (51.0 kg and 47.0 kg [standard deviation = 6.0 kg]). A total of 37 calves per group was required to achieve 80% power (β) with 95% confidence (α = 0.05). We estimated that calves fed electrolytes would likely lose more body weight than calves fed MR.

Blood sampling. The sample size calculation was performed a priori based on hematological outcomes outlined by Knowles et al. (1999). Based on estimated NEFA concentrations that would occur in calves that were fed milk replacer compared with those fed an ORS during a rest period (335 µmol/L vs 514 µmol/L, respectively [standard deviation = 144 mmol/L]), a total of 12 calves per group were required to achieve 80% power (β) with 95% confidence (α = 0.05).

Statistical Analysis.

Data were imported into Stata 17 from Microsoft Excel (StataCorp LP, College Station, TX) for statistical analysis. Calf was the experimental unit for all analyses. The assumption of independence was evaluated using Spearman Rank coefficients for each of the variables and determined to be fulfilled if values were <0.6. A univariable regression analysis was conducted between each outcome and predictor variable. Linearity between the outcome and each predictor variable was assessed visually. If a variable did not have a linear relationship with the outcome, it was categorized into quartiles. Predictor variables with $P < 0.2$ were included for assessment in the multivariable models. Stepwise backward elimination was used to build the final models, in which variables with $P < 0.05$ were included in the final models. Statistical significance was reported at $P < 0.05$. Tendencies were reported at $0.05 < P < 0.10$. Normality of residuals was evaluated for each of the models visually using residual plots. In all models, calves fed MR were set as the referent group; results are presented as margins of difference. In models using repeated measures, the interaction between treatment and sample time was included as a fixed effect and calf was included as a random effect. In each model, treatment group was forced into the model as a fixed effect and transportation cohort was included as a random effect. In lieu of multivariable models, chi-squared tests were used for categorical outcomes and a Fisher’s exact test was used for outcomes with ≤5 observations per category. In models assessing blood gas and clinical health outcomes, dehydration at baseline was included as a covariate to account for the increased number of calves dehydrated at baseline in the ORS-D group. Models used for the analysis of health and behavior outcomes are outlined in Table 2. To account for type I error, a Bonferroni adjustment was made for each repeated measures model.

Blood parameters. For each blood parameter, a repeated measures mixed linear regression model was created to identify factors associated with each outcome over time. Predictor variables included treatment, baseline body weight (weight after unloading at the holding facility), breed (dairy-beef cross or Holstein), and percentage of body weight lost during transportation; transportation cohort (1 – 4) and calf were included as a random effects and sample time was included as the repeated measure. Baseline dehydration and baseline values for each blood parameter were forced into each model as a covariate.

Additionally, mixed effects Poisson regression models were created to assess the number of times each calf was categorized with metabolic and respiratory acidosis from unloading at the holding facility to 48 h after arrival to the calf-raiser (5 sampling time points). Predictor variables included treatment, baseline body weight (weight after unloading at the holding facility), and breed (dairy-beef or Holstein). The presence or absence of dehydration at baseline in the ORS-D group. In the final model, treatment group and baseline body weight were included as fixed effects; transportation cohort (1 – 4) and calf were included as a random effects.

Health outcomes. To determine the effect of the treatments on health outcomes, predictor variables included treatment (MR, ORS-D, ORS-T), body weight at unloading at the holding facility (baseline), and breed (dairy-beef or Holstein). The presence or absence of diarrhea at unloading at the holding facility (baseline) and the presence or absence of dehydration at unloading at the holding facility (baseline) were also included as predictor variables in the models assessing occurrence of dehydration, a sunken flank, and diarrhea.

A repeated measure logistic regression model was created to identify factors associated with dehydration. In the final model, treatment group, presence or absence of dehydration at baseline, and the interaction between treatment group and sample time were included as fixed effects; transportation cohort (1 – 4) and calf were included as a random effects and sample time was included as a repeated measure. A repeated measure logistic regression model was created to identify factors associated with the presence of a sunken flank. In the
final model, treatment group, breed, and the interaction between treatment group and sample time were included as fixed effects; transportation cohort (1 – 4) and calf were included as a random effects and sample time was included as a repeated measure. A repeated measure logistic regression model was created to identify factors associated with the presence of a fever. In the final model, treatment group, breed, and the interaction between treatment group and sample time were included as fixed effects; transportation cohort (1 – 4) and calf were included as random effects and sample time was included as a repeated measure.

Mixed effects Poisson regression models were created to assess the number of days calves had diarrhea and signs of respiratory disease during the 14 d after transportation. In the final model, treatment group, presence or absence of disease (diarrhea or respiratory disease) at baseline, and the interaction between treatment group and sample time were included as fixed effects; transportation cohort (1 – 4) was included as a random effect.

**Growth outcomes.** Mixed linear regression models were created to identify factors associated with the percentage of body weight lost during transportation, ADG over the first 14 d after arrival to the calf-raising facility, and ADG over 8 weeks. Predictor variables included treatment group (MR, ORS-D, ORS-T), body weight at unloading at the holding facility (baseline), and breed (dairy-beef or Holstein). In the final models for both percentage of body weight lost and growth over the different periods, treatment group, transportation cohort (1 – 4), and body weight at baseline were included as fixed effects; transportation cohort (1 – 4) was included as a random effect.

