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Effects of pre- and post-transport feeding protocols on the metabolism and physiological status of veal calves

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ABSTRACT

This experimental study aimed to investigate the effects of pre- and post-transport feeding protocols with varying volumes of milk replacer on metabolic and physiological variables of unweaned calves during and after long-distance transport by ferry and road. We monitored 2 commercial shipments of male dairy and beef × dairy calves (n = 116). Pre-transport, calves (n = 58/treatment) were fed 2 L the morning of transport or 6 L divided over 2 feeds the evening before and the morning of transport. Post-transport, calves were fed a conventional restricted protocol or 25% more volume per day (3.2 or 4.0 L/d, increasing to 5.8 or 7.2 L/d over 3 wk). We obtained blood samples and BW at 5 time points: (1) pre-transport at an assembly center (AC) in Ireland, (2) after 24 h of ferry and road transport at a lairage in France, (3) after a 13-h rest stop and 16.5 h of road transport at 2 veal farms in the Netherlands, (4) on d 12 and (5) on d 21 post-transport (total transport duration 53.5 h). Blood samples were analyzed for markers of energy balance (glucose, nonesterified fatty acids [NEFA], BHB), hydration (urea, electrolytes, albumin, hematological variables), physiological stress (cortisol), and muscle fatigue (lactate, creatine kinase). Linear mixed models were used to assess the effects of feeding protocols on physiological variables. Calves fed more milk replacer twice pre-transport had a better energy balance with significantly higher blood glucose at the lairage (estimated marginal mean [95% CI]: 3.68 [3.47–3.88] vs. 3.32 [3.13–3.51] mmol/L) and significantly less fat mobilization than calves fed less at the AC (NEFA: 0.10 [0.09–0.11] vs. 0.20 [0.18–0.23] mmol/L, BHB: 0.06 [0.05–0.07] vs. 0.11 [0.10–0.12] mmol/L) and lairage (NEFA: 0.60 [0.54–0.66] vs. 0.69 [0.62–0.76] mmol/L, BHB: 0.26 [0.22–0.29] vs. 0.35 [0.30–0.39] mmol/L), but not upon arrival at the veal farm (NEFA: 0.83 [0.75–0.92] vs. 0.65 [0.59–0.72] mmol/L). Calves fed more were also significantly less dehydrated throughout transport, with lower chloride levels overall (97.2 [96.3–98.0] vs. 95.6 [94.8–96.5] mmol/L) and lower urea (2.60 [2.21–2.99] vs. 3.68 [3.13–4.22] mmol/L) and potassium (6.67 [6.50–6.83] vs. 6.87 [6.71–7.04] mmol/L) at the lairage. By d 12 (next sampling after arrival), all blood variables except sodium and cortisol had normalized for all treatments. Post-transport, ADG was significantly increased for calves fed 25% more (0.47 [0.43–0.51] vs. 0.40 [0.36–0.43] kg/d). We conclude that feeding calves two 3-L milk feeds compared with one 2-L milk feed before long-distance transport reduced the negative impacts of prolonged fasting, especially regarding energy balance and hydration status. However, these positive effects were evident at the mid-transport lairage but were not sustained throughout the multiday journey. Calves fed more also experienced energy depletion, hypoglycemia, and dehydration, suggesting that even 6 L of milk replacer pre-transport does not fulfill the metabolic needs of calves during long-distance transport. These findings highlight significant welfare concerns with current transport practices, underscore the need to reassess feeding protocols during rest stops, and point to the importance of future research focused on shortening fasting periods and optimizing feeding protocols.
Key words: transport, feed provision, biomarkers, calves

INTRODUCTION

Long fasting periods are one of the main welfare concerns for calves undergoing long-distance transportation. Under non-transport conditions, unweaned calves must be fed twice a day in the European Union (Council of the European Union, 2008) and should receive approximately 20% of their BW in milk or milk replacer (**MR**) per day to meet their metabolic needs (Khan et al., 2011).

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However, during long-distance transport, defined in the European Union as all journeys exceeding 8 h (Council of the European Union, 2004), feed is often withheld for prolonged periods (>12 h), which has been shown to result in lowered glucose levels and a negative energy balance, causing calves to experience hunger and stress (Todd et al., 2000, Fisher et al., 2014; Marcato et al., 2020a). Extended fasting also contributes to weight loss and dehydration (Knowles et al., 1997), which are associated with an increased risk of disease and mortality on veal farms (Mormede et al., 1982; Renaud et al., 2018a). The additional energy needed for thermoregulation and maintaining balance in a moving vehicle aggravates energy loss during transport (Todd et al., 2000; Bernardini et al., 2012). In commercial practice, calves often do not receive appropriate feeds at auction markets, assembly centers (**AC**), or farms of origin due to mismatched feeding and pick-up schedules, extending fasting periods past actual transport times (Roadknight et al., 2021a).

Ireland exports more than 120,000 mostly bull calves of less than 6 wk of age to mainland Europe annually to be reared on beef or veal farms (Department of Agriculture, Food and the Marine, 2013–2022). In the European Union, unweaned calves must be fed an appropriate quantity of quality milk (replacer) after a maximum period of 19 h when transported (Council of the European Union, 2004); however, these requirements are waived for transport from remote European Union member states like Ireland, where export necessarily includes an 18 to 20 h ferry journey, during which calves cannot be fed (Council of the European Union, 2004, Article 30[7]). Including transport to and from the ferry, this leads to fasting periods routinely exceeding 24 h (European Commission, 2023). Transport can span several days including rest periods. Calves are transported directly from the farm of origin or via auction markets to an AC, where they are fed before continuing transport by truck and roll-on, roll-off ferry; they are unloaded and fed again at a control post ("lairage") upon arrival in France and rested for about 13 h before further road transport to rearing facilities.

Pre-transport feeding practices could be an effective way to mitigate the detrimental impacts of long fasting periods on calf welfare. Generally, data from several non-transport-related studies suggest that higher planes of nutrition improve immune function in calves, potentially reducing the risk of disease and mortality during and after transport (Nonnecke et al., 2003; Ballou, 2012). In the specific context of transport, Marcato et al. (2020a) found that calves fed 1.5 L of MR instead of electrolytes before a 6-h transport had higher blood glucose levels at arrival and resorted less to fat mobilization as a source of energy, showing that pre-transport diet is an important factor in avoiding hunger and improving calf welfare. Milk-fed calves also exhibited lower cortisol levels post-transport and increased numbers of monocytes (involved in the innate immune defense against pathogens) compared with calves fed electrolytes (Marcato et al., 2021). Correspondingly, Bajus et al. (2024) found that calves fed MR during an 8-h rest stop in between 12 and 6 h of transport showed less fat mobilization and tendencies for reduced signs of respiratory disease and diarrhea after transportation than calves fed electrolyte solution. To the best of the authors' knowledge, no previous studies to date have examined the effects of volume of milk fed pre-transport on calf metabolic and physiological status.

After transportation, the return of metabolic and physiological variables to normal levels is dependent on the nature of the biomarker and duration of the event. Metabolic variables such as blood glucose, BHB, and nonesterified fatty acids (**NEFA**) often normalize within 24 h after 6 to 18 h of transport (Marcato et al., 2020a). Other blood parameters are more variable. Cortisol has been reported to return to pre-transport values within 5 to 48 h (Bernardini et al., 2012; Masmeijer et al., 2019) and it can take markers of hydration such as blood urea and albumin from 48 h to several weeks to normalize, depending on transport duration and conditions (Bernardini et al., 2012; Marcato et al., 2020a). In most European veal production systems, calves are usually either fed electrolytes or MR (depending on time of arrival) after being unloaded and placed in individual boxes. During the early fattening period, calves are then fed a restrictive diet to prevent possible digestive upset (Mormede et al., 1982; Marcato et al., 2020b), and the amount of MR provided is less than what a calf would normally drink from its mother (3–4 L per day vs. >10 L per day, McPherson et al., 2024). Feeding larger volumes of milk at the veal farm the first weeks after transport might contribute to a quick recovery. Milk allowance is also linked to weight gain, and several studies have shown that growth rates in young calves increase linearly as MR intake increases (Diaz et al., 2001; Bartlett et al., 2006). Optimizing post-transport feeding strategies, particularly the provision of adequate milk volumes, could therefore enhance calf welfare as well as growth performance.

