**Application.** This study emphasizes the importance of feeding unweaned calves adequate amounts of milk replacer before transport to reduce dehydration, but shows that long-distance transport compromises calf physiology regardless of age or pre-transport diet, highlighting the need for more comprehensive strategies to improve welfare during transportation.

**Introduction.** Long-distance transport poses many challenges to calf welfare and physiology, including extended fasting intervals, which may lead to depleted energy levels, dehydration, and elevated stress. Older calves with more body reserves may be able to better cope with long fasting periods, and feeding calves higher volumes of milk replacer before departure may aid in maintaining a positive energy balance and hydration status throughout transport. The aim of this study was to investigate how calf age and pre-transport diet affect the physiological status of unweaned calves during and after long-distance transport via ferry and road*.*

**Materials and Methods.** We followed a commercial shipment of 138 male calves (118 Holstein-Friesian, 20 Holstein-Friesian crossbreds) from an assembly centre (AC) in Ireland via road and ferry to a lairage (LA) in France (24.5h + 13h rest stop) and then via road to a veal farm (VF) in the Netherlands (13.5h). The study had a 2 x 2 factorial design with factor 1) calf age: 2-3 weeks (“younger”) or 4-5 weeks (“older”), and factor 2) pre-transport diet: calves were fed 2L or 4L of milk replacer the morning of transport. Blood samples were collected at the AC and LA, upon arrival at the VF and on Day 8 after arrival. Body weight (BW) was recorded at the same time points and on Day 22 after arrival. Blood samples were analysed for variables indicating energy balance (glucose, β-hydroxybutyrate, non-esterified fatty acids), hydration status (urea, total protein, albumin, sodium, potassium, chloride, haematocrit), muscle fatigue (creatine kinase, lactate), and stress (cortisol). Linear mixed models with age, diet, time points and their interactions as fixed effects and animals and farms of origin as random effects were used to analyse all variables.

**Results.** Younger calves had higher glucose levels than older calves at the AC (4.51 ± 0.09 vs 4.16 ± 0.09 mmol/L, p < 0.01), arrival on the VF (4.52 ± 0.09 vs 4.13 ± 0.09 mmol/L, p < 0.01), and Day 8 (5.59 ± 0.09 vs 5.28 ± 0.09 mmol/L, p = 0.01). Calves fed 2L had higher urea levels at the LA than calves fed 4L (4.01 ± 0.17 vs 3.10 ± 0.13 mmol/L, p < 0.001). Younger calves had lower sodium levels at arrival on the VF (139.9 ± 0.3 vs 140.8 ± 0.3 mmol/L, p= 0.03), and lower chloride levels at the AC (97.41 ± 0.29 vs 99.01 ± 0.29 mmol/L,p < 0.001) and LA (96.60 ± 0.28 vs 97.50 ± 0.29 mmol/L, p < 0.01). Indicators of muscle fatigue and stress were not affected by age or diet. Age had an overall effect on BW, with younger animals weighing less than older animals over the entire study period (49.8 ± 0.9 vs 53.0 ± 0.9 kg, p < 0.01), but neither age nor diet affected changes in BW between time points. Mean values for all treatments were outside reference limits for healthy calves for 7/13 blood variables at AC (and glucose for older calves), 9/13 at LA (and urea for 2L calves), 7/13 at arrival (and glucose for older calves, potassium for 2L calves), and 2/13 on Day 8 after arrival (and creatine kinase for older calves).

**Conclusions.** Calves were notably challenged by long-distance transport, with more than half of all blood variables outside reference limits during transport regardless of treatment, but normal calf physiology was mostly restored by Day 8 after transport. Calf age affected energy balance, hydration status and BW: younger calves had higher glucose levels and showed fewer signs of dehydration (lower levels of electrolytes) during transport but weighed less throughout the study period. Feeding calves 4L of milk replacer instead of 2L before transport had limited positive effects on their hydration status: urea levels were higher and outside reference limits for calves only fed 2L after ferry transport, but none of the other hydration variables were affected by diet.