A repeated measures mixed linear regression model was created to identify factors associated with body weight at each sampling time. In the final model, treatment (MR, ORS-D, ORS-T), transportation cohort (1 – 4), sample time (unloading at holding facility, reloading at holding facility, arrival to calf-raising facility, 24 h, 48 h, 5 d, 7 d, 10 d, 14 d, 8 wk), and the interaction between treatment and sample time were included as fixed effects; transportation cohort (1 – 4) and calf were included as random effects.

**Behavior outcomes.** To analyze the association of treatment (MR, ORS-D, ORS-T) with calf behavior, body weight at unloading at the holding facility (baseline), breed, transportation cohort, presence or absence of diarrhea at unloading at the holding facility (baseline), presence or absence of respiratory disease at unloading at the holding facility (baseline), and number of diseases (0 = none, 1 = diarrhea or respiratory disease, 2 = diarrhea and respiratory disease) at unloading at the holding facility (baseline) were included as predictor variables. A mixed linear regression model was used to assess the percentage of lying time (%), lying bouts (no.), and activity index during transportation. In each model, treatment group and was included as a fixed effect and transportation cohort (1 – 4) was included as a random effect. Repeated measures mixed linear regression models were used to assess daily lying time (h/d), daily lying bouts (no./d), and daily activity index. In each model, treatment group, baseline body weight, transportation cohort, and the interaction between treatment group and sample time were included as fixed effects; transportation cohort (1 – 4) and calf were included as random effects and day was the repeated measure.

**RESULTS**

**Descriptive Statistics**

A total of 129 surplus dairy calves were enrolled in the study and transported in 4 cohorts. Each cohort had 35 calves, except the third cohort, which had 24 calves. The sex of calves within the study was not recorded. The exact age of calves at transportation was unknown, however all calves were under 14 d of age. Holstein (n = 70) and dairy-beef cross (n = 59) calves were included in the study. The average IgG concentration at unloading at the holding facility (baseline) was 18.3 g/L ± 7.6, 18.2 g/L ± 7.7, and 18.7 g/L ± 6.3 for calves fed MR, ORS-D, and ORS-T, respectively (Table 3). The average body weight at unloading at the holding facility (baseline) was 43.9 kg ± 5.9, 43.7 kg ± 6.5, and 45.0 kg ± 4.5 for calves fed MR, ORS-D, and ORS-T, respectively (Table 3). There were no differences found between treatment groups for baseline IgG concentration (P = 0.40) or body weight (P = 0.54), as determined by a chi-squared test. No calves had an abnormal or swollen joint at any of the time points evaluated and, therefore, this outcome was not statistically analyzed.

**Treatment Refusals.** In the first cohort, 1 calf in the ORS-D treatment group refused 0.25 L during the first feeding. In the second cohort, 2 calves in the ORS-D treatment group refused 2 L and 1 calf in the ORS-T refused 0.25 L during the second feeding. In the third cohort, 1 calf in the ORS-D refused 0.5 L during the second feeding. No calves refused treatments in the fourth cohort.

**Blood Parameters**

**Fat and energy mobilization.** At arrival to the calf-raising facility, calves fed ORS-D had higher con-
Table 2: Description of the models used to analyze health and behavior outcomes