Overall, relatively little is known about the impact of feeding practices on the metabolic and physiological status of calves during and after transport, especially in the context of long-distance transport spanning several days. Specifically, no research to date has addressed pre-transport feeding protocols on combined ferry and road journeys such as calf export from Ireland to mainland Europe. Additionally, much of the previous research regarding calf transport has been conducted in purely experimental or observational settings. To make results more representative of actual transport conditions, the present experimental study was conducted on commer-

cial calf shipments. The objective of this study was to investigate the effects of pre- and post-transport feeding protocols with different planes of nutrition on metabolic and physiological variables of unweaned calves during and after long-distance transport via ferry and road. We hypothesized that providing calves with two 3-L milk feeds before transport, rather than one 2-L milk feed, would enable them to better maintain functional physiology during transport (i.e., improved energy and water balance, fewer signs of fatigue and stress, and higher BW). Additionally, we hypothesized that feeding calves a higher daily volume of MR in the weeks following transport would facilitate and accelerate the recovery of their pre-transport metabolic state.

MATERIALS AND METHODS

Ethical Approval

This experiment was approved by the Teagasc Animal Ethics Committee (Fermoy, Ireland; approval number TAEC2021-325) and the Health Products Regulatory Authority (Dublin, Ireland; approval number AE19132/P153).

Experimental Design and Animals

This experimental study was conducted in April and May 2022 on 2 consecutive commercial shipments (**S1**, **S2**) of 58 calves each between Ireland and the Netherlands with a 4-wk interval in between. The study comprised a transport phase (from AC to arrival on veal farm) and a post-transport phase (the first 3 wk after arrival on the veal farm). We applied a pre-transport feeding protocol treatment with 2 levels, resulting in 2 treatment groups: pre-transport feeding protocol alternative (**preALT**, fed 3 L of MR the evening before transport and again 3 L the morning of transport) and control (**preCTRL**, fed 2 L the morning of transport, not fed the evening before) during the transport phase. A post-transport feeding protocol treatment with 2 additional levels was applied after arrival: post-transport feeding protocol alternative (**postALT**, fed 25% more volume of MR than the control group at each feed, i.e., 2.0 L twice daily, gradually increasing to 3.6 L over 3 wk) and control (**postCTRL**, fed 1.6 L twice daily, gradually increasing to 2.9 L over 3 wk). Pre- and post-transport feeding protocol treatments resulted in 4 treatment groups during the post-transport phase, arranged as a 2 × 2 factorial design. The control treatments (pre- and post-transport) were chosen to reflect current standard practices in Irish AC, European control posts or lairages, and Dutch veal farms (based on personal experience of the researchers as well as personal communications with industry experts and commercial collaborators); the alternative treatments were designed to improve upon the standard feeding practices by offering calves more volume of MR to be able to cope better with the challenges of transport, especially the long fasting periods, and aid in recovery post-transport. The exact feeding protocols for all treatments are shown in Table 1.

Sample size was calculated using the software G*Power 3.1.9.7 (Faul et al., 2009) based on an expected difference of 0.8 ± 1 SD mmol/L (3.4 vs. 4.2 mmol/L) in plasma glucose between treatments during transport, which was observed in a small-scale pilot study conducted during a similar transport. We required 26 calves per treatment to achieve 80% power with a significance level of $\alpha = 0.05$ and chose to include 30 calves per treatment to allow for dropouts.

Study calves were randomly chosen by AC staff from the available stock at the AC in Wicklow, Ireland, the evening before transport. Age, breed, and farm of origin of all calves were obtained by scanning their electronic identification tags (eID). We roughly estimated the distance each calf had been transported to the AC that day by calculating the shortest route between farm of origin and AC using Google Maps (Google LLC, Mountain View, CA). These distances are approximate, as the exact transport routes were not known. Calves were then blocked by age and breed to minimize confounding effects and allocated to treatment groups by one researcher using a random number generator in Microsoft Excel (Microsoft Corp., Redmond, WA). Ear tags with 4 different colors, each corresponding to a treatment group, were placed on the existing calf ear tags to be able to distinguish between groups. However, all study personnel collecting and analyzing blood samples were blinded to the treatment groups. To be eligible for the study, calves had to be at least 14 d old and weigh ≥40 kg on the day of departure and consume at least 75% of their allotted volume of MR. Before departure from the AC, all calves underwent a routine veterinary inspection (conducted by the Irish Department of Agriculture, Food and the Marine) to assess their fitness for transport, which is standard procedure for all calves that are transported out of Ireland. Calves were not transported if they exhibited pronounced respiratory distress, diarrhea, painful navel inflammation, lameness, or decreased responsiveness.

Feeding and Management During and After Transport

The transport schedule including treatments and sampling moments is presented in Figure 1. The night before transport, 10.5 h before departure from the AC (S1: 0000–0100 h, S2: 0300–0400 h), preALT calves were fed 3 L of MR at 40°C (125 g of milk powder/L; 21% CP, 17% crude fat, 8.4% crude ash, 0.2% crude fiber). The morning of transport, 5.5 h before departure

Table 1. Overview of pre- and post-transport feeding protocols per day during and following long-distance transportation of unweaned calves from Ireland to the Netherlands

Feeding protocol	Day relative to arrival (d 1)	Location	L/d (MR or electrolytes)	g/L (MR or electrolytes)	g/d (muesli[1])	ME/d[2] (Mcal/d)	ME per kg of BW[2] (kcal/kg per d)
preALT	−3[3]	AC	3 (MR[4])	125	—	1.81[3]	—
	−2	AC	3 (MR[4])	125	—	1.81	32
	−1	Lairage	3 (MR[5])	90	—	1.33	26
	1	Veal farm (arrival)	2 (electrolytes[5])	20	—	—	—
preCTRL	−3[3]	AC	—	—	—	-[3]	—
	−2	AC	2 (MR[4])	125	—	1.21	22
	−1	Lairage	3 (MR[5])	90	—	1.33	26
	1	Veal farm (arrival)	2 (electrolytes[6])	20	—	—	—
postALT	2	Veal farm	2 × 2.0 (MR[7])	130	—	2.55	48
	3–7	Veal farm	2 × 2.0 (MR[7])	130	—	2.55	—
	8–10	Veal farm	2 × 2.3 (MR[7])	130	200	3.59	—
	11–14	Veal farm	2 × 2.8 (MR[7])	130	200	4.23	—
	15–17	Veal farm	2 × 3.3 (MR[7])	130	200	4.87	—
	18–20	Veal farm	2 × 3.6 (MR[7])	130	300	5.58	—
	21	Veal farm	2 × 3.6 (MR[7])	130	300	5.58	90
postCTRL	2	Veal farm	2 × 1.6 (MR[7])	130	—	2.04	38
	3–7	Veal farm	2 × 1.6 (MR[7])	130	—	2.04	—
	8–10	Veal farm	2 × 1.8 (MR[7])	130	200	2.95	—
	11–14	Veal farm	2 × 2.2 (MR[7])	130	200	3.47	—
	15–17	Veal farm	2 × 2.6 (MR[7])	130	200	3.98	—
	18–20	Veal farm	2 × 2.9 (MR[7])	130	300	4.69	—
	21	Veal farm	2 × 2.9 (MR[7])	130	300	4.69	77

[1]Muesli mixed with 15% chopped wheat straw; 13% CP, 4% crude fat, 5.1% crude ash, 7.7% crude fiber, ME = 3,280 kcal/kg.

[2]Metabolizable energy (Mcal or kcal); BW of calves was only available on d −2, −1, 1, and 21; BW on d 1 was used for the calculation of ME per kg BW on d 2 because they were assumed to be sufficiently similar.

[3]Day before departure: Calves arrived at AC in evening (preALT calves were fed, preCTRL calves were not fed that evening); we do not know if and what or how much calves were fed before leaving their farm of origin that same day, so this ME/day value is only an assumption.

[4]Milk replacer; 21% CP, 17% crude fat, 8.4% crude ash, 0.2% crude fiber; ME = 4,820 kcal/kg.

[5]Milk replacer; 22% CP, 19% crude fat, 8.7% crude ash, 0.1% crude fiber; ME = 4,940 kcal/kg.

[6]Sodium bicarbonate–based electrolyte mix; ME not available.