<table>
<thead>
<tr>
<th>Model</th>
<th>Model Type</th>
<th>Outcome(s)</th>
<th>Follow-up period</th>
<th>Fixed Effects</th>
<th>Random Effects</th>
<th>Repeated Measure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mixed effects logistic regression model with</td>
<td>Proportion of calves with dehydration per day</td>
<td>72 h</td>
<td>Treatment group, baseline dehydration, interaction between treatment group and sample time</td>
<td>Calf, transportation cohort</td>
<td>Sample time</td>
</tr>
<tr>
<td></td>
<td>repeated measures</td>
<td></td>
<td></td>
<td></td>
<td>Calf, transportation cohort</td>
<td>Sample time</td>
</tr>
<tr>
<td>2</td>
<td>Mixed effects logistic regression model with</td>
<td>Proportion of calves with a sunken flank per day</td>
<td>72 h</td>
<td>Treatment group, breed, interaction between treatment group and sample time</td>
<td>Calf, transportation cohort</td>
<td>Sample time</td>
</tr>
<tr>
<td></td>
<td>repeated measures</td>
<td></td>
<td></td>
<td></td>
<td>Calf, transportation cohort</td>
<td>Sample time</td>
</tr>
<tr>
<td>3</td>
<td>Mixed effects logistic regression model with</td>
<td>Proportion of calves with a fever per day</td>
<td>14 d</td>
<td>Treatment group, breed, interaction between treatment group and sample time</td>
<td>Calf, transportation cohort</td>
<td>Sample time</td>
</tr>
<tr>
<td></td>
<td>repeated measures</td>
<td></td>
<td></td>
<td></td>
<td>Calf, transportation cohort</td>
<td>Sample time</td>
</tr>
<tr>
<td>4</td>
<td>Mixed effects Poisson regression</td>
<td>Number of days with diarrhea</td>
<td>14 d</td>
<td>Treatment group, baseline disease, interaction between treatment group and sample time</td>
<td>Transportation cohort</td>
<td>—</td>
</tr>
<tr>
<td>5</td>
<td>Mixed effects Poisson regression</td>
<td>Number of days with signs of respiratory disease</td>
<td>14 d</td>
<td>Treatment group, baseline disease, interaction between treatment group and sample time</td>
<td>Transportation cohort</td>
<td>—</td>
</tr>
<tr>
<td>6</td>
<td>Mixed effects linear regression model</td>
<td>Percentage of body weight lost during transportation</td>
<td>6 h</td>
<td>Treatment group, transportation cohort, baseline body weight</td>
<td>Transportation cohort</td>
<td>—</td>
</tr>
<tr>
<td>7</td>
<td>Mixed effects linear regression model</td>
<td>ADG over the first 14 d after arrival</td>
<td>14 d</td>
<td>Treatment group, transportation cohort, baseline body weight</td>
<td>Transportation cohort</td>
<td>—</td>
</tr>
<tr>
<td>8</td>
<td>Mixed effects linear regression model</td>
<td>ADG over 56 d after arrival</td>
<td>8 weeks</td>
<td>Treatment group, transportation cohort, baseline body weight</td>
<td>Transportation cohort</td>
<td>—</td>
</tr>
<tr>
<td>9</td>
<td>Mixed effects linear regression model with</td>
<td>Body weight at each sampling time</td>
<td>8 weeks</td>
<td>Treatment group, sample time, interaction between treatment and sample time</td>
<td>Calf, transportation cohort</td>
<td>Sample time</td>
</tr>
<tr>
<td></td>
<td>repeated measures</td>
<td></td>
<td></td>
<td></td>
<td>Calf, transportation cohort</td>
<td>Sample time</td>
</tr>
<tr>
<td>10</td>
<td>Mixed effects linear regression model</td>
<td>Percentage of time spent lying down, number of lying bouts, and activity index during transportation</td>
<td>6 h</td>
<td>Treatment group</td>
<td>Transportation cohort</td>
<td>—</td>
</tr>
<tr>
<td>11</td>
<td>Mixed effects linear regression with</td>
<td>Daily lying time, lying bouts, and activity index during transportation</td>
<td>3 d</td>
<td>Baseline body weight, and the interaction between treatment group and day</td>
<td>Calf, transportation cohort</td>
<td>Day</td>
</tr>
<tr>
<td></td>
<td>repeated measures</td>
<td></td>
<td></td>
<td></td>
<td>Calf, transportation cohort</td>
<td>Day</td>
</tr>
</tbody>
</table>
centrations of NEFA (Δ = +0.2 mmol/L, P < 0.01, 95% CI: 0.1 to 0.3) (Figure 1a) compared with those fed MR. With respect to BHB, calves in ORS-D (Δ = +48.7 µmol/L, P < 0.01, 95% CI: 14.6 to 82.7) and ORS-T (Δ = +39.9 µmol/L, P < 0.01, 95% CI: 6.8 to 72.9) (Figure 1b) had higher BHB concentrations than calves fed MR. There was no effect of treatment on anion gap (Figure 2d), pCO2 (Δ = +2.8 mmol/L; P > 0.10; Figure 2c), or blood pH (Figure 2f).

**Blood gas and blood acid-base.** Before reloading at the holding facility, there was evidence that calves fed ORS-D had a higher blood BE (Δ = +2.4 mmol/L; P = 0.07, 95% CI: −0.09 to 4.93; Figure 2a) and calves fed ORS-D had higher HCO3 (Δ = +2.7 mmol/L; P < 0.01, 95% CI: 0.6 to 4.9; Figure 2b) than calves fed MR. Additionally, at reloading at the holding facility, both ORS-D had higher concentrations of tCO2 (ORS-D: Δ = +3.1 mmol/L, P = 0.01, 95% CI: 0.4 to 5.8; Figure 2c).

At arrival to the calf-raising facility, calves fed ORS-T had a higher BE concentration (Δ = +3.02 mmol/L; P < 0.01, 95% CI: 0.53 to 5.50) than calves fed MR. At arrival to the calf-raising facility, calves fed ORS-D and ORS-T both had higher concentrations of tCO2 (ORS-D: Δ = +2.7 mmol/L, P = 0.045, 95% CI: 0.03 to 5.5; ORS-T: Δ = +3.6 mmol/L, P < 0.01, 95% CI: 1.0 to 6.3) and HCO3 (ORS-D: Δ = +2.2 mmol/L, P = 0.045, 95% CI: 0.03 to 4.5; ORS-T: Δ = +3.1 mmol/L; P < 0.01, 95% CI: 0.9 to 5.3) than calves fed MR. There was no effect of treatment on anion gap (Figure 2d), pCO2 (Figure 2e), or blood pH (Figure 2f).