[7]Milk replacer; 21% CP, 18.5% crude fat, 8.2% crude ash, 0.05% crude fiber; ME = 4,910 kcal/kg.

from the AC, study calves were fed 3 L (preALT) or 2 L (preCTRL) of MR with the same composition and temperature as the previous feeding. Calves were fed from feed buckets with individual compartments and failure to drink the expected amount was recorded. Approximately 90 min after feeding, calves were weighed with a calibrated digital weigh-scale and blood sampled (see details in "Blood Collection and Analyses" section). Calves rested for another 2 h before being loaded onto a livestock truck with deep straw bedding. The 3 pens on the bottom deck were filled with 4 or 5 calves from each treatment group (recognizable by ear tag color) that were randomly selected from pens by AC staff (n = 19/20 per pen, space allowance in accordance with minimum legal requirements: 0.3 m^2/calf, see Council of the European Union, 2004), whereas the remaining pens were filled with non-study calves. Calves remained on the truck for the next 22.5 h (S2: 24 h), including a 17 h (S2: 18 h) ferry journey from Dublin, Ireland (S2: Rosslare, Ireland), to Cherbourg, France. Immediately after offloading at a control post (lairage) in Tollevast, France, calves were again weighed and blood sampled. Afterward, all calves were fed 3 L of MR at 40°C (90 g of milk powder/L; 22% CP, 19% crude fat, 8.7% crude ash, 0.1% crude fiber) according to standard lairage procedures and then allowed to rest on straw bedding in 2 group pens (n = 29 per pen). After a total of 13 h spent at the lairage, study personnel reloaded the calves, again randomly selecting 4 or 5 calves from each treatment group (identified by ear tag color) to fill the 3 pens on the bottom deck. Calves were not necessarily assigned to the same pen as during the previous transport. The 2 shipments traveled onward (S1 for 13 h, S2 for 16.5 h) to 2 separate veal farms in the province of Gelderland, the Netherlands. Immediately after arrival, calves were weighed and blood sampled, and then fed a sodium bicarbonate–based electrolyte mix dissolved in 2 L of water at approximately 40°C. The total transport duration was 48.5 h for S1 and 53.5 h for S2. Calves were fasted for 31.5 h (S2: 32 h) from AC to the lairage and 25 h (S2: 28 h) from the lairage to arrival on the veal farm.

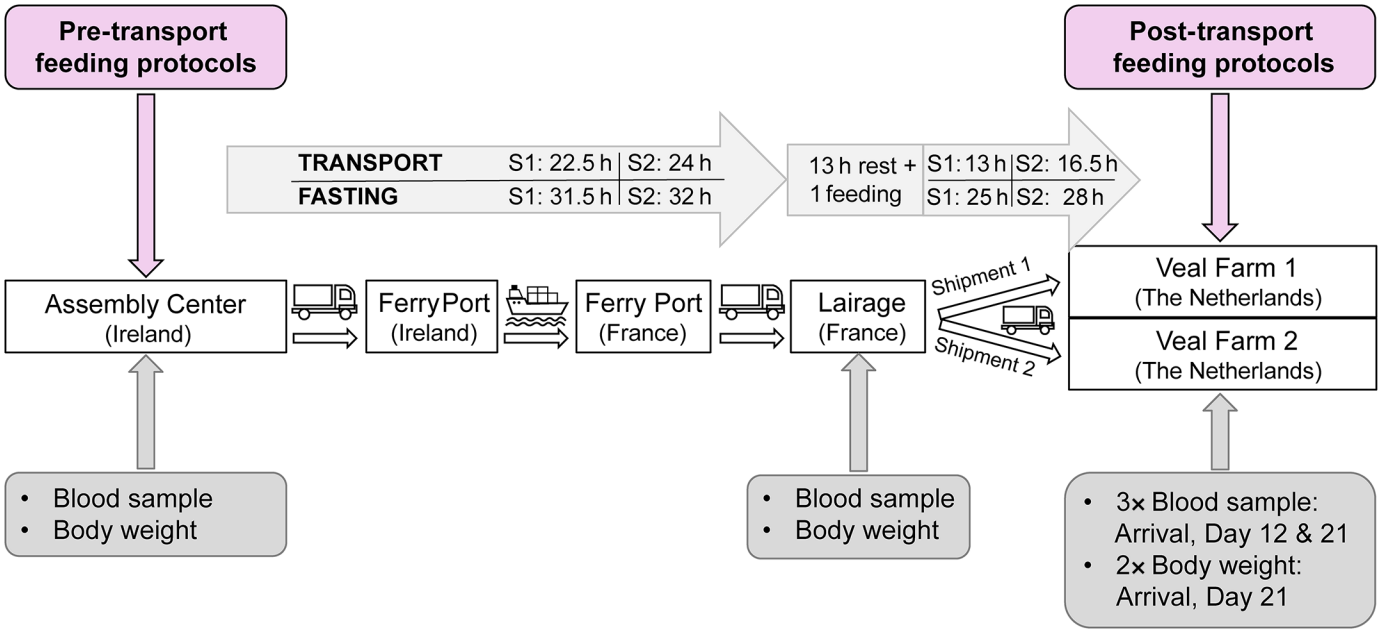

Figure 1. Transport schedule for 2 shipments (S1, S2) of young calves between Ireland and the Netherlands, including pre- and post-transport feeding protocol treatments, sampling moments, and transport and fasting durations.

At the veal farm, calves were individually housed in "calf-boxes" (100 cm width × 180 cm length, space allowance in accordance with minimum legal requirements: 1.8 m^2/calf; see Council of the European Union, 2008) with wooden slats and fenced partitions that allowed social contact with other calves. Each pen, comprising 4 to 5 such boxes, included only one treatment (pre-transport × post-transport feeding protocol) to facilitate the post-transport feeding treatment, but treatments were randomly distributed across the barn. Starting the day after arrival (d 2), calves were fed MR twice daily (45°C; 130 g of milk powder/L; 21% CP, 18.5% crude fat, 8.2% crude ash, 0.05% crude fiber) according to their treatment groups: postCTRL calves were fed according to the standard veal farm protocol (1.6 L per feeding, gradually increasing to 2.9 L over 3 wk) and postALT calves received 25% more volume of MR than the standard protocol (2.0 L per feeding, increasing to 3.6 L). Starting on d 8, all calves were provided muesli (d 8–17: 200 g/d, d 18–21: 300 g/d; 13% CP, 4% crude fat, 5.1% crude ash, 7.7% crude fiber) mixed with 15% chopped wheat straw. Blood samples were taken on d 12 and 21 after arrival, and calves were weighed on d 21. After conclusion of the study, the fenced partitions were removed, and calves were housed in groups (Figure 1).

Blood Collection and Analyses

Blood was collected via jugular venipuncture at 5 time points (AC, lairage, arrival, and d 12 and 21 on the veal farm) into 4 BD Vacutainer tubes per calf: 6 mL of EDTA, 6 mL of lithium heparin, 6 mL of glycolytic inhibitor, and 8.5-mL serum-separating tubes using a 20-gauge, 1-inch (2.54 cm) BD Vacutainer needle (Becton, Dickinson and Co., Plymouth, UK). Red blood cell (**RBC**) count and hematocrit and hemoglobin concentrations were quantified in EDTA whole-blood samples using automated hematology analyzers within a few hours after collection. Whole-blood samples taken at the AC were analyzed at Teagasc Grange (Dunsany, Ireland) using the ADVIA 2120 (Siemens Healthcare Diagnostics, Eschborn, Germany), whereas all subsequent whole-blood samples were analyzed at Rimondia (Elspeet, the Netherlands) using the XT-1800i (Sysmex Europe, Norderstedt, Germany). Interassay coefficients of variation (%) for RBC, hematocrit, and hemoglobin were 1.26, 1.47, and 1.31 for AC samples and 0.94, 1.19, and 0.81, respectively. All other samples were centrifuged at 1,300 × *g* at room temperature for 10 min (serum-separating tubes) or 15 min (heparin, glycolytic inhibitor), and the separated serum or plasma was frozen at −20°C until analysis. We used the AU480 Chemistry Analyzer (Beckman Coulter, CA) to determine plasma glucose, NEFA, BHB, urea, albumin, lactate, and creatine kinase and serum sodium with colorimetric methods, and serum potassium and chloride with ion selective electrodes; this analyzer ensures a maximum interassay coefficient of variation of 10%. Plasma cortisol concentrations were estimated using a commercial ELISA kit (Enzo Life Sciences, Brussels, Belgium).

Statistical Analyses

All statistical analyses were performed in R 4.3.1 (R Core Team, 2024). Blood variables and BW were analyzed using linear mixed models in the R package 'lme4,' with individual calf as the experimental unit. First, we specified a model to analyze data collected in the transport phase. It comprised the fixed effects pre-transport feeding protocol (preCTRL, preALT) and time point (AC, lairage, arrival on veal farm) as well as their interaction, shipment (S1, S2), and breed (Holstein-Friesian [**HF**], Hereford × Holstein-Friesian crossbreds [**HEX**], beef × Holstein-Friesian crossbreds [**BeefX**]). Additionally, the model included calf age at departure in days and distance traveled from farm of origin to the AC in kilometers as continuous covariates. Calf was entered as a random effect to correct for repeated measurements. Pen on the truck from AC to the lairage and pen on the truck from lairage to veal farm were included as random effects to account for lack of independence between calves transported in the same pen.

A second, similar model was used to analyze blood data spanning the post-transport phase. Pre-transport feeding protocol (preALT, postALT), post-transport feeding protocol (postALT, postCTRL), time point (d 12 and 21 after arrival), shipment (S1, S2), and breed (HF, HEX, BeefX) were specified as fixed effects, and 2- and 3-way interactions between pre- and post-transport feeding protocol and time point were included. As covariates, we entered age at departure in days and difference between a calf's BW at arrival on the veal farm and the mean BW of a calf's pre-transport feeding protocol treatment group at arrival to correct for individual differences in BW at the beginning of the post-transport phase while minimizing collinearity with the pre-transport treatment. Random effects for this model were calf and pen at the veal farm. Average daily gain between arrival and d 21 on the veal farm was calculated for each calf and analyzed using the same model, with the exception that time point was not included as a fixed effect.

For all models, residuals were examined for normality and homogeneity of variance with the R package 'performance,' and variables were log-transformed to meet model assumptions when necessary (transport and post-transport phase: BHB, urea, cortisol, creatine kinase, lactate; only transport phase: glucose, NEFA). Fixed effects and interactions were tested for significance ($P < 0.05$) using approximate F-tests (Kenward and Roger, 1997) with the Anova function in the R package 'car.' Interactions that were not significant were stepwise excluded from the model and subsequent pairwise comparisons of estimated marginal means were calculated with the 'emmeans' R package. Because all comparisons were between only 2 treatment means (preALT and preCTRL or postALT and postCTRL) within specific time points, contrasts were calculated without adjusting for multiple comparisons.

RESULTS

Descriptive Statistics

Four calves were declared unfit for transport by a veterinarian at the AC and were excluded from the study before transport due to bloat (1 × preCTRL × postALT, S1), increased respiratory rate (1 × preALT × postCTRL, S2), and decreased responsiveness (2 × preCTRL × postCTRL, S1 and S2). As a result, the study included 116 male calves in total (59 preALT, 57 preCTRL; 59 postALT, 57 postCTRL). Calves had a mean age of 29 ± 7 d and included 47 HF, 49 HEX, and 20 various other BeefX. They originated from dairy farms across Ireland, and the distance a calf traveled from its farm of origin to the AC on the day before international transport commenced was estimated to range from 7 to 347 km, with a median distance of 262 km. Estimated median (range) distances traveled from dairy farms of origin to the AC were 267 (7–347), 250 (7–340), 258 (7–338), and 266 (11–340) km for calves in the preALT × postALT, preALT × postCTRL, preCTRL × postALT, and preCTRL × postCTRL experimental treatment groups, respectively. There were no refusals during the pre-transport or post-transport feeding. Two calves from the preCTRL group did not consume ~0.25 L of the 2-L feed on the morning of transport but were not identified as outliers or influential observations for any variables and so remained in the dataset.

Effects of Pre-Transport Feeding Protocols During Transport

For the transport phase (AC to arrival), only the effects of the pre-transport feeding protocol treatment are described, as the post-transport feeding protocol treatment was only applied after arrival. During transport, significant interactions between pre-transport feeding protocol and time point were found for indicators of energy balance, namely glucose ($P = 0.015$), NEFA ($P < 0.0001$), and BHB ($P < 0.0001$) (Figure 2A–C). The preALT treatment (estimated marginal mean [95% CI]: 3.68 [3.47, 3.88] mmol/L) had higher glucose concentrations than the preCTRL treatment (3.32 [3.13, 3.51] mmol/L, $P = 0.003$) at the lairage. Conversely, both NEFA and BHB levels were higher for preCTRL calves than preALT calves at the AC (NEFA: 0.20 [0.18, 0.23] vs. 0.10 [0.09, 0.11] mmol/L, $P < 0.0001$; BHB: 0.11 [0.10, 0.12] vs. 0.06 [0.05, 0.07] mmol/L, $P < 0.0001$)

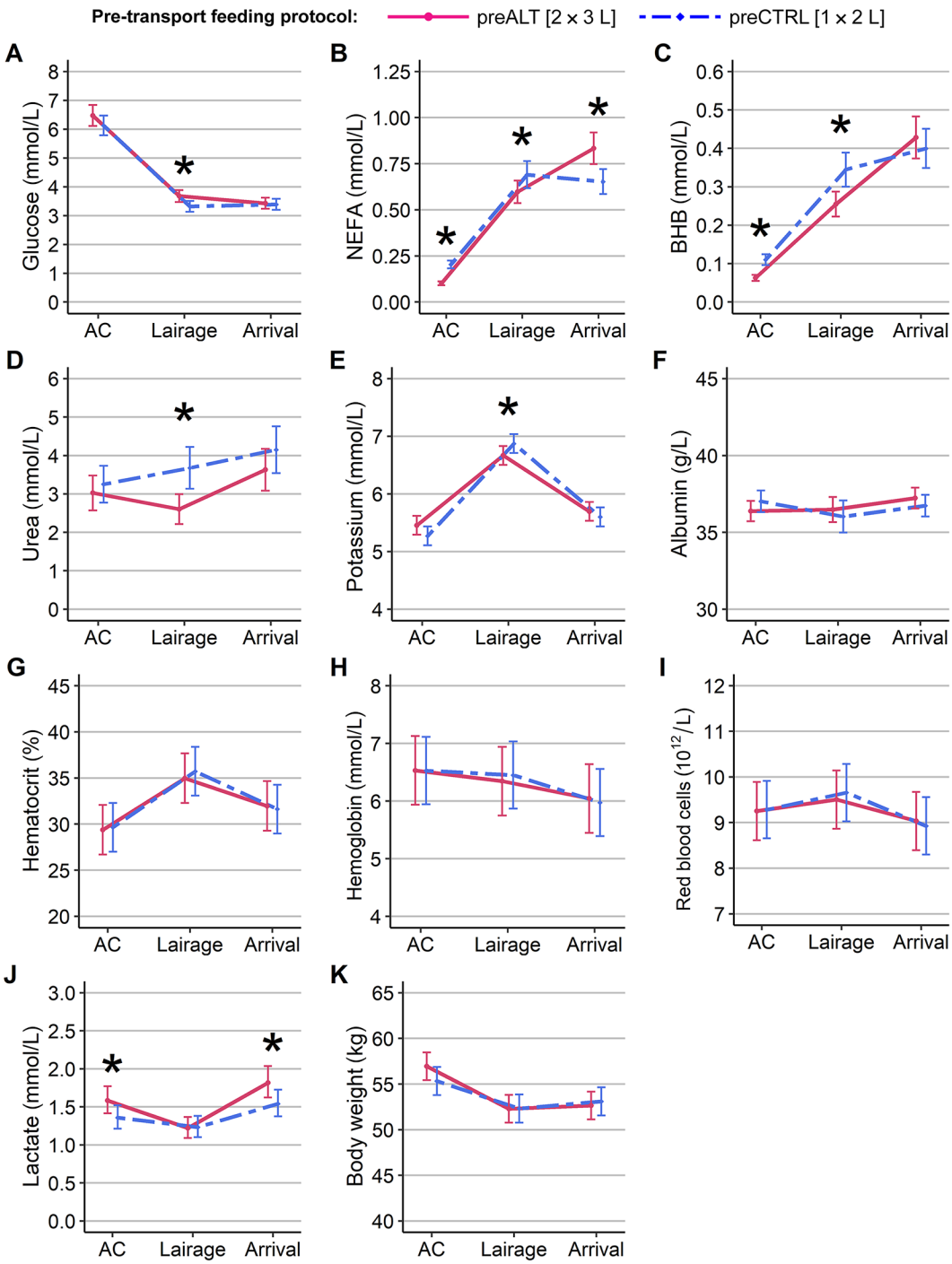

Figure 2. Interactions between pre-transport feeding protocol (preALT: fed 2 × 3 L of MR before transport; preCTRL: fed 1 × 2 L of MR before transport) and time point (AC = assembly center in Ireland; lairage = 13-h rest stop in France; arrival = arrival at veal farm in the Netherlands) for glucose (A), NEFA (B), BHB (C), urea (D), potassium (E), albumin (F), hematocrit (G), hemoglobin (H), red blood cells (I), lactate (J), and BW (K) in veal calves (n = 116). Estimated marginal means ± 95% CI derived from a linear mixed model are depicted. Asterisks indicate significant differences ($P \leq 0.05$) between treatments within a sampling moment.

and at the lairage (NEFA: 0.69 [0.62, 0.76] vs. 0.60 [0.54, 0.66] mmol/L, $P = 0.049$; BHB: 0.35 [0.30, 0.39] vs. 0.26 [0.22, 0.29] mmol/L, $P = 0.001$). Upon arrival on the veal farm, NEFA levels were higher in preALT than in preCTRL calves (0.83 [0.75, 0.92] vs. 0.65 [0.59, 0.72] mmol/L, $P = 0.002$).