**Blood electrolytes.** Before reloading at the holding facility, calves fed the ORS-D had higher concentrations of sodium (Δ = +3.4 mmol/L; P = 0.01, 95% CI: 0.5 to 6.3; Figure 3a) than calves fed MR. There was also evidence that those in ORS-D had a higher concentration of sodium at arrival to the calf raising facility (Δ = +2.8 mmol/L; P = 0.07, 95% CI: −0.1 to 5.8) compared with calves fed MR. There was no effect of treatment on chloride and potassium concentrations (P > 0.10; Figure 3b,c).

**Metabolic and respiratory acidosis.** At unloading at the holding facility (baseline), 4/43 MR, 3/43 ORS-D, and 0/42 ORS-T calves had metabolic acidosis. In addition, upon arrival to the calf-raising facility, 0/43 MR, 1/43 ORS-D, and 1/42 ORS-T calves had metabolic acidosis. From unloading at the holding facility until 48 h after arrival to the calf-raiser, metabolic acidosis was identified in 6/43 MR, 8/43 ORS-D, and 0/42 ORS-T calves. There was no effect of treatment on the proportion of sampling times with metabolic acidosis (P > 0.10; Figure 4a).

At unloading at the holding facility (baseline), 4/43 MR, 2/43 ORS-D, and 2/42 ORS-T calves had respiratory acidosis, whereas upon arrival to the calf-raising facility, 1/43 MR, 2/43 ORS-D, and 1/42 ORS-T calves had respiratory acidosis. From unloading at the holding facility until 48 h after arrival to the calf-raiser (5 sampling time points), respiratory acidosis was identified in 4/43, 3/43, and 2/42 calves in the MR, ORS-D, and ORS-T groups, respectively. There was no treatment effect on the proportion of sampling times with respiratory acidosis (P > 0.10; Figure 4b).

**Tissue damage.** There was no effect of treatment on LDH or CK concentrations at any of the sampling time points (P > 0.10; Figure 5a,b).

**Other parameters.** There was no effect of treatment on BUN, hematocrit, or hemoglobin concentrations at any of the sampling time points (P > 0.10; Figure 6a,b,c).

**Health Outcomes**

**Mortality.** Within the first 14 d of arrival to the calf-raising facility, the mortality rate was 9.4% (12/128). The mortality rate for calves fed MR, ORS-D, or ORS-T was 4.7% (2/43), ORS-D 11.6% (5/43), and 11.9% (5/42), respectively, and there were no differences in mortality rate by treatment (P > 0.10), as determined by a Fisher’s exact test. The mortality rate for cohorts 1, 2, 3, and 4 was 9.6% (3/35), 5.7% (2/35), 0% (0/24), and 20% (7/35), respectively. Calves died on average on d 5.1 ± 2.0 after arrival.

**Attitude.** In the 14 d after arrival to the calf-raising facility, 57.8% (n = 74/128) of calves were scored as slightly depressed or depressed at least once. In the MR, ORS-D, and ORS-T groups, 58.1% (n = 25/43), 53.5% (n = 23/43), and 61.9% (n = 26/42) scored as slightly depressed or depressed at least once, respectively. There were no statistical differences found between groups, as determined by a chi-squared test (P > 0.10).

**Weakness.** In the 14-d period after arrival to the calf-raising facility, 76.6% (n = 98/128) calves were...
scored as weak at least once. In the MR, ORS-D, and ORS-T groups, 79.1% (n = 34/43), 67.4% (n = 29/43), and 81.4% (n = 35/42) scored as weak at least once, respectively, within the first 14 d after arrival. Calves in the MR, ORS-D, and ORS-T were scored as weak for an average of 39% ± 30.8, 34% ± 31.0, and 39.5% ± 27.9 of days within the first 14 d after arrival, respectively, and there were no differences between groups, as determined by a chi-squared test (P > 0.10).

**Dehydration.** At unloading at the holding facility (baseline), more calves that were fed ORS-D were dehydrated than calves that were fed MR (Δ = +1.6% (25 vs. 11 calves); P = 0.03, 95% CI: 0.10 to 3.1; Figure 7). From immediately after arrival to 72 h after arrival to the calf-raiser, calves were scored once per day per calf. Dehydration was observed 73/210, 81/212, and 83/208 times during health scoring for calves fed MR, ORS-D, and ORS-T, respectively (calves may have been scored as dehydrated multiple days in a row). There were no treatment effects on dehydration at any sampling time point (P > 0.10) other than the baseline.

**Sunken flank.** At arrival to the calf-raising facility, 10, 7, and 7 calves in the MR, ORS-D, and ORS-T groups had a sunken flank, respectively. In the 72 h following arrival to the calf-raising facility, a sunken flank was identified 49/210, 35/212, and 50/208 times (a sunken flank could be identified multiple days in a row) in calves in the MR, ORS-D, and ORS-T groups.