Six of 8 parameters indicating hydration status showed significant interactions between pre-transport feeding protocol and time point (Figure 2D–I). The treatment and time interaction was significant for urea ($P < 0.001$), and preCTRL calves had higher urea levels at the lairage (3.68 [3.13, 4.22] vs. 2.60 [2.21, 2.99] mmol/L, $P = 0.002$) compared with preALT calves, while a smaller potential difference was observed upon arrival (4.15 [3.54, 4.76] vs. 3.63 [3.08, 4.17] mmol/L, $P = 0.082$). Potassium showed a significant treatment and time interaction ($P = 0.002$). Both treatment groups reached peak potassium concentrations after the ferry journey and subsequently decreased to nearly pre-transport levels on arrival. At the lairage, preCTRL calves had significantly higher potassium levels compared with preALT calves (6.87 [6.71, 7.04] vs. 6.67 [6.50, 6.83] mmol/L, $P = 0.036$), whereas at the AC, potential evidence of a reversed relationship was observed (preCTRL: 5.27 [5.11, 5.43] mmol/L, preALT: 5.45 [5.29, 5.62] mmol/L, $P = 0.061$). Neither sodium ($P = 0.548$) nor corrected chloride ($P = 0.247$) showed significant interactions between pre-transport treatment and time point. While pre-transport feeding protocol had no effect on overall sodium levels (preALT: 142.0 [8.61, 9.92] mmol/L, preCTRL: 142.0 [8.64, 9.93] mmol/L, $P = 0.540$), corrected chloride was higher in preCTRL calves (97.2 [96.3, 98.0] mmol/L) than in preALT calves (95.6 [94.8, 96.5] mmol/L, $P < 0.0001$) during transport. Albumin ($P = 0.043$) and the hematological variables (hematocrit: $P = 0.039$; hemoglobin: $P = 0.048$; RBC: $P = 0.049$) all showed overall significant interactions between pre-transport feeding protocol and time point, but no differences were detected between them at specific sampling moments or overall (albumin: preALT: 36.7 [36.1, 37.3] g/L, preCTRL: 36.6 [36.0, 37.2] g/L, $P = 0.795$; hematocrit: preALT: 32.1% [29.3%, 34.9%], preCTRL: 32.3% [29.6%, 35.1%], $P = 0.833$; hemoglobin: preALT: 6.31 [5.70, 6.92] mmol/L, preCTRL: 6.32 [5.72, 6.91] mmol/L, $P = 0.962$; RBC: preALT: 9.26 [8.61, 9.92] 10^{12}/L, preCTRL: 9.29 [8.64, 9.93] 10^{12}/L, $P = 0.916$).

Regarding parameters related to stress and muscle fatigue, only lactate showed a significant interaction between treatment and time point ($P = 0.047$; Figure 2J). Whereas lactate levels in preCTRL calves displayed relatively minor fluctuations over time, preALT calves showed greater lactate concentrations at the AC (1.59 [1.43, 1.76] vs. 1.36 [1.21, 1.50] mmol/L, $P = 0.040$) and again upon arrival (1.83 [1.64, 2.02] vs. 1.55 [1.39, 1.71] mmol/L, $P = 0.017$). For cortisol ($P = 0.680$) and creatine kinase ($P = 0.515$), no significant interactions between pre-transport feeding protocol and time point were observed. Pre-transport feeding protocol also had no overall effect on cortisol (preALT: 8.9 [6.3, 12.3] ng/mL, preCTRL: 10.5 [7.6, 14.4] ng/mL, $P = 0.311$) or creatine kinase (preALT: 163 [131, 200] U/L, preCTRL: 184 [148, 225] U/L, $P = 0.258$).

Body weight did not differ between treatment groups overall (preALT: 54.0 [51.2, 56.8] kg, preCTRL: 53.5 [50.9, 56.3] kg, $P = 0.718$) or at any sampling point during transport, even though an overall interaction between treatment and time point was detected ($P < 0.0001$; Figure 2K). Over the course of transport, calves lost 7.5% (preALT) or 4% (preCTRL) of their initial BW, mostly between AC and lairage.

Effects of Pre- and Post-Transport Feeding Protocols After Transport

The effects of pre- and post-transport feeding protocol during the post-transport phase (d 12–21 post-arrival) are summarized in Table 2. No significant interactions were observed between treatments or between treatment and time point (all $P > 0.05$); therefore, treatment means are reported separately for each feeding protocol treatment for the entire post-transport period.

Pre-transport feeding protocol affected only one blood variable during the post-transport period, with preCTRL calves showing higher BHB levels than preALT calves (0.11 [0.10, 0.12] vs. 0.09 [0.08, 0.10] mmol/L, $P = 0.005$). Post-transport feeding protocol affected 2 blood variables: postCTRL had higher urea (2.90 [2.72, 3.08] vs. 2.65 [2.49, 2.82] mmol/L, $P = 0.034$) and corrected chloride levels (96.9 [96.9, 97.3] vs. 96.2 [95.8, 96.5] mmol/L, $P = 0.003$) than postALT calves. Additionally, post-transport feeding protocol influenced ADG from arrival to d 21, with postALT (0.47 [0.43, 0.51] kg/d) calves displaying greater growth than postCTRL calves (0.40 [0.36, 0.43] kg/d, $P = 0.008$; Table 2).

DISCUSSION

This study was designed to investigate the effects of different pre- and post-transport feeding protocols on the metabolic and physiological responses of calves during and after long-distance transport from Ireland to the Netherlands. While some experimental studies have examined feeding practices surrounding transport, this is the first study to investigate the effects of 2 limited feeding protocols on calf metabolic and physiological variables under commercial transport conditions on a journey spanning several days and combining ferry and road transport. Our results demonstrate that offering calves an extra milk feed at the AC as well as a larger volume of MR before transport (2 × 3 L instead of 1 × 2 L) improves their metabolic and physiological condition during the journey, particularly in terms of calf energy balance and hydration status. However, it is important to note that this positive effect was not

Table 2. Effects of pre-transport feeding protocol (preALT: 2 × 3 L vs. preCTRL: 1 × 2 L of MR before transport) and post-transport feeding protocol (postALT: 2.0 L twice daily, gradually increasing to 3.6 L over 3 wk vs. postCTRL: 1.6 L twice daily, gradually increasing to 2.9 L over 3 wk) on blood variables and ADG of young calves (n = 116) after long-distance transport from Ireland to the Netherlands (post-transport phase = from d 12 to 21 after arrival for blood variables and from arrival to d 21 for ADG)[1]