![Figure 1](https://example.com/figure1.png)

**Figure 1.** A margins plot of the predicted probability of the interaction between treatment group (MR, ORS-D, and ORS-T) and sampling time on (A) NEFA concentrations, (B) BHB concentrations, and (C) glucose concentrations in surplus dairy calves (n = 65). Calves fed MR were used as the referent group. Pairwise comparisons were performed at each time point; ORS-D and ORS-T were compared with MR (referent). The signifiers “a” and “b” denote a difference (P < 0.05) in NEFA or BHB concentrations between surplus dairy calves fed ORS-D or ORS-T, respectively, compared with MR. Rest period is defined as RP in the graphs.
Figure 2. A margins plot of the predicted probability of the interaction between treatment group (MR, ORS-D, and ORS-T) and sampling time on (A) BE, (B) HCO₃⁻, (C) tCO₂, (D) anion gap, (E) pCO₂ concentrations, and (F) blood pH in surplus dairy calves (n = 65). Pairwise comparisons were performed at each time point; ORS-D and ORS-T were compared with MR (referent). The signifiers “a” and “b” denote a difference (P < 0.05) in BE, HCO₃⁻, tCO₂, anion gap, pCO₂ concentrations, or blood pH between surplus dairy calves fed ORS-D or ORS-T, respectively, compared with MR. Rest period is defined as RP in the graphs.
There was no effect of treatment on presence or absence of a sunken flank at any sampling time point \((P > 0.10;\) Figure 8).

**Fever.** In the 14-d period after arrival to the calf-raising facility, 99.2% (127/128) of calves had a fever at least once. There were no differences in the proportion of calves with a fever between calves fed ORS-T and ORS-D and MR \((P > 0.10)\) on any of the 14 d evaluated (Figure 9).

**Diarrhea.** In the 14-d period after arrival to the calf-raising facility, 97.6% (125/128) of calves had diarrhea at least once. In the first week after arrival \((d 0 – d 7)\), 120/128 calves had diarrhea at least once, while in the second week after arrival \((d 8 – d 14)\), 105/117 calves had diarrhea at least once. Calves had their first bout of diarrhea on \(d 3.1 \pm 2.6\) after arrival to the calf raiser. The median (range) number of days with diarrhea was 5 (0 to 12), 5 (0 to 10), and 6 (0 to 11) d for calves fed MR, ORS-D, and ORS-T, respectively. There were no differences in the number of days with diarrhea found between calves fed ORS-D vs. MR \(+0.03 d, P = 0.07, 95\% CI: −0.18 to 0.34;\) Figure 10); however, there was evidence that calves fed ORS-T had a higher number of days with diarrhea than calves fed MR \(+0.16 d, P = 0.09, 95\% CI: −0.02 to 0.34)\).

**Respiratory disease.** In the 14 d after arrival to the calf-raising facility, 76.6% (98/128) of calves presented outward signs of respiratory disease (i.e., met
the criteria outlined using the scoring guide) at least once. In the first week after arrival (d 0 to d 7), 77/128 calves had outward signs of respiratory disease at least once, while in the second week after arrival (d 8 to d 14), 86/117 calves had outward signs of respiratory disease at least once. On average, calves had their first day of respiratory disease on 7.8 ± 3.0 d after arrival to the calf raiser. The median (range) number of days with respiratory disease was 2 (0 to 11), 2 (0 to 9), and 3 (0 to 11) for calves fed MR, ORS-D, and ORS-T, respectively. There were no differences between calves fed ORS-D and MR (+0.10 d; \( P = 0.44 \), 95% CI: −0.36 to 0.16; Figure 11); however, there was evidence that calves fed ORS-T had a higher proportion of days with respiratory disease than calves fed MR (+0.22 d; \( P = 0.06 \), 95% CI: −0.01 to 0.46).

Antimicrobial and NSAID use. Throughout the 14 d after arrival to the calf-raiser, 18.0% (23/128) of calves were treated with an antimicrobial and 35.2% (45/128) of calves were treated with an NSAID, at least once. In the MR, ORS-D, and ORS-T groups, 30.2% (13/43), 20.9% (9/43), and 9.5% (4/42) calves were treated with an antimicrobial at least once, respectively, however no differences were observed between

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**Figure 4.** Box and whisker plot showing the effect of treatment fed (MR, ORS-D, ORS-T) on the proportion of sampling times with (A) metabolic acidosis or (B) respiratory acidosis in surplus dairy calves (n = 65). Calves fed ORS-D or ORS-T were compared with calves fed MR (referent).

**Figure 5.** A margins plot of the predicted probability of the interaction between treatment group (MR, ORS-D, and ORS-T) and sampling time on concentration of (A) LDH or (B) creatine kinase (CK) in surplus dairy calves (n = 65). Calves fed ORS-D or ORS-T were compared with calves fed MR (referent). Rest period is defined as RP in the graphs.
groups, as determined by a chi-squared test \((P > 0.10)\). Additionally, in the MR, ORS-D, and ORS-T groups, 46.5 \((20/43)\), 37.2 \((16/43)\), and 20.9 \((9/43)\) calves were treated with an NSAID at least once, respectively, and no differences were observed, as determined by a chi-squared test \((P > 0.10)\).