	Pre-transport feeding protocol			Post-transport feeding protocol		
Variable	preALT	preCTRL	*P*-value	postALT	postCTRL	*P*-value
Glucose (mmol/L)	4.87 (4.73, 5.02)	4.79 (4.64, 4.93)	0.355	4.90 (4.75, 5.04)	4.77 (4.62, 4.91)	0.178
NEFA (mmol/L)	0.12 (0.11, 0.13)	0.1 (0.10, 0.13)	0.630	0.12 (0.11, 0.13)	0.11 (0.10, 0.13)	0.438
BHB (mmol/L)	0.09 (0.08, 0.10)	0.11 (0.10, 0.12)	0.005	0.10 (0.09, 0.11)	0.11 (0.09, 0.12)	0.178
Urea (mmol/L)	2.74 (2.57, 2.91)	2.81 (2.64, 2.99)	0.532	2.65 (2.49, 2.82)	2.90 (2.72, 3.08)	0.034
Sodium (mmol/L)	142.2 (141.6, 142.8)	142.5 (141.9, 143.1)	0.457	142.1 (141.5, 142.7)	142.6 (141.9, 143.2)	0.304
Corrected chloride (mmol/L)	96.5 (96.2, 96.8)	96.6 (96.3, 97.0)	0.460	96.2 (95.8, 96.5)	96.9 (96.6, 97.3)	0.003
Potassium (mmol/L)	5.09 (5.01, 5.17)	5.04 (4.96, 5.12)	0.344	5.03 (4.95, 5.11)	5.10 (5.02, 5.18)	0.216
Albumin (g/L)	34.5 (33.9, 35.1)	34.4 (33.8, 35.0)	0.825	34.5 (33.9, 35.1)	34.3 (33.7, 34.9)	0.547
Hematocrit (%)	31.1 (29.3, 32.8)	31.3 (29.6, 33.1)	0.827	31.3 (29.6, 33.0)	31.1 (29.3, 32.8)	0.849
Hemoglobin (mmol/L)	5.93 (5.55, 6.30)	5.96 (5.59, 6.34)	0.876	5.96 (5.59, 6.34)	5.93 (5.55, 6.30)	0.889
RBC (10^{12}/L)	9.16 (8.70, 9.62)	9.20 (8.74, 9.67)	0.889	9.23 (8.76, 9.69)	9.13 (8.67, 9.60)	0.757
Lactate (mmol/L)	1.33 (1.24, 1.43)	1.25 (1.16, 1, 33)	0.159	1.27 (1.18, 1.36)	1.31 (1.22, 1, 40)	0.462
Cortisol (ng/mL)	7.4 (5.8, 9.0)	8.3 (6.3, 10.2)	0.461	7.2 (5.6, 8.8)	8.5 (6.5, 10.4)	0.287
Creatine kinase (U/L)	136 (118, 153)	138 (120, 155)	0.871	134 (117, 151)	140 (122, 158)	0.614
ADG (kg/d)	0.43 (0.39, 0.47)	0.44 (0.40, 0.48)	0.755	0.47 (0.43, 0.51)	0.40 (0.36, 0.43)	0.008

[1]Estimated marginal means (95% CI) derived from a linear mixed model and *P*-values from pairwise comparisons between treatment means.

sustained until arrival at the destination farm, likely because even calves that received an additional feed before transport did not consume enough milk to meet their metabolic needs during the prolonged journey. To better meet calves' metabolic needs, it is essential to increase pre-transport feeding and either implement a second feeding during lairage in France or reduce lairage duration, although the latter would necessitate revisions to existing European Union policy (Council of the European Union, 2004). We also found that feeding calves 25% more volume of MR than is common practice in the Dutch veal industry (4.0 L/d increasing to 7.2 L/d over 3 wk instead of 3.2 L/d increasing to 5.8 L/d) following transport had only limited effects on calf physiology, but did lead to higher BW gain.

Effects of Pre-Transport Feeding Protocols During Transport

Energy Balance. In this study, pre-transport feeding protocol had effects on 8 of 14 blood variables. The most notable differences between the pre-transport feeding treatments were evident in blood parameters reflecting calf energy balance. Plasma concentrations of NEFA and BHB typically increase as a result of fat mobilization or in stressful situations when not enough easily available energy is provided by feed (Marcato et al., 2020a; Pisoni et al., 2022; Goetz et al., 2023). A negative energy balance is thus characterized by a low plasma glucose concentration accompanied by elevated levels of NEFA and BHB, which is commonly observed in calves as a consequence of long fasting periods during transport (Marcato et al., 2018; Roadknight et al., 2021a). In the present study, those calves fed a higher volume of MR before transport had lower BHB and NEFA levels before (at AC) and after the ferry transport (at the lairage), where they also had higher glucose levels. This indicates that they experienced a less severe negative energy balance during the first section of the journey than calves that received only one 2-L feed. This aligns with the findings of Marcato et al. (2020a), who reported higher glucose levels and lower NEFA concentrations upon arrival in calves that were fed

milk compared with those fed electrolytes before a 6-h transport. Similarly, Bajus et al. (2024) found that calves fed MR rather than an oral rehydration solution during a mid-transportation rest period had lower NEFA and BHB concentrations upon arrival at the calf-raising facility. However, upon arrival at the destination farm, those positive effects were no longer apparent, and conversely, NEFA levels were significantly higher in preALT calves. In addition, despite the aforementioned differences, both treatments fell below the reference limit for glucose in healthy young calves (4.3 mmol/L, suggested reference ranges for healthy male calves, 11 to 30 d old, Dillane et al., 2018), indicating they experienced hypoglycemia and exceeded the upper reference limit at the lairage and upon arrival for NEFA (0.29 mmol/L, suggested reference range for healthy male Holstein calves, 5 wk old, Yu et al., 2019) and BHB (0.15 mmol/L, suggested reference ranges for healthy calves, 27 d old, Knowles et al., 2000). These findings suggest that feeding larger volumes of MR before transport improves calves' ability to counteract the detrimental effects of the prolonged fasting period to some extent but does not provide enough energy to maintain a positive energy balance over a 31-h fasting period (and 22-h transport), and much less over further transport (even when calves are fed again before transport commences). This is supported by Todd et al. (2000) who reported that calves who were fed a single milk feed (50 mL/kg BW) and then fasted for 30 h (which included 12 h of transportation by truck) entered a negative energy balance after approximately 18 h. These results make it evident that to avoid hypoglycemia and energy depletion, fasting periods during long-distance transport must be shortened, and larger feeds should be offered before and during transport. This highlights the need for innovations that enable feeding calves during transport or logistical solutions to reduce fasting duration. This could be achieved by either providing a second feed at the lairage or shortening the rest period to limit the overall time without access to nutrition. Fasting duration can also be minimized by feeding calves only 2 to 3 h before departure from the AC, which still gives calves time for digestion before transport (Marahrens and Schrader, 2020).

Hydration Status. Dehydration is a common and well-documented consequence of long-distance transport for young calves, caused primarily by prolonged fasting and insufficient access to water before and during transport (Knowles et al., 1997; Pempek et al., 2017; Roadknight et al., 2021b; England et al., 2023). In this study, calves fed a higher volume of MR before transport showed fewer signs of dehydration throughout the journey, but most differences between treatments were recorded at the lairage, indicating that the alternative feeding protocol was able to compensate for fluid deficiency mostly during the first section of transport from AC to lairage. Here preALT calves had lower urea, potassium, and corrected chloride, but there was no effect of pre-transport feeding protocol on sodium, albumin, and RBC-related parameters. When compared with reference ranges of un-transported calves (Knowles et al., 2000; Mohri et al., 2007; Dillane et al., 2018), there was an increase in signs of dehydration over the course of transport regardless of pre-transport feeding protocol. At the AC, lairage, and arrival, 1 (sodium), 2 (sodium, potassium), and 4 variables (urea, sodium, potassium, and albumin) exceeded the upper reference limit for both treatments, respectively. Maintaining water homeostasis involves complex physiological mechanisms, and (de-) hydration status can be linked to many clinical (skin elasticity, sunken eyes, and capillary refill, Kells et al., 2020) and physiological markers associated with, among others, blood volume, electrolyte and mineral balance, and protein homeostasis (reviewed by Marcato et al., 2018). In our study, hematological variables were relatively unaffected, whereas urea and electrolyte levels increased over transport, which could indicate that the latter blood components respond more quickly to fluid loss. Contrary to our findings, Bernardini et al. (2012) found that, after a 19-h transport, urea and electrolyte levels remained relatively consistent, whereas hematocrit and RBC increased. It has also been suggested that clinical indicators, such as the skin tent test, are more sensitive to dehydration than blood parameters, and could be measured simultaneously to more accurately capture calf hydration status (Goetz et al., 2023).