**Growth outcomes**

**Body weight at each sampling time.** There was no effect of treatment on body weight at any sampling point \((P > 0.10;\) Figure 12).

**Body weight loss during transportation.** The average body weight loss was 4.1\% ± 6.8, 2.0\% ± 8.2, and 3.6\% ± 6.7 for calves in the MR, ORS-D, and ORS-T groups, respectively. There was no effect of treatment on body weight loss during transportation \((P > 0.10;\) Figure 13a).

**Average daily gain.** The mean ADG over the first 14 d after arrival to the calf-raising facility was 0.75 ± 0.13 kg, 0.72 ± 0.13 kg, and 0.77 ± 0.12 kg for calves in the MR, ORS-D, and ORS-T groups, respectively. There was no effect of treatment on ADG during this period \((P > 0.10;\) Figure 13b). The mean ADG of calves over the first 56 d after arrival to the calf-raising facility was 0.84 ± 0.27 kg, 0.88 ± 0.30 kg, and 0.84 ± 0.17 kg for calves in the MR, ORS-D, and ORS-T groups, respectively. There was no effect of treatment on long-term ADG \((P > 0.10;\) Figure 13c).

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Figure 6. A margins plot of the predicted probability of the interaction between treatment group (MR, ORS-D, and ORS-T) and sampling time on concentration of (A) blood urea nitrogen, (B) hematocrit, or (C) hemoglobin in surplus dairy calves \((n = 65)\). Pairwise comparisons were made at each time point; calves fed ORS-D or ORS-T were compared with calves fed MR (referent). Rest period is defined as RP in the graphs.
Behavior outcomes

**During transportation.** There were no treatment effects on the amount of time spent lying down and number of lying bouts during transportation ($P > 0.10$). However, the activity index was lower during transportation in ORS-T compared with MR ($\Delta = -308.1; P = 0.03, 95\% CI: -576.9$ to $-39.4$; Figure 14).

**Lying behavior following transportation.** There were no treatment effects on the amount of time spent lying down and lying bouts in the days following transportation (d 0 to d 3; $P > 0.10$; Figure 15a; Figure 15b). In addition, calves fed ORS-D ($\Delta = -800.8; P = 0.04, 95\% CI: -1593.4$ to $-8.1$; Figure 15c) and ORS-T transportation in ORS-T compared with MR ($\Delta = -308.1; P = 0.03, 95\% CI: -576.9$ to $-39.4$; Figure 14).

**Figure 7.** A margins plot of the predicted probability of the interaction between treatment group (MR, ORS-D, and ORS-T) and sampling time on the proportion of surplus calves with dehydration ($n = 128$) from unloading at the holding facility to 72 h after arrival to the calf-raising facility. Calves fed MR were used as the referent group. Pairwise comparisons were performed at each time point; ORS-D and ORS-T were compared with MR (referent). The signifier “a” denotes a difference ($P < 0.05$) in the proportion of surplus calves with dehydration between surplus dairy calves fed ORS-D compared with MR. Rest period is defined as RP in the graphs.

**Figure 8.** A margins plot of the predicted probability of the interaction between treatment group (MR, ORS-D, and ORS-T) and sampling time on the proportion of surplus calves with a sunken flank ($n = 128$) from unloading at the holding facility to 72 h after arrival to the calf-raising facility. Pairwise comparisons were made at each time point; calves fed ORS-D or ORS-T were compared with calves fed MR (referent). Rest period is defined as RP in the graphs.

**Figure 9.** A margins plot of the predicted probability of the interaction between treatment group (MR, ORS-D, and ORS-T) and sampling time on the proportion of surplus calves with a fever ($n = 128$) from unloading at the holding facility to 14 d after arrival to the calf-raising facility. Pairwise comparisons were made at each time point; calves fed ORS-D or ORS-T were compared with calves fed MR (referent).

**Figure 10.** Box and whisker plot showing the effect of treatment fed (MR, ORS-D, ORS-T) on the proportion of days with diarrhea in surplus dairy calves ($n = 128$). Calves fed ORS-D or ORS-T were compared with calves fed MR (referent). The signifiers “b” denotes evidence for a difference ($0.05 < P < 0.1$) in the proportion of days with diarrhea between surplus dairy calves fed ORS-T compared with MR.
The aim of this study was to investigate the effect of feeding MR, ORS-D, or ORS-T to surplus dairy calves during a mid-transportation rest period on metabolic and clinical health indicators, growth, and activity behavior. Our study showed that calves fed MR had less fat mobilization and were more active on the day of transportation than calves fed either ORS-D or ORS-T. There was also evidence that calves fed MR had a lower number of days with diarrhea and respiratory disease than calves fed ORS-T. There was no treatment effect on the incidence of metabolic or respiratory acidosis, growth outcomes, or the time spent lying down during transportation and in the days following transportation.