Elevated levels of urea following transport have been found in several studies (Mormede et al., 1982; Knowles et al., 1997; Roadknight et al., 2021b) and are caused by an overall loss of plasma volume resulting from fluid loss or increased protein catabolism as a response to energy depletion through fasting and other transport-related stressors (Knowles et al., 1999a). In our study, urea levels were significantly higher in preCTRL calves at the lairage and also above the upper reference limit (Knowles et al., 2000), and although by arrival both treatment groups exceeded the reference range, urea in preCTRL was still notably higher than in preALT calves, suggesting that dehydration and energy loss was significantly reduced by the alternative feeding treatment. This aligns with the findings of Knowles et al. (1997), who reported higher urea levels in unfed calves than in calves fed before a 24-h transport, and Marcato et al. (2020a), who found higher urea level in calves fed electrolytes before an 18-h transport compared with milk-fed calves.

Elevated electrolyte levels can be a sign of a general decline in plasma volume through fluid loss, caused by prolonged feed and water withdrawal (Schaefer et al., 1997; Minka and Ayo, 2010). A large increase in potassium levels was observed at the lairage and was more marked in preCTRL calves, whereas chloride levels were higher

for preCTRL calves throughout transport. However, both treatment groups exceeded upper reference limits of sodium and potassium (139.3 and 5.37 mmol/L, Dillane et al., 2018) at all 3 time points (except potassium for preCTRL at the AC), indicating that calf hydration status was already somewhat compromised before international transport. Similarly, Marcato et al. (2020a) reported that 70% of calves at a German AC were already dehydrated before transport and also exhibited signs of an impaired energy balance. In this study, calves fed an additional time the evening they arrived at the AC showed fewer signs of fat mobilization compared with calves fed only once at the AC. These findings highlight that calves often begin long-distance journeys in an already physiologically vulnerable state and underscore the need to revise feeding protocols at AC to adequately prepare calves for further transport. Moreover, our findings show that even though feeding preALT calves a higher volume of MR before transport counteracted some fluid loss during the ferry journey, they still experienced dehydration. Bajus et al. (2024) found lower levels of sodium at arrival in calves fed MR compared with calves fed a rehydration solution during a mid-journey rest stop, but no effect on potassium or chloride concentrations. Marcato et al. (2020a) found no effect of feeding either 1.5 L of electrolytes or MR before 6 or 18 h of transport on sodium concentrations. These findings support the idea that greater volumes of MR before transport and during rest stops may prevent excessive fluid loss and aid in maintaining a normal electrolyte balance.

Albumin concentrations have been shown to both increase (Mormede et al., 1982; Knowles et al., 1997; Earley et al., 2013) and decrease (Marcato et al., 2020a) as a result of transport, whereby higher albumin levels result from dehydration due to water and food deprivation, and lower albumin levels may signal the onset of inflammation (Petersen et al., 2004). In our study, albumin levels rose narrowly above the upper reference limit for both treatments at arrival (36.5 g/L, suggested reference range for healthy Holstein calves, 28 d old, Mohri et al., 2007) but showed no effect of pre-transport feeding protocol.

We also found no effect of pre-transport feeding protocol on the hematological variables hematocrit, hemoglobin, and RBC. This mirrors the findings of Marcato et al. (2020a) and Bajus et al. (2024), who reported no differences in hematocrit, hemoglobin, and RBC during 6- to 18-h journeys between calves fed MR or electrolytes before transport or during a mid-transportation rest period, respectively. These consistent outcomes suggest that hematological variables during transport are largely unaffected by pre-transport feeding. Contrary to previous studies that reported elevated levels of hematocrit and RBC count following transport due to dehydration and stress responses (Kent and Ewbank, 1986; Bernardini et al., 2012), these variables were overall only marginally affected by transport in the present study and always remained within reference limits (Knowles et al., 2000; Dillane et al., 2018).

Stress. Cortisol is primarily responsible for activating the physiological stress response and the marker used most often to indicate stress in transport situations (Goetz et al., 2022). Here, cortisol levels were not influenced by pre-transport feeding protocol, and both treatments were above the reference limit at all sampling points (3.0 ng/mL, Knowles et al., 2000), reaching their peak at the lairage. This outcome was somewhat expected, as transport includes many social and environmental stressors in addition to prolonged fasting (which may be mitigated by feeding strategies), such as handling, commingling with unknown calves in new environments, novel sounds and movements, and in the context of this study, also additional handling and restraining for the purpose of sampling, which may all invoke stress responses (Roadknight et al., 2021a). Situations involving multiple stressors, such as those that occur during transport, can lead to a ceiling effect in cortisol responses. Consequently, plasma cortisol concentrations may offer limited sensitivity for assessing animal stress (Molony and Kent, 1997; Coetzee et al., 2008). There was also large variability in cortisol levels among calves, which may originate from individual previous experiences, general fearfulness, or differences in handling during unloading and sampling. A less invasive and thus potentially more conclusive way to measure stress in future may be cortisol from saliva, hair, or feces (Wenker et al., 2022; Lei et al., 2023; Vogt et al., 2023).

Muscle Fatigue. Elevated plasma creatine kinase and lactate concentrations are a result of muscle cell damage caused by exertion or trauma and are commonly reported to increase during transport when calves stand for extended periods and may bruise during loading and unloading or when losing balance (Todd et al., 2000; Fisher et al., 2014; Jongman and Butler, 2014). In line with these previous studies, creatine kinase levels increased over transport, but were not affected by feeding protocol. Both treatments were above upper reference limits at all time points during transport (130 U/L, Knowles et al., 2000). Lactate, in contrast, showed inconsistent changes and was higher for preALT calves before transport, where they also exceeded the reference limit, and at arrival, where both treatments rose above reference limits (1.37 mmol/L, suggested reference range for healthy calves, 1–12 mo old, Šoltésová et al., 2015). It is not clear how pre-transport feeding protocol may have contributed to these differences between treatments. Lactate is also known to increase in the presence of respiratory diseases and diarrhea (Coghe et al., 2000), both of which are frequently found in veal calves and can be exacerbated by transport stress (Pardon et al., 2013; Pempek et al.,

2017). It is possible that diseases were more prevalent in the preALT treatment; however, this was not reflected in other blood parameters.

Body Weight. Lower BW at arrival to calf-raising facilities is associated with higher incidences of respiratory disease and early mortality (Winder et al., 2016; Renaud et al., 2018a), and an improved pre-transport feeding protocol may be able to reduce weight loss over transport and improve body condition upon arrival. In this study, BW was not affected by feeding protocol: Calves in both treatments lost weight between the AC (preALT: 57.0 kg, preCTRL: 55.3 kg) and lairage (preALT: 52.3 kg, preCTRL: 52.3 kg) and largely maintained their BW during the following transport until arrival (preALT: 52.7 kg, preCTRL: 53.1 kg). This weight loss most likely occurred mainly due to loss of fluid through excretion and accounted for 7.5% (preALT) and 4% (preCTRL) of initial BW, which falls within the range of previously reported values for long-distance transport (Earley and Murray, 2010; Bernardini et al., 2012; Marcato et al., 2020a). These results suggest that the alternative feeding protocol was not sufficient to prevent or reduce BW loss over prolonged transport. Although calves were weighed shortly *after* feeding at the AC and again *before* feeding at the lairage, which may have influenced the magnitude of recorded weight loss and may explain why calves fed more lost more weight between AC and lairage, this weighing schedule effectively reflects the real-time physiological impact of transport, capturing BW (almost) immediately before loading and upon unloading.

Overall, effects of the pre-transport feeding protocol were most pronounced at the lairage rest stop, where the impact of fasting was less notable in calves fed more for all indicators of energy balance and a third of the hydration parameters. Upon arrival at the veal farm, only 2 parameters differed between treatments, with calves fed more now showing higher NEFA and lactate levels. At this point, both treatments were outside reference ranges for 10 out of 14 blood variables. This outcome is not surprising, as between the application of the feeding treatments at the AC and arrival at the destination farm, the calves had spent over 24 h without feed twice and over 48 h in transport and at the lairage, where both groups received the same amount of feed. Two 3-L feeds before transport only positively affected calf physiology during the first half of the journey. At the lairage, calves were fed after sampling, approximately 2 to 3 h after arrival, and then rested for an additional 10 to 11 h before the second transport, which brought their total fasting time for this section of the journey to 25 to 28 h. Goetz and Renaud (2024) found that calves that had an 8-h rest stop midway through a 16-h transport lost less weight and exhibited higher post-transport ADG compared with calves transported for 16 h without rest. However, calves were fed twice during the rest period, whereas in the current study, calves were only fed once after arrival at the lairage, extending the fasting period during the second section of the journey and potentially negating the positive effects of the resting period. Calves that were rested also show more signs of exhaustion and fatigue after transport, suggesting that the additional time spent in transit and corresponding loading and unloading events during an extended rest stop add to the strain of transport (Goetz and Renaud, 2024). Considering these findings as well as the demonstrated benefits of the enhanced pre-transport feeding protocol, future studies should investigate the possibility of either increasing the volume of MR fed at the lairage, offering a second feed before departure, or shortening the rest period to reduce overall time spent in transit. When considering a shorter rest period or multiple feedings, calves should ideally rest for 2 to 3 h after feeding before reloading to prevent digestive upset (Marahrens and Schrader, 2020); however, if necessary, shorter resting periods (≥1 h) may be acceptable (Goetz and Renaud, 2024).