In this study, we found that calves fed ORS-D had greater concentrations of NEFA and BHB immediately upon arrival to the calf-raising facility compared with calves fed MR. Furthermore, calves fed ORS-T had higher BHB concentrations at arrival to the calf-raising facility. NEFA, BHB, and glucose are commonly used as indicators of fat and energy mobilization in transported cattle (reviewed by Roadknight et al., 2021). In a similar study, calves fed ORS before loading had higher NEFA and BHB concentrations compared with calves fed MR after 6 h of transportation, which agrees with the findings in this study (Marcato et al., 2020a). Also in agreement with this study’s findings, calves that were fed 1.5 L of an ORS and then feed deprived for 19 h had higher NEFA and BHB concentrations on the day of feed deprivation than calves that had been fed 2.5 L of MR before 19 h of feed deprivation (Pisoni et al., 2022). Transporting and fasting calves can cause increases in indicators of fat mobilization in the blood (Marcato et al., 2020a, Goetz et al., 2023a,b; Pisoni et al., 2022). No differences were found with respect to glucose concentrations, which aligns with Marcato et al. (2020a) who found that dairy calves fed MR or an ORS before 18 h of transportation had similar glucose concentrations immediately after arrival. Hence, feeding calves MR rather than an ORS before transportation (Marcato et al., 2020a) and during a mid-transportation rest period could be better at minimizing fat mobilization in the calves, but may not influence glucose concentrations. Additionally, future studies should investigate formulating an ORS that provides a similar glucose concentration as MR.

Very few animals in this study developed respiratory or metabolic acidosis after transportation and there was no difference between treatment groups. Metabolic acidosis commonly occurs in calves that are feed deprived for long periods of time, such as up to 60 h, as found in a study by Parker et al. (2003), and those that experience diarrhea (Smith, 2009). Previous research has also found that transported calves experienced metabolic acidosis after long durations of transportation (Parker...
et al., 2003; Goetz et al., 2023b). We found very few animals developed respiratory or metabolic acidosis after transportation and there was no difference between the proportion of sampling times with metabolic or respiratory acidosis. As calves in this study were sourced from a single dairy farm, it is possible that the calves included in this study had better fitness for transportation than those included in other studies (Goetz et al. 2023a) and therefore less likely to suffer alteration in the respiratory system or gastrointestinal (GI) tract that could result in acid-base abnormalities.

Based on the results of this study, feeding ORS with a high strong ion difference (SID) during a mid-transportation period may mitigate the severity of metabolic acidosis in calves. However, it is unclear why the timing of increase in blood acid-base parameters was not the same between the 2 ORS treatments. Additionally, we found that calves fed ORS-D had a higher concentration of both sodium and chloride in blood than calves fed MR before reloading at the holding facility. Differences in sodium and chloride concentrations between treatment groups may be explained by the higher sodium and chloride intake from the ORS-D compared with MR. Sodium, in particular, plays an important role in maintaining acid-base balance and correcting metabolic acidosis (Mohri et al., 2008; Smith, 2009). However, it was found that there were no treatment effects on the incidence metabolic or respiratory acidosis, therefore further conclusions cannot be drawn. It is also important to note that both ORS treatments increased blood acid-base parameters which may have mitigated the occurrence of metabolic or respiratory acidosis. Further

Figure 13. Box and whisker plot showing the effect of treatment fed (MR, ORS-D, ORS-T) on the percentage of body weight lost during transportation (A), average daily gain over 14 d (B), and average daily gain over 8 weeks (C) in surplus dairy calves (n = 128). Calves fed ORS-D or ORS-T were compared with calves fed MR (referent).
research should be done on feeding calves MR or an ORS during mid-transportation rest periods and their effect on maintaining acid-base balance.

There was evidence that calves fed ORS-T have a greater proportion of days with diarrhea than calves fed MR. Comparatively, Marcato et al. (2020b) found that more calves experienced diarrhea within 3 wk of arrival to a veal farm when fed MR before 6 h and 18 h of transportation than calves fed an ORS. As results from this study conflict with those of Marcato et al. (2020b), more research is needed to investigate this finding. This study also found evidence that ORS-T fed calves had a higher proportion of days with respiratory disease than calves fed MR. There is no evidence to suggest that feeding calves an ORS has a positive or negative impact on bovine respiratory disease. However, previous research has found a correlation between feeding MR and immune function. For example, Bach et al. (2013) found that feeding calves higher volumes of MR decreases relapse of respiratory disease. In another study, better immune function was found in Jersey calves fed a higher volume of MR (Ballou, 2012), which may be due to higher amounts of energy. Although not fully representative of feed deprivation in regard to transportation, these studies may provide insight into the effect of feeding calves MR to maintain immune system function during metabolic stress. Further research is needed to assess the impact of feeding MR or an ORS on diarrhea and respiratory disease after transportation to calf-raisers.

In this study, calves fed MR had a higher activity index than calves fed ORS-T and ORS-D during transportation and on the day of transportation. Activity index is a measure of total overall activeness, including rate of acceleration of the leg, and total steps (Silper et al., 2015; Gladden et al., 2020). Calves with compromised health status, and calves that are pre-clinically sick have been associated with lower activity indices than healthy calves (Cantor and Costa, 2022; Cantor et al., 2022), suggesting that low activity indices in calves may not reflect normal biological function. As calves fed MR had a higher activity index, but no treatment differences in lying time, our results suggest that feeding calves MR during a mid-transportation rest period results in more activity that did not compromise lying time. Furthermore, a high activity index has been associated with play bouts in calves (Gladden et al., 2020). Future research should investigate if calves with a high activity index during transport are playing or investigating their environment within the trailer more often than lower index calves.

**Limitations**

During the sample size calculations, clustering within cohorts was not accounted for, which may have resulted in a larger sample size being needed to complete the study. Therefore, this may have influenced the power to detect statistical significance between treatment groups for some of the outcomes within this study. Upon arrival to the holding facility, more calves in the ORS-D group were dehydrated than in the groups fed MR or ORS-T. Dehydration has been shown to increase morbidity and mortality after arrival to calf-raisers (Renaud et al., 2018) and may have affected disease and blood gas and acid-base outcomes in these calves. As such, when conducting analyses, dehydration at baseline was included as a covariate in several models assessing blood gas and clinical health outcomes. Furthermore, calves did not have access to free-choice water during the rest period as the facility was not equipped to provide this. Although calves in this study were not fasted or limit-fed, water intake during an 8-h rest period would likely be limited based on previous studies showing that, when offered after birth, calves consumed an average of 0.75 kg of water per day (Wickramasinghe et al., 2019). In this study, calves were fed 1 h before being loaded for transportation. Although 1 h may not be sufficient to complete the digestion process, several studies have transported calves 1 h after feeding MR with no major apparent effects on calf health (Goetz et al., 2023a,b; Marcato et al., 2020a,b). However, future research may

![Figure 14](image_url)

**Figure 14.** Box and whisker plot showing the effect of treatment fed (MR, ORS-D, or ORS-T) on the activity index of surplus dairy calves (n = 87) during transportation. Calves fed MR were used as the referent group. An “a” denotes a difference (P < 0.05) in the activity index during transportation of calves fed ORS-T compared with calves fed MR. Calves fed ORS-D or ORS-T were compared with MR (referent). The signifier “a” denotes a difference (P < 0.05) in the activity index of surplus dairy calves fed ORS-D or ORS-T compared with MR.
be warranted to investigate this theory and determine the optimal time of feeding before transportation. An additional limitation is that due to the high mortality rate within the 4th cohort, calves were group-treated with an antimicrobial which may have affected the proportion of diarrhea that calves experienced within this group. However, disease (Svensson et al., 2003; Pardon et al., 2013) and high mortality (Winder et al., 2016) at veal and calf-raiser is not uncommon. The levels of disease and mortality represented in this study are similar to those of previous studies (Scott et al., 2019, Goetz et al., 2023a). Therefore, despite the higher mortality of our 4th cohort, the general population of surplus calves was still accurately represented. Sex was not recorded in this study and calves were sourced from a single dairy farm, which may decrease external validity as calf management often varies between farms and sexes. Further, as one of the researchers was not blinded to treatment and it is unlikely that all of the chalk markings on the calves became invisible after 24 h, imperfect blinding may have occurred in this study. Lastly, there were many outcomes assessed within this paper and, therefore, the chance of finding statistical significance due to chance could have occurred leading to a type I error.

Figure 15. A margins plot of the predicted probability of the interaction between treatment fed (MR, ORS-D, or ORS-T) and day relative to transportation (d 0 to d 3) on daily lying time (A), the number of daily lying bouts (B), and daily activity index (C) that surplus dairy calves (n = 87). Pairwise comparisons were performed at each time point; ORS-D and ORS-T were compared with MR (referent). The signifier “a” and “b” denote a difference (P < 0.05) in daily lying time, the number of daily lying bouts, and the daily activity index of surplus dairy calves fed ORS-D or ORS-T, respectively, compared with MR. Rest period is defined as RP in the graphs.
CONCLUSION

Calves fed ORS-D or ORS-T had greater fat mobilization than calves fed MR after the rest period. Additionally, there was evidence that calves fed ORS-T had a higher proportion of days with diarrhea and respiratory disease in the 14 d after arrival to the calf-raising facility than calves fed MR. During transportation, calves fed ORS-T were less active than calves fed MR which was also found on the day of transportation, where calves fed ORS-T had a lower activity index than calves fed MR. Calves fed ORS-D also had a lower activity index. Although feeding an ORS seemed to improve acid-base balance in this study, our results suggest that feeding MR rather than an ORS during a mid-transportation rest period could minimize negative lying behavior during transportation, negative health outcomes, and fat mobilization, but does not affect growth outcomes after arrival to calf-raisers. Therefore, based on these results, it appears that feeding calves adequate amounts of MR may be a better option than ORS during a mid-transportation rest period. Further research should be done to investigate other strategies to implement before transportation and during mid-transportation rest periods to improve surplus dairy calf success after arrival to calf-raising facilities.

REFERENCES


Bajus et al.: NUTRITIONAL STRATEGIES TO IMPROVE PERFORMANCE OF TRANSPORTED CALVES