Our study indicates that although an improved pre-transport feeding protocol has some benefits, its positive impact is limited by prolonged transport durations and the accumulated adverse effects of transport-associated stress. Regardless of pre-transport feeding protocol, calves exhibited signs of dehydration, stress, hypoglycemia, and substantial energy depletion. Evidence suggests that subjective experiences such as thirst, hunger, and fatigue may occur before measurable physiological or clinical indicators emerge, potentially affecting overall welfare (Roadknight et al., 2021a). Based on these observations, providing only 2 L of milk before extended transport does not appear to meet acceptable welfare standards (FAWAC, 2020; NASEM, 2021; EFSA AHAW Panel et al., 2022), and even offering two 3-L feeds does not represent best practice.

Effects of Pre- and Post-Transport Feeding Protocols After Transport

Overall, both pre- and post-transport feeding protocol had few effects on calf physiology during the post-transport period, but the alternative post-transport feeding protocol positively affected calf growth. Although postALT calves received 25% more MR per feed, their glucose levels were comparable to the control group, and all treatments displayed a positive energy balance and hydration status on d 12 and 21 after transport. Calves in the postALT treatment had lower BHB levels during the post-transport period compared with those in the preCTRL group. Additionally, the alternative post-transport feeding protocol had some minor positive effects on hydration, as indicated by lower urea and chloride levels in

postALT calves. However, all blood variables associated with energy metabolism and hydration had improved to levels comparable or even better than pre-transport values by d 12 and had also returned to within normal range (except for sodium, which was slightly above the reference limit but close to pre-transport values). Other notable exceptions from this pattern were cortisol levels, which continued to exceed the reference limit throughout the post-transport period for all treatments, and creatine kinase and lactate levels, which increased from d 12 to 21. As during transport, these measurements may have partly been due to the acute stress of handling and sampling, but a restrictive diet can also be a source of chronic stress for calves (Mattiello et al., 2002). We can infer from these findings that all calves had physiologically recovered from transport by d 12 after arrival regardless of treatment group, and that the conventional post-transport feeding protocol was adequate for calves to maintain a functional metabolism. Nevertheless, the increased alternative pre- and post-transport feeding protocols offered some benefits in the post-transport phase in terms of calf energy balance and hydration but did not seem to mitigate stress.

Additionally, weight gain in the first 3 wk after arrival was notably improved by feeding 25% more volume of MR per feed. Calves fed the alternative post-transport feeding protocol gained more weight from arrival to d 21 (0.47 vs. 0.40 kg/d for control calves). This finding supports prior observations that increasing the amount of MR fed to unweaned calves results in higher growth rates (Diaz et al., 2001; Rosenberger et al., 2017). The ADG of both groups was below recommended targeted growth recommendations for young calves (NASEM, 2021), but within the range of reported growth for veal calves in the first weeks after arrival on the veal (0.2–0.8 kg/d, Timmerman et al., 2005; Pardon et al., 2015; Webb et al., 2015), which is likely a result of the restricted milk allowance typical for the European veal industry at the beginning of the fattening period (Pardon et al., 2015). Growth performance in veal calves is also negatively associated with multiple factors such as low BW and dehydration at arrival and poor health (Renaud et al., 2018b), all of which may be exacerbated by long transport (Rot et al., 2022).

It should be considered that in the current study, we measured blood variables immediately and 12 d after arrival on the veal farm. Although this allows us to conclude that most variables had returned to pre-transport levels by d 12 post-transport, it is likely that these changes occurred within the first hours to days after arrival. Calves have been reported to recover a positive energy balance within as little as 4 to 24 h (Knowles et al., 1999b; Marcato et al., 2020a) and a normal hydration status within the first week post-transport (Knowles et al., 1997; Marcato et al., 2020a). It is possible that the alternative feeding protocol advanced or accelerated calves' physiological recovery in the days following arrival, and further studies with more frequent sampling immediately following transport are needed to track recovery times and processes and the role of post-transport feeding protocols in aiding recovery. It would also be useful to compare behavioral expressions of hunger between feeding treatments to assess the adequacy of post-transport feeding protocols.

Limitations

Potential criticism of this study may relate to its experimental design, particularly transport durations and choice of feeding protocols. Because this study was deliberately conducted under commercial conditions, all calves were subjected to extended fasting periods (32 h followed by another 28 h the next day). Previous research has demonstrated that the negative effects of transport on calf welfare—including dehydration, negative energy balance, and exhaustion—worsen with longer transport durations and, consequently, longer fasting periods (Marcato et al., 2020b; Roadknight et al., 2021b). Under non-transport conditions, unweaned calves require at least 6 L of milk or MR per day to meet their metabolic needs (NASEM, 2021). Given these recommendations, both the pre-transport control (2 L) and alternative (6 L) feeding protocols are insufficient for calves undergoing long-distance transport. Similarly, the alternative post-transport treatment, although an improvement over the control treatment which reflects standard Dutch practices, still represents a restrictive feeding protocol (2 to 3.6 L twice daily). This is particularly concerning when considering that calves are already metabolically compromised from transport.

Conducting research in a commercial setting alongside multiple industry stakeholders—including livestock exporters, truck drivers, veal farmers, and the Dutch veal industry—limited experimental flexibility and introduced constraints on the study design. However, this approach also provided several key advantages. Although our feeding regimen was more restricted than in previous experimental transport studies, our control groups accurately reflect current commercial calf transport conditions in Europe, allowing us to assess the impacts of real-world industry practices. Research conducted in actual commercial conditions also ensures high external validity, meaning our findings are directly applicable to industry settings. It also allowed us to evaluate the feasibility of practical improvement strategies for commercial calf transport. Moreover, the collaborative approach of this study is likely to increase industry acceptance of our findings and facilitate implementing feasible recommendations for improvement. Given that many

management and feeding practices in veal farming have remained largely consistent over the years, meaningful change will require evidence-based, industry-supported interventions. Additionally, this study establishes an important baseline for both industry stakeholders and researchers, serving as a reference point for future comparisons with alternative feeding strategies. Future research should explore further improvements such as feeding higher volumes of MR, providing multiple feedings at rest stops and lairages or shortening rest periods, investigating different MR formulations as well as technology that would allow feeding warm MR to calves while loaded onto a truck or ferry.

CONCLUSIONS

This study demonstrates that pre-transport feeding protocols influence calf metabolism but do not fully prevent adverse effects of prolonged transport. Calves fed two 3-L milk replacer meals before commercial transport between Ireland and the Netherlands exhibited higher glucose levels, reduced fat and protein mobilization, and fewer signs of dehydration after 24 h compared to those fed a single 2-L meal. However, these benefits diminished as transport continued, with both groups showing signs of energy depletion, hypoglycemia, and dehydration. Thus, while increased pre-transport feeding offers clear improvements, it is still insufficient under current multiday transport conditions. Reducing the fasting interval during lairage by providing an additional feed or shortening the rest period may offer further welfare gains. Post-transport, a 25% increase in milk replacer for 3 wk improved growth, but normal metabolism and physiology were restored within 12 d regardless of post-transport feeding protocol. Overall, these findings highlight the challenges posed by current transport practices and emphasize the urgent need to optimize feeding strategies before, during, and after transport to better support calf welfare during long-distance journeys.

NOTES

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Nonstandard abbreviations used: AC = assembly center; BeefX = beef × Holstein-Friesian crossbreed; HEX = Hereford × Holstein-Friesian crossbreed; HF = Holstein-Friesian; MR = milk replacer; NEFA = non-esterified fatty acids; postALT = post-transport feeding protocol alternative; postCTRL = post-transport feeding protocol control; preALT = pre-transport feeding protocol alternative; preCTRL = post-transport feeding protocol control; RBC = red blood cells; S1 and S2 = shipment 1 and 2.

